Cover: Georgette van Oosten

ISBN: 90-8559-021-3

Printed by Optima Grafische Communicatie, Rotterdam

Clinical research in HIV-1 infected children

Patiëntgebonden onderzoek bij HIV-1 geïnfecteerde kinderen

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof.dr. S.W.J. Lamberts
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
woensdag 26 januari 2005 om 15:45 uur
door

Pieter Leendert Alex Fraaij

geboren te Delft

Promotiecommissie

Promotor:	Prof.dr. R. de Groot
Overige leden:	Prof.dr. J.J.M. van Dongen
	Prof.dr. A.D.M.E Osterhaus
	Dr. D.M. Burger

The studies described in this thesis were financially sponsored by the Aidsfonds, Netherlands, Merck Sharp & Dohme, The Netherlands, Abbott, The Netherlands and GlaxoSmithKline, The Netherlands

Printing of this thesis was financially supported by:
GlaxoSmithKline B.V.
Abbott B.V.
Boehringer Ingelheim B.V.
Merck Sharp & Dohme B.V.
Bristol-Myers Squibb B.V.

Contents

Chapter 1	**General Introduction**	7
Chapter 2	**Outline of this thesis**	13
	Part one: Clinical, virological and immunological aspects of Highly Active Antiretroviral Therapy in HIV-1 infected children	
Chapter 3	Efficacy of highly active antiretroviral therapy in HIV-1 infected children. *A.M.C. van Rossum, P.L.A. Fraaij, R. de Groot*	19
Chapter 4	Sustained viral suppression and immune recovery after 4 years treatment of HIV-1 infected children with HAART *P.L.A. Fraaij, G. Verweel, A.M.C. van Rossum, E.G. van Lochem, M. Schutten, C.M.R. Weemaes, N.G. Hartwig, D.M. Burger, R. de Groot*	43
Chapter 5	Indinavir/low dose ritonavir containing HAART in HIV-1 infected children has potent antiretroviral activity, but is associated with side effects and frequent discontinuation of treatment. *P.L.A. Fraaij, G.Verweel, A.M.C. van Rossum, N.G. Hartwig, D.M. Burger, R. de Groot*	53
Chapter 6	Safety and efficacy of an abacavir based HAART regimen after pre-treatment with protease inhibitors in children with an undetectable HIV-1 viral load. *P.L.A. Fraaij, A.S. Bergshoeff, G. Verweel, L. van der Knaap, A.M.C. van Rossum, N.G. Hartwig, D.M. Burger, R. de Groot*	61
Chapter 7	Safety and efficacy of a NRTI-sparing HAART regimen of efavirenz and lopinavir/ritonavir in HIV-1 infected children *P.L.A. Fraaij, J. Neubert, A.S. Bergshoeff, A.M.C. van Rossum, N.G. Hartwig, H. Schroten, K.Korn, D.M. Burger, R. de Groot, T. Niehues*	73
Chapter 8	Initiation of highly active antiretroviral therapy leads to an HIV specific immune response in a seronegative infant. *P.L.A. Fraaij, A.M.C. van Rossum, K.C. Wolthers, A.C.T.M. Vossen, Y.M. Jeucken, J.H.S.A.M. Kuijpers, E.G. van Lochem, M.T.L. Roos, R. de Groot, N.G. Hartwig*	79
Chapter 9	The CD38 expression level on CD8+ T-cells does not predict viral response to HAART in HIV-1 infected children. *P.L.A. Fraaij, A.M.C. van Rossum, K.C. Wolthers, J.H.S.A.M. Kuijpers, C.M.R. Weemaes, A.M. van Furth, N.G. Hartwig, H. Hooijkaas, R. de Groot, E.G. van Lochem*	83
	Part two: Pharmacology of antiretroviral agents in children with HIV-1 infection	
Chapter 10	Therapeutic drug monitoring in children with HIV/AIDS *P.L.A. Fraaij, N. Rakhmanina, D.M. Burger, R. de Groot*	99
Chapter 11	A new method for analysis of AZT-triphosphate and nucleotide-triphosphates *J.J.A. van Kampen, P.L.A. Fraaij, V. Hira, A.M.C. van Rossum, N.G. Hartwig, R. de Groot, T. Luider*	109

Contents

Chapter 12	Pharmacokinetics of nelfinavir in children: Influencing factors and dose implications *A.S. Bergshoeff, P.L.A. Fraaij, A.M.C. van Rossum, T.W.F. Wolfs, S.P.M. Geelen, R. de Groot, D.M. Burger*	121
Chapter 13	Pharmacokinetics of indinavir combined with low-dose ritonavir in Human Immunodeficiency Virus type 1-infected children *A.S. Bergshoeff, P.L.A. Fraaij, A.M.C. van Rossum, G. Verweel, L.H. Wynne, G.A. Winchell, R.Y. Leavitt, B-Y. T. Nguyen, R. de Groot, D.M. Burger*	135
Chapter 14	Increased dose of lopinavir/ritonavir compensates for efavirenz-induced drug-drug interaction in HIV-1 infected children. *A.S. Bergshoeff, P.L.A. Fraaij, J. Neubert, G. Verweel, N.G. Hartwig, T. Niehues, R de Groot and D.M. Burger*	145
Chapter 15	Plasma levels of zidovudine twice compared to thrice daily in six HIV-1 infected children *A.S. Bergshoeff, P.L.A. Fraaij, C. Verweij, A.M.C. van Rossum, G.Verweel, N.G. Hartwig, R. de Groot, D.M. Burger*	159
Chapter 16	Changes in indinavir exposure over time: a case study in six HIV-1 infected children *P.L.A. Fraaij, A.S. Bergshoeff, A.M.C. van Rossum, N.G. Hartwig, D. Burger, R. de Groot*	167
Chapter 17	Pharmacokinetics of antiretroviral therapy in HIV-1 infected children *P.L.A. Fraaij, J.J.A. van Kampen, D.M. Burger, R. de Groot*	175
	Part three: Psychosocial aspects of the treatment of HIV-1 infected children	
Chapter 18	An analysis of the social situation of HIV-1 affected families *P.L.A. Fraaij, J.B.G. Rijkschroeff, L. van der Knaap, N.G. Hartwig, R. de Groot*	205
Chapter 19	A guideline on interventions in HIV-1 infected children with therapy failure *P.L.A. Fraaij, R de Groot, J.B.G. Rijkschroeff, L. van der Knaap, M.J. Blondeau, T. Segers, J.L. van Es, N.G. Hartwig*	215
Chapter 20	**Summary and discussion**	225
Chapter 21	**Conclusions and future perspectives**	235
Chapter 22	**Samenvatting**	241
	Dankwoord	247
	Curriculum Vitae	250
	List of publications	251

General introduction

Chapter 1

General introduction

Acquired immune deficiency syndrome (AIDS) was described for the first time in 1981. Two years later the previously unknown human immunodeficiency virus (HIV) was identified as the causative agent. (1-3) HIV has been included in the genus *Lentiviruses* of the Retroviridae family. Two types are recognized: HIV-1 and HIV-2. Of these, HIV-1 is the primary etiologic agent of the current pandemic. HIV probably originates from simian immunodeficiency virus (SIV) which is endemic in African monkey species. Cross species transition may have occurred trough preparation and eating of monkey meat. Even today more than one-fifth of the monkey meat sold in the markets of Cameroon is infected with SIV. (4) The available evidence suggests that SIV entered the human population from multiple zoonotic infections. The last common ancestor of the main group of HIV-1 is dated in the first quarter of the twentieth century. (5) Since the eighties of last century a devastating pandemic has developed. At the end of 2003, 40 million people were infected by HIV/AIDS of which 5 million people had been newly infected in that year alone. Ninety-five percent of the new infections occur in the developing countries and 50% in women with child-baring potential. (6) Since mother to child transmission (MTCT) is the main route for transmission of HIV-1 in children, the high number of HIV infected mothers imposes a global health thread to children. Indeed in 2003, 500,000 children died from HIV/AIDS and another 700,000 were newly infected. Besides imposing a direct health risk to children HIV also causes major social and economic dilemmas. HIV mostly affects young adults, killing one or both parents of the children of AIDS victims. Between 10 and 15 million children have become orphans. Hence by destroying human capital and the mechanisms that generate human capital formation HIV/AIDS undermines the basis of economic growth. If nothing is done to fight the current epidemic HIV-affected countries face economic collapse. In addition, children and families affected by AIDS often face rejection and social isolation. (6)

MTCT of HIV can occur before (intrauterine), during (intrapartum) or after delivery through breastfeeding. Most infections occur intrapartum (60-75 %). (7-9) In the western world reported MTCT rates in untreated HIV-infected women are between 15 to 25%. In developing countries this percentage is higher ranging from 21 to 43%. This difference is thought to be caused by breastfeeding and more frequent occurrence of untreated sexually transmittable diseases in mothers in developing countries. (10) The maternal HIV viral load at delivery is the most important predictor of vertical transmission. In the western world the widespread use of zidovudine to suppress the viral load and to prevent vertical transmission has resulted in a decrease of perinatal transmission to 4%. (11) The subsequent use of Highly Active Antiretroviral Therapy (HAART) has reduced MTCT rates to below 1%. (12) A breakthrough for the developing world may be the use of nevirapine to prevent MTCT. In the HIVNET study the use of nevirapine administered once to the mother and once to the child resulted in a reduction of MTCT risk of nearly 50% during the first 14-16 weeks of life. (13) Unfortunately, despite the development of cheaper and easier to use medication antiretroviral therapy regimens to prevent MTCT and screening facilities for pregnant women are still not widely available in

developing countries. This is mostly because of budgetary constraints. Therefore MTCT will remain a problem in the near future and preventable HIV infections will continue to occur.
Disease progression to AIDS is more rapid in children than in adults. Early cohort studies have shown that 20 to 25% of the HIV infected children die within the first 2 years of age. (14, 15) This rapid progression correlates with a higher viral burden and faster depletion of CD4+ T-cells in infants and children than in adults. Without therapy most pediatric patients will die before adolescence. (16-19) However, since the first description of AIDS in children in 1983 the face of the disease in the industrialized world has changed drastically. The introduction of HAART has resulted in reduction of disease progression to AIDS and an improved life expectancy for HIV-1 infected children. (18, 20) Antiretroviral therapy can be divided in four different classes according to their mode of action: nucleotide reverse transcriptase inhibitors (NRTI), non nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI) and fusion inhibitors (FI). HAART generally consists of three or more antiretroviral drugs. Normally a PI and/or NNRTI combined with two NRTI, although no strict definition exists. The same medication is used in children as in adults, although antiretroviral drugs are often not registered for (young) children and are not available in child friendly formulation.

The use of HAART in children results in viral load suppression and in normalisation of the CD4+ T-cell counts independent of age. (21-25) However, the virological response rates in children are frequently inferior to those observed in adults. (26-32) This is probably due to the complexity of institution of HAART in children. Ample room for improvement still exists. First, dosing of antiretroviral drugs is difficult by changes in pharmacokinetic parameters due to growth and development. Furthermore, the large interpatient and intrapatient variability in the pharmacokinetics of antiretroviral drugs complicate optimal dosing. In addition, pediatric dose recommendations are not available for some of the newer antiretroviral drugs. Moreover, if available dose recommendations in the different guidelines show substantial differences. Secondly, adherence to the medication regimen is difficult to maintain. Complicated dosing regimens, poor palatability, intake of medication during sleeping time or school hours, food requirements, side effects, unwillingness of young children and adolescents to take the medication, and psychosocial issues related to HIV-infection need to be dealt with. New approaches for the treatment of HIV-1 infected children should deal with these problems to ensure maintenance of therapy success in the future. Easier to take and more potent antiretroviral medication has been developed in recent years and can improve future treatment results. In addition, new methods such as therapeutic drug monitoring (TDM) should be evaluated in children. Novel combinations of HAART need to be developed for use in children with HIV resistant to the conventional HAART regimens. Adherence to therapy needs to be ensured through education of the patients and social support of families affected by HIV/AIDS. Finally, a structured approach to therapy failure should be developed to ensure quick and optimal support of the patient and to prevent the development of viral resistance.

The complicated problems indicated above have been recognized in the multidisciplinary study group for HIV-1 infected children, the Rotterdam pediatric HIV cohort. Children in the

Rotterdam cohort were enrolled from the outpatient clinics of the Erasmus MC-Sophia Children's Hospital Rotterdam, University Medical Center St. Radboud and the VU medical Center Amsterdam.

New treatment protocols were developed and implemented to optimize HAART in children in the Rotterdam pediatric HIV cohort. In addition, studies were performed in collaboration with the Heinrich Heine University in Düsseldrof, Germany. This thesis contains the results of these studies on the clinical care and management of HIV-1 infected children.

References

1. Gottlieb MS, Schroff R, Schanker HM, Weisman JD, Fan PT, Wolf RA, et al. Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. N Engl J Med 1981;305(24):1425-31.
2. Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT, Chamaret S, Gruest J, et al. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science 1983;220(4599):868-71.
3. Gallo RC, Sarin PS, Gelmann EP, Robert-Guroff M, Richardson E, Kalyanaraman VS, et al. Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). Science 1983;220(4599):865-7.
4. Peeters M, Courgnaud V, Abela B, Auzel P, Pourrut X, Bibollet-Ruche F, et al. Risk to human health from a plethora of simian immunodeficiency viruses in primate bushmeat. Emerg Infect Dis 2002;8(5):451-7.
5. Korber B, Muldoon M, Theiler J, Gao F, Gupta R, Lapedes A, et al. Timing the ancestor of the HIV-1 pandemic strains. Science 2000;288(5472):1789-96.
6. UNAIDS. AIDS epidemic update: 2003. Geneva: UNAIDS/WHO; 2003 december 2003.
7. Mock PA, Shaffer N, Bhadrakom C, Siriwasin W, Chotpitayasunondh T, Chearskul S, et al. Maternal viral load and timing of mother-to-child HIV transmission, Bangkok, Thailand. Bangkok Collaborative Perinatal HIV Transmission Study Group. AIDS 1999;13(3):407-14.
8. Chouquet C, Burgard M, Richardson S, Rouzioux C, Costagliola D. Timing of mother-to-child HIV-1 transmission and diagnosis of infection based on polymerase chain reaction in the neonatal period by a non-parametric method. AIDS 1997;11(9):1183-4.
9. Bertolli J, St Louis ME, Simonds RJ, Nieburg P, Kamenga M, Brown C, et al. Estimating the timing of mother-to-child transmission of human immunodeficiency virus in a breast-feeding population in Kinshasa, Zaire. J Infect Dis 1996;174(4):722-6.
10. Rates of mother-to-child transmission of HIV-1 in Africa, America, and Europe: results from 13 perinatal studies. The Working Group on Mother-To-Child Transmission of HIV. J Acquir Immune Defic Syndr Hum Retrovirol 1995;8(5):506-10.
11. Connor EM, Sperling RS, Gelber R, Kiselev P, Scott G, O'Sullivan MJ, et al. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N Engl J Med 1994;331(18):1173-80.
12. Ioannidis JP, Abrams EJ, Ammann A, Bulterys M, Goedert JJ, Gray L, et al. Perinatal transmission of human immunodeficiency virus type 1 by pregnant women with RNA virus load <1000 copies/ml. J Infect Dis 2001;183(4):539-45.
13. Musoke P, Guay LA, Bagenda D, Mirochnick M, Nakabiito C, Fleming T, et al. A phase I/II study of the safety and pharmacokinetics of nevirapine in HIV-1-infected pregnant Ugandan women and their neonates (HIVNET 006). AIDS 1999;13(4):479-86.
14. Tovo PA, de Martino M, Gabiano C, Cappello N, D'Elia R, Loy A, et al. Prognostic factors and survival in children with perinatal HIV-1 infection. The Italian Register for HIV Infections in Children. Lancet 1992;339(8804):1249-53.
15. Dunn D. Short-term risk of disease progression in HIV-1-infected children receiving no antiretroviral therapy or zidovudine monotherapy: a meta-analysis. Lancet 2003;362(9396):1605-11.

16. Scott GB, Hutto C, Makuch RW, Mastrucci MT, O'Connor T, Mitchell CD, et al. Survival in children with perinatally acquired human immunodeficiency virus type 1 infection. N Engl J Med 1989;321(26):1791-6.
17. Buehler JW, Berkelman RL, Curran JW. Reporting of AIDS: tracking HIV morbidity and mortality. JAMA 1989;262(20):2896-7.
18. Gortmaker SL, Hughes M, Cervia J, Brady M, Johnson GM, Seage GR, 3rd, et al. Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. N Engl J Med 2001;345(21):1522-8.
19. Palumbo PE, Raskino C, Fiscus S, Pahwa S, Fowler MG, Spector SA, et al. Predictive value of quantitative plasma HIV RNA and CD4+ lymphocyte count in HIV-infected infants and children. JAMA 1998;279(10):756-61.
20. de Martino M, Tovo PA, Balducci M, Galli L, Gabiano C, Rezza G, et al. Reduction in mortality with availability of antiretroviral therapy for children with perinatal HIV-1 infection. Italian Register for HIV Infection in Children and the Italian National AIDS Registry. JAMA 2000;284(2):190-7.
21. Starr SE, Fletcher CV, Spector SA, Yong FH, Fenton T, Brundage RC, et al. Combination therapy with efavirenz, nelfinavir, and nucleoside reverse-transcriptase inhibitors in children infected with human immunodeficiency virus type 1. Pediatric AIDS Clinical Trials Group 382 Team. N Engl J Med 1999;341(25):1874-81.
22. Vigano A, Dally L, Bricalli D, Sala N, Pirillo M, Saresella M, et al. Clinical and immuno-virologic characterization of the efficacy of stavudine, lamivudine, and indinavir in human immunodeficiency virus infection. J Pediatr 1999;135(6):675-82.
23. van Rossum AM, Geelen SP, Hartwig NG, Wolfs TF, Weemaes CM, Scherpbier HJ, et al. Results of 2 years of treatment with protease-inhibitor--containing antiretroviral therapy in dutch children infected with human immunodeficiency virus type 1. Clin Infect Dis 2002;34(7):1008-16.
24. Saez-Llorens X, Violari A, Deetz CO, Rode RA, Gomez P, Handelsman E, et al. Forty-eight-week evaluation of lopinavir/ritonavir, a new protease inhibitor, in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2003;22(3):216-224.
25. van Rossum AM, Scherpbier HJ, van Lochem EG, Pakker NG, Slieker WA, Wolthers KC, et al. Therapeutic immune reconstitution in HIV-1-infected children is independent of their age and pretreatment immune status. AIDS 2001;15(17):2267-75.
26. Mueller BU, Sleasman J, Nelson RP, Jr., Smith S, Deutsch PJ, Ju W, et al. A phase I/II study of the protease inhibitor indinavir in children with HIV infection. Pediatrics 1998;102(1 Pt 1):101-9.
27. Pelton SI, Johnson D, Chadwick E, Baldwin Z, Yogev R. A one year experience: T cell responses and viral replication in children with advanced human immunodeficiency virus type 1 disease treated with combination therapy including ritonavir. Pediatr Infect Dis J 1999;18(7):650-2.
28. Thuret I, Michel G, Chambost H, Tamalet C, Giraud P, Brunet C, et al. Combination antiretroviral therapy including ritonavir in children infected with human immunodeficiency. AIDS 1999;13(1):81-7.
29. Melvin AJ, Mohan KM, Arcuino LA, Edelstein RE, Frenkel LM. Clinical, virologic and immunologic responses of children with advanced human immunodeficiency virus type 1 disease treated with protease inhibitors. Pediatr Infect Dis J 1997;16(10):968-74.
30. Wintergerst U, Hoffmann F, Solder B, al e. Comparison of two antiretroviral triple combinations including the protease inhibitor indinavir in children infected with human immunodeficiency virus. Pediatr Infect Dis J 1998;17:495-9.
31. Jankelevich S, Mueller BU, Mackall CL, Smith S, Zwerski S, Wood LV, et al. Long-term virologic and immunologic responses in human immunodeficiency virus type 1-infected children treated with indinavir, zidovudine, and lamivudine. J Infect Dis 2001;183(7):1116-20.
32. Teglas JP, Quartier P, Treluyer JM, Burgard M, Gregoire V, Blanche S. Tolerance of efavirenz in children. AIDS 2001;15(2):241-3.

Outline of this thesis

Chapter 2

Outline of this thesis

In **part one** selected clinical aspects of HAART in HIV-1 infected children are described. **Chapter 3** reviews the efficacy of HAART in children. In **chapter 4** long term data on the clinical efficacy of PI use in HIV-1 infected children are described. Although HAART is efficacious in children viral failure may occur. This failure is often associated with non-adherence to the medication. Therefore easier to use regimens have been developed. The clinical parameters of an indinavir, ritonavir boosted regimen are described in **chapter 5** and of an abacavir containing regimen in **chapter 6**. Despite efforts to prevent viral failure resistance to antiretroviral therapy does develop in HIV-1 infected children. In **chapter 7** the efficacy of a salvage regimen consisting of lopinavir/ritonavir and efavirenz for children infected with viruses which are highly resistant to NRTI is described.

In **chapter 8** we describe that the initiation of HAART leads to a HIV specific immune response in an initial seronegative child. The CD38 expression level on CD8+ T-cells as a marker for therapy outcome of HAART is evaluated in **chapter 9**.

Part two contains several studies on the pharmacokinetics of antiretroviral agents in HIV-1 infected children. This is an important issue in the treatment of HIV-1 infection, because the level of viral suppression and drug toxicity are associated with the plasma drug concentrations. An overview of studies on the use of Therapeutic Drug Monitoring (TDM) in HIV-1 infected children is presented in **chapter 10**. Two of the three classes of antiretroviral agents are suitable for TDM: the NNRTIs and the PIs, but not the NRTIs. NRTIs are intracellularly converted to their active NRTI-triphosphate form. Intracellular NRTI triphosphate levels may correlate better with antiviral efficacy than NRTI plasma levels. However, the methods developed for the analysis of intracellular NRTI levels require large volumes of blood and are therefore not suitable for use in children. We present a novel method using MALDI-TOF mass spectrometry to measure intracellular NRTI-TP levels in **chapter 11**.

Risk factors for subtherapeutic plasma levels of the PI nelfinavir are described in **chapter 12**. In **chapter 13** we discuss the pharmacokinetic profile of the combination of indinavir with low-dose ritonavir in children. The development of resistance of HIV to different antiretroviral regimens necessitates the development of new combinations antiretroviral drugs to form HAART. In **chapter 14** we describe the pharmacokinetics of such a regimen: lopinavir/ritonavir combined with efavirenz. Currently no pharmacokinetic data are available on two or three times daily zidovudine use in children. In **chapter 15** the pharmacokinetics of zidovudine used three times daily are compared to those of zidovudine used twice daily. Changes in indinavir exposure over time are studied in **chapter 16.** In the final chapter of part 2, **chapter 17** we review the currently available data on the pharmacokinetics of antiretroviral drugs in children.

Children and families affected by AIDS often face rejection and social isolation. In addition, in the Rotterdam cohort most patients are not of Dutch origin and some stay in the Netherlands illegally. **Part three** of this thesis deals with social aspects of the treatment of children with

HIV/AIDS in the Rotterdam cohort. **Chapter 18** reports on the social support structure of the families of HIV-1 infected children. In **chapter 19** we present a treatment protocol to improve adherence to HAART. In this protocol treatment is optimized by changes in medication as well as the introduction of social support to the families.

Chapters 20 summarizes the above described studies and brings the data in perspective. In **chapter 21** we briefly present the conclusions of our studies and discuss new ideas concerning the future directions of care and research in children with HIV/AIDS. Finally, in **chapter 22** the findings presented in this thesis are summarized in Dutch.

Part one

Clinical, virological and immunological aspects of Highly Active Antiretroviral Therapy in HIV-1 infected children

Efficacy of highly active antiretroviral therapy in HIV-1 infected children

Annemarie M.C. van Rossum, Pieter L.A. Fraaij, Ronald de Groot

Lancet Infect Dis 2002;2(2):93-102

Chapter 3

Abstract

Introduction: Although the reduction in HIV-1-related deaths is similar in adults and children, the extent of the changes in two important surrogate markers (HIV-1 RNA levels and CD4+ T-cell counts) differs widely. In most pediatric studies virological response rates to highly active antiretroviral therapy (HAART) are inferior to those in adults. This review provides an overview of the pediatric clinical studies using HAART and seeks to improve the understanding of factors, which may contribute to success or failure of HAART in children.

Methods: An overview of all current articles on pediatric clinical trials using HAART is provided. Twenty-three papers were available. HIV-1 RNA loads and CD4+ T-cell counts were used as primary outcome measures.

Results: Virological response rates were highly variable both between the different antiretroviral drugs but also between different studies using the same medication. Four studies in which dosages of the administrated PI were adjusted after pharmacokinetic evaluation had superior virological response rates compared to those in which fixed dosages were used. Immunological response rates were more uniform than virological responses. In almost all studies increases of CD4+ T-cell counts are reported independent of the extent of the virological response. Side-effects of HAART were generally mild, transient and of gastro-intestinal origin. Significant percentages of patients with serum lipid abnormalities were reported in three pediatric studies. However, signs of clinical lipodystrophy were not observed.

Conclusion: The inferior virological response rates, which have been reported in HIV-1 infected children treated with HAART form a reflection of the challenges which are encountered in the treatment of these children. Difficulties with adherence and with the pharmacokinetics of protease inhibitors in children require an intensive, child adjusted approach. A practical approach to therapy in institutions without tertiary top-reference care facilities may be induction therapy with a lopinavir containing regimen to reduce high viral load levels followed by an easily tolerated maintenance regimen for example containing abacavir or nevirapine.

Introduction

Since the introduction of highly active antiretroviral therapy (HAART) a reduction in the rate of progression of AIDS and HIV-1-related deaths has been observed among adults living in the Western world. (1,2) The effectiveness of HAART in infants and children to reduce HIV-1-related deaths is at least similar, or even greater than that observed in adults. (1)

The measurement of two surrogate markers, HIV-1 RNA levels and CD4+ T-cell counts, has become the basis for the prediction of clinical, virological and immunological responses in both HIV-1 infected adults and children treated with HAART. (2,3) Although the reduction in HIV-1-related deaths is similar in adults and children, the extent of the changes in these parameters differs widely. In most pediatric studies virological response rates to HAART are inferior to those in adults. (4-12) Since virological suppression is associated with long-term success of HAART, this may have major implications for the future health of these children. (13-15)

The institution of optimal treatment regimens in HIV-1 infected children poses an enormous challenge. First, large interindividual differences of pharmacokinetics of antiretroviral drugs, especially of protease inhibitors, complicate optimal dosing of antiretroviral drugs in children. Secondly, it is difficult to maintain adherence to combination therapy during many years. Problems such as the intake of evening medication during sleeping time or afternoon medication during school, unwillingness of young children and adolescents to take medication, poor palatability and side-effects of medication have to be dealt with. Thirdly, different viral dynamics may complicate optimal suppression of HIV-1. Viral load reduction in children following the introduction of HAART has a slower phase II decay rate in children in comparison with adults. (16) Baseline viral loads in children are higher, which may be a barrier to reach undetectable viral loads. (17-19)

Despite the difficulties in the treatment of HIV-1 infected children and the poor virological responses in many studies, it has become clear that a similar rate of suppression of HIV-1 replication may be obtained in children during the first year of treatment as compared to that in adults. (20-26) The reasons for the highly variable therapy results in children are not well understood. This review provides an overview of the pediatric clinical studies using HAART and seeks to improve the understanding of factors, which may contribute to success or failure of HAART in children.

Methods

An overview of all current articles on pediatric clinical trials using HAART is provided. Search strategy: The Pubmed database (www.ncbi.nlm.nih.gov) was used to search for articles on pediatric clinical trials using HAART.
Selection criteria: articles not written in English were excluded. No selection on date was made. Articles with text word combinations: HIV-1 and children, HIV-1 infection and children, ritonavir and children, indinavir and children, nelfinavir and children, saquinavir and children, nevirapine and children and abacavir and children were selected.

Twenty-three papers were available. HIV-1 RNA loads and CD4+ T-cell counts were used as primary outcome measures.

Ritonavir

In March 1996, ritonavir (RTV) was the first protease inhibitor approved by the US Food and Drug Administration (FDA) for the treatment of HIV-1 infected children (age 2 to 16 years). RTV has been available in a pediatric formulation (liquid) and leads when used as a single agent to a marked and rapid decline of plasma HIV-1 RNA levels. (27) However, the HIV-1 RNA levels gradually return toward baseline values after a few weeks of treatment, presumably because of the development of resistance. Virological response rates were sustained for a 24-week period after administration of the highest dose (400 mg/m^2 q12h). (27) The virological response rates of combinations of RTV with one or two nucleosides were also modest with 16%, 14%, 32% and 42% below 400 copies HIV-1 RNA/ml after 24, 52, 72 and 48 weeks respectively. (10,11,22,27) CD4+ T-cells significantly increased in most children irrespective of the extent of the virological suppression. Toxicity by RTV consisted mainly of gastro-intestinal symptoms (nausea, vomiting). These symptoms were frequently reported as mild and transient although in 4-23% more severe gastro-intestinal symptoms, fever or rash were observed. (10,11,22,27) Thuret et al. reported an increase in the serum levels of triglycerides and cholesterol of 33% and 61% respectively after at least 12 months of therapy, although no clinical signs of lipodystrophy were reported. (11) In Table 1 the study results with RTV are summarized. The poor virological efficacy in children, the serious side-effects which are seen in a high percentage of the children and the poor taste of the liquid formulation, form in important disadvantages in the use of this PI.

Indinavir

In March 1996 the protease inhibitor indinavir (IDV) was registrated by the FDA for use in HIV-1 infected adults. Although IDV thus far only has been available in a capsule formula, it is possible to dissolve IDV in water for use in infants and young children. (24) Indinavir has to be administered to a fasting child at least two hours before or one hour after the intake of food.
Virological response rates of IDV combination therapy with two NRTIs differ widely. Mueller et al. reported that 6% of their 54 patients had an HIV-1 RNA load below the detection limit of 200 copies/ml after 16 weeks. 28 After 96 weeks only 4 (12%) of the 33 patients that completed a follow-up of 96 weeks. (29) This very low response rate was probably due to the administration of IDV as monotherapy during the first 12 weeks. Van Rossum et al. and Vigano et al. reported higher virological response rates: 70% <500 copies/ml and 87% <400 copies/ml after 24 and 72 weeks respectively. (20, 24) In all these studies irrespective of the virological response rate immunological improvement was reported. Renal side-effects by IDV due to crystallisation of indinavir in the kidney were reported in 11 to 80% of the patients and varied from crystalluria and hematuria to nephrolithiasis. Nephrolithiasis was documented in 2 to 28% of the children. (4,5,7-9,20,24,28,29) Gastro-intestinal symptoms formed the other major side-effects of indinavir. (4,5,7-9,20,24,28,29)

Van Rossum et al. demonstrated that the administration of IDV with low-dose ritonavir in a twice daily regimen results in a higher AUC and in higher trough levels of indinavir independent of food intake. (30) Pediatric studies on the efficacy of a twice daily regimen with indinavir and low-dose ritonavir in children are ongoing. In Table 2 the pediatric studies with IDV are summarized. The results of these studies differ widely. The use of IDV has been associated with a virological response rate which is comparable to that obtained in studies with this protease inhibitor in adults. However, nephrotoxicity is a major side-effect of IDV. Since this side-effect is associated with high drug levels of IDV, pharmacokinetic monitoring is necessary to reduce the risk for nephrotoxicity. (31) Urinalysis should be routinely performed in children treated with IDV. Frequent dosing and food restrictions may result in poorer adherence. The combination of IDV and low-dose RTV results in a simplified regimen and should therefore be further explored in children.

Nelfinavir

Nelfinavir (NFV) was the third protease inhibitor approved by the FDA for use in HIV-1 infected children in March 1997. NFV has been available in a pediatric formulation (powder 50 mg/g). NFV has to be administered with food to guarantee an optimal absorption. NFV has been studied in combination with two NRTIs and with two NRTIs and a NNRTI. (21,23,32) The virological response rates in children treated with a combination of NFV and 2 NRTIs vary from 69% <400 copies/ml and 44% <50 copies/ml in an intention to treat analysis (23) to 44% <400 copies/ml (33). Administration of NFV plus a NNRTI (nevirapine or efavirenz) and two NRTIs is associated with excellent virological responses. (21,33,34) Wiznia et al compared the effectiveness of NFV, NVP, d4T and 3TC to NFV, d4T and 3 TC. The virological and immunological responses were superior with the four-drug regimen. (21,33,34) Moderate to severe (≥ grade 2) side-effects were frequently observed in 25% to 77% of the patients. Adverse events included diarrhea, nausea, vomiting and rash. A transient or persistent increase in the serum levels of triglycerides or cholesterol was observed in 50% and 30% respectively, although no clinical signs of lipodystrophy were reported.

Schuster et al. and Wiznia et al. reported that the administration of NFV in a twice daily regimen results in pharmacokinetic parameters which are comparable to those found in a three times daily regimen. (33,34) However, pediatric studies on the efficacy of a twice daily regimen are not available. In Table 3 the pediatric studies with NFV are summarized. Nelfinavir is the only protease inhibitor with a pediatric formulation (powder) that is easy to use in very young infants as well as in older children. However, the co-administration with food may result in problems in children that refuse to eat or in children that have to be awakened for an evening dose. Again, the study results with NFV differ widely. In one study a virological response rate was demonstrated comparable to that obtained in adults. Combination treatment of NFV with EFV resulted in an excellent virological response rate. However, when children fail on this regimen, few alternatives are left, because of cross-resistance within these groups of antiretroviral medication.

Table 1. Pediatric studies using ritonavir (RTV).

Author	Regimen	RTV dose (q12h)	Inclusion criteria	Number of patients	Follow –up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Mueller et al.	RTV+ after 12 weeks ZDV and/or ddI	250, 300, 350 or 400 mg/m^2	Age 0.5-18 years, Naive, CDC stage 2, 3, B or C	48	24	↓ 2 $\log_{10}$ cop/ml (with 400 mg/m^2) 16% <200 cop/ml	Med ↑ 79 cells/mm^3	Mild and transient nausea, diarrhea, abdominal pain (98%)	27
Pelton et al.	RTV+d4T or ZDV with of without 3TC	350 mg/m^2	PI naive, HIV-1 RNA> 50,000 copies/ml and/or severely ↓ CD4+ cell count	43	52	↓ 1.69 $\log_{10}$ cop/ml 14% <400 cop/ml	Med ↑ 588 cells/mm^3	Vomiting, ↑ liver enzymes (9%) necessitating discontinuation	10
Thuret et al.	RTV+ZDV or d4T+3TC	350-400 mg/m^2	PI naive	22	72	↓ 1.5 $\log_{10}$ cop/ml 32% <400 cop/ml	Med ↑ 472 cells/mm^3	4% gastro-intestinal symptoms necessitating discontinuation ↑ triglycerides or cholesterol in 33% and 61% respectively	11
Nachmann et al.	RTV+ZDV+3TC or d4T/RTV or AZT/3TC	350 mg/m^2	age 2-17 years, PI naive, Clinically stable	297	48	42% <400 cop/ml (RTV/ZDV/3TC) and 27% (RTV/d4T)	Med ↑ 818 cells/mm^3 =33% of total T-cells (RTV/ZDV/3TC) Med ↑ 767 cells/mm^3 =29% of total T-cells (RTV/d4T)	Grade 3: 23% (RTV/d4T) and 17% (RTV/ ZDV/3TC): nausea, vomiting, rash, fever, neutropenia	22

Table 2. Pediatric studies using Indinavir (IDV).

Author	Regimen	IDV dose (q8h)	Inclusion criteria	Number of patients	Follow -up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Rutstein et al.	IDV+2 NRTIs (n=19) or RTV+2 NRTIs (n=9)	480 (range: 333-571) mg/m^2	PI naive	28 (19 on IDV)	24	↓ 1.99 log_{10} cop/ml (IDV =RTV) 25% <400 cop/ml	Med ↑ 202 cells/mm^3	IDV: 44% renal side effects. 28% nephrolitiasis	4
Melvin et al.	IDV+2 NRTIs (n=5) or RTV+2 NRTIs (n=4)	500 mg/m^2	PI naive	9 (5 on IDV)	28-52	↓ 1.7 log_{10} cop/ml 22% <400 cop/ml	Med ↑ 499 cells/mm^3	80% (40% when misdosage is excluded): renal complications	5
Monpoux et al.	IDV, d4T, 3TC	500 mg/m^2	Severe immunodeficiency, High viral load, clinically stable PI naive	7	24	↓ 0.6 log_{10} cop/ml 14% <400 cop/ml	Med ↑ 132 cells/mm^3	Vomiting (n=1), mild ↑ bilirubin	9
Mueller et al.	IDV+ after 12 weeks: ZDV+3TC	250 mg/m^2 or 350 mg/m^2 or 500 mg/m^2	Age 6 months-18 years, CDC stage B, C, 1, 2, Normal hematological and chemistry parameters, Clinically stable PI naive	54	16	↓ 0.07 log_{10} cop/ml (250 and 350 mg/m^2), ↓ 0.76 log_{10} cop/ml (500 mg/m^2), 6% <200 cop/ml (on triple)	Med ↑ 60 cells/mm^3	Generally well tolerated. 13% hematuria, 2% nephrolithiasis	28
Kline et al.	IDV+d4T+ddI	500 mg/m^2	Symptomatic HIV disease or immuno - suppression ≥ 1 year NRTIs	12	48	↓ 2 log_{10} cop/ml	Med ↑ 317 cells/mm^3	33% nausea, vomiting 50% crystalluria. 42% hematuria, 80% pyuria, 17% transient jaundice	8

Wintergerst et al.	IDV+ZDV+3TC or IDV+d4T+3TC	500 mg/m^2	Age <18 years PI naive	15	24	↓ 1.6 $\log_{10}$ cop/ml 40% <400 cop/ml	Med ↑ 101% above baseline	NA	7
Vigano et al.	IDV+d4T+3TC	500 mg/m^2	Symptomatic HIV disease, Immuno-suppression and prior NRTI use PI naive	25	72	87% <400 cop/ml	Med ↑ 360 cells/mm^3 =10% of total T-cells (at month 12)	24% renal symptoms 4% transient jaundice	20
Van Rossum et al.	IDV+ZDV+3TC	400, 500, 600 or 660 mg/m^2	PI naive, HIV-1 RNA >5000 cop/ml Immuno-suppression Age 3 months-18 years	28	24	70% <500 cop/ml 48% <40 cop/ml	Med ↑ 100 cells/mm^3 =27% in relation to normal values	41% side-effects, mainly gastro-intestinal Renal symptoms in 11%	24
Jankelevich et al.	IDV+ after 12 weeks: ZDV+3TC	250, 350, or 500 mg/m^2 After week 58: 350 mg/m^2	Age 6 months-18 years, CDC stage B, C, 1, 2, Normal hematological and chemistry parameters, Clinically stable PI naive	33	96	↓ 0.74 $\log_{10}$ cop/ml 12% <200 cop/ml (on triple)	Med ↑ 199 cells/mm^3	NA	29

Table 3. Pediatric studies using nelfinavir (NFV).

Author	Regimen	NFV dose (q8h)	Inclusion criteria	Number of patients	Follow -up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Krogstad et al.	NFV+2 NRTIs	20-30 mg/kg	Age 0-13 years, PI naive, Clinically stable	62	54 (median 42)	71% ≥ ↓ 0.7 log_{10} cop/ml	Remained constant	25% grade 1 or 2 transient diarrhea Grade 1 nausea, flatulence, anorexia, epistaxis, fever, neutropenia, abdominal pain, anemia and rash	32
Funk et al.	NFV+ZDV+3TC or NFV+d4T+ddI	20-30 mg/kg	Antiretroviral naive, CDC B, C, 2 or 3, HIV-1 RNA >20,000 (> 2 years) HIV-1 RNA >100,000 (< 2 years)	16	48	Med ↓ 2.8 log_{10} cop/ml 69% <400 cop/ml (ITT) 44% <50 cop/ml (ITT)	Med ↑ 157 cells/mm^3 =33% of total T-cells	Initial transient diarrhea, lack of concentration and rash. Transient or persistent ↑ triglycerides or cholesterol in 50% and 31% respectively	23
Starr et al.	NFV+EFV+2 NRTIs	20-30 mg/kg	Age < 16 years, HIV-1 RNA >400 cop/ml, PI and NNRTI naive, Ability to swallow capsules	48	57	Med ↓ 2.7 log_{10} cop/ml 81% <400 cop/ml (AT) 61% <400 cop/ml (ITT) 70% <50 cop/ml (AT) 53% <50 cop/ml (ITT)	Med ↑ 74 cells/mm^3 =3% of total T-cells	25% moderate, 9% severe , 2% life threatening. Most common:rash, diarrhea, neutropenia, biochemical abnormalities	21

Wiznia et al.	NFV+d4T+ 3TC (1) or NFV+d4T+N VP (2) or NFV+d4T+ 3TC+NVP (3) or RTV+d4T+N VP (4)	30 mg/kg 55 mg/kg q12h (n=12)	Age 4 months-17 years, Stable CDC 1 or 2 cat. PI, NNRTI, d4T, 3TC naive, Clinically stable	193	24	1: 44% <400 cop/ml 2: 50% <400 cop/ml 3: 63% <400 cop/ml 4: 46% <400 cop/ml	1: Med ↑ 105 cells/mm^3 2: Med ↑ 87 cells/mm^3 3: Med ↑ 294 cells/mm^3 4: Med ↑ 254 cells/mm^3	77% ≥grade 2 Most common: rash, nausea, vomiting, fever	33

Table 4. Pediatric studies using saquinavir (SQV).

Author	Regimen	PI Dose	Inclusion criteria	Number of patients	Follow -up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Hoffmann et al.	A: SQV+RTV+≥ 1NRTI (n=6) or B: SQV+NFV+≥ 1NRTI (n=5)	SQV+RTV: 15-30mg/kg +250-400 mg/m^2 q12h SQV+NFV: 15-30mg/kg +30-35 mg/kg q8h	Failure of prior therapy including at least 1 PI, Age <14 years	11	24	A: Med ↓ 1.4 $\log_{10}$ cop/ml 20% <200 cop/ml (AT) 0% <50 cop/ml B: Med ↓ 0.2 $\log_{10}$ cop/ml 0% <200 cop/ml (AT)	A: Med ↑ 23% above baseline = Med ↑ 281 cells/mm^3 B: Med ↑ 7% above baseline = Med ↑ 3 cells/mm^3	45% grade 1 or 2 diarrhea 91% ↑increase of triglycerides above normal 55% mild increase lever enzymes	35
Kline et al.	1: SQV-SGC+2NRTIs 2: SQV-SGC+NFV+2 NRTIs	1: SQV-SGC 33mg/kg q8h 2: SQV-SGC 33mg/kg + NFV 30mg/kg q8h	Age 3-16 years, PI naive, Naive to ≥1 prescribed NRTI CDC stage A, B, C, 1 or 2, Normal hematological and biochemical parameters	1: 14 2: 13	1: 72 2: 48	1: Med ↓2.12 $\log_{10}$ cop/ml 36% <50 cop/ml 2: Med ↓2.58 $\log_{10}$ cop/ml 62% <50 cop/ml	1: Med ↑292 cells/mm^3 2: Med ↑154 cells/mm^3	No differences between 1 and 2. Generally mild: diarrhea (36%), abdominal discomfort (16%), headache (16%)	36

Table 5. Pediatric studies using nevirapine (NVP).

Author	Regimen	NVP Dose	Inclusion criteria	Number of patients	Follow -up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Luzuriaga et al.	NVP	1.120 mg/m^2/day or 2. 240 mg/m^2/day or 3.120 mg/m^2/day first 14 days followed by 240 mg/m^2/day or 4. <9years: 120 mg/m^2/day first 14 days followed by 400 mg/m^2/day	Age 2 months-18 years, Immunosuppression, CDC stage A, B, C Prior antiretroviral treatment <6 weeks	21	8	NA	43% ≥5% increase	5% Rash	40
Luzuriaga et al.	NVP+ZDV+3TC	120 mg/m^2/day first 28 days followed by 400 mg/m^2/day	Age 2-24 months Immuno-suppression, CDC stage A, B, C Prior antiretroviral treatment <6 weeks	8	24	38% ↓1.5 log_{10} cop/ml 25% <400 cop/ml	Stable of slight increase (88% no immuno-suppression)	NA	6

Hainaut et al.	1. ZDV+ddI+ 3TC 2. ZDV+3TC+ NVP	120 mg/m^2/day first 14 days followed by 300-400 mg/m^2/day	Children born to HIV-1 infected mothers that became HIV-1 positive during perinatal follow-up	4	NA	50% <50 cop/ml	Persistent normal CD4+ T-cell counts	No side-effects	41

Table 6. Pediatric studies using efavirenz (EFV).

Author	Regimen	EFV Dose	Inclusion criteria	Number of patients	Follow -up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Teglas et all	1. EFV + NRTI (n= 24) 2. EFV +PI +NRTI (n=9)	Median dose 13.3 mg/kg	inclusion criteria not included in paper	33	24 weeks	Med ↓1.2 log_{10} cop/ml 48 % below 200 copies/ml 27 % below 50% copies/ml	Med ↑ 128 cells/mm^3	42% of the children had at least one distinguishable side effect. Of all children 15% suffered of cutaneous side effects, 30% of nervous system side effects and 6% of both. 7 children stopped the study due to intolerance	43
Starr et al.	NFV+EFV+2 NRTIs	Mean dose at 2 weeks 11.7 mg/kg Mean dose at 6 weeks 13.3 mg/kg	Age < 16 years, HIV-1 RNA >400 cop/ml, PI and NNRTI naive, Ability to swallow capsules	48	57	Med ↓ 2.7 log_{10} cop/ml 81% <400 cop/ml (AT) 61% <400 cop/ml (ITT) 70% <50 cop/ml (AT) 53% <50 cop/ml (ITT)	Med ↑ 74 cells/mm^3 =3% of total T-cells	25% moderate, 9% severe , 2% life threatening. Most common:rash, diarrhea, neutropenia, biochemical abnormalities	21

Table 7. Pediatric study using abacavir (ABC).

Author	Regimen	ABC Dose	Inclusion criteria	Number of patients	Follow -up (weeks)	Outcome HIV-1 RNA	Outcome CD4+ T-cells	Toxicity	Reference
Sáez-Llorens et al.	1. ZDV+3TC (n=103) 2. ABC+ZDV+3TC (n=102)	8 mg/kg q12h	Age 3 months-13 years, CDC stage N,A,B,C, PI discontinuation ≥2 weeks before enrollment, Normal hematology and chemistry parameters, CD4+ % >15%	205	48	1. Med ↓0.21 log_{10} cop/ml 1. 6% <400 cop/ml 2. Med ↓0.61 log_{10} cop/ml 2. 11% <400 cop/ml	1: Med ↑-14 cells/mm^3 =0.8% of total T-cells 2: Med ↑99 cells/mm^3 =3.1% of total T-cells	Nausea, vomiting and cough more frequent in 2 (46% and 46^ versus 30% and 25% resp.) Fever, diarrhea, nasal signs, rashes and ear/nose/throat infections occurred in ≥15% Grade 3 or 4 infrequently	45

Gastro-intestinal side-effects are frequently associated with the use of NFV, but are mainly transient. Increases in triglycerides and cholesterol have been reported in 31 to 91% of the children which may have important consequences for their health. The efficacy of a twice-daily regimen, which seems possible from a pharmacokinetic point of view, should be studied.

Saquinavir

Saquinavir hard gelatin capsule (SQV-HGC) was the first HIV-1 protease inhibitor approved for use in HIV-1-infected adults by the FDA. The use of SQV-HGC has been restricted by poor bioavailability (4% uptake of a single oral dose taken with food). To improve this pharmacokinetic profile SQV has been combined with RTV or with NFV. Both PI's are inhibitors of cytochrome P450 3A isoenzymes and therefore co-administration results in markedly higher plasma concentrations of SQV.
Hoffmann et al. studied the efficacy of SQV with RTV and of SQV with NFV in 11 children with failure on prior therapy including at least one PI. (35) The antiretroviral effect (HIV-1 RNA reduction and CD4+ T-cell increase) was more pronounced with the combination SQV/RTV than when SQV/NFV were given. This was probably due to the stronger inhibition of cytochrome p459 3A isoenzymes by ritonavir thus leading to higher trough levels of SQV in combination with RTV. Complete suppression of viral replication below quantifiable levels could not be achieved. Both regimens were well tolerated. Recently a new formulation of SQV that improves the low oral bioavailability of SQV-HGC, saquinavir soft gelatin capsule (SQV-SGC) has been approved for use in HIV-1 infected adults. Kline et al. evaluated the pharmacokinetics, tolerance, safety and activity of SQV-SGC with NFV in children older than 3 years and reported good tolerance, safety, and virological and immunological response rates. 36 In Table 4 the results of pediatric studies with SQV are summarized. The combination of SQV-SGC with NFV results in a very promising virological suppression in the absence of serious side-effects.

Nevirapine

Nevirapine (NVP) is a potent non-nucleoside inhibitor of HIV-1 reverse transcriptase with a favorable pharmacokinetic profile allowing twice daily administration. (37,38) In adults once daily dosing results in similar exposure to NVP. (39) However, pharmacokinetic evaluation has not yet been performed in children. NVP is available in a pediatric formulation (liquid).
Luzuriaga et al. studied the pharmacokinetics, safety and activity in HIV-1 infected children in 1996. (40) Administration of NVP leads to a rapid and profound (≤ 50% of baseline) reduction in plasma p24 antigen levels in 10 of 12 children. However, at the lower dosage (120 mg/m^2/day) antiviral activity was lost rapidly and appeared to be associated with the isolation of viruses with decreased in vitro sensitivity. At higher dosages a more durable antiretroviral activity was observed during the 8 weeks of monotherapy. Rash was the most common side-effect in 5% of the patients. Because the use of NVP in combination with other antiretroviral agents would likely provide more potent antiretroviral activity, Luzuriaga et al. studied combination treatment with NVP, zidovudine and lamivudine in 8 infants younger than 16 months. In this study a more durable response was found. (6) However, only 25% of the

patients reached an HIV-1 RNA load <400 copies/ml. Viruses with decreased sensitivity to nevirapine were isolated during the treatment period from five infants. (6) Hainaut et al. treated 2 vertically infected infants with NVP, ZDV and 3TC. In both infants an HIV-1 RNA load <50 copies was observed. (41)

Table 5 summarizes the pediatric studies with NVP. The development of class resistance with single step mutations in the reverse transcriptase gene remains a major therapeutic problem with this class of antiretrovirals. Since HIV-1 infected children tend to have higher baseline viral loads with a higher replication rate than adults, this is a major disadvantage in the treatment of children with a high baseline viral load. (42) However, the favorable pharmacokinetic profile allowing twice and possibly once daily dosing, a pediatric formulation with a taste that children like and the absence of major side-effects makes this drug a promising antiretroviral component for children with lower baseline viral loads.

Efavirenz

Efavirenz (EFV) was the second NNRTI to be approved for the use in children. A pediatric (liquid) formulation is currently available on a compassionate use basis. The pharmacokinetic profile of EFV allows for once daily dosing. In spite of this promising feature, little experience has been obtained with the treatment of HIV-1 infected children with EFV. Star et all. conducted a study in children receiving EFV in combination with NFV and NRTI. (21) A total of 57 HIV-1-infected children were included. After 48 weeks of treatment 76% of the children had plasma levels below 400 copies and 63% below 50 copies. High viral loads at start of the study decreased the likelihood that plasma viral loads would become undetectable. Adverse effects of at least moderate severity included rash (30%), diarrhea (18%), neutropenia (12%) and biochemical abnormalities (12%). Mild central nervous system toxicity was found in 14% children, which resolved once EFV was given at bedtime rather than in the morning. Since the patients in this study received a combination of NFV and EFV it is impossible to discriminate which of both drugs induced this toxicity. Teglas et all. conducted a study in 24 children receiving EFV and a NRTI and 9 children receiving EVF, a NRTI and a PI. (43) After 6 months of treatment the viral load was below 200 copies in 48% of the children and below 50 copies in 27% of the children. Fifteen children (42%) suffered of at least one clinically apparent side effect. Five (15%) had mild diffuse cutaneous eruptions, 10 (30%) children suffered from nervous system side effects and two had both. The treatment was discontinued due to intolerance in seven children. Younger children experienced more side effects than older children. Table 6 summarizes the results of studies with EFV.

In children the use of efavirenz has been associated with serious adverse events. Rash and serious neurological problems are frequent side-effects. High viral loads at start of the study decreased the likelihood that plasma viral loads would become undetectable.

Therefore, in our opinion efavirenz is not a first choice drug in children.

Abacavir

Abacavir (ABC) is a potent nucleoside reverse transcriptase inhibitor (NRTI) that in combination with two other NRTIs results in a viral load of <400 copies/ml in 74% of treatment-naive adults

after 48 weeks of therapy. (44) It is available in a pediatric formulation (liquid). Sáez-Llorenz et al performed a randomized, double-blind study of ABC/ZDV/3TC versus ZDV/3TC in 205 antiretroviral therapy-experienced HIV-1 infected children. (45) Virological, immunological and clinical response rates over the 48 weeks of the study indicate that the addition of ABC to ZDV/3TC provided increased antiviral activity over that provided by ZDV/3TC. However, as expected in antiretroviral therapy-experienced participants many of whom had received previous therapy with ZDV with of without 3TC, the degree of viral suppression provided by the ABC/ZDV/3TC regimen was modest, while improvement in immune response was moderate. Nausea/vomiting and cough occurred more frequently among children receiving ABC/ZDV/3TC. The hypersensitivity syndrome associated with the use of ABC was not observed in this study. Thus far only one child (3%) with a hypersensitivity reaction has been reported in a phase I study of ABC in HIV-1 infected children. (46) In Table 7 the results of the pediatric studies with ABC are summarized. The well tolerated pediatric formulation, the possibility to administrate this medication twice-daily and the low incidence of serious side-effects result in favorable qualities of ABC. However, studies on the efficacy of this drug in naive children are lacking. Since in adults poorer virological outcome has been associated with a high baseline viral load and children tend to have higher baseline viral loads, the efficacy of ABC in children should be studied. (42,47)

Discussion

This review provides an overview of all current articles on pediatric clinical trials using HAART. Twenty-three papers mostly with a small number of patients were available using 4 PI's (RTV, IDV, NFV and SQV), 1 NNRTI (NVP) and 1 NRTI (ABC). HIV-1 RNA loads and CD4+ T-cell counts were used as primary outcome measures, because these parameters have been demonstrated to be independent predictors of the clinical course in HIV-1 infected infants and children. (3) In addition, these two surrogate measures were measured in all studies, while other clinical progression markers such as growth were unfortunately often not available. (48)

Virological response rates were highly variable both between the different antiretroviral drugs but also between different studies using the same medication. The studies with the highest percentages of children reaching a viral load below the detection limits of 400 copies/ml were reported by Starr et al: NFV+EFV+2 NRTIs, Vigano et al.: IDV+d4T+3TC, Kline et al.: SQV+NFV+2 NRTIs, Van Rossum et al.: IDV+ZDV+3TC, Funk et al.: NFV+2NRTIs and Wiznia et al.: NFV+NVP+2 NRTIs. (20,21,23,24,33,36) The percentages of viral loads below the detection limit of 400 copies/ml varied between 63% and 87%, which is comparable with the data on HAART in adults. (25,26) However, the majority of pediatric studies showed inferior virological response rates in comparison with those in adults. In studies using RTV, NFV, IDV, NVP or ABC virological success rates varied from 11% to 50% of the patients with a HIV-1 RNA load <400 copies/ml. (4-11,22,27-29,33,45)
Several factors may be associated with virological failure. These include pharmacokinetic parameters (low plasma blood levels of protease inhibitors are associated with virological failure, inadequate adherence to antiretroviral therapy and differences in baseline

characteristics (prior antiretroviral treatment, younger age, and high baseline viral load). (49,50) Since studies were often not comparable with respect to these factors, comparison of the results of the different studies is complicated. It is striking that all four studies in which dosages of the administrated PI were adjusted after pharmacokinetic evaluation resulted in superior virological response rates compared to studies in which fixed dosages were used. (21,23,24,36) Interindividual pharmacokinetic differences result in inadequate plasma PI levels in a part of the children treated with a fixed dose. (27,34,51-53) Since virological response is associated with plasma protease inhibitor levels, these inadequate plasma PI levels may be partly responsible for the differences in virological response rates. (27,28,32,51,52) In our opinion, it is therefore imperative to measure PI pharmacokinetics in all children treated with a PI to determine the individual dosage necessary for pharmacokinetic values comparable with adult values.

The combination of nelfinavir with either a NNRTI or SQV and two NRTIs resulted in optimal virological responses in a high percentage of the patients. These twice daily regimens may result in an improved adherence to HAART compared to three daily medication schemes. (21,33,36) Lopinavir/ritonavir, which was recently approved by the FDA is also a very promising compound because of twice daily dosing and the excellent virological response rates. (54) Future possibilities for once daily -or even less frequent- dosing are very important to improve adherence to antiretroviral therapy. Administration of indinavir with two NRTIs also leads to a good virological response rate in a high percentage of the patients. (20,24) However, in a majority of the studies with indinavir virological response rates were suboptimal. (4,5,7-9,28,29) Since these studies differed substantially in follow-up time, baseline characteristics of the patients and study design these variable results are not well understood.

Analyses of T-cell repopulation in groups of children with different ages are hampered by the fact that CD4+ T-cell counts are highly dependent on the age of the patients. (55,56) The calculation of CD4+ T-cells as a percentage of the total T-cell counts is therefore used in many pediatric studies. However, data generated in this way are influenced by the major changes in the numbers of CD8+ T-cells in HIV-1-infected patients. (57-59) Immunological outcome parameters of the different pediatric studies are therefore not comparable. Nevertheless, immunological response rates were more uniform than the virological responses. In almost all studies increases of CD4+ T-cell counts are reported independent of the extent of the virological response. CD4+ T-cell numbers in HIV-1-infected children on HAART recover more rapidly than CD4+ T-cells in HIV-1-infected adults. (12,57,60-63) The good immunological response to HAART has been attributed to the large volume of the thymus, which is still present in young children. (64,65) The observation that CD4+ T-cell counts recover despite the presence of virological failure may possible be explained by the selection of certain viral variants with resistance to protease inhibitors that have in-vitro impaired replicative capacity. (66) Douek et al. also reported an increase in peripheral CD4+ T-cell counts in both virologic responders and non-responders on antiretroviral therapy. (67) However, they observed that the recovery of thymic function was affected by the degree to which virus suppression was

achieved when thymic function was measured by quantifying T-cell receptor rearrangement excision circles in peripheral blood.

Side-effects of HAART were generally mild, transient and mainly of gastro-intestinal origin. Lipodystrophy syndrome is commonly reported in HIV-1 infected adults treated with antiretroviral therapy. The occurrence of this syndrome consisting of a fat-wasting condition associated with abnormalities of lipid metabolism and impaired glucose tolerance has been reported in 1.5-83% of HIV-1-infected adults. (68,69) Only two groups investigated the incidence of lipodystrophy in children. The incidences in these studies were 29% and 33%. (70,71) In both studies lipodystrophy was associated with advanced disease at baseline. Jaquet et al. observed a combination of peripheral atrophy and central adipositas in only two of a group of 39 pubertal children. In the other children only one of these features was observed. (71) Significant percentages of patients with serum lipid abnormalities were reported in three pediatric studies. (11,23,35) However, the investigators did not report signs of clinical lipodystrophy. The consequences of the lipodystrophy syndrome for the future treatment of HIV-1 infected children are not yet clear, because little is known about the pathophysiology, although it is generally believed that lipodystrophy has multiple causes and modes of presentation. Furthermore, it still remains to be clarified whether this syndrome is attributable to antiretroviral drugs. Another concern in long-term treatment of HIV-1 infected children is the observation of an increased rate of bone turnover causing bone mineral density decrease. The severity of osteopenia seems to be related to lipodystrophy. (72) Long-term data on the comparison between PI containing regimens versus NNRTI containing medication schemes with respect to lipodystrophy and bone mineral loss are necessary to evaluate the contribution of the different antiretroviral drugs to the development of these abnormalities.

The inferior virological response rates, which have been reported in HIV-1 infected children treated with HAART form a reflection of the challenges which are encountered in the treatment of children with HIV-1/AIDS. Difficulties with adherence and with the pharmacokinetics of protease inhibitors in children demand an intensive, child adjusted approach. Since HIV-1 infection in children is a rare disease in the Western world, and experience in the treatment is often insufficient, it may be more practical to treat children with an adjusted regimen that is not dependent of pharmacokinetic evaluation. This could be achieved by initiating therapy with a potent regimen to reduce the viral load to undetectable levels. Lopinavir/ritonavir is a very potent protease inhibitor and could thus be part of this induction regimen. However, long-term treatment with lopinavir/ritonavir in children is complicated by the poor taste of lopinavir/ritonavir and by serious changes of lipid metabolism. (54) After viral load has been significantly reduced, an easy to use maintenance regimen with less side-effects could be introduced to maintain long-term adherence. This maintenance regimen may contain for example nevirapine or abacavir. Future studies with induction-maintenance regimens are needed. The goal of these studies is to ensure similar efficacy and toxicity compared to those reported in adults.

References

1. de Martino M, Tovo PA, Balducci M, et al. Reduction in mortality with availability of antiretroviral therapy for children with perinatal HIV-1 infection. Italian Register for HIV Infection in Children and the Italian National AIDS Registry. JAMA 2000;284(2):190-7.
2. Fiscus SA, Hughes MD, Lathey JL, et al. Changes in virologic markers as predictors of CD4 cell decline and progression of disease in human immunodeficiency virus type 1-infected adults treated with nucleosides. AIDS Clinical Trials Group Protocol 175 Team. J Infect Dis 1998;177(3):625-33.
3. Palumbo PE, Raskino C, Fiscus S, et al. Predictive value of quantitative plasma HIV RNA and CD4+ lymphocyte count in HIV-infected infants and children. JAMA 1998;279(10):756-61.
4. Rutstein RM, Feingold A, Meislich D, Word B, Rudy B. Protease inhibitor therapy in children with perinatally acquired HIV infection. AIDS 1997;11(12):F107-11.
5. Melvin AJ, Mohan KM, Arcuino LA, Edelstein RE, Frenkel LM. Clinical, virologic and immunologic responses of children with advanced human immunodeficiency virus type 1 disease treated with protease inhibitors. Pediatr Infect Dis J 1997;16(10):968-74.
6. Luzuriaga K, Bryson Y, Krogstad P, et al. Combination treatment with zidovudine, didanosine, and nevirapine in infants with human immunodeficiency virus type 1 infection. N Engl J Med 1997;336(19):1343-9.
7. Wintergerst U, Hoffmann F, Solder B, al e. Comparison of two antiretroviral triple combinations including the protease inhibitor indinavir in children infected with human immunodeficiency virus. Pediatr Infect Dis J 1998;17:495-9.
8. Kline MW, Fletcher CV, Harris AT, et al. A pilot study of combination therapy with indinavir, stavudine (d4T), and didanosine (ddI) in children infected with the human immunodeficiency virus. J Pediatr 1998;132(3 Pt 1):543-6.
9. Monpoux F, Sirvent N, Cottalorda J, Mariani R, Lefbvre JC. Stavudine, lamivudine and indinavir in children with advanced HIV-1 infection: preliminary experience [letter]. AIDS 1997;11(12):1523-5.
10. Pelton SI, Johnson D, Chadwick E, Baldwin Z, Yogev R. A one year experience: T cell responses and viral replication in children with advanced human immunodeficiency virus type 1 disease treated with combination therapy including ritonavir. Pediatr Infect Dis J 1999;18(7):650-2.
11. Thuret I, Michel G, Chambost H, et al. Combination antiretroviral therapy including ritonavir in children infected with human immunodeficiency. AIDS 1999;13(1):81-7.
12. Essajee SM, Kim M, Gonzalez C, et al. Immunologic and virologic responses to HAART in severely immunocompromised HIV-1-infected children. AIDS 1999;13(18):2523-32.
13. Kempf DJ, Rode RA, Xu Y, et al. The duration of viral suppression during protease inhibitor therapy for HIV-1 infection is predicted by plasma HIV-1 RNA at the nadir. AIDS 1998;12(5):F9-14.
14. Raboud JM, Montaner JS, Conway B, et al. Suppression of plasma viral load below 20 copies/ml is required to achieve a long-term response to therapy. AIDS 1998;12(13):1619-24.
15. Raboud JM, Rae S, Hogg RS, et al. Suppression of plasma virus load below the detection limit of a human immunodeficiency virus kit is associated with longer virologic response than suppression below the limit of quantitation. J Infect Dis 1999;180(4):1347-50.
16. Melvin AJ, Rodrigo AG, Mohan KM, et al. HIV-1 dynamics in children. J Acquir Immune Defic Syndr Hum Retrovirol 1999;20(5):468-73.
17. Shearer WT, Quinn TC, LaRussa P, et al. Viral load and disease progression in infants infected with human immunodeficiency virus type 1. Women and Infants Transmission Study Group. N Engl J Med 1997;336(19):1337-42.
18. Dickover RE, Dillon M, Leung KM, et al. Early prognostic indicators in primary perinatal human immunodeficiency virus type 1 infection: importance of viral RNA and the timing of transmission on long-term outcome. J Infect Dis 1998;178(2):375-87.
19. Spector SA, Hsia K, Yong FH, et al. Patterns of plasma human immunodeficiency virus type 1 RNA response to highly active antiretroviral therapy in infected children. J Infect Dis 2000;182(6):1769-73.
20. Vigano A, Dally L, Bricalli D, et al. Clinical and immuno-virologic characterization of the efficacy of stavudine, lamivudine, and indinavir in human immunodeficiency virus infection. J Pediatr 1999;135(6):675-82.
21. Starr SE, Fletcher CV, Spector SA, et al. Combination therapy with efavirenz, nelfinavir, and nucleoside reverse-transcriptase inhibitors in children infected with human immunodeficiency virus type 1. Pediatric AIDS Clinical Trials Group 382 Team. N Engl J Med 1999;341(25):1874-81.

22. Nachman SA, Stanley K, Yogev R, et al. Nucleoside analogs plus ritonavir in stable antiretroviral therapy-experienced HIV-infected children: a randomized controlled trial. Pediatric AIDS Clinical Trials Group 338 Study Team. JAMA 2000;283(4):492-8.
23. Funk MB, Linde R, Wintergerst U, et al. Preliminary experiences with triple therapy including nelfinavir and two reverse transcriptase inhibitors in previously untreated HIV- infected children. AIDS 1999;13(13):1653-8.
24. Van Rossum AM, Niesters HG, Geelen SP, et al. Clinical and virologic response to combination treatment with indinavir, zidovudine, and lamivudine in children with human immunodeficiency virus-1 infection: A multicenter study in The Netherlands. J Pediatr 2000;136(6):780-788.
25. Bart PA, Rizzardi GP, Tambussi G, et al. Immunological and virological responses in HIV-1-infected adults at early stage of established infection treated with highly active antiretroviral therapy. AIDS 2000;14(13):1887-97.
26. Gulick RM, Mellors JW, Havlir D, et al. 3-year suppression of HIV viremia with indinavir, zidovudine, and lamivudine. Ann Intern Med 2000;133(1):35-9.
27. Mueller BU, Nelson RP, Jr., Sleasman J, et al. A phase I/II study of the protease inhibitor ritonavir in children with human immunodeficiency virus infection. Pediatrics 1998;101(3 Pt 1):335-43.
28. Mueller B, Sleasman J, Nelson R, al e. A phase I/II study of the protease inhibitor indinavir in children with HIV infection. Pediatrics 1998;102:100-109.
29. Jankelevich S, Mueller BU, Mackall CL, et al. Long-term virologic and immunologic responses in human immunodeficiency virus type 1-infected children treated with indinavir, zidovudine, and lamivudine. J Infect Dis 2001;183(7):1116-20.
30. van Rossum AM, de Groot R, Hartwig NG, Weemaes CM, Head S, Burger DM. Pharmacokinetics of indinavir and low-dose ritonavir in children with HIV-1 infection. AIDS 2000;14(14):2209-10.
31. Dieleman JP, Gyssens IC, van der Ende ME, de Marie S, Burger DM. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. AIDS 1999;13(4):473-8.
32. Krogstad P, Wiznia A, Luzuriaga K, et al. Treatment of human immunodeficiency virus 1-infected infants and children with the protease inhibitor nelfinavir mesylate. Clin Infect Dis 1999;28(5):1109-18.
33. Wiznia A, Stanley K, Krogstad P, et al. Combination nucleoside analog reverse transcriptase inhibitor(s) plus nevirapine, nelfinavir, or ritonavir in stable antiretroviral therapy-experienced HIV-infected children: week 24 results of a randomized controlled trial--PACTG 377. Pediatric AIDS Clinical Trials Group 377 Study Team. AIDS Res Hum Retroviruses 2000;16(12):1113-21.
34. Schuster T, Linde R, Wintergerst U, et al. Nelfinavir pharmacokinetics in HIV-infected children: a comparison of twice daily and three times daily dosing. AIDS 2000;14(10):1466-8.
35. Hoffmann F, Notheis G, Wintergerst U, Eberle J, Gurtler L, Belohradsky BH. Comparison of ritonavir plus saquinavir- and nelfinavir plus saquinavir-containing regimens as salvage therapy in children with human immunodeficiency type 1 infection. Pediatr Infect Dis J 2000;19(1):47-51.
36. Kline MW, Brundage RC, Fletcher CV, et al. Combination therapy with saquinavir soft gelatin capsules in children with human immunodeficiency virus infection. Pediatr Infect Dis J 2001;20(7):666-71.
37. Montaner JS, Reiss P, Cooper D, et al. A randomized, double-blind trial comparing combinations of nevirapine, didanosine, and zidovudine for HIV-infected patients: the INCAS Trial. Italy, The Netherlands, Canada and Australia Study. JAMA 1998;279(12):930-7.
38. Koup RA, Merluzzi VJ, Hargrave KD, et al. Inhibition of human immunodeficiency virus type 1 (HIV-1) replication by the dipyridodiazepinone BI-RG-587. J Infect Dis 1991;163(5):966-70.
39. van Heeswijk RP, Veldkamp AI, Mulder JW, et al. The steady-state pharmacokinetics of nevirapine during once daily and twice daily dosing in HIV-1-infected individuals. Aids 2000;14(8):F77-82.
40. Luzuriaga K, Bryson Y, McSherry G, et al. Pharmacokinetics, safety, and activity of nevirapine in human immunodeficiency virus type 1-infected children. J Infect Dis 1996;174(4):713-21.
41. Hainaut M, Peltier CA, Gerard M, Marissens D, Zissis G, Levy J. Effectiveness of antiretroviral therapy initiated before the age of 2 months in infants vertically infected with human immunodeficiency virus type 1. Eur J Pediatr 2000;159(10):778-82.
42. Palumbo PE, Kwok S, Waters S, et al. Viral measurement by polymerase chain reaction-based assays in human immunodeficiency virus-infected infants. J Pediatr 1995;126(4):592-5.
43. Teglas JP, Quartier P, Treluyer JM, Burgard M, Gregoire V, Blanche S. Tolerance of efavirenz in children. AIDS 2001;15(2):241-3.

44. Fischl MA. Antiretroviral therapy in 1999 for antiretroviral-naive individuals with HIV infection. AIDS 1999;13 Suppl 1:S49-59.
45. Saez-Llorens X, Nelson RP, Jr., Emmanuel P, et al. A randomized, double-blind study of triple nucleoside therapy of abacavir, lamivudine, and zidovudine versus lamivudine and zidovudine in previously treated human immunodeficiency virus type 1-infected children. The CNAA3006 Study Team. Pediatrics 2001;107(1):E4.
46. Kline MW, Blanchard S, Fletcher CV, et al. A phase I study of abacavir (1592U89) alone and in combination with other antiretroviral agents in infants and children with human immunodeficiency virus infection. AIDS Clinical Trials Group 330 Team. Pediatrics 1999;103(4):e47.
47. Staszewski S, Keiser P, Montaner J, et al. Abacavir-lamivudine-zidovudine vs indinavir-lamivudine-zidovudine in antiretroviral-naive HIV-infected adults: A randomized equivalence trial. Jama 2001;285(9):1155-63.
48. Ogino MT, Dankner WM, Spector SA. Development and significance of zidovudine resistance in children infected with human immunodeficiency virus. J Pediatr 1993;123(1):1-8.
49. Burger DM, Hoetelmans RMW, Hugen PWH, et al. Low plasma concentrations of indinavir are related to virological treatment failure in HIV-1-infected patients on indinavir-containing triple therapy. Antivir Ther 1998;3:215-20.
50. Harris M, Durakovic C, Rae S, et al. A pilot study of nevirapine, indinavir, and lamivudine among patients with advanced human immunodeficiency virus disease who have had failure of combination nucleoside therapy. J Infect Dis 1998;177(6):1514-20.
51. Gatti G, Vigano A, Sala N, et al. Indinavir pharmacokinetics and parmacodynamics in children with human immunodeficiency virus infection. Antimicrob Agents Chemother 2000;44(3):752-5.
52. Burger DM, van Rossum AM, Hugen PW, et al. Pharmacokinetics of the protease inhibitor indinavir in HIV type 1-infected children. Antimicrob Agents Chemother 2001;45(3):701-5.
53. Fletcher CV, Brundage RC, Remmel RP, et al. Pharmacologic characteristics of indinavir, didanosine, and stavudine in human immunodeficiency virus-infected children receiving combination therapy. Antimicrob Agents Chemother 2000;44(4):1029-34.
54. Cahn P, Violari A, Saez-Llorenz X, et al. Kaletra (ABT-378/ritnavir) in HIV-infected children at 48 weeks. 5th International Congress on Drug Therapy in HIV Infection 2000, Glasgow, UK.
55. Hulstaert F, Hannet I, Deneys V, et al. Age-related changes in human blood lymphocyte subpopulations. II. Varying kinetics of percentage and absolute count measurements. Clin Immunol Immunopathol 1994;70(2):152-8.
56. Comans-Bitter WM, de Groot R, van den Beemd R, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr 1997;130(3):388-93.
57. Sleasman JW, Nelson RP, Goodenow MM, et al. Immunoreconstitution after ritonavir therapy in children with human immunodeficiency virus infection involves multiple lymphocyte lineages. J Pediatr 1999;134(5):597-606.
58. Autran B, Carcelain G, Li TS, et al. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Science 1997;277(5322):112-6.
59. Pakker NG, Notermans DW, de Boer RJ, et al. Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation. Nat Med 1998;4(2):208-14.
60. Bohler T, Walcher J, Holzl-Wenig G, et al. Early effects of antiretroviral combination therapy on activation, apoptosis and regeneration of T cells in HIV-1-infected children and adolescents. AIDS 1999;13(7):779-89.
61. Cohen Stuart JW, Slieker WA, Rijkers GT, et al. Early recovery of CD4+ T lymfocytes in children on highly active antiretroviral therapy. AIDS 1998;12(16):2155-9.
62. Vigano A, Vella S, Saresella M, et al. Early immune reconstitution after potent antiretroviral therapy in HIV-infected children correlates with the increase in thymus volume. AIDS 2000;14(3):251-61.
63. Gibb DM, Newberry A, Klein N, de Rossi A, Grosch-Woerner I, Babiker A. Immune repopulation after HAART in previously untreated HIV-1-infected children. Paediatric European Network for Treatment of AIDS (PENTA) Steering Committee [letter]. Lancet 2000;355(9212):1331-2.
64. Vigano A, Vella S, Principi N, et al. Thymus volume correlates with the progression of vertical HIV infection. AIDS 1999;13(5):F29-34.

65. Mackall CL, Fleisher TA, Brown MR, et al. Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. N Engl J Med 1995;332(3):143-9.
66. Zennou V, Mammano F, Paulous S, Mathez D, Clavel F. Loss of viral fitness associated with multiple Gag and Gag-Pol processing defects in human immunodeficiency virus type 1 variants selected for resistance to protease inhibitors in vivo. J Virol 1998;72(4):3300-6.
67. Douek DC, Koup RA, McFarland RD, Sullivan JL, Luzuriaga K. Effect of HIV on Thymic Function before and after Antiretroviral Therapy in Children. J Infect Dis 2000;181(4):1479-1482.
68. Qaqish RB, Fisher E, Rublein J, Wohl DA. HIV-associated lipodystrophy syndrome. Pharmacotherapy 2000;20(1):13-22.
69. Garcia F, Ortega M, Cruceta A, al. e. Incidence of metabolic changes and lipodystrophy in two trials comparing double versus triple antiretroviral therapy in early HIV disease. 1st international workshop on adverse drug reactions and lipodystrophy in HIV 1999, San Diego, CA.
70. Arpadi SM, Cuff PA, Horlick M, Wang J, Kotler DP. Lipodystrophy in HIV-infected children is associated with high viral load and low CD4+ -lymphocyte count and CD4+ -lymphocyte percentage at baseline and use of protease inhibitors and stavudine. J Acquir Immune Defic Syndr 2001;27(1):30-4.
71. Jaquet D, Levine M, Ortega-Rodriguez E, et al. Clinical and metabolic presentation of the lipodystrophic syndrome in HIV-infected children. AIDS 2000;14(14):2123-8.
72. Mora S, Sala N, Bricalli D, Zuin G, Chiumello G, Vigano A. Bone mineral loss through increased bone turnover in HIV-infected children treated with HAART. AIDS 2001;15(14):1823-9.

Sustained viral suppression and immune recovery after 4 years treatment of HIV-1 infected children with HAART

Pieter L.A. Fraaij, Gwenda Verweel, Annemarie M.C. van Rossum. Ellen G. van Lochem, Martin Schutten, Corry M.R. Weemaes, Nico G. Hartwig, David M. Burger, Ronald de Groot

Clin Infect Dis: in press

Chapter 4

Abstract

We here report the long-term data of 31 HIV-1 infected children treated with HAART. A high proportion of the children had undetectable HIV-1 RNA levels. CD4+ T-cell counts recovered and remained stable. Adverse events were observed frequently but were mostly mild.

Introduction

Prospective studies on the use of 'highly active antiretroviral therapy' (HAART) in HIV-1 infected children show viral suppression and recovery of the immune system. However, response rates are highly variable and frequently inferior to those observed in adults. (1) Most studies in HIV-1 infected children do not exceed an observation period of 48 weeks. Therefore little is known about the durability of the viral suppression and the reconstitution of the immune system. (1) We here report the 192 weeks (4 year) results of a prospective, open, cohort study on the clinical, immunological and virological response to therapy with HAART in 31 HIV-1 infected children.

Materials and methods

Study protocol

PI-naive HIV-1 infected children with a viral load above 5,000 copies/ml (measured on two consecutive occasions) and/or a CD4 cell count below their age-specific reference value were treated with indinavir or nelfinavir and 2 nucleoside reverse transcriptase inhibitors (NRTI). In all patients, steady-state intensive plasma PK sampling of the used PI was performed. PK sampling was repeated until area under the plasma concentration-time curve (AUC) reached target values.(2) Selected clinical data and laboratory values were obtained during regular visits to the outpatient department. Adherence to the medication regimen was assessed by interviews with parents and patients, medication diaries and by measurements of plasma drug levels. All patients who started medication before January first 2000 were included in this study. The study protocol was approved by the medical ethical committee of the Erasmus MC. Written informed consent was obtained from parents and patients

Statistical analysis

At each time point the percentage of the patients with HIV-1 RNA levels below the detection limit was calculated. For missing data the response value was considered as greater than the detection limit. Because absolute CD4+ T-cell and CD8 + T-cell counts are age related, CD4+ and CD8+ T-cells counts as percentage of age reference values were calculated. Hereto the patient's individual values at the different time-points were divided by the median of the age-specific reference values for T-cell subpopulations at that time-point. (3-5) To evaluate growth standard deviation scores (SDS) were obtained from the Dutch reference curves.

Differences between paired variables were analyzed with the Wilcoxon signed rank test and between groups with the Mann-Whitney-U test. The relation between response to therapy and baseline HIV-1 RNA levels and age were evaluated using exact binary logistic regression analysis. For statistical analysis SPSS© 10, LogXact© 4.1, Microsoft Excel© 97 and Growth analyzer© software were used.

Results

Between January 1997 and January 2004,31 HIV-1 infected children started HAART in our cohort. Their baseline characteristics are summarized in Table 1. None of the children had received prior treatment with PI or NNRTIs.

Medication use during the 192 weeks follow-up

At baseline 28 children started indinavir and 3 nelfinavir containing HAART. Therapy was changed a total of 38 times in 28 children. Reasons to change therapy included therapy failure (n=20), drug toxicity (n=7), simplification of therapy (n=7), refusal/non-tolerance for the medication regimen (n=3) and failure to obtain appropriate PK values (n= 1). During follow-up patients used a median of 2 different HAART regimes (range 1-5).

Table 1. Baseline characteristics of the 31 children.

Characteristics				
Sex (male/female)				16/15
Ethnicity (white/non-white)				4/27
Age in years*				5.1 (0.2-16.4)
Route of infection	Vertical			22
	Blood products			4
	Unknown			5
Clinical stage (CDC-classification)†				
	N1: 2	A1: 3	B1: 2	C1:
	N2: 3	A2: 5	B2: 5	C2: 2
	N3: 1	A3: 1	B3: 1	C3: 6
No prior NRTI treatment				15
Prior treatment with NRTI	Zidovudine:			11
	Zidovudine, zalcitabine:			2
	Zidovudine, lamivudine			1
	Zidovudine, didanosine			1
	Zidovudine, didanosine, lamivudine			1
Start HAART	IDV/AZT/3TC			27
	IDV/3TC/ddI			1
	NFV/AZT/3TC			1
	NFV/d4T/ddI			2
HIV-1 RNA levels *				87.350 (725-2.195.000)
CD4+ T-cells*	Absolute (10^6 cells/ml)			480 (0-3.580)
	% of normal			47 (0-143)
CD8+ T-cells*	Absolute (10^6 cells/ml)			1.240 (180-5.436)
	% of normal			155 (27.5-745)

* Median (range), † Clinical and Immunological categories as defined by the US Centers for Disease Control and Prevention (CDC).

In 13 (41% of all patients) children HAART was changed at least once because of viral failure. Six patients were lost to follow-up. Reasons for study termination included death (n=1), emigration (n=2), failure to come to the appointments (n=2) and age > 18 years (n=1).

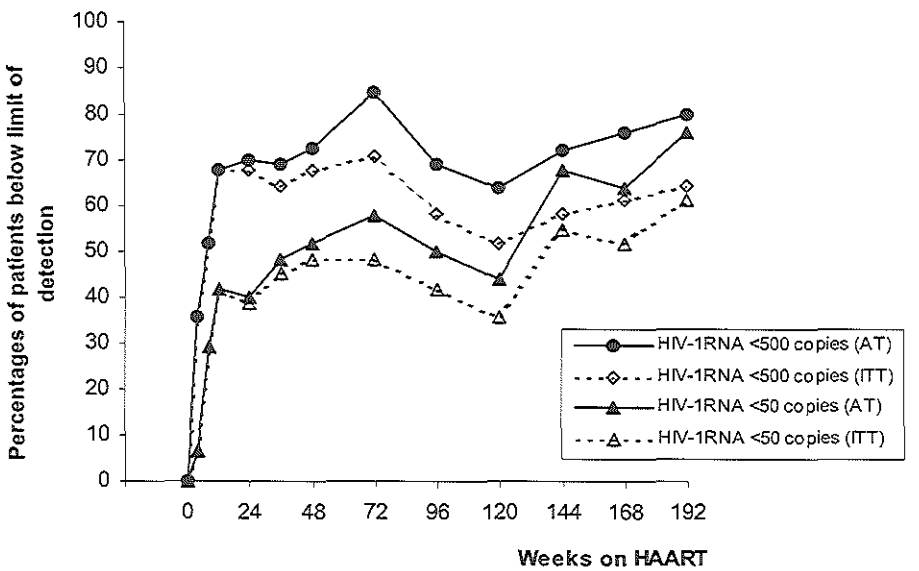
Clinical results

One child died of serious invasive opportunistic infections one year after start of therapy. Three children showed progression in their CDC-classification stage, none of these suffered from serious pathology. The other children in the cohort were in good health and a significant increase in growth was observed after start of HAART. (Median Body Mass Index (BMS) ΔSDS = 0.44; p = 0.019 and median lenght ΔSDS = 0.32; p = 0.05).

HIV-1 virologic response

Figure 1 shows the proportion of patients with HIV-1 RNA levels below the detection limits in time assessed using an 'as treated (AT)' and an 'intention to treat (missing = failure)' analysis. After 4 years 80% and 76% of the children had HIV-1 levels below 500 and 50 copies/ml with the AT-analysis and 65% and 61% of the children with the ITT-analysis. Of the 25 children on treatment after 4 years, 7 (28%) reached HIV-1 RNA levels below 500 copies/ml at week 12 and maintained HIV-1 RNA levels below 500 copies/ml during the whole follow up. These 7 patients were all considered to be adherent to the medication regimen, whereas 11 of the 18 who did not have complete viral suppression were defined at least once as non-adherent.

Figure 1. The proportion of children whose HIV-1 RNA levels decreased to less than 500 and less than 50 copies/ml in an as treated (AT) and an intention to treat missing = failure analysis (ITT).

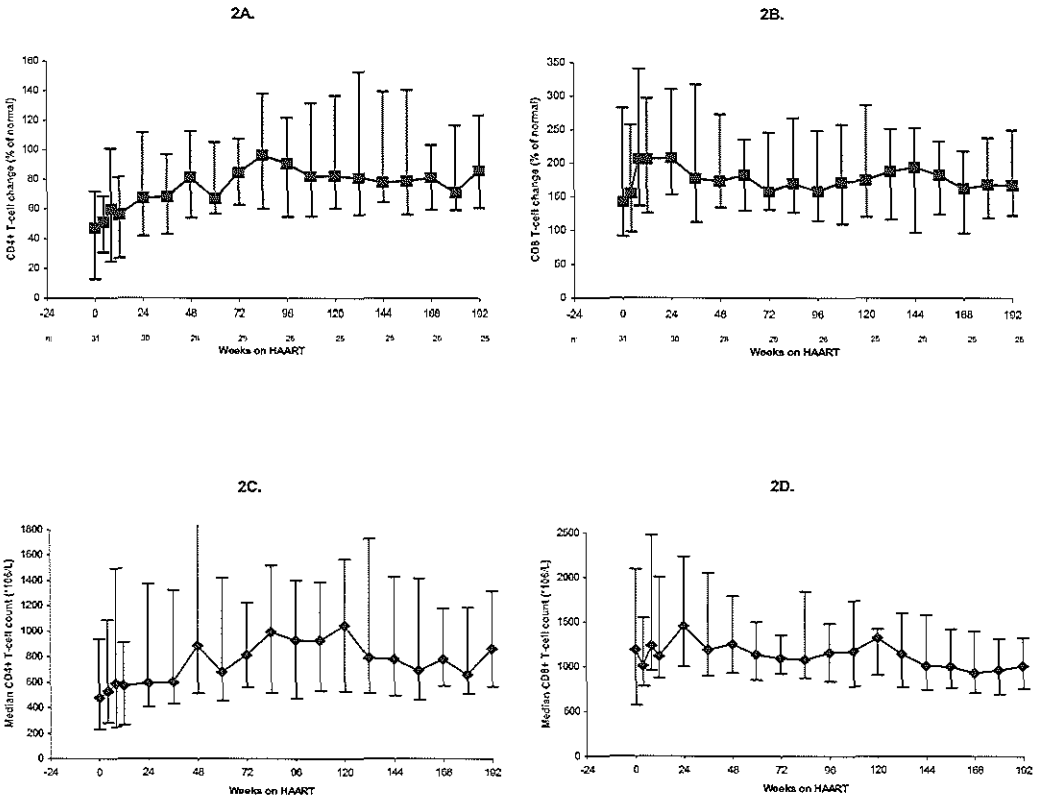


Binary logistic regression of the parameters age and baseline HIV-1 RNA levels and viral response revealed no relation between the baseline HIV RNA level and therapy response p=0.222, β = -0.58), but a significant negative relation between age and viral response rate was found (p = 0.04, β = -0.62).

CD4+ and CD8+ T-cell counts

In Figure 2A and C the median CD4+ T-cell count relative to normal and the absolute CD4+ T-cells counts (IQR) are depicted in time. Both the median relative to normal and the absolute T-cell counts were significantly higher at week 192 than at baseline. (p= 0.01 for the relative CD4+ T-cell count and p = 0.025 for the absolute CD4 T-cell count).

Figure 2A and **B:** Median relative (%normal) CD4+ and CD8+ T-cell counts of all patients.
Figure 2C and **2D:** Median absolute CD4+ and CD8+ T-cell counts.



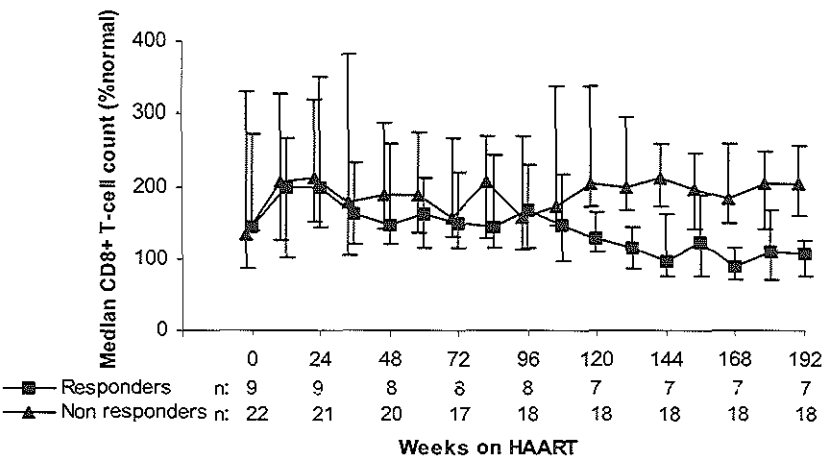
In figure 2B and D the median relative to normal and absolute CD8 T-cell counts (IQR) are depicted. Both remained high throughout the follow-up period and did not change significantly between baseline and week 192. (p = 0.96 and p=0.216 respectively).

T-cell function was analysed by means of proliferative response to CD3 mAb plus CD28 mAb in whole blood lymphocyte culture.(3, 6) The level of ^{3}H-thymidine incorporation increased after stimulation from of a median (IQR) 345 (134-865) counts/min /10^3 T-cells at baseline to 1186 (640-1528) counts/min /10^3 T-cells at week 192. (p = 0.07). Expressed as percentages of the median of 3450 adult healthy donors an increase the T-cell response increased form a median (IQR) of 17% (7-43) at baseline to 59% (32-76) at week 192 (p=0.07).

The CD4+ and CD8+ T-cell response and virologic response

Reconstitution of CD4+ T-cells (absolute CD4+ T-cell count and CD4+ T-cell count as percentage of normal) was not significantly different for virologic responders and virologic non responders. Likewise the absolute CD8+ T-cell count did not significantly differ between responders and non responders. However, when normalized for age from 96 weeks of therapy on the median relative CD8+ T-cell count was lower in viral responders compared to viral non responders. (Figure 3) With a median CD8+ T-cell count (IQR) at week 192 of 107% (65-126) in responders and 205% (161-255) in non responders (p= 0.017).

Figure 3: Change from baseline of the CD8+ T-cell count as percentage of normal in virologic responders and non responders.



Adverse events

During follow-up 24 patients (77%) reported clinical adverse events. These were mostly mild and of gastro-intestinal origin. In 7 children the medication regime was changed because of toxicity. In all cases the toxicity was associated with the use of indinavir. In one patient medication was changed for skin rash in all other cases because of nephrotoxicity, including hematuria and flank pain (n=4) and silent nephrolithiasis found on ultrasound examination (n=2).(7) Grade 3 or 4 laboratory adverse events were observed and included trombocytopenia

(n=3), increased amylase levels (n=2) and increased GGT levels (n=1). None of these resulted in a change of therapy.

In 2 cases lipoatrophy was suspected based on anthropometric measurements. Both children used stavudine, but different PIs (nelfinavir or indinavir). At the 4 year time point fasting triglycerid and cholesterol levels could be obtained in 17 of the 25 children still on treatment. Both were not markedly increased with only 1 and 4 patients with cholesterol or triglycerides above the ULN respectively.

Discussion

In this study a high proportion of children had a suppressed viral load after 4 year of treatment. Despite these good results, viral failure occurred often and frequently required changes in therapy. Strikingly, the proportion of children with suppressed viral load increased during follow-up. We feel that this may be due to therapy changes and more intensive intervention when non-adherence was suspected.

In contrast to other studies in children with an equal baseline age as in our cohort a negative relation between age and viral response was found. (8, 9) We speculate that puberty related problems interfering with the intake of HAART were more likely to occur due to the longer follow-up in our study.

After an initial increase the CD4+ T-cell counts remained stable throughout the follow-up period. Indicating a durable effect of HAART use on the CD4+ T-cell population. The median CD8+ T-cell count and relative age specific reference count remained high throughout the entire follow-up period, indicating ongoing immune stimulation. This is different from data obtained in adults where CD8+ T-cells counts returned to baseline levels or even decreased below baseline levels after initiation of HAART. (10, 11) Interestingly, a difference in CD8+ T-cell numbers as percentages of age related reference values was observed for patients who had viral suppression throughout the study period and those who had not. This difference may be the result of decreased antigenic stimulation. However the exact reason remains to be unclear and should be subject to further study.

In conclusion, an excellent response to HAART was observed. A high proportion of the children had undetectable HIV-1 RNA levels. CD4+ T-cell counts and T-cell function recovered and remained stable throughout the follow-up period. Adverse events occurred frequently, but were mostly mild.

Acknowledgements

The authors thank all children and their families participating in this study and Katja Wolthers and Marijke Roos for helpful discussion.

References

1. van Rossum AM, Fraaij PL, de Groot R. Efficacy of highly active antiretroviral therapy in HIV-1 infected children. Lancet Infect Dis 2002;2(2):93-102.
2. Burger DM, van Rossum AM, Hugen PW, Suur MH, Hartwig NG, Geelen SP, et al. Pharmacokinetics of the protease inhibitor indinavir in human immunodeficiency virus type 1-infected children. Antimicrob Agents Chemother 2001;45(3):701-5.
3. van Rossum AM, Scherpbier HJ, van Lochem EG, Pakker NG, Slieker WA, Wolthers KC, et al. Therapeutic immune reconstitution in HIV-1-infected children is independent of their age and pretreatment immune status. AIDS 2001;15(17):2267-75.
4. Comans-Bitter WM, de Groot R, van den Beemd R, Neijens HJ, Hop WC, Groeneveld K, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr 1997;130(3):388-93.
5. Shearer WT, Rosenblatt HM, Gelman RS, Oyomopito R, Plaeger S, Stiehm ER, et al. Lymphocyte subsets in healthy children from birth through 18 years of age: the Pediatric AIDS Clinical Trials Group P1009 study. J Allergy Clin Immunol 2003;112(5):973-80.
6. Roos MT, Prins M, Koot M, de Wolf F, Bakker M, Coutinho RA, et al. Low T-cell responses to CD3 plus CD28 monoclonal antibodies are predictive of development of AIDS. AIDS 1998;12(14):1745-51.
7. van Rossum AM, Dieleman JP, Fraaij PL, Cransberg K, Hartwig NG, Gyssens IC, et al. Indinavir-associated asymptomatic nephrolithiasis and renal cortex atrophy in two HIV-1 infected children. AIDS 2001;15(13):1745-7.
8. van Rossum AM, Geelen SP, Hartwig NG, Wolfs TF, Weemaes CM, Scherpbier HJ, et al. Results of 2 years of treatment with protease-inhibitor–containing antiretroviral therapy in dutch children infected with human immunodeficiency virus type 1. Clin Infect Dis 2002;34(7):1008-16.
9. Starr SE, Fletcher CV, Spector SA, Yong FH, Fenton T, Brundage RC, et al. Combination therapy with efavirenz, nelfinavir, and nucleoside reverse-transcriptase inhibitors in children infected with human immunodeficiency virus type 1. Pediatric AIDS Clinical Trials Group 382 Team. N Engl J Med 1999;341(25):1874-81.
10. Pakker NG, Kroon ED, Roos MT, Otto SA, Hall D, Wit FW, et al. Immune restoration does not invariably occur following long-term HIV-1 suppression during antiretroviral therapy. INCAS Study Group. AIDS 1999;13(2):203-12.
11. Pakker NG, Notermans DW, de Boer RJ, Roos MT, de Wolf F, Hill A, et al. Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation. Nat Med 1998;4(2):208-14.

Indinavir/low dose ritonavir containing HAART in HIV-1 infected children has potent antiretroviral activity, but is associated with side effects and frequent discontinuation of treatment

Pieter L.A. Fraaij, Gwenda Verweel, Annemarie M.C. van Rossum, Nico G. Hartwig, David M. Burger, Ronald de Groot

Submitted

Chapter 5

Abstract

We here present the study results of 21 HIV-1 infected children who were treated with indinavir plus low dose ritonavir and 2 NRTIs for 48 weeks. Although this q12h HAART regimen had potent antiretroviral activity, it was frequently associated with side effects and discontinuation of therapy.

Introduction

Indinavir is a potent protease inhibitor (PI), which has successfully been used in adults and children in combination with nucleoside reverse transcriptase inhibitors (NRTIs) to suppress HIV-1 infection. (1, 2) Durable suppression of HIV requires good adherence to the HAART regimen. Discontinuation or irregular use of HAART results in viral rebound, resistance of HIV to the medication and ultimately AIDS. (3) HAART regimens containing indinavir are difficult to adhere to. Indinavir, when used as a sole protease inhibitor, must be administered three times daily (q8h), thus interfering with the sleeping time and school hours of children. In addition, food restrictions further complicate the regimen, since the drug needs to be taken on an empty stomach or with a low-caloric meal.

To ensure adherence to an indinavir containing HAART regimen, less frequent and less complicated dosing schemes are required. Addition of low-dose ritonavir to indinavir increases plasma levels of indinavir allowing for a twice daily medication scheme without the necessity for food restrictions. (4, 5) Currently, no information on the long term clinical efficacy and safety of this combination in children is available. Therefore we performed an open uncontrolled pilot study in 21 HIV-1 infected children who were treated with indinavir and low-dose ritonavir containing HAART.

Methods

Children included in the Rotterdam cohort and treated with q8h dosed HAART including indinavir as the sole PI were offered to change therapy to q12h dosed HAART with indinavir and low-dose ritonavir. (6) In addition, between 2001 and 2002 HAART containing indinavir and low dose ritonavir was initiated in children naive to antiretroviral medication with a mean HIV RNA level > 5.000 copies/ml and a CD4+ T-cell count below age-specific reference values. The study protocol was approved by the medical ethical committee of the Erasmus MC. Written and informed consent was obtained from parents and patients. The patients' medical histories, physical examinations and laboratory values were analysed before the start of the medication and after 4, 8, 12, 24, 36 and 48 weeks of treatment. After minimally 2 weeks of treatment the steady state pharmacokinetics of indinavir were determined. (7) This procedure was repeated when dosage adjustment of indinavir was necessary to normalize the area under the plasma concentration-time curve (AUC) to adult values. Age-specific reference values for the CD4+ T-cells were calculated by dividing the patient's individual value at the different time-points by the median of the reference value at the different time-points. For statistical analysis of the data, SPSS©10 and Microsoft Excel©97 software were used.

Results

A total of 21 children were enrolled in the study of which 16 completed the 48 weeks study period. The baseline characteristics of all patients are summarized in Table 1. Four children were naive to antiretroviral therapy (Group A) and 17 were PI experienced. Of these, 9 had baseline HIV-1 RNA levels below 500 copies/ml (Group B1) and 8 had HIV-1 RNA levels above

500 copies/ml.(Group B2). Indinavir was started at a median dosage of 400 mg/m^2 (range, 300-600) and ritonavir was started at a median dosage of 125 mg/m^2 (range, 100-125).

Table 1. Baseline characteristics of the 21 children.

Characteristics		
Sex (male/female)		7/14
Ethnicity (white/non-white)		2/19
Age in years*		7 (0.4-16.3)
Route of infection	Vertical	15
	Blood products	3
	Unknown	3
Treatment history	- Naive to HAART (**Group A**)	4
	- Pretreated with PI containing HAART HIV RNA< 500 copies/ml (**Group B1**)	9
	- Pretreated with PI containing HAART HIV RNA > 500 copies/ml (**Group B2**)	8
Baseline HIV-1 RNA levels# (copies/ml)	Group A	168,750 (48,975 – 589,500)
	Group B2	4,650 (2,360 –14,794)
PI dosage*	indinavir (mg/m^2)	400 (300-600)
	ritonavir (mg/m^2)	125 (100-125)
NRTI backbone	AZT/3TC, q12h	20
	ddI/d4T, 12h	1

* Median (range); # median Interquartile range (IQR).

The clinical condition of the patients during the study period was good. The course of the surrogate markers viral load and CD4+ T-cell counts during follow-up are depicted in Table 2. Overall after 48 weeks of treatment 13 out of the 16 children still on treatment had HIV-1 RNA levels < 500 copies/ml and 11 < 50 copies/ml. In *Group A*, 3 out of 4 patients had HIV-1 RNA levels < 50 copies after 48 weeks of treatment. In *Group B1*, 7 out of 8 patients had HIV-1 RNA levels < 50 copies. In *Group B2*, 3 of 4 children had HIV-1 RNA levels < 500 copies/ml, but only

Table 2. Clinical and immunological parameters during 1 year follow up of 21 children treated with indinavir/ritonavir.

		Week 0	Week 12	Week 24	Week 36	Week 48
Total group	On treatment	21	18	16	16	16
	HIV-1 RNA levels < 500	9 (43%)	13 (72%)	13 (81%)	14 (88%)	13 (81%)
	HIV-1 RNA levels < 50	6 (29%)	8 (44%)	10 (63%)	11 (69%)	11 (69%))
	Median %CD4*	37 (23-54)	40 (32-61)	41 (35-61)	40 (32-62)	40 (30-62)
	Median CD4% normal*	61 (38-105)	72 (47-117)	78 (55-122)	67 (57-135)	68 (54-128)
Group A	On treatment:	4	4	4	4	4
	HIV-1 RNA levels < 500	0	3 (75%)	4 (100%)	3 (75%)	3 (75%)
	HIV-1 RNA levels < 50	0	1 (25%)	2 (50%)	3 (75%)	3 (75%)
	Median %CD4*	28 (7-41)	39 (15-46)	38 (26-58)	36 (21-56)	40 (26-58)
	Median CD4% normal*	39 (8-123)	45 (27-95)	71 (42-149)	61 (49-115)	57 (42-139)
Group B1	On treatment	9	8	8	8	8
	HIV-1 RNA levels < 500	9 (100%)	6 (75%)	8 (100%)	8 (100%)	7 (88%)
	HIV-1 RNA levels < 50	6 (67%)	6 (75%)	8 (100%)	6 (75%)	7 (88%)
	Median %CD4*	45 (38-48)	46 (38-59)	43 (37-53)	42 (37-57)	40 (32-56)
	Median CD4% normal*	96 (60-118)	76 (69-134)	78 (67-126)	87 (58-141)	80 (57-121)
Group B2	On treatment:	8	7	4	4	4
	HIV-1 RNA levels < 500	0	4 (75%)	1 (25%)	3 (75%)	3 (75%)
	HIV-1 RNA levels < 50	0	1 (15%)	0	2 (50%)	1 (25%)
	Median %CD4*	24 (19-46)	33 (29-61)	34 (22-53)	40 (27-53)	41 (25-52)
	Median CD4% normal*	53 (27-68)	69 (51-124)	83 (48-122)	100 (54-143)	90 (49-205)

*Median (interquartile range). Group A: children naive to HAART, Group BI: PI experienced patients with HIV-1 RNA < 500 copies on baseline, Group BII: PI experienced patients with HIV-1 RNA > 500 copies on baseline

one had HIV-1 RNA levels < 50 copies/ml. Over time an increase in CD4+ T-cells was observed, albeit not significant for any treatment group. (p-value baseline vs. week 48 for the total group CD4% total T-cells 0.234 and for CD4% normal 0.14).

Most children (n=19) reported side effects related to the medication. These side effects were often mild and of gastrointestinal origin. However, serious adverse were reported in 7 children. These included nephrolithiasis (n=2), silent nephrolithiasis found upon ultrasound research (n=2), jaundice (n=1), impaired liver functions, vomiting and malaise (n=1) and dehydration due to vomiting (n=1).

Five patients stopped therapy during the follow-up period. In one additional patient medication was discontinued on the last study day. Discontinuation happened 4 times because of drug toxicity (1 nephrolithiasis, 1 silent nephrolithiasis, 1 rash, 1 hepatitis) and twice on patient request.

No significant differences were found between the PK parameters of children with or without side effects (data not shown). However, in 2 children renal side effects resolved after a decrease of the indinavir or ritonavir dosage. In the 4 children who discontinued medication because of side effects dosage adjustments were not performed. This was because of the seriousness of the side effects and hesitation in the patients and their parents to restart indinavir.

Discussion

The studied regimen had potent antiretroviral activity with suppression of HIV in most children who remained on therapy for one year. However, side effects occurred frequently. No clear relation between the height of PK parameters and side effects or discontinuation of therapy were found. Still, most children in our study received indinavir/ritonavir 400/125 mg/m^2 q12h which has been shown to result in significantly higher indinavir exposure compared to reference data of indinavir q8h in both adults and children. (7) This increased AUC may result in a higher incidence of side effects, especially when instructions to increase fluid intake are not sufficiently followed. This is of major concern since the occurrence of side effects poses a major threat to the adherence, whilst maintenance of adherence was the most important reason to perform this medication change. In addition, patients reported that the combination was not easy to take: the high pill burden and the bad taste of ritonavir liquid were considered as negative aspects of the new regimen. Side effects and intolerance to the medication resulted in a high number of children that discontinued the study regimen as compared to studies in children with new and easier to use HAART regimens. In a study on lopinavir/ritonavir containing HAART in children only 2 out of 100 patients prematurely discontinued treatment. (8) However, one should be cautious to compare both studies, since the numbers of patients are relatively small and the studied patient population selected differently. Interestingly, a large proportion of children that discontinued medication in our study had detectable viral loads at baseline. Possibly, preexisting problems with adherence may have

influenced our data. Still, because of the high number of side effects and patients that prematurely discontinued medication we feel that newer and easier to use HAART regimens than indinavir/low dose ritonavir should be used to treat HIV-1 infected children. However in resource pour settings were these costly new drugs may not be available indinavir combined with ritonavir should be considered as a treatment option for HIV-1 infected children.

References

1. van Rossum AM, Geelen SP, Hartwig NG, Wolfs TF, Weemaes CM, Scherpbier HJ, et al. Results of 2 years of treatment with protease-inhibitor–containing antiretroviral therapy in dutch children infected with human immunodeficiency virus type 1. Clin Infect Dis 2002;34(7):1008-16.
2. Gulick RM, Mellors JW, Havlir D, Eron JJ, Meibohm A, Condra JH, et al. 3-year suppression of HIV viremia with indinavir, zidovudine, and lamivudine. Ann Intern Med 2000;133(1):35-9.
3. Deeks SG. Treatment of antiretroviral-drug-resistant HIV-1 infection. Lancet 2003;362(9400):2002-11.
4. Hsu A, Granneman GR, Cao G, Carothers L, Japour A, El-Shourbagy T, et al. Pharmacokinetic interaction between ritonavir and indinavir in healthy volunteers. Antimicrob Agents Chemother 1998;42(11):2784-91.
5. van Rossum AM, de Groot R, Hartwig NG, Weemaes CM, Head S, Burger DM. Pharmacokinetics of indinavir and low-dose ritonavir in children with HIV-1 infection. AIDS 2000;14(14):2209-10.
6. Burger DM, van Rossum AM, Hugen PW, Suur MH, Hartwig NG, Geelen SP, et al. Pharmacokinetics of the protease inhibitor indinavir in human immunodeficiency virus type 1-infected children. Antimicrob Agents Chemother 2001;45(3):701-5.
7. Bergshoeff AS, Fraaij PL, van Rossum AM, Verweel G, Wynne LH, Winchell GA, et al. Pharmacokinetics of indinavir combined with low-dose ritonavir in human immunodeficiency virus type 1-infected children. Antimicrob Agents Chemother 2004;48(5):1904-7.
8. Saez-Llorens X, Violari A, Deetz CO, Rode RA, Gomez P, Handelsman E, et al. Forty-eight-week evaluation of lopinavir/ritonavir, a new protease inhibitor, in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2003;22(3):216-224.

Safety and efficacy of an abacavir based HAART regimen after pre-treatment with protease inhibitors in children with an undetectable HIV-1 viral load

Pieter L.A. Fraaij, Alina S. Bergshoeff, Gwenda Verweel, Linda van der Knaap, Annemarie M.C. van Rossum, Nico G. Hartwig, David M. Burger, Ronald de Groot

Submitted

Chapter 6

Abstract

Objective: To assess the antiviral efficacy, safety and pharmacokinetic parameters of the replacement of protease inhibitors (PIs) for the nucleoside reverse transcriptase inhibitor (NRTI) abacavir in pediatric patients with HIV-1 RNA levels < 500 copies/ml.

Methods: Pediatric patients receiving 2 NRTIS and at least one PI with HIV-1 RNA levels < 500 copies/ml were offered a medication change to abacavir containing HAART in an open label study. Clinical assessment included plasma RNA levels, lymphocyte counts, chemistry, hematology, and adverse events monitoring. In addition, intensive plasma pharmacokinetic sampling of abacavir was performed.

Results: In intention to treat analysis considering switch as failure, 10/11 enrolled patients had HIV-1 RNA levels < 50 copies/ml after 48 weeks of treatment. In one child, medication was changed because of viral failure at week 12. This child was one of 4 enrolled patients who had received NRTI treatment prior to HAART. Plasma pharmacokinetic parameters of abacavir were similar to historical controls in HIV-1 infected children.

The abacavir containing combination treatment was well tolerated and most side effects were mild. A significant reduction in cholesterol plasma levels, but not triglycerides, was observed after initiation of abacavir.

Conclusion: Replacement of PIs by abacavir in HIV-1 infected children with HIV-1 RNA levels < 500 copies/ml provides continued viral suppression and improvement of lipid abnormalities. The regimen should be applied with caution in patients who have been extensively pretreated with NRTIs.

Introduction

After the introduction of highly-active antiretroviral therapy (HAART), an impressive reduction in the rate of disease progression to AIDS and HIV-1 related deaths has been observed in adults, adolescents and children. (1, 2) Still, institution of optimal treatment poses a major challenge. Large interindividual differences in the pharmacokinetics of antiretroviral drugs, different viral dynamics in children, a developing immature immune system and difficulties in adherence to the medication regimen complicate therapy. (3-5) In 1997, we initiated a Dutch multicenter study on the treatment of HIV-1 infected children using protease inhibitor (PI) based HAART. Our study had favourable results in children using either indinavir or nelfinavir with viral response rates of 69% HIV-1 RNA levels < 500 copies /ml and 50% < 50 copies/ml after 96 weeks. (6) Despite these good results, the PI based medication regimens that were used have disadvantages. These include frequent dosing, sometimes during sleeping time and school hours, poor palatability of medication, food restrictions during intake of medication, and a high pill burden. In addition, side effects such as abnormal distribution of fat tissue (lipodystrophy syndrome) have been found to be associated with PI usage. (7, 8)

We sought to simplify the medication regimen in children to ensure a maximal degree of adherence without jeopardising antiretroviral efficacy. The nucleoside reverse transcriptase inhibitor (NRTI) abacavir has been proposed as a new component of HAART allowing for such simplification of therapy. (9) It is administered twice daily, has a low pill burden and has acceptable palatability. Abacavir as part of a triple NRTI regimen may not be the first choice of medication in children, since it has reduced efficacy with high viral loads, which are characteristic for pediatric HIV infection. (10, 11) However, after the viral load has been reduced by initial PI based treatment, abacavir can possibly replace the PI. In addition, changing a PI based regimen to an abacavir based regimen may lower abnormal serum lipids and possibly decrease the risk lipodystrophy. (12, 13)

Studies in adults show that in patients previously on well controlled PI based HAART, replacement of the PI for abacavir is generally well tolerated and effective, reducing the chance for therapy discontinuation. (9, 13, 14) In addition a significant decrease was observed in cholesterol and triglyceride levels.

We here report on a study in children with undetectable HIV-1 RNA levels while on a PI based regimen, in whom the PI was replaced for abacavir.

Materials and methods

Patients

This was an open label study, in which HIV-1 infected children treated in the Rotterdam cohort aged between 1 and 18 years using HAART containing the PIs indinavir or nelfinavir and 2 NRTIs with viral load < 500 HIV-1 RNA copies at least 4 weeks before baseline could be enrolled. Prior treatment with NRTI before HAART was allowed. The study protocol was

approved by the medical ethical committee of the ErasmusMC. Written and informed consent was obtained from patients and/or parents. Physical examinations and laboratory measurements were performed within 4 weeks before start of the abacavir containing medication regimen (baseline) and after 4, 8, 12, 24, 36 and 48 weeks of treatment. Plasma HIV-1 RNA levels were measured by an in vitro nucleic acid amplification test (Amplicor 1.5 HIV-1 Monitor test (Roche Diagnostic Systems, Branchburg, US)) with a lower limit of quantification of 50 copies/ml. On baseline, week 12, 24, 36 and 48, lymphocyte subsets were analyzed with the FACSCount System (Bencton Dickinson Immunocytometry Systems, San Jose , CA, USA).

Medication and pharmacokinetics

Medication was prescribed in the following doses: abacavir 8 mg/kg q12h (max. 300 mg q12h), zidovudine: 180 mg/m^2 q12h (max 300 mg q12h), lamivudine 4 mg/kg q12h (max 150 mg q12h), didanosine 120 mg/m^2 q12h (max 200 mg q12h) and stavudine 1 mg/kg q12h (if body weight <60 kg, not exceeding 30 mg q12h; if bodyweight $\geq$ 60 kg, not exceeding 40 mg q12h). There were no food restrictions, except for didanosine. Abacavir was administered as oral solution containing 20 mg/ml abacavir, tablets of 300 mg or customised pharmacy prepared capsules. The dose of 8 mg per kg could deviate if the maximum dosage was attained or if necessary to enable the use of a whole tablet formulation (patient or pharmacy request).

All patients were admitted to the hospital to determine steady state 12h pharmacokinetics of abacavir. Blood samples were obtained 0, 30, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480, 600 and 720 minutes after ingestion of the medication. Plasma was separated by centrifugation (10 min at 3000 x *g*) and samples were stored at -20 °C until analysis. Abacavir concentrations were determined in plasma by validated method of HPLC (unpublished data). The lower limit of quantification was 0.015 mg/L with accuracy of 97-100%. Within-day and between-day variability was 1.1-1.9% and 0.16-2.3%, respectively.

Pharmacokinetic parameters were calculated in Microsoft Excel© 97 using non-compartmental methods. (15) The abacavir peak plasma level (Cmax), the trough level at the 12-h time point (Cmin) and the time to peak plasma concentration (Tmax) were determined visually from the plasma concentration-time curve. Area under the plasma concentration-time curve 0-12h (AUC0-12) was calculated using the trapezoidal rule. Apparent oral clearance (Cl/F) of abacavir was calculated as dose (mg)/AUC0-12h. Relative apparent oral clearance (Cl/F*kg) was calculated as dose (mg)/AUC0-12 * body weight (kg).

Adverse events

During the whole study period, adverse events were scored. They were defined as any clinical sign or symptom, or meaningful laboratory test abnormality, possibly or probably related to the study medication, excluding HIV-related disorders. Severity, relation to medication and hospitalization were scored. When possible, grading of severity of adverse events was performed conform to the NIH division of AIDS toxicity table for grading severity of pediatric

adverse experiences. During visits, patients and their parents or caretakers were asked to report on the patient's well-being, activity and appetite. Blood samples for serum triglycerides and total cholesterol were obtained at baseline, when using PI based HAART and week 4 after therapy change. Hereafter, the patients' lipid levels were analysed once every 6 months according to the center's treatment protocol. For analysis of late term effects of abacavir on cholesterol and triglycerides, the last sample obtained after a minimal duration of abacavir therapy of 1 year was used. Samples for cholesterol and triglyceride levels and were taken after a fasting period of at least three hours.

Statistics

For statistical analysis of the data, SPSS© version 10.0 (SPSS, IL, U.S.) and Microsoft Excel© 97 software were used. All statistical analyses were based on non-parametric tests. Comparison of paired data from patients at different times of follow-up was performed with the Wilcoxon signed rank test. Viral response was defined as the percentage of patients with HIV-1 RNA levels below 500 or 50 copies/ml. For analysis of efficacy, intention to treat (ITT) analysis was used. ITT analysis was applied in two different ways: all exposed patients included (regardless of therapy switch) or as switch equaling failure (in which change of medication was considered as viral failure). Because absolute CD4+ T-cell and CD8+ T-cell counts are age-dependent, relative CD4+ and CD8+ T-cell counts were calculated. Hereto, at the different time-points, age-specific reference values were calculated by dividing the patient's individual value by the median age-related reference value at that time point. (16) The Friedman test was used for differences in CD4+ and CD8+ percentages of normal measurements in time. All p-values are two tailed and p-values below 0.05 were considered statistically significant.

Results

Patient characteristics

A total of 11 children were included in the study between November 2000 and January 2003. One child refused enrolment, because caregivers and the child were satisfied with her nelfinavir containing HAART regimen. The baseline characteristics of the 11 enrolled patients are shown in Table 1. Although some children had experienced progressive disease and significant immunosuppression in the past, none of the patients showed signs or symptoms of AIDS-defining illness at baseline. Five children had been pre-treated with indinavir containing HAART, 5 had received both indinavir and nelfinavir and one child was pre-treated with nelfinavir alone. Four children had received monotherapy with zidovudine prior to the start of HAART. One patient had received zidovudine, stavudine and lamivudine prior to the start of HAART. The median duration on HAART was 4.5 years (range: 1.9-4.8 years). At baseline, all patients had HIV-1 RNA levels below 500 copies/ml and 10 of the 11 children had HIV-1 RNA levels < 50 copies/ml, one child had a RNA level of 478 copies/ml.

Table 1. Baseline patient characteristics.

Number of patients	11
Age in years (median (range))	8.9 (3.1-17.8)
Route of infection (number of cases)	
Vertical	8
Blood products	1
Unknown	2
Clinical stage (CDC-classification)	N2: 1
	A2: 3
	B2: 3
	B3: 1
	C2: 1
	C3: 2
Prior treatment with mono NRTI (number of cases):	
None	7
Monotherapy AZT	3
Monotherapy AZT, later d4T, 3TC	1
Prior HAART	
IDV/AZT/3TC/(RTV)	5
IDV/AZT/3TC/(RTV), AZT/3TC/NFV	4
NFV/d4T/ddI	1
IDV/AZT/3TC, NFV/d4T/ddI	1
HIV-1 RNA levels at baseline	
HIV-1 RNA < 500 copies/ml	11
HIV-1 RNA < 50 copies/ml	10
CD4+ T-cells Median (IQR)	
Absolute counts (106 cells/ml)	880 (530-1656)
% of age specific reference value	88 (66-165)
CD8+ T-cells Median (IQR)	
Absolute counts (106 cells/ml)	1070 (810-1656)
% of age specific reference value	165 (158-208)

Abbreviations : NRTI : nucleoside reverse transcriptase inhibitor; 3TC: lamivudine ; AZT: zidovudine; d4T: stavudine; ddI: didanosine; IDV: indinavir; NFV: nelfinavir; (RTV): ritonavir boosting.

Pharmacokinetics of abacavir

In all 11 children included in the study, intensive plasma pharmacokinetic analysis was performed for abacavir (Figure1). The median dose of abacavir was 7.9 mg/kg (range: 5.1-9.8). Pharmacokinetic parameters are listed in Table 2. Pharmacokinetic parameters were in accordance with historical controls of children using abacavir. (17)

Figure 1. Individual pharmacokinetic profiles of abacavir in 11 children.

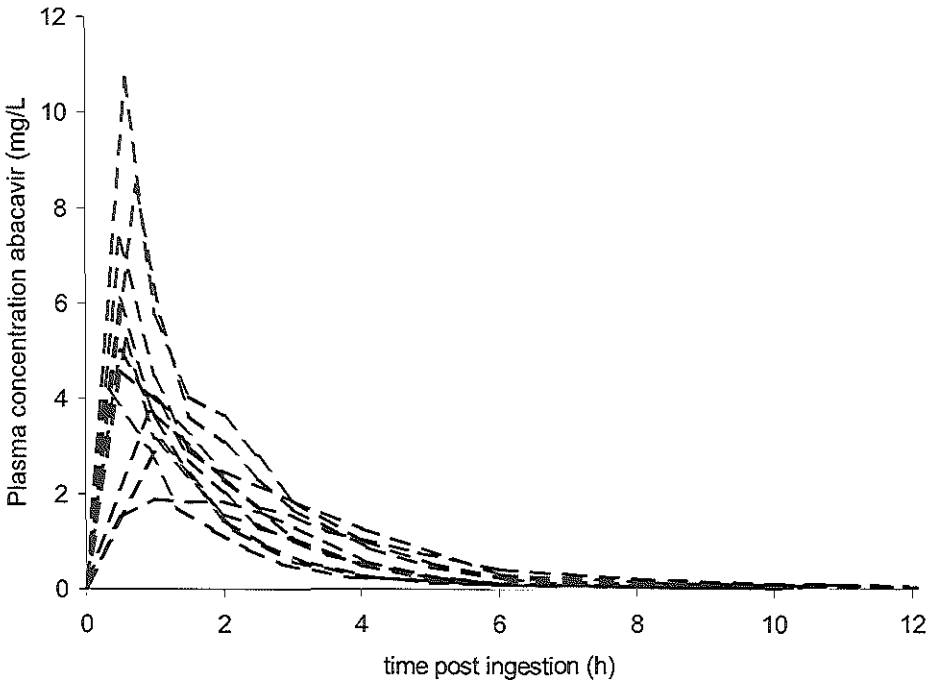


Table 2. Steady-state pharmacokinetic parameters of 11 children using abacavir in combination with 2 NRTI.

Pharmacokinetic parameter	Current study (median (interquartile range)) (N=11)	Historical data of abacavir in children (mean (coefficient of variation)) (N=45) (15)
AUC0-12 (mg/L*h)	9.3 (7.1-11.5)	9.8 (47)
Cmax (mg/L)	5.0 (4.1-6.7)	3.71 (37)
Tmax (h) (median)	0.58 (0.50-0.88)	N.A.
Cmin (mg/L)	0.04 (0.04-0.07)	N.A.
Cl/F (L/h)	21.4 (18.0-29.4)	19 (62)
Cl/F*kg (L/h*kg)	0.85 (0.67-1.12)	N.A.
T1/2 (h)	2.3 (2.1-3.6)	1.28 (33)

N.A.: not available

Clinical observationss

No changes were observed in the patient's overall well being, activity and appetite as reported by the children and the caregivers. None of the patients showed disease progression. No AIDS defining signs or symptoms were observed. All children were still on study after 48 weeks of treatment.

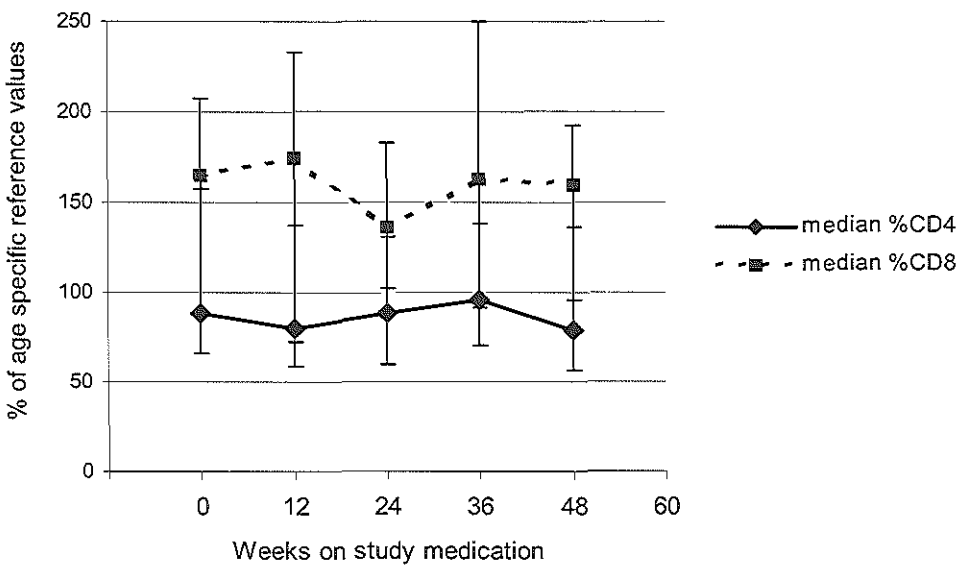
HIV-1 virological and immunological response

In ITT analysis all exposed patients included, at 48 weeks after the switch to abacavir, all 11 children had HIV-1 RNA levels below 50 copies/ml. When using the switch equals failure analysis, 10 of the 11 children (91%) had successful treatment outcome at 48 weeks.

In one child, HIV-1 RNA levels increased from < 50 copies/ml at baseline to 236 copies/ml at week 8 and 554 at week 12. On patient request, medication was changed to lopinavir/r and efavirenz, which resulted in a viral load decrease to below 50 HIV-1 RNA copies/ml at week 24. This patient had received extensive NRTI treatment with zidovudine, stavudine and lamivudine before initiation of HAART. The 3 other patients who had received mono NRTI treatment prior to HAART responded well to the abacavir containing regimen with viral loads below 50 copies/ml 48 weeks after initiation of abacavir containing treatment.

In another child, which was enrolled into the study with a viral load above 50, but below 500 copies/ml, viral load was suppressed to below 50 copies/ml at week 4 after therapy switch to abacavir containing HAART.

Figure 2. CD4+ and CD8+ T-cells (median (interquartile range)) as percentage of age-specific reference values.



Median CD4+ and CD8+ T-cell counts as percentage of normal fluctuated throughout the study period (Table 1; Figure 2). At baseline, median CD4+ percentage of age specific reference values was 88 (interquartile range (IQR): 66-165) At week 48, median CD4+ percentage of age specific reference values was 79 (IQR: 56-136). Changes in the CD4+ and CD8+ T-cell counts as percentage of age specific reference values in time were not statistically significant (p-value for CD4+ percentages = 0.243 and p-value for CD8+ percentages = 0.544).

Table 3. Virological response in 11 children who switched PI based HAART to abacavir in combination with 2 NRTI. Intention to treat analysis on all exposed patients and using switch equals failure.

Weeks on treatment	All exposed patients. Viral responders: HIV-1 RNA < 500 copies/ml (% of total)	All exposed patients. HIV-1 RNA < 50 copies/ml (% of total)	Switch equals failure: HIV-1 RNA < 500 copies/ml (% of total)	Switch equals failure: HIV-1 RNA < 50 copies/ml (% of total)
0	11/11 (100%)	10/11 (91%)	11/11 (100%)	10/11 (91%)
4	11/11 (100%)	11/11 (100%)	11/11 (100%)	11/11 (100%)
8	11/11 (100%)	10/11 (91%)	11/11 (100%)	10/11 (91%)
12	10/11 (91%)	10/11 (91%)	10/11 (91%)	10/11 (91%)
24	11/11 (100%)	11/11 (100%)	10/11 (91%)	10/11 (91%)
36	11/11 (100%)	11/11 (100%)	10/11 (91%)	10/11 (91%)
48	11/11 (100%)	11/11 (100%)	10/11 (91%)	10/11 (91%)

Adverse events

Abacavir containing combination treatment was well tolerated by all children. The adverse events were mild and all transient in nature. None of the patients changed medication due to adverse events. Therapy related side effects included diarrhoea (2 patients), abdominal pain (2 patients), abdominal and flank pain (1patient), malaise (1 patient), fever (1 patient), eczema/dry skin (1 patient) and a skin rash (1 patient). This rash was transient and was reported upon the study visit at the time it had already resolved. Medication was not discontinued. In one child, platelet counts decreased to 24,000 109/l (NIH toxicity grade 4), but restored on a subsequent measurement. The cause for this transient thrombocytopenia remained unknown. One child had a transient grade 3 elevation of serum amylase. No other grade 3 or 4 events were documented. Grade 1 and 2 laboratory adverse events included anaemia (2 cases), low erythrocyte count (8 cases), raised potassium level (2 cases), raised amylase levels (4 cases)

raised calcium levels (1 case) and abnormal liver function: ALT raised (2 cases), AST raised (3 cases) and γ-GT raised (7 cases). Four children had increased levels of LDH. Creatinine was increased in one child (48 µmol/L) and urea in two children (7.0 and 7.5 µmol/L, respectively).
Of nine children, plasma cholesterol levels could be analysed in follow up. Two children were excluded from the analysis. One child was excluded since insufficient amounts of blood could be obtained. Also, the patient who had changed medication to a PI based regimen was excluded from this analysis. The median time between start of abacavir and the last obtained lipid plasma level was 60 (IQR: 49-106) weeks. Median total cholesterol plasma levels decreased from 5.2 mmol/l (IQR: 3.7-5.45) to 4.1 (IQR: 3.3-4.95) at week 4 and 3.8 (3.15-4.5) in the last sample, after a follow-up of at least 48 weeks (p-value of baseline vs. 48 weeks follow-up was 0.011). Median (IQR) plasma triglycerides decreased from 0.88 mmol/l (0.74-1.28) to 0.66 (0.43-1.08) at week 4, but increased again to 0.74 (0.55-1.11) in the last sample, after a follow-up of at least 48 weeks (p-value of baseline vs. week 48 was 0.138). One child was suspected for lipoatrophy on baseline, which did not change based on visual inspection. No other cases of lipodystrophy of lipoatrophy were reported while on abacavir.

Discussion

Children living with HIV/AIDS face the challenge of life-long complex antiretroviral therapy regimens. Watson et al. showed in children with HIV-1 that non-adherence is common and might be the major impediment to successful treatment. (5) Simplification of antiviral therapy in HIV-1 infected children with viral suppression on PI based HAART may increase adherence or prevent possible future non-adherence.

In our study population of 11 HIV-1 infected children, the abacavir based regimen had potent antiviral activity. However, in one child the regimen failed. This was the patient who had received extensive therapy with NRTIs prior to HAART became available. Viral resistance tests of this child during NRTI treatment were not available and could not be retrospectively performed since no samples were available. After initiation of HAART viral resistance tests were not feasible since HIV-1 RNA was no longer detectable. In adults similar observations have been made in which sub-optimal mono or dual NRTI treatment in the past resulted in therapy failure of abacavir based regimens (13, 14) The finding that 3 out of 4 children responded well to the abacavir containing regimen despite pre-treatment with mono NRTI therapy prior to HAART is encouraging. Nevertheless, this regimen should be applied with caution in patients who have received prior suboptimal NRTI treatment and in whom no data on earlier acquired viral resistance are available.
The regimen was well tolerated and no serious side effects were seen, especially hypersensitivity reaction (HSR) was not observed. However, HSR to abacavir occurs only seldom (in <5% of the users) and may have been missed due to the limited number of children included in our study. (18) The risk for HSR should be taken into consideration when children are prescribed abacavir. Interestingly, plasma cholesterol decreased sharply immediately after the medication change. This indicates that the PI used at baseline had a direct effect on lipid

metabolism that was quickly and with sustained effect reversed after its removal. Similar observations have been made in adults in whom medication was changed from a PI based regimen to abacavir. (9, 13, 14) For triglycerids, in our study, no such a decrease at 48 weeks was observed, despite an initial decrease after change of medication. An explanation for this observation could be the small sample size of this study.

The plasma exposure to abacavir in our group was somewhat higher than the exposure reported in adults but data were in accordance with previously reported pediatric data. (17, 19, 20) Most children used zidovudine as part of their abacavir combination treatment. In adults, the co-administration of zidovudine with abacavir produced a small and inconsistent effect on the pharmacokinetic parameters of abacavir. (19) While this phenomenon is of unclear significance, it may have increased the interindividual variability in our pharmacokinetic data.

Clearly, our study was performed in a selected population. All children had viral load levels below 500 copies/ml, which suggests that adherence was good. In addition all children included in our study were older than 2 years of age, which has been found to be associated with a favourable outcome of HAART in children. (7) In this population, our study showed that a difficult to use PI based regimen can be changed to the more easy to use abacavir based regimen. Whether viral load suppression of this regimen is similar to a PI based regimen needs to be further investigated in a larger scale, comparative study.

In conclusion, replacement of PI by abacavir in a HAART regimen in HIV-1 infected children with suppressed viral load provides continued viral suppression and improvement of lipid abnormalities. The regimen should be applied with caution in patients who have been extensively pretreated with NRTIs, in whom resistance to NRTI is likely to be present.

Footnote

Financial disclosure

The authors participated in studies financially supported by Merck Sharp & Dome, GlaxoSmithKline and the Dutch Aids Foundation from 1997 to 1999. From 2002 until 2003 the studies received financial support by Merck Sharp & Dome, GlaxoSmithKline and Abbott. No other associations of the authors that might pose a conflict of interest are reported. There was no involvement of the previously mentioned sponsors with data collection, data analysis, the writing of this article and article submission.

References

1. Palella FJ, Jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998;338(13):853-60.
2. Gortmaker SL, Hughes M, Cervia J, Brady M, Johnson GM, Seage GR, 3rd, et al. Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. N Engl J Med 2001;345(21):1522-8.
3. Burger DM, van Rossum AM, Hugen PW, Suur MH, Hartwig NG, Geelen SP, et al. Pharmacokinetics of the protease inhibitor indinavir in human immunodeficiency virus type 1-infected children. Antimicrob Agents Chemother 2001;45(3):701-5.
4. Bergshoeff AS, Fraaij PL, van Rossum AM, Wolfs TF, Geelen SP, de Groot R, et al. Pharmacokinetics of nelfinavir in children: influencing factors and dose implications. Antivir Ther 2003;8(3):215-22.

5. Watson DC, Farley JJ. Efficacy of and adherence to highly active antiretroviral therapy in children infected with human immunodeficiency virus type 1. Pediatr Infect Dis J 1999;18(8):682-9.
6. van Rossum AM, Geelen SP, Hartwig NG, Wolfs TF, Weemaes CM, Scherpbier HJ, et al. Results of 2 years of treatment with protease-inhibitor–containing antiretroviral therapy in dutch children infected with human immunodeficiency virus type 1. Clin Infect Dis 2002;34(7):1008-16.
7. van Rossum AM, Fraaij PL, de Groot R. Efficacy of highly active antiretroviral therapy in HIV-1 infected children. Lancet Infect Dis 2002;2(2):93-102.
8. McComsey G, Bhumbra N, Ma JF, Rathore M, Alvarez A. Impact of protease inhibitor substitution with efavirenz in HIV-infected children: results of the First Pediatric Switch Study. Pediatrics 2003;111(3):e275-81.
9. Clumeck N, Goebel F, Rozenbaum W, Gerstoft J, Staszewski S, Montaner J, et al. Simplification with abacavir-based triple nucleoside therapy versus continued protease inhibitor-based highly active antiretroviral therapy in HIV-1-infected patients with undetectable plasma HIV-1 RNA. AIDS 2001;15(12):1517-26.
10. Palumbo PE, Raskino C, Fiscus S, Pahwa S, Fowler MG, Spector SA, et al. Predictive value of quantitative plasma HIV RNA and CD4+ lymphocyte count in HIV-infected infants and children. JAMA 1998;279(10):756-61.
11. Staszewski S, Keiser P, Montaner J, Raffi F, Gathe J, Brotas V, et al. Abacavir-lamivudine-zidovudine vs indinavir-lamivudine-zidovudine in antiretroviral-naive HIV-infected adults: A randomized equivalence trial. JAMA 2001;285(9):1155-63.
12. Carr A, Samaras K, Burton S, Law M, Freund J, Chisholm DJ, et al. A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors. AIDS 1998;12(7):F51-8.
13. Martinez E, Arnaiz JA, Podzamczer D, Dalmau D, Ribera E, Domingo P, et al. Substitution of nevirapine, efavirenz, or abacavir for protease inhibitors in patients with human immunodeficiency virus infection. N Engl J Med 2003;349(11):1036-46.
14. Opravil M, Hirschel B, Lazzarin A, Furrer H, Chave JP, Yerly S, et al. A randomized trial of simplified maintenance therapy with abacavir, lamivudine, and zidovudine in human immunodeficiency virus infection. J Infect Dis 2002;185(9):1251-60.
15. Gibaldi M. Compartmental and noncompartmental pharmacokinetics. 4th edition ed. Philadelphia; 1991.
16. Comans-Bitter WM, de Groot R, van den Beemd R, Neijens HJ, Hop WC, Groeneveld K, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr 1997;130(3):388-93.
17. Kline MW, Blanchard S, Fletcher CV, Shenep JL, McKinney RE, Jr., Brundage RC, et al. A phase I study of abacavir (1592U89) alone and in combination with other antiretroviral agents in infants and children with human immunodeficiency virus infection. AIDS Clinical Trials Group 330 Team. Pediatrics 1999;103(4):e47.
18. Clay PG. The abacavir hypersensitivity reaction: a review. Clin Ther 2002;24(10):1502-14.
19. McDowell JA, Lou Y, Symonds WS, Stein DS. Multiple-dose pharmacokinetics and pharmacodynamics of abacavir alone and in combination with zidovudine in human immunodeficiency virus-infected adults. Antimicrob Agents Chemother 2000;44(8):2061-7.
20. Hughes W, McDowell JA, Shenep J, Flynn P, Kline MW, Yogev R, et al. Safety and single-dose pharmacokinetics of abacavir (1592U89) in human immunodeficiency virus type 1-infected children. Antimicrob Agents Chemother 1999;43(3):609-15.

Safety and efficacy of a NRTI-sparing HAART regimen of efavirenz and lopinavir/ritonavir in HIV-1-infected children

Pieter L.A. Fraaij, Jennifer Neubert, Alina S. Bergshoeff, Annemarie M.C. van Rossum, Nico G. Hartwig, Horst Schroten, Klaus Korn, David M. Burger, Ronald de Groot, Tim Niehues

Antivir Ther 2004;9:297-299

Chapter 7

Abstract

We studied a nucleoside reverse transcriptase inhibitor (NRTI)-sparing regimen for the treatment of children infected with NRTI-resistant HIV-1. The combination of lopinavir/ritonavir and efavirenz suppressed HIV-1 levels for a prolonged period and resulted in a significant increase in CD4+ T- cell numbers despite an extensive prior treatment with NRTI (>4 years). Observed side effects were transient with the exception of dyslipidemia.

Introduction

It is unclear which treatment regimen should be used in HIV-1 infected children with nucleoside reverse transcriptase inhibitor (NRTI)-resistant virus. Both lopinavir/ritonavir- and efavirenz-containing regimens are potent and safe in children. (1, 2) We hypothesised that a combination of lopinavir/ritonavir and efavirenz without NRTIs would be safe and achieve maximal viral suppression. At the same time, unnecessary NRTI related side effects could be prevented.

Table 1. HIV-1 bDNA levels in the 8 individual HIV-1 infected children after start of efavirenz and lopinavir/ritonavir.

	Baseline	Week 4	Week 8	Week 12	Week 24	Week 36	Week 48
1.	47,975	129	<50	<50	<50	<50	51
2.	238	<50	<50	<50	<50	<50	<50
3.	1711	702	<50	<50	<50	<50	<50
4.	2985	<50	<50	<50	<50	1341	156
5.	5379	191	<50	<50	<50	<50	56
6.	8215	<50	<50	<50	<50	<50	<50
7.	3051	80	<50	<50	<50	<50	<50
8.	36,385	ND	22,607	148	Lost to follow-up		

ND = Not done

Design

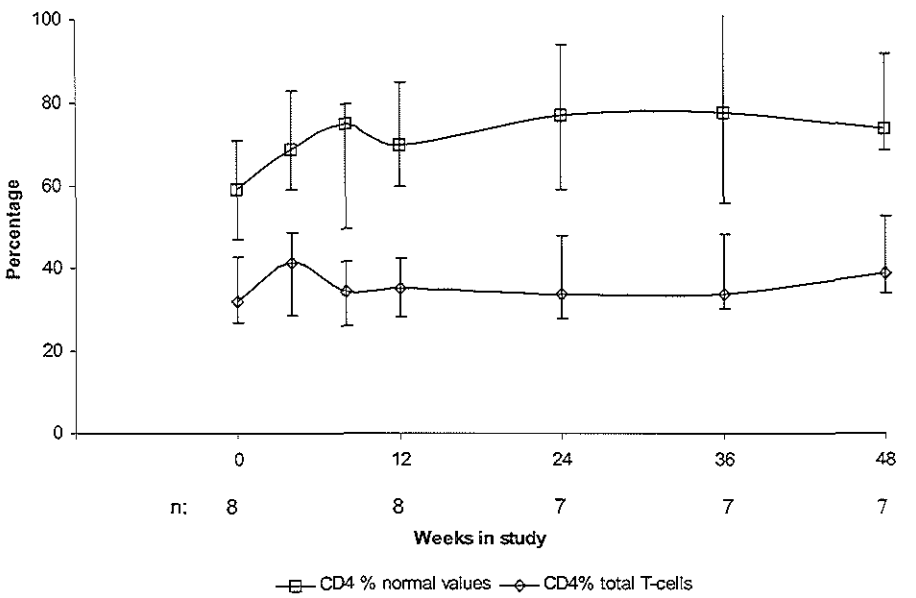
Inclusion criteria were genotypic resistance to NRTIs, a CD4 cell count less than the age-specific reference value and/or HIV-1 bDNA > 1,000 copies/ml. Eight children (age: 5.6-15 years) with a mean prior NRTI treatment duration of 4.4 years (range: 2.6-6.1 years) were enrolled. Treatment was changed to a combination of efavirenz (14 mg/kg q24h) and lopinavir/ritonavir (300/75 mg/m^2 q12h). Intensive pharmacokinetic sampling was performed. The medication dose was adjusted when necessary to obtain plasma levels within adult population reference values. The clinical response, HIV-1 bDNA levels, adherence to the medication regimen (measured by medication charts, TDM and MEMS caps) and immune reconstitution were studied in a non-controlled open label study.

Results

No changes were observed in the patients´ overall well-being, activity and appetite. In all patients viral load levels decreased directly after the medication change. One child was lost to follow up at week 12. After 48 weeks of therapy 7 of 7 children had HIV-1 levels < 500 bDNA copies/ml and 4 of 7 < 50 bDNA copies/ml. (see Table 1.) Two of the 3 children with bDNA levels > 50 copies/ml were non-adherent (patient 4 and 5). The median absolute CD3+ CD4+ cell count increased from 530 (Interquartile (I.Q.) range: 373-655) at baseline to 665 (I.Q. range: 580-738) at week 48 (p = 0.018). (median age specific values 59% (I.Q. range 47-71) at

baseline and 74% (I.Q. range 70-92%) at week 48). The median absolute CD3+CD8+ cell count remained high with 849 (IQ range: 532-1416) at baseline and 884 (I.Q. range: 702-1295) at week 48. (Median age specific values 166% (I.Q. range 133-211) at baseline and 176% (I.Q. range 133-216) at week 48). See Figure 1 for detailed data on age specific CD4 values and CD4 as percentage of total T-cells. Adverse events included a rash (n=5), an increase in liver enzymes and an epileptic seizure (n=1). After initiation of the study medication cholesterol levels increased, with 4 of 7 children experiencing levels above the upper limit of normal (ULN) during follow-up. In these children low-density lipoprotein levels had increased as well. No deviating high-density lipoprotein levels were observed. At baseline 3 of 8 children had triglyceride levels above the ULN, 5 of 7 at week 48. No signs of lipodystrophy were observed.

Figure 1. The immune reconstitution of CD4+ T-cells after initiation of the study medication. Data are presented as age specific reference values (CD4 % normal) and as percentage of total T-cells (CD4%).



Conclusion

We conclude that the combination of lopinavir/ritonavir and efavirenz used in this small observational, non-comparative pilot study shows promising results. We found a potent antiviral activity and reconstitution of the immune system even after a long pretreatment with NRTIs. Side effects were observed frequently, but were mostly transient with the exception of

dyslipidemia. We feel that these findings allow for a larger, preferably comparative study on this combination in children resistant to NRTIs or in children with NRTI-related side effects.

Acknowledgements

We like to thank Marie-Theres Düsterhues and Gabriele Hagedorn for their help with the collection of patient samples and support of the children.

References

1. Saez-Llorens X, Violari A, Deetz CO, Rode RA, Gomez P, Handelsman E, Pelton S, Ramilo O, Cahn P, Chadwick E, Allen U, Arpadi S, Castrejon MM, Heuser RS, Kempf DJ, Bertz RJ, Hsu AF, Bernstein B, Renz CL, Sun E. Forty-eight-week evaluation of lopinavir/ritonavir, a new protease inhibitor, in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2003;22(3):216-224.
2. Starr SE, Fletcher CV, Spector SA, Yong FH, Fenton T, Brundage RC, Manion D, Ruiz N, Gersten M, Becker M, McNamara J, Mofenson LM, Purdue L, Siminski S, Graham B, Kornhauser DM, Fiske W, Vincent C, Lischner HW, Dankner WM, Flynn PM. Combination therapy with efavirenz, nelfinavir, and nucleoside reverse-transcriptase inhibitors in children infected with human immunodeficiency virus type 1. Pediatric AIDS Clinical Trials Group 382 Team. N Engl J Med 1999;341(25):1874-81.

Initiation of highly active antiretroviral therapy leads to an HIV specific immune response in a seronegative infant

Pieter L.A. Fraaij, Annemarie M.C. van Rossum, Katja C. Wolthers, Ann C.T.M. Vossen, Yvonne M. Jeucken, Jac H.S.A.M. Kuijpers, Ellen G. van Lochem, Marijke T.L. Roos, Ronald de Groot, Nico G. Hartwig

AIDS 2003;17(1):138-40

Chapter 8

Case report

This is a report of an HIV-1-infected child with absent HIV-specific antibodies, in whom the appearance of HIV-specific antibodies followed after initiation of highly active antiretroviral therapy (HAART). An 11-month-old girl with a history of oral candidiasis, anemia and failure to thrive was hospitalised for dyspnoea, tachypnoea and diarrhoea. Pneumonia was diagnosed by chest X-ray. Blood cultures were positive for *Salmonella typhimurium*. HIV-1 infection was considered and enzyme immunoassay (EIA) and Western blot were performed; HIV-1-specific-antibodies were not detected. Shortly afterwards the child was diagnosed with hypertrophic cardiomyopathy and referred to a tertiary center. The diagnosis HIV/AIDS was again considered and HIV-1-specific polymerase chain reaction and p24-antigen tests were performed, which were positive. The child had been breastfed for 6 weeks. Other possible routes of transmission besides vertical transmission could not be identified. Genotyping revealed an A/B mosaic virus, which normally yields a detectable antibody response. Before start of HAART, CD4+ T and B-cell numbers were 0.19 and 0.021 x $10E^9$/l respectively, with >90% of the B-cells expressing CD5 phenotype. Immunoglobulin levels were 9.9 g/l and adequate levels of specific antibodies against poliomyelitis and pertussis were found after vaccination, indicating that the immune system was capable to mount a specific antibody response. Dissociation of antigen-antibody complexes did not result in the detection of HIV-1-specific antibodies in EIA and Western blot. However, p24-levels increased in the dissociated samples. The patient's clinical situation gradually improved and she was discharged from the hospital after 1.5 months. B-cell counts normalized after 3 months of therapy (150% median normal value) and T-cells normalized between 6 and 9 months (80% and 110% of median normal values respectively). T-cell function was studied during treatment in whole blood lymfocyte culture. The proliferative response to CD3 mAb plus CD28 mAb was measured *in vitro* by means of incorporation of ^{3}H-thymidine. (1) Steadily an increase in T cell reactivity was seen, from <50 at baseline to 12,100 at week 48 and 33,000 cpm at week 96. (95% confidence limit in normal adults > 17,000). HIV-1-specific antibodies could be detected for the first time after 6 months of therapy. The Western blot showed antibodies specific for gp24 and gp160. (For more detailed virological and immunological parameters during 2 year follow-up see Table 1.)

Discussion

This patient suffered from a rapidly progressive HIV-1 infection, with a striking absence of HIV-1-specific antibodies. Possible hypotheses for this finding were evaluated:

The capture of antibodies within immune complexes was not responsible for the absence of HIV-specific antibodies. However after dissociation p24 levels increased. This may have been caused by the dissociation of low avidity HIV-specific antibodies.

HIV-1-specific in utero induced immunotolerance as described by Siegrist is a possibility, but one would not expect an HIV-1-specific immune response after initiating HAART. (2) *In utero*

Table 1. Virological and immunological parameters during 2 year follow-up.

Months after start therapy	Day 0	Week 1	Week 2	Month 1	Month 2	Month 3	Month 5	Month 6	Month 9	Month 12	Month 24
HIV-1-specific antibodies	Negative	Negative	Negative	-	-	-	Negative	Positive	Positive	-	-
Viral load (copies/ml)	252,000	300,000	317,000	115,000	22,700	8190	-	3230	1530	783	50
IgG (g/l)	9.9	-	-	-	12.3	12.9	-	10,7	8,3	7,7	13.1
CD4+-cells (x $10E^9$/l)§	0.19	0.19	0.20	0.44	0.49	0.44	-	1.76	2.45	3.19	2.94
CD4% median*	8 (↓)	8 (↓)	9 (↓)	19 (↓)	21 (↓)	19 (↓)	-	80 (N)	111 (N)	145 (N)	226 (↑)
CD8+-cells (x $10E^9$/l) §	1.42	1.02	1.00	2.23	2.50	1.63	-	3.69	3.30	3.87	3.15
CD8 % median*	29 (N)	93 (N)	91 (N)	202 (N)	227 (N)	148 (N)		307 (↑)	275 (↑)	323 (↑)	394 (↑)
CD19+ total (x $10E^9$/l) §	-	0.021	0.027	0.037	0.109	2.108	-	2.204	2.974	3.090	2.500
CD19% median*	-	2 (↓)	2 (↓)	3 (↓)	8 (↓)	150 (N)	-	170 (N)	229 (↑)	238 (↑)	313 (↑)
%CD5+CD19+-cells	-	96	91	63	98	99	-	96	96	90	55

Percentage of median age specific reference values (↓ = below normal range, ↑ = above normal range, N = within normal range) Normal values are derived from Comans-Bitter et al. (5) § Absolute counts

developed tolerance for HIV would have resulted in the absence of HIV-specific antibodies for many years.The presence of B-cell dysfunction has previously been observed in HIV-1 infected patients.(3) Despite the low number of B-cells normal concentrations of immunoglobulins were present and the immune system was capable to mount a specific immune response after vaccination. This implied a specific immuno tolerance to HIV. Three months after the start of therapy, B-cell numbers reached normal levels, but still no HIV-1-specific antibodies could be detected. These were not detected until 6 months after the start of therapy when the CD4+ T-cell numbers normalized. Therefore the absence of HIV-1-specific antibodies may have been caused by a selective defect in T-B cell interaction. On initiation of therapy 96% of the B-cells expressed CD5 (normally 50-75%). This subpopulation dominates in cord blood and can be found in elevated levels in patients with autoimmune disease. They are thought to produce low affinity polyreactive antibodies. (4) These may have caused the increase in p24-levels after dissociation of immune complexes. During follow up the percentage of B-cells that expressed CD5 remained high and only decreased to normal levels 2 years after the start of therapy. We speculate that as a consequence of the undetectable viral load, the CD4 T-cell function fully restored resulting in further maturation of the B cell compartment.

In conclusion p24-antigen and HIV-1-specific PCR tests should always be performed in seronegative children who are clinically suspected for AIDS. The initial absence of HIV-1-specific antibodies in this child may have been due to a selective defect in T-B cell interaction.

References

1. Bloemena E, Roos MT, Van Heijst JL, Vossen JM, Schellekens PT. Whole-blood lymphocyte cultures. J Immunol Methods. 1989;122(2):161-7.
2. Siegrist CA, Wyler CA, Gerritsen EJ, Perrin L, Speiser D, Suter S. Specific tolerance to HIV-1 antigens in an infant with rapid progression to AIDS. AIDS. 1993;7(12):1683-4.
3. Ammann AJ, Schiffman G, Abrams D, Volberding P, Ziegler J, Conant M. B-cell immunodeficiency in acquired immune deficiency syndrome. JAMA. 1984;251(11):1447-9.
4. Erkeller-Yuksel FM, Deneys V, Yuksel B, Hannet I, Hulstaert F, Hamilton C, et al. Age-related changes in human blood lymphocyte subpopulations. J Pediatr. 1992;120(2 Pt 1):216-22.
5. Comans-Bitter WM, de Groot R, van den Beemd R, Neijens HJ, Hop WC, Groeneveld K, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr. 1997;130(3):388-93.

The CD38 expression level on CD8+ T-cells does not predict viral response to HAART in HIV-1 infected children

Pieter L.A. Fraaij, Annemarie M.C. van Rossum, Katja C. Wolthers, Jac H.S.A.M. Kuijpers, Corry M.R. Weemaes. A M. van Furth, Nico G. Hartwig, Herbert Hooijkaas, Ronald de Groot, Ellen G. van Lochem

Submitted

Chapter 9

Abstract

Introduction: CD38 is both a marker of activation and immaturity on T and B-cells. Absolute cell counts and the proportion of CD8+CD38+ T-cells have been proposed as prognostic marker for disease progression to AIDS and therapy outcome of HAART in adults. However, results on the use on CD38 expression in children are contradicting. We speculate that this is due to the broad expression of CD38 on cells of different lineages and the heterogenicity of CD38 expression per cell. We therefore analysed the expression levels of CD38 on CD8+ T-cells and not absolute cell counts or proportions of CD38+CD8+ T-cells.

Methods: We studied longitudinally the level of CD38 expression on CD8+ T-cells from 36 HIV-1 infected children using HAART. CD38 expression levels on CD8+ T-cells were measured on a flow cytometer at baseline and after start of therapy.

Results: After initiation of HAART the expression level of CD38 on CD8+ T-cells decreased significantly in all patients. (p-values baseline vs. week 48 for responders 0.001 and non-responders 0.011). The median CD38+ expression level was not significantly different at any time point for responders and non-responders. CD38 expression levels at baseline did not differ between viral responders and non responders at any time point in the study.

Conclusions: After initiation of HAART the expression level of CD38 on CD8+ T-cells decreased in all patients The median expression level of CD38 on CD8+ T-cells did not correlate with outcome of antiviral therapy and can therefore not be utilized as marker of therapy outcome.

Introduction

Since the introduction of highly active antiretroviral therapy (HAART) a reduction of the rate of disease progression to AIDS and HIV related deaths has been observed in adults, adolescents and children living with HIV-1. (1, 2) Still, the institution of optimal treatment regimes in HIV-1 infected children poses an enormous challenge. Inter individual differences of pharmacokinetics of antiretroviral drugs, failure to adhere to the medication, extremely high viral load levels and an immature, naive immune system complicate antiviral therapy in children. To optimize the outcome of HAART in children predictive factors for therapy success or failure are necessary. In cohort studies in HIV-1 infected adults not using HAART elevated percentages and absolute counts of CD8+CD38+ T-cells and the intensity of CD38 expression on CD8 cells were found to be prognostic factors of disease progression to AIDS and death. (3-6) Moreover, observations in adults suggest that expression of CD38 may be a useful tool for monitoring HAART, since decreased proportions of CD8+CD38+ T-cell as soon as one month after start of therapy were found to correlate with a favourable response to HAART. (7, 8)

For children the role of CD38 remains unresolved. Several conflicting studies have been published on the use of CD38 as prognostic marker in HIV-1 infected children. Plaeger-Marshall et al. and Sherman et al. reported that both high CD38 expression levels on CD8+ T cells and high percentages of CD38+CD8+ T-cells correlated with disease progression. (9, 10) In contrast, two other studies reported a relation between low absolute CD8+CD38+ T-cell numbers and percentage of CD8+CD38+ T-cells and disease progression. (11, 12) Furthermore, contradictory results on the correlation between high percentages of CD8+ T-cells expressing CD38 and high viremia in HAART were found: Vigano et al. reported that a higher percentage of CD8+CD38+ T-cells predicted maintenance of high viremia in children treated with HAART, whereas Caselli et al could not confirm this correlation. (13, 14) Recently, Resino et al. reported an negative correlation between the percentage of CD8+CD38+ T-cells and median relative CD38 fluorescence at the moment viral suppression was obtained and the duration of the viral response. In addition in a substudy children with a percentage of CD8+CD38+ T-cells above the median percentage of the total group were more at risk for viral failure. (15) For an overview of the different studies see table 1.

Several factors complicate the use of CD38 as a prognostic marker in children. The immature naive immune system in children complicates the use of CD38 as activation marker, since CD38 is expressed on immature T and B-cells. (16, 17) Therefore, in children CD38 is both a marker of immaturity and a marker of immune activation. (8) Furthermore, the CD38 molecule is distributed ubiquitously on cells of different lineage, with heterogeneous expression levels. Thus lymphocytes that express CD38 cannot be clearly distinguished from those who do not. Subpopulations of CD38+ and CD38- lymphocytes can therefore only be defined by setting an arbitrary marker. This may partly explain the contradictory results of the previously described studies on the percentages of CD38+ T-cells.

Table 1. Summary of previously performed studies in HIV-1 infected children.

Author	Marker analysed	Therapy	Follow-up	Conclusion	Number of subjects
Plaeger-Marshall et al.[9]	Cross sectional analysis. Relative (as percentage of total CD8+ T-cells) number of CD8+CD38+ T-cells.	No	No	Brighter CD38/HLA DR expression is associated with disease progression	Controls: 40 Patients: 26
De Martino et al.[12]	Absolute and relative (as percentage of total CD8+ T-cells) number of CD8+CD38+ T-cells in 5 year survivors	12 children received treatment with NRTI	One year	Lower percentage and absolute count of CD8+CD38+ T-cells is associated with disease progression	Controls: 12 Patients: 25
Schlesinger et al.[11]	Absolute and relative (as percentage of blood leukocytes) number of CD8+CD38+ T-cells in relation to survival	No	Three years	Lower absolute count of CD8+CD38+ T-cells is associated with disease progression	Patients: 71
Sherman et al.[10]	Relative (as percentage of total CD8+ T-cells) number of CD8+CD38+ T-cells and the quantity of CD38 expression on CD8+ T-cells	No	One year	Higher percentage CD8+CD38+ T-cells and an increased CD38 expression level per CD8+ T-cell is related to disease progression	Controls: 11 Patients: 49
Vigano et al.[13]	Relative number CD8+CD38+ T-cells in relation with outcome of HAART	Yes	One year	Higher percentage of CD8+CD38+ T-cells predicts viral failure	Patients: 16
Caselli et al.[14]	Relative (as percentage of blood leukocytes) number of CD8+CD38+ T-cells in relation with outcome of HAART	Yes	One year	No relation between percentage of CD8+CD38+ T-cells and viral failure	Patients: 103
Resino et al.[15]	Relative number of CD8+CD38+ T-cells and CD38 fluoresce intensity.	Yes, no strict approach to ART	Four years	A: Negative correlation between the duration of viral suppression and the percentage and fluorescence intensity of CD8+CD38+ T-cells at the moment of first undetectable viral load (uVL) B: Association between increased percentages of CD8+CD38+ T-cells at the moment of uVL and viral failure.	Patients A: 42 B: 17

Changes in CD38 expression in a population of CD8+ T-cells can be measured by analyzing the intensity of CD38 expression per CD8+ T cell. When implementing this approach, Sherman et al. found a more profound correlation between disease progression and an increased CD38 expression on CD8+ T-cells in vertically HIV infected children. (10) Since CD38 expression was proposed as a marker for the outcome of HAART, we studied longitudinally the median intensity of CD38 expression on CD8+ T-cells from HIV-1 infected children using HAART.

Methods

Patient inclusion

Between 1997 and 2002, 43 HIV-1 infected children were enrolled in a prospective open uncontrolled study to evaluate the clinical, immunological and virological response to HAART. Patients were recruited from the combined Pediatric HIV/AIDS cohorts of the Erasmus-MC/Sophia Children's Hospital, University Medical Center Nijmegen and VU Medical Center Amsterdam. Children were enrolled in this CD38 expression study if both samples from baseline and at least week 12 were present. Based on these criteria 36 children were enrolled in the study.

After patients started HAART, steady state intensive plasma pharmacokinetic (PK) sampling of indinavir or nelfinavir was performed. PK sampling was repeated until the $AUC_{0\text{-}8h}$ reached adult target values. The study protocol was approved by the medical ethical committee of the Erasmus MC. Written informed consent was obtained from parents and patients.

Plasma HIV-1 RNA determination

Plasma HIV-1 RNA levels were measured by an in vitro nucleic acid amplification test (AMPLICOR 1.5 HIV-1 MONITOR test (Roche Diagnostic Systems)) with a lower limit of quantification of 500 copies/ml. Patients were determined to be a viral responder if HIV-1 RNA levels < 500 copies/ml were reached at week 12 after the initiation of HAART, which maintained during follow-up.

Immunophenotyping

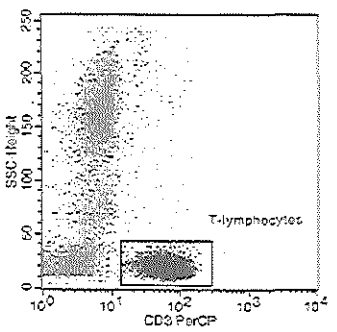
Cell samples

Heparinized peripheral blood samples for CD38 expression level analysis were obtained at baseline, week 12, 24, 36 and 48 on regular outpatient visits. Blood was kept at room temperature for a maximal of 24 hours after sampling.

Flow cytometric analysis

In short, 50 µl whole blood was incubated with optimally titrated fluorescein isothiocyanate (FITC), phyco-erythrin (PE), peridinin chlorophyll protein (PerCP) and allophycocyanin (APC)-conjugated monoclonal antibodies (MAb) for 20 minutes at room temperature. After lysing of red blood cells (RBCs) by ammonium chloride (10 minutes at room temperature) and washing of the cells twice with phosphate-buffered salin pH 7.8 (PBS)/ 0.2% bovine serum albumin (BSA) 20,000 CD3+ events were acquired on a dual laser FACS Calibur™ flow cytometer

(Becton Dickinson, San Jose, CA, USA). The following MAb were used: CD4-FITC (SK3; BD Bioscience), CD38-PE (HB7; BD Bioscience), HLA-DR-PerCP (243; BD Bioscience) CD8-APC (SK1; BD Bioscience). The flow cytometer was checked on a weekly basis using Sphero califlowkit™ calibration beads. Standardized settings were used for data acquisition of the CD38 expression level on CD8+ T-cells.



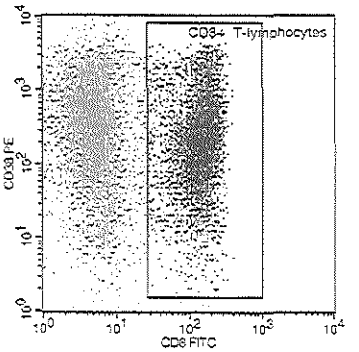
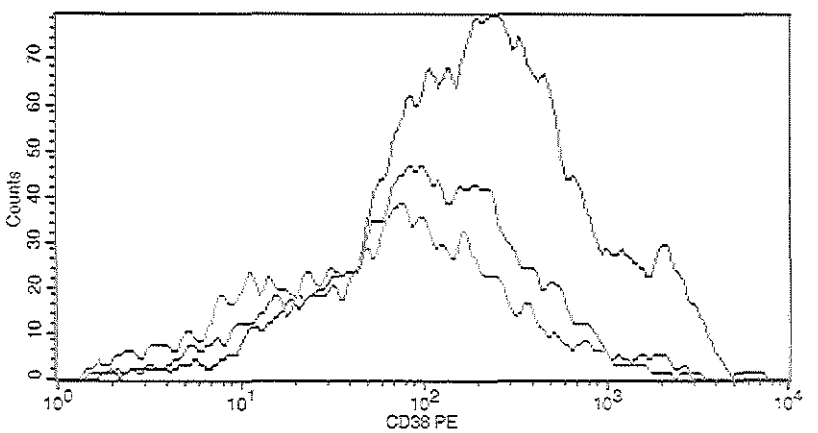


Figure 2A. Side scatter versus CD3 expression is used to gate the T-lymphocytes **Figure 2B.** Representative CD8/CD38 dot plot of an individual patient in our cohort at baseline. CD8+ T-cells (CD3-gated) can be selected from this dot plot to" analyse the CD38 expression level on CD8 T-cells in a histogram. Clearly no subpopulation of CD38+ T-cells can be established without setting an arbitrary marker. **Figure 2C.** Changes in the level of CD38 expression of an individual patient after initiation of antiviral therapy at baseline (black), 3 months (green) and 12 months (red) after treatment.



Data analysis

CELL Quest™ software (BD Bioscience) was used to analyse the CD38 expression level on CD3+CD8+ gated lymphocytes. In a semi-quantitative approach the median fluorescence intensity of FL-2 (CD38-PE) on CD3+CD8+ T-cells was used to compare CD38 expression levels between different patient samples over time.

Statistical methods

All children with data on the CD38 expression level available on baseline and after at least 12 weeks of follow-up were included for analysis (n=36). Because absolute CD4+ T-cell and CD8 + T-cell counts are age-dependent, relative CD4+ and CD8+ T-cells counts were calculated. Hereto the patient's individual value at the different time-points was divided by the median of the respective reference value. CD4 and CD8 T-cell numbers are expressed as percentage of normal. (18, 19) The Spearman test was used to analyze the correlation on baseline and week 48 between HIV-1 RNA levels, age and CD38 expression levels. Differences between paired variables were analyzed with the Wilcoxon signed rank test and between groups with the Mann-Whitney U test. All p-values are two tailed and p-values smaller than 0.05 were considered significant. For statistical analysis SPSS© 10 and Microsoft Excel© 97 software were used.

Results

The baseline characteristics of the thirty-six patients are presented in table 2. The median age of the children was 6.1 years (range 0.3-16.5 years). Twenty-two (61%) of 36 patients had not received prior treatment and 14 of 36 (39%) patients had received prior treatment with one or more nucleoside reverse transcriptase inhibitors (NRTI) (mostly zidovudine). The median HIV-1 RNA level was significantly higher in the group of children that did not receive prior antiviral therapy (p=0.001), but no differences were found for absolute CD4+ or CD8+ T-cell numbers and the relative numbers as percentages of age specific reference values. Most children started HAART consisting of indinavir q8h, zidovudine and lamivudine. From the year 2000 on this was converted to a q12h regime: indinavir q12h, ritonavir, zidovudine and lamivudine. Four children received nelfinavir, zidovudine and lamivudine. After 48 weeks two children were lost to follow up due to emigration and of four children week 48 samples were not obtained.

Plasma HIV-1 RNA level and relative CD4+ and CD8+ T-cell numbers

The medians and interquartile ranges of the viral load after start of HAART and the percentage of children with a viral load below 500 copies/ml for responders and non-responders are depicted in figure 1A and 1B. In total 17 children were determined non-responders and 19 responders. The relative CD4+ and CD8+ T-cell numbers as percentages of age related reference values are depicted in figure 1C and D.

CD38 expression levels on CD8+ T-cells in HIV-1 infected children treated with HAART

CD38 is heterogeneously expressed on CD8+ T-cells, without clearly distinguishable negative and positive subpopulations (figure 2A and 2B). Therefore the proportion of CD38+CD8+ T-

cells cannot be determined unless an arbitrary marker is set. To compare changes in the intensity of expression of CD38 on CD8+ T-cells during treatment, the median fluorescence intensity of CD38 on CD8+ T-cells was analysed (figure 2C). The median and the interquartile range (IQR) of the CD38 expression level for responders and non-responders are depicted in figure 3. After initiation of HAART the expression level of CD38 on CD8+ T-cells decreased significantly in all patients. (p-values baseline vs. week 48 for responders 0.001 and non-responders 0.011). The median CD38+ expression level was not significantly different at any time point for responders and non-responders. CD8+ T-cells of children who received prior treatment with NRTI displayed a significant lower median CD38+ expression, compared to CD8+ T-cells of children with no prior treatment (p-value = 0.019). Figure 4 depicts the absence of a relation between the HIV-1 RNA level and the median CD38 in an individual non-responder. The median CD38 expression level clearly follows the decrease in the HIV-1 RNA levels. After a subsequent increase in HIV-1 RNA levels the CD38 expression does not longer follow viral load. Despite the fact that HIV-1 RNA levels return to baseline levels the CD38 expression levels remain below baseline levels through the follow up period do not.

Table 2: Baseline characteristics of the study patients

Characteristics	N:		
Number of patients	36		
Age in years*	6.1 (0.3-16.5)		
Route of infection			
Vertical	24		
Blood products	5		
Unknown	7		
Clinical stage (CDC-classification)			
N1: 2	A1: 1	B1: 3	C1: 0
N2: 3	A2: 5	B2: 7	C2: 4
N3: 2	A3: 2	B3: 1	C3: 6
Prior treatment with NRTI	14		
No prior treatment	22		
HAART:			
IDV/AZT/3TC/(RTV)	32		
NFV/d4T/ddI	4		
Median HIV-1 RNA (copies/ml)*	123,000 (2680-24.5E6)		
CD4+ T-cells Median (IQR)			
Absolute (10^6 cells/ml)*	382 (145-903)		
% of normal*	37 (11-64)		
CD8+ T-cells			
Absolute (10^6 cells/ml)*	1104 (570-17403)		
% of normal*	138 (90-261)		

* Median (range)

Correlation between age, HIV-1 RNA levels and the median CD38 expression level

A correlation has previously been described between age, the HIV-1 RNA level and the mean absolute number of CD38+CD8+ T cells.(10) At baseline this observation could be confirmed. Both a correlation between the HIV-1 RNA level and CD38 expression levels and between age and CD38 expression levels were observed (correlation coefficient: 0.564; $p < 0.001$ and -0.435; $p = 0.08$, respectively). However, during treatment the correlation between the HIV-1

RNA level and CD38 expression levels, as well as the correlation between age and CD38 expression levels were lost (at week 48 correlation coefficient: -0.35; $p < 0.854$ and -0.4173; $p = 0.359$, respectively).

Figure 1A. The median and interquartile ranges (IQR) of HIV-1 RNA levels in virologic responders after initiation of HAART. **Figure 1B.** The median and IQR of HIV-1 RNA levels in non-responders. **Figure 1C.** Median CD4 and CD8 T-cell count as percentage of age related reference values in responders. **Figure 1D.** Median CD4 and CD8 T-cell count as percentage age related reference values in non-responders.

Fig 1.A Responders

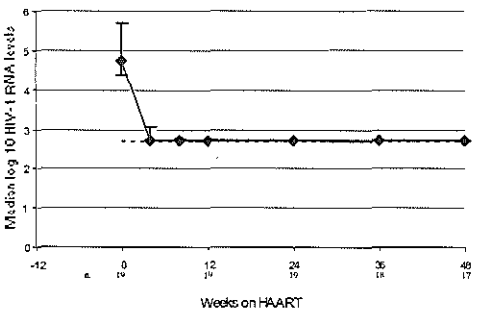


Fig 1B. Non responders

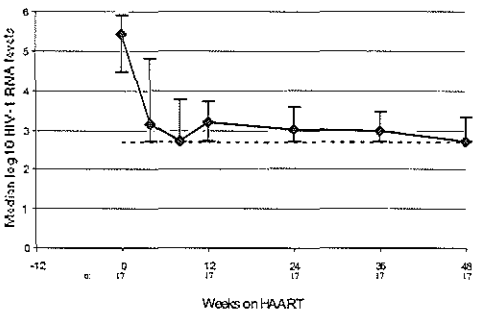


Fig 1C. Responders

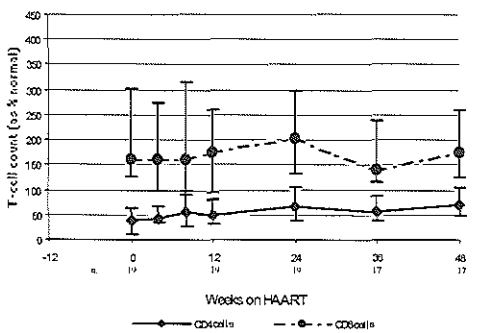
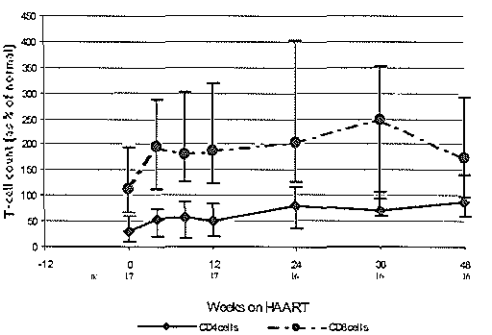


Fig 1D. Non responders



CD38 expression levels on CD8+ T-cells as a prognostic marker for response to HAART in HIV-1 infected children

To determine if the level of CD38 expression on CD8+ T-cells can act as a prognostic marker for response to HAART in HIV-1 infected children, we compared CD38 expression levels on CD8+ T cells from responders and non-responders. Already at baseline no significant

Figure 3. CD38+ expression level on CD8+ T-cells after initiation of HAART for viral responders and viral non-responders.

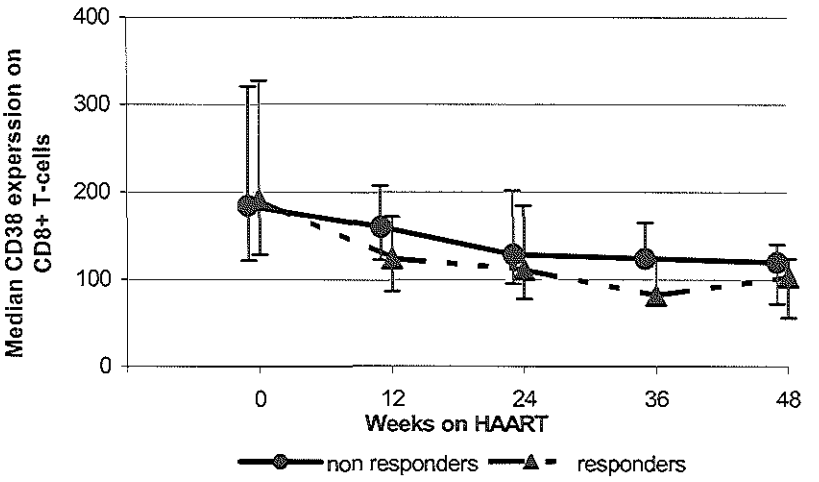
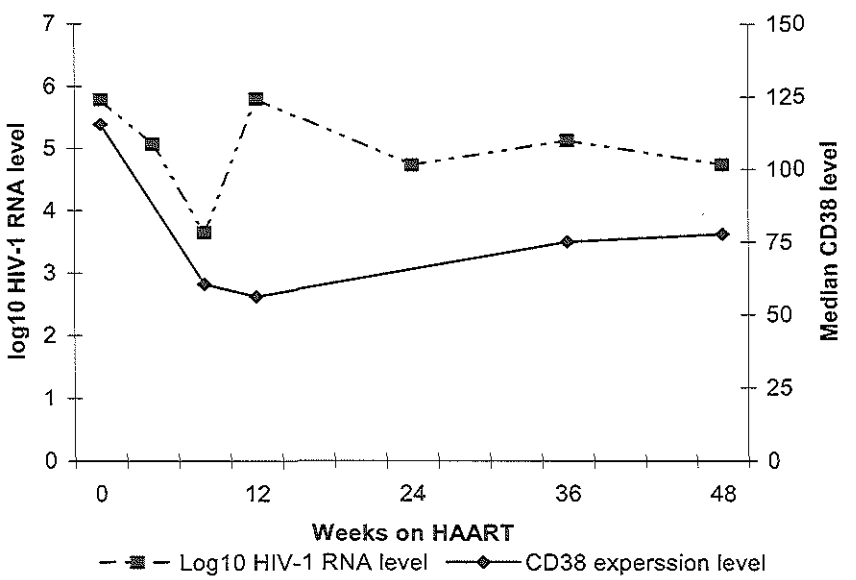


Figure 4. Changes in CD38 expression in an individual patient on HAART. CD38 expression levels follow the HIV-1 RNA decrease and do not return to baseline values despite ongoing viral replication.



difference in CD38 expression could be found between the CD8+ T-cells of the defined groups of responders and non-responders (Figure 3). Figure 5 depicts the relation between the median CD38 expression on CD8+ T-cells in the individual children at baseline and the level of virus suppression at the different time points under HAART. Two children with detectable viral loads at week 12 and 24 had high levels of CD38 expression on their CD8+ T cells, while in the other children with detectable HIV-1 RNA levels the level of CD38 expression at baseline did not differ from responders. At week 48, the viral load of one of the two children with high CD38 expression at baseline became undetectable. No significant differences could be found in median CD38+ expression levels on CD8+ T-cells at baseline between the children with undetectable or detectable viral load during HAART on week 12, 24 and 48. To exclude the influence of pre-treatment on CD38 expression levels, the same analysis was performed in the 22 children that had not received prior treatment. Again, no significant differences were observed in the median levels of CD38 expression at baseline in the group of children with HIV-1 RNA levels above 500 copies/ml and the group of children with HIV-1 RNA levels below 500 copies/ml on week 12, 24 and 48. (p-values 0.337, 0.223 and 1.00, respectively). To minimize the influence of age on the level of CD38 expression on baseline and viral response on week 12, 24 and 48 we again analyzed the CD38 expression levels in children older than 2 years only (n = 27). Again no significant differences could be found for the levels of CD38 at baseline expression and outcome of antiviral therapy on week 12 and 48. (p-values: 0.641 and 0.447, respectively).

Discussion

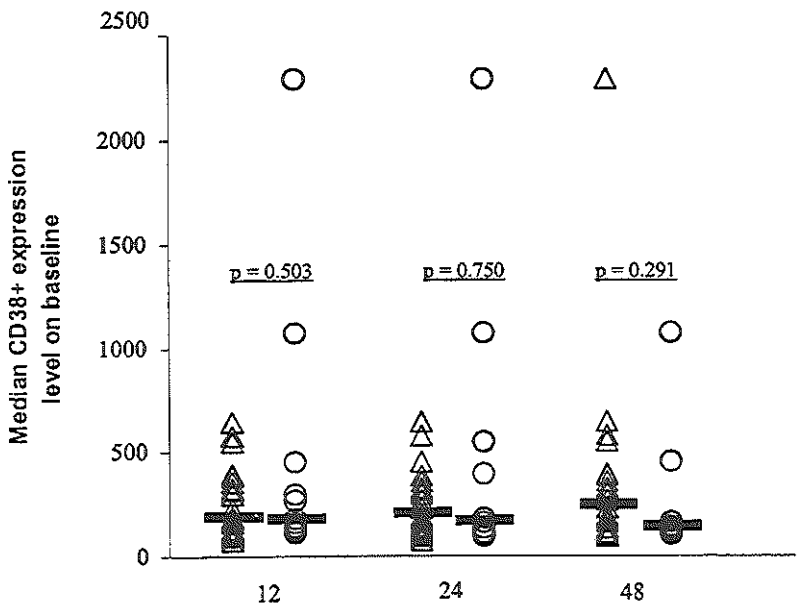
In this study we longitudinally analyzed the median level of CD38 expression on CD8+ T-cells from 36 HIV-1 infected children. The CD38 molecule is expressed heterogeneously on lymphocytes. Therefore subpopulations either negative or positive for the CD38 molecule can only be defined if one sets an arbitrary threshold. We chose not to do so and analysed the CD38 expression level per cell. Since the group of children described here was longitudinally followed we could both analyse the predictive value of the CD38 expression level at baseline, before HAART and after initiation of therapy in time.

Maturation, differentiation and activation of the immune system all influence the expression level of CD38 on CD8+ T cells from HIV-1 infected children. Indeed, before start of therapy, a relation between the level of CD38 expression, viral load and age was observed, confirming the data published by Sherman et al. (10). The positive correlation we found between CD38 expression levels on CD8+ T cells and viral load before therapy supports the view of CD38 as a prognostic marker for disease progression in children not treated with HAART. (10)

After start of treatment, a significant decrease in CD38 expression levels was observed on CD8+ T cells from all patients. This is not likely to be due to the increment of age in the population, since a decrease in CD38 expression could already be observed after 12 weeks of therapy. More likely, the decreased expression levels of CD38 on CD8+ T-cells may reflect a

decreased immunoactivation due to a decrease in HIV-1 RNA levels by HAART. Interestingly, this decrease in CD38 expression was observed to the same level in children responding to therapy with undetectable viral load as well as in children that did not. Despite detectable HIV-1 RNA levels in the non-responder group the HIV-1 RNA levels did decrease substantially, thereby probably reducing immune activation and thus CD38 expression. Remarkably, CD8+ T-cell count remained high throughout the study period in both the responder as the non-responder group. If the loss of activated CD8+ T cells due to suppression of viral replication is compensated by influx of cells with a more naïve immunophenotype, decrease of CD38 expression levels might partly be compensated by the expression levels of CD38 on immature CD8 T cells.

Figure 5. Median CD38+ expression levels on CD8+ T-cells at baseline in children with a viral load > 500 copies/ml compared to children with a viral load below 500 copies/ml on a certain time point after initiation of HAART. Symbols depict individual values. Depicts the median value.



△ VL< 500 c/ml	n = 25	26	26
○ VL> 500 c/ml	n = 11	10	8

At baseline, no significant differences could be observed in the median CD38 expression levels between children responding to HAART and children not responding to HAART. Nor was a relation observed in the baseline CD38 expression and the viral outcome on week 12, 24 and 48. To eliminate the possibility that high CD38 expression levels in young children biased our data we stratified the patient group for age. Although the sample size was too small to definitively exclude the influence of age, again the level of CD38 did not predict success or failure of therapy on any of the designated time points.

We studied a relative small group of patients in which most had a favourable virological outcome after 48 weeks of therapy. A possible bias may be that CD38 expression was already low at baseline in our study population compared to the normal population. A relatively low CD38 expression may have resulted in favourable viral outcome after initiation of HAART as postulated by Vigano et al. (13) Still, viral failure did occur with relatively low CD38 expression in our group of patients.

The application of CD38 expression on CD8+ T-cells as prognostic marker is limited in the pediatric population. The sensitivity of our described method may improve if CD38 expression is measured on HIV specific CD8+ T-cells. This can be performed by gating T-cells with HLA peptide HIV specific tetrameric complexes as previously reported by van Baarle et al. (20) Still, even if measured on HIV-1 specific T-cells maturation of the immune system and frequent intercurrent infections associated with childhood will influence the obtained results, complicating the use of activation markers in children.

In conclusion CD38 expression on lymphocytes should not be measured as proportions of cell populations. In our study the baseline median expression level of CD38 on CD8+ T-cells did not correlate with outcome of antiviral therapy. Furthermore, in children, changes in CD38 expression levels not only reflect immune activation but can also be due to changes in T cell subpopulations after HAART.

References

1. Gortmaker SL, Hughes M, Cervia J, et al. Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. N Engl J Med 2001;345(21):1522-8.
2. Palella FJ, Jr., Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998;338(13):853-60.
3. Liu Z, Cumberland WG, Hultin LE, Prince HE, Detels R, Giorgi JV. Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. J Acquir Immune Defic Syndr Hum Retrovirol 1997;16(2):83-92.
4. Giorgi JV, Liu Z, Hultin LE, Cumberland WG, Hennessey K, Detels R. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. J Acquir Immune Defic Syndr 1993;6(8):904-12.
5. Lenkei R, Bratt G, Holmberg V, Muirhead K, Sandstrom E. Indicators of T-cell activation: correlation between quantitative CD38 expression and soluble CD8 levels in asymptomatic HIV+ individuals and healthy controls. Cytometry 1998;33(2):115-22.

6. Liu Z, Hultin LE, Cumberland WG, et al. Elevated relative fluorescence intensity of CD38 antigen expression on CD8+ T cells is a marker of poor prognosis in HIV infection: results of 6 years of follow-up. Cytometry 1996;26(1):1-7.
7. Burgisser P, Hammann C, Kaufmann D, Battegay M, Rutschmann OT. Expression of CD28 and CD38 by CD8+ T lymphocytes in HIV-1 infection correlates with markers of disease severity and changes towards normalization under treatment. The Swiss HIV Cohort Study. Clin Exp Immunol 1999;115(3):458-63.
8. Savarino A, Bottarel F, Malavasi F, Dianzani U. Role of CD38 in HIV-1 infection: an epiphenomenon of T-cell activation or an active player in virus/host interactions? AIDS 2000;14(9):1079-89.
9. Plaeger-Marshall S, Hultin P, Bertolli J, et al. Activation and differentiation antigens on T cells of healthy, at-risk, and HIV-infected children. J Acquir Immune Defic Syndr 1993;6(9):984-93.
10. Sherman GG, Scott LE, Galpin JS, et al. CD38 expression on CD8(+) T cells as a prognostic marker in vertically HIV-infected pediatric patients. Pediatr Res 2002;51(6):740-5.
11. Schlesinger M, Peters V, Jiang JD, Roboz JP, Bekesi JG. Increased expression of activation markers on CD8 lymphocytes in children with human immunodeficiency virus-1 infection. Pediatr Res 1995;38(3):390-6.
12. de Martino M, Rossi ME, Azzari C et al. Different meaning of CD38 molecule expression on CD4+ and CD8+ cells of children perinatally infected with human immunodeficiency virus type 1 infection surviving longer than five years. Pediatr Res 1998;43(6):752-8.
13. Vigano A, Saresella M, Rusconi S, et al. Expression of CD38 on CD8 T cells predicts maintenance of high viraemia in HAART-treated HIV-1-infected children. Highly active antiretroviral therapy. Lancet 1998;352(9144):1905-6.
14. Caselli D, Comolli G, Maccabruni A, et al. CD38/CD8 expression and HAART failure. Lancet 1999;353(9155):840-1.
15. Resino, S., J.M. Bellon, M.D. Gurbindo, et al., CD38 expression in CD8+ T cells predicts virological failure in HIV type 1-infected children receiving antiretroviral therapy. Clin Infect Dis, 2004. 38(3): p. 412-7.
16. Malavasi F, Funaro A, Roggero S, et al. Human CD38: a glycoprotein in search of a function. Immunol Today 1994;15(3):95-7.
17. Mehta K, Shahid U, Malavasi F. Human CD38, a cell-surface protein with multiple functions. Faseb J 1996;10(12):1408-17.
18. van Rossum AM, Scherpbier HJ, van Lochem EG, et al. Therapeutic immune reconstitution in HIV-1-infected children is independent of their age and pretreatment immune status. AIDS 2001;15(17):2267-75.
19. Comans-Bitter WM, de Groot R, van den Beemd R, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr 1997;130(3):388-93.
20. Van Baarle D, Kostense S, Hovenkamp E., et al. Lack of Epstein-Barr virus and HIV-specific CD27- CD8+ T cell is associated with progression to viral disease in HIV-infection. AIDS 2002, 16:2001-11.

Part two

Pharmacology of antiretroviral agents in children with HIV-1 infection

Therapeutic drug monitoring in children with HIV/AIDS

Pieter L.A. Fraaij, Natella Rakhmanina, David.M. Burger, Ronald de Groot

Ther Drug Monit 2004;26(2):122-6

Chapter 10

Abstract

In this paper we present an overview on the use of TDM in the treatment of HIV-1 infected children. The processes of growth and development have a significant impact on drug metabolism. The use of TDM allows to optimize plasma drug concentrations of antiretroviral drugs. This is important when one considers that the levels of viral suppression and drug toxicity in adults and children are associated with the plasma concentration of PIs and NNRTIs. Indeed in clinical practice the use of TDM in the treatment of HIV-1 infected children has favourable results. However, there is a serious shortage on population reference values of antiretroviral medication in children. Targeting plasma drug levels in children to adult reference values may be insufficient because of the unique features of HIV infection in children. Apart from its primary function for dose optimisation, TDM can also be used as a tool to assess adherence to antiviral medication. One should however be cautious to base assumptions on plasma levels alone, since aberrant plasma levels may also be the result of other factors such as changes in nutritional habits, drug-drug interactions or changing gastric motility. We conclude that TDM is a useful tool in the treatment of HIV-1 infected children. Additional data are needed to establish child-specific reference values and to assess the optimal method of TDM.

Introduction

The ongoing HIV/aids pandemic has devastating consequences in both children and adults. At the end of 2002 approximately 3.2 million children (age < 15 year) were estimated to be infected by HIV. In 2002 610,000 children died from HIV/AIDS and another 800,000 were newly infected. Most of these new infections and HIV-related deaths occur in developing countries.(1) In the Western world the situation is different: An impressive reduction in the rate of disease progression to AIDS and HIV-1 related deaths has been observed in adults, adolescents and children after the introduction of Highly Active Antiretroviral Therapy (HAART). (2,3) In addition, preventive strategies have resulted in a sharp decline in the mother to child transmission rate (4,5). Yet, institution of optimal treatment in children poses a major challenge. First, large interindividual differences in the pharmacokinetics of antiretroviral drugs, especially in protease inhibitors, complicate dosing. Secondly, adherence to the medication regimen is difficult to maintain. Complicated dosing regimens, poor palatibility, intake of evening medication during sleeping time or intake of medication during school hours, food requirements, side effects, unwillingness of young children and adolescents to take the medication and psychosocial issues related to HIV-infection in the children and the care-takers need to be dealt with. Thirdly, the different viral dynamics and the developing immature immune system of children complicate therapy.

In children HAART is composed of the same drugs as those in adults, although often antiviral medication has not been registered for young children whereas dose recommendations are frequently not available. HAART usually encompasses two classes of antiretroviral therapy: a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and a protease inhibitor (PI) or an combination of two NRTIs and a non nucleoside reverse transcriptase inhibitor (NNRT). Treatment with HAART in children is associated with favourable results in some studies. (5-9) However, the virological response rates in children are frequently inferior to those in adults. (10-16)

Pediatric pharmacology

Growth and development are dynamic continuously changing parameters during childhood. This partly explains the differences in pharmacokinetics between adults and children.(17-19) The gastrointestinal absorption of drugs in children is different from that in adults. Until the age of 5 years the pH in the stomach of children is thought to be high compared with the pH in adults. Furthermore, gastric emptying time changes with age. In neonates the gastric emptying time is delayed compared to adults, whereas in infants and older children the gastric emptying time is more rapid than in adults. Neonates also have a reduced intestinal motility and biliary function. Despite these maturational changes in the gastrointestinal tract overall bioavailability of most orally administered medication in neonates and young infants is adequate. However, additional problems may occur while administering antiretroviral drugs because of food restrictions associated with the use of antiviral therapy. Fasting or in contrast a high fat meal

are often advised to optimize absorption of the medication. Both may pose problems in young children who need regular meals and often cannot tolerate high fat meals.

In children the distribution of drugs in the body changes due to age-specific differences in water content, plasma protein concentrations and permeability of specific compartments for drugs. The body water content changes significantly with age. At birth the total body water (TBW) as a percentage of total weight in neonates is 77% (TBW can be up to 90% in preterm neonates). This decreases to 55-60% at 1 year and to 20% in adults. In addition, extracellular fluid volumes (EFC) in neonates are 35-45% of total bodyweight as compared to 20% in adults. Therefore drug dosage per kg commonly needs to be higher in prepubertal children than in adults to obtain comparable plasma levels.

Changes in hepatic enzyme activity and maturation of the kidney function influence the elimination of drugs in children. Drug metabolism occurs through hepatic enzymes in two phases: The phase I reaction (oxidation, reduction and hydrolysis) and phase II reaction (reduction). Neonatal activity of the P450 enzyme, a mixed function oxidase system, is approximately 20-70% of adult values. This increases to adult levels by 6-12 months, subsequently exceeds adult levels at 1-4 years and finally declines to normal adult levels at the end of puberty. Of the enzymes playing a role in the Phase II reaction glycuronidation is decreased at birth and reaches adult levels after 3 years. However, other enzymes tend to have higher levels in children. Drug excretion is also influenced by age. The glomerular filtration and tubular secretory capacity are decreased at birth and reach adult values after 7 months. Peak renal functions occur at 3-5 years of age and decline to average values in time.

Therapeutic Drug Monitoring (TDM) and the treatment for HIV infection

TDM has been proposed as a useful tool for the optimisation of antiviral therapy in both adults and children. In some HIV treatment centers the use of serum drug concentrations has become part of routine treatment of HIV-1 infected individuals. The use of TDM allows for effective drug concentrations while it may at the same time prevent toxicity.

Two of the three classes of antiretroviral agents are suitable for TDM: the NNRTIs and the PIs. NRTIs are pro-drugs which are intracellularly converted to active NRTI-triphosphates (NRTI-TP). (20) Therefore, plasma concentrations of NRTIs may not be a good indicator of viral activity. Indeed plasma concentrations of zidovudine correlate poorly with anti-HIV activity. (21) Intracellular levels of NRTI triphosphate levels may correlate better with antiviral efficacy. (20,22,23) However, the active intracellular levels of the NRTIs can only be measured in specialised research laboratories. Moreover, the methods developed for the analysis of intracellular NRTI levels require large volumes of blood and are therefore not suitable for use in children. In contrast, small volumes of plasma can be used to measure concentrations of PIs and NNRTIs. For both drugs pharmacokinetic analysis appears to be particularly important. (24) Considerable inter-individual variability in plasma levels and high likelihood of drug interactions may lead to excessively high or low PI or NNRTI levels (for review see Van

Heeswijk et al., Back et al. and Burger et al. (24-26)) This is even more important when one considers that the level of viral suppression in adults and children is associated with the plasma concentration of PIs and NNRTIs. (27-32) In addition, high plasma levels may correlate with drug toxicity of both PIs and NNRTIs. (31,33-35)

TDM can be used in different ways. TDM can be performed shortly after the initiation of therapy of HIV-1 infected children. The dosage of the medication may subsequently be adjusted to meet population reference values in adults. One may also choose to use TDM only after medication failure. TDM may also be used to identify suspected drug interactions or non-compliance. Different PK parameters may be assessed. Trough levels (Cmin), peak levels (Cmax) and the area under the plasma concentration-time curve (AUC) may all be used for the assessment of TDM. The AUC is probably the most accurate measurement. However, analysis of the AUC is invasive, time consuming and complex.

Experiences with TDM in children in the clinical practice

Since 1997 HAART naive HIV-1 infected children with a viral load above 5,000 copies/mL and/or a CD4 cell count below their age-specific reference start HAART containing at least one PI in our institute. In all patients, steady state intensive plasma PK sampling of PI is performed within 4 weeks as standard of care. When dose adjustment is necessary to normalise the AUC values to normal adult values this procedure is repeated until the AUC reaches the reference values. Since 2001 random single sample plasma PI and NRTI levels are also part of the routine care for the monitoring of HIV-1 infected children in our hospital.

Large interindividual differences were observed in the pharmacokinetics of the different PIs. For instance, in children using 33 mg/kg indinavir metabolic weight q8h up to an 18-fold variances in the AUC were observed, (2.8-51 mg/L*hr). (29) Interestingly, in this study in 5 of the 11 children with a lower $AUC_{0\text{-}8h}$ (<20 mg/L*h) viral load could be detected, whereas this was not the case in children with a higher $AUC_{0\text{-}8h}$. In addition, in adults several studies have demonstrated that suboptimal plasma concentrations of the PI nelfinavir correlated with virological failure. In children this relation was studied in the pharmacokinetic substudy of the PENTA 5 trial. Differences in virological response were compared in children with a nelfinavir through concentration below or above the consensus minimum. Children with low nelfinavir trough concentrations were more likely to experience virological failure. (36)

The approach as described above resulted in favourable results in children using either indinavir or nelfinavir participating in the Dutch study group for children with HIV-1 infection. In this study group 69% of the children had HIV-1 RNA levels < 500 copies /mL and 50% < 50 copies/ml after 96 weeks of treatment. In addition the CD4 cell count as age specific reference value had increased from 44% to 96%. (8) Furthermore, HAART was shown to have a sustained positive effect on growth. (37)

In May 2003 49 children (aged 8 months – 18 years) from 5 different centers were included in the Rotterdam cohort. At that time point the children in the cohort had used HAART between 2-75 months. Cross- sectional analysis revealed that 43 (88%) children had HIV-1 RNA levels < 500 copies/ mL and 40 (82%) HIV-1 levels below < 50 copies/mL. Although this is a cross sectional description and children used different antiviral therapy regimens for different time periods these data show that high rates of viral response can be obtained in children, similar to those in adults.

The effects of long term use of PI in children

Animal and human studies indicate that exposure to PIs may gradually decrease over time. (38,39) Currently there is little information on changes in the pharmacokinetic parameters after prolonged PI use in children. However, changes over time may be expected in children, since growth and development have a significant impact on pharmacokinetic parameters. We therefore performed a case study in six HIV-1 infected children using indinavir for more than 2 years. We observed changes in indinavir exposure. A decrease in the $AUC_{0\text{-}8h}$ was seen in 4 out of 6 patients. In 3 patients the AUC had decreased below the designated target value. In 2 of these 3 patients the HIV-1 RNA levels were > 500 copies/mL. (41)

Adherence and TDM

Apart from its primary function as dose optimisation, TDM can also be used as a tool to assess compliance. Children living with HIV/AIDS face the challenge of life-long complex antiretroviral therapy regimens. Paterson et al. showed that patients need to take at least 95% of their medication to optimize the virological response. (41) Using pharmacy records Watson et al. showed that non-adherence is common in children with HIV-1 and may be the major impediment tot successful treatment. (42) Clearly knowledge on the level of compliance is important when treating patients. However, there is no golden standard to measure adherence. All available methods have limitations. Self-reported or medical caregiver-reported non-adherence scores, electronic monitoring systems (Medical Event Monitoring System (MEMS)) pill counts, serum levels of drugs and the refill history have been used in the past. (40-46)

Previously in adults PI concentrations outside the limits of the reference population values were found to correlate with non-adherence and therapy failure. (47) Van Rossum et al. performed a study to assess adherence using this method in children. (48) Of 40 HIV-1 infected children blood samples were taken at regular outpatients visits and analysed for plasma levels of indinavir and nelfinavir and HIV-1 RNA levels. For each sample a concentration ratio was calculated by dividing the concentration in that sample by the time adjusted population value. The percentage of samples fulfilling the criteria for compliance was assessed for each child using three different methods 1) Concentration ratios below or above concentration ratio limits (CORAL), consisting of the 5^{th} and 95^{th} percentile of population data obtained in adults, were determined indicative of non-compliance, 2) concentration ratios below the lower CORAL were determined indicative of non-compliance or 3) only children with plasma sample levels below

the limit of quantification (<0.04 mg/L) were considered non- compliant. Compliance rates calculated using method 2 were significantly higher in children responding to therapy than in children who experienced viral failure. Compliance rates calculated with methods 2 and 3 were significantly lower in children using indinavir compared to children using nelfinavir. These data indicate that TDM is an additional objective tool for the assessment of non-compliance in children.

Discussion

TDM can be a useful tool in the treatment of HIV-1 infected children. Interindividual differences in pharmacokinetic parameters require intensive monitoring. The use of TDM reduces the risk for underdosing and subsequent therapy failure or over dosing. Indeed our tailor-made approach in children starting HAART has resulted in optimal viral suppression. Interestingly the studies in which the medication dosage was adjusted based on TDM are among those with the best viral outcome. (49) A major complication in the use of TDM in HIV-1 infected patients is that, although reference values have been defined for some PIs and NRTIs, significant variation occurs in plasma drug levels of individual patients. There is a serious shortage in population reference values of antiretroviral medication in children. Therefore in clinical practice plasma drug levels in children are targeted to adult reference values, which may be insufficient due to the unique features of HIV infection in children such as the high viral load and the presence of a developing immature immune system. On the other hand children may also be more prone for side effects in which case adult plasma levels of drugs may be to high. At this moment no data are available as to which parameter is the best for the measurement of pharmacokinetics in children and how often PK analysis should be performed. Future studies in this field are required.

The use of TDM to measure adherence to therapy has led to promising results. However one should be cautious to base assumptions of non- compliance on plasma levels alone, since aberrant plasma levels may also be the result of pharmacokinetic effects not related to non-compliance such as changes in the intake or composition of food, gastrointestinal motility or drug-drug interactions. Alternatively compliance may improve prior to the hospital visit, resulting in adequate plasma levels despite poor overall compliance. Hugen et al. reported that adherence in adults is best measured by a combination of methods including pill counts, MEMS, adherence questionnaires. (50)

In conclusion TDM clearly has a place in the treatment of HIV infected children. Adjustment of antiretroviral therapy based on TDM may prevent medication failure or toxicity in children. However, more data are needed to establish child specific reference values for antiretroviral medication and to assess the optimal method of TDM, thus avoiding over treatment or insufficient analysis.

Acknowledgements

The authors would like to thank Annemarie van Rossum for helpful discussion and critical review of the manuscript.

References

1. UNAIDS. AIDS epidemic update: 2002. Geneva: UNAIDS/WHO; june 2002.
2. Gortmaker SL, Hughes M, Cervia J., et al. Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. N Engl J Med 2001;345(21):1522-8.
3. Palella FJ, Jr., Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998;338(13):853-60.
4. Connor EM, Sperling RS, Gelber R,, et al. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. N Engl J Med 1994;331(18):1173-80.
5. Ioannidis JP, Abrams EJ, Ammann A, et al. Perinatal transmission of human immunodeficiency virus type 1 by pregnant women with RNA virus load <1000 copies/ml. J Infect Dis 2001;183(4):539-45.
6. Starr SE, Fletcher CV, Spector SA, et al. Combination therapy with efavirenz, nelfinavir, and nucleoside reverse-transcriptase inhibitors in children infected with human immunodeficiency virus type 1. Pediatric AIDS Clinical Trials Group 382 Team. N Engl J Med 1999;341(25):1874-81.
7. Vigano A, Dally L, Bricalli D, et al. Clinical and immuno-virologic characterization of the efficacy of stavudine, lamivudine, and indinavir in human immunodeficiency virus infection. J Pediatr 1999;135(6):675-82.
8. van Rossum AM, Geelen SP, Hartwig NG, et al. Results of 2 years of treatment with protease-inhibitor--containing antiretroviral therapy in dutch children infected with human immunodeficiency virus type 1. Clin Infect Dis 2002;34(7):1008-16.
9. Saez-Llorens X, Violari A, Deetz CO, et al. Forty-eight-week evaluation of lopinavir/ritonavir, a new protease inhibitor, in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2003;22(3):216-224.
10. Mueller BU, Nelson RP, Jr., Sleasman J, et al. A phase I/II study of the protease inhibitor ritonavir in children with human immunodeficiency virus infection. Pediatrics 1998;101(3 Pt 1):335-43.
11. Pelton SI, Johnson D, Chadwick E, et al. A one year experience: T cell responses and viral replication in children with advanced human immunodeficiency virus type 1 disease treated with combination therapy including ritonavir. Pediatr Infect Dis J 1999;18(7):650-2.
12. Thuret I, Michel G, Chambost H, et al. Combination antiretroviral therapy including ritonavir in children infected with human immunodeficiency. AIDS 1999;13(1):81-7.
13. Melvin AJ, Mohan KM, Arcuino LA, et al. Clinical, virologic and immunologic responses of children with advanced human immunodeficiency virus type 1 disease treated with protease inhibitors. Pediatr Infect Dis J 1997;16(10):968-74.
14. Wintergerst U, Hoffmann F, Solder B, et al. Comparison of two antiretroviral triple combinations including the protease inhibitor indinavir in children infected with human immunodeficiency virus. Pediatr Infect Dis J 1998;17:495-9.
15. Jankelevich S, Mueller BU, Mackall CL, et al. Long-term virologic and immunologic responses in human immunodeficiency virus type 1-infected children treated with indinavir, zidovudine, and lamivudine. J Infect Dis 2001;183(7):1116-20.
16. Teglas JP, Quartier P, Treluyer JM, et al. AIDS 2001;15(2):241-3.
17. Ufkes JGR. Farmacotherapie bij kinderen, bejaarden en zwangeren. In: Ufkes JGR, Stolk ML, editors. Farmacotherapie op recept. Alphen aan de Rijn: Van Zuiden Communications; 1995. p. 100-26.
18. Reed MD, Gall P. Principles of drug therapy. In: Behrman RE, Kliegman RM, Jenson HB, editors. Nelson textbook of pediatrics. Philadelphia: W.B. Saunders company; 2000. p. 2229-2234.

19. King JR, Kimberlin DW, Aldrovandi GM, et al. Antiretroviral Pharmacokinetics in the Paediatric Population: A Review. Clin Pharmacokinet 2002;41(14):1115-1133.
20. Gao WY, Shirasaka T, Johns DG, et al. Differential phosphorylation of azidothymidine, dideoxycytidine, and dideoxyinosine in resting and activated peripheral blood mononuclear cells. J Clin Invest 1993;91(5):2326-33.
21. Sale M, Sheiner LB, Volberding et al. Zidovudine response relationships in early human immunodeficiency virus infection. Clin Pharmacol Ther 1993;54(5):556-66.
22. Perno CF, Yarchoan R, Cooney DA, et al. Inhibition of human immunodeficiency virus (HIV-1/HTLV-IIIBa-L) replication in fresh and cultured human peripheral blood monocytes/macrophages by azidothymidine and related 2',3'-dideoxynucleosides. J Exp Med 1988;168(3):1111-25.
23. Fletcher CV, Acosta EP, Henry K, et al. Concentration-controlled zidovudine therapy. Clin Pharmacol Ther 1998;64(3):331-8.
24. Back D, Gatti G, Fletcher C, et al. Therapeutic drug monitoring in HIV infection: current status and future directions. AIDS 2002;16 Suppl 1:S5-37.
25. Van Heeswijk RP. Critical issues in therapeutic drug monitoring of antiretroviral drugs. Ther Drug Monit 2002;24(3):323-31.
26. Burger DM, Aarnoutse RE, Hugen PW. Pros and cons of therapeutic drug monitoring of antiretroviral agents. Curr Opin Infect Dis 2002;15(1):17-22.
27. Burger DM, Hoetelmans RM, Hugen PW, et al. Low plasma concentrations of indinavir are related to virological treatment failure in HIV-1-infected patients on indinavir-containing triple therapy. Antivir Ther 1998;3(4):215-20.
28. Stein DS, Fish DG, Bilello JA, et al. A 24-week open-label phase I/II evaluation of the HIV protease inhibitor MK-639 (indinavir). AIDS 1996;10(5):485-92.
29. Burger DM, van Rossum AM, Hugen PW, et al. Pharmacokinetics of the protease inhibitor indinavir in human immunodeficiency virus type 1-infected children. Antimicrob Agents Chemother 2001;45(3):701-5.
30. Durant J, Clevenbergh P, Garraffo R, et al. Importance of protease inhibitor plasma levels in HIV-infected patients treated with genotypic-guided therapy: pharmacological data from the Viradapt Study. AIDS 2000;14(10):1333-9.
31. Marzolini C, Telenti A, Decosterd LA, et al. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. AIDS 2001;15(1):71-5.
32. Veldkamp AI, Weverling GJ, Lange JM, et al. High exposure to nevirapine in plasma is associated with an improved virological response in HIV-1-infected individuals. AIDS 2001;15(9):1089-95.
33. van Rossum AM, Dieleman JP, Fraaij PL, et al. Persistent sterile leukocyturia is associated with impaired renal function in human immunodeficiency virus type 1-infected children treated with indinavir. Pediatrics 2002;110(2 Pt 1):e19.
34. Dieleman JP, Gyssens IC, van der Ende ME, et al. Urological complaints in relation to indinavir plasma concentrations in HIV-infected patients. AIDS 1999;13(4):473-8.
35. Gonzalez de Requena D, Nunez M, Jimenez-Nacher I, et al. Liver toxicity caused by nevirapine. AIDS 2002;16(2):290-1.
36. Burger DM, Bergshoeff AS, de Groot R, et al. Maintaining the nelfinavir through concetration above 0.8 mg/L significantly improves virological response in HIV-infected children. Submitted, 2003.
37. Verweel G, van Rossum AM, Hartwig NG, et al. Treatment with highly active antiretroviral therapy in human immunodeficiency virus type 1-infected children is associated with a sustained effect on growth. Pediatrics 2002;109(2):E25.
38. Huang L, Wring SA, Woolley JL, et al. Induction of P-glycoprotein and cytochrome P450 3A by HIV protease inhibitors. Drug Metab Dispos 2001;29(5):754-60.
39. Gisolf EH, van Heeswijk RP, Hoetelmans RW, et al. Decreased exposure to saquinavir in HIV-1-infected patients after long-term antiretroviral therapy including ritonavir and saquinavir. AIDS 2000;14(7):801-5.
40. Fraaij PL, Bergshoeff AS, Van Rossum AM, et al. Changes in indinavir exposure over time: a case study in six HIV-1-infected children. J Antimicrob Chemother 2003. E- publication before print.
41. Paterson DL, Swindells S, Mohr J, et al. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. Ann Intern Med 2000;133(1):21-30.
42. Watson DC, Farley JJ. Efficacy of and adherence to highly active antiretroviral therapy in children infected with human immunodeficiency virus type 1. Pediatr Infect Dis J 1999;18(8):682-9.

43. Demas PA, Webber MP, Schoenbaum EE, et al. Maternal adherence to the zidovudine regimen for HIV-exposed infants to prevent HIV infection: a preliminary study. Pediatrics 2002;110(3):e35.
44. Van Dyke RB, Lee S, Johnson GM, et al. Reported adherence as a determinant of response to highly active antiretroviral therapy in children who have human immunodeficiency virus infection. Pediatrics 2002;109(4):e61.
45. Liu H, Golin CE, Miller LG, et al. A comparison study of multiple measures of adherence to HIV protease inhibitors. Ann Intern Med 2001;134(10):968-77.
46. Murri R, Ammassari A, Gallicano K, et al. Patient-reported nonadherence to HAART is related to protease inhibitor levels. J Acquir Immune Defic Syndr 2000;24(2):123-8.
47. Hugen PW, Burger DM, Aarnoutse RE, et al. Therapeutic drug monitoring of HIV-protease inhibitors to assess noncompliance. Ther Drug Monit 2002;24(5):579-87.
48. van Rossum AM, Bergshoeff AS, Fraaij PL, et al. Therapeutic drug monitoring of indinavir and nelfinavir to assess adherence to therapy in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2002;21(8):743-7.
49. van Rossum AM, Fraaij PL, de Groot R. Efficacy of highly active antiretroviral therapy in HIV-1 infected children. Lancet Infect Dis 2002;2(2):93-102.
50. Hugen PW, Langebeek N, Burger DM, et al. Assessment of adherence to HIV protease inhibitors: comparison and combination of various methods, including MEMS (electronic monitoring), patient and nurse report, and therapeutic drug monitoring. J Acquir Immune Defic Syndr 2002;30(3):324-34.

A new method for analysis of AZT-triphosphate and nucleotide-triphosphates

Jeroen J.A. van Kampen, Pieter L.A. Fraaij, Vishal Hira, Annemarie M.C. van Rossum, Nico G. Hartwig, Ronald de Groot, Theo M. Luider

Biochem Biophys Res Commun 2004:315(1):151-159

Chapter 11

Abstract

We have developed a new method based on Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry (MS) for analysis of zidovudine-triphosphate and (deoxy)nucleotide-triphosphates, which ultimately can be used for Nucleoside Reverse Transcriptase Inhibitor (NRTI) treatment monitoring in HIV-1 infected children and adults. Four different matrices were compared for sensitivity and reproducibility of zidovudine-triphosphate detection and anthranilic acid mixed with nicotinic acid (AA/NA) was selected as most suitable matrix. Solutions of zidovudine-triphosphate, ATP and dGTP were detected up to 0.5 femtomole per sample. Furthermore, intracellular zidovudine-triphosphate, ATP and dGTP were detected in Peripheral Blood Mononuclear Cells (PBMCs). Zidovudine-triphosphate, ATP and dGTP yield identical mass spectra, however MALDI-TOF Post Source Decay analysis can be used for discrimination between these compounds. We conclude that this method based on MALDI-TOF MS can be used for analysis of intracellular zidovudine-triphosphate and (deoxy)nucleotide-triphosphates in PBMCs.

Introduction

Institution of optimal treatment of HIV-1 infected patients poses a major challenge. Treatment normally consists of a combination of three classes of antiretroviral drugs: the protease inhibitors (PIs), the non-nucleoside reverse transcriptase inhibitors (NNRTIs) and the nucleoside reverse transcriptase inhibitors (NRTIs). A major feature of these three drug classes is that large inter-individual and intra-individual differences are observed in their pharmacokinetics (1-5). This is even more important when one considers the relation between viral suppression and plasma concentration of PIs and NNRTIs (1, 3, 6-9). Therefore, we routinely perform pharmacokinetic analyses of PIs and NNRTIs in HIV-1 infected children and adjust the dose of PIs and NNRTIs to maintain optimal plasma concentrations. This approach has resulted in favorable results with 69 % viral response after 2 years of treatment (10).

However, such an approach is not possible for NRTIs. NRTI plasma concentrations correlate poorly with HIV-1 activity, since NRTIs are prodrugs that are intracellularly converted to active NRTI-triphosphates (NRTI-TPs) using the kinases from the host cell (11). Intracellularly, the NRTI-TP competes with its corresponding endogenous deoxynucleotide-triphosphate (dNTP) for incorporation into viral DNA by HIV reverse transcriptase. Incorporation of NRTI-TP terminates elongation of viral DNA, thus prevents viral replication. *In vitro* studies with Peripheral Blood Mononuclear Cells (PBMCs) show that HIV-1 activity of NRTIs correlates more closely to the ratio of the intracellular NRTI-TP concentration and the corresponding intracellular dNTP concentration than to the intracellular NRTI-TP concentration alone (12-14). In addition, a clinical study with HIV-1 infected adults shows that the intracellular NRTI-TP concentration alone correlates to HIV-1 activity (15).

Current methods for quantification of NRTI-TPs, such as Radio Immunoassays, require large blood samples and do not allow high throughput analysis (16, 17). This complicates patient related studies, which require analysis of multiple blood samples of a large group of HIV-1 infected patients. Methods have been developed, such as HPLC coupled to Electro Spray Ionization (ESI) MS, which allow for high throughput analysis of NRTI-TPs (18-22). These methods still require at least 7 ml of blood to obtain sufficient material for analysis. This amount of blood allows for patient related studies in HIV-1 infected adults, but still complicates NRTI-TP studies in HIV-1 infected children, since such studies require multiple blood samples from one individual on a single day.

We aimed to develop a relative easy method for analysis of intracellular NRTI-TP, which ultimately can be used for studies in HIV-1 infected children and adults. We studied the usability of Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry (MS) for this method, since: 1) MALDI-TOF MS methods have been developed for quantification of drugs, such as antibiotics and intracellular tetraphenylphosphonium (23-25). 2) MALDI-TOF MS is able to detect compounds at very low concentrations, which allows for analysis when only small amounts of material can be obtained (26). 3) MALDI-TOF MS is more tolerant to salts and other contaminants compared to other forms of MS (26). This allows for reduction in sample preparation steps, which reduces sample loss and sample preparation

time. 4) MALDI-TOF MS allows for rapid and fully automated analysis of large amounts of samples, which allows for high throughput use (27, 28).
We studied the use of MALDI-TOF MS for analysis of the triphosphate form of the most widely used NRTI zidovudine (AZT-TP). We also examined if MALDI-TOF MS is capable of detecting dNTPs (dGTP), since *in vitro* studies show that HIV-1 activity correlates better to the ratio of intracellular NRTI-TP and dNTP concentration than to the intracellular NRTI-TP concentration alone [12-14]. In addition, we studied the analysis of NTPs (ATP, CTP, GTP and UTP) by MALDI-TOF MS, since these compounds share many features (e.g. size, triphosphate group) with dNTPs and NRTI-TPs and could cause interferences. To our knowledge, this is the first time MALDI-TOF MS is used for analysis of NRTI-TP and endogenous (d)NTPs. Therefore, no suitable matrices are known for MALDI-TOF analysis of these compounds. We studied the usability of four different matrices, which are currently used in MALDI-TOF analysis of related compounds such as oligonucleotides. Subsequently, the most suitable matrix was used for analysis of intracellular AZT-TP in PBMCs. Finally, we studied the use of MALDI-TOF Post Source Decay (MALDI-PSD) analysis for discrimination of AZT-TP from ATP and dGTP, since these three compounds have the same molecular weight, which can cause interferences when using a method based on mass spectrometry.

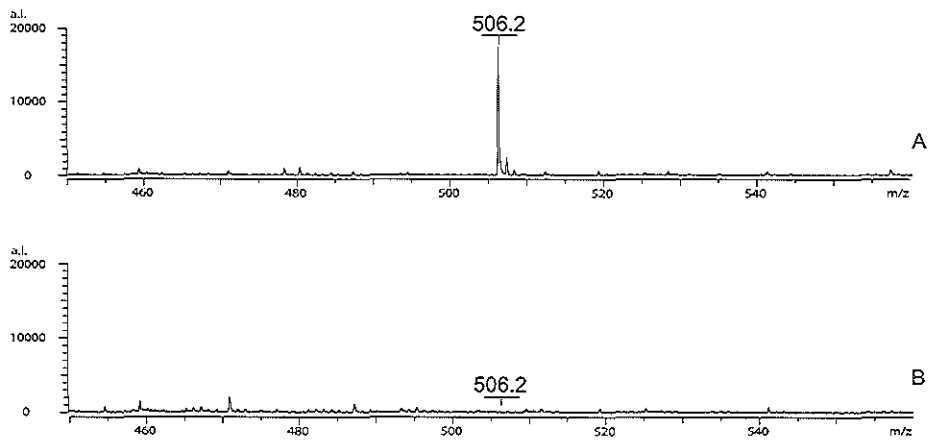
Materials and Methods

Standard nucleotide solutions: ATP (molecular weight 507.2 Da), CTP (483.1 Da), GTP (523.3 Da), dGTP (507.2 Da), UTP (484.1 Da) (Amersham, Sweden) and AZT-TP (507.2 Da) (Calbiochem, Germany) were diluted with HPLC-grade water and stored at -80 °C until analysis.
Preparation of matrix solutions: Solution I: 45 mM anthranilic acid (AA) (Fluka, Switzerland) was mixed with 45 mM nicotinic acid (NA) (Fluka, Switzerland) and 55 mM diammoniumhydrogencitrate (DAHC) (Fluka, Switzerland) in 45 % acetonitrile (Aldrich, Germany). *Solution II:* 3-hydroxy picolinic acid (3-HPA) (Aldrich, Germany) was mixed with DAHC and HPLC-grade water, until the solution had a concentration of 3 mg/ml 3-HPA and 9 mg/ml DAHC. *Solution III:* 5-methoxy salicylic acid (5-MSA) was saturated in one part acetonitrile and one part DAHC mixed with HPLC-grade water (50 mM). Subsequently, saturated 5-MSA solution was ten times diluted with DAHC mixed with HPLC-grade water (50 mM). *Solution IV:* 2,5-dihydroxybenzoic acid (2,5-DHB) was mixed with DAHC and HPLC-grade water, until the solution had a concentration of 3 mg/ml 2,5-DHB and 9 mg/ml DAHC. Fresh matrix solutions were prepared in Teflon tubes on the day of analysis.
Comparison of matrix solutions: Matrix solutions I - IV were compared for reproducibility and limit of detection of AZT-TP analysis in standard solution.
Relation between AZT-TP concentration and signal-to-noise ratio: Different AZT-TP dilutions were analyzed to assess the effect of the AZT-TP concentration on the signal-to-noise ratio. The signal-to-noise ratio (S/N) was calculated by dividing the maximal signal height to the local noise level. Samples were only measured if sample crystallization was observed by light microscopy (needle crystallization).

PBMC isolation: Venous blood from healthy volunteers was collected in Vacutainer CPT tubes (Becton Dickinson). PBMCs were separated by centrifugation at 2,000 rpm for 20 minutes and washed twice with PBS (1,500 rpm for 10 minutes and 1,200 rpm for 15 minutes, respectively). PBS was discarded and 3 ml growth media (RPMI supplemented with 10 % heat-inactivated fetal calf serum, 10000 IU/ml penicillin, 10000 IU/ml streptomycin and 20 % DMSO) was added.

PBMC incubation with AZT: Growth media was added until a final concentration of 1×10^6 PBMCs per ml was reached. 1 µl AZT solution (Fluka, Switzerland) was added to reach a final concentration of 10 µM. An equal amount of deionized water was added for the creation of "negative control" samples. Cultures were incubated at 37 °C for 3 hours. After incubation, cells were centrifuged at 3800 rpm for 5 minutes and washed with PBS (3800 rpm for 5 minutes). "Positive controls" were created by adding 200 femtomole AZT-TP to the PBMC pellet just before extraction.

Figure 1. Measurement of 500 femtomole AZT-TP (A). An expected signal was found at m/z 506.2. The negative control sample (B) yielded no signal at m/z 506.2. AA/NA was used for matrix. a.i. = absolute intensity. m/z = mass-to-charge ratio.



Nucleotide extraction from PBMCs: To the PBMC pellet 500 µl cold MeOH (60 %) was added and nucleotides were extracted at 4 °C overnight. After extraction, samples were centrifuged (10,000 rpm for 5 minutes), supernatants were collected and lyophilized with a SpeedVac (Savant, USA) for 45 minutes. Residues were stored at - 80 °C until analysis. Per sample 0.85×10^6 PBMCs were used for intracellular AZT-TP analysis.

MALDI-TOF analysis: Matrix solution (0.5 µl) was pipetted onto an Anchor Chip target plate (Bruker Daltonics, Germany) and dried at room temperature. Subsequently 0.5 µl sample was pipetted onto the crystallized matrix and dried at room temperature. Analysis was performed by a BIFLEX III MALDI-TOF mass spectrometer (Bruker Daltonics, Germany) using the negative

reflectron mode. Laser attenuation was set at 40 (UNIX operating system). Hundred shots were used for each mass spectrum. Post Source Decay (PSD) analysis was performed on PBMC samples and on standard solutions of AZT-TP, ATP and dGTP by the BIFLEX III MALDI-TOF mass spectrometer in the negative mode.

Prediction of fragmentation pattern of AZT-TP, ATP and dGTP: MS Fragmenter software from ACD/Labs was used for prediction of the fragmentation pattern of AZT-TP, ATP and dGTP.

Results and discussion

Comparison of different matrices for AZT-TP analysis: We tested four different matrices on their sensitivity and reproducibility of AZT-TP detection. Both the AA/NA (solution I) and 3-HPA (solution II) matrices were able to detect the expected mass signal of AZT-TP up to 0.5 femtomole per sample (figure 1 and 2).

The reproducibility of the mass spectra, however, is much better when AA/NA is used compared to the use of 3-HPA. The expected mass signal of AZT-TP was not detected when 25 femtomole of AZT-TP was analyzed with the 5-MSA matrix (solution III).The 2,5-DHB matrix (solution IV) itself yielded a signal at *m/z* of 505.3, which can interfere with the detection of AZT-TP. AA/NA is therefore the most suitable matrix for analysis of AZT-TP.

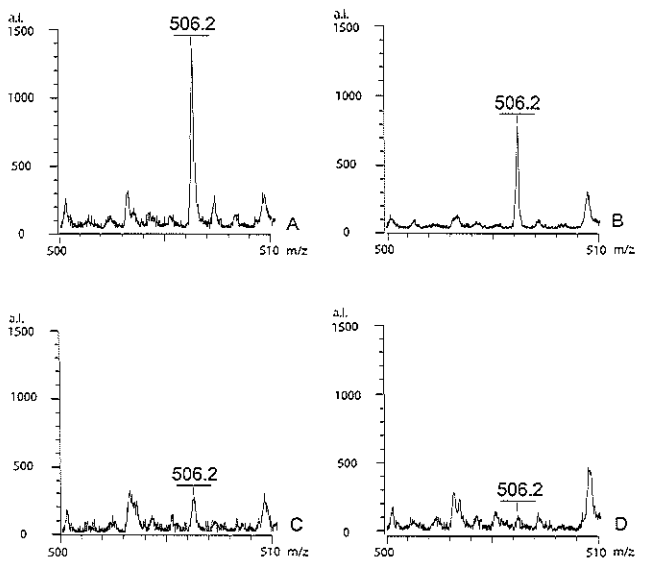


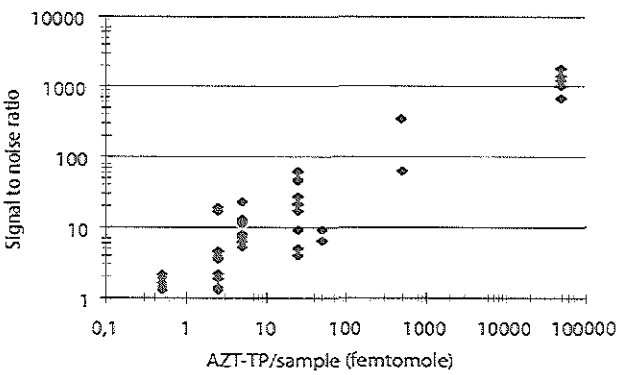
Figure 2. Measurement of 5.0 (A), 2.5 (B) and 0.5 (C) femtomole AZT-TP. An expected signal was found at m/z 506.2 when AZT-TP was diluted up to 0.5 femtomole per sample. The negative control sample (D) yielded no signal at m/z 506.2. AA/NA was used for matrix.

The effect of the AZT-TP concentration on the signal-to-noise ratio: Known quantities of AZT-TP were measured to analyze the relation with the signal-to-noise ratio. Figure 3 shows a clear linear relation between the signal-to-noise ratio and AZT-TP concentration. This allows for rough estimation of the AZT-TP concentration. However, more exact values of the intracellular AZT-TP concentration are required for patient related research, which can not be obtained by estimation based on the AZT-TP concentration versus signal-to-noise ratio curve. There are

more accurate ways for quantification by MALDI-TOF MS. By adding a known concentration of a compound (internal standard) to the sample, one can compare signal-to-noise ratio of the analyte of interest with the signal-to-noise ratio of the internal standard. This method has resulted in an accurate quantification of different compounds, as published earlier (22-24).

Detection of (d)NTPs in standard solution: dGTP was used for exploring the usability of MALDI-TOF MS for dNTP analysis. The expected mass signal of dGTP (*m/z* 506.2) was detected up to a dilution of 0.5 femtomole per sample when using the AA/NA matrix. This shows that MALDI-TOF MS is able to detect dNTP, which is necessary for exploring its ability of measuring the ratio of intracellular NRTI-TP and corresponding dNTP in PBMCs. It is likely that other dNTPs, such as dTTP, can be detected by MALDI-TOF MS, since related compounds (NTPs, dGTP, AZT-TP) can be detected up to 0.5 femtomole per sample. ATP, CTP, GTP and UTP were used for exploring the usability of MALDI-TOF MS for NTP analysis. The expected mass signal of ATP (*m/z* 506.2) was detected up to a dilution of 0.5 femtomole per sample when using the AA/NA matrix. Samples of 500 and 50 picomole of ATP, CTP, GTP and UTP were analyzed using the 3-HPA matrix. All mass signals were detected at the expected mass-to-charge ratios (*m/z* 506.2, *m/z* 482.1, *m/z* 522.3 and *m/z* 483.1 respectively). This shows that MALDI-TOF MS is able to detect NTP, which is necessary for predicting possible interference of such compounds when analyzing AZT-TP. ATP, dGTP and AZT-TP yield a mass signal at the same mass-to-charge ratio (*m/z* 506.2) in standard nucleotide solutions. This complicates future experiments for AZT-TP quantification. Therefore, we studied if ATP and dGTP also interfered with the detection of AZT-TP in PBMCs.

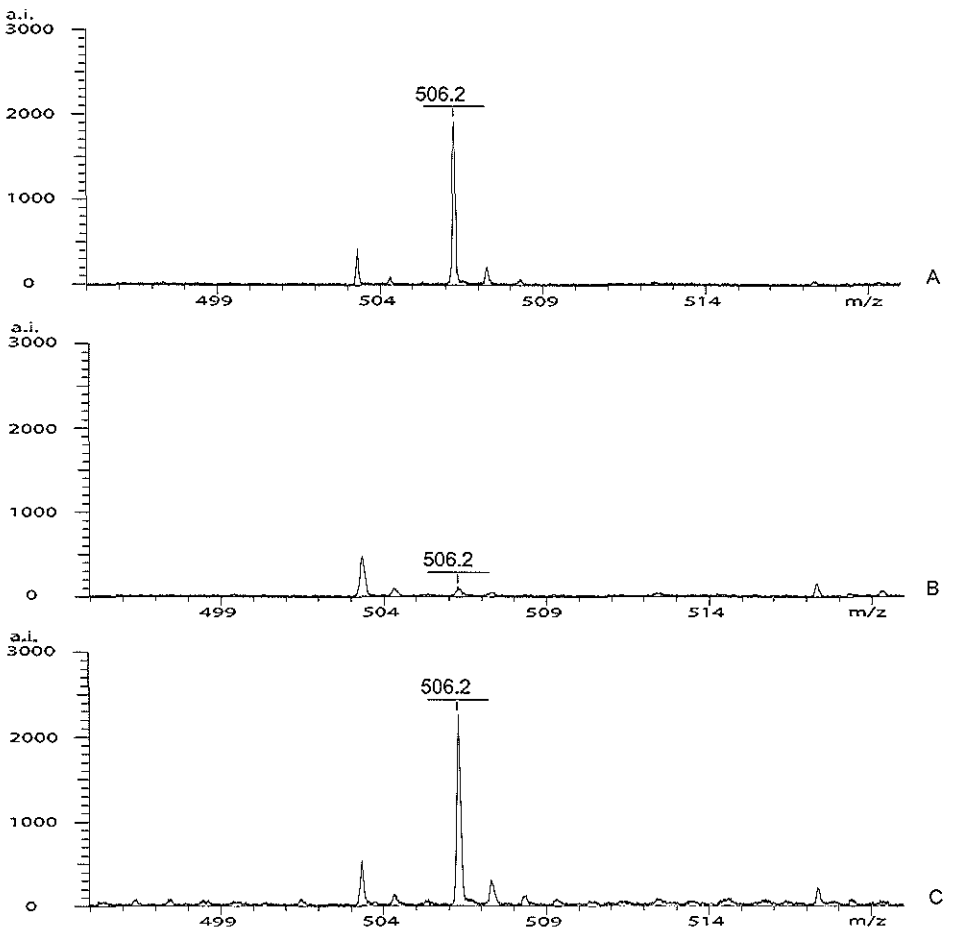
Figure 3. A linear relation was found between AZT-TP concentration and signal-to-noise ratio. AA/NA was used for matrix.



Intracellular AZT-TP in PBMCs: The analysis of the extract of PBMCs incubated with AZT revealed a mass signal at the expected *m/z* of 506.2 (figure 4). This expected mass signal was

again detected when 200 femtomole AZT-TP was added to the pellet of PBMCs not incubated with AZT (positive control). The extract of PBMCs not incubated with AZT (negative control)

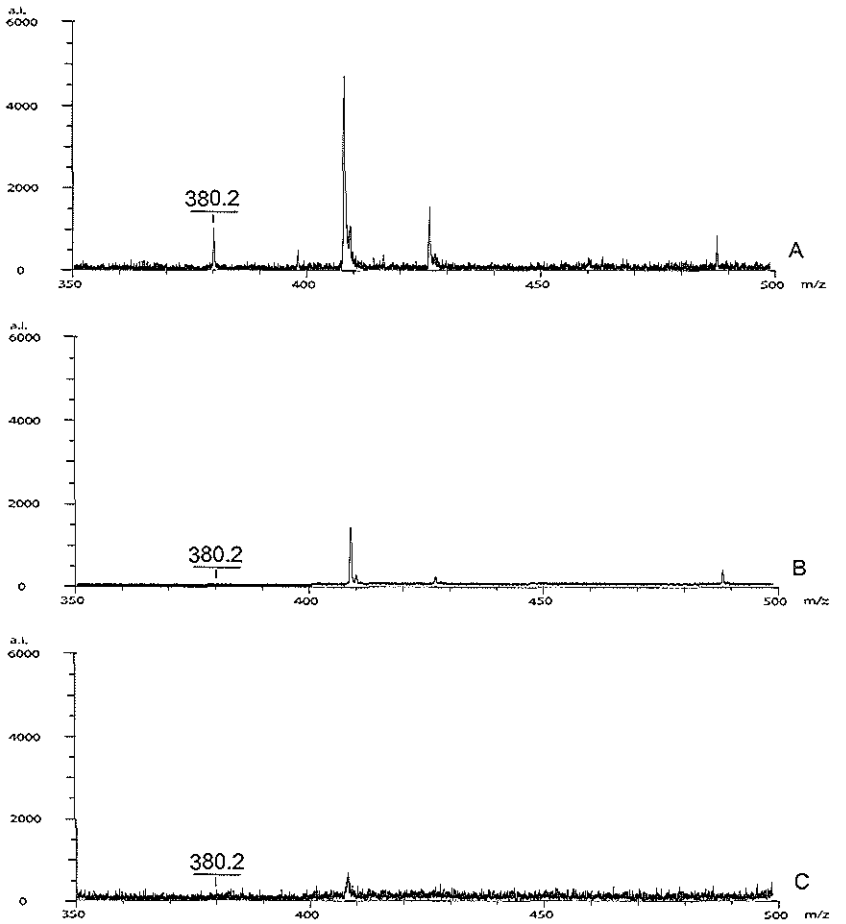
Figure 4. Mass signals of intracellular ATP, dGTP and AZT-TP were detected at the expected m/z 506.2 in PBMCs incubated with AZT (A) Mass signals of intracellular ATP and dGTP were detected at the expected m/z 506.2 in PBMCs not incubated with AZT (negative control) (B). Mass signals of intracellular ATP, dGTP and AZT-TP are found at the expected m/z 506.2 in PBMCs not incubated with AZT and spiked with 200 femtomole AZT-TP (positive control) (C). Per sample 85x106 PBMCs were used. AA/NA was used for matrix.



also yielded a mass signal at *m/z* of 506.2. This shows that AZT-TP, ATP and dGTP can be detected in PBMCs by MALDI-TOF. However, ATP and dGTP interfere with AZT-TP detection in PBMCs. It is not possible to estimate the AZT-TP concentration by comparing signal-to-noise ratio of the mass signal at *m/z* 506.2 in negative controls and in PBMCs incubated with AZT.

AZT affects the nucleotide pool size, thus we cannot assume that ATP and dGTP are present in the same concentrations in negative controls and in PBMCs incubated with AZT (29). Therefore, we have searched for a method to discriminate AZT-TP from ATP and dGTP.

Figure 5. MALDI-PSD analysis of 0.5 femtomole AZT-TP revealed a unique mass signal at m/z 380.2 (A). This mass signal was not found by MALDI-PSD analysis of 5.0 nanomole ATP (B) and dGTP (C). AA/NA was used for matrix.

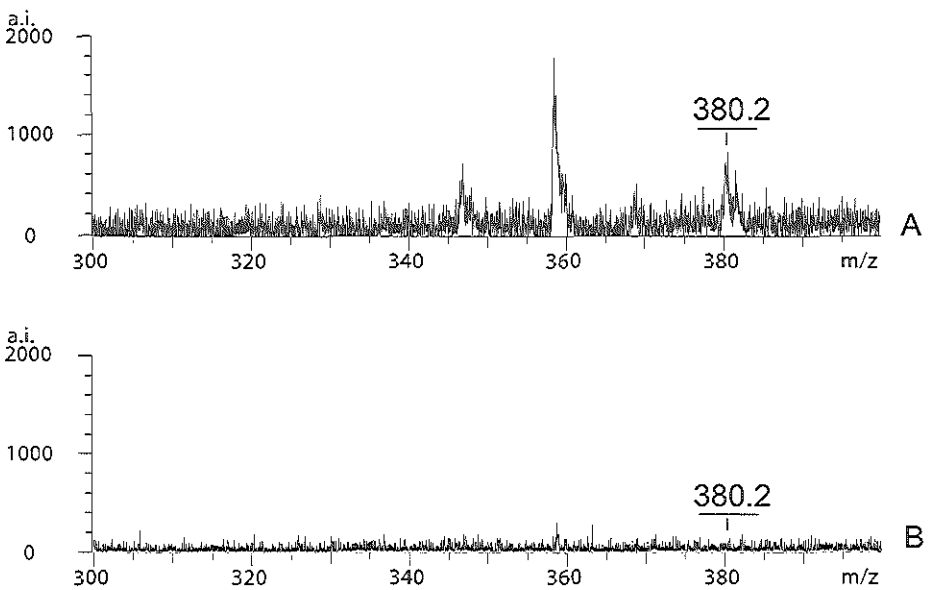


Fragmentation of AZT-TP, ATP and dGTP in standard nucleotide solutions: MALDI-PSD analysis was used for detection of the unique AZT-TP fragment. As predicted by software, the MALDI-PSD analysis of AZT-TP, ATP and dGTP in standard solution revealed a unique mass signal for AZT-TP at *m/z* 380.2 (figure 5). This signal was not observed when analyzing ATP and dGTP by MALDI-PSD. Subsequently, we explored the ability of MALDI-PSD analysis for detecting the AZT-TP fragment in PBMCs.

Fragmentation of AZT-TP present in PBMCs: Figure 6 depicts the detection of the mass signal at the expected *m/z* 380.2 in PBMCs incubated with AZT. This signal was not observed in the analysis of PBMCs not incubated with AZT. Thus, MALDI-PSD analysis is able to discriminate AZT-TP from ATP and dGTP in standard nucleotide solutions and in PBMCs.

The development of a method for quantification of NRTI-TP in HIV-1 infected children requires that the analysis can be performed on small amounts of PBMCs. Approximately one million PBMCs can be derived from one ml blood, which is an acceptable blood sample size for studies in HIV-1 infected children. Font *et al.* showed that the intracellular AZT-TP concentration ranges from 38 to 193 femtomole per million PBMCs in HIV-1 infected adults (21). The signal-to-noise ratio of AZT-TP detection in standard solution obtained by our method is larger than 10 in this range of measurement. However, the limit of detection of the unique AZT-TP fragment in PBMCs is probably higher than the limit of detection of AZT-TP in standard solutions. Furthermore, we have to explore if MALDI-PSD analysis can be used for accurate quantification of AZT-TP by using an internal standard.

Figure 6. The MALDI-PSD analysis of PBMCs incubated with AZT (A) revealed the expected signal of the unique fragment of AZT-TP at m/z 380.2. This signal was not detected in the MALDI-PSD analysis of PBMCs not incubated with AZT (B).



We conclude that: 1) AA/NA is a suitable matrix for MALDI-TOF analysis of NRTI-TPs, NTPs and dNTPs. 2) AZT-TP, ATP and dGTP can be detected up to 0.5 femtomole per sample by MALDI-TOF MS. 3) CTP, GTP and UTP can be detected by MALDI-TOF MS. 4) Intracellular AZT-TP, ATP and dGTP can be detected in PBMCs by MALDI-TOF MS. 5) Discrimination of

AZT-TP from ATP and dGTP can be obtained by MALDI-PSD analysis in standard nucleotide solutions and in PBMCs. 6) Our developed method could be useful for NRTI-TP studies in HIV-1 infected children and adults.

Acknowledgements

We like to acknowledge the SKZ Foundation for financial support.

References

1. A.S. Bergshoeff, P.L. Fraaij, A.M. van Rossum, et al. Pharmacokinetics of nelfinavir in children: influencing factors and dose implications, Antiviral Therapy 8 (2003) 215-222.
2. J.R. King, D.W. Kimberlin, G.M Aldrovandi et al. Antiretroviral pharmacokinetics in the paediatric population: a review, Clin Pharmacokinet 41 (2002) 1115-33.
3. D.M. Burger, A.M. van Rossum, P.W. Hugen, et al. Dutch Study Group for Children with HIV-1 Infection, Pharmacokinetics of the protease inhibitor indinavir in human immunodeficiency virus type 1-infected children, Antimicrob Agents Chemother 45 (2001) 701-5.
4. A.M. van Rossum, R. de Groot, N.G. Hartwig,et al. Pharmacokinetics of indinavir and low-dose ritonavir in children with HIV-1 infection, AIDS 14 (2000) 2209-10.
5. P.L. Fraaij, A.S. Bergshoeff, A.M. van Rossum, et al. Changes in indinavir exposure over time: a case study in six HIV-1 infected children, accepted Journal of Antimicrobial Chemotherapy.
6. D.M. Burger, R.M. Hoetelmans, P.W. Hugen, et al. Low plasma concentrations of indinavir are related to virological treatment failure in HIV-1-infected patients on indinavir-containing triple therapy, Antivir Ther 3 (1998) 215-20.
7. C. Marzolini, A. Telenti, L.A. Decosterd, et al. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients, AIDS 15 (2001) 71-5.
8. A.I. Veldkamp, G.J. Weverling, J.M. Lange, et al. High exposure to nevirapine in plasma is associated with an improved virological response in HIV-1-infected individuals, AIDS 15 (2001) 1089-95.
9. M. Pfister, L. Labbe, S.M. Hammer, et al. Adult AIDS Clinical Trial Group Study 398, Population pharmacokinetics and pharmacodynamics of efavirenz, nelfinavir, and indinavir: Adult AIDS Clinical Trial Group Study 398, Antimicrob Agents Chemother 47 (2003) 130-7.
10. A.M. van Rossum, S.P. Geelen, N.G. Hartwig, et al. Results of 2 years of treatment with protease-inhibitor-containing antiretroviral therapy in dutch children infected with human immunodeficiency virus type 1, Clin Infect Dis 34 (2002) 1008-16.
11. M. Sale, L.B. Sheiner, P.Volberding, et al., Zidovudine response relationships in early human immunodeficiency virus infection, Clin Pharmacol Ther 54 (1993) 556-66.
12. W.Y. Gao, T. Shirasaka, D.G. Johns et al., Differential phosphorylation of azidothymidine, dideoxycytidine, and dideoxyinosine in resting and activated peripheral blood mononuclear cells, J Clin Invest 91 (1993) 2326-33.
13. C.F. Perno, R. Yarchoan, D.A. Cooney, et al. Inhibition of human immunodeficiency virus (HIV-1/HTLV-IIIBa-L) replication in fresh and cultured human peripheral blood monocytes/macrophages by azidothymidine and related 2',3'- dideoxynucleosides, J Exp Med 168 (1988) 1111-25.
14. E.S. Arner, S. Eriksson, Deoxycytidine and 2',3'-dideoxycytidine metabolism in human monocyte- derived macrophages. A study of both anabolic and catabolic pathways, Biochem Biophys Res Commun 197 (1993) 1499-504.
15. C.V. Fletcher, E.P. Acosta, K. Henry, et al. Concentration-controlled zidovudine therapy, Clin Pharmacol Ther 64 (1998) 331-8.
16. H. Kuster, M. Vogt, B. Joos, et al. A method for the quantification of intracellular zidovudine nucleotides, J Infect Dis 164 (1991) 773-6.
17. R.L. Robbins, B.H. Waibel, A. Fridland, Quantitation of intracellular zidovudine phosphates by use of combined cartridge-radioimmunoassay methodology, Antimicrob Agents Chemother 40 (1996) 2651-4.

18. S. Kewn, P.G. Hoggard, S.D. Sales, et al. Development of enzymatic assays for quantification of intracellular lamivudine and carbovir triphosphate levels in peripheral blood mononuclear cells from human immunodeficiency virus-infected patients, Antimicrob Agents Chemother 46 (2002) 135-43.
19. F. Becher, D. Schlemmer, A. Pruvost, et al. Development of a direct assay for measuring intracellular AZT triphosphate in humans peripheral blood mononuclear cells, Anal Chem 74 (2002) 4220-7.
20. J.D. Moore, G. Valette, A. Darque, et al. Simultaneous quantitation of the 5'-triphosphate metabolites of zidovudine, lamivudine, and stavudine in peripheral mononuclear blood cells of HIV infected patients by high-performance liquid chromatography tandem mass spectrometry, J Am Soc Mass Spectrom 11 (2000) 1134-43.
21. E. Font, O. Rosario, J. Santana, et al. Determination of zidovudine triphosphate intracellular concentrations in peripheral blood mononuclear cells from human immunodeficiency virus- infected individuals by tandem mass spectrometry, Antimicrob Agents Chemother 43 (1999) 2964-8.
22. F. Becher, A. Pruvost, J. Gale, et al. A strategy for liquid chromatography/tandem mass spectrometric assays of intracellular drugs: application to the validation of the triphosphorylated anabolite of antiretrovirals in peripheral blood mononuclear cells, J Mass Spectrom. 38 (2003) 879-90.
23. D.A. Rideout, Bustamante, G. Siuzdak, Cationic drug analysis using matrix-assisted laser desorption/ionization mass spectrometry: application to influx kinetics, multidrug resistance, and intracellular chemical change, Proc Natl Acad Sci U S A 90 (1993) 10226-9.
24. D.J. Harvey, Quantitative aspects of the matrix-assisted laser desorption mass spectrometry of complex oligosaccharides, Rapid Commun Mass Spectrom 7 (1993) 614-9.
25. Y.C. Ling, L. Lin, Y.T. Chen, Quantitative analysis of antibiotics by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Rapid Commun Mass Spectrom 12 (1998) 317-27.
26. G. Siuzdak, Mass spectrometry for biotechnology, Academic Press, 1996.
27. J. Leushner, N.H. Chiu, Automated mass spectrometry: a revolutionary technology for clinical diagnostics, Mol Diagn. 5 (2000) 341-8.
28. D.A. van Ausdall , W.S. Marshall, Automated high-throughput mass spectrometric analysis of synthetic oligonucleotides, Anal Biochem. 256 (1998) 220-8.
29. A. Fridland, M.C. Connelly, R. Ashmun, Relationship of deoxynucleotide changes to inhibition of DNA synthesis induced by the antiretroviral agent 3'-azido-3'-deoxythymidine and release of its monophosphate by human lymphoid cells (CCRF-CEM), Mol Pharmacol. 37 (1990) 665-70.

Pharmacokinetics of nelfinavir in children: influencing factors and dose implications

Alina S. Bergshoeff, Pieter L.A. Fraaij, Annemarie M.C. van Rossum, Tom F.W. Wolfs, Sibyl P.M. Geelen, Ronald de Groot, David M Burger

Antivir Therapy 2003; 8(3):215–22

Chapter 12

Abstract

Objectives: To describe the pharmacokinetics (PK) of the protease inhibitor nelfinavir and its active metabolite M8 in children, and to evaluate the influence of patient-related factors on plasma levels of nelfinavir.

Methods: HIV-1 infected children treated with nelfinavir q8h were eligible for inclusion in this retrospective study. 0-8h intensive plasma PK sampling was performed at steady state. Nelfinavir Cmax, AUC0-8, C8 and relative apparent oral clearance (Cl*F/kg) were calculated.

Results: 24 children (median age: 4.5 years, median nelfinavir dose: 28 mg/kg q8h) were included. Nelfinavir PK were highly variable. 10/24 children had an AUC0-8 below the value of 12.5 mg/L*h which has earlier been associated with increased virologic failure rate in children. With age < 2 years and a dose of 20 mg/kg q8h, a non-significant trend was observed to more AUC0-8 < 12.5 mg/L*h (OR (95% CI): 2.44 (0.41-14.7) and 8.7 (0.79-95), respectively). Nelfinavir C8 correlated strongly with AUC0-8 ($r=0.89$, $p < 0.001$). C8 > 0.69 mg/L predicted an AUC0-8 > 12.5 mg/L*h with 71% sensitivity and 80% specificity. Dose of nelfinavir per body surface area was a better predictor of AUC0-8 than dose per body weight.

Conclusions: Nelfinavir PK show high interindividual variability in children. Children < 2 years old tend to be at increased risk for low nelfinavir levels. These data show that the nelfinavir dose of 20 mg/kg q8h is inadequate in most children. Also, these data suggest that pediatric dosing of nelfinavir based on body surface area should be considered. Therapeutic drug monitoring can detect abnormal plasma levels and is therefore of importance to optimize therapy with nelfinavir in HIV infected children. However, further research is needed to more firmly establish a therapeutic range for nelfinavir in children.

Introduction

With the introduction of highly active antiretroviral therapy (HAART), HIV infection has become a chronic disease with strongly improved survival. However, management of HIV infection is often complicated by several factors, such as adverse events related to medication, complex medication schedules, which result in non-compliance and emergence of resistant mutants of HIV. HIV protease inhibitors, which are often part of HAART, show highly variable pharmacokinetics. Since plasma levels of protease inhibitors have been related to virological efficacy, this may have important consequences for the success of HIV treatment. (1) Insight in the pharmacokinetics of protease inhibitors and the factors contributing to variability in their pharmacokinetics is valuable, e.g. for the correct application of therapeutic drug monitoring (TDM).

In children, additional factors, such as poor palatability of medication, the absence of pediatric dose forms, and changes in drug disposition and elimination due to physiological maturation may complicate an optimal response to HAART. The protease inhibitor nelfinavir is frequently prescribed for treatment of pediatric HIV infection. It has shown effective suppression of HIV combined with good tolerability in children > 2 years old. (2, 3) Despite its wide use, data on pharmacokinetics of nelfinavir in children, especially in those < 2 years old, are sparse. Even less is known about the pharmacokinetics of M8, the active metabolite of nelfinavir, in the pediatric population. The major objective of this study was to describe the pharmacokinetics of both nelfinavir and its active metabolite M8 in HIV-1 infected children. In particular, attention was paid to the pharmacokinetics of children < 2 years old. Furthermore, the association between patient related factors and low plasma levels of nelfinavir has been investigated.

Materials and methods

Patients

HIV infected children between 0-18 years old treated with nelfinavir q8h + 2 nucleoside analogues were eligible for inclusion in this retrospective, observational two-center study. Comedication was allowed if it was not expected to interfere with the PK of nelfinavir. Informed consent was obtained from all patients or care-givers prior to start of antiretroviral therapy. Nelfinavir dose was calculated as mg nelfinavir/kg body weight. The standard adult nelfinavir dose of 750 mg q8h was not exceeded.

Pharmacokinetic sampling

In all children, intensive pharmacokinetic sampling was performed at steady state (> 1 week after start of nelfinavir). The procedure was part of standard patient care in our clinics. Pharmacokinetic sampling was performed at the day care unit. Medication ingestion was directly observed and with food. For infants, medication was mixed with formula. Older children received medication with a meal. Blood samples were drawn at time points 0 (pre-dose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, and 8 h post ingestion. Within 24 hours after collection, samples were centrifuged and plasma was stored at –20 °C. Plasma concentrations of nelfinavir and M8 were

determined by validated HPLC assay with UV detection (lower limit of quantification: 0.04 mg/L). (4)

Nelfinavir and M8 pharmacokinetics

Nelfinavir pharmacokinetic parameters were calculated using non-compartmental methods (5). Nelfinavir plasma peak level (Cmax) and trough level at the 8-h time point (C8) were determined. Area under the plasma concentration-time curve 0-8h was calculated using the trapezoidal rule. Apparent oral clearance of nelfinavir was calculated as dose (mg)/AUC0-8. Relative apparent oral clearance (Cl/kg*F) was calculated as dose (mg)/AUC0-8*body weight (kg). M8 plasma levels were also measured and Cmax, C8 and AUC0-8 of M8 were calculated. The association of different patient related factors with nelfinavir pharmacokinetic parameters and with the ratio of M8 AUC0-8 / nelfinavir AUC0-8 was estimated. A nelfinavir AUC0-8 of 12.5 mg/L*h was used as a breakpoint in data analysis, since AUC0-8s of nelfinavir below this value have been associated with an increased virologic failure rate in children. (6) To evaluate the relation between nelfinavir dose and nelfinavir AUC0-8, dose strata were formed of 20, 30 and 40 mg/kg nelfinavir q8h, respectively.

All patients were monitored as part of standard patient care. HIV-1 RNA load (copies/mL) at start and after 6 months of nelfinavir therapy was used to measure virological efficacy. Viral load was determined by PCR (limit of quantification 500 copies/mL, HIV-1 specific PCR (Roche Diagnostics, Brandenburg, NY, USA). Virologic response was defined as a viral load below 500 copies/mL at 6 months after start of therapy.

Statistical methods

All statistical tests were performed using SPSS (SPSS, Chicago, IL, U.S.A., version 10.0). Spearman's rank correlation was calculated to evaluate the association between nelfinavir pharmacokinetic parameters and patient related factors. A t test was used to test significance. Linear regression after ln transformation of (AUC0-8, C8) was calculated. Receiver Operating Characteristic (ROC) curves were constructed to estimate sensitivity and specificity with which C8 or nelfinavir dose (mg/m^2) could predict an AUC0-8 > 12.5 mg/L*h. For this purpose, of patients with C8 or nelfinavir dose above a given value, % sensitivity (% true positives) was plotted against % (1- specificity) (% false positives). Sensitivity was defined as the number of patients with an AUC0-8 > 12.5 and C8 or dose above a given value /total number of patients with AUC0-8 > 12.5. 1-specificity was defined as % patients with AUC0-8 < 12.5 and C8 or dose above a given value /total number of patients with AUC0-8 < 12.5. Odds ratio calculation and Fisher's Exact Test were performed to estimate the association of patient characteristics with nelfinavir AUC0-8 < 12.5 mg/L*h. For this purpose, patient characteristics and the occurrence of nelfinavir AUC0-8 < 12.5 mg/L*h were binary scaled. The Mann-Whitney U Test and Kruskal-Wallis test were used to compare medians. A p-value of <0.05 was considered statistically significant.

Results

24 HIV infected children between 5 months and 18 years of age were included. Patients using comedication known to cause a pharmacokinetic interaction with nelfinavir and patients in whom intensive pharmacokinetic sampling was incomplete were excluded. Patient characteristics are given in tables 1A and 1B.

Treatment with nelfinavir q8h + 2 nucleoside analogues was initiated between November 1997 and August 2000. Doses of nelfinavir were chosen upon the physician's discretion. The median nelfinavir dose was 28 mg/kg q8h (IQR: 26-31 mg/kg q8h), with a maximum absolute dose of 750 mg qh8. Patients used nelfinavir either in tablets or in the powder formulation. 14 patients were naive to treatment with protease inhibitors, 9 were non-naive and of one patient, prior treatment was unknown. All protease inhibitor non-naive patients had been pretreated with the protease inhibitor indinavir. They had switched therapy to nelfinavir for several reasons (Table 1B). Two patients received the non-nucleoside analogues nevirapine and efavirenz respectively, in addition to their nelfinavir-containing regimen. Since earlier findings did not suggest nevirapine or efavirenz to markedly influence nelfinavir or M8 plasma levels, these pharmacokinetic data were not excluded from data analysis. (7, 8) In all children, PK sampling was performed at steady state and nelfinavir and M8 plasma concentrations were determined. Overall PK parameters of nelfinavir and M8 are described in Table 2.

Figure 1: Scatter plot of ln(AUC0-8) versus ln(C8) of nelfinavir

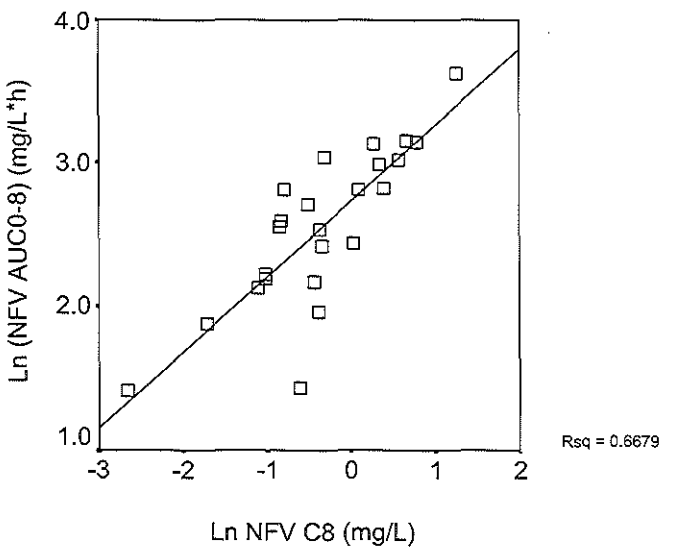


Table 1A. Demographic characteristics overall and in age groups < and ≥ 2 years old

	age < 2 years	age ≥ 2 years	overall
age (years) median (IQR)	0.5 (0.5-0.58)	6.6 (3.8-8.7)	4.5 (0.71-7.1)
Female (n)	3/7	6/17	9/24
body weight (kg) median (IQR)	7.0 (6.0-8.0)	17.0 (13.5-28.2)	14 (9.5-22)
BSA (m2) median (IQR)	0.37 (0.34-0.44)	0.72 (0.59-1.0)	0.63 (0.45-0.86)
Ethnicity (n)			
African		9	9
Caucasian	2	1	3
Asian		1	1
Hispanic		2	2
Mixed	5	4	9

IQR: inter quartile range; n: cases; BSA: body surface area

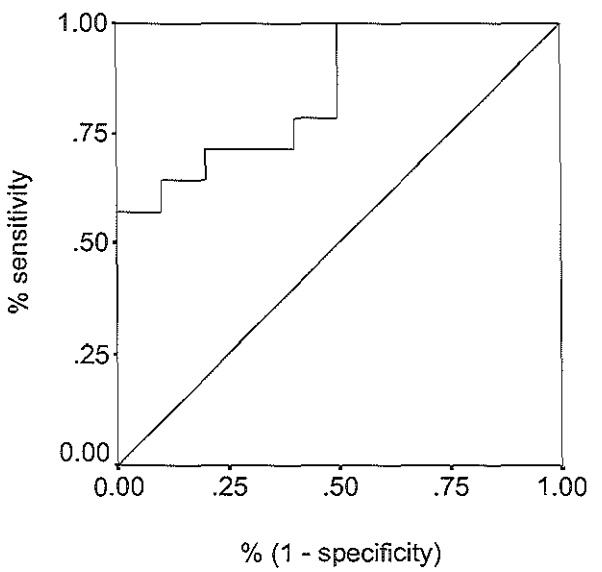
Table 1B. Clinical and pharmacological characteristics overall and in age groups < and ≥ 2 years old

	age < 2 years	age ≥ 2 years	overall
number of PK curves	7	17	24
dose (mg/kg) median (IQR)	29 (26-37)	28 (25-31)	28 (26-31)
HIV RNA median (IQR) at start nelfinavir	750,000 (5,280-750,000)	45,700 (12,870-389,500)	98,650 (10,905-692,750)
protease inhibitor naïve (n)	5/7	9/17*	14/24*
Reasons for switch to nelfinavir if non-naïve (n)		2	2
virological failure		3	3
nephrotoxicity	1		1
hypersensitivity	1		1
low drug levels		1	1
non-compliance		1	1
other/unknown			
antiretroviral comedication (n)			
AZT/3TC	3	12	15
d4T/3TC	2	2	4
d4T/ddI		3	3
other	2		2

n: cases; PK: pharmacokinetic; IQR: inter quartile range; AZT: zidovudine; 3TC: lamivudine; d4T: stavudine; ddI: didanosine. *of one patient no data available

After stratification to dose groups of 20 (n=5), 30 (n=14) and 40 (n=5) mg/kg nelfinavir q8h, nelfinavir AUC0-8 showed a less than dose proportional increase (median AUC0-8s of 8.7, 16.6 and 12.5 mg/L*h, respectively). Both Cmax and C8 of nelfinavir and M8 correlated strongly with the AUC0-8 of nelfinavir or M8, respectively (all p-values < 0.001 and r > 0.8) Figure 1 depicts the correlation of (lnC8, lnAUC0-8). Linear regression of (Ln AUC0-8,Ln C8) showed a nelfinavir C8 of 0.67 mg/L corresponding with an AUC0-8 of 12.5 mg/L*h. A C8 > 0.69 mg/L predicted a nelfinavir AUC0-8 > 12.5 mg/L*h with optimal sensitivity (71%) and specificity (80 %) (Figure 2).

Figure 2: Receiver Operating Characteristic curve describing sensitivity versus 1-specificity for C8 of nelfinavir to predict AUC > 12.5 mg/L*h.



While median nelfinavir dose was similar in children below and above 2 years of age (medians 29 and 28 mg/kg q8h, respectively), the younger group showed a tendency to lower AUC0-8, Cmax and C8 of nelfinavir (Table 2). Also, Cl/kg*F tended to be higher in children < 2 years old than in older children (2.8 vs. 1.9 L/h). None of these differences reached statistical significance (p values all > 0.1), except a significantly higher absolute Cl/F of nelfinavir in older children. A non-significant trend to more lower AUC0-8s was also observed in younger children if 12.5 mg/L*h was used as a breakpoint for AUC0-8 (Table 3). In total, ten out of 24 children (42%) had a nelfinavir AUC0-8 < 12.5 mg/L*h, and these rates were 4/7 (57%) and 6/17 (35%), respectively, in children < 2 and ≥ 2 years old (p > 0.1). OR (95% CI) for an AUC0-8 < 12.5 mg/L*h with age < 2 years was 2.44 (0.41-14.7) (not significant).

Table 2. PK parameters (median, IQR) overall and in age groups < and ≥ 2 years old.

PK parameter	age < 2 years	age ≥ 2 years	overall	
	n=7	n=17	n=24	P-value
C max (mg/L)	2.2 (1.9-4.8)	3.6 (2.7-4.5)	3.5 (2.2-4.5)	> 0.1
C8 (mg/L)	0.43 (0.36-0.73)	0.69 (0.50-1.44)	0.69 (0.43-1.4)	> 0.1
AUC0-8 (mg/L*h)	11.2 (8.9-20.8)	15.0 (7.9-20.1)	13.1 (8.8-20.2)	> 0.1
Cl/F (L/h)	15.1 (8.4-27.4)	37.4 (24.4-71.5)	32 (19.6-52.3)	0.002#
Cl/F*kg (L/h*kg)	2.8 (1.4-3.4)	1.9 (1.5-3.1)	2.1 (1.5-3.2)	> 0.1
M8/nelfinavir AUC0-8 ratio	0.31 (0.21-0.43)	0.29 (0.20-0.43)	0.29 (0.20-0.44)	> 0.1

#statistically significant; n: cases; Cmax: peak plasma level; C8: plasma level at the 8-h time point; AUC0-8: area under the plasma concentration-time curve 0-8h; Cl/F : apparent oral clearance; Cl/F*kg : relative apparent oral clearance

Table 3. Patient characteristics of children with nelfinavir AUC0-8 < vs. > 12.5 mg/L*h

	nelfinavir AUC0-8 < 12.5 mg/L*h	nelfinavir AUC0-8 > 12.5 mg/L*h	
	n=10	n=14	P-value
age (yr.) median (IQR)	2.9	4.8	> 0.1
Female (n)	4/10	5/14	> 0.1
dose (mg/kg) median (IQR)	28 (17-39)	30 (28-37)	> 0.1
dose (mg/m2) median (IQR)	551(391-835)	727 (628-809)	0.04#
body weight (kg) median (IQR)	12 (6-40)	14 (9-23)	> 0.1
BSA (m2) median (IQR)	0.56 (0.38-1.25)	0.63 (0.42-0.89)	> 0.1
HIV RNA median baseline (IQR)	77,350 (13,920-750,000)	106,150 (6955-578,250)	> 0.1
protease inhibitor naïve (n)	8/10	6/14*	> 0.1
Virologic response week 48 (HIV-RNA < 500 copies/mL) (n (%))	8/10 (80)	10/14 (71)	> 0.1

*of one patient, no data available, #statistically significant, n: cases; IQR: inter quartile range; BSA: body surface area

A nelfinavir dose of 20 mg/kg q8h yielded the highest percentage of nelfinavir AUC0-8 < 12.5 mg/L*h, although this difference was not significant (80% versus 29 and 40%, respectively, in dose groups of 30 and 40 mg/kg nelfinavir q8h p> 0.1). OR (95% CI) for AUC0-8 < 12.5 mg/L*h with a dose of 20 mg/kg q8h compared to higher doses was 8.7 (0.79-91) (not significant). While receiving a similar median nelfinavir dose in mg/kg, children with a nelfinavir AUC0-8 < 12.5 mg/L*h received a statistically significant lower median nelfinavir dose in mg/body surface

area (m^2), than children with a nelfinavir AUC0-8 > 12.5 mg/L*h (p=0.04) (Table 3). A nelfinavir dose > 650 mg/m^2 predicted a nelfinavir AUC0-8 > 12.5 mg/L*h with optimal sensitivity (79%) and specificity (67%). No apparent association was found between the occurrence of nelfinavir AUC0-8 < 12.5 mg/L*h and gender.

Of the 5 children in whom the maximum adult dose of 750 mg q8h had been attained, 3 (60%) had nelfinavir AUC0-8s below 12.5 mg/L*h vs. 7/19 (37%) of children who had not reached the dose of 750 mg q8h (OR (95% CI): 2.57 (0.34-19.3)).

The M8/nelfinavir ratio ranged between 0.1 and 0.8, (not shown in Table 2), except for two patients, in whom plasma levels of M8 were below the limit of quantification. No relation was found between M8/NFV ratio and dose of nelfinavir, age, gender, ethnicity or pre-treatment (p values all > 0.1).

Twenty-two of twenty-four children completed 6 months of nelfinavir containing therapy. The two remaining children had switched on their parents' request to q12h regimens containing abacavir and lopinavir/ritonavir, respectively. Of the 22 children who completed 6 months of treatment with nelfinavir, sixteen (73%) showed virologic response (viral load < 500 copies/mL). Virologic response rates at 6 months in nelfinavir dose categories of 20, 30 and 40 mg/kg q8h were 50, 71 and 100%, respectively (p>0.1). In children with a nelfinavir AUC < 12.5 mg/L*h, the virologic response rate at 6 months was 80%, while in children with nelfinavir AUC0-8 > 12.5 mg/L*h, a percentage of 71% was found (p > 0.1) (Table 3). In none of the patients, serious adverse events were reported.

Discussion

This study provides a description of the PK of nelfinavir in a relatively large number of children in whom intensive PK sampling was performed. In adults, plasma levels of nelfinavir have been associated with virological efficacy. (9-11) In children, a target value of 10 mg/L*h has been used for nelfinavir AUC0-8, but studies correlating nelfinavir levels with efficacy are limited. (3, 12) In the present study, a nelfinavir AUC0-8 of 12.5 mg/L*h was used as a threshold in data analysis. This was at the time of the study the only reported PK cut-off value for virologic response to nelfinavir in pediatric patients. (6) However, this value was derived from a naive population of HIV-infected children and has not yet been validated in pretreated patients.

Risk factors for low nelfinavir levels

Age < 2 years and a dose of 20 mg/kg q8h both showed a trend to lower plasma concentrations of nelfinavir, and higher rate of AUC0-8s below 12.5 mg/L*h. Also, children in whom the maximal (adult) dose of nelfinavir was reached tended to have higher rates of AUC0-8s below 12.5 mg/L*h. Although none of these differences was statistically significant, these findings are relevant for clinical practice.

Age below 2 years has previously been associated with an increased risk for lower than average, possibly subtherapeutic nelfinavir plasma levels. (6,12-14) In infants < 4 months old, even nelfinavir doses up to 40 mg/kg q8h have resulted in plasma levels far below adult values

(14). This may be explained by factors such as higher metabolic clearance in young children, impaired absorption, and lower amount of alpha-acid glycoprotein. (15)
The very high rate of nelfinavir AUC0-8s < 12.5 mg/L*h in the dose category of 20 mg/kg q8h nelfinavir when compared to doses of 30 and 40 mg/kg q8h suggests, that the nelfinavir dose of 20 mg/kg q8h is insufficient and should not be applied in children. The considerable rate of nelfinavir AUC0-8s < 12.5 mg/L*h (60%) in children who had reached the adult nelfinavir dose indicates that in some children, the adult nelfinavir dose needs to be exceeded in order to normalize plasma levels of nelfinavir. At the time of enrolment in this study, the adult nelfinavir dose was recommended in children with body weight > 23 kg. Currently, guidelines recommend the adult nelfinavir dose in children > 13 years old, which corresponds with a higher body weight. (16) Nevertheless, in the present study, 2/3 children using 750 mg q8h nelfinavir and showing low nelfinavir AUC0-8s were below 13 years old, suggesting that an actual age limit probably should be below 13 years.
Interestingly, a significantly lower nelfinavir dose/body surface area was found in children with AUC0-8 < 12.5 mg/L*h when compared to children with AUC0-8 > 12.5 mg/L*h, while both groups were receiving the same median dose per body weight. This suggests, that when nelfinavir dose is calculated per body weight, (younger) children with a relatively high body surface area are at increased risk for lower plasma levels. Body surface area has been described as a more accurate measure of metabolic activity, and might therefore be more appropriate in children. (15) Our data indicate that a nelfinavir dose > 650 mg/m^2 q8h would be needed to obtain an AUC0-8 > 12.5 mg/L*h. No association was found between gender and low nelfinavir plasma levels, which is in accordance with previous data. (17) Assuming, that gender-related differences in PK of protease inhibitors are related to hormonal differences between males and females, they would mainly be expected after sexual maturation. These differences were difficult to distinguish in this relatively young pediatric population (median age: 4.5 years). (15)

PK of nelfinavir and M8

A strong correlation was found for both nelfinavir and M8 between Cmax, or C8 and AUC0-8. This correlation could largely simplify TDM by using one time point from a PK curve (e.g. C8) as a predictor of total exposure. Ln-linear regression showed a C8 of 0.67 mg/L corresponding with a nelfinavir AUC0-8 of 12.5. Similarly, a C8 > 0.69 mg/L predicted nelfinavir AUC0-8 > 12.5 mg/L*h with optimal sensitivity and specificity. Both values are in accordance with efficacy thresholds for twice daily nelfinavir in adults. (9-11) Earlier data found a strong correlation between the 2-h plasma concentration and AUC0-8 of nelfinavir. (18) However, these findings warrant further investigation.
While previous studies in pediatric and adult patients assumed a dose-proportional increase of nelfinavir AUC0-8, in the present study, AUC0-8 showed a less than dose proportional increase. The effect of a dosage increase can efficiently be monitored using TDM. However, these findings suggest that some patients with low plasma levels of nelfinavir might not benefit from a dosage increase, i.e. would not experience sufficiently higher plasma levels after a dosage adjustment.

In adults, the ratio between M8 and nelfinavir plasma levels is relatively constant at a nelfinavir dose of 1250 mg q12h and a M8/NFV ratio of 0.3 is in accordance with findings in adult patients (19, 20). In children, also lower M8/nelfinavir ratios have been reported, which we could not confirm. (13)

Factors contributing to variability in nelfinavir PK

Nelfinavir absorption strongly improves when nelfinavir is taken with food. (21) In the present study, infants took their medication with formula, while older children received a standard meal. However, difficulties with ingestion of medication are common in children. For example, very young children, who take medication with formula, may be unable to take in the total medication dose at once, which may alter the time to peak level. While special attention was paid to complete medication ingestion in the presence of a substantial amount of food, interindividual variability of PK due to food effects and difficulties with medication ingestion could not totally be ruled out in this pediatric population. Nelfinavir is principally metabolised by cytochrome enzymes (CYP) 3A4 and 2C19. CYP-inducing comedication is known to decrease nelfinavir plasma levels, but in this study, except for the two patients who used nevirapine or efavirenz, no other CYP-modifying comedication was used (21). While NVP and EFV are known to affect CYP3A4, the effect on metabolism of nelfinavir appears absent for nevirapine, and very slight for efavirenz. EFV, but not NVP has shown to slightly decrease M8 concentrations. (7, 8) Of interest, the child who used EFV showed a high AUC0-8 of nelfinavir (38 mg/L*h) and a M8/NFV AUC ratio of 0.14, while the child who used NVP showed a low AUC0-8 of nelfinavir (9 mg/L*h) and a M8/NFV ratio of 0.3. The relevance of these individual cases remains uncertain. Genetic polymorphism of CYP2C19 might have caused additional variability of nelfinavir and M8 PK. CYP2C19 polymorphism is especially found in Asians and Caucasians, with 18-22 and 2-6% of slow metabolizers, respectively. (17) However, the effect of CYP2C19 polymorphism was not likely, since most children were of African or mixed African origin and Asians and Caucasians were poorly represented (Table 1). It should also be remarked, that CYP2C19 is not a unique pathway of nelfinavir metabolism and impact of CYP2C19 polymorphism on nelfinavir plasma levels may be moderate. Furthermore, polymorphism in P-glycoprotein expression, of which nelfinavir is a substrate and possibly an inductor, may have influenced the PK of nelfinavir. (22-24) These factors were not examined, but might also explain part of the observed variability of nelfinavir PK.

Virologic outcome

An overall virologic response of 73% was measured after 6 months of nelfinavir containing HAART. Both similar and considerably lower success rates have been reported in children using protease inhibitor containing treatment. (2, 3 ,25-30) The difference between virologic responses in the dose group of 20 vs. 30 and 40 mg/kg nelfinavir q8h would be in accordance with the high rates of AUC0-8 < 12.5 mg/L*h in the lowest dose category. Meanwhile, in this study, no significant relationship was found between plasma levels of nelfinavir and virologic efficacy, and response rate was not significantly different between children with an AUC < 12.5 mg/L*h and children with an AUC > 12.5 mg/L*h. However, it should be noted that children

naive and non-naive for protease inhibitors were not equally distributed between these groups, since most children with a nelfinavir AUC < 12.5 mg/L*h (8/10) were naive, while this was the case in only 6/14 (43%) of children with a AUC > 12.5 mg/L*h. Taking into account, that naive patients tend to better respond to antiretroviral therapy than pretreated patients, this may have biased our findings. Finally, as stated before, the cut-off value of 12.5 mg/L*h for AUC0-8 of nelfinavir has been proposed for naive children, while a different cut-off value may be needed for non-naive children, depending on e.g. the presence of viral resistance and differences in clinical condition in these patients.

Conclusion

PK of nelfinavir in children showed high interindividual variability in this population heterogeneous with regard to pre-treatment, age and dose. Children < 2 years old tend to be at higher risk for low plasma levels of nelfinavir. A nelfinavir dose of 20 mg/kg q8h yielded a low plasma levels in most of the children. Although this findings were not statistically significant, they suggest that children using nelfinavir should be closely monitored by TDM. Also, the dose of 20 mg/kg q8h nelfinavir is insufficient and should not be used. The strong correlation between C8 and nelfinavir AUC0-8 could simplify PK sampling. The maximum dose of 750 mg q8h is frequently suboptimal and needs to be exceeded in children. Nelfinavir dose based on body surface area, rather than body weight should be considered in children. While low plasma levels of nelfinavir have been related with virologic outcome in adults, we were not able to find such an association in this highly heterogeneous pediatric study population. Meanwhile, in children, TDM of nelfinavir is expected to be of similar importance as in adults, e.g. since it can detect abnormal plasma levels and allows handling in order to prevent toxicity or virologic failure. However, further research is strongly needed to more firmly establish a therapeutic range for nelfinavir in children.

Acknowledgements

Corrien Verweij, Marga van de Graaff and Jackie Droste are kindly acknowledged for processing and analysis of plasma samples for nelfinavir and M8. Nike Nauta is kindly acknowledged for providing patient data.

References

1. Acosta EP, Kakuda TN, Brundage RC, et al. Pharmacodynamics of human immunodeficiency virus type 1 protease inhibitors. Clinical Infectious Diseases 2000; 30 Suppl 2:S151-S159.
2. Krogstad P, Wiznia A, Luzuriaga K, et al. Treatment of human immunodeficiency virus 1-infected infants and children with the protease inhibitor nelfinavir mesylate. Clinical Infectious Diseases 1999; 28:1109-1118.
3. Starr SE, Fletcher CV, Spector SA, et al. Combination therapy with efavirenz, nelfinavir, and nucleoside reverse- transcriptase inhibitors in children infected with human immunodeficiency virus type 1. Pediatric AIDS Clinical Trials Group 382 Team. New England Journal of Medicine 1999; 341(25):1874-1881.
4. Hugen PWH, Verwey-van Wissen CPWGM, et al. Simultaneous determination of the HIV-protease inhibitors indinavir, nelfinavir, saquinavir and ritonavir in human plasma by reversed-phase high-

performance liquid chromatography. Journal of Chromatography B Biomedical Sciences and Applications 1999; 727:139-149.

5. Gibaldi M. Biopharmaceutics and Clinical Pharmacokinetics. Fourth ed. Philadelphia: Lea & Febiger, 1999.
6. Hsyu PH, Capparelli EV, Amantea M, et al. Population Pharmacokinetics (PK) of TID Nelfinavir (NFV) and Correlation to Efficacy in Pediatric Patients. 1st IAS Conference on HIV Pathogenesis and Treatment, 2001, abstract 348.
7. Fiske WD, Benedek IH, White SJ, et al. Pharmacokinetic Interaction between Efavirenz (EFV) and Nelfinavir Mesylate (NFV) in Healthy Volunteers. 5th Conference on retroviruses and opportunistic infections, February 1998, Chicago.Abstract no.349.
8. Acosta EP, Nachman S, Wiznia A, et al. Pharmacokinetic (PK) Evaluation of Nelfinavir (NFV) in Combination with Nevirapine (NVP) or Ritonavir (RTV) in HIV-Infected Children - PACTG 403. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Canada, September 17-20, 2000 Abstract no.1642.
9. Powderly WG, Saag MS, Chapman S, et al. Predictors of optimal virological response to potent antiretroviral therapy. AIDS 1999; 13(14):1873-1880.
10. Burger DM, Hugen PWH, Aarnoutse RE, et al. Treatment failure of nelfinavir-containing triple therapy can largely be explained by low nelfinavir plasma concentrations. AIDS 2000; 14(S4):S89.
11. Pellegrin I, Breilh D, Montestruc F, et al. Virologic response to nelfinavir-based regimens: pharmacokinetics and drug resistance mutations (VIRAPHAR study). AIDS 2002; 16(10):1331-1340.
12. Brundage RC, Fletcher CV, Fenton T, et al. Efavirenz (EFV) and Nelfinavir (NFV) Pharmacokinetics (PK) in HIV-Infected Children under 2 Years of Age. 7th Conference on Retroviruses and Opportunistic Infections, 30 January-2 February 2000, San Francisco, CA, USA, abstract 719.
13. Capparelli EV, Sullivan JL, Mofenson L, et al. Pharmacokinetics of nelfinavir in human immunodeficiency virus-infected infants. Pediatric Infectious Disease Journal 2001; 20(8):746-751.
14. Litalien C, Ciaquinto C, Faye A, et al. Nelfinavir doses should be increased in infants less than 3 months. XIII International AIDS Conference, Durban, 2000, abstract MoPeB2213.
15. Yaffe SJ, Aranda JV. Pediatric Pharmacology: Therapeutic Principles in Practice, 2nd Edition. WB Saunders Company, 1992.
16. USA Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. http://www.hivatis.org.
17. Jackson KA, Rosenbaum SE, Kerr BM, et al. A population pharmacokinetic analysis of nelfinavir mesylate in human immunodeficiency virus-infected patients enrolled in a phase III clinical trial. Antimicrobial Agents and Chemotherapy 2000; 44(7):1832-1837.
18. Regazzi MB, Villani P, Seminari E, et al Clinical pharmacokinetics of nelfinavir combined with efavirenz and stavudine during rescue treatment of heavily pretreated HIV-infected patients. Journal of Antimicrobial Chemotherapy 2000; 45:343-347.
19. Zhang KE, Wu E, Patick AK, et al. Circulating metabolites of the human immunodeficiency virus protease inhibitor nelfinavir in humans: structural identification, levels in plasma, and antiviral activities. Antimicrobial Agents and Chemotherapy 2001; 45(4):1086-1093.
20. Baede-van Dijk PA, Hugen PWH, Verwey-van Wissen CPWGM, et al. Analysis of variation in plasma concentrations of nelfinavir and its active metabolite M8 in HIV-positive patients. AIDS 2001; 15(8):991-998.
21. Bardsley-Elliot A, Plosker GL. Nelfinavir: an update on its use in HIV infection. DRUGS 2000; 59(3):581-620.
22. Meyer UA. Pharmacogenetics and adverse drug reactions. The Lancet 2000; 356(november):1667-1671.
23. Huang L, Wring SA, Woolley JL, et al. Induction of P-glycoprotein and cytochrome P450 3A by HIV protease inhibitors. Drug Metabolism and Disposition 2001; 29(5):754-760.
24. Choo EF, Leake B, Wandel C, et al. Pharmacological inhibition of P-glycoprotein transport enhances the distribution of HIV-1 protease inhibitors into brain and testes. Drug Metabolism and Disposition 2000; 28(6):655-660.
25. Spector SA, Hsia K, Yong FH, et al. Patterns of plasma human immunodeficiency virus type 1 RNA response to highly active antiretroviral therapy in infected children. Journal of Infectious Diseases 2000; 182(6):1769-1773.

26. Funk M, Linde R, Wintergerst U, et al. Preliminary experiences with triple therapy including nelfinavir and two reverse transcriptase inhibitors in previously untreated HIV-infected children. AIDS 1999; 13(13):1653-1658.
27. Krogstad P, Lee S, Johnson G, et al. Nucleoside-analogue reverse-transcriptase inhibitors plus nevirapine, nelfinavir, or ritonavir for pretreated children infected with human immunodeficiency virus type 1. Clinical Infectious Diseases 2002; 34(7):991-1001.
28. Fletcher CV, Fenton T, Powell C, et al. Pharmacologic Characteristics of Efavirenz (EFV) and Nelfinavir (NFV) Associated with Virologic Response in HIV-Infected Children. 8th Conference on Retrovirus and Opportunistic Infections, Chicago, Ill, February 4-8, 2001, abstract 259.
29. Della Negra M, Bologna R, Lopez E, et al. Long Term (48 Weeks) Efficacy and Safety of Antiretroviral Therapy with Nelfinavir (NFV) in HIV-infected Children 3 months to 12 years of age. 1st IAS Conference on HIV Pathogenesis and Treatment, 2001, abstract 776.
30. Nadal D, Steiner F, Chesaux J-J, et al. Responses to antiretroviral treatment including ritonavir or nelfinavir in children infected with the HIV type 1. XIII International AIDS conference, Durban South Africa, abstract TuPeB3239.

Pharmacokinetics of indinavir combined with low-dose ritonavir in Human Immunodeficiency Virus type 1-infected children

Alina S. Bergshoeff, Pieter L.A. Fraaij, Annemarie M.C. van Rossum, Gwenda Verweel, Linda H. Wynne, Gregory A. Winchell, Randi Y. Leavitt, Bach-Yen T. Nguyen, Ronald de Groot, David M. Burger

Antimicrob Agents Chemother 2004;48(5):1904-7

Chapter 13

Abstract

So far, no pediatric doses for combined use of indinavir with ritonavir have been defined yet. This study evaluated the pharmacokinetics of 400 mg/m2 indinavir with 125 mg/m2 q12h ritonavir in 14 HIV-1 infected children. AUC0-24 and Cmin of indinavir were similar to data of indinavir/ritonavir 800/100 mg q12h in adults, while Cmax was slightly decreased (GMRs (90%CI)): 1.1 (0.87-1.3), 0.96 (0.60-1.5) and 0.80 (0.68-0.94), respectively.

Research Letter

The HIV-protease inhibitor indinavir has been licensed since 1996 for the treatment of HIV-1 infection in adults and children of 4 years and older. Indinavir, if used as a sole protease inhibitor, should be administered three times daily (q8h), preferably on an empty stomach or with a low-caloric meal. The addition of the HIV protease inhibitor ritonavir to indinavir containing therapy is known to increase plasma levels of indinavir, especially the 12-h trough level (Cmin). (2,11) The increased Cmin of indinavir, when combined with ritonavir, can potentially improve antiretroviral efficacy of the regimen. (1)

Table 1. Baseline characteristics of 14 enrolled patients.

Gender (n (%))	Male 6/14 (43%)
Age (years)	8.4 (4.3)
Race (n (%))	
White	2 (14)
Black	9 (64)
Asian	1 (7)
Other	2 (14)
Dose indinavir in mg/m2 q12h	392 (34)
Dose ritonavir in mg/m2 q12h	124 (8)
Patients with one or more prior antiretroviral therapies (%)	10/14 (71)
Antiretroviral prior treatment (n=10)	
Indinavir q8h	8/10 (80)
Indinavir/ritonavir q12h	1/10 (10)
Nelfinavir	1/10 (10)
Zidovudine + Lamivudine	10/10 (100)
CD4 cell count	
Cells/mm3	824 (613)
% of age specific median cell counts	75 (44)
Plasma HIV RNA viral load (Log10 copies/mL),	3.60 (1.05)
Concomitant treatment (n)	
Zidovudine/lamivudine	12
Didanosine/stavudine	2
Fluconazole	1
Terbinafine	1
Co-trimoxazole	2

Data are mean values (+ standard deviations) of 14 observations

Data on the use of indinavir as a component of HAART in HIV-infected children are relatively limited and often based on small sample size. (4,6,14-18,22-24) Children show generally lower 8-h trough levels (Cmin) of indinavir than adults and may therefore be at higher risk of virologic failure. (4,6) In HIV-infected children, the combination of indinavir with ritonavir is promising, in view of its higher Cmin, and the possibility of twice daily (q12h) administration. However, this combination has been very little explored in children

This was a prospective, one-armed, one period, open label study in HIV-1 infected children between 2 and 18 years of age. After enrollment, patients started study medication consisting of indinavir 400 mg/m2 q12h with 125 mg/m2 ritonavir q12h on Study Day 1. On the pharmacokinetic sampling day, 2-4 weeks after Study Day 1, a 12-h pharmacokinetic curve to obtain plasma levels of indinavir and ritonavir was recorded. Pharmacokinetic parameters of indinavir and ritonavir were calculated using non-compartmental methods. Safety was assessed at baseline (before start of study medication) and on the PK day. All statistical tests were performed using SPSS (SPSS, Chicago, IL, U.S.A., version 10.0).

Figure 1A. AUC0-24 of indinavir in 14 patients.

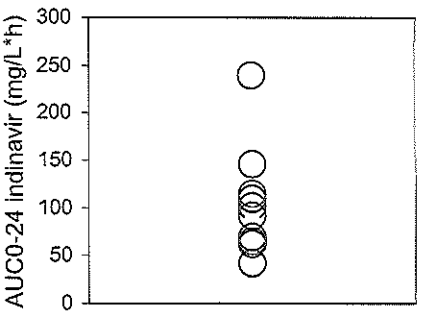


Figure 1B. Cmax of indinavir in 14 patients.

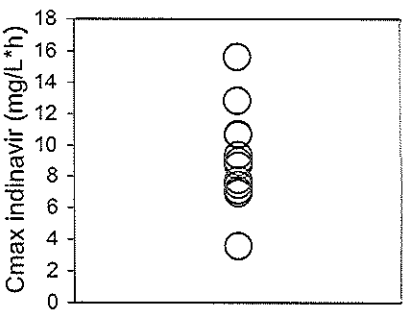


Figure 1C. Cmin of indinavir in 14 patients.

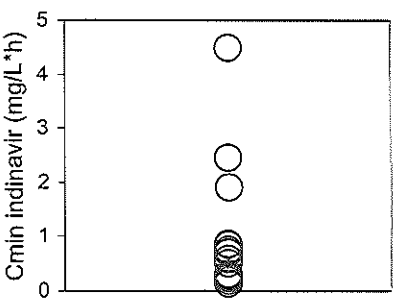


Table 2. Pharmacokinetic parameters of indinavir and ritonavir (Geometric Means + 90% confidence interval) of 14 children using indinavir with low-dose ritonavir and compared to historical controls (Geometric Mean Ratios).

Indinavir	AUC0-24	Cmax	Cmin	Tmax (median (IQR))
Patient data (geometric means) (N=14)	92.9 (75.4-114.5)	8.6 (7.3-10.1)	0.63 (0.40-1.0)	2.8 (1.8-3.1)
GMR versus data of 500 mg/m2 q8h in children[1]	1.8 (1.5-2.3)#	1.1 (0.97-1.4)	8.4 (5.3-13.4)#	N.A.
GMR versus data of indinavir 800 mg q8h in adults[2]	1.8 (1.4-2.2)#	1.2 (0.99-1.4)	4.9 (3.1-7.9)#	N.A.
GMR versus data of 800 mg indinavir + 100 mg ritonavir q12h in adults[3]	1.1 (0.87-1.3)	0.80 (0.68-0.94)#	0.96 (0.60-1.5)	N.D.
Ritonavir	**AUC0-24**	**Cmax**	**Cmin**	**Tmax (median (IQR))**
Patient data (geometric means) (N=14)	76.8 (61.0-96.7)	6.0 (4.7-7.8)	1.1 (0.84-1.6)	3.6 (1.5-4.2)
GMR versus data of 800 mg indinavir + 100 mg ritonavir q12h in adults[3]	3.8 (3.0-4.8)#	2.9 (2.2-3.7)#	3.2 (2.4-4.3)#	N.D.

[1]combined protocols Merck 068 and PACTG395; [2]Merck protocol 021; [3]Arnaiz et al., 2003; N.A.: not available; N.D.: not determined; GMR: geometric mean ratio; #statistically significant; IQR: interquartile range

Baseline patient characteristics of the fourteen HIV-infected children that were enrolled. are given in Table 1. Pharmacokinetic parameters of indinavir and ritonavir for the total of 14 patients are summarized in Table 2. Overall, the regimen of indinavir/ritonavir 400/125 mg/m2 q12h resulted in significantly higher AUC0-24 of indinavir than reference data of indinavir q8h in both adults and children. This increase in AUC0-24 was reflected in significantly higher geometric mean Cmin, while Cmax was only marginally and non-significantly increased. In none of the patients, Cmin of indinavir was below the value of 0.1 mg/L, which has been associated with increased virologic failure rate (figure 1).(3) Compared to the pharmacokinetic data of 800 mg indinavir with 100 mg ritonavir q12h in adults, geometric means of AUC0-24 and Cmin of indinavir were not significantly different, while geometric mean Cmax was modestly, but significantly decreased.

Ritonavir AUC0-12, Cmax, and Cmin were significantly higher than historical references of 100 mg ritonavir q12h combined with indinavir in adults (Table 2).

Of the 14 children enrolled, 8 (57%) suffered from a clinical or laboratory adverse experience between Study Days 1 and 2. In total, 22 adverse experiences were listed (Table 3).

Table 3. Description of adverse experiences (cases (% of the total of 22 adverse experiences))

Adverse experience	Frequency
Gastrointestinal disorders	3 (14%)
Ear, nose and throat infections	1 (5%)
Skin disorders	2 (9%)
Urinary tract disorders	2 (9%)
(nephrolithiasis and leucocyturia)	
Jaundice/hepatomegaly	2 (9%)
Abnormal liver function tests	4 (18%)
Abnormal blood cell counts	1 (5%)
Increased cholesterol/triglyceride levels	5 (23%)
Increased amylase levels	1 (5%)
Decreased platelet count	1 (5%)
Total of all adverse events	22 (100%)

Published data in HIV-infected children have indicated that a pediatric dose of indinavir q8h should be in the order of 500–600 mg/m2 q8h. (4, 17) The pharmacokinetics of indinavir as a sole protease inhibitor in children are characterized by a higher Cmax than in adults, followed by relatively high drug clearance, often resulting in lower Cmin than in adults. (4-6,15) In substantial numbers of children, Cmin of indinavir is below the concentration of 0.1 mg/L, which has been associated with virologic efficacy and approximates the *in vitro* 95% inhibitory concentration (IC95) of indinavir for wild type virus. (1, 3) While doses of indinavir above 500-600 mg/m2 q8h result in higher Cmin and possibly better clinical response, toxicity of indinavir seems to be dose limiting in children. (4, 20) In the 14 HIV-1 infected children described here, the regimen of 400 mg/m2 indinavir q12h with 125 mg/m2 ritonavir q12h resulted in higher AUC0-24 and Cmin compared to pharmacokinetic data of indinavir q8h in both adults and children. The explored dose combination resulted in AUC0-24 and Cmin of indinavir approximately similar to the pharmacokinetic data of indinavir with low-dose ritonavir in adults. (2)

When compared to published data from a case series on 4 children between 0.8 and 10 years old treated with 500/100 mg/m2 q12h indinavir with ritonavir, AUC0-24 and Cmax of indinavir were dose-proportionally lower in our study. (21) In contrast, Cmin of indinavir was approximately 40% higher, and not lower, as might have been expected, than found in the case series. In comparison with preliminary pharmacokinetic data from a pediatric study in 11

patients between 3 and 11 years old who used 350 mg/m2 q12h indinavir with 125 mg/m2 ritonavir, in our study, AUC0-24 and Cmin of indinavir were 59% and 50% higher, respectively, while Cmax was 16% higher (E. G. Chadwick, J. H. Rodman, P. Samson, T. Fenton, E. J. Abrams, B. Nowak, S. I. Pelton, S. Lavoie, K. Knapp, M. Bambji, R. Yogev, 10th Conference on Retroviruses and Opportunistic Infections, abstract 875, 2003). Especially the differences in AUC0-24 and Cmin were remarkable, since the 2 regimens were only distinguished from each other by a slight difference (14%) in indinavir dose (400 vs. 350 mg/m2. Considering our data with respect to the other data on pharmacokinetics of indinavir with ritonavir in children, it should be taken into account that differences in outcome may be partly due to high interindividual variability and limited sample size of these studies. In conclusion, the investigated doses of indinavir with ritonavir resulted in higher than expected AUC0-24, combined with a favorably increased Cmin of indinavir, resulting in values of Cmin > 0.1 mg/L in all children. While the short-term tolerability of study medication was generally good, data on long-term safety and efficacy of this regimen in children are needed for a better evaluation

Acknowledgements:

The study-nurses and medical students of the Erasmus MC /Sophia Children's Hospital, Division of Pediatric Infectious Diseases and Immunology, Rotterdam are kindly acknowledged for collection of the samples. The technicians of the Department of Clinical Pharmacy, University Medical Center Nijmegen are kindly acknowledged for analysis of the plasma samples. Financial support was received from Merck & Co., Inc., West Point, PA, U.S.A.

References

1. Acosta, E. P., T. N. Kakuda, R. C. Brundage, P. L. Anderson, and C. V. Fletcher. 2000. Pharmacodynamics of human immunodeficiency virus type 1 protease inhibitors. Clin Infect Dis 30 Suppl 2:S151-S159.
2. Arnaiz, J. A., J. Mallolas, D. Podzamczer, J. Gerstoft, J. D. Lundgren, P. Cahn, G. Fatkenheuer, A. D'Arminio-Monforte, A. Casiro, P. Reiss, D. M. Burger, M. Stek, and J. M. Gatell. 2003. Continued indinavir versus switching to indinavir/ritonavir in HIV-infected patients with suppressed viral load. AIDS 17:831-840.
3. Burger, D. M., R. M. W. Hoetelmans, P. W. H. Hugen, J. W. Mulder, P. L. Meenhorst, P. P. Koopmans, K. Brinkman, M. Keuter, W. Dolmans, and Y. A. Hekster. 1998. Low plasma concentrations of indinavir are related to virological treatment failure in HIV-1 infected patients on indinavir-containing triple therapy. Antivir Ther 3:215-220.
4. Burger, D. M., A. M. van Rossum, P. W. Hugen, M. H. Suur, N. G. Hartwig, S. P. Geelen, H. J. Scherpbier, R. M. Hoetelmans, A. G. Vulto, and R. de Groot. 2001. Pharmacokinetics of the protease inhibitor indinavir in human immunodeficiency virus type 1-infected children. Antimicrob Agents Chem 45:701-705.
5. Fletcher, C. V., R. C. Brundage, R. P. Remmel, L. M. Page, D. Weller, N. R. Calles, C. Simon, and M. W. Kline. 2000. Pharmacologic characteristics of indinavir, didanosine, and stavudine in human immunodeficiency virus-infected children receiving combination therapy. Antimicrob Agents Chem 44:1029-1034.
6. Gatti, G., A. Vigano, N. Sala, S. Vella, M. Bassetti, D. Bassetti, and N. Principi. 2000. Indinavir pharmacokinetics and pharmacodynamics in children with human immunodeficiency virus infection. Antimicrob Agents Ch 44:752-755.

7. Gulick, R. M., J. W. Mellors, D. Havlir, J. J. Eron, C. Gonzalez, D. McMahon, L. Jonas, A. Meibohm, D. Holder, W. A. Schleif, J. H. Condra, E. A. Emini, R. Isaacs, J. A. Chodakewitz, and D. D. Richman. 1998. Simultaneous vs sequential initiation of therapy with indinavir, zidovudine, and lamivudine for HIV-1 infection: 100-week follow-up. JAMA 280:35-41.

8. Gulick, R. M., J. W. Mellors, D. Havlir, J. J. Eron, C. Gonzalez, D. McMahon, D. D. Richman, F. T. Valentine, L. Jonas, A. Meibohm, E. A. Emini, and J. A. Chodakewitz. 1997. Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. New Engl J Med 337:734-739.

9. Gulick, R. M., J. W. Mellors, D. Havlir, J. J. Eron, A. Meibohm, J. H. Condra, F. T. Valentine, D. McMahon, C. Gonzalez, L. Jonas, E. A. Emini, J. A. Chodakewitz, R. Isaacs, and D. D. Richman. 2000. 3-year suppression of HIV viremia with indinavir, zidovudine, and lamivudine. Ann Intern Med 133:35-39.

10. Hammer, S. M., K. Squires, M. D. Hughes, J. M. Grimes, L. M. Demeter, J. S. Currier, J. J. Eron, J. E. Feinberg, H. H. Balfour, L. R. Deyton, J. A. Chodakewitz, and M. A. Fischl. 1997. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. N Engl J Med 337:725-733.

11. Hsu, A., G. R. Granneman, G. Cao, L. Carothers, A. Japour, T. El-Shourbagy, S. Dennis, J. Berg, K. Erdman, J. M. Leonard, and E. Sun. 1998. Pharmacokinetic interaction between ritonavir and indinavir in healthy volunteers. Antimicrob Agents Chem 42:2784-2791.

12. Hugen, P. W., D. M. Burger, H. J. ter Hofstede, P. P. Koopmans, M. Stek, Y. A. Hekster, P. Reiss, and J. M. Lange. 2000. Dose-finding study of a once-daily indinavir/ritonavir regimen. J Acq Immun Defic Synd 25:236-245.

13. Hugen, P. W. H., C. P. W. G. M. Verwey-van Wissen, D. M. Burger, E. W. Wuis, P. P. Koopmans, and Y. A. Hekster. 1999. Simultaneous determination of the HIV-protease inhibitors indinavir, nelfinavir, saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 727:139-149.

14. Jankelevich, S., B. U. Mueller, C. L. Mackall, S. Smith, S. Zwerski, L. V. Wood, S. L. Zeichner, L. Serchuck, S. M. Steinberg, R. P. Nelson, J. W. Sleasman, B. Y. Nguyen, P. A. Pizzo, and R. Yarchoan. 2001. Long-term virologic and immunologic responses in human immunodeficiency virus type 1-infected children treated with indinavir, zidovudine, and lamivudine. J Infect Dis 183:1116-1120.

15. Kline, M. W., C. V. Fletcher, A. T. Harris, K. D. Evans, R. C. Brundage, R. P. Remmel, N. R. Calles, S. B. Kirkpatrick, and C. Simon. 1998. A pilot study of combination therapy with indinavir, stavudine (d4T), and didanosine (ddI) in children infected with human immunodeficiency virus. J Pediatr 132:543-546.

16. Monpoux, F., N. Sirvent, J. Cottalorda, R. Mariani, and J. C. Lefbvre. 1997. Stavudine, lamivudine and indinavir in children with advanced HIV-1 infection: preliminary experience. AIDS 11:1523-1525.

17. Mueller, B. U., J. Sleasman, R. P. Nelson, S. Smith, P. Deutsch, W. Ju, S. M. Steinberg, F. M. Balis, P. F. Jarosinski, P. Brouwers, G. Mistry, G. Winchell, S. Zwerski, S. Sei, L. V. Wood, S. Zeichner, and P. A. Pizzo. 1998. A phase I/II study of the protease inhibitor indinavir in children with HIV infection. Pediatrics 102:101-109.

18. Rutstein, R., A. Feingold, D. Meislich, B. Word, and B. Rudy. 1997. Protease inhibitor therapy in children with perinatally acquired HIV infection. AIDS 11:F107-F111.

19. Saah, A. J., G. A. Winchell, M. L. Nessly, M. A. Seniuk, R. R. Rhodes, and P. J. Deutsch. 2001. Pharmacokinetic profile and tolerability of indinavir-ritonavir combinations in healthy volunteers. Antimicrob Agents Chem 45:2710-2715.

20. van Rossum, A. M., J. P. Dieleman, P. L. Fraaij, K. Cransberg, N. G. Hartwig, D. M. Burger, I. C. Gyssens, and R. de Groot. 2002. Persistent sterile leukocyturia is associated with impaired renal function in human immunodeficiency virus type 1-infected children treated with indinavir. Pediatrics 110:e19.

21. van Rossum, A. M. C., R. de Groot, N. G. Hartwig, C. M. Weemaes, S. Head, and D. M. Burger. 2000. Pharmacokinetics of indinavir and low-dose ritonavir in children with HIV-1 infection. AIDS 14:2209-2219.

22. van Rossum, A. M. C., H. G. M. Niesters, S. P. M. Geelen, H. J. Scherpbier, N. G. Hartwig, C. M. Weemaes, A. J. P. Veerman, M. H. Suur, E. R. De Graeff-Meeder, W. A. T. Slieker, W. C. J. Hop, A. D. M. E. Osterhaus, D. M. Burger, and d. R. Groot. 2000. Clinical and virologic response to combination treatment with indinavir, zidovudine, and lamivudine in children with human immunodeficiency virus-1 infection: A multicentre study in the Netherlands. J Pediatr 136:780-788.

23. Vigano, A., L. Dally, D. Bricalli, N. Sala, M. Pirillo, M. Saresella, D. Trabattoni, S. Vella, M. Clerici, and N. Principi. 1999. Clinical and immuno-virologic characterization of the efficacy of stavudine, lamivudine, and indinavir in human immunodeficiency virus infection. J Pediatr 135:675-682.

24. Wintergerst, U., F. Hoffmann, B. Solder, G. Notheis, T. Petropoulou, J. Eberle, L. Gurtzler, and B. H. Belohradsky. 1998. Comparison of two antiretroviral triple combinations including the protease inhibitor indinavir in children infected with human immunodeficiency virus. Pediatr Infect Dis J 17:495-499.

25. Yaffe, S. J. and J. V. Aranda. 1992. Pediatric Pharmacology: Therapeutic Principles in Practice, 2nd Edition. WB Saunders Company, 1992.

Increased dose of lopinavir/ritonavir compensates for efavirenz induced drug-drug interaction in HIV-1 infected children

Alina S. Bergshoeff, Pieter L.A. Fraaij, Jennifer Neubert, Gwenda Verweel, Nico G. Hartwig, Tim Niehues, Ronald de Groot, David M. Burger

J Acquir Immune Defic Syndr: in press

Chapter 14

Abstract

Nucleoside reverse transcriptase inhibitor (NRTI) sparing regimens have not yet been systematically evaluated in children. The non-nucleoside reverse transcriptase inhibitors (NNRTI) nevirapine and efavirenz lower plasma levels of protease inhibitors (PI) in adults and children. Therefore, co-administration of lopinavir/ritonavir with nevirapine and efavirenz necessitates a 30% increase in the dose of lopinavir/ritonavir in adults. In children, the extent of the pharmacokinetic interaction between efavirenz and lopinavir/ritonavir has not yet been studied.

Aim: To investigate the pharmacokinetics of increased dose (300/75 mg/m^2 q12h) lopinavir/ritonavir with normal dose (14 mg/kg q24h) efavirenz in HIV-1 infected children.

Methods: Steady-state pharmacokinetics of lopinavir and efavirenz were determined and compared to historical data.

Results: Fifteen children of median age (range) 11.8 (5.7-16.3) years were included. Area under the plasma concentration-time curve (AUC_{0-12}), peak levels (C_{max}) and trough levels (C_{min}) of lopinavir were similar to historical data in adults and children. Medians (interquartile range) were: 92.3 (43.5-138.5) mg/L*h, 12.5 (6.9-16.7) mg/L and 5.7 (1.3-8.0) mg/L, respectively. Efavirenz pharmacokinetics approximated previous data in adults and children.

Conclusion: The increased dose of 300/75 mg/m^2 q12h lopinavir/ritonavir compensates for the enzyme-inducing effect of efavirenz in HIV-infected children.

Introduction

The introduction of HAART (highly-active antiretroviral therapy) has considerably improved efficacy and outcome of treatment of HIV-1 infected adults and children. HAART, including 2 NRTIs with either an HIV protease inhibitor or an NNRTI, has a high potency with regard to virological suppression and immune restoration and is generally recommended in both adults and children (1-4). In addition, novel regimens consisting of protease inhibitors with an NNRTI offer a good treatment alternative, particularly for patients in whom viral resistance to NRTIs has developed or who experience intolerance to this class of drugs (5-8). Lopinavir is a potent HIV protease inhibitor, which is produced in co-formulation with a low dose of ritonavir. In this combination, ritonavir acts as a pharmacokinetic booster of lopinavir by increasing its plasma levels. When combined with the NNRTIs nevirapine or efavirenz, lopinavir/ritonavir has displayed good antiretroviral efficacy in both naive and pretreated HIV-1 infected adults (9). Importantly, pharmacokinetic data on these latter regimens have indicated that both efavirenz and nevirapine lower plasma levels of lopinavir while the pharmacokinetics of nevirapine and efavirenz remain unaffected by lopinavir/ritonavir. This interaction also occurs with other protease inhibitors and is attributed to induction of cytochrome (CYP) 3A4, the major enzyme involved in metabolism of most protease inhibitors, by nevirapine and efavirenz. (10, 11) In adults, a 33% increased dose of lopinavir/ritonavir (533/133 mg q12h) used with nevirapine or efavirenz, yields plasma levels equal to lopinavir alone. (12, 13) In children using nevirapine, a 30% increased lopinavir/ritonavir dose is needed to obtain plasma levels of this drug similar to those without nevirapine. (14,15) An interaction between lopinavir/ritonavir and efavirenz, analogous to the interaction with nevirapine is very likely to occur also in children but this has to be confirmed yet.

We investigated the pharmacokinetics of the combination of a 30% increased dose (300/75 mg/m^2 q12h) of lopinavir/ritonavir with a normal dose (14 mg/kg q24h) of efavirenz in HIV-1 infected children.

Methods

Patients

HIV-1 infected children between 3 months and 18 years old treated with lopinavir/ritonavir and efavirenz were included in the study. Children were enrolled from two medical centers. Baseline characteristics are shown in Table 1. Half of these children were enrolled in RODU-01, a study which investigated the pharmacokinetics, pharmacodynamics, safety and tolerability of lopinavir/ritonavir with efavirenz in HIV-1 infected children who were extensively pretreated with single or dual NRTIs. (16) The other patients were treated with the combination as part of a treatment protocol in one of the centers and had been pretreated with HAART including a (ritonavir boosted) protease inhibitor or NNRTI in addition to NRTIs and had developed multiple resistance to NRTIs. Informed consent was obtained from all patients or care-givers prior to enrollment into the study or prior to start of antiretroviral treatment, respectively.

Medication

Lopinavir was prescribed in capsules of 133/33 mg lopinavir/ritonavir each and/or oral liquid containing 80 mg lopinavir and 20 mg ritonavir per mL. Efavirenz was prescribed in capsules of 50, 100 or 200 mg and/or oral liquid containing 30 mg efavirenz per mL. All co-medication was recorded in patient files and on forms used for pharmacokinetic sampling. Co-medication was only allowed if it was not expected to interfere with the pharmacokinetics of either of the antiretroviral drugs. Selected doses were 300/75 mg/m^2 lopinavir/ritonavir twice daily (q12h) and 14 mg/kg efavirenz once daily (q24h), respectively. The dose of lopinavir/ritonavir was 30% higher with regard to the regular pediatric dose (230/58 mg/m^2 lopinavir/ritonavir q12h), and similar to the dose recommended by the manufacturer when used with nevirapine in children (17). Since pharmacokinetic data in adults did not show any effect of lopinavir/ritonavir on efavirenz plasma levels, lopinavir/ritonavir was not expected to interfere with efavirenz plasma levels in children. Therefore, the normal pediatric dose of 14 mg/kg q24h efavirenz was selected.

Patients and/or caretakers were instructed to take/administer efavirenz in the evening to reduce the risk of central nervous system related adverse experiences. Also, it was emphasized to patients and/or caretakers to take medication at regular time intervals as prescribed for both drugs, and to take medication with food in order to enhance absorption of lopinavir/ritonavir.

Pharmacokinetic sampling

In all children, intensive pharmacokinetic sampling was performed at steady state (> 2 weeks after start of medication). In the morning of the day of sampling, children were admitted to the day care unit of the hospital. The ingestion of the morning dose of lopinavir/ritonavir was directly observed. Medication was taken with a regular, bread-containing breakfast. Blood samples were drawn at time points 0 (pre-dose), 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10 and 12 h post ingestion of lopinavir/ritonavir. Time of ingestion of the last dose of efavirenz was recorded. Within 24 hours after collection, samples were centrifuged and plasma was stored at –20 °C. Plasma concentrations of lopinavir and efavirenz were determined by validated HPLC assays with UV detection (lower limits of quantification: 0.10 mg/L for both compounds). Inter- and intraday assay variation was lower than 5.6% and 2.8% for lopinavir and efavirenz, respectively. (18, 19)

Pharmacokinetics of lopinavir and efavirenz

Pharmacokinetic parameters of lopinavir were calculated with non-compartmental methods using Microsoft Excel 2000®. (20) The plasma peak level (C_{max}) and trough level at the 12-h time point (C_{min}) were determined. Area under the plasma concentration-time curve 0-12h (AUC_{0-12}) was calculated using the trapezoidal rule. Relative apparent oral clearance (Cl/m^{2}*F) was calculated as dose (mg)/ AUC_{0-12}*body surface area (m^2). Time to C_{max} (T_{max}) was determined visually from the pharmacokinetic curve. To determine the terminal half-life ($t_{1/2}$), the elimination coefficient (K_{el}) was calculated as (slope of the terminal phase of the log-transformed plasma concentration-time curve of lopinavir) /ln10. Slope was determined by

linear regression of at least 3 time points of the log-transformed plasma concentration-time curve. $T_{1/2}$ was derived by dividing ln2 by K_{el}.

Since patients took efavirenz in the evening and plasma sampling was performed during daytime, plasma concentrations of efavirenz were obtained only from the second half of the dosing interval. These plasma concentrations were used to calculate the average plasma concentration (C_{avg}), i.e., for a q24h regimen, the plasma concentration at the 12-h time point (20). C_{avg} of efavirenz was derived directly from the plasma concentration-time curve, or calculated by inter- or extrapolation. From C_{avg}, the area under the plasma concentration-time curve 0-24h (AUC_{0-24}) for efavirenz was estimated by the following equation: $C_{avg} = D*F/Cl*\tau = AUC_{0-24}/\tau$, in which D is efavirenz dose in mg; F = oral bioavailability; τ is the dose interval (24h for efavirenz) and Cl = apparent oral clearance (L/h). In patients in whom the curve exhibited a terminal elimination phase, K_{el} was calculated similarly as for lopinavir and the 24-h trough concentration (C_{24}) was determined by extrapolation.

Pharmacokinetic parameters of lopinavir were compared with historical data of lopinavir alone and of increased dose of lopinavir with nevirapine in children, and with data of lopinavir with efavirenz in adults. (14, 17, 21) Efavirenz data were compared to historical data in children (only AUC_{0-24} and C_{24}) and adults. (11 ,19 ,22)

Safety and efficacy

Safety and efficacy were intensively monitored, either in accordance with the RODU-1 study protocol, or as a part of routine patient care. Results of safety and efficacy in both patient groups have been published elsewhere. (16)

Statistical methods

All statistical tests were performed using SPSS (SPSS®, Chicago, IL, U.S.A., version 10.0). Spearman's rank correlation was calculated to evaluate the relationship of pharmacokinetic parameters to each other and to clinical characetristics (age, body weight, body surface area). A t test was used to test significance. The Fisher's Exact Test was performed to estimate the association of nominally scaled patient characteristics with pharmacokinetic parameters. The Mann-Whitney U Test was used to compare medians of continuously scaled variables. A two-sided p-value of <0.05 was considered statistically significant.

Results

Baseline patient characteristics

16 HIV-1 infected children were enrolled in the study between October 2001 and April 2003. Pharmacokinetic data from one patient were excluded due to strong suspicion of non-compliance, based on several unquantifiable plasma levels of lopinavir and efavirenz in samples taken at regular patient visits and an unquantifiable pre-dose level at intensive pharmacokinetic sampling. Patient demographic characteristics of the remaining 15 patients are listed in Table 1. Median age (range) of these patients (9 girls, 6 boys) was 11.8 (5.7-16.3) years. All 15 evaluable children had received prior antiretroviral treatment; 7 had received a protease inhibitor with 2 NRTIs, 7 a solely nucleoside based regimen consisting of one or two

NRTIs, and one patient had been pretreated with an NNRTI with 2 NRTIs. All 15 patients had multiple genotypic resistance to NRTIs at baseline. None of the patients used co-medication that was expected to interfere with the pharmacokinetics of lopinavir/ritonavir and/or efavirenz. Intensive pharmacokinetic sampling was performed 2-8 weeks (median 4 weeks) after start of the regimen.

Table 1: Patient baseline characteristics (N=15).

Age (median, range)	11.8 (5.7-16.3)
Gender (female/male)	9/6
Body weight (kg) (median, IQR)	32.5 (26.0-36.5)
Body surface area (m^2) (median, IQR)	1.1 (1.0-1.2)
Ethnicity (numbers (%))	
Asian	2 (13)
African	9 (60)
Caucasian	1 (7)
Hispanic	1 (7)
Other	2 (13)
Previous antiretroviral treatment (numbers (%))	
Protease inhibitor + 2 NRTIs	7 (47)
Nevirapine + 2 NRTIs	1 (7)
Mono- or dual NRTIs	7 (47)
Number of different previous antiretroviral regimens per patient (median (range))	1 (1-3)
Lopinavir dose (mg/m^2 q12h) (median (IQR))	300 (290-341)
Efavirenz dose (mg/kg q24h) (median (IQR))	12.9 (12.1-13.9)

IQR: interquartile range

Pharmacokinetics

Lopinavir

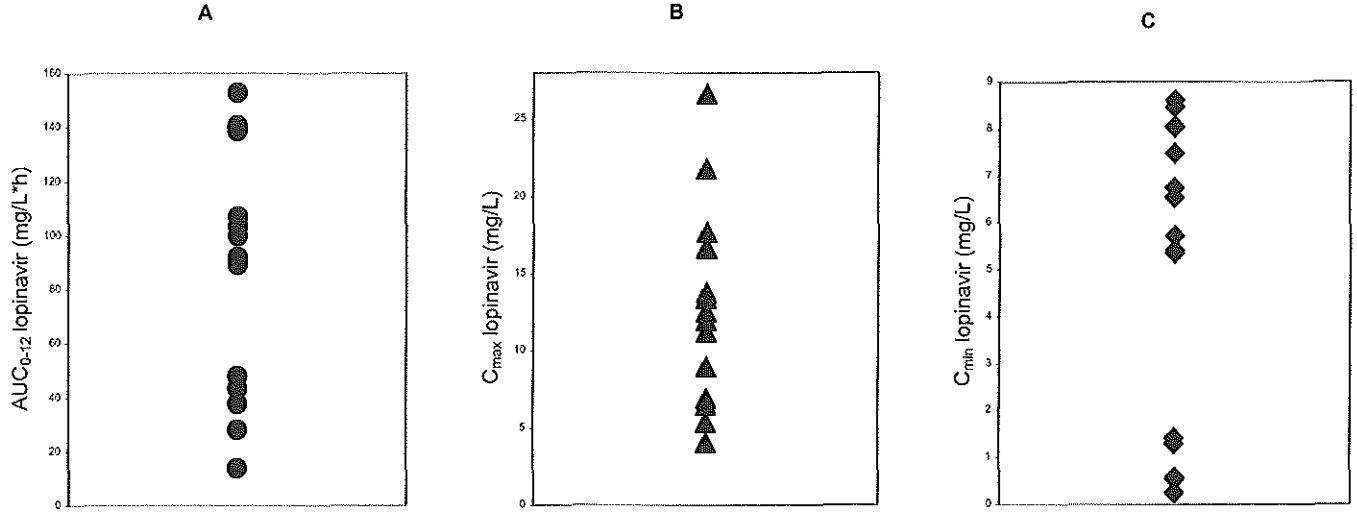
Median lopinavir AUC_{0-12}, C_{max} and C_{min} levels were similar to historical references of lopinavir/ritonavir alone, increased dose lopinavir/ritonavir with nevirapine in children, and increased dose of lopinavir/ritonavir with efavirenz in adults (Table 2). T_{max} and Cl/m^2*F (median, interquartile range) were 3.2 (1.6-5.2) h and 3.1 (2.5-7.0) $L/h*m^2$, respectively. AUC_{0-12}, C_{max} and C_{12} of lopinavir showed 11, 9 and 34-fold interindividual variation, respectively. None of the pharmacokinetic parameters (AUC_{0-12}, C_{max}, C_{min}, Cl/m^2*F, T_{max} or $t_{1/2}$) of lopinavir was related with gender, age or weight (p values all > 0.1).

Table 2. Pharmacokinetic parameters of lopinavir in children using lopinavir/ritonavir with efavirenz (N=15), compared to historical data of children using lopinavir/ritonavir alone or increased dose lopinavir/ritonavir with nevirapine and historical data of adults using lopinavir/ritonavir alone or increased dose lopinavir/ritonavir with efavirenz.

	Present study N=15 (median (IQR))	Historical data of children using lopinavir/ritonavir 230/58 mg/m^2 q12h alone [17]	Historical data ofchildren using lopinavir/ritonavir 300/75 mg/m^2 q12h with nevirapine [14]	Historical data of adults using lopinavir/ritonavir 533/133 mg q12h with efavirenz 600 mg q24h [21]	Historical data of ladults using lopinavir/ritonavir 400/100 mg q12h alone [17]
		Mean ± SD and (ratio present/historical value) N=12	Mean ± SD and (ratio present/historical value) N=12	Mean ± SD and (ratio present/historical value) N=26	Mean ± SD and (ratio present/historical value) N=21
AUC_{0-12} (mg/L*h)	92.3 (43.5-138.5)	72.6 ± 31.1 (1.27)	85.8 ± 36.9 (1.08)	89.8 ± 65.4	82.8 ± 44.5 (1.11)
C_{max} (mg/L)	12.5 (6.9-16.7)	8.16 ± 2.94 (1.53)	10.04 ± 3.26 (1.25)	10.7 ± 6.5	9.6 ± 4.4 (1.30)
C_{12} (mg/L)	5.7 (1.3-8.0)	3.35 ± 2.14 (1.70)	3.56 ± 3.45 (1.60)	4.1 ± 4.0	5.5 ± 4.0$^{\#}$ (1.03)
t1/2	5.8 (2.7-6.7)	5.8 ± 3.0 (1.00)	4.7 ± 4.5 (1.23)	N.A.	5.8 ± 2.4 (1.0)

$^{\#}$: morning trough level; IQR: interquartile range; SD: standard deviation

Figure 1. (A) AUC_{0-12}, (B) C_{max} and (C) C_{min} of lopinavir in children using lopinavir/ritonavir 300/75 mg/m^2 q12h with 14 mg/kg efavirenz (N=15).



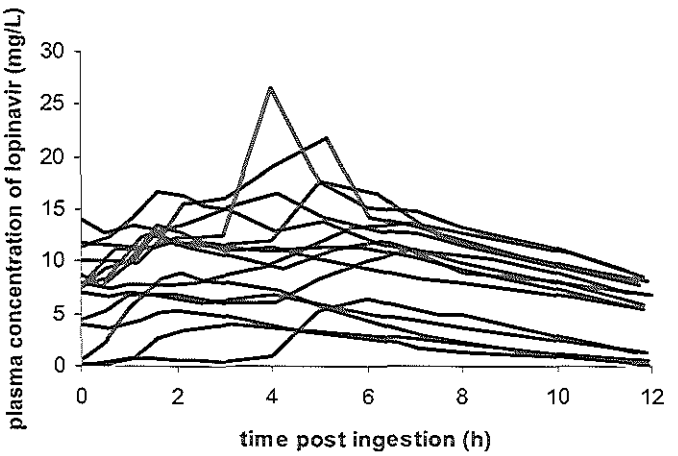
Visual inspection of the scatters of individual pharmacokinetic parameters and pharmacokinetic curves showed lower plasma levels of lopinavir in five children (figures 1 and 2). These five children showed significantly lower AUC_{0-12}, C_{max} and C_{min} of lopinavir than the 10 remaining children (group medians were 37.7 vs. 105.4 mg/L*h; 6.5 vs. 15.2 mg/L; 0.58 vs. 7.1 mg/L, respectively) (p values all 0.002, Figures 1 and 2, Table 2) and lower plasma levels with regard to the historical references (Table 2). Also, the absolute difference between median C_{max} and C_{min} of lopinavir was smaller in the children with low plasma levels (p=0.04). In 3/5 children with low plasma levels of lopinavir, C_{12} of lopinavir was below the value of 1.0 mg/L. This plasma level approximates 15 times the 50% inhibitory concentration (IC_{50}) of lopinavir for wild type virus in a serum containing medium, and is considered a lower limit for C_{min} in patients naïve to antiretroviral therapy. (23,24) Also, $t_{1/2}$ of lopinavir was significantly shorter and Cl/m^2*F significantly higher in the 5 children with lower plasma levels (p values both 0.002).
The 5 patients with lower plasma levels of lopinavir were not significantly different from the 10 patients with higher plasma levels with regard to median age (10.5 vs. 11.9 years), body weight (33.4 vs. 31.7 kg), gender (2/5 vs. 4/10 males), lopinavir dose (306 vs. 296 mg/m^2 q12h), efavirenz dose (12.1 vs. 12.9 mg/kg q24h) or C_{avg} or AUC_{0-24} of efavirenz (p values all > 0.1). The only difference in patient characteristics between these groups of children was ethnical background. While all 5 children with lower lopinavir levels were of African origin, ethnicity of the 10 children with higher plasma levels was as follows: Asian: n=2, African: n=4, Caucasian: n=2, other: n=2.

Efavirenz
Analysis of plasma levels of efavirenz showed a C_{avg} (median (interquartile range)) of 3.9 (3.0-5.9) mg/L in the whole group of children, which tended to be higher than has been reported in adults. (19, 22) In 11/15 patients, the pharmacokinetic curve of efavirenz showed a terminal elimination phase. In these patients, median (interquartile range) $t_{1/2}$ and C_{24} were 25.5 (12.3-64.9) hour and 2.1 (1.5-3.1) mg/L, respectively, which was in accordance with historical data in adults and children. (11, 19, 22) Estimated AUC_{0-24} of efavirenz (median (interquartile range)) was 93.9 (73.8-130.2) mg/L*h, which tended to be higher than these historical data.

Discussion

In 15 HIV-1 infected children, the increased dose of 300/75 mg/m^2 q12h lopinavir/ritonavir combined with 14 mg/kg q24h efavirenz resulted in median AUC_{0-12}, C_{max} and C_{min} of lopinavir similar to historical data of both lopinavir/ritonavir alone and increased dose lopinavir/ritonavir with nevirapine (children) or efavirenz (adults). This suggests that the investigated doses provided adequate systemic lopinavir exposure in most children. Meanwhile, plasma levels of lopinavir showed high interindividual variability. Efavirenz levels, measured only during the second half of the dose interval, resulted in $t_{1/2}$ and C_{24} approximately similar to historical data in children and adults, while C_{avg} and estimated AUC_{0-24} tended to be higher than historical controls. (11, 19, 22)

Figure 2. Individual pharmacokinetic curves of lopinavir in children using 300/75 mg/m^2 q12h lopinavir/ritonavir with 14 mg/kg q24h efavirenz



While treatment options for HIV-1 positive children have increased during the past decade, several factors may still impose limitations. Viral resistance and drug-related toxicity are frequently encountered, especially after long-term treatment. All children in this study had received prior antiretroviral treatment containing at least one NRTI and had developed multiple resistance against this class of drugs. A regimen containing lopinavir/ritonavir with efavirenz has shown good efficacy in heavily preatreated HIV-1 infected adults and may therefore be of interest in HIV-1 infected children too, when NRTIs do not offer a treatment option anymore. (25, 26) Also, the regimen has a relatively limited pill burden. Finally, liquid formulations of both drugs are available, enabling their use by children who cannot swallow capsules.

In our study, in the majority of the children, lopinavir pharmacokinetic parameters were similar to historical controls. Although in most children the explored lopinavir/ritonavir dose was adequate, interestingly, in 5/15 patients, $AUC_{0\text{-}12}$, C_{max} and C_{min} of lopinavir were significantly lower than in the other children. This finding may reflect the presence of a subpopulation of patients with lower plasma levels of lopinavir. In 3 out of these 5 patients, C_{min} fell below 1.0 mg/L, a value equaling 15 times the IC_{50} of lopinavir for wild-type virus as determined in the presence of 50% human serum and 10% fetal calf serum, i.e. an inhibitory quotient (IQ) of 15. An IQ above 15 for lopinavir has been associated with significantly improved antiviral response. (21, 24) Especially in protease inhibitor pretreated patients, virus may have become less susceptible to lopinavir, and in that case, the IQ of 15 is only reached at plasma levels above 1.0 mg/L. Consequently, children with C_{min} below 1.0 mg/L in our study possibly had subtherapeutic plasma levels of lopinavir.

In the 5 patients with lower plasma levels, $t_{1/2}$ of lopinavir was significantly shorter reflecting, a higher elimination rate when compared to the 10 remaining children. This finding could result from an increased enzyme inducing effect of efavirenz. Induction of hepatic CYP3A4 has been correlated with plasma levels of efavirenz. (27) However, in the present study, this could not be confirmed, since in the children with lower levels of lopinavir, levels of efavirenz were not significantly higher. Also, in these children, Cl/m^2*F was significantly increased, which could reflect both decreased absorption and increased elimination. The findings that not only C_{min}, but also C_{max} were lower and that the absolute difference between C_{max} and C_{min} was smaller in these children suggest also decreased intestinal absorption of lopinavir. Consequently, in this study, lower plasma levels of lopinavir were likely to have been caused by decreased absorption combined with an increased hepatic clearance of lopinavir. Absorption of lopinavir is known to be increased with food and therefore, all patients ingested medication with a standardized breakfast on the day of pharmacokinetic sampling. However, it cannot entirely be excluded that patients with lower plasma levels ingested medication with a smaller amount of food. An explanation for increased elimination, and possibly also for decreased absorption of lopinavir, could be lower plasma levels of its pharmacokinetic booster ritonavir, which is both a substrate and an inhibitor of CYP3A4, and, at least in vitro, inhibits activity of the membrane efflux protein P-glycoprotein (PgP). (28-31) The boosting effect by ritonavir on plasma levels of lopinavir and other protease inhibitors has been found to depend on plasma levels of ritonavir. (32)

Remarkably, the only difference between the children with higher and lower plasma levels of lopinavir was ethnicity, African children being more strongly represented in the group of children with lower plasma levels than in the group of children with higher plasma levels of lopinavir. Genetic polymorphism of CYP3A4, the major enzyme involved in metabolism of lopinavir and ritonavir, has been found more frequently in Africans than in other ethnic groups, but does not seem to be of remarkable influence on CYP3A4 related metabolism. (33, 34) Also, polymorphism of the gene (MDR1) coding for PgP has been found significantly more frequent in Africans than in other races. (35, 36) Genetic polymorphism may influence metabolism of PgP substrates, but its effects *in vivo* still remain to be elucidated. (37) In addition, activity of CYP or PgP is known to be influenced by several external factors, such as concomitant medication and nutritional patterns. While no relevant co-medication was registered during the study, the regular use of food containing modifiers of CYP or PgP may have influenced plasma levels of lopinavir or efavirenz. The impact of genotypic parameters and plasma levels of ritonavir on the pharmacokinetics of lopinavir could not be evaluated in the present study, since none of these factors were determined.

The pharmacokinetics of efavirenz were approximately similar to historical data in adults and children, except for the estimated $AUC_{0\text{-}24}$ and C_{avg}, which tended to be higher than these data. (11, 19, 22) The relevance of this latter finding may be low, especially for $AUC_{0\text{-}24}$, since by measuring plasma levels of efavirenz during the second part of the pharmacokinetic curve, only a rough estimate of $AUC_{0\text{-}24}$ could be made. The sampling design thus provided for limited

estimates of efavirenz pharmacokinetic parameters. Furthermore, earlier data in adults did not shown any influence of lopinavir/ritonavir on plasma levels of efavirenz. (13)

In conclusion, these data indicate that the investigated doses of 300/75 mg/m^2 q12h lopinavir/ritonavir with 14 mg/kg q24h efavirenz in the majority of children result in pharmacokinetic parameters similar to historical data of lopinavir/ritonavir alone, and of increased dose lopinavir/ritonavir with nevirapine or efavirenz in both children and adults, respectively. Plasma levels of efavirenz were approximately similar to historical data, enabling the use of the pediatric standard dose of efavirenz (14 mg/kg q24h). These results suggest that the evaluated doses of lopinavir/ritonavir and efavirenz are appropriate for most children. However, regarding the high interindividual variability in plasma levels of in particular lopinavir, therapeutic drug monitoring should be applied to detect individual patients who display abnormal plasma levels.

Acknowledgements: The study-nurses and/or medical students of the Erasmus MC /Sophia, Division of Pediatric Infectious Diseases and Immunology, Rotterdam and of the Heinrich Heine University Hospital, Department of Pediatrics, Düsseldorf, Germany, are kindly acknowledged for collection of the samples.
The technicians of the Department of Clinical Pharmacy, University Medical Center Nijmegen are kindly acknowledged for processing and analysis of plasma samples.

References

1. van Rossum AM, Fraaij PL, de Groot R. Efficacy of highly active antiretroviral therapy in HIV-1 infected children. Lancet Infect Dis 2002;2:93-102.
2. Gortmaker SL, Hughes M, Cervia J, et al. Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. N Engl J Med 2001;345:1522-28.
3. Anonymous. Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. www.aidsinfo.nih.gov/guidelines/pediatric . 2003.
4. Sharland M, Blanche S, Castelli G, et al. PENTA guidelines for the use of antiretroviral therapy, 2004. HIV Med 2004;5 Suppl 2:61-86.
5. Seminari E, Maggiolo F, Villani P, et al. Efavirenz, nelfinavir, and stavudine rescue combination therapy in HIV-1-positive patients heavily pretreated with nucleoside analogues and protease inhibitors. J Acquir Immune Defic Syndr 1999;22:453-60.
6. Piketty C, Race E, Castiel P, et al. Efficacy of a five-drug combination including ritonavir, saquinavir and efavirenz in patients who failed on a conventional triple-drug regimen: phenotypic resistance to protease inhibitors predicts outcome of therapy. AIDS 1999;13:F71-F77.
7. Albrecht MA, Bosch RJ, Hammer SM, et al. Nelfinavir, efavirenz, or both after the failure of nucleoside treatment of HIV infection. N Engl J Med 2001;345:398-407.
8. Joly V, Descamps D, Yeni P. NNRTI plus PI combinations in the perspective of nucleoside-sparing or nucleoside-failing antiretroviral regimens. AIDS Rev 2002;4:128-39.
9. Cvetkovic R, Goa K. Lopinavir/Ritonavir : A Review of its Use in the Management of HIV Infection. Drugs 2003;63:769-802.
10. Viramune® Summary of Product Characteristics. Boehringer Ingelheim International GmbH, Ingelheim am Rhein, Germany . 1999.
11. Stocrin® Summary of Product Characteristics. Merck Sharp & Dohme Limited, Hertfordshire, United kingdom . 2002.

12. Bertz, R, Foit, C., Burt, D., et al. Assessment of the effect of nevirapine on the pharmacokinetics of lopinavir/ritonavir (Kaletra (R) after multiple dosing in HIV-infected adults. XIVth International AIDS Conference, July 7-12 2002, Barcelona, Spain, abstract TuPeB4565 . 2002.
13. Bertz, R, Lam, W., Hsu, A., al. Assessment of the Pharmacokinetic Interaction between ABT-378/Ritonavir (ABT-378/r) and Efavirenz (EFV) in Healthy Volunteers and in HIV+ Subjects. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Canada, September 17-20, 2000, abstract 424 . 2000.
14. Hsu, A., Bertz, R, Lam, W., et al. Assessment of Pharmacokinetic Interactions Between Kaletra™ (lopinavir/ritonavir or ABT-378/r) and Nevirapine in Pediatric Subjects. 5th International Congress on Drug Therapy in HIV Infection,Glasgow, UK, October 2000, abstract 440, poster. 2000.
15. Saez-Llorens X, Violari A, Deetz CO, et al. Forty-eight-week evaluation of lopinavir/ritonavir, a new protease inhibitor, in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2003;22:216-24.
16. Fraaij PL, Neubert J, Bergshoeff AS, al. Safety and efficacy of a NRTI-sparing HAART regimen of efavirenz and lopinavir/ritonavir in HIV-1-infected children. Antivir Ther 2004;9:297-99.
17. Product information Kaletra®. Abbott, Chicago, IL, USA 2000.
18. Droste JAH, Verweij-van Wissen CPWGM, Burger D.M. Simultaneous Determination of the HIV Drugs Indinavir, Amprenavir, Saquinavir, Ritonavir, Lopinavir, Nelfinavir, the Nelfinavir hydroxymetabolite M8 and Nevirapine in Human Plasma by Reversed Phase High Performance Liquid Chromatography. Ther Drug Monit 2003;25:393-99.
19. Aarnoutse RE, Grintjes KJ, Telgt DS, et al. The influence of efavirenz on the pharmacokinetics of a twice-daily combination of indinavir and low-dose ritonavir in healthy volunteers. Clin Pharmacol Ther 2002;71:57-67.
20. Gibaldi M. Compartmental and noncompartmental pharmacokinetics. Biopharmaceutics and clinical pharmacokinetics. Philadelphia, London: Lea & Febiger, 1991:14-23.
21. Hsu A, Isaacson J, Brun S, et al. Pharmacokinetic-pharmacodynamic analysis of lopinavir-ritonavir in combination with efavirenz and two nucleoside reverse transcriptase inhibitors in extensively pretreated human immunodeficiency virus-infected patients. Antimicrob Agents Chemother 2003;47:350-359.
22. Villani P, Regazzi MB, Castelli F, et al. Pharmacokinetics of efavirenz (EFV) alone and in combination therapy with nelfinavir (NFV) in HIV-1 infected patients. Br J Clin Pharmacol 1999;48:712-15.
23. Anonymous. Optimising TDM in HIV clinical care. A practical guide to performing therapeutic drug monitoring (TDM) for antiretroviral agents. *Available: http://www* HIVpharmacology com Last visited at May 24, 2004
24. Molla A, Vasavanonda S, Kumar G, et al. Human serum attenuates the activity of protease inhibitors toward wild-type and mutant human immunodeficiency virus. Virology 1998;250:255-62.
25. Kempf DJ, Isaacson JD, King MS, et al. Analysis of the virological response with respect to baseline viral phenotype and genotype in protease inhibitor-experienced HIV-1-infected patients receiving lopinavir/ritonavir therapy. Antivir Ther 2002;7:165-74.
26. Masquelier B, Breilh D, Neau D, et al. Human immunodeficiency virus type 1 genotypic and pharmacokinetic determinants of the virological response to lopinavir-ritonavir-containing therapy in protease inhibitor-experienced patients. Antimicrob Agents Chemother 2002;46:2926-32.
27. Mouly S, Lown KS, Kornhauser D, et al. Hepatic but not intestinal CYP3A4 displays dose-dependent induction by efavirenz in humans. Clin Pharmacol Ther 2002;72:1-9.
28. Profit L, Eagling VA, Back DJ. Modulation of P-glycoprotein function in human lymphocytes and Caco-2 cell monolayers by HIV-1 protease inhibitors. AIDS 1999;13:1623-27.
29. Drewe J, Gutmann H, Fricker G, et al. HIV protease inhibitor ritonavir: a more potent inhibitor of P-glycoprotein than the cyclosporine analog SDZ PSC 833. Biochem Pharmacol 1999;57:1147-52.
30. Olson DP, Scadden DT, D'Aquila RT, et al. The protease inhibitor ritonavir inhibits the functional activity of the multidrug resistance related-protein 1 (MRP-1). AIDS 2002;16:1743-47.
31. Garraffo, R., Durant, J., Chaillou, S., et al. In *Vitro* and *In Vivo* Protease Inhibitors (PI) Kinetics in Human Cells Expressing or not MDR1. Role of Ritonavir (RI) in Intracellular PI Accumulation. 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Canada, September 17-20, 2000, abstract 1167 . 2000.
32. Guiard-Schmid JB, Poirier JM, Meynard JL, et al. High variability of plasma drug concentrations in dual protease inhibitor regimens. Antimicrob Agents Chemother 2003;47:986-90.

33. Wandel C, Witte JS, Hall JM, et al. CYP3A activity in African American and European American men: population differences and functional effect of the CYP3A4*1B5'-promoter region polymorphism. Clin Pharmacol Ther 2000;68:82-91.
34. Ball SE, Scatina J, Kao J, et al. Population distribution and effects on drug metabolism of a genetic variant in the 5' promoter region of CYP3A4. Clin Pharmacol Ther 1999;66:288-94.
35. Kim RB, Leake BF, Choo EF, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin Pharmacol Ther 2001;70:189-99.
36. Schaeffeler E, Eichelbaum M, Brinkmann U, et al. Frequency of C3435T polymorphism of MDR1 gene in African people. Lancet 2001;358:383-84.
37. Lin JH, Yamazaki M. Role of P-glycoprotein in pharmacokinetics: clinical implications. Clin Pharmacokinet 2003;42:59-98.

Plasma levels of zidovudine twice compared to thrice daily in six HIV-1 infected children

Alina S. Bergshoeff, Pieter L.A. Fraaij, Corrien Verweij, Annemarie M.C. van Rossum, Gwenda Verweel, Nico G. Hartwig, Ronald de Groot, David M. Burger

J Antimicrob Chemother: in press

Chapter 15

Abstract

Objectives: Zidovudine is frequently administered every 12h in HIV-infected children but so far no pharmacokinetic data of this agent administered every 12h are available. We have evaluated the plasma pharmacokinetics of zidovudine administered every 8h vs every 12h in HIV-1 infected children.

Methods: In HIV-1 infected children who switched from zidovudine every 8h to every 12h, a pharmacokinetic curve was recorded both before and after the switch. Zidovudine plasma levels were measured by HPLC. Pharmacokinetic parameters were calculated by noncompartmental methods.

Results: Six HIV-1 infected children (median age (range) 7.8 (2.5 – 13.4) years) were included. In these patients, geometric mean ratios of $AUC_{0\text{-}24}$ and C_{max} for zidovudine every 12h vs. every 8h were not significantly different from 1.0.

Conclusions: Plasma pharmacokinetic parameters of zidovudine taken every 8h and every 12h were not significantly different and therefore suggest bioequivalence of these two dose frequencies.

Introduction

Zidovudine was the first drug licensed for treatment of HIV infection. It is recommended as a component of highly-active antiretroviral treatment (HAART) and in the prophylaxis of perinatal transmission of HIV. (1, 2) Current guidelines such as PENTA indicate a pediatric dose range for zidovudine of 90-180 mg/m^2 q6h or every 8h. Meanwhile, zidovudine is also increasingly used every 12h. However, in children, except for neonates and infants, no published data exist on the pharmacokinetics of zidovudine every 8h or every 12h. (3-5) Considering their common use in children, the pharmacokinetics of these regimens should be characterized in pediatric patients. We here report the plasma pharmacokinetics of zidovudine every 8h and every 12h in six HIV-1 infected children.

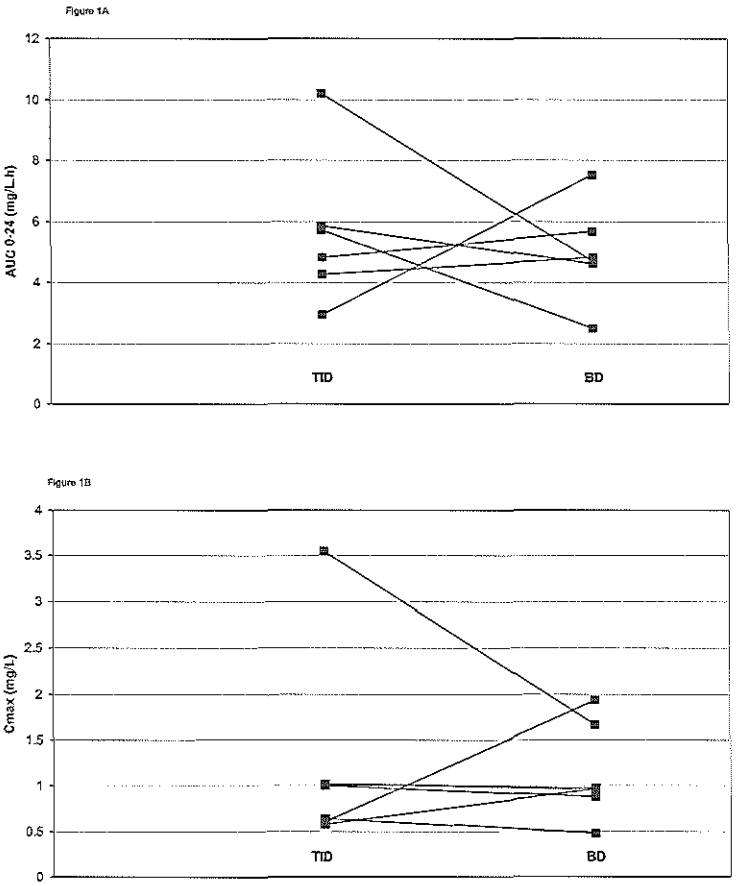
Methods

This was a retrospective analysis of pharmacokinetic data from HIV-1 infected children between 1 and 18 years old, who were included in an ongoing study on the simplification of HAART conducted in our center (inclusion August 2000 - January 2003). Briefly, children were offered the possibility of changing their every 8h HAART into fully every 12h HAART. Antiretroviral medication prior to switch consisted of zidovudine 120 mg/m^2 every 8h with indinavir 600 mg/m^2 every 8h and 4 mg/kg every 12h lamivudine. After switch, children received zidovudine 180 mg/m^2 every 12h with indinavir/ritonavir 500/100 mg/m^2 every 12h and lamivudine 4 mg/kg every 12h. Zidovudine was administered as capsules of 100mg or 250mg, tablets of 300mg, or oral solution containing 10mg/mL (Retrovir, AZT®). Written informed consent was obtained from patients or caretakers prior to enrollment. Intensive pharmacokinetic sampling of all antiretroviral drugs was performed at steady state, prior to and > 2 weeks after switch to the every 12h regimen. Here are described the pharmacokinetics of zidovudine in patients who changed zidovudine from every 8h to every 12h.

Plasma concentrations of zidovudine were determined by validated HPLC assay with UV detection (lower limit of quantification: 0.017 mg/L (accuracy 99-101%, intra- and interday coefficients of variation 1.5-2.0% and 1.5-2.2%, respectively (unpublished data)). Data were included from patients in whom both a every 8h and every 12h curve of at least 5 evaluable time points was available and absolute total daily zidovudine doses differed less than 25% between the every 12h and every 8h regimen. Furthermore, pharmacokinetic samplings had to be less than six months apart.

Estimated area under the plasma concentration-time curve (AUC_{0-24}), peak level (C_{max}), trough level (C_{min}), relative apparent oral clearance ($Cl/F*m^2$) and terminal plasma half-life ($t_{1/2}$) of zidovudine were calculated using non-compartmental methods. (6) To compare regimens, within-patient ratios of pharmacokinetic parameters for zidovudine every 12h versus every 8h were calculated, from which geometric mean ratios (GMR) with 90% confidence intervals (CI) were obtained. A 90% CI of GMR containing 1.0 was considered as reflecting similarity of both regimens. Results were compared with literature data on adults.

Figure 1: (A) AUC_{0-24} and (B) C_{max} of zidovudine every 12h (squares) vs. every 8h (rounds) in 6 children who switched zidovudine every 8h to every 12h.



Results

Six patients were enrolled (5 girls, 1 boy) of median age 7.8 years (range 2.5 – 13.4). Median number of samples per pharmacokinetic curve was 7 for every 8h regimens, and 7.5 for every 12h regimens. GMRs of pharmacokinetic parameters of zidovudine every 12h versus every 8h did not show significant differences between both regimens, but were characterized by wide CI. In our study, except for C_{max} of the every 12h regimen, zidovudine levels were slightly higher than in adults using zidovudine every 8h or every 12h (Table 1, Figure 1) (7, 8). Zidovudine every 12h did not result in a higher C_{max} than every 8h. No correlation was found between zidovudine pharmacokinetic parameters (AUC_{0-24},C_{max} and Cl/F^*m^2) and age, body weight or body surface area (p-values all > 0.05). However, zidovudine $t_{1/2}$ inversely correlated with age for both the every 12h and every 8h regimen (r^2: -0.78 and –0.69, p values 0.019 and 0.042, respectively).

Table 1: Pharmacokinetics of zidovudine every 12h and every 8h in HIV-1 infected children and historical data of zidovudine in adults.

Pharmacokinetic parameter	Children using zidovudine 120 mg/m^2 every 8h in current study (GM + 90% CI) N=6	Children using zidovudine 180 mg/m^2 every 12h in current study (GM + 90% CI) N=6	Zidovudine every 12h vs. every 8h in current study (GMR + 90% CI) N=6	Zidovudine 200 mg every 8h (7) (mean ± SEM) N=20-25	Zidovudine 300 mg every 12h (8) (GM + 95% CI) N=12
AUC_{0-24} (mg/L*h)	5.24 (3.73-7.35)	4.72 (3.50-6.36)	0.90 (0.52-1.56)	3.06 +- 0.13	2.92 (2.22-3.82)
C_{max} (mg/L)	0.96 (0.55-1.70)	1.04 (0.69-1.57)	1.08 (0.62-1.88)	0.44 +-0.024	1.15 (0.71-1.85)
C_{min} (mg/L) (median)	< 0.017	<0.017	N.D.	N.A.	N.A.
Cl/F*m^2 (L/h*m^2)	63.3 (46.6-85.8)	79.5 (60.3-104.8)	1.26 (0.83-1.90)	N.A.	N.A.
$T_{1/2}$ (h)	1.31 (0.99-1.72)	1.15 (0.90-1.47)	0.88 (0.71-1.10)	N.A.	N.A.

N.D.: not determined; GM: geometric mean; GMR: geometric mean ratio; CI: confidence interval; SEM: standard error of the mean; AUC_{0-24}: estimated 0-24h area under the plasma concentration-time curve; C_{max}: peak plasma level; C_{min}: plasma trough level; Cl/F*m^2: relative apparent oral clearance; $t_{1/2}$: terminal plasma half life

Discussion

This study presents the first pharmacokinetic data of zidovudine every 12h compared to every 8h in children above the infant age. Pharmacokinetic parameters of zidovudine every 12h compared to every 8h did not reveal significant differences, suggesting equivalence of both regimens in terms of plasma pharmacokinetics. The observed tendency for higher plasma levels in children than in adults could be due to the higher pediatric zidovudine dose per body weight: 360 mg/m^2/day equals 13 mg/kg/day in an average child with 1 m^2 body surface area weighing 28 kg, which is higher than the adult dose (600 mg/day = 8.6 mg/kg/day in an adult weighing 70 kg). Also, zidovudine every 12h did not result, as expected with lower dose frequency, in a higher C_{max} than every 8h. (7, 8) Pharmacokinetic parameters of zidovudine were highly variable, probably as a result of small sample size. Therefore, these findings should be confirmed in a larger number of patients. Zidovudine AUC_{0-24},C_{max} and Cl/F*m^2 were independent of age, body weight or body surface area. This is in accordance with literature data, which generally indicate that zidovudine Cl/F*m^2 most strongly increases during first weeks of life, reaching adult levels at the age of 2 years, and all children in our study were above this age. (5, 9, 10) In contrast, a significantly increased elimination rate of zidovudine was found with age, which would suggest further maturation of zidovudine metabolism during childhood. Remarkably, as stated above, this higher elimination rate was not reflected in lower AUC_{0-24} or C_{max} and higher Cl/F*m^2 in older children. While this finding should be considered cautiously because of the small number of patients in our study, an explanation, also mentioned for premature infants, could be decreased zidovudine absorption or increased first-pass metabolism in younger children. (5) resulting in no net difference of AUC_{0-24}, C_{max} and Cl/F*m^2 between younger and older children.

In conclusion, in this group of six HIV-1 infected children, pharmacokinetics of zidovudine every 12h were not statistically different from every 8h, suggesting bioequivalence of both regimens. These findings need confirmation in studies of larger sample size. Finally, efficacy of both regimens in children should be evaluated in a comparative study.

Acknowledgements: Technicians of the Department of Clinical Pharmacy, University Medical Center, Nijmegen, are kindly acknowledged for processing and analysis of the plasma samples. Study nurses of the Department of Pediatrics, Erasmus MC-Sophia Children's hospital, Rotterdam, are kindly acknowledged for blood drawings. No financial support was received for this study

References

1. Anonymous. Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. www.aidsinfo.nih.gov/guidelines/adult . 2003.
2. Anonymous. Guidelines for the Use of Antiretroviral Agents in Pediatric HIV Infection. www.aidsinfo.nih.gov/guidelines/pediatric . 2003.
3. Mirochnick M, Capparelli E, Dankner W, et al. Zidovudine pharmacokinetics in premature infants exposed to human immunodeficiency virus. Antimicrob Agents Chemother 1998; 42:808-812.

4. Moodley D, Pillay K, Naidoo K, et al. Pharmacokinetics of zidovudine and lamivudine in neonates following coadministration of oral doses every 12 hours. J Clin Pharmacol. 2001; 41:732-741.
5. Capparelli EV, Mirochnick M, Dankner WM, et al. Pharmacokinetics and tolerance of zidovudine in preterm infants. J Pediatr. 2003; 142:47-52.
6. Gibaldi M. Compartmental and noncompartmental pharmacokinetics.In: Biopharmaceutics and clinical pharmacokinetics, Fourth Edition, Philadelphia, London: Lea & Febiger, 1991: 14-23.
7. Vanhove GF, Kastrissios H, Gries JM, et al. Pharmacokinetics of saquinavir, zidovudine, and zalcitabine in combination therapy. Antimicrob Agents Chemother. 1997; 41:2428-2432.
8. Cremieux AC, Katlama C, Gillotin C, et al. A comparison of the steady-state pharmacokinetics and safety of abacavir, lamivudine, and zidovudine taken as a triple combination tablet and as abacavir plus a lamivudine-zidovudine double combination tablet by HIV-1-infected adults. Pharmacotherapy 2001; 21:424-430.
9. Mirochnick M, Capparelli E, Connor J. Pharmacokinetics of zidovudine in infants: a population analysis across studies. Clin Pharmacol Ther. 1999; 66:16-24.
10. Capparelli EV, Englund JA, Connor JD, et al. Population pharmacokinetics and pharmacodynamics of zidovudine in HIV-infected infants and children. J Clin Pharmacol. 2003; 43:133-140.

Changes in indinavir exposure over time: a case study in six HIV-1-infected children

Pieter L.A. Fraaij, Alina S. Bergshoeff, Annemarie M.C. van Rossum, Nico G. Hartwig, David M. Burger, Ronald de Groot

J Antimicrob Chemother 2003;52(4):727-730

Chapter 16

Abstract

Objective: To study changes in indinavir exposure over time in HIV-1 infected children.

Methods: Protease inhibitor (PI) naive HIV-1 infected children were treated with indinavir, zidovudine and lamivudine. Steady-state plasma pharmacokinetic (PK) sampling was carried out as standard of care. The AUC_{0-8} was targeted between 15 and 30 mg/L*hr. PK sampling was repeated after dosage adjustment until the AUC_{0-8} reached target values. Patients were included when the time interval between PK samplings was ≥ 2 years and differences in dosage/m^2 < 10% between PK samplings 1 and 2. Corrections of dose for changes in body size were performed.

Results: Six children were enrolled with a median age of 5.2 years (range 1.7-13.6 years). All had a viral load below 500 copies/ml. The geometric mean (GM) of the AUC_{0-8h} decreased from 25.3 mg/L*h at the first PK-day to 19.1 mg/L*hr at the second PK-day (GMR (Geometric Mean Ratio): 0.76 (95%C.I.: 0.48-1.20)). The GM of Cmax decreased from 11.8 to 10.4 mg/L (GMR: 0.88 (95%C.I.: 0.59-1.32). The GM of Cmin decreased from 0.08 to 0.07 mg/L (GMR: 0.86 (95%C.I.: 0.62-1.18). All children had an AUC_{0-8h} above 15 mg/L*hr on the first PK-day; three had an AUC_{0-8h} below 15 mg/L*hr on the second PK-day. In two of these three children the plasma viral load was > 500 copies/ml.

Conclusion: Changes in indinavir exposure were observed in time. In two patients decreased indinavir exposure was associated with virological failure. Therapeutic drug monitoring should be carried out over time since this may prevent subtherapeutic dosing in children.

Introduction

Since the introduction of highly active antiretroviral therapy (HAART) the life expectancy of HIV-1 infected children has improved dramatically. (1) Still, institution of optimal treatment poses a major challenge. In children, large inter individual differences are observed in the pharmacokinetics of antiretroviral drugs, especially in protease inhibitors (PI). For example Burger et al showed 18-fold variability (2.8-51 mg/L*hr) in the AUC_{0-8h} of indinavir in children treated with a dosage of 33 mg/kg metabolic weight. (2) This is even more important when one considers that the level of viral suppression and the plasma concentration of indinavir are associated in adults and children. (3, 4) Therefore, we routinely carried out pharmacokinetic analysis of plasma concentrations of PI in our hospital. Our approach resulted in favourable results with 69% viral response after 2 years of treatment. (5) Yet, viral failure occurs in some of the children. Data in animals and adults indicate that exposure to PI may gradually decrease over time. (6, 7) Currently there is no information on changes in the pharmacokinetic parameters after prolonged PI use in children. However, changes over time can be expected in children, since the processes of growth and development have a significant impact on drug absorption, distribution and clearance. (8) Decreased drug exposure over time may lead to viral rebound, selection of resistant mutants and ultimately to treatment failure. We here present a case study on the effects of time on indinavir exposure in six HIV-1 infected children.

Methods

Patients

PI-naive HIV-1 infected children with a viral load above 5,000 copies/ml and/or a CD4+ T-cell count below their age-specific reference value started HAART consisting of indinavir 400 mg/m^2 q8h, zidovudine 120 mg/m^2 q8h and lamivudine 4 mg/kg q12h. In all patients, steady-state intensive plasma PK sampling of indinavir was performed as standard of care. The AUC_{0-8h} was targeted between 15 mg/L*h and 30 mg/L*hr. (2) PK sampling was repeated until the AUC_{0-8h} reached target values. Hereafter, PK sampling was not routinely repeated. However, in case of viral failure single sample indinavir plasma levels were considered. Children were eligible for inclusion in this study, when data were available from both the first intensive PK sampling with the dose resulting in an adequate AUC_{0-8} (= PK-day 1), and second intensive pharmacokinetic sampling (= PK-day 2) on this (fixed) dose/m^2, with a minimum interval of 2 years. Dose adjustments < 10% of dosage in mg/m^2 body surface area (BSA) were allowed between PK samplings. Corrections of dose for changes in body size were performed for indinavir and for Nucleoside Reverse Transcriptase Inhibitors (NRTI). Selected clinical data were obtained during regular visits to the outpatient department. The study protocol was approved by the medical ethical committee of the Erasmus MC. Written informed consent was obtained from parents and patients.

Pharmacokinetics

Patients took indinavir on an empty stomach and blood samples were collected at time points 0 (pre-dose) and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7 and 8 hours post ingestion. Plasma was

separated by centrifugation (10 min at 3000 x *g*) and samples were stored at -20 °C until analysis. Indinavir concentrations were determined in plasma by HPLC, as previously reported. (9) The assay has intra-assay and inter-assay variances below 7.48% and 3.46%, respectively. (10) Pharmacokinetic parameters were calculated in Microsoft Excel© 97 by non-compartmental methods. (11)

For comparison of the various PK parameters the geometric mean (GM) of the ratio between PK-day 1 and 2 was calculated (GMR). For analysis of the pharmacokinetic data, SPSS© 10 and Microsoft Excel© 97 software were used.

Table 1. The individual changes in AUC_{0-8} and HIV-1 RNA levels between PK day 1 and 2

Patient	Race	Age at PK-day 1 (years)	Time between PK-days (years)	AUC_{0-8h} PK-day 1 (mg/L*h)	HIV-1 RNA PK-day 1 (copies/ml)	AUC_{0-8} PK-day 2 (mg/L*h)	HIV-1 RNA PK-day 2 (copies/ml)	AUC-II/ AUC-I (%)
1.A03	Black	4.4	2.8	37.7	< 500	17.5	< 500	46%
2.A05	Caucasian / Black	1.7	2.7	27.7	< 500	30.7	< 500	111%
3.A10	Caucasian	6.1	3.5	15.4	< 500	11.1	< 500	72%
4.A13	Black	13.6	2.6	20.6	< 500	10.2	5110	50%
5.A14	Black	10.9	2.6	20.4	< 500	14.8	3620	73%
6.A23	Black	3.1	2.0	38.7	<500	54.3	< 500	140%

Results

Between 1997 and 2001, 35 children started indinavir q8h in our hospital. The children had a median age 3.2 years range (0.2-13.6 year). Fifty-four percent of the patients was female. Sixty-three percent of the children had a least one parent originating from a sub-Saharan country (n = 22). At the time of this study (2000-2001), 8 children were still eligible for enrollment. Reasons not to include the remaining 27 patients in this case study were: loss to follow up (n = 5), a change in indinavir dosage per m^2 > 10% (n = 1) and a medication change before this study was initiated (n = 21). Patients changed medication before the study because of toxicity (n = 5), patient request (n = 1), failure to obtain acceptable PK values (n = 3), medication failure (n = 5) or medication was changed to a twice daily indinavir/ritonavir containing regimen after less than two year of treatment with indinavir q8h (n = 7). Of the 8 patients that could be enrolled in the study two were not. One as a result of suspected non-compliance on PK-day 2 and the other because of difficulties with obtaining PK-data owing to autism. The median age of the 6 included patients was 5.2 years (range 1.7-13.6) at the first PK-day and most (n=5) had at least one patient originating from a sub-Saharan country. The time period to obtain the optimal dosage of indinavir was 1 to 9 months after start of IDV 400 mg/m^2 (median 6 months). The median time between the first and the second PK-day was 2.5 years (range 2.0-3.5 years). On the second PK-day the median percentage of the original dosage per m^2 was 98% (range 93%-109%). The absolute median dosage (mg) of indinavir increased from 350 mg (range 300-800) to 450 mg (range 400-800). Three patients used co-

medication on the first PK-day (amphotericin B/co-trimoxazole, fluconazole/co-trimoxazole and co-trimoxazole). None of the patients used co-medication on the second PK-day.

In four of the six children the AUC_{0-8} had decreased on the second PK-day. In two children the AUC_{0-8} had increased. For data of the individual patients see Table 1. The GM of the AUC_{0-8}, Cmax, Cmin and $t_{1/2}$, all decreased on the second PK-day compared with the first PK-day. The pharmacokinetic parameters for the study group are summarized in Table 2.

All children had an AUC_{0-8h} above 15 mg/L*hr on the first PK-day. On the second PK-day the AUC_{0-8h} had decreased to below 15 mg/L*hr in three of the six children. These three children also had the lowest AUC_{0-8h} on the first PK-day. In two of these children virus could be detected on the second PK-day, whereas all patients had a viral load below 500 copies/ml on the first PK-day. (See Table 1) The median CD4 cell count as percentage of their age-specific reference value had increased from 66% (range 43-131) to 106% (range: 51-165). No clinically relevant abnormalities were observed in blood chemistry parameters for liver and kidney functions on PK-day 1 and PK-day 2.

Table 2. Changes in pharmacokinetic parameters in time in HIV-1 infected children (n = 6). Values for the first and second PK-day are presented as geometric mean (GM).

Parameter	First PK-day G.M. (range)	Second PK-day G.M. (range)	GMR II/I (range)	95% C.I.
AUC_{0-8h}	25.3 (15.4-38.7)	19.1 (10.2-54.3)	0.76 (0.46-1.4)	0.48-1.2
Cmax	11.8 (8.8-17.0)	10.4 (6.5-21.0)	0.88 (0.5-1.6)	0.59-1.32
Cmin	0.08 (0.04-0.21)	0.07 (0.04-0.15)	0.86 (0.71-1.5)	0.62-1.18
T1/2	1.67 (0.8-2.4)	1.45 (0.9-2.0)	0.87 (0.5-1.9)	0.49-1.54
Cl/F	16.3 (7.4-38.8)	27.4 (7.4-78.3)	1.67 (1.0-2.7)	1.13-2.48
$Cl/F*m^2$	20.6 (13.5-28.4)	30.9 (11-64)	1.5 (0.79-4.72)	0.75-2.98
Vd/F	39.3 (12.4-100)	62.5 (11.7-217.4)	1.59 (0.5-5.2)	0.66-3.84
$Vd/F*m^2$	49.7 (15.9-97.5)	64.7 (17.4-142.4)	1.3 (0.45-8.98)	0.40-4.3

Discussion

Currently, no information is available on changes in PK parameters of PI after long-term PI use in children. We therefore carried out a case study in HIV-1 infected children using indinavir for a

prolonged period and observed a decrease in the AUC_{0-8h} between PK-day 1 and PK-day 2 in 4 out of 6 children.

The differences found between the two PK curves cannot be explained by changes in the techniques, since we used the same methodology for all PK curves. Inter-assay variability is not likely to be responsible for the observed changes in indinavir exposure, since the changes in AUC_{0-8h} exceeded the inter-assay variance by far. (10) We did not observe clinically relevant abnormalities in blood chemistry parameters. Therefore, the changes in indinavir exposure were not the result of changes in organ function due to indinavir usage. Also the co-medication used on the first PK-day was not expected to have caused a difference in PK parameters, because it does not interfere with the metabolism of indinavir. (12)

It is unlikely that growth influenced the results, since the medication dosage was based on square meters of body surface and adjusted when length or weight had changed. Interestingly, in one child (A13) the absolute dose was not increased, and still her clearance and volume of distribution had markedly increased. Hypothetically, the decreased indinavir exposure may have been caused by a decrement in indinavir exposure with age. However, this is not expected since younger children have an increased hepatic enzymatic activity compared to older children and adults. (8) Duration of therapy per se did not seem to influence the change in indinavir exposure, since both increased and decreased exposure could be shown in four children who were on therapy for approximately the same time period (2.5 years). Still, mechanisms such as the induction of P-glycoprotein and CYP 450 levels after prolonged PI usage as also shown *in vitro* by Huang et al. may have resulted in the decreased indinavir exposure. (7)

Remarkably, the three children with AUC II below the 15 mg/L*hr threshold were the older ones, suggesting that older children may be more prone to develop subtherapeutic indinavir levels in time.

An alternative explanation for the observed failure of therapy after prolonged use of indinavir, may be non-compliance. Yet, in one of the children with a viral load > 500 copies/ml, the Cmin corresponded with the C_0, indicating that at least the preceding dose was taken in time. However, non-compliance obviously influences the antiviral efficacy of indinavir and may thus have influenced our findings. (13)

Clearly, our study is limited by the small sample size of 6 children. Still, the included patients were representative for age and race for the patient population using indinavir in our hospital. A difference existed for sex, since all patients in the study population were female. In this study a confounding factor may be the selection of patients with decreasing exposure to indinavir, because children with increasing exposure to indinavir are more likely to suffer from complications and to discontinue treatment before a second PK-sampling can be performed. As a result, these children would not have been included in this type of study. However, we do not

expect this phenomenon to be a major confounder, since only in a small group medication change due to toxicity had happened.

At the time of this case study, random single indinavir plasma levels were not obtained in our hospital as part of the routine care for HIV-1 infected children. It was considered after viral failure occurred, mostly to check for compliance. Currently, in out hospital both full PK samplings for PI levels and random single sample plasma PI levels are part of the routine care for HIV-1 infected children, allowing for optimal dosing and early detection of changed exposure of PI.

In conclusion, our data suggest an effect of time on indinavir exposure in HIV-1 infected children. Both increased and decreased indinavir exposure was observed over time. Sub-therapeutic plasma levels of indinavir were found, which in 2 of 3 cases were associated with viral failure. Regular monitoring of drug levels may prevent subtherapeutic PI dosing in children receiving HAART.

References

1. de Martino M, Tovo PA, Balducci M, Galli L, Gabiano C, Rezza G, et al. (2000). Reduction in mortality with availability of antiretroviral therapy for children with perinatal HIV-1 infection. Italian Register for HIV Infection in Children and the Italian National AIDS Registry. JAMA 284, 190-7.
2. Burger D. M., van Rossum A. M., Hugen P. W., et al. Pharmacokinetics of the protease inhibitor indinavir in human immunodeficiency virus type 1-infected children. (2001). Antimicrob Agents Chemother 45, 701-5
3. Burger D. M., Hoetelmans R. M., Hugen P. W., et al. (1998). Low plasma concentrations of indinavir are related to virological treatment failure in HIV-1-infected patients on indinavir-containing triple therapy. Antivir Ther 3, 215-20.
4. Stein D. S., Fish D. G., Bilello J. A., et al. (1996). A 24-week open-label phase I/II evaluation of the HIV protease inhibitor MK-639 (indinavir). AIDS 10, 485-92.
5. van Rossum A. M., Geelen S. P., Hartwig N. G., et al. (2002). Results of 2 years of treatment with protease-inhibitor–containing antiretroviral therapy in dutch children infected with human immunodeficiency virus type 1. Clin Infect Dis 34, 1008-16.
6. Gisolf E. H., van Heeswijk R. P., Hoetelmans R. W., et al. (2002). Decreased exposure to saquinavir in HIV-1-infected patients after long-term antiretroviral therapy including ritonavir and saquinavir. AIDS 14, 801-5.
7. Huang L., Wring S. A., Woolley J. L., et al. (2001). Induction of P-glycoprotein and cytochrome P450 3A by HIV protease inhibitors. Drug Metab Dispos 29, 754-60.
8. King J. R., Kimberlin D. W., Aldrovandi G. M., et al. (2002). Antiretroviral Pharmacokinetics in the Paediatric Population: A Review. Clin Pharmacokinet 41, 1115-1133.
9. Burger D. M., de Graaff M., Wuis E. W., et al. (1997). Determination of indinavir, an HIV-protease inhibitor, in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 703, 235-41.
10. Hugen P. W., Verweij-van Wissen C. P., Burger D. M., et al. (1999). Simultaneous determination of the HIV-protease inhibitors indinavir, nelfinavir, saquinavir and ritonavir in human plasma by reversed-phase high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl 727, 139-49.
11. Gibaldi, M. (1991). Compartmental and noncompartmental pharamcokinetics. In Biopharmaceutics and Clinical Pharmacokinetics, 4th edn,, pp 14-23. Lea & Febiger, Philadelphia, VS.
12. Burger, D. M. Drug interactions. [Online] http://www.hivpharmacology.com (April 8th 2003, date last accessed)

13. van Rossum A. M., Bergshoeff A. S., Fraaij P. L., et al. (2002) Therapeutic drug monitoring of indinavir and nelfinavir to assess adherence to therapy in human immunodeficiency virus-infected children. Pediatr Infect Dis J 21, 743-7.

Pharmacokinetics of antiretroviral therapy in HIV-1 infected children

Pieter L.A. Fraaij, Jeroen J. A. van Kampen, David M. Burger, Ronald de Groot

Clin Pharmacokinet: in press

Chapter 17

Abstract

Introduction: The initiation of antiretroviral therapy (ART) has resulted in an impressive reduction in the rate of disease progression to AIDS and HIV-1 related deaths in children. However, there are still several major challenges to improve therapy. A major topic that need to be dealt with is to establish the optimal dosage of ART for children. This review presents an overview on the currently available data on the pharmacokinetics of antiretroviral drugs in children and seeks to summarize these data in relation to the currently available guidelines.

Methods: An overview on the currently available peer reviewed articles on the pharmacokinetics of antiretroviral therapy in children is provided. Only original papers in English were used for data collection.

Results: For a substantial number of antiretroviral drugs and especially in young children dose recommendation are still absent. The recommended drug dosages in the guidelines are often different from the dosage in the officially approved drug product label and may deviate between the different guidelines. The groups of children included in the pharmacokinetic studies were often small. High inter- and intrapatient variability for the pharmacokinetic data were observed for all available antiretroviral drugs. The sometimes extremely high variability in pharmacokinetic data seriously hampers the application of fixed antiretroviral drug dosages. This is important concerning that outcome of antiretroviral therapy was found to correlate to plasma drug levels. Therefore therapeutic drug monitoring (TDM) should be considered to optimize of HIV therapy in children.

Conclusions: Dosing of antiretroviral therapy is complicated in children because of high inter- and intrapatient variability of drug levels. In addition, often no or little pharmacokinetic data are available. Application of TDM to optimize the ART dosage should be considered when treating HIV-1 infected children.

Contents

1. Aspects of pediatric HIV-1 infection and antiretroviral therapy
2. Nucleoside and Nucleotide Reverse Transcriptase Inhibitors (NRTI)
    2.1 Zidovudine
    2.2. Lamivudine
    2.3. Didanosine
    2.4. Stavudine
    2.5. Zalcitabine
    2.6. Abacavir
    2.7. Tenofovir
3. Non Nucleoside Reverse Transcriptase Inhibitors (NNRTI)
    3.1 Nevirapine
    3.2. Efavirenz
4. Protease Inhibitors (PI)
    4.1 Indinavir
    4.2 Nelfinavir
    4.3 Ritonavir
    4.4 Saquinavir
    4.5 Amprenavir
    4.6 Lopinavir/ritonavir
5. Fusion Inhibitors (FI)
6. Discussion and conclusions

1. Pediatric HIV-1 infection and antiretroviral therapy

Since the first transition of the human immunodeficiency virus (HIV) from monkey to men in the first quarter of the twentieth century a devastating pandemic has developed.(1, 2) At the end of 2003, approximately 2.5 million children (age < 15 year) were infected by HIV. In 2003 alone, 500,000 children died from HIV/AIDS and another 700,000 were newly infected. Most of these new infections and HIV-related deaths occur in developing countries.(3) Two subtypes of HIV have currently been identified: HIV-1 and HIV-2. In this review we will discuss only subjects who are treated for infection by HIV-1.

The clinical features and laboratory parameters of HIV-1 infection in children are different from those in adults. In children, HIV-1 infection is associated with extremely high viral loads. The development of disease in the infant period is further complicated by the immature stage of the immune system.(4) Consequently, disease progression to AIDS in perinatally infected children is faster than in adults. Virtually all patients die before adolescence when therapy is not started in time.(5-8) The use of highly active antiretroviral therapy (HAART) has resulted in an impressive reduction of disease progression to AIDS.(8, 9)

Antiretroviral therapy (ART) can be divided in four different classes according to their mode of action: nucleoside and nucleotide reverse transcriptase inhibitors (NRTI), non nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI) and fusion inhibitors (FI). HAART generally consists of three or more antiretroviral drugs: mostly a PI and/or NNRTI combined with two NRTI, although no strict definition exists.

We here present a review on the available peer reviewed pharmacokinetic data of antiretroviral drugs in pediatric patients. A literature search was performed using WWW.PUBMED.COM. The search strategy was "HIV and children and 'generic drug name'" (Last search November 29th 2003). Only original papers in English were used for data collection. Data from abstracts and package inserts were not included in this review.

2. Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

The NRTIs were the first drug to treat HIV infection. NRTIs are prodrugs that require intracellular modification to active NRTI-triphosphates (NRTI-TPs) using the kinases from the host cell. The NRTI-TP competes intracellularly with its corresponding endogenous deoxynucleotide-triphosphate for incorporation into viral DNA by HIV reverse transcriptase. Incorporation of NRTI-TP terminates elongation of viral DNA. Hence replication of HIV is prevented.(10) Therefore, intracellular levels of NRTI triphosphate may correlate better with clinical outcome.(11-13) However, intracellular levels of the NRTI-TPs can only be measured in specialized research laboratories and the assays require large amounts of blood unsuitable for use in children. NRTIs are mostly renally excreted. Most other antiretroviral drugs are hepatically metabolized, therefore drug-drug interactions are less common.

2.1.Zidovudine

Zidovudine is rapidly absorbed and peak levels (C_{max}) are observed in less than one hour (T_{max}). Clearance is rapid and biexponential with a terminal half-life ($t_{1/2\beta}$) of 1.5 hours.(14, 15) Zidovudine has good central nervous system (CNS) penetration and the steady state cerebral spinal fluid (CSF)/plasma ratio is 0.24 (±0.07)(16) Balis *et al.* performed pharmacokinetic analysis in 16 children receiving intravenous and/or oral doses of zidovudine. The absolute bioavailability of zidovudine in these patients was 0.68 (SD ±0.25). After 4-8 weeks of intravenous therapy three small groups of patients (n = 2 to 7) started oral zidovudine at 120, 180 and 240 mg/m^2 q6h. Indeed the 50% dosage increase after the conversion of intravenous to oral medication resulted in plasma levels that were comparable to those at the intravenous infusion. In a separate phase I trial the proposed zidovudine dosage for further use of zidovudine was set to 180 mg/m^2 q6h, because this dosage was well tolerated with limited toxic effects.(17) In the study of Balis *et al.* this schedule resulted in a mean AUC of 10.9 µmol*h/l. However, the authors state that this dosing schedule may be inadequate, because plasma concentrations remained above the *in vitro* established threshold of ≥ 1 µmol/l for less than half the dosing interval.(14) Mueller *et al.* studied the pharmacokinetic parameters of orally administered zidovudine using dosages ranging from 60 to 180 mg/m^2 in groups of 10 to 15

patients. The data found were comparable to those in the previously described study by Balis *et al.* The mean AUC increased proportionally with the dose and ranged from 4.89 µmol*h/l at the 60 mg/m^2 dosage level to 11 µmol*h/l at the 180 mg/m^2 dosage level. The intrapatient and interpatient variability in the pharmacokinetics was high (median coefficient of variance 15% and 33%, respectively). Prolonged use of zidovudine did not change drug exposure. No apparent relationship was found between surrogate markers for clinical outcome (CD4+ T-cell count, p24 antigen or neuropsychometric findings) and plasma drug concentrations.(18)

A different zidovudine dosing interval was studied by Bakshi *et al.* In this trial, zidovudine was administered in a 240 mg/m^2 q8h schedule as monotherapy or in combination with zalcitabine in a total of 250 children. The zidovudine mean Cmax was 7.18 (SD ±3.93) µmol/l with a population AUC of 13.5 (SD ±5.4) µmol/l*h, comparable to the AUCs observed in the q6h studies.(19)

In all 3 studies mentioned above, the investigators could not find a relationship between age and plasma levels of zidovudine.(14, 18, 19) This may be due to the restrictions in inclusion of children to those older than 1 year of age. It is possible that younger children are more at risk for deviating plasma levels due to immature renal function. Boucher *et al.* studied the pharmacokinetic differences between infants and neonates. The average clearance (CL) of zidovudine in patients < 14 days was 10.9 ml/min/kg compared to 19 ml/min/kg in older children ($p < 0.001$) and the $t_{1/2\beta}$ was significantly longer (3.12 vs. 1.87 h; $p = 0.0002$)(20) Mirochnick *et al.* used population analysis to estimate zidovudine pharmacokinetics in newborns and infants and found that zidovudine elimination was slow immediately after birth and rapidly increased in the first weeks of life.(21) In another study premature neonates were at risk for increased zidovudine levels because the clearance was strongly diminished. Therefore an initial reduction of the zidovudine dosage to 1.5 mg q12h was recommended for preterm neonates.(22)

Summary

Zidovudine is widely used in HIV-1 infected children. Still only limited data is available on the pharmacokinetics and pharmacodynamics of this drug. Most of the published studies used the now abandoned q6h dosing schedule. Bakshi *et al.* reported on a q8h based regimen, in which the AUC was comparable to those observed in the q6h studies. However, the zidovudine dosage used was higher than currently advised in the guidelines. No data are available on q12h use of zidovudine in children, even though this is currently the most commonly used schedule in clinical practice. Neonates appear to be more prone to high levels of zidovudine than older children.

2.2. Lamivudine

Lamivudine suppresses both the replication of HIV and Hepatitis B virus. In HIV-1 infected children 6 lamivudine dosages, ranging from 1-10 mg/kg, using both intravenous and oral formulation were studied.(23, 24) The terminal half-life ($t_{1/2\beta}$) of lamivudine was 1.7h (range,

0.9-4.2) after intravenous administration. Absorption was rapid (median T_{max} 1.5 h, range 0.5-4) and median fraction of the oral dose absorbed was 0.7 (0.15 – 1.26). C_{max} and AUC increased linearly to the dose for intravenous as well as oral administration.(23, 24) CSF lamivudine concentrations also increased proportionally to the dose, with a median CSF to serum ratio of 0.12 (range, 0.0-0.46). These ratios were not obtained at steady state and may reflect drug exposure in CSF inaccurately.(24) All dose levels attained serum levels above the IC_{50}. Still, the q12h dosing interval of lamivudine is inappropriate in respect to the considerable lower half-life of the pro-drug. C_{trough} levels drawn 12-h after the last dose were below the limit of detection at all dose levels. However, the intracellular triphosphate half life of the drug is 10.5 to 15.5 h in vitro in HIV infected peripheral blood lymphocytes, which may allow for the q12h dosing interval.(24) Patients receiving ≥ 4 mg/kg q12h lamivudine were found to have slightly better virologic response. However, no relationship was found between pharmacokinetic parameters, toxicity and viral response.(23, 24) Based on preliminary data from the concomitant adult trial, the dosages of all children was set to 4 mg/kg q12h.(23, 24)

Age was inversely correlated to clearance, when normalized for body weight (CL/kg) (r = 0.425; p = 0.0022). However when normalized to BSA (CL/m^2), this relation was not observed (r = 0.096; p = 0.50).(24) The inverse relation between age and CL/kg was also found by Sokal *et al* in HBV infected children.(25) In neonates, the pharmacokinetics of lamivudine changed markedly over a period of 7 days: CL/F increased 1.6 fold and the day7/day 1 ratios for C_{max} and AUC were 0.13 and 0.64 respectively. Still the currently advised 2 mg/kg q12h appears to be safe and effective.(26, 27)

Summary

The current lamivudine dosage of 4 mg/kg q12h has been studied in two small groups of children (n=7 to 11) and published by three different investigators.(23-25) Age does not influence lamivudine pharmacokinetic parameters in older children when normalized for BSA. Neonates appear to be more prone to high levels of lamivudine.

2.3. Didanosine

The acid liability of didanosine requires administration with antacids. An enteric-coated (EC) formulation of didanosine is available as alternative for the chewable buffered tablet. Single dose pharmacokinetic parameters of the EC formulation were studied in 8 children and appeared to be similar to those found for the buffered formulation.(28)

Forty-eight patients received a single intravenous dose of didanosine ranging from 20-180 mg/m^2 followed by oral doses ranging from 20-180 mg/m^2 q8h.(29) The plasma clearance of didanosine after 1-h infusion was rapid and biexponential, with a $t_{1/2\alpha}$ of 12 min and $t_{1/2\beta}$ of 0.8h. Didanosine was rapidly absorbed after a dose of antacid, with a T_{max} of 0.5h. The mean bioavailability was 0.19 (SD ±0.17). For 2 patients in the lowest dosage category, didanosine concentrations remained undetectable for the 8 hours after dose. Didanosine had inferior CFS penetration with undetectable didanosine levels in 17 out of 20 CFS samples. The C_{max} and the

AUC increased proportionally with the dose for oral or intravenous administration. For the oral formulation, the mean AUC increased from 0.6 µmol/l*h at 20 mg/m^2 to 6.1 µmol/l*h at 180 mg/m^2. A correlation between plasma concentrations of didanosine, decline of p24 antigen levels and improvement of intelligence quotient score was found.(29)

A study on the long-term use of didanosine with comparable dosage as above in combination with stavudine was performed in 85 children.(18) Despite high interpatient variability, comparable pharmacokinetic results were found. In patients with very low or undetectable plasma levels doubling of the amount of antacid increased the AUC. The pharmacokinetic assessment of didanosine was repeated in 12 patients with the same dose of didanosine and antacids 3 to 23 months after start of therapy. After this period, the AUC had increased in 9 patients (median increase from 2.57 to 4.23 µM*h (p= 0.08)). In contrast to the previous study, Mueller *et al.* found no relation between didanosine concentration and clinical outcome.(30) However, the didanosine AUC did correlate with outcome in another study on didanosine as part of HAART.(31)

Studies in adults suggest that didanosine can be dosed in a once daily schedule.(32, 33) Abreu *et al.* compared the bioavailability of didanosine at 180 mg/m^2 once daily to that at 90 mg/m^2 twice daily in 24 children. They found that the relative bioavailability was 0.95 ±0.49. The authors suggest that once daily dosing of didanosine may be applied in the pediatric population as well.(34)

Both in the study of Balis *et al.* and Muller *et al.* no relation was found between age and pharmacokinetic parameters for children > 3 years old.(18, 29) Two studies were performed in neonates. In both studies highly variable pharmacokinetics were found.(35, 36) Rongkavilit *et al.* reported that the pharmacokinetics of didanosine do not change between week 2 and week 4 post gestational age. However, a trend towards lower systemic clearance in neonates compared to older children was found. Still, the authors of this study do not recommend dose reductions, because of the small sample size and markedly observed variability.(36)

In HIV-1 infected adults, the AUC for didanosine was reduced by approximately 50% with food compared to fasting conditions.(37, 38) Therefore didanosine needs to be ingested on an empty stomach. In children, didanosine levels were determined during fasting and with food in two groups of patients receiving 50 mg/m^2 q12h or 150 mg/m^2 q12h didanosine. Administration of food significantly reduced the mean absorbed fraction of didanosine (0.19 (SD ±0.09) vs. 0.27 (SD ±0.13), p< 0.0001). However, the lower fraction absorbed with food was offset by the absorption rate becoming rate limiting for elimination, resulting in similar AUCs. (Mean AUC normalized to 100 mg/m^2 when fasted 835.9 µg/L*h (SD ±465.8) vs. with food 796.3 µg/L*h (SD ±367.5; p= 0.22)).(39)

Interactions between didanosine and other NRTIs were studied. Didanosine can be coadministered with stavudine without affecting each others pharmacokinetics. In contrast,

zidovudine coadministration reduced the didanosine AUC by 19% (5.9 vs. 4.8 µmol/l*h; $p < 0.05$).(18, 40)

Summary

The analysis of the pharmacokinetic parameters of didanosine is complicated by the high inter- and intrapatient variability. Most pharmacokinetic data on the use of didanosine are obtained from patients using the buffered form. The currently frequently prescribed EC-coated form of didanosine appears to be bioequivalent. However, this was found in a very small study group. Limited pharmacokinetic evidence is available on once daily use of didanosine in children as suggested in the European guidelines. Bioequivalence between once and two daily dosing schedules was published. Still a high variability was observed and the dosage was different from the dose recommended in the guidelines. In children the didanosine exposure did not change significantly when administered with or without food. Therefore the currently advised food restriction may be unnecessary. A trend towards lower systemic clearance was found in neonates.

2.4. Stavudine

Stavudine was administered at doses of 0.125 to 4 mg/kg/day in 37 children. Each subject was given a single intravenous dose (0.5-2 mg/kg) before oral medication. After intravenous administration, the elimination of stavudine was rapid with a $t_{1/2}$ of 1.24h for 1 mg/kg and 0.82h for 2 mg/kg. The total drug exposure was linearly related to the intravenous and oral dose administered. Absolute bioavailability ranged from 61% to 78%. The median time to T_{max} was less than 1 hour for both the first oral dose and at week 12. Plasma accumulation of the drug between day 1 and week 12 did not occur. CFS stavudine concentrations obtained in 7 patients ranged from 16% to 97% of the concomitant plasma concentrations. Compared with adult patients receiving the same weigh-adjusted doses, children had a lower C_{max} and AUC, and more rapid stavudine elimination. Children taking 1 or 2 mg/kg/day (mean AUCs: 628 µg*h/l (SD ±255) and 1629 µg*h/l (SD ± 480)) had similar mean stavudine exposure as adults using 0.5 or 1 mg/kg/day (mean AUCs: 565 µg*h/l (SD ±103) and 1173 µg*h/l (SD +/- 370)).(41, 42)

In eight neonates using 1 mg/kg stavudine q12h combined with didanosine and nelfinavir the plasma drug concentration was comparable to the data observed in older children.(36)

Kline *et al.* found a possible relationship between the AUC of stavudine and viral response.(43) In contrast, Fletcher *et al.* found no relation between viral response and plasma levels of stavudine when used as a composite of HAART.(31)

Summary

The peer reviewed data on stavudine are based on a mg per kg schedule and not on fixed dosages above 30 kg as recommended in the guidelines and FDA approved drug label. A dosage for all children based on BW may therefore be more appropriate. In a small group of neonates stavudine exposure was comparable to that in older children.

2.6. Abacavir

Twenty-two patients received single doses of abacavir of 4 mg/kg and 8 mg/kg. Interpatient variability in pharmacokinetics was high for both dosing groups. Abacavir was rapidly absorbed with a T_{max} < 1.5 hours. This is comparable to data in adults using a similar dosage, suggesting equal absorption rates in children and adults. However, the AUC was 45 to 48% lower and $t_{1/2\beta}$ was approximately 21 to 33% shorter in children.(44) In a study by Kline *et al* the steady state pharmacokinetic parameters of abacavir at 4 mg/kg and 8 mg/kg q12h were evaluated in 38 and 45 children respectively. Drug exposure in children was comparable to that in adults. Abacavir AUC values at both doses were similar across the age range, indicating absence of age-dependency on the pharmacokinetic behavior of the drug. The 8 mg/kg dose yielded a mean AUC value of 9.8 mg/L*h, comparable to data reported in adults using a dose of 300 mg. However, considerable variability was observed with a coefficient of variation for the mean AUC of 47%.(45)

Summary

Two studies in comparatively large numbers of patients have evaluated the pharmacokinetics of abacavir in children. The recommended abacavir dose of 8 mg/kg q12h results in pharmacokinetic parameters comparable to those in adults receiving 300mg q12h. Currently, no data are available on abacavir use in neonates.

2.7 Tenofovir

Tenofovir disoproxil fumarate (DF) is a potent nucleotide analog reverse transcriptase inhibitor. NtRTI, already possess one phosphate group and are intracellularly converted to their active NtRTI-diphosphate form.

Hazra et al. performed a study on tenofovir DF pharmacokinetics in18 treatment experienced HIV-infected children requiring a change in ART. The age range in this study was narrow since the children were required to swallow pills (median age 11.9 years, range 6.2 to 16.2 years). Single-dose (day 0) and steady state (week 4) tenofovir DF pharmacokinetics were obtained in 18 and 16 patients respectively. Steady state pharmacokinetics were studied after other ART was added. As target dose, 175 mg/m^2, was chosen to match the approved 300 mg dose in adults. The median day 0 dose was 208 mg/m^2. Oral tenofovir was rapidly absorbed with a median T_{max} of 1.3h. The total geometrical mean single dose drug exposure was 2.50 µg*h/L (range 1.06-3.63). At 48 hours after administration the mean plasma concentration of tenofovir was 9.87 µg/L, and plasma levels were detectable in 16 out of the 17 patients. Even though the first dose administered to children was higher than the target adult-equivalent, the mean AUC was 34% lower compared to the values reported in adults. The steady-state geometrical mean AUC was 2.920 µg*h/L (range 1.8-5.59), which is significantly higher than the AUC obtained at the single dose evaluation ($p < 0.001$) and comparable to that in adults.(46) It can not be excluded that the increase in the AUC was the result of drug accumulation. However, a study in adults treated with tenofovir TD monotherapy did not demonstrate unexpected drug

accumulation.(47) The higher drug exposure may well be attributable to an effect of the additional ART (i.e. ritonavir) present at steady state.

Summary

Pharmacokinetics obtained at a single median dose of 208 mg/m^2 revealed lower drug exposure in children compared to that reported in adults. In contrast, steady state pharmacokinetic data in combination with other ART were comparable to those observed in adults. Further studies on a possible interaction between other ART and tenofovir FD need to be performed. Currently, no data are available on the use of tenofovir FD in children less than 6 years of age.

3. Non Nucleoside Reverse Transcriptase Inhibitors (NNRTI)

NNRTIs are potent inhibitors of HIV-1 replication, but generally not effective against HIV-2. They act by non-competitive, allosteric inhibition of HIV-1 reverse transcriptase. NNRTI are hepatically metabolized by CYP enzymes, in particular CYP3A and CYP2B6. Furthermore, efavirenz and nevirapine induce CYP 3A, which can result in significant drug-drug interactions.(48) Three NNRTIs are currently available: nevirapine, efavirenz and delavirdine. Peer reviewed studies are available of nevirapine and efavirenz use in children, but not of delavirdine.

3.1. Nevirapine

Luzuriaga *et al.* studied the pharmacokinetics of nevirapine in children receiving single doses of 7.5, 30 and 120 mg/m^2 (n= 9) and multiple doses between 120-240 mg/m^2/day (n = 21). The C_{max} and AUC increased proportional to the administered single dose. In the multiple dose group a 1.5 to 2-fold increase in the nevirapine clearance was found compared to the single dose group. This resulted in lower than expected $C_{through}$ levels. Subsequently the nevirapine dosage was increased to 200 mg/m^2 q12 in children less than 9 years old and to 120 mg/m^2 q12h children over 9 years to attain C_{trough} levels comparable to those observed in adults. Nevirapine clearance was found to be more rapid in young children and to decrease with age. Overall the clearance remained high in children compared to adults.

Considerable inter- and intrapatient variability was observed in C_{trough} levels. The C_{trough} levels in responders (median 10, range, 8.9-21.5 µM) tended to be higher than in non responders (median 8, range, 5.9-31.4 µM). However, these differences were not statistically significant and the three highest mean nevirapine C_{trough} levels were measured in non-responders.(49)

In contrast to older children, the elimination was found to be diminished in infants, with a $t_{1/2}$ ranging from 36.8 to 65.7 h, after their mothers received a single dose of 200 mg nevirapine during labour. When infants also received 2 mg/kg nevirapine as a single dose 48-72 h after birth, plasma concentrations maintained > 100 µg/L (10 times IC_{50}) up to 7 days of life.(50) However, when maternal nevirapine administration started at 38 weeks of gestation and continued through labour, plasma nevirapine concentrations of the infants were lower than

expected, with plasma levels in half of the infants falling below the 100 µg/L target at day 7. This was possibly caused by *in utero* induction of CYP3A in the fetus. Therefore an additional nevirapine dose on day 4 or 5 after birth was suggested for infants of mothers who use nevirapine during pregnancy.(51)

Summary

No peer reviewed data are available on nevirapine dosing based on BW as suggested in the US guidelines and the FDA approved drug label. Single dose and steady state pharmacokinetics of nevirapine have been assessed in small study groups using different nevirapine dosages based on BSA. Because of the rapid clearance in children compared to adults the dosage needed to be increased to 120-200 mg/m^2. In neonates nevirapine clearance is diminished, resulting in plasma levels above 100 µg/L up to 7 days. However, prepartum maternal nevirapine use may lead to accelerated nevirapine metabolism in the fetus necessitating an extra dose to maintain target plasma drug concentrations.

3.2. Efavirenz

Efavirenz pharmacokinetics have been studied in 50 children who also received nelfinavir and NRTIs. Both nelfinavir and efavirenz are metabolised by the CYP450-enzyme system, which could potentially bias the pharmacokinetic data. In this study, the efavirenz dosage was calculated to an adult dosage of 600 mg q24h. The dosage was hereafter adjusted to target adult reference values of the AUC. The AUC was determined at weeks 2 and 6. In an intention to treat analysis, the AUC values were within the target range in 44% of the children at week 2 (mean dose 11.7 mg/kg/day) and 56 % of the children at week 6 (mean dose 14.2 mg/kg/day).(52)

The same study design was used in another analysis of the pharmacokinetics of the liquid formulation of efavirenz (n=18).(53) Efavirenz was administered at 120% of the capsule dose, based on an expected 20% lower bioavailability as observed in adult volunteers. Upon pharmacokinetic analysis at mean dosage of 15.7 mg/kg/day 4 of the 18 children had AUC values within the target range. In 11 children the dosage needed to be increased. In this study efavirenz or nelfinavir AUC values did not correlate with virologic success.(53) In contrast, Marzolini *et al.* did find a relation in adults between the efavirenz plasma levels and viral response rate.(54)

Summary

A dosage of approximately 14 mg/kg per day resulted in drug levels within the adult references ranges. This dosage corresponds with the advise in the guidelines. However, at this dosage level not all patients had adequate drug levels. The liquid formulation of efavirenz has lower bioavailability than the capsules and requires dosages over 120% of the capsulated formulation. No information is present on the effect of age on pharmacokinetic parameters. Efavirenz has not been studied in children less than 3 years of age.

Table 1. Dose recommendations for nucleoside reverse transcriptase inhibitors.

Medication	Guidelines USA[1]	Guidelines Europe[2]	FDA approved prescription[3]
Abacavir (tablet, solution)	1m-3m: under study 8 mg/kg q12h.		
	Children: 8 mg/kg q12h.	Children: idem.	Children (3m-16y): idem.
Didanosine (tablet, delayed release capsule, pediatric powder and buffered powder for solution)	< 90d: 50 mg/m^2 q12h.		2w-8m: 100 mg/m^2 q12h.
	Children: 120 mg/m^2 q12h (range 90-150 mg/m^2 q12h).	Children: 240 mg/m^2/day (q24h or q12h). Children (> 60kg): 400 mg/day (q24h or q12h).	Children (> 8m): 120 mg/m^2 q12h.
	No dose recommendations for delayed release capsule.	Delayed release capsule q24h for older children.	Delayed release capsule not approved.
Emtricitabine (capsule)	No dose recommendations.	Idem.	Not approved.
lamivudine (tablet, solution)	< 30d: 2 mg/kg q12h.	< 30d: idem.	
	Children: 4 mg/kg q12h.	Children: idem. Children (> 60kg): 150 mg q12h.	Children (3m-16y): idem.
stavudine (capsule, extended release capsule, solution)			< 13d: 0.5 mg/kg q12h
	Children (< 30kg): 1 mg/kg q12h. Children (30-60kg): 30 mg q12h. Children (≥ 60kg): 40 mg q12h.	Children: idem.	Children: idem.
	No dose recommendations for extended release capsule.	Idem.	Extended release capsule not approved.
tenofovir (tablet)	No dose recommendations.	Idem.	Not approved.
zalcitabine (tablet)	0.01 mg/kg q8h.	0.015 mg/kg q12h.	Not approved.
zidovudine (tablet, capsule, solution, IV fluid)	Premature infants: 2 mg/kg q12h (po) or 1.5 mg/kg q12h (IV). Increase dosing to q8h at 2w (g.a. ≥ 30w) or at 4w of age (g.a. < 30w).		
	Neonates: 2 mg/kg q6h (po) or 1.5 mg/kg q6h (IV).	Neonates: 2 mg/kg q6h.	Neonates: 2 mg/kg q6h (po) or 1.5 mg/kg q6h (IV).
	Children: 160 mg/m^2 q8h (range 90-180 mg/m^2 q6h to q8h) or 120 mg/m^2 q6h (IV) or 20 mg/m^2/h (IV).	Children: 180 mg/m^2 q12h (po) or 120 mg/m^2 q6h (IV) or 20 mg/m^2/h (IV).	Children (6w-12y): 160 mg/m^2 q8h.

[1] Guidelines for the use of antiretroviral agents in pediatric HIV infection, 20 January 2004, http://www.aidsinfo.nih.gov

[2] PENTA guidelines for the use of antiretroviral therapy in paediatric HIV infection, HIV Medicine 2002 3(3) 215-26

[3] Latest approved drug label (last search 02-03-2004), http://www.accessdata.fda.gov/scripts/cder/drugsatfda

Table 2. Dose recommendations for non nucleoside reverse transcriptase inhibitors.

Medication	Guidelines USA[1]	Guidelines Europe[2]	FDA approved prescription[3]
delavirdine (tablet)	No dose recommendations.	Idem.	Not approved.
efavirenz (capsule, tablet)	< 3y: no dose recommendations.	Idem.	Not approved.
	10 to < 15kg: 200 mg q24h. 15 to < 20kg: 250 mg q24h. 20 to < 25kg: 300 mg q24h. 25 to < 32.5kg: 350 mg q24h. 32.5 to < 40kg: 400 mg q24h. ≥ 40kg: 600 mg q24h.	Idem.	Idem.
nevirapine (tablet, solution)	< 2m: under study 5 mg/kg or 120 mg/m^2 q24h for 14 days, followed by 120 mg/m^2 q12h for 14 days, followed by 200 mg/m^2 q12h.		
	Children: 120 mg/m^2 q24h for 14 days, followed by 120-200 mg/m^2 q12h.	Children: 150-200 mg/m^2 q24h for 14 days, followed by 150-200 mg/m^2 q12h.	Children (2m-8y): 4 mg/kg q24h for 14 days, followed by 7 mg/kg q12h. Children (> 8y): 4 mg/kg q24h for 14 days, followed by 4 mg/kg q12h.
	Or Children (< 8y): 7 mg/kg q24h for 14 days, followed by 7 mg/kg q12h. Children (> 8y): 4 mg/kg q24h for 14 days, followed by 4 mg/kg q12h.		

[1] Guidelines for the use of antiretroviral agents in pediatric HIV infection, 20 January 2004, http://www.aidsinfo.nih.gov

[2] PENTA guidelines for the use of antiretroviral therapy in paediatric HIV infection, HIV Medicine 2002 3(3) 215-26

[3] Latest approved drug label (last search 02-03-2004), http://www.accessdata.fda.gov/scripts/cder/drugsatfda/

Table 3. Dose recommendations for protease inhibitors.

Medication	Guidelines USA[1]	Guidelines Europe[2]	FDA approved prescription[3]
amprenavir (capsule, solution)	Not recommended for children < 4y		Not approved for children < 4y
	4-12y and 13-16y (< 50kg): 22.5 mg/kg q12h or 17 mg/kg q8h (solution), or 20 mg/kg q12h or 15 mg/kg q8h (capsule).	Children: 20 mg/kg q12h. Increase dose for solution.	4-12y and 13-16y (< 50kg): 22.5 mg/kg q12h or 17 mg/kg q8h (solution), or20 mg/kg q12h or 15 mg/kg q8h (capsule). 13-16y (> 50kg): 1400 mg q12h (solution).
atazanavir (capsule)	No dose recommendations.	Idem.	Not approved.
fosamprenavir (capsule)	No dose recommendations.	Idem.	Not approved.
indinavir (capsule)	Not recommended for neonates.	Idem.	Not approved.
	Children: under study 500 mg/m^2 q8h. Patients with small BSA may require 300-400 mg/m^2 q8h.	Children: 500 mg/m^2 q8h	
lopinavir/ritonavir (capsule, solution)	6m-12y (7-15kg): 12/3 mg/kg q12h. 6m-12y (15-40kg): 10/2.5 mg/kg q12h. > 40kg or > 12y: 400/100 mg q12h. Or 230/57.5 mg/m^2 q12h.	225/56.25 to 300/75 mg/m^2 q12h.	6m-12y (7-15kg): 12/3 mg/kg q12h. 6m-12y (15-40kg): 10/2.5 mg/kg q12h. > 40kg or > 12y: 400/100 mg q12h.
nelfinavir (tablet, powder)	Neonate/infant: under study 40 mg/kg q12h.	Infants: 150 mg/kg/day.	
	Children: 20-30 mg/kg q8h (up to 45 mg/kg q8h is routinely used). Children (> 6y): under study 50-55 mg/kg q12h.	Children: 55-60 mg/kg q12h.	Children (2-13y): 20-30 mg/kg q8h.
ritonavir (capsule, solution)		Infants: 900 mg/m^2/day.	
	Children: 250 mg/m^2 q12h and stepwise increase to 400 mg/m^2 q12h over 5 days.	Children: idem.	Children: idem.
saquinavir (hard capsule, soft capsule)	Under study 50 mg/kg q8h.	50 mg/kg q8h (soft capsule).	Not approved.

[1] Guidelines for the use of antiretroviral agents in pediatric HIV infection, 20 January 2004, http://www.aidsinfo.nih.gov

[2] PENTA guidelines for the use of antiretroviral therapy in paediatric HIV infection. HIV Medicine 2002 3(3) 215-26

[3] Latest approved drug label (last search 02-03-2004), http://www.accessdata.fda.gov/scripts/cder/drugsatfda/

g.a. = gestational age

4. Protease Inhibitors (PI)

HIV protease enzyme is required for post-translational cleavage of the Gag and Gag-Pol polyproteins into smaller functional proteins. PIs block this enzyme, leading to non-infectious immature virion formation.(55) PIs are hepatically metabolized by CYP enzymes and undergo oxidative metabolism by CYP3A. Additional CYP enzymes may further metabolize individual PIs. In addition, PIs act as inducers or inhibitors of the CYP system and are therefore highly susceptible to drug-drug interactions.(56)

4.1. Indinavir

Mueller *et al* assessed the pharmacokinetic profile of indinavir suspension and capsules at different dosages in 43 children. Indinavir was rapidly absorbed after administration of both the capsules and solution (T_{max} between 0.5 and 2 hours). The AUC in the three patients treated with capsules at 500 mg/m^2 q8h was comparable to values obtained in adults treated with 800 mg q8h. The $t_{1/2}$, however, was shorter in children, resulting in lower C_{trough} levels. Pharmacokinetic analysis of the indinavir solutions revealed marked interpatient variability and a substantially lower bioavailability compared to the capsules. Subsequently, therapy was changed to capsules in all children. The indinavir dosage was administered at 500 mg/m^2 q8h for the entire cohort. However, due to the occurrence of hematuria the dosage was decreased to 350 mg/m^2 q8h.(57)

In another study, indinavir pharmacokinetics were analyzed in 12 children receiving 500 mg/m^2 indinavir q8h in combination with stavudine and didanosine. Indinavir doses were adjusted to maintain extrapolated C_{trough} levels at 0.1 mg/L. Nine patients needed dose adjustment to 500 mg/m^2 q6h, because of low C_{trough} levels. A high interpatient variability in pharmacokinetics was observed with a CL/F ranging from 0.6 to 3.5 L/h/kg. CFS indinavir concentrations were obtained in 4 children and levels ranged from 0.15 to 0.98 mg/l.(58)

In another study by Gatti *et al* the BSA and AUC were negatively correlated in 11 children receiving 500 mg/m^2 indinavir q8h in combination with lamivudine and zidovudine (r = 0.73; p = 0.012). Still the median C_{min} was lower than reported in adults. The authors suggest that a dose reduction or more frequent dose interval is appropriate in children with small BSA.(59) In contrast, Burger *et al.* found a significant higher clearance in children below 6 years of age than in older children. (2.5 vs.1.0 L/h*kg; p = 0.03).(60) The C_{max} was higher in patients with side effects, although this was statistically not significant (15.3 (S.D.+/-8.2) vs. 9.8 (S.D.+/-4.4 mg/l)). A trend toward higher immunological efficacy in patients with higher indinavir exposure was observed. Plasma levels and viral efficacy could not be compared, because all patients had a good viral response.(59)

Burger *et al.* studied the indinavir pharmacokinetics in 27 children when coadministered with zidovudine and lamivudine. Indinavir doses were based on the metabolic weight (MW). MW was chosen on the assumption that in children the clearance is higher than in adults, even when corrected for body weight. Dose changes were made and pharmacokinetic analysis

repeated if patients had an indinavir AUC below or above adult reference values. Most children started indinavir at an initial dosage of 33 mg/kg MW, which resulted in low AUC values in 11 children. Subsequently, the dosage was increased to 50 mg/kg MW q8h. In 5 children the dosage needed to be even further increased to 67 mg/kg MW. After 6 months of therapy, 5/11 children with an AUC < 20 mg/l*h had a detectable viral load against none of the 11 children with an AUC > 20 mg/l*h. The authors conclude that it is preferable to adjust indinavir dosage to obtain target AUCs, rather than to give a fixed dosage.(60)

To study changes in indinavir exposure in time, pharmacokinetic analysis was repeated in 6 children after at least 2 years of treatment with indinavir. All children had an AUC above the target level of 15 mg/h*l on the first day, but at the second pharmacokinetic analysis 3 children had an AUC below 15 mg/h*l. This was associated with viral failure in 2 patients. The investigators suggest to routinely perform TDM to prevent viral failure.(61)

The combination of low dose ritonavir with indinavir allows for decreased indinavir dosing and thus an easier to take medication regimens. Indinavir (500 mg/m^2) plus ritonavir (100 mg/m^2) was evaluated in 4 children. This regimen resulted in adequate values for the AUC, C_{max} and C_{min} in three children, but in extremely high levels in one child. Two children had significant side effects. The authors conclude that the combination from a pharmacological point of view is a good alternative. However, they warn that the substantial toxicity needs further follow-up.(62)

Summary

Pharmacokinetic parameters were obtained in a relatively small group of patients using the same dosage of indinavir. The guidelines currently recommend an indinavir dosage of 500 mg/m^2 q8h. However, in the studies using this dosage, dose adjustments were frequently necessary, because of toxicity or suboptimal drug levels. In a pilot study, addition of ritonavir to indinavir reduced the dosing interval of indinavir and adequate drug levels were obtained. Extremely high plasma levels in one child and significant toxicity were observed in 2 of the 4 children. The high variability in drug levels complicates the use of indinavir. TDM is compulsory for safe use of indinavir in children.

4.2. Nelfinavir

Krogsted *et al.* studied single-dose followed by multiple-dose pharmacokinetics of nelfinavir powder formulation in children. Single doses were started and sequentially escalated to achieve AUC values between 50 and 200% of the median AUC observed in adults using 500 or 750 mg nelfinavir. AUC values within this target were obtained in children receiving 20 mg/kg q8h nelfinavir. Pharmacokinetic parameters of the powder and the tablet formulation were compared in 6 children using nelfinavir dosages of 10 or 20 mg/kg. The bioavailability of both formulations was equal with a powder to tablet ratio for the AUC of 1.04 (SD ±0.68). Subsequently, multiple dosing using both nelfinavir formulations was started at 20–30 mg/kg q8h. Pharmacokinetic sampling was again performed in 19 children. With a mean dose of 20 ±3 mg/kg, a median AUC of 16 (IQR 13-20) was obtained, comparable to the data in the single

dose study. Thus on a body weight adjusted basis, children require higher nelfinavir doses than adults receiving 500-750 mg nelfinavir.(63) Starr *et al.* found comparable nelfinavir pharmacokinetics in 50 patients using a combination of nelfinavir, efavirenz and NRTIs. At an average nelfinavir dosage of 24.6 mg/kg q8h (mean AUC 19.9 mg*h/L) 40 of the 50 patients had an AUC above the minimum adult target range of 10 mg*h/L.(52)

Bergshoeff *et al* studied factors influencing nelfinavir pharmacokinetics in 24 children. Children less than 2 years of age showed a tendency towards lower nelfinavir exposure compared to children over 2 years of age. Based on adult data a minimum target AUC of 12.5 mg/l*h was set. Retrospectively children were stratified in three dose groups (20, 30 and 40 mg/kg q8h). In the lowest dose group 80% of the children had AUC values below the cut off value, compared to 29% and 40% in the 30 and 40 mg/kg dose group ($p > 0.1$). While receiving a similar dosage in mg/kg, children with a nelfinavir AUC < 12.5 mg/l*h received a significant lower median nelfinavir dosage when based on BSA ($p = 0.04$). Moreover, a nelfinavir dosage > 650 mg/m^2 q8h predicted a AUC value > 12.5 mg/l*h with optimal sensitivity (79%) and specificity (67%)(64).

Litalien *et al* performed pharmacokinetic analysis on nelfinavir in 14 infants, who underwent 18 intensive pharmacokinetic samplings. In this study nelfinavir was dosed higher than in older children (120 mg/kg/day). Still, all AUC values in the first 4 infants enrolled (all < 4 months) were well below those observed in adults and the nelfinavir dosage was further increased to 150 mg/kg/day. At the same time the dose interval was changed to twice daily. Hereafter, C_{min}, C_{max} and AUC were still below the 10th percentile for adults in 9, 3 and 5 individual samplings, respectively.(65)

In order to simplify therapy, the nelfinavir regimen may be changed from q8h to q12h. In the first study in 18 children on this subject, older children had significantly higher drug levels in the q12h regimen compared to the q8h regimen. However such a difference was not observed in the younger children (< 25 kg).(66) In another investigation comparing a q8h nelfinavir (30 mg/kg) regimen to a q12h regimen (45 mg/kg) in 12 patients the AUC in the q8h group was 90.5 mg*h/l vs. 71.9 mg*h/l in the q12h group. The nelfinavir levels were all well above adult levels. However, a 7-fold interpatient variability was observed for drug exposure, indicating that dose adjustments based on plasma levels may be necessary for both regimens.(67) Finally, Gatti *et al* investigated $C_{through}$ and C_{max} levels for nelfinavir administered 2 (50 mg/kg) or 3 times daily (20-30 mg/kg) in 35 patients. No significant differences were found between both parameters. However, 7 of 14 children in the q12h group had $C_{through}$ levels below 1 mg/L (estimated IC_{50} cut off value), compared to only 1 of the 11 children in the q8h group. The clinical importance of this finding is unclear, since no relation between the pharmacokinetic parameters and viral outcome was found.(68)

Interestingly in three studies in children nelfinavir levels did not relate to therapy outcome.(52, 64, 68) In contrast, in adults a strong relationship between nelfinavir levels and viral

suppression has been described.(69, 70) Moreover, in adults adjustment of nelfinavir levels based on TDM improved the virological outcome.(71)

Summary

A substantial amount of data is present on pharmacokinetic parameters of nelfinavir in children. Children require higher nelfinavir doses than adults and a dosage range between 20 and 30 mg/kg q8h is recommended. However, to obtain drug levels within reference values the lowest dosage levels appear to be inappropriate. Young children are more at risk for low drug levels than older children. This is especially the case in neonates in whom the nelfinavir dosage should be substantially increased. Nelfinavir pharmacokinetics allow for twice daily dosing with adequate drug levels. However, marked interpatient variability complicates dosing. Because of the marked differences in drug levels TDM is proposed.

4.3. Ritonavir

The pharmacokinetics of ritonavir solution were evaluated at four dose levels (250-400 mg/m^2) in 44 children. Pharmacokinetic analysis was performed twice, at day 7 and 28. No significant changes between both study days were observed. The T_{max} of ritonavir was obtained within 2 to 4 hours and exceeded the EC_{90} of 2.1 mg/L in all dose levels. Increases in plasma concentrations were proportional with the dose. In the 10 patients included in the 400 mg/m^2 dose group, the median plasma concentrations were above the EC_{90} during the whole 12 hour period. However, considerable interpatient variability was observed, with a range for the AUC values of 21.4 to 219.3 mg*h/l. No significant effects of age, gender or BW were observed for children over 2 years old. In two 18 months old children the pharmacokinetic parameters were comparable to those in older children. However, two 6 months old infants treated with 250 mg/m^2 ritonavir had very high CL/F values and subsequent low drug exposure.

In a different study the relation between ritonavir plasma levels and viral efficacy was studied in 31 children. Patients received HAART including ritonavir at a dosage between 300 to 400 mg/m^2. Plasma levels were obtained twice. The first time 4 weeks after start of ritonavir (observation 1) and the second time after at least 3 months of ritonavir use (observation 2). For each observation C_{trough} and plasma levels 2 hours after intake (T2-levels) were obtained. A significant increase in the C_{trough} between both observations was found (1.64 mg/l (range, 0-6.36 mg/l) vs. 5.9 mg/l (range, trace-18.1 mg/l, p = 0.0228). No difference was found for the T2-levels. The authors explain the increase in C_{trough} levels by better compliance or by metabolic or kinetic variation, related to disease evolution or a decrease in enzymatic reduction. The ritonavir C_{trough} levels were compared according to viral response. Patients were considered complete responders, partial responders or non-responders. The median C_{trough} levels for each group were 3.17 (range, 0.29-7.4), 2.52 (range, 0.15-7.85) and 1.04 (range, 0-5.04) mg/l, respectively. A wide range in plasma levels and intra individual variability were observed. Still, a significant difference between the C_{trough} in the responder and partial responder group compared to the non-responder group was found. No differences were observed for the T2-levels. Interestingly, despite the relation between C_{trough} and viral response, the C_{trough} in the

responder group often did not reached the EC_{90} concentration of 2.4 mg/l. (72)

Summary

The ritonavir dosages have been studied in a relative small group of children. The dosage of 400 mg/m^2 resulted in median drug levels above the target value. However, considerable variability in drug levels was observed and TDM appears to be imperative. Ritonavir is cleared more rapidly in children less than one year old.

4.4.Saquinavir

Two saquinavir formulations are available: soft and hard gel capsule. The hard gel capsule is not to be used as a sole protease inhibitor. In a complex study in 35 children by Grub *et al.* pharmacokinetic data were obtained for saquinavir alone and in combination with nelfinavir after single dose, short term steady state and long term use. In this study, saquinavir was initially started at a dose of 33 mg/kg, twice the dose recommended for adults on a mg/kg base. However, after analysis of the single dose pharmacokinetics a high clearance was found and the dosage was increased to 50 mg/kg. Despite this dose increment the geometrical mean (GM) AUC of 1.93 mg/l*h (range, 0.59-7.16) obtained after 4 weeks of treatment was again below adult reference values. This suboptimal drug exposure is probably due to lower bioavailability and higher CL/F of saquinavir in children. After approximately 2 years of treatment the CL/F remained unchanged, but the CL/F/kg decreased 25%, increasing the GM AUC to 2.52 mg/l*h (1.07-5.23). The combination of saquinavir and nelfinavir decreased the saquinavir clearance, resulting in 2- to 3-fold higher steady state AUC levels of saquinavir. (GM AUC: 3.69 (0.52-19.2). For all dosages a significant correlation between the average C_{trough} concentration and durable viral load suppression was found, with a mean C_{trough} concentration above 0.2 mg/l resulting in optimal viral suppression.(73)

The pediatric dose used in this study was 50 mg/kg with as maximum the adult dose of 1200 mg. Therefore children > 24 kg were treated with a lower dose based on BW. The impact of the upper dose limit of plasma exposure was investigated in a substudy. Patients received either 1200 mg saquinavir or a weight adjusted dose without upper limit. For the unrestricted group, this resulted in an average dose of 2250 mg (range, 1400-3600 mg). Unrestricted dosing resulted in higher saquinavir exposure with a GM AUC $_{0\text{-}12h}$ of 5.473 (2.16-12.3) mg*h/ml compared to 2.957 (119-5.71) for the fixed dose.(73)

Summary

The pharmacokinetics of saquinavir are different in children from those in adults. Saquinavir alone at a dosage of 50 mg/kg q12h as recommended in the guidelines does not result in desired plasma drug levels. Addition of a second PI improves drug levels. Saquinavir can only be safely used when TDM is applied.

4.5. Amprenavir

Due to the quantity of polypropylene glycol, amprenavir cannot be used in younger children. The original data of amprenavir pharmacokinetics in children have been obtained but were not presented in a peer reviewed paper. In the review by King *et al.*, pharmacokinetic parameters of amprenavir after single and multiple dose in 20 children were presented. In the multiple dose group, patients received 15 mg/kg q8h amprenavir resulting in mean AUC of 8.7 mg/l*h. In both the single and multiple dosage groups, the C_{min} was around the EC_{90} (estimated to be 0.23 mg/l).(74) Boosting of amprenavir drug levels with ritonavir has been suggested to increase the C_{min} and decrease the pill burden and thus the amount of polypropylene glycol.(75)

Currently, amprenavir is mostly used as salvage therapy. A study on the possible interactions in a salvage regimen was performed in a small group of children using amprenavir in combination with efavirenz or delavidine and NRTI.(76) Efavirenz decreased amprenavir levels to below the limit of detection within 4 hours. Plasma levels improved drastically after ritonavir was added. In combination with delavirdine 10-fold higher amprenavir levels were observed compared to values found in adults using amprenavir monotherapy.(76)

Summary

Only limited data is available on amprenavir use in children. Amprenavir is mostly used in salvage regimens. Due to the high probability of interaction with other ART, drug levels should always be monitored. Amprenavir can not be used in young children.

5.1. Lopinavir/ritonavir

Lopinavir/ritonavir is the first commercially available drug formulation including both an active PI and ritonavir as booster. In a study by Seaz-Ilorenz *et al.* NNRTI naive children received either 230/75.5 mg/m^2 or 300/75 mg/m^2 q12h liquid formulation lopinavir/ritonavir combined with NRTI. In addition, ART experienced patients also received nevirapine. An intensive pharmacokinetic evaluation was performed on the first 26 patients in the 230/75.5 mg/m^2 dose group and in 27 patients in the 300/75 mg/m^2 dose group. Based on analysis of efficacy, safety and pharmacokinetic data, the dosage for the whole study group was set to 300/75 mg/m^2. However, in the final analysis of the complete pharmacokinetic data, it became apparent that nevirapine reduced lopinavir exposure. When co-administered with nevirapine, the pharmacokinetic data of the 300/75 mg/m^2 group (mean AUC 85.9, SD ±36.9 mg*h/L) were comparable to those obtained in adults using lopinavir/ritonavir without nevirapine. In the absence of nevirapine, the observed concentrations of lopinavir in the 230/75.5 mg/m^2 group (mean AUC 72.6, SD ±31.1 mg*h/L) were similar to those observed in adults. Within the range of 6 month to 12 years the pharmacokinetic parameters did not seem to be dependent on age.

ART experienced patients in the 230/75.5 mg/m^2 dose group tended to have a lower virologic response rate at week 12 compared to the higher dose group (17/27 vs. 21/29; p= 0.570). However, this difference was not significant and influenced by concomitant nevirapine use (77)

Summary

One study has been performed on the use of lopinavir/ritonavir in a reasonably large group of children. Considerable variation in pharmacokinetic data was observed. A dose of 230/75.5 mg/m^2 lopinavir/ritonavir resulted in plasma concentrations comparable to adult values. The lopinavir/ritonavir dosage should be increased to 300/75.5 mg/m^2 when used in combination with nevirapine. Age did not influence drug exposure. No data are available on lopinavir/ritonavir use in children less than 6 months of age.

5. Fusion inhibitors

Fusion inhibitors are a new different class of antiretroviral agents. A major disadvantage of fusion inhibitors is that they need to be administered subcutaneously. The first fusion inhibitor available for use in children is enfuvirtide (T-20).

Enfuvirtide was administered at a dosage of 30 and 60 mg/m^2 q12h. After 7 days, C_{trough} levels were 870 (range, 470-1223) and 2363 (range, 268-4875) ng/ml for the 30 and 60 mg/m^2 group respectively. In only one out of four children in the 30 mg/m^2 group, the C_{trough} was above the designated target of 1000 ng/ml. Of six out of eight patients in the 60 mg/m^2 group evaluable C_{trough} levels were obtained. All were above 1000 ng/ml.

6. Discussion and conclusions

Since the introduction of HAART the perspectives for HIV-1 infected children have improved dramatically. In time new more potent and easier to use medication has been developed. However, there is still ample room for improvement. Major topics that need to be dealt with are the issues concerning the dosage of the available antiretroviral drugs. Table 4 gives an overview on these issues. For a substantial number of drugs, dose recommendation are still absent. Especially in young children, a serious shortage in pharmacokinetic data and evidence based dose recommendation exists. Remarkably, the recommended drug dosages in the guidelines are often different from the dosage in the officially approved drug product label. Even so strikingly, the dose recommendations provided by the different guidelines may deviate. In addition the range of the dosages advised in the guidelines is often broad. Thus, while attaining to the official guidelines, patients may receive highly divergent doses of medication. Distinct problems are associated with the dosing of ART in HIV-1 infected children: Initially dose recommendations were based on tolerability and safety of the drug. Later drug levels were targeted to the *in vitro* drug inhibitory concentrations or targeted to adult plasma drug levels. The groups of children included in these pharmacokinetic studies were often small. In addition, the patients were divided in divergent groups using different dose levels and/or formulations. Thus, the number of children using one specific dosage and formulation of ART was further reduced. This is a serious problem, concerning the inter- and intrapatient variability of the pharmacokinetic data observed for all antiretroviral drugs in children. The sometimes extremely high range of pharmacokinetic data seriously hampers the application of a fixed ART. Drug levels are especially important when one considers that the level of viral

suppression is associated with the plasma concentration of some of the ART drugs. In addition, high plasma drug levels can also correlate with drug toxicity.(78, 79) Plasma concentrations of NRTIs may not be a good indicator of viral activity, because these are pro-drugs. Studies on intracellular NRTI-TP levels are required for evidence based dosages of NRTI in children. Still, as described in this review, for both ddI and d4T a relation between plasma drug concentrations and outcome have been described. Small volumes of plasma can be used to measure concentrations of PIs an NNRTIs in blood. This allows for the use of TDM. TDM allows for effective drug concentrations while it may at the same time prevent toxicity. In some HIV treatment centers the use of plasma drug concentrations has become part of routine treatment of HIV-1 infected children with favorable results.(80) In the clinical practice plasma drug levels in children are targeted to adult reference values, since little or no target drug levels are available for children. In table 5 target values for adults are summarized as proposed on the www.hivpharmacology.com website.

Table 4. Factors complicating dosing of ART in HIV-1 infected children.

Name ART	Do dose recommendation in children exist?	FDA approved dose?	Does the FDA approved dose match the U.S. uidelines?	Do European and U.S. guidelines agree?	Are peer reviewed studies on PK in children available?	Has medication been studied in children < 6 months?	Inter patient variability of PK substantial?	Plasma drug levels or dosage in children found to correlate to viral efficacy?
Abacavir	Yes	Yes	Yes	Yes	Yes	No	Yes	ND
Amprenavir	Yes	Yes	Yes	Yes	No	No	ND	ND
Atazanavir	No	No	NA	NA	No?	No	ND	ND
Didanosine	Yes	Yes	No	No	Yes	Yes	Yes	Yes
Delaverdine	No	No	NA	NA	No	No	ND	ND
Efavirenz	Yes	Yes	Yes	Yes	Yes	No	Yes	No
Emtricitabine	No	No	NA	NA	No	No	ND	ND
Indinavir	Yes	No	Yes	Yes	Yes	No	Yes	Yes
Lamivudine	Yes	Yes	Yes	No	Yes	Yes	Yes	No
Lopinavir	Yes	Yes	No	No	Yes	No	Yes	?
Nelfinavir	Yes	No	No	No	Yes	Yes	Yes	No
Nevirapine	Yes	Yes	No	No	Yes	Yes	Yes	?
Ritonavir	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Saquinavir	Yes	No	NA	Yes	Yes	No	Yes	Yes
Stavudine	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Tenofovir	No	NA	NA	NA	Yes	No	Yes	ND
Zalcitabine	Yes	No	NA	No	Yes	No	Yes	NA
Zidovudine	Yes	Yes	No	No	Yes	Yes	Yes	No

ND = No data available, NA = Not applicable, ? not significant or inconclusive data

Drug resistance of HIV is of growing concern when treating HIV-1 infected individuals.(81) The individual assessment of the drug susceptibility of the viral isolates may optimize the use of TDM. This concept can be quantified by means of the inhibitory quotient (IQ) defined as the ratio of a drug C_{min} value to the drug concentration required to inhibit viral replication *in vitro*.(82) An easier to use and more cost effective approach may be the genotypic IQ (GIQ).(83) The GIQ is defined as the ratio of the C_{min} to the number of mutations. In treatment experienced adults these inhibitory quotients predicted the viral response to PIs.(84-88) As yet individualization of the dosage based on the IQ or GIQ has not been performed. However in the near future application of these methods can improve the treatment of patients with drug resistant HIV.

Table 5. Therapeutic ranges in adults for antiretroviral drugs.[#]

Medication	Minimum trough level (mg/l)	Maximum peak level (mg/l)
efavirenz[§]	1.0	4.0
nevirapine[§]	3.4	nd
Amprenavir	0.4 / 1.2*	nd
indinavir	0.1	10
lopinavir / ritonavir	1.0 / 4.0*	nd
nelfinavir	0.8	nd
ritonavir	2.1	nd
saquinavir	0.1	nd

* Treatment experienced patients, [§] Due to long terminal $t_{1/2}$ changes in drug levels are minimal and sampling time is less important, [#] This table was obtained from www.hivpharmacology.com and based on the following reviews: Back *et al*, AIDS 2002; 16, Suppl 1 S5-S37; Burger *et al*, Curr Opin Inf Dis 2002; Acosta *et al*, AIDS Res Hum Retrovir 2002.(79, 93, 94)

Finally, host pharmacogenomics can be instrumental to further optimize dosing of antiretroviral drugs in HIV-1 infected patients. Host genetic polymorphism for drug metabolizing enzymes or drug transporter proteins are thought to influence plasma and intracellular drug concentrations. For individual patients with a distinct polymorphism alternative dosage may be required.(89) Currently there is no data available on this subject in children. Promising results have been obtained in adults on the relation between host polymorphism and drug levels. However, these data are sparse and often in conclusive.(90-92) Before clinical application more studies on the subject need to be preformed.

In conclusion the dosing of antiretroviral therapy is complicated in children because of high inter and intrapatient variability of drug levels. In addition often no or little pharmacokinetic data are available. Especially in young children data are sparse. Application of TDM to optimize the ART dosage should be considered when treating HIV-1 infected children.

References

1. Peeters M, Courgnaud V, Abela B, Auzel P, Pourrut X, Bibollet-Ruche F, et al. Risk to human health from a plethora of simian immunodeficiency viruses in primate bushmeat. Emerg Infect Dis 2002;8(5):451-7.

2. Korber B, Muldoon M, Theiler J, Gao F, Gupta R, Lapedes A, et al. Timing the ancestor of the HIV-1 pandemic strains. Science 2000;288(5472):1789-96.
3. UNAIDS. AIDS epidemic update: 2003. Geneva: UNAIDS/WHO; 2003 december 2003.
4. Palumbo PE, Raskino C, Fiscus S, Pahwa S, Fowler MG, Spector SA, et al. Predictive value of quantitative plasma HIV RNA and CD4+ lymphocyte count in HIV-infected infants and children. JAMA 1998;279(10):756-61.
5. Scott GB, Hutto C, Makuch RW, Mastrucci MT, O'Connor T, Mitchell CD, et al. Survival in children with perinatally acquired human immunodeficiency virus type 1 infection. N Engl J Med 1989;321(26):1791-6.
6. de Martino M, Tovo PA, Galli L, Gabiano C, Chiarelli F, Zappa M, et al. Puberty in perinatal HIV-1 infection: a multicentre longitudinal study of 212 children. AIDS 2001;15(12):1527-34.
7. Buehler JW, Berkelman RL, Curran JW. Reporting of AIDS: tracking HIV morbidity and mortality. JAMA 1989;262(20):2896-7.
8. Gortmaker SL, Hughes M, Cervia J, Brady M, Johnson GM, Seage GR, 3rd, et al. Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. N Engl J Med 2001;345(21):1522-8.
9. Palella FJ, Jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998;338(13):853-60.
10. Stretcher BN. Pharmacokinetic optimisation of antiretroviral therapy in patients with HIV infection. Clin Pharmacokinet 1995;29(1):46-65.
11. Gao WY, Shirasaka T, Johns DG, Broder S, Mitsuya H. Differential phosphorylation of azidothymidine, dideoxycytidine, and dideoxyinosine in resting and activated peripheral blood mononuclear cells. J Clin Invest 1993;91(5):2326-33.
12. Perno CF, Yarchoan R, Cooney DA, Hartman NR, Gartner S, Popovic M, et al. Inhibition of human immunodeficiency virus (HIV-1/HTLV-IIIBa-L) replication in fresh and cultured human peripheral blood monocytes/macrophages by azidothymidine and related 2',3'-dideoxynucleosides. J Exp Med 1988;168(3):1111-25.
13. Fletcher CV, Acosta EP, Henry K, Page LM, Gross CR, Kawle SP, et al. Concentration-controlled zidovudine therapy. Clin Pharmacol Ther 1998;64(3):331-8.
14. Balis FM, Pizzo PA, Eddy J, Wilfert C, McKinney R, Scott G, et al. Pharmacokinetics of zidovudine administered intravenously and orally in children with human immunodeficiency virus infection. J Pediatr 1989;114(5):880-4.
15. Wintergerst U, Rolinski B, Vocks-Hauck M, Wahn V, Debatin KM, Notheis G, et al. Pharmacokinetics of orally administered zidovudine in HIV-infected children and adults. Infection 1995;23(6):344-8.
16. Pizzo PA, Eddy J, Falloon J, Balis FM, Murphy RF, Moss H, et al. Effect of continuous intravenous infusion of zidovudine (AZT) in children with symptomatic HIV infection. N Engl J Med 1988;319(14):889-96.
17. McKinney RE, Jr., Maha MA, Connor EM, Feinberg J, Scott GB, Wulfsohn M, et al. A multicenter trial of oral zidovudine in children with advanced human immunodeficiency virus disease. The Protocol 043 Study Group. N Engl J Med 1991;324(15):1018-25.
18. Mueller BU, Pizzo PA, Farley M, Husson RN, Goldsmith J, Kovacs A, et al. Pharmacokinetic evaluation of the combination of zidovudine and didanosine in children with human immunodeficiency virus infection. J Pediatr 1994;125(1):142-6.
19. Bakshi SS, Britto P, Capparelli E, Mofenson L, Fowler MG, Rasheed S, et al. Evaluation of pharmacokinetics, safety, tolerance, and activity of combination of zalcitabine and zidovudine in stable, zidovudine-treated pediatric patients with human immunodeficiency virus infection. AIDS Clinical Trials Group Protocol 190 Team. J Infect Dis 1997;175(5):1039-50.
20. Boucher FD, Modlin JF, Weller S, Ruff A, Mirochnick M, Pelton S, et al. Phase I evaluation of zidovudine administered to infants exposed at birth to the human immunodeficiency virus. J Pediatr 1993;122(1):137-44.
21. Mirochnick M, Capparelli E, Connor J. Pharmacokinetics of zidovudine in infants: a population analysis across studies. Clin Pharmacol Ther 1999;66(1):16-24.
22. Mirochnick M, Capparelli E, Dankner W, Sperling RS, van Dyke R, Spector SA. Zidovudine pharmacokinetics in premature infants exposed to human immunodeficiency virus. Antimicrob Agents Chemother 1998;42(4):808-12.

23. Lewis LL, Venzon D, Church J, Farley M, Wheeler S, Keller A, et al. Lamivudine in children with human immunodeficiency virus infection: a phase I/II study. The National Cancer Institute Pediatric Branch-Human Immunodeficiency Virus Working Group. J Infect Dis 1996;174(1):16-25.
24. Mueller BU, Lewis LL, Yuen GJ, Farley M, Keller A, Church JA, et al. Serum and cerebrospinal fluid pharmacokinetics of intravenous and oral lamivudine in human immunodeficiency virus-infected children. Antimicrob Agents Chemother 1998;42(12):3187-92.
25. Sokal EM, Roberts EA, Mieli-Vergani G, McPhillips P, Johnson M, Barber J, et al. A dose ranging study of the pharmacokinetics, safety, and preliminary efficacy of lamivudine in children and adolescents with chronic hepatitis B. Antimicrob Agents Chemother 2000;44(3):590-7.
26. Moodley J, Moodley D, Pillay K, Coovadia H, Saba J, van Leeuwen R, et al. Pharmacokinetics and antiretroviral activity of lamivudine alone or when coadministered with zidovudine in human immunodeficiency virus type 1-infected pregnant women and their offspring. J Infect Dis 1998;178(5):1327-33.
27. Moodley D, Pillay K, Naidoo K, Moodley J, Johnson MA, Moore KH, et al. Pharmacokinetics of zidovudine and lamivudine in neonates following coadministration of oral doses every 12 hours. J Clin Pharmacol 2001;41(7):732-41.
28. King JR, Nachman S, Yogev R, Hodge J, Aldrovandi G, Damle B, et al. Single-dose pharmacokinetics of enteric-coated didanosine in HIV-infected children. Antivir Ther 2002;7(4):267-70.
29. Balis FM, Pizzo PA, Butler KM, Hawkins ME, Brouwers P, Husson RN, et al. Clinical pharmacology of 2',3'-dideoxyinosine in human immunodeficiency virus-infected children. J Infect Dis 1992;165(1):99-104.
30. Mueller BU, Butler KM, Stocker VL, Balis FM, Brouwers P, Jarosinski P, et al. Clinical and pharmacokinetic evaluation of long-term therapy with didanosine in children with HIV infection. Pediatrics 1994;94(5):724-31.
31. Fletcher CV, Brundage RC, Remmel RP, Page LM, Weller D, Calles NR, et al. Pharmacologic characteristics of indinavir, didanosine, and stavudine in human immunodeficiency virus-infected children receiving combination therapy. Antimicrob Agents Chemother 2000;44(4):1029-34.
32. Hoetelmans RM, van Heeswijk RP, Profijt M, Mulder JW, Meenhorst PL, Lange JM, et al. Comparison of the plasma pharmacokinetics and renal clearance of didanosine during once and twice daily dosing in HIV-1 infected individuals. AIDS 1998;12(17):F211-6.
33. Keiser P, Turner D, Ramilo O, Kvanli MB, Smith JW, Nassar N. An open-label pilot study of the efficacy and tolerability of once-daily didanosine versus twice-daily didanosine. Clin Infect Dis 1998;27(2):400-1.
34. Abreu T, Plaisance K, Rexroad V, Nogueira S, Oliveira RH, Evangelista LA, et al. Bioavailability of once- and twice-daily regimens of didanosine in human immunodeficiency virus-infected children. Antimicrob Agents Chemother 2000;44(5):1375-6.
35. Wang Y, Livingston E, Patil S, McKinney RE, Bardeguez AD, Gandia J, et al. Pharmacokinetics of didanosine in antepartum and postpartum human immunodeficiency virus--infected pregnant women and their neonates: an AIDS clinical trials group study. J Infect Dis 1999;180(5):1536-41.
36. Rongkavilit C, Thaithumyanon P, Chuenyam T, Damle BD, Limpongsanurak S, Boonrod C, et al. Pharmacokinetics of stavudine and didanosine coadministered with nelfinavir in human immunodeficiency virus-exposed neonates. Antimicrob Agents Chemother 2001;45(12):3585-90.
37. Shyu WC, Knupp CA, Pittman KA, Dunkle L, Barbhaiya RH. Food-induced reduction in bioavailability of didanosine. Clin Pharmacol Ther 1991;50(5 Pt 1):503-7.
38. Knupp CA, Milbrath R, Barbhaiya RH. Effect of time of food administration on the bioavailability of didanosine from a chewable tablet formulation. J Clin Pharmacol 1993;33(6):568-73.
39. Stevens RC, Rodman JH, Yong FH, Carey V, Knupp CA, Frenkel LM. Effect of food and pharmacokinetic variability on didanosine systemic exposure in HIV-infected children. Pediatric AIDS Clinical Trials Group Protocol 144 Study Team. AIDS Res Hum Retroviruses 2000;16(5):415-21.
40. Gibb D, Barry M, Ormesher S, Nokes L, Seefried M, Giaquinto C, et al. Pharmacokinetics of zidovudine and dideoxyinosine alone and in combination in children with HIV infection. Br J Clin Pharmacol 1995;39(5):527-30.
41. Kline MW, Dunkle LM, Church JA, Goldsmith JC, Harris AT, Federici ME, et al. A phase I/II evaluation of stavudine (d4T) in children with human immunodeficiency virus infection. Pediatrics 1995;96(2 Pt 1):247-52.
42. Kaul S, Kline MW, Church JA, Dunkle LM. Determination of dosing guidelines for stavudine (2',3'-didehydro-3'-deoxythymidine) in children with human immunodeficiency virus infection. Antimicrob Agents Chemother 2001;45(3):758-63.

43. Kline MW, Fletcher CV, Federici ME, Harris AT, Evans KD, Rutkiewicz VL, et al. Combination therapy with stavudine and didanosine in children with advanced human immunodeficiency virus infection: pharmacokinetic properties, safety, and immunologic and virologic effects. Pediatrics 1996;97(6 Pt 1):886-90.
44. Hughes W, McDowell JA, Shenep J, Flynn P, Kline MW, Yogev R, et al. Safety and single-dose pharmacokinetics of abacavir (1592U89) in human immunodeficiency virus type 1-infected children. Antimicrob Agents Chemother 1999;43(3):609-15.
45. Kline MW, Blanchard S, Fletcher CV, Shenep JL, McKinney RE, Jr., Brundage RC, et al. A phase I study of abacavir (1592U89) alone and in combination with other antiretroviral agents in infants and children with human immunodeficiency virus infection. AIDS Clinical Trials Group 330 Team. Pediatrics 1999;103(4):e47.
46. Hazra R, Balis FM, Tullio AN, DeCarlo E, Worrell CJ, Steinberg SM, et al. Single-dose and steady-state pharmacokinetics of tenofovir disoproxil fumarate in human immunodeficiency virus-infected children. Antimicrob Agents Chemother 2004;48(1):124-9.
47. Deeks SG, Barditch-Crovo P, Lietman PS, Hwang F, Cundy KC, Rooney JF, et al. Safety, pharmacokinetics, and antiretroviral activity of intravenous 9-[2-(R)-(Phosphonomethoxy)propyl]adenine, a novel anti-human immunodeficiency virus (HIV) therapy, in HIV-infected adults. Antimicrob Agents Chemother 1998;42(9):2380-4.
48. Smith PF, DiCenzo R, Morse GD. Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors. Clin Pharmacokinet 2001;40(12):893-905.
49. Luzuriaga K, Bryson Y, McSherry G, Robinson J, Stechenberg B, Scott G, et al. Pharmacokinetics, safety, and activity of nevirapine in human immunodeficiency virus type 1-infected children. J Infect Dis 1996;174(4):713-21.
50. Mirochnick M, Fenton T, Gagnier P, Pav J, Gwynne M, Siminski S, et al. Pharmacokinetics of nevirapine in human immunodeficiency virus type 1-infected pregnant women and their neonates. Pediatric AIDS Clinical Trials Group Protocol 250 Team. J Infect Dis 1998;178(2):368-74.
51. Mirochnick M, Siminski S, Fenton T, Lugo M, Sullivan JL. Nevirapine pharmacokinetics in pregnant women and in their infants after in utero exposure. Pediatr Infect Dis J 2001;20(8):803-5.
52. Starr SE, Fletcher CV, Spector SA, Yong FH, Fenton T, Brundage RC, et al. Combination therapy with efavirenz, nelfinavir, and nucleoside reverse-transcriptase inhibitors in children infected with human immunodeficiency virus type 1. Pediatric AIDS Clinical Trials Group 382 Team. N Engl J Med 1999;341(25):1874-81.
53. Starr SE, Fletcher CV, Spector SA, Brundage RC, Yong FH, Douglas SD, et al. Efavirenz liquid formulation in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2002;21(7):659-63.
54. Marzolini C, Telenti A, Decosterd LA, Greub G, Biollaz J, Buclin T. Efavirenz plasma levels can predict treatment failure and central nervous system side effects in HIV-1-infected patients. AIDS 2001;15(1):71-5.
55. Roberts NA, Martin JA, Kinchington D, Broadhurst AV, Craig JC, Duncan IB, et al. Rational design of peptide-based HIV proteinase inhibitors. Science 1990;248(4953):358-61.
56. Acosta EP, Kakuda TN, Brundage RC, Anderson PL, Fletcher CV. Pharmacodynamics of human immunodeficiency virus type 1 protease inhibitors. Clin Infect Dis 2000;30 Suppl 2:S151-9.
57. Mueller BU, Sleasman J, Nelson RP, Jr., Smith S, Deutsch PJ, Ju W, et al. A phase I/II study of the protease inhibitor indinavir in children with HIV infection. Pediatrics 1998;102(1 Pt 1):101-9.
58. Kline MW, Fletcher CV, Harris AT, Evans KD, Brundage RC, Remmel RP, et al. A pilot study of combination therapy with indinavir, stavudine (d4T), and didanosine (ddI) in children infected with the human immunodeficiency virus. J Pediatr 1998;132(3 Pt 1):543-6.
59. Gatti G, Vigano A, Sala N, Vella S, Bassetti M, Bassetti D, et al. Indinavir pharmacokinetics and parmacodynamics in children with human immunodeficiency virus infection. Antimicrob Agents Chemother 2000;44(3):752-5.
60. Burger DM, van Rossum AM, Hugen PW, Suur MH, Hartwig NG, Geelen SP, et al. Pharmacokinetics of the protease inhibitor indinavir in human immunodeficiency virus type 1-infected children. Antimicrob Agents Chemother 2001;45(3):701-5.
61. Fraaij PL, Bergshoeff AS, Van Rossum AM, Hartwig NG, Burger DM, De Groot R. Changes in indinavir exposure over time: a case study in six HIV-1-infected children. J Antimicrob Chemother 2003;52(4):727-30.
62. van Rossum AM, de Groot R, Hartwig NG, Weemaes CM, Head S, Burger DM. Pharmacokinetics of indinavir and low-dose ritonavir in children with HIV-1 infection. AIDS 2000;14(14):2209-10.

63. Krogstad P, Wiznia A, Luzuriaga K, Dankner W, Nielsen K, Gersten M, et al. Treatment of human immunodeficiency virus 1-infected infants and children with the protease inhibitor nelfinavir mesylate. Clin Infect Dis 1999;28(5):1109-18.
64. Bergshoeff AS, Fraaij PL, van Rossum AM, Wolfs TF, Geelen SP, de Groot R, et al. Pharmacokinetics of nelfinavir in children: influencing factors and dose implications. Antivir Ther 2003;8(3):215-22.
65. Litalien C, Faye A, Compagnucci A, Giaquinto C, Harper L, Gibb DM, et al. Pharmacokinetics of nelfinavir and its active metabolite, hydroxy-tert-butylamide, in infants perinatally infected with human immunodeficiency virus type 1. Pediatr Infect Dis J 2003;22(1):48-55.
66. Schuster T, Linde R, Wintergerst U, Funk MB, Kurowski M, Kreuz W, et al. Nelfinavir pharmacokinetics in HIV-infected children: a comparison of twice daily and three times daily dosing. AIDS 2000;14(10):1466-8.
67. van Heeswijk RP, Scherpbier HJ, de Koning LA, Heymans HS, Lange JM, Beijnen JH, et al. The pharmacokinetics of nelfinavir in HIV-1-infected children. Ther Drug Monit 2002;24(4):487-91.
68. Gatti G, Castelli-Gattinara G, Cruciani M, Bernardi S, De Pascalis CR, Pontali E, et al. Pharmacokinetics and pharmacodynamics of nelfinavir administered twice or thrice daily to human immunodeficiency virus type 1-infected children. Clin Infect Dis 2003;36(11):1476-82.
69. Durant J, Clevenbergh P, Garraffo R, Halfon P, Icard S, Del Giudice P, et al. Importance of protease inhibitor plasma levels in HIV-infected patients treated with genotypic-guided therapy: pharmacological data from the Viradapt Study. AIDS 2000;14(10):1333-9.
70. Burger DM, Hugen PW, Aarnoutse RE, Hoetelmans RM, Jambroes M, Nieuwkerk PT, et al. Treatment failure of nelfinavir-containing triple therapy can largely be explained by low nelfinavir plasma concentrations. Ther Drug Monit 2003;25(1):73-80.
71. Burger D, Hugen P, Reiss P, Gyssens I, Schneider M, Kroon F, et al. Therapeutic drug monitoring of nelfinavir and indinavir in treatment-naive HIV-1-infected individuals. AIDS 2003;17(8):1157-65.
72. Dumon C, Solas C, Thuret I, Chambost H, Lacarelle B, Michel G, et al. Relationship between efficacy, tolerance, and plasma drug concentration of ritonavir in children with advanced HIV infection. Ther Drug Monit 2000;22(4):402-8.
73. Grub S, Delora P, Ludin E, Duff F, Fletcher CV, Brundage RC, et al. Pharmacokinetics and pharmacodynamics of saquinavir in pediatric patients with human immunodeficiency virus infection. Clin Pharmacol Ther 2002;71(3):122-30.
74. King JR, Kimberlin DW, Aldrovandi GM, Acosta EP. Antiretroviral Pharmacokinetics in the Paediatric Population: A Review. Clin Pharmacokinet 2002;41(14):1115-1133.
75. Scott T, Garris C, Rogers M, Graham N, Garrett L, Pedneault L. Safety profile and tolerability of amprenavir in patients enrolled in an early access program. Clin Ther 2001;23(2):252-9.
76. Wintergerst U, Engelhorn C, Kurowski M, Hoffmann F, Notheis G, Belohradsky BH. Pharmacokinetic interaction of amprenavir in combination with efavirenz or delavirdine in HIV-infected children. AIDS 2000;14(12):1866-8.
77. Saez-Llorens X, Violari A, Deetz CO, Rode RA, Gomez P, Handelsman E, et al. Forty-eight-week evaluation of lopinavir/ritonavir, a new protease inhibitor, in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2003;22(3):216-224.
78. van Rossum AM, Dieleman JP, Fraaij PL, Cransberg K, Hartwig NG, Burger DM, et al. Persistent sterile leukocyturia is associated with impaired renal function in human immunodeficiency virus type 1-infected children treated with indinavir. Pediatrics 2002;110(2 Pt 1):e19.
79. Back D, Gatti G, Fletcher C, Garaffo R, Haubrich R, Hoetelmans R, et al. Therapeutic drug monitoring in HIV infection: current status and future directions. Aids 2002;16 Suppl 1:S5-37.
80. Fraaij PL, Rakhmanina N, Burger DM, De Groot R. Therapeutic drug monitoring in children with HIV/AIDS. Ther Drug Monit 2004;26(2):in press.
81. Deeks SG. Treatment of antiretroviral-drug-resistant HIV-1 infection. Lancet 2003;362(9400):2002-11.
82. Aarnoutse RE, Schapiro JM, Boucher CA, Hekster YA, Burger DM. Therapeutic drug monitoring: an aid to optimising response to antiretroviral drugs? Drugs 2003;63(8):741-53.
83. Marcelin AG, Lamotte C, Delaugerre C, Ktorza N, Ait Mohand H, Cacace R, et al. Genotypic inhibitory quotient as predictor of virological response to ritonavir-amprenavir in human immunodeficiency virus type 1 protease inhibitor-experienced patients. Antimicrob Agents Chemother 2003;47(2):594-600.
84. Shulman N, Zolopa A, Havlir D, Hsu A, Renz C, Boller S, et al. Virtual inhibitory quotient predicts response to ritonavir boosting of indinavir-based therapy in human immunodeficiency virus-infected patients with ongoing viremia. Antimicrob Agents Chemother 2002;46(12):3907-16.

85. Duval X, Lamotte C, Race E, Descamps D, Damond F, Clavel F, et al. Amprenavir inhibitory quotient and virological response in human immunodeficiency virus-infected patients on an amprenavir-containing salvage regimen without or with ritonavir. Antimicrob Agents Chemother 2002;46(2):570-4.
86. Hsu A, Isaacson J, Brun S, Bernstein B, Lam W, Bertz R, et al. Pharmacokinetic-pharmacodynamic analysis of lopinavir-ritonavir in combination with efavirenz and two nucleoside reverse transcriptase inhibitors in extensively pretreated human immunodeficiency virus-infected patients. Antimicrob Agents Chemother 2003;47(1):350-9.
87. Casado JL, Moreno A, Sabido R, Marti-Belda P, Antela A, Dronda F, et al. Individualizing salvage regimens: the inhibitory quotient (Ctrough/IC50) as predictor of virological response. AIDS 2003;17(2):262-4.
88. Gonzalez de Requena D, Gallego O, Valer L, Jimenez-Nacher I, Soriano V. Prediction of virological response to lopinavir/ritonavir using the genotypic inhibitory quotient. AIDS Res Hum Retroviruses 2004;20(3):275-8.
89. Back DJ, Khoo SH. The role of clinical pharmacology in optimizing antiretroviral therapy. Br J Clin Pharmacol 2003;55(5):473-6.
90. Fellay J, Marzolini C, Meaden ER, Back DJ, Buclin T, Chave JP, et al. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetics study. Lancet 2002;359(9300):30-6.
91. Nasi M, Borghi V, Pinti M, Bellodi C, Lugli E, Maffei S, et al. MDR1 C3435T genetic polymorphism does not influence the response to antiretroviral therapy in drug-naive HIV-positive patients. Aids 2003;17(11):1696-8.
92. Haas DW, Wu H, Li H, Bosch RJ, Lederman MM, Kuritzkes D, et al. MDR1 gene polymorphisms and phase 1 viral decay during HIV-1 infection: an adult AIDS Clinical Trials Group study. J Acquir Immune Defic Syndr 2003;34(3):295-8.
93. Burger DM, Aarnoutse RE, Hugen PW. Pros and cons of therapeutic drug monitoring of antiretroviral agents. Curr Opin Infect Dis 2002;15(1):17-22.
94. Acosta EP, Gerber JG. Position paper on therapeutic drug monitoring of antiretroviral agents. AIDS Res Hum Retroviruses 2002;18(12):825-34.

Part three

Psychosocial aspects of the treatment of HIV-1 infected children

An analysis of the social situation of HIV-1 affected families

Pieter L.A. Fraaij, Juul B.G. Rijkschroeff, Linda van der Knaap,
Nico G. Hartwig, Ronald de Groot

Submitted

Chapter 18

Abstract

Goal: To obtain insight in the social situation of families of HIV-1 infected children.

Methods: From 1997 until January 2004, 59 children were treated in the Rotterdam cohort. Selected social demographic data of the families were obtained during follow-up. In addition, from 2000 on, detailed data were obtained on the social support network of the caregivers by means of the standardized social support network card, developed by the department of Social Psychiatry of the University of Maastricht.

Results: HIV-1 infection in children was found to be associated with serious psychosocial problems in the family. In 16 of the 59 (27%) children one or both parents had died. During the follow-up period 14 of the 59 (24%) children were cared for by others than the biological parents. Child protection services were involved with 16 of the 59 (27%) children. The social support network of the caregivers was limited, with a mean 13 (range, 4-43) members (> 16 years of age) reported. Strikingly, a high percentage (30%) of the reported social support network members was acquired through contacts with social services (hospital, social work etc.). In addition, social support network members were often not living in the Netherlands. The mean number of social support network members living outside the Netherlands was 2.5 (range, 0-24). To assess the number of people the caregiver had access to, their social support network was calculated excluding network members living aboard and network members obtained from social services. This resulted in a mean social network of 7 (0-31) members.

Conclusions: A normal social network consists of 33 to 55 persons. In comparison caregivers of HIV infected children treated in the Rotterdam cohort possess a limited social support network. This was found to be associated with serious psychological, economical and social problems. Physicians treating HIV infected individual should be aware of these problems.

Analyse van de sociale omstandigheden van gezinnen met HIV

Samenvatting

Doel: Het verwerven van inzicht in de sociale omstandigheden van gezinnen met HIV-geïnfecteerde kinderen.

Methoden: Sinds 1997 zijn 59 kinderen met een HIV-1 infectie behandeld in het Rotterdam Kinder HIV-cohort. Sociaal demografische gegevens van de ouders/verzorgers en kinderen werden verzameld. Tevens werd vanaf 2000 een sociaal netwerk analyse uitgevoerd bij de ouders/verzorgers van HIV-1 geïnfecteerde kinderen.

Resultaten: Een HIV infectie bij kinderen gaat vaak gepaard met ernstige psychosociale en gezinsproblematiek. Bij 16 van de 59 (27%) kinderen waren één of beide ouders overleden. Veertien van de 59 (24%) kinderen werden gedurende de gehele follow-up periode of een deel daarvan door andere personen dan de biologische ouders opgevoed. Jeugdbeschermings instanties hadden betrokkenheid bij 16 van de 59 (27%) kinderen. De ouders/verzorgers van HIV-1 geïnfecteerde kinderen beschikken over een sociaal netwerk van een zeer geringe omvang. De gemiddelde grootte van het sociale netwerk (netwerkleden > 16 jaar) was 13 personen (spreiding: 4-43). Hierbij valt op dat dat netwerkleden uit de sector maatschappelijke diensten een groot aandeel hebben (30%). Bovendien leeft een aanzienlijk deel van de leden van het sociale netwerkleden (gemiddeld 2.5) niet in Nederland.

Conclusies: De ouders/verzorgers van HIV geïnfecteerde kinderen beschikken over een gering sociaal netwerk. Dit gaat gepaard met ernstige economische en psychosociale problematiek. Bij de behandeling van en de zorg voor HIV geïnfecteerde patiënten dient hiermee rekening gehouden te worden.

Introductie

Het HIV behandelteam van het ErasmusMC/Sophia kinderziekenhuis in Rotterdam behandelt sinds 1997 HIV-1 geïnfecteerde kinderen met krachtige antiretrovirale combinatietherapie. Sinds die tijd zijn slechts 2 kinderen overleden aan de gevolgen van aids en bevinden de meeste patiënten zich in een goede klinische conditie. (1)

Om goede behandel resultaten te verkrijgen is een goede therapie trouw een essentiële factor. (2) Tijdens de polikliniekbezoeken van de patiënten werd vaak ernstige psychosociale problematiek vermoed bij bij de ouders en verzorgers. Deze problematiek kan nadelige gevolgen hebben voor de psychosociale toestand van het kind en voor de therapietrouw. De kans dat de ouders/verzorgers zich niet goed aan het medicatie schema houden neemt toe in de aanwezigheid van psychosociale problematiek en bij het ontbreken van een sociaal netwerk. (3, 4) Het is om die reden van groot belang een goed inzicht te verkrijgen in de sociale structuur van de gezinnen waarin deze kinderen opgroeien. Hiertoe onderzochten wij de sociaal demografische kenmerken van de gezinnen van HIV-1 geïnfecteerde kinderen. Tevens werd de omvang van het sociale netwerk van de ouders/verzorgers bestudeerd dmv een sociale netwerkkaart (M.S.N.A sociale diagnostiek) ontwikkeld door de afdeling Sociale Psychiatrie,Universiteit Maastricht.

Methoden

Sociaal demografische gegevens

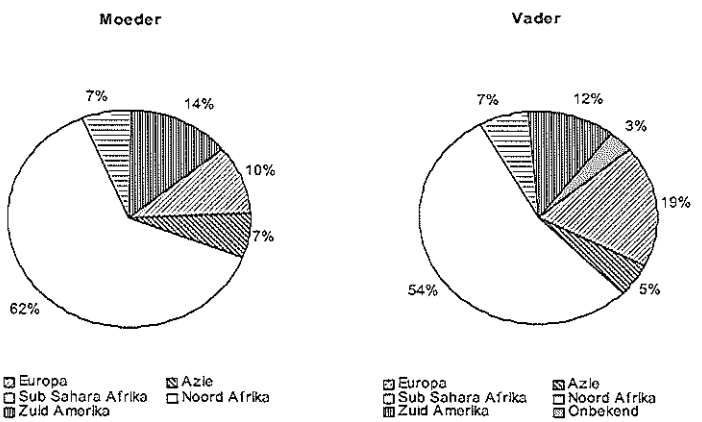
De hier beschreven kinderen werden behandeld in het ErasmusMC-Sophia Kinderziekenhuis of bij één van de 3 samenwerkende centra (Universitair Medisch Centrum St Radboud, VU Medisch Centrum Amsterdam en Leeuwarden Medisch Centrum). Gezamenlijk vormt deze groep kinderen het Rotterdamse Kinder HIV-cohort. Naast klinische, virologische en immunologische parameters werden ook sociaal demografische gegevens van de ouders/verzorgers en kinderen bijgehouden. Ook werd systematisch genoteerd of de Raad voor de Kinderbescherming of Jeugdbeschermings instanties betrokken waren bij de zorg voor de kinderen.

Sociaal netwerk analyse

Vanaf 2000 werd gestart met het afnemen van een gedetailleerde sociaal netwerk analyse bij de ouders/verzorgers van HIV geïnfecteerde kinderen. Het persoonlijke sociaal netwerk van ouders/verzorgers van HIV-geïnfecteerde kinderen werd m.b.v. de gestandaardiseerde sociaal netwerk analyse (M.S.N.A. sociale diagnostiek), ontwikkeld door de afdeling Sociale Psychiatrie, universiteit van Maastricht, in kaart gebracht. (5) Deze kaart werd door de medisch maatschappelijk werker tijdens een interview met de ouder/verzorger ingevuld. De sociale kaart werd afgenomen bij de ouder/verzorger die in de dagelijkse praktijk verantwoordelijk was voor de medicatie gift aan het kind. Wanneer beide ouders verantwoordelijk waren voor deze zorg werd de sociale kaart bij de moeder afgenomen. Bij het benoemen van de sociaal netwerk leden werden deze ingedeeld binnen de sectoren verwanten, vriendschappelijke betrekkingen,

collega's, buren of maatschappelijke diensten. Voor ieder sociaal netwerk lid werd de leeftijd en de geografische bereikbaarheid voor de focale persoon gescoord. Kinderen jonger dan 16 jaar werden wel tot het totale sociale netwerk gerekend, maar niet tot het ondersteunend sociaal netwerk. Personen waarmee langer dan 1 jaar geen contact meer was geweest werden niet tot het sociale netwerk gerekend.

Afbeelding 1. Gebied van herkomst van de biologische ouders van 59 HIV-1 geïnfecteerde kinderen.



Resultaten

Algemene sociaal demografische gegevens van het totale HIV cohort

Tussen 1997 en december 2003 werden 59 kinderen in het Rotterdam cohort voor een HIV infectie behandeld. Deze 59 kinderen hadden 56 ouder paren (3 maal betrof het kinderen in hetzelfde gezin). Het merendeel van de kinderen (n = 55) was nieuw gediagnosticeerd. Tien patiënten waren per 1 januari 2004 niet meer in follow-up. Twee patiënten waren overleden aan de gevolgen van aids, 2 patiënten waren ouder dan 17 jaar geworden en werden aansluitend behandeld door een internist, 4 patiënten waren geëmigreerd en bij 2 patiënten was de zorg door een ander HIV behandel centrum overgenomen.

Het merendeel van de biologische ouders van de kinderen in het Rotterdam cohort was niet afkomstig uit Nederland. In afbeelding 1 wordt het gebied van herkomst van de ouders weergegeven. In 39 van de 59 (66%) gevallen leefden de kinderen samen met een ander HIV geïnfecteerd familie lid (meestal één van de ouders). Veertien van de 59 (24%) kinderen werden gedurende de follow-up periode geheel of tijdelijk (langer dan een half jaar) door andere personen dan de biologische ouders opgevoed. De redenen hiervoor waren: overlijden van een of beide ouders (n = 7), een jeugdbescherming maatregel (n = 5), of een vlucht uit een oorlogsgebied naar Nederland zonder ouders (n = 2).

Tabel 1. De sociaal demografische gegeven van de ouder/verzorgers op het moment van afname van de sociale kaart.

Geslacht (m/v)		8/26
Biologische ouder (ja/nee)		28/6
HIV-geïnfecteerd (ja/nee/onbekend)		22/11/1
Leeftijd	25-34 jaar	18 (53%)
	35-44 jaar	13 (38%)
	> 45 jaar	3 (9%)
Regio afkomst focaal persoon	Europa	8 (24%)
	Noord Afrika	1 (3%)
	Zuid Amerika	3 (9%)
	Subsahara Afrika	22 (65%)
Burgerlijke staat	Ongehuwd/geen partner	6 (18%)
	Gehuwd/ partner	19 (56%)
	Gescheiden	7 (21%)
	Partner overleden	2 (6%)
Woonsituatie op	Samen met partner	18 (53%)
	Zonder partner	16 (47%)
Opleiding	Geen onderwijs	1 (3%)
	Basis onderwijs	14 (41%)
	VMBO	5 (15%)
	MBO/HAVO	7 (21%)
	HBO/VWO	4 (12%)
	Universiteit	3 (9%)
Deelname arbeidsproces	Nee	23 (68%)
	Ja	10 (29%)
	Studie	1 (3%)

Slechts van 31 van de 59 kinderen (52%) kinderen waren beide ouders in leven. Van 11 (19%) kinderen was één van de ouders overleden en van 5 (8%) kinderen beide. In 12 (20%) gevallen was het onduidelijk of één of beide ouders nog in leven waren.

Bij 16 kinderen van de 59 kinderen (27%) was de Raad voor de Kinderbescherming of een Jeugdzorg instelling betrokken bij de zorg. In het merendeel van de gevallen was dit in verband met psychosociale problematiek (n = 13). In de overige gevallen was betrokkenheid van jeugdzorg instanties noodzakelijk na het overlijden van de verzorgende ouder.

Sociaal netwerk analyse ouder/verzorger

De sociaal netwerk kaart werd bij 38 ouders/verzorgers van 39 patiënten afgenomen. Bij 18 ouder(s) verzorgers van 20 patiënten gebeurde dit niet. Redenen hiervoor waren 1) Het niet langer in follow-up zijn van de patiënt vanaf 2000 (n = 6); 2) weigering van medewerking door

de ouder/verzorger (n = 8); 3) een te korte behandelduur of frequente grote veranderingen in de gezinssituatie (n = 3) en 4) een te grote reisafstand voor maatschappelijk werk (n = 3).

Tabel 2. Gemiddelde en mediane omvang van het sociale netwerk van 34 ouders/ verzorgers uit een gezin met één of meer HIV-1 geïnfecteerde kinderen.

Totaal aantal personen sociaal netwerk inclusief kinderen < 16 jaar Gemiddeld (spreiding)	15 (5-46)
Ondersteunend sociaal netwerk (exclusief kinderen < 16 jaar) Gemiddeld (spreiding)	13 (4-43)
Ondersteunend sociaal netwerk waarbij netwerkleden woonachtig in Nederland Gemiddeld (spreiding)	11 (3-43)
Ondersteunend sociaal netwerk zonder netwerkleden uit de sector maatschappelijke diensten Gemiddeld (spreiding)	9 (1-32)
Vrij bereikbaar sociaalnetwerk* Gemiddeld (spreiding)	7 (0-31)

* Vrij bereikbaar sociaalnetwerk, is het ondersteunend sociaal netwerk exclusief, netwerkleden buiten Nederland en netwerkleden uit het cluster maatschappelijke diensten.

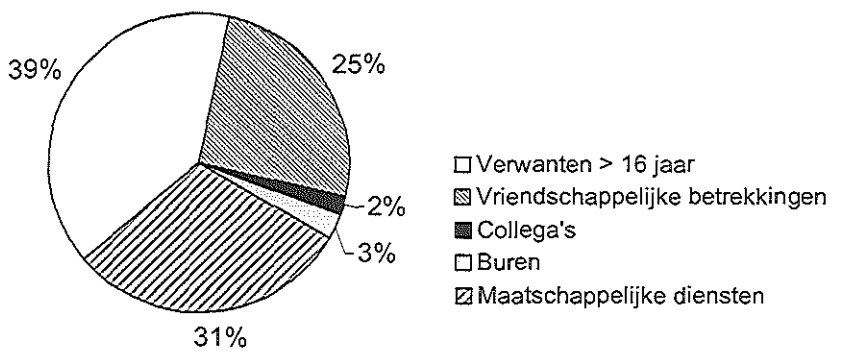
Van de 38 verkregen sociale kaarten waren 4 niet bruikbaar voor analyse. Dit door onoverkoombare communicatie problemen of angst bij de ouder verzorgers om illegaal in Nederland verblijvende netwerkleden te noemen. De gegevens van deze ouders/verzorgers werden niet gebruikt voor data analyse. Derhalve bleven uiteindelijk 34 ouders/verzorgers over voor analyse.

De sociaal demografische gegevens deze personen zijn samengevat in tabel 1. Zes van de 34 geïnterviewde focale personen waren niet de biologische ouder van de patiënt, maar een pleegouder. Het merendeel van de focale personen was tussen de 25 en 34 jaar oud en afkomstig uit sub Sahara Afrika. De helft van de ondervraagden woonde niet met een partner samen en voedde de kinderen in een éénoudergezin op. Het opleidingsniveau varieerde aanzienlijk van geen opleiding tot een universitaire graad. Het grootste gedeelte van de ouders/verzorgers was laag opgeleid waarbij het merendeel (44%) geen voorgezet onderwijs gevolgd had. Meer dan de helft (n = 23 (68%)) van de ondervraagden nam niet actief deel aan het arbeidsproces. In 16 (27%) van de 59 gezinnen moest rondgekomen worden van een uitkering. In 10 gevallen waren beide ouders werkeloos en in 6 gevallen betrof het een werkeloze alleenstaande moeder. (n = 6).

In tabel 2 wordt de gemiddelde en mediane omvang van het sociale netwerk van de ouders/verzorgers weergegeven. De gemiddelde grootte van het gerapporteerde sociale netwerk (netwerkleden > 16 jaar) was 13 personen (spreiding, 4-43). In figuur 2 wordt de

verdeling in de verschillende sectoren van het sociaal netwerk van de 34 ouders/verzorgers weergegeven. Hieruit blijkt dat een groot gedeelte van het sociale netwerk (30%) van de ouders/verzorgers personen wordt verkregen via maatschappelijke diensten. Als deze leden van het sociaal netwerk niet mee gerekend worden valt het gemiddelde sociaal netwerk aanzienlijk lager uit met 9 (1-32) netwerkleden. Eenentwintig focale personen gaven aan dat één of meerdere leden van hun sociaal netwerk buiten Nederland leefde. Om beter inzicht in de bereikbaarheid en beschikbaarheid van het sociale netwerk te krijgen werd berekend hoe groot het sociaal netwerk was zonder de in het buitenland levende netwerkleden. Na het weg laten van deze netwerkleden kwam het een gemiddelde sociale netwerk van ouders/verzorgers van HIV geïnfecteerde kinderen op 7 (spreiding 0-31).

Afbeelding 2. Samenstelling van het ondersteunend netwerk van 34 ouders/verzorgers van HIV-1 geïnfecteerde kinderen. Wenselijk is een sociaal netwerk waarbij vriendschappelijke betrekkingen, collega's/buren en verwanten gelijk verdeeld zijn, met een minimale inbreng van de maatschappelijke diensten.



Discussie

Uit onze studie blijkt dat een HIV infectie bij kinderen vaak gepaard gaat met psychosociale problematiek in het gezin. Hierbij valt op dat veel gezinnen zich in een ernstig sociaal isolement bevinden. Baars et al. geven in hun "Sociale netwerkstrategieën in de sociale psychiatrie" aan dat een normaal sociaal netwerk varieert tussen de 33 en 55 personen. (5) De door ons geïnterviewde ouders scoorden over het algemeen een veel geringer aantal. Opvallend hierbij is het grote aandeel dat de sociaal netwerkleden uit de sector maatschappelijke diensten hebben in het totale netwerk. Dit is een zorgwekkende ontwikkeling. Wenselijk is een sociaal netwerk waarbij vriendschappelijke betrekkingen, collega's/buren en verwanten gelijk verdeeld zijn, met een minimale inbreng van de maatschappelijke diensten. De oververtegenwoordiging van sociaal netwerkleden uit de sector maatschappelijke diensten kan er op duiden dat de HIV

infectie een belangrijk gedeelte van het dagelijkse leven binnen het gezin uit maakt en dat het gezin mogelijk gemedicaliseerd geraakt is. Behalve de geringe omvang en de afwijkende samenstelling van het sociale netwerk valt ook op dat een groot aantal van de sociaal netwerkleden buiten Nederland leeft. Gezien het grote aantal van origine niet Nederlandse ouders en het belang van verwanten voor het sociale netwerk is dit niet verwonderlijk. Dit heeft echter wel grote gevolgen voor de beschikbaarheid van het sociale netwerk en het sociale isolement van de ouders/verzorgers en kan dit ook leiden tot praktische problemen rond de zorg van de kinderen. Immers de groep mensen waarbij de ouder/verzorger bij praktische problemen om hulp kon vragen is slechts zeer gering.

Het sociale isolement van gezinnen van HIV-1 geïnfecteerde kinderen heeft waarschijnlijk meerdere oorzaken. Over het algemeen is niet alleen het kind geïnfecteerd, maar ook de ouders en ander gezinsleden. Deze ziekte ging in het verleden gepaard met significante morbiditeit en sterfte. De HIV infectie zelf kan ook aanleiding zijn tot een sociaal isolement. Een bekend verschijnsel bij HIV infectie is sociale stigmatisatie van de geïnfecteerden. (6) Het bekend worden van de diagnose HIV buiten het gezin wordt vaak door de ouders/verzorgers met grote zorg vermeden. (3) Dit kan sociale isolatie tot gevolg hebben. Ook de afkomst van de ouders/verzorgers kan bijdragen tot de beschreven problematiek. Over het algemeen zijn de verwanten en vrienden achter gebleven in het land van herkomst. Een nieuw sociaal netwerk opbouwen in een ander land dan het geboorte land kost vaak tijd en is een moeizaam proces. Tevens valt op dat een groot gedeelte van de focale personen niet deelneemt aan het arbeidsproces en dus ook geen contacten met collega's heeft. Het niet deelnemen aan het arbeidsproces heeft ook tot gevolg dat de financiële middelen van de gezinnen beperkt zijn, waardoor het ontmoeten van mensen buiten de leefomgeving bemoeilijkt wordt.

De hier gepresenteerde gegevens kunnen zijn beïnvloed door het feit dat niet alle patiënten voor data analyse beschikbaar waren. Het valt te verwachten dat netwerkleden uit de sector maatschappelijke dienstverlening een aanzienlijk minder groot deel van het sociaal netwerk uit maken van ouders/verzorgers die niet deel wilden nemen aan deze analyse. Ook is het mogelijk dat culturele verschillen onze gegevens beïnvloed hebben. Echter het vaak ontbreken van verwanten en in sommige gevallen zelfs het geheel afwezig zijn van een sociaal netwerk doen vermoeden dat de hier beschreven resultaten een reëel probleem vormen bij de zorg voor HIV-geïnfecteerde patiënten.

Zoals hier boven beschreven gaat de zorg voor HIV-geïnfecteerde kinderen gepaard met en veelheid van problemen. De behandeling van HIV geïnfecteerde kinderen dient derhalve in teamverband te gebeuren. De kern van een dergelijk team bestaat uit een gespecialiseerde kinderarts, HIV-verpleegkundige en medisch maatschappelijk werk. Tevens is het noodzakelijk een psycholoog deel uit te laten maken van een dergelijk team.

Referenties

1. Fraaij PL, Verweel G, van Rossum AM, Van Lochem EG, Schutten M, Weemaes CM, et al. Sustained viral suppression and immune recovery after 4 years treatment of HIV-1 infected children with protease inhibitor containing HAART. submitted Clin. Infec. Dis. 2004.
2. van Rossum AM, Geelen SP, Hartwig NG, Wolfs TF, Weemaes CM, Scherpbier HJ, et al. Results of 2 years of treatment with protease-inhibitor--containing antiretroviral therapy in dutch children infected with human immunodeficiency virus type 1. Clin Infect Dis 2002;34(7):1008-16.
3. Reddington C, Cohen J, Baldillo A, Toye M, Smith D, Kneut C, et al. Adherence to medication regimens among children with human immunodeficiency virus infection. Pediatr Infect Dis J 2000;19(12):1148-53.
4. Demas PA, Webber MP, Schoenbaum EE, Weedon J, McWayne J, Enriquez E, et al. Maternal adherence to the zidovudine regimen for HIV-exposed infants to prevent HIV infection: a preliminary study. Pediatrics 2002;110(3):e35.
5. Baars H, Uffing H, G. D. Sociale netwerkstrategieen in de sociale psychiatrie. Houten: Bohn Stafleu Van Loghum; 1990.
6. Lau JT, Tsui HY, Li CK, Chung RW, Chan MW, Molassiotis A. Needs assessment and social environment of people living with HIV/AIDS in Hong Kong. AIDS Care 2003;15(5):699-706.

A guideline on interventions in HIV-1 infected children with therapy failure

Pieter L.A. Fraaij, Ronald de Groot, Juul B.G. Rijkschroeff, Linda van der Knaap, Marie-José Blondeau, Tosca Segers, Hans L. van Es, Nico G. Hartwig

Medisch Contact 2004;59(45):1792-5

Chapter 19

Abstract

Introduction

After the introduction of 'highly active antiretroviral therapy' (HAART) a decrease was observed in HIV-1 related morbidity and mortality. In most children HAART results in full suppression of HIV-1 RNA levels and normalisation of the CD4+ T-cell counts. However, viral rebound, resistance of HIV to the medication and ultimately progression to AIDS occurs occasionally. Treatment failure is often caused by non adherence to HAART. Subsequently the treatment team will initiate interventions to improve adherence of the children. A complicated medical and ethical situation may arise when the caregiver(s) are unwilling or unable to administer the medication or do not accept these interventions. In order to deal with these problems we established a multidisciplinary working group. In this working group members of the HIV treatment team, the child protection service in Rotterdam, youth care organizations, a jurist and a juvenile court magistrate are represented. Members of the working group agreed that a standardized approach was required to deal with therapy failure in HIV-1 infected children. We therefore developed a guideline "Interventions in HIV-1 infected children with therapy failure".

Summary of the guideline

In the guideline "Interventions in HIV-1 infected children with therapy failure" the actions to reinitiate successful therapy are delineated. In short, after therapy failure (defined as an insufficient decrease or an increase of the HIV-1 RNA levels at more than one time point) the reasons for failure are assessed by means of a standardized questionnaire. This questionnaire is completed by the members of the treatment team together with patients and their caregivers. Depending on the results of this query selected interventions will be started. When therapy failure is thought to be related to non-adherence to HAART these interventions include an increase in the frequency of visits to the outpatient clinic, home visits by the HIV nurse, (economical) support by social worker and temporary directly administrated antiretroviral therapy (DAART) by a home care organization. Child protection services will be notified when the patients and their caregivers are unwilling or unable to give the medication correctly or comply with the interventions. After notification the child protection service will investigate the case. Depending on the results the juvenile court magistrate can be asked to impose a youth protection measure. These measures can vary from the appointment of a family custodian to (temporarily) care for the child in a foster-family. When a youth protection measure is imposed youth care organizations will always be involved to support the patients and their caregivers. To preserve the specific knowledge on HIV treatment this task will be performed by one nationally operating youth care organization.

First results after initiation of the guideline

Between 2000 and 2003 the guideline was used for intervention in 9 patients. Of these 9 children 8 were non-adherent to HAART. One of the 8 children 1 left the Netherlands to Africa before further action could be taken. For 3 of the 7 remaining children the treatment team asked the child protection service to investigate in the case, since parents were unwilling to give the medication and to comply with the interventions. The child protection service

requested a youth protection measure in 2 of the 3 children. In these 2 case the juvenile court magistrate decided on the appointment of a family custodian for one child and on further care in a foster-family for the other.

The interventions improved adherence in all 7 patients and resulted in undetectable viral loads in 6 of the 7 children one year after the first intervention. In one child HIV-1 RNA levels did decrease significantly but remained detectable. In this child viral resistance to HAART complicated full viral suppression.

Conclusion

A standardized approach to therapy failure resulted in an increase in adherence to the medication in children and subsequent suppression of HIV-1 RNA levels. Because of these favorable results we feel that the guidelines should be implemented nationally to improve the care for HIV-1 infected children.

Protocol gezamenlijke aanpak therapieontrouw

Multidisciplinaire richtlijn verbetert therapietrouw bij HIV-1 geïnfecteerde kinderen

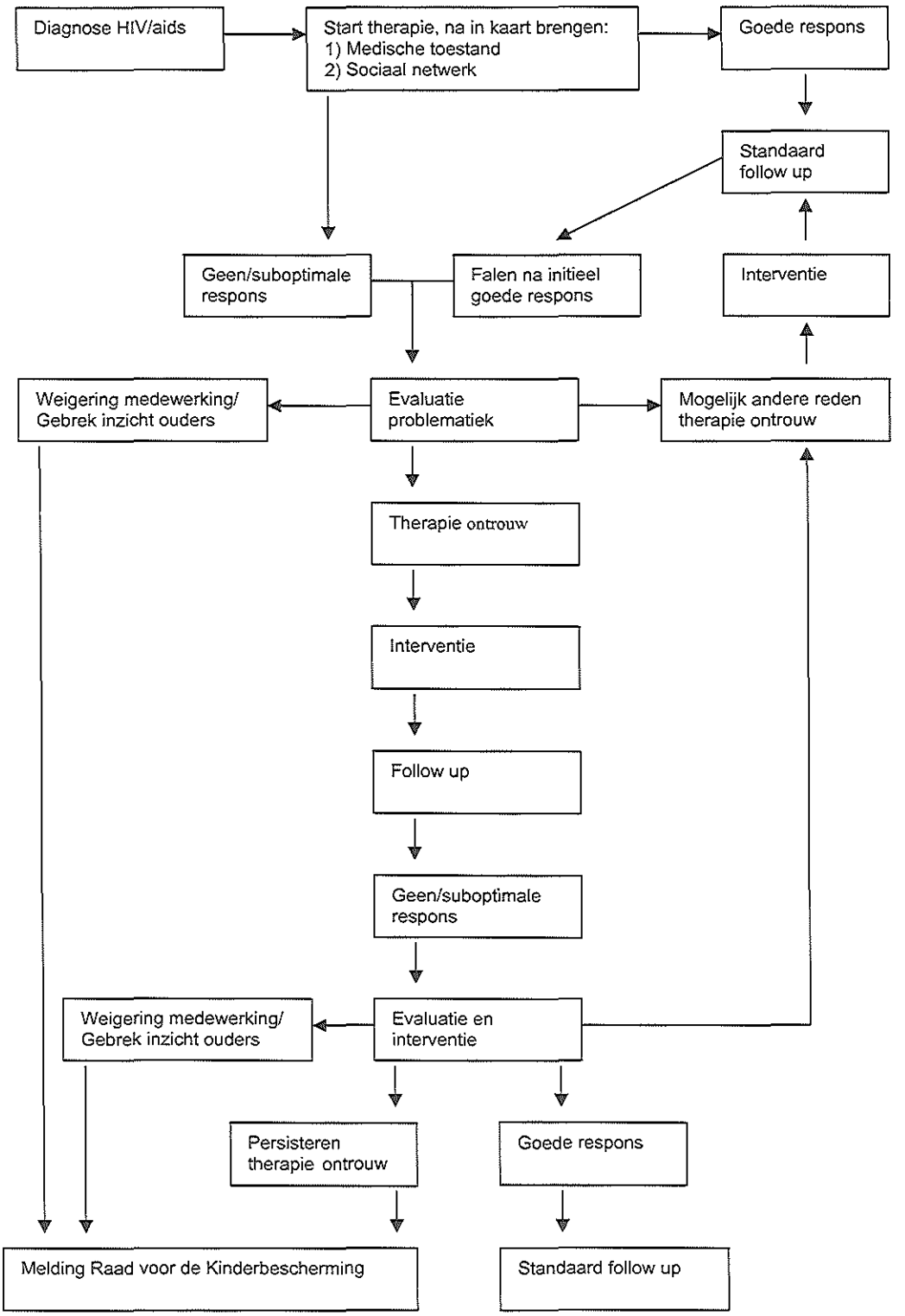
Het falen van de therapie bij HIV-geïnfecteerde kinderen door medicatieontrouw is een groot probleem. Het gebruik van multidisciplinaire richtlijn kan hierin verbetering brengen.

Sinds het beschikbaar komen van krachtige antiretrovirale medicatie, Highly Active Antiretroviral Therapy (HAART), is het toekomstperspectief voor patiënten met HIV in de westerse wereld sterk verbeterd. (1,2) Voor het slagen van therapie is therapietrouw van essentieel belang. Uit een studie bij volwassenen blijkt dat 95% van de medicatie goed ingenomen moet worden om een optimaal behandelresultaat te garanderen. (3) Het levenslang innemen van HAART is geen eenvoudige opgave. Voor de meeste geneesmiddelen is geen kindvriendelijke formulering beschikbaar. De tabletten, capsules of drank moeten vaak in grote hoeveelheden worden ingenomen en ze hebben over het algemeen een onaangename smaak. Het op vaste tijden innemen van medicatie is moeilijk vol te houden. Ook treden veelvuldig bijwerkingen op.

Inzicht therapieontrouw

Vanaf 1997 wordt in het Rotterdamse Erasmus MC-Sophia Kinderziekenhuis HIV-1 cohort follow-up onderzoek verricht naar de behandeling van HIV-1 geïnfecteerde kinderen. Het merendeel van de kinderen bleek na vier jaar behandeling in een goede klinische conditie te verkeren, met volledige onderdrukking van HIV. Een goede therapietrouw en een optimale dosering van de gebruikte medicatie waren belangrijke voorwaarden voor een succesvolle behandeling. (4) Het inzicht in de therapietrouw kan worden verbeterd met behulp van medicatiedagboeken, gesprekken tussen de behandelaar en de patiënten/ouders en de bepaling van medicatiespiegels in het bloed.
Het merendeel van de kinderen is voor therapieinname mede afhankelijk van de ouders. In het geval van therapieontrouw moet het behandelteam de ouders dan ook actief betrekken bij de benadering van de problemen. Gezien het belang van de antiretrovirale therapie voor de gezondheid van het kind ondernam het behandelteam veelvuldig pogingen om de therapietrouw te verbeteren. Hoewel deze interventies vaak succes hadden, bleek de vrijwillige basis van deze ondersteuning niet altijd voldoende. Het behandelteam werd in die gevallen geconfronteerd met complexe ethische en juridische vraagstellingen met betrekking tot de mogelijkheden tot interventie. Om de zorg voor HIV-geïnfecteerde kinderen zo optimaal mogelijk te organiseren, werd een bredere overlegstructuur opgezet. Hierin konden deze problemen worden besproken. Dit resulteerde in het Multidisciplinair HIV-overleg waarin vertegenwoordigers zitting hadden van de Raad voor de Kinderbescherming, de jeugdzorg, het arrondissementparket Rotterdam en het HIV-behandelteam van het Erasmus MC-Sophia. Tijdens de besprekingen achtten alle deelnemers een gestandaardiseerde aanpak van therapiefalen noodzakelijk. Dit om onnodige vertraging in de behandeling en onduidelijkheden tussen verschillende instanties te voorkomen.

Figuur 1. Schematisch overzicht handelswijze pediatrische HIV-infectie



Richtlijnen

In figuur 1 worden de richtlijnen rond therapiefalen schematisch uiteengezet. In het kort is de benadering als volgt: met behulp van een vragenlijst inventariseert het HIV- behandelteam zowel patiënt gerelateerde als medicatie gerelateerde factoren, die kunnen bijdragen aan het falen van de medicatie. Deze vragenlijst wordt door de behandelaren en de ouder/verzorgers ingevuld. Vervolgens worden de uitkomsten en de eventuele interventies besproken. Deze interventies houden in:

1. Een verhoging van de frequentie van het aantal contacten met leden van het behandelteam. Tijdens deze extra bezoeken wordt uitgebreid gesproken over het belang van therapietrouw en de door de patiënt en ouders ervaren problemen met het nemen van de medicatie.
2. Huisbezoeken door de HIV-verpleegkundige. Tijdens deze huisbezoeken kan waardevolle informatie over de sociaal-economische omstandigheden en de woonsituatie van het gezin worden verkregen. Tevens kan de medicatiegift in de thuissituatie worden geobserveerd en kunnen handreikingen worden gedaan om de inname te verbeteren.
3. Sociale en economische ondersteuning van het gezin. Met behulp van maatschappelijk werk kan worden getracht situaties die therapietrouw bemoeilijken (bijvoorbeeld onregelmatige werktijden in een eenoudergezin) of die de aandacht van de ouder/verzorger te veel afleiden (bijvoorbeeld schulden) te verbeteren.
4. Tijdelijke toediening van de medicatie door een thuiszorginstantie. Deze zogenoemde directly administrated antiretroviral therapy (DAART), is van tijdelijke aard. In deze overbruggingsperiode worden de ouders ontlast en kunnen de eerder genoemde interventies worden ondernomen. Tevens krijgt het behandelteam feedback van de thuisverpleegkundige. Dit levert vaak een duidelijker inzicht in de motivatie en de problemen van de ouders op.

In de loop van dit traject worden de interventies en de behaalde resultaten bij ieder bezoek met de ouders geëvalueerd. Mocht hierbij blijken dat gemaakte afspraken niet of onvoldoende worden nageleefd, dan zal het behandelteam deze patiënt aanmelden bij een AMK of in de regio Rotterdam direct bij de Raad voor de Kinderbescherming. De motivatie voor deze directe melding is dat in de ogen van het behandelteam een medisch onacceptabele acute levensbedreigende situatie is ontstaan voor het kind. Dit kan uiteindelijk leiden tot ernstige ziekte of de dood. Indien na het onderzoek van het AMK of de Raad voor de Kinderbescherming blijkt dat de bereidheid tot het adequaat toedienen van de medicatie afwezig blijft, zal de kinderrechter gevraagd worden een jeugdbeschermingsmaatregel op te leggen. Over het algemeen zal dit uiteindelijk resulteren in een (voorlopige) ondertoezichtstelling (V)OTS. Bij het uitspreken van deze maatregel wordt (tijdelijk) het gezag over de kinderen met de ouders gedeeld door een jeugdbeschermingsinstelling. Wegens de specifieke problemen en het geringe aantal patiënten dat in verband met de HIV-behandeling uiteindelijk met jeugdbescherming in contact komt, achtte de werkgroep het van groot belang deze kinderen bij één landelijk opererende jeugdbescherming instelling aan te melden. Hiertoe werd de William Schrikker Groep betrokken bij het overleg. Mocht een (V)OTS niet afdoende worden geacht of onvoldoende effect hebben, dan kan de Raad voor de Kinderbescherming of

de instelling voor jeugdbescherming aan de kinderrechter toestemming vragen om het kind onder te brengen op een plaats waar de verstrekking van de medicijnen wel is gegarandeerd. De duur van deze regeling hangt af van de uitspraak van de kinderrechter en of ouders alsnog in staat zijn en bereid blijken de medicatie te geven. De werkgroep benadrukt dat het niet in het belang van het kind wordt geacht om het kind uit huis te plaatsen. Alleen bij uiterste noodzaak moet hiertoe worden overgegaan.

Toepassing protocol

Tussen november 2001 en april 2003 werd het ontwikkelde protocol bij negen kinderen met een episode van therapiefalen toegepast. Na evaluatie door middel van de vragenlijst werd in acht gevallen vastgesteld dat het falen van de therapie berustte op therapieontrouw. Na het invullen van het vragenformulier en de vaststelling van therapieontrouw onttrokken de ouders van één patiënt zich aan de zorg. Voordat verdere actie kon worden ondernomen, vertrok dit gezin naar het buitenland. Eén jaar na het invullen van de vragenlijst was bij zes van de zeven overgebleven kinderen met therapieontrouw HIV niet meer aantoonbaar in het bloed. Bij één patiënt was wel sprake van een daling, maar bleef het virus aantoonbaar ondanks inname van de medicatie onder toezicht van de thuiszorg. Het virus was reeds resistent geworden voor de medicijnen, zodat het niet meer volledig onderdrukt kon worden. In vier van de zeven gevallen hadden de eerder beschreven interventies door het HIV-behandelteam een volledige onderdrukking van HIV tot gevolg. Bij twee kinderen werd na onvoldoende medewerking van de ouders en het uitblijven van behandelresultaten ondanks de interventies uiteindelijk besloten tot melding bij het AMK of de Raad voor de Kinderbescherming. Eén kind werd onmiddellijk na het gesprek met de ouder gemeld bij de Raad voor de Kinderbescherming.
Deze drie meldingen hadden tot gevolg dat één patiënt uit huis werd geplaatst en één patiënt ondertoezicht werd gesteld. In het laatste geval werd besloten geen verdere actie te ondernemen nadat moeder alsnog instemde met thuiszorg.

Medicatieontrouw

Het falen van therapie door medicatieontrouw is een groot probleem in de behandeling van HIV-geïnfecteerde kinderen. Aangezien kinderen afhankelijk zijn van hun ouders/verzorgers voor de medicatiegift, ligt de kern van het probleem ook vaak bij deze personen. De achtergrond van medicatieontrouw kan verschillend zijn: onmacht, onwil en ongeloof bij de ouders/verzorgers, verschil van inzicht met de behandelaren of de aanwezigheid van andere problemen die de aandacht van de ouders te veel afleiden. Daarnaast speelt angst voor het bekend raken van de diagnose een belangrijke rol.
Het vaststellen van medicatieontrouw is een complex proces. (5 ,6, 7) Bij afwezigheid van medicatie in het bloed kan worden gesteld dat de medicatie niet is in genomen. Omgekeerd is het aantreffen van medicatie in het bloed geen zekerheid dat de medicatie ook volgens het voorscheven schema is in genomen. Een goede relatie tussen de ouders/verzorgers en behandelaars is noodzakelijk om informatie over de medicatieinname te verkrijgen. Het toepassen van het faalprotocol heeft effecten op dit contact. Enerzijds kan een potentiële

melding aan de Raad voor de Kinderbescherming de ouders huiverig maken tot openheid omtrent de medicatietrouw. Anderzijds wordt duidelijkheid gecreëerd over de te nemen stappen. Op deze wijze worden onaangename verrassingen en argwaan voorkomen.
Een van de belangrijkste mogelijkheden om de medicatietrouw te garanderen, is tijdelijk de medicatie onder toezicht van een thuiszorgorganisatie te geven. Het daadwerkelijk toelaten van de thuiszorg binnen het gezin is een voorwaarde om de werkelijk gevraagde zorg te leveren.

Specifieke problemen

De onduidelijke verblijfstatus van de patiënt en zijn/haar familieleden kan problemen op leveren. Voorbeelden hiervan zijn: onduidelijkheden rond vergoedingen, problemen met indicatiestelling van thuiszorg en verwarring rond de rechtspositie van de kinderen en ouders/verzorgers. De werkgroep is van mening dat de verblijfstatus van een patiënt geen enkele rol mag spelen bij de behandeling. Naast bovengenoemde obstakels leidt het gebruik van nog niet-geregistreerde medicatie eveneens tot onduidelijkheden. In de praktijk kan het met enige regelmaat voorkomen dat de enige beschikbare effectieve medicatie nog niet geregistreerd is en de ouders dit weigeren te geven of geen zorgdragen voor een adequate inname. Juridisch staat het ouders en patiënten vrij om gebruik van een niet-geregistreerd geneesmiddel te weigeren. Toch concludeert de werkgroep dat gezien het levensreddende karakter van de therapie een dergelijk geval moet worden gemeld bij het AMK of de Raad voor de Kinderbescherming.

Het ontbreken van kennis rond pediatrische HIV en antiretrovirale therapie bij organisaties als de thuiszorg, het AMK en de Raad voor de Kinderbescherming, kan leiden tot vertragingen en miscommunicatie tussen de verschillende organisaties, met suboptimale antiretrovirale behandeling als gevolg. De hier beschreven richtlijn beoogt deze onduidelijkheden weg te nemen en optimale begeleiding van de patiënt en de ouders veilig te stellen.

Beperkingen

De door ons opgestelde richtlijn kent ook beperkingen. HIV kan door suboptimale therapie ongevoelig worden voor de gegeven medicatie. Dit is een ernstig risico gezien de lange wachttijd die optreedt voordat een plaats in een pleeggezin is gerealiseerd. Een andere beperking is het ontbreken van een aanpak van therapieontrouw bij pubers tussen de twaalf en zeventien jaar. Verplichte thuiszorg of uithuisplaatsing is voor deze groep vaak geen reële optie. In deze leeftijdscategorie nemen de kinderen vaak uit eigen beweging de medicatie niet goed in en hebben ze het recht mee te beslissen over de behandeling. Voor deze specifieke groep moet een andere benadering worden ontwikkeld. Ondanks deze tekortkomingen concluderen we dat toepassing van het faalprotocol tot zeer gunstige resultaten kan leiden. Een regio overstijgend protocol waarin behandelaars, AMK, Raad voor de Kinderbescherming en de William Schrikker Groep deelnemen kan een effectievere zorg voor kinderen met therapieontrouw bewerkstelligen.

Samenvatting

Bij het mislukken van de HIV-behandeling van een kind door onmacht of onwil van de ouders ziet het HIV-behandelteam zich geconfronteerd met een complexe ethische en juridische vraagstelling.
Om de zorg voor HIV-geïnfecteerde kinderen zo optimaal mogelijk te organiseren, werd het Multidisciplinair HIV-overleg opgericht.
Dit overleg leidde uiteindelijk tot het opstellen van het protocol Werkwijze bij therapie ontrouw van HIV-geïnfecteerde kinderen.

Referenties

1. de Martino M, Tovo PA, Balducci M, et al. Reduction in mortality with availability of antiretroviral therapy for children with perinatal HIV-1 infection. Italian Register for HIV Infection in Children and the Italian National AIDS Registry. JAMA 2000;284(2):190-7.
2. Gortmaker SL, Hughes M, Cervia J, et al. Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. N Engl J Med 2001;345(21):1522-8.
3. Paterson DL, Swindells S, Mohr J, et al. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. Ann Intern Med 2000;133(1):21-30.
4. van Rossum AM, Geelen SP, Hartwig NG, et al. Results of 2 years of treatment with protease-inhibitor–containing antiretroviral therapy in dutch children infected with human immunodeficiency virus type 1. Clin Infect Dis 2002;34(7):1008-16.
5. Van Dyke RB, Lee S, Johnson GM, et al Reported adherence as a determinant of response to highly active antiretroviral therapy in children who have human immunodeficiency virus infection. Pediatrics 2002;109(4):e61.
6. Reddington C, Cohen J, Baldillo A, et al. Adherence to medication regimens among children with human immunodeficiency virus infection. Pediatr Infect Dis J 2000;19(12):1148-53.
7. Demas PA, Webber MP, Schoenbaum EE, et al. Maternal adherence to the zidovudine regimen for HIV-exposed infants to prevent HIV infection: a preliminary study. Pediatrics 2002;110(3):e35.

Summary and discussion

Chapter 20

In **chapter 1** we describe the background of the studies presented in this thesis. The initial studies in HIV-1 infected children show that use of HAART results in viral suppression and normalization of the CD4+ T-cell count. (1-4) However, failure of therapy does occur and is associated with increasing HIV-1 RNA levels, viral resistance to the medication, decreased immune function and the subsequent development of AIDS. In addition, little is known about the long-term effects of HAART in children. Most prospective studies in HIV-1 infected children do not exceed an observation period of 48 weeks. To study novel approaches to HAART in children the multidisciplinary study group for HIV-1 infected children was established. Children included in the Rotterdam cohort are treated at the outpatient clinics of the Erasmus MC-Sophia Children's Hospital Rotterdam, University Medical Center St. Radboud and the VU medical Center Amsterdam. In addition studies were performed in collaboration with the Heinrich Heine University in Düsseldorf, Germany.

Chapter 2 provides an overview of the contents of this thesis. Studies were performed on 1) clinical, virological and immunologocal aspects of HAART in HIV-1 infected children, 2) the clinical pharmacology of HAART in children and 3) the psychosocial aspects of the treatment of HIV-1 infected children.

Clinical, virological and immunological aspects of HAART in HIV-1 infected children

Prospective studies on the use of HAART in HIV-1 infected children show a good viral suppression and a gradual recovery of the immune system. However, viral response rates are highly variable and frequently inferior to those observed in adults. (**Chapter 3**).

As discussed in **chapter 3** most prospective studies in HIV-1 infected children do not exceed an observation period of 48 weeks. Therefore little is known about the durability of the viral suppression and the reconstitution of the immune system. In **chapter 4** the 192 weeks (4 year) results of a prospective, open, cohort study on the clinical, immunological and virological response rates to therapy with protease inhibitor (PI) containing HAART in 31 HIV-1 infected children are reported. Throughout the follow-up period most children were in good clinical condition. Adverse events were observed frequently but were mild. After 192 weeks of treatment 80% and 76% of the patients had an HIV-1 RNA level below 500 and 50 copies/ml respectively. Despite these good results, viral failure occurred often and frequently required changes in therapy. The proportion of children with a suppressed viral load increased during follow-up. We propose that this may be due to therapy changes and more intensive intervention when non-adherence was suspected. The relative CD4+ T-cell counts in relation to the age specific reference values increased significantly after start of therapy and remained at 80% of normal values. Throughout the entire follow-up period CD8+ T-cells remained high, indicating an ongoing immune stimulation. This is different from data obtained in adults where CD8+ T-cells counts returned to baseline levels or even decreased below baseline levels after initiation of HAART. (5) Interestingly, a difference in CD8+ T-cell numbers as percentages of age related reference values was observed for patients who had viral suppression throughout the study period and those who had not. This may be the result of decreased antigenic stimulation.

Whether the decrease in CD8+ T-cells in the responder group reflects normalization of the CD8+ T-cell repertoire and loss of previously described clonal expansion of CD8+ T-cells due to HIV-1 infection is not yet clear.

Simplification of therapy is urgently needed to maintain adherence. Therefore two HAART regimens which could be used twice daily instead of thrice daily were evaluated. In **chapter 5** a study on the treatment of 21 HIV-1 infected children with indinavir plus low dose ritonavir and 2 NRTIs is presented. We found that this HAART regimen has potent antiretroviral activity, but is also frequently associated with side effects and premature discontinuation of therapy. This is of major concern since the occurrence of side effects poses a major threat to the adherence, whilst maintenance of adherence was the most important reason to perform this medication change. (6) Studies in children with new and easier to use HAART regimens show less side effects and a lower proportion of patients with premature study discontinuation (see **chapter 6).** Therefore we feel that newer and easier to use HAART regimens than indinavir/low dose ritonavir should be used to treat HIV-1 infected children. However, when these drugs are not available indinavir combined with ritonavir can be considered as a treatment option for HIV-1 infected children. In contrast, a combination of 2 NRTIs and abacavir seemed to be well tolerated as shown in **chapter 6.** In this chapter the antiviral efficacy, safety and pharmacokinetic parameters of the replacement of PIs for abacavir in children with HIV-1 RNA levels < 500 copies/ml were studied. This easy to use q12h dosed combination has only mild adverse events and none of he patients changed medication because adverse events. All patients had HIV-1 RNA levels < 50 copies/ml after 48 weeks of treatment. However, in one child a medication change was necessary because of viral failure. This child had received mono NRTI treatment prior to HAART.

Although HAART in children has potent activity viral rebound leading to resistance of HIV to the medication continues to occur. One of the most important reasons for viral resistance was the subsequent introduction of mono and dual NRTI treatment and finally HAART. Thus in the pre-HAART era significant resistance to NNRTI has evolved in patients. (7) It is unclear which treatment regimen should be used in children infected with NRTI-resistant virus. Both lopinavir/ritonavir and efavirenz containing regimens are potent and safe in children. (1, 4) We hypothesized that a combination of lopinavir/ritonavir and efavirenz without NRTIs would be safe and achieve maximal viral suppression, while at the same time, unnecessary NRTI related side effects could be prevented. In **chapter 7** a combination of lopinavir/ritonavir and efavirenz for the treatment of children infected with NRTI resistant HIV-1 is presented. The NRTI sparing regimen suppressed HIV-1 levels for a prolonged period and resulted in a significant increase in CD4+ T- cell numbers despite an extensive prior treatment with NRTI (>4 years). Side-effects were transient with the exception of dyslipidemia. After initiation of the study medication cholesterol levels increased, with 4 out of 7 children experiencing levels above the upper limit of normal (ULN) during follow-up. In these children LDL levels had increased as well. At baseline 3 out of 8 children had triglyceride levels above the ULN. This increased to 5 out of 7 at week 48. We conclude that the combination of lopinavir/ritonavir and efavirenz has potent

antiviral activity. However, dyslipidemia needs to be monitored carefully and addition data on effects of lopinavir and efavirenz use should be obtained. Risk factors for dyslipidemia and possible interventions should be studied.

In **chapter 8** we present the case history of an HIV-1 infected child with absent HIV-specific antibodies, in whom the appearance of HIV-specific antibodies followed after initiation of HAART. The absence of HIV specific antibodies was most likely due to a selective defect in T-B cell interaction. We speculate that as a consequence of the undetectable viral load, the CD4 T-cell function fully restored, which resulted in further maturation of the B cell compartment allowing the production of HIV-1 specific antibodies.

To optimize the outcome of HAART in children predictive factors for therapy success or failure are needed. In cohort studies in HIV-1 infected adults CD8+CD38+ T-cells and the intensity of CD38 expression on CD8 cells were found to be prognostic factors of disease progression to AIDS and a useful tool for the monitoring of HAART. (8-11) The role of CD38 in children remains unresolved. Several conflicting studies have been published. Both increased and decreased CD8+CD38+ T-cell counts and percentages were found to correlate with a favourable prognosis. (12-15) Furthermore, Vigano et al. reported that a higher percentage of CD8+CD38+ T-cells predicted maintenance of high viremia in children treated with HAART, whereas Caselli et al. could not confirm this correlation. (16, 17) We speculate that these contradicting results are caused by several factors: in children CD38 is both a marker of immaturity and a marker of immune activation. (11) Furthermore, the CD38 molecule is distributed ubiquitously on cells of different lineage, with heterogeneous expression levels. Thus lymphocytes that express CD38 cannot be clearly distinguished from those who do not. Subpopulations of CD38+ and CD38- lymphocytes can therefore only be defined by setting an arbitrary marker. To avoid selection we analysed the expression levels of CD38 on CD8+ T-cells and not absolute cell counts or proportions of CD38+CD8+ T-cells in HIV-1 infected children using HAART. Results of this approach are reported in **chapter 9.** After initiation of HAART the expression level of CD38 on CD8+ T-cells decreased significantly in all patients, both in responders and non-responders. We speculate that the decrease in CD38 expression in the non-responders may have been the result of decreasing immune activation. The median CD38+ expression level was not significantly different at any time point for responders and non-responders. In addition, CD38 expression levels at baseline did not differ between viral responders and non-responders at any time point in the study. We conclude that the usefulness of analysis of CD38 expression on CD8+ T-cells as prognostic marker in children with HIV/AIDS is limited. The sensitivity of our method may improve if CD38 expression is measured on HIV specific CD8+ T-cells. Still, even if measured on HIV-1 specific T-cells maturation of the immune system and frequent infections associated with childhood will influence the obtained results.

Pharmacology of antiretroviral agents in children

In **chapter 10** we present an overview on the use of therapeutic drug monitoring (TDM) in the treatment of HIV-1 infected children. The use of TDM allows to optimize plasma drug concentrations of antiretroviral drugs. This is important when one considers that the levels of viral suppression and drug toxicity in adults and children are associated with the plasma concentration of PIs and NNRTIs. (18-20) Indeed in clinical practice the use of TDM has favorable results. (See also **chapter 3**) However, there is a serious shortage on population pharmacokinetic reference values of antiretroviral medication in children. Therefore plasma drug levels in children are often targeted to adult reference values. This may be insufficient because of the extremely high HIV-1 RNA levels and immature immune system associated with HIV infection in children. Currently different pharmacokinetic (PK) parameters can be used to perform TDM: trough levels (Cmin), peak levels (Cmax) and full drug exposure by means of analysis of the area under the plasma concentration-time curve (AUC). At this moment no data are available as to which parameter is the best for the measurement of pharmacokinetics in children and how often PK analysis should be performed.

Apart from its primary function for dose optimisation, TDM can also be used as a tool to assess adherence to antiviral medication. One should however be cautious to base assumptions on plasma levels alone, since aberrant plasma levels may also be the result of other factors such as changes in nutritional habits, drug-drug interactions or changing gastric motility. (21) Overall it is concluded that TDM is a useful tool in the treatment of HIV-1 infected children. However, additional data are needed to establish child-specific reference values and to assess the optimal method of TDM.

Two of the three classes of antiretroviral agents are suitable for TDM: the NNRTIs and the PIs. NRTIs are pro-drugs which are intracellularly converted to active NRTI-triphosphates (NRTI-TP). (22) Therefore, plasma concentrations of NRTIs may not be a good indicator to predict antiviral activity. Indeed intracellular levels of NRTI triphosphate level seem to correlate better with antiviral efficacy. (22-24) However, measurement of the active intracellular levels of the NRTIs is laborious and needs to be performed in specialised research laboratories. Moreover the currently available techniques require large volumes of blood and are therefore not suitable for use in children. In **Chapter 11** we report on a new method based on Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry (MS) for analysis of zidovudine-triphosphate (AZT-TP) and (deoxy)nucleotide-triphosphates, which may be used in the future for NRTI treatment monitoring in HIV-1 infected children and adults. AZT-TP and dNTPs were detected up to 0.5 femtomole per sample. Furthermore, intracellular AZT-TP, ATP and dGTP were detected in Peripheral Blood Mononuclear Cells (PBMCs). A complicating factor is that zidovudine-triphosphate, ATP and dGTP yield identical mass spectra. MALDI-TOF Post Source Decay analysis can be used for discrimination between these compounds. However, application of Post Source Decay analysis may seriously increase the limit of detection. Addition of enzymes that specifically degrade ATP and dGTP or derivatization of AZT-TP may allow for a better limit of detection. Studies on this topic are still in progress. In the

near future a MALDI TOF MS method for accurate quantification of all NRTI-TP in PBMC can be developed, requiring only small amounts of blood. This method can be useful for studies on NRTI-TP levels in HIV-1 infected children and adults.

The danger of applying a fixed dosage of antiretroviral therapy in children is illustrated in **chapter 12.** In this chapter the pharmacokinetics (PK) of the protease inhibitor nelfinavir and its active metabolite M8 in children and influence of patient-related factors on plasma levels of nelfinavir are described. Nelfinavir pharmacokinetics were highly variable. Ten out of 24 children had an AUC0-8 below the value of 12.5 mg/L*h, which has previously been associated with increased virologic failure rate in children. (25) Age < 2 years and a dose of 20 mg/kg q8h both showed a trend to lower plasma concentrations of nelfinavir, and higher rate of AUC0-8 below 12.5 mg/L*h. Young age (<2 years) previously has been reported to be associated with suboptimal nelfinavir levels. (26) This may be explained by factors such as higher metabolic clearance in young children, impaired absorption, and lower amount of alpha-acid glycoprotein (27). Because of the risk for suboptimal nelfinavir plasma levels the nelfinavir dosage of 20 mg/kg q8h can better not be used in children. Data from our study indicate that nelfinavir should be dose based on BSA , rather than body weight (BW). Interestingly no significant relationship was found between plasma levels of nelfinavir and virologic efficacy. However, it should be noted that children naive and non-naive for protease inhibitors were not equally distributed between the groups. Most children in the group with nelfinavir levels below 12.5 mg/L*h were naive and naive patients tend to respond better to antiretroviral therapy than pretreated patients. Additional studies to establish the therapeutic range of nelfinavir in children are required.

The PK profile of 400 mg/m^2 indinavir with 125 mg/m^2 q12h ritonavir in 14 HIV-1 infected children is explored in **chapter 13.** Addition of the HIV protease inhibitor ritonavir to indinavir containing therapy increases plasma levels of indinavir, allowing for a twice daily medication scheme without the necessity for food restrictions. (28, 29) In none of the patients, C_{min} of indinavir was below the value of 0.1 mg/L, which has been associated with increased virologic failure rate. (30) The regimen resulted in significantly higher AUC_{0-24} of indinavir than reference data of indinavir q8h in both adults and children. The explored dose combination resulted in AUC_{0-24} and C_{min} of indinavir approximately similar to the pharmacokinetic data of indinavir with low-dose ritonavir in adults.

PIs and NNRTIs are both substrates for of cytochrome P450 (CYP) isoenzymes. Thus the pharmacokinetics of these drugs are likely to interact with each other. In children using nevirapine a 30% increased lopinavir dosage was needed to obtain plasma levels comparable to plasma levels without nevirapine. A similar dose increase is recommended when lopinavir is combined with efavirenz. However, no data are available whether this increase is sufficient. In **chapter 14** the pharmacokinetics of increased dose lopinavir (300/75 mg/m^2 q12h) with normal dose efavirenz (14 mg/kg q24h) are studied. We concluded that the pharmacokinetic data were generally similar to historical data of lopinavir alone or in combination of NNRTI in adults and

children. However, plasma levels of lopinavir showed a high interindividual variability. Interestingly, 5 of the 15 children had distinctly lower plasma levels of lopinavir compared to the other children. This finding may indicate that a subpopulation exists which is prone to have low lopinavir plasma levels. However, the number of children included in our study was small (n= 15). Additional data in a larger group of children with comparatively low lopinavir plasma levels are necessary to definitively identify this group and study the underlying mechanisms that result in the decreased lopinavir plasma levels.

The NRTI backbones of the HAART regimens are in clinical practice mostly dosed q12h or q24h to simplify the available regimens. However, with the exception of neonates and infants, there are no published data on the pharmacokinetics of zidovudine q12h in children (31, 32). In a small group of 6 children we studied the pharmacokinetics of zidovudine dosed q12h (**Chapter 15**). In these children, geometric mean ratios of $AUC_{0\text{-}24}$ and C_{max} for zidovudine q12h vs. q8h were not significantly different from 1.0, suggesting bioequivalence. However, pharmacokinetic parameters were highly variable. Therefore our data need to be confirmed in a larger number of patients. As mentioned earlier NRTI require intracellular phosphorylation for activation. We feel that intracellular pharmacokinetics of AZT should be measured as well in future studies to evaluate the exact dosage interval of zidovudine.

In all patients in the Rotterdam cohort steady state intensive plasma PK sampling of PIs is performed. Dose changes are made and PK sampling is repeated until the (adult) target PK values are reached. Thus suboptimal plasma levels due to an inadequate dosage regimen may prevented. Little information is available on changes in the pharmacokinetic parameters after prolonged PI use in children. These changes may be expected, since growth and development have a significant impact on drug absorption, distribution and clearance. (27) In addition, studies in adults indicate that indinavir exposure decreases after prolonged use. (33) To study changes in indinavir exposure in time, PK analysis was repeated in 6 children after at least 2 years of treatment with indinavir. Results of this study are presented in **chapter 16**, all children had an AUC above the target level of 15 mg /h*l on the first day, but at the second pharmacokinetic analysis 3 children had an AUC below 15 mg /h*l. This decreased exposure to indinavir was associated with viral failure in 2 patients. The exact mechanism responsible for this decrease in plasma levels remains unclear. However, we suggest to routinely perform TDM to prevent viral failure because of changes in drug metabolism over time.

In **chapter 17** we review the currently available data on the pharmacokinetics and pharmacodynamics of antiretroviral drugs in children. For a substantial number of drugs, dose recommendations are absent for use in children. Especially in young children, a serious shortage in pharmacokinetic data and evidence based dose recommendation exists. Different guidelines recommend different dosages and the range of the dosages advised often is broad. Thus HIV-1 infected patients receive highly divergent doses of medication.

The groups of children included in the pharmacokinetic studies were often small. In addition, the patients were divided in divergent groups using different dose levels and/or formulations. Thus, the number of children using one specific dosage and formulation of ART was further reduced. This is a serious problem, concerning that the inter- and intrapatient variability of the pharmacokinetic data observed for all antiretroviral drugs in children is high. Because of the absence of clearly established fixed dosages these findings clearly support our vision that TDM should be part of the routine care of HIV-infected children to avoid therapy failure and toxicity.

Psychosocial aspects of the treatment of HIV-1 infected children

Adherence to the HAART regimen is pivotal for a HAART regimen to succeed. (3, 34) The social situation of a family may have a tremendous impact on the ability to consistently access care and adhere to medication. Previous studies have shown that caregivers with a limited social support network are more likely to be non adherent to antiretroviral therapy. (35) In **chapter 18** the social situation of HIV-1 affected families is reported. We found that HIV-1 infection of children is associated with serious psychosocial problems in the family. In 16 of the 59 (27%) children one or both parents had died. During the follow-up period 14 of the 59 (24%) children were cared for by others than the biological parents. Child protection services were involved in 16 of the 59 (27%) children. The social support network of the caregivers was limited, with a mean of 13 (range, 4-43) social network members (> 16 years of age). Strikingly, a high number (30%) of the reported social support network members was obtained through community services. The accessibility of the social network members was of serious concern , since a high number of the social support network members were not living in the Netherlands. We speculate that these problems are the result of HIV/aids related morbidity of the caregivers, fear of exposing the diagnosis to other people and the fact that the parents of our patients are generally not born in the Netherlands. It is often difficult to obtain a solid social support network in another country than the country of birth. Physicians treating HIV infected individuals should be aware of these problems.

Treatment failure is often induced by non-adherence to the HAART regimen. (7) Therefore, upon failure of therapy the treatment team will initiate interventions to improve adherence of the patients. These interventions include an increase in the frequency of visits to the out patient clinic, home visits by the HIV nurse, (economical) support by social work and directly administrated antiretroviral therapy (DAART) by home care organizations. Children are often dependent of their caregivers for the administration of therapy. A complicated medical and ethical situation may arise when the caregiver(s) are unwilling to administer the medication or do not accept the interventions initiated by the treatment team. In order to deal with these problems a multidisciplinary working group was established. In this working group members of the HIV treatment team, child protection service, youth care organisations and juridical experts were represented. The initiative resulted in the composition of the guideline "Interventions in HIV infected children with therapy failure". In this guideline all interventions are clearly and stepwise set out. In **chapter 19** the guideline and the results obtained after implementation of the guideline in the Rotterdam cohort are presented. Between 2000 and 2003 the guideline

was used for intervention in 9 patients. Of these 9 children 8 were non-adherent to the medication. In 7 out of these 8 children interventions conform the guidelines improved adherences. One year after start of the interventions 6 of the 7 children had an undetectable viral load. Because of these favorable results we feel that the guidelines should be implemented nationally to improve the care for HIV infected children.

1. Starr SE, Fletcher CV, Spector SA, et al. Combination therapy with efavirenz, nelfinavir, and nucleoside reverse-transcriptase inhibitors in children infected with human immunodeficiency virus type 1. Pediatric AIDS Clinical Trials Group 382 Team. N Engl J Med 1999;341(25):1874-81.
2. Vigano A, Dally L, Bricalli D, et al. Clinical and immuno-virologic characterization of the efficacy of stavudine, lamivudine, and indinavir in human immunodeficiency virus infection. J Pediatr 1999;135(6):675-82.
3. van Rossum AM, Geelen SP, Hartwig NG, et al. Results of 2 years of treatment with protease-inhibitor--containing antiretroviral therapy in dutch children infected with human immunodeficiency virus type 1. Clin Infect Dis 2002;34(7):1008-16.
4. Saez-Llorens X, Violari A, Deetz CO, et al. Forty-eight-week evaluation of lopinavir/ritonavir, a new protease inhibitor, in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2003;22(3):216-224.
5. Pakker NG, Notermans DW, de Boer RJ, et al. Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation. Nat Med 1998;4(2):208-14.
6. Max B, Sherer R. Management of the adverse effects of antiretroviral therapy and medication adherence. Clin Infect Dis 2000;30 Suppl 2:S96-116.
7. Deeks SG. Treatment of antiretroviral-drug-resistant HIV-1 infection. Lancet 2003;362(9400):2002-11.
8. Giorgi JV, Liu Z, Hultin LE,. et al. Elevated levels of CD38+ CD8+ T cells in HIV infection add to the prognostic value of low CD4+ T cell levels: results of 6 years of follow-up. The Los Angeles Center, Multicenter AIDS Cohort Study. J Acquir Immune Defic Syndr 1993;6(8):904-12.
9. Liu Z, Hultin LE, Cumberland WG, et al. Elevated relative fluorescence intensity of CD38 antigen expression on CD8+ T cells is a marker of poor prognosis in HIV infection: results of 6 years of follow-up. Cytometry 1996;26(1):1-7.
10. Liu Z, Cumberland WG, Hultin LE, et al. Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR and CD38 expression. J Acquir Immune Defic Syndr Hum Retrovirol 1997;16(2):83-92.
11. Savarino A, Bottarel F, Malavasi F, et al.. Role of CD38 in HIV-1 infection: an epiphenomenon of T-cell activation or an active player in virus/host interactions? AIDS 2000;14(9):1079-89.
12. de Martino M, Rossi ME, Azzari C, et al. Different meaning of CD38 molecule expression on CD4+ and CD8+ cells of children perinatally infected with human immunodeficiency virus type 1 infection surviving longer than five years. Pediatr Res 1998;43(6):752-8.
13. Schlesinger M, Peters V, Jiang JD, et al. Increased expression of activation markers on CD8 lymphocytes in children with human immunodeficiency virus-1 infection. Pediatr Res 1995;38(3):390-6.
14. Sherman GG, Scott LE, Galpin JS, et al. CD38 expression on CD8(+) T cells as a prognostic marker in vertically HIV-infected pediatric patients. Pediatr Res 2002;51(6):740-5.
15. Plaeger-Marshall S, Hultin P, Bertolli J, et al. Activation and differentiation antigens on T cells of healthy, at-risk, and HIV-infected children. J Acquir Immune Defic Syndr 1993;6(9):984-93.
16. Vigano A, Saresella M, Rusconi S, et al. Expression of CD38 on CD8 T cells predicts maintenance of high viraemia in HAART-treated HIV-1-infected children. Highly active antiretroviral therapy. Lancet 1998;352(9144):1905-6.
17. Caselli D, Comolli G, Maccabruni A, et al. CD38/CD8 expression and HAART failure. Lancet 1999;353(9155):840-1.
18. Back D, Gatti G, Fletcher C, et al. Therapeutic drug monitoring in HIV infection: current status and future directions. AIDS 2002;16 Suppl 1:S5-37.

19. Van Heeswijk RP. Critical issues in therapeutic drug monitoring of antiretroviral drugs. Ther Drug Monit 2002;24(3):323-31.
20. Burger DM, Aarnoutse RE, Hugen PW. Pros and cons of therapeutic drug monitoring of antiretroviral agents. Curr Opin Infect Dis 2002;15(1):17-22.
21. van Rossum AM, Bergshoeff AS, Fraaij PL, et al. Therapeutic drug monitoring of indinavir and nelfinavir to assess adherence to therapy in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2002;21(8):743-7.
22. Gao WY, Shirasaka T, Johns DG, et al.. Differential phosphorylation of azidothymidine, dideoxycytidine, and dideoxyinosine in resting and activated peripheral blood mononuclear cells. J Clin Invest 1993;91(5):2326-33.
23. Perno CF, Yarchoan R, Cooney DA, et al. Inhibition of human immunodeficiency virus (HIV-1/HTLV-IIIBa-L) replication in fresh and cultured human peripheral blood monocytes/macrophages by azidothymidine and related 2',3'-dideoxynucleosides. J Exp Med 1988;168(3):1111-25.
24. Fletcher CV, Acosta EP, Henry K, et al. Concentration-controlled zidovudine therapy. Clin Pharmacol Ther 1998;64(3):331-8.
25. Hsyu P, Capparelli E, Amantea M, et al. Pharmacokinetics (PK) of TID nelfinavir (NFV) and correlation to efficacy in pediatric patients. In: 1st IAS Conference on HIV Pathogenesis and treatement; 2001; 2001.
26. Litalien C, Faye A, Compagnucci A, et al. Pharmacokinetics of nelfinavir and its active metabolite, hydroxy-tert-butylamide, in infants perinatally infected with human immunodeficiency virus type 1. Pediatr Infect Dis J 2003;22(1):48-55.
27. King JR, Kimberlin DW, Aldrovandi GM, et al. Antiretroviral Pharmacokinetics in the Paediatric Population: A Review. Clin Pharmacokinet 2002;41(14):1115-1133.
28. Hsu A, Granneman GR, Cao G, et al. Pharmacokinetic interaction between ritonavir and indinavir in healthy volunteers. Antimicrob Agents Chemother 1998;42(11):2784-91.
29. van Rossum AM, de Groot R, Hartwig NG, et al. Pharmacokinetics of indinavir and low-dose ritonavir in children with HIV-1 infection. AIDS 2000;14(14):2209-10.
30. Burger DM, Hoetelmans RM, Hugen PW, et al. Low plasma concentrations of indinavir are related to virological treatment failure in HIV-1-infected patients on indinavir-containing triple therapy. Antivir Ther 1998;3(4):215-20.
31. Mirochnick M, Capparelli E, Dankner W, et al.. Zidovudine pharmacokinetics in premature infants exposed to human immunodeficiency virus. Antimicrob Agents Chemother 1998;42(4):808-12.
32. Moodley D, Pillay K, Naidoo K, et al. Pharmacokinetics of zidovudine and lamivudine in neonates following coadministration of oral doses every 12 hours. J Clin Pharmacol 2001;41(7):732-41.
33. Gisolf EH, van Heeswijk RP, Hoetelmans RW, et al. Decreased exposure to saquinavir in HIV-1-infected patients after long-term antiretroviral therapy including ritonavir and saquinavir. AIDS 2000;14(7):801-5.
34. Watson DC, Farley JJ. Efficacy of and adherence to highly active antiretroviral therapy in children infected with human immunodeficiency virus type 1. Pediatr Infect Dis J 1999;18(8):682-9.
35. Demas PA, Webber MP, Schoenbaum EE, et al. Maternal adherence to the zidovudine regimen for HIV-exposed infants to prevent HIV infection: a preliminary study. Pediatrics 2002;110(3):e35.

Conclusions and future perspectives

Chapter 21

Conclusions and future perspectives

In this chapter we briefly summarize the conclusions from our research and present ideas concerning the future directions of care and research in children with HIV/AIDS. The major conclusions of this thesis are summarized in **Table 1** and future directions for research and care in **Table 2.**

Almost all of the patients described in this thesis are participants in the Rotterdam cohort. The principle design of the Rotterdam cohort is that care of HIV-1 infected children is organized in a structured way and research projects predominantly serve to improve the quality of care for HIV-1 infected children. Between 1997 and 2004, 59 HIV-1 infected children have been treated in this cohort. Care for these children is provided by a multidisciplinary team, consisting of an HIV-nurse, social worker, research physicians and pediatricians specialised in infectious diseases and immunology. The major objective of the treatment is to obtain durable suppression of HIV below the detection limit of 50 HIV-1 RNA copies/ml. Durable suppression can only be obtained when adherence to the medication regimen is ensured. This is done by structured discussions with patients and parents, provision of age-adequate information on the disease, social and economic support of the families and the development of easier to use highly active antiretroviral therapy (HAART) regimens. For structured follow-up patients are treated according to a study protocol and the patients' medical history, physical examination and laboratory values are analysed every 3 months. In case of viral failure patients and their parents are contacted immediately and subsequently interventions are performed. Soon after the start of treatment of HIV infected children with HAART the importance of therapeutic drug monitoring (TDM) was acknowledged and became part of the routine care for children with HIV/AIDS. In each patient an intensive pharamacokinetic analysis is performed. If required individual dose changes are done based on the results of this analysis. This procedure is repeated until acceptable plasma drug levels are obtained. In addition, from 2000 on random plasma drug samples are analyzed every visit to our outpatient clinic to analyse the presence of changes in drug metabolism and to monitor adherence.

This approach has contributed to the excellent clinical outcome in our patients. After 4 years of treatment the virological response rates are high (80% <500 copies/m and 76% <50 copies/ml) and fully comparable to those obtained in adults. In the 4 year follow-up period CD4+ T-cell numbers recover and remain stable. New HAART regimens were developed, which are easier to take and more effective for use in children with viral resistance to the medication. However, medication needed to be changed often because of viral failure. In addition, with time the proportion of children that encountered difficulties to adherence to the medication regimen increased. To deal with medication failure guidelines on interventions in HIV-1 infected children with therapy failure were constructed in collaboration with child protection services, jurists and youth care institutions. The first results of this study on the implementation of these guidelines are promising. Of major concern are the serious psychosocial problems encountered in many of the HIV affected families. These problems pose a serious treat for the development of HIV-1

infected children. Therefore we strongly recommend to include a psychologist as a permanent member of the HIV treatment team.

Most studies in this thesis are pilot experiments and therefore performed in small numbers of patients. The findings from these studies need confirmation in studies with a larger sample size. Randomized trials need to be performed to compare the efficacy and side-effects of the different HAART regimens in this thesis.

Finally, in our studies we show that the treatment of HIV-1 infected children under conditions as mentioned above is highly successful. Treatment leads to an optimal clinical response, viral suppression and recovery of the immune system. Adverse events occur but are generally mild and transient.

Table 1. Major conclusions from this thesis[$]

Part one: Clinical aspects of HAART in children	**Evidence obtained from chapter(s)[$]**
Virological response rates to HAART are durable and comparative to those in adults	3, 4, 6, 7
Application of therapeutic drug monitoring improves efficacy of HAART	3, 4, 10,16, 17
Poor adherence is associated with viral failure	4, 7
Simplified q12h administered HAART regimens can be used for the treatment of HIV-1 infected children	5, 6, 7,13, 14, 15
A NRTI sparing regimen of Lopinavir/r and efavirenz fully suppresses HIV-1 RNA levels in NRTI resistant HIV-infected children.	7
Immunological aspects of HAART in children	
Initiation of HAART results in durable reconstitution of the immune system of HIV-1 infected children	4, 8
CD8+ T-cell counts of HIV-1 Infected children treated with HAART decrease only after prolonged viral suppression	4, 7, 9
After initiation of HAART the expression level of CD38 on CD8+ T-cells decreases in all patients	9
The median expression level of CD38 on CD8+ T-cells does not correlate with outcome of antiviral therapy	9
Part two: Pharmacology of antiretroviral agents in children with HIV-1 infection	
The application of therapeutic drug monitoring is imperative for the treatment of HIV-1 infected children	3, 4, 10, 12, 16, 17
A serious shortage of antiretroviral medication PK reference values exists for in the pediatric population	10, 12, 17
The dosage of antiretroviral therapy is based on limited peer reviewed information	12, 14, 17
Part three: Psycho-social aspects of HIV treatment	
Caregivers of HIV infected children possess a limited social support network. This is associated with serious psychological, economical and social problems	18
Structured interventions upon treatment failure results in increased adherence to the medication and subsequent viral suppression	19

[$] Level of evidence in most of the here presented studies is graded level 3 (non-comparative research) on a scale form 1 to 4 (1 = evidence provided by 2 or more independent randomized trials of sufficient sample size, whereas 4 = expert opinion)

Table 2. Recommendations on future directions for research and care for HIV-1 infected children.

Child specific pharmacokinetic (PK) reference values are required to optimize therapeutic drug monitoring (TDM) in children. In addition the optimal PK parameter(s) to evaluate antiretroviral therapy in children need to be identified • Shortly, the Paediatric European Network for Treatment of AIDS (PENTA) will start a controlled study to evaluate the effect of TDM on clinical and virological outcome in children with HIV/AIDS (PENTA-14). • This trial will also serve to determine the optimal parameter(s) to evaluate the pharmacokinetics of PIs in children. • In addition, child-specific reference values for different PIs will be generated
Increased knowledge on the intracellular metabolism of antiretroviral drugs is required • The MALDI TOF MS method to measure intracellular (IC) drug levels will be extended to NNRTIs and PIs. • IC drug concentration in different cell types and IC drug-drug interactions will be studied *in vitro*. • The relationship between the intracellular drug concentrations, viral suppression and drug toxicity will be studied *in vivo* both in adults and children
Easier to use HAART regimens should be developed to simplify therapy and sustain adherence to the medication • Currently the RONDO study is ongoing in which a once daily administered HAART regimen composed of lopinavir/r, lamivudine and abacavir is evaluated
Further studies on the occurrence of dyslipidemia in larger group of children using HAART are required.
Studies on CD8+ T-cell receptor Vβ repertoire or HLA peptide HIV specific tetrameric complexes may elucidate why the CD8+ T-cell count decreases in responders after pronlonged viral suppression
Adherence to the medication must be improved by age-specific education and support for the patients and their families (psychologist, interactive computer program and adolescent discussion groups). • Currently in Rotterdam cohort children are educated by a specialized HIV-nurse on their diagnosis by means of inter-active computer program. In addition a national adolescent discussion group has been established. • Considering the significant psychological problems associated with an HIV-1 infection a psychologist should be a permanent member of the HIV treatment team. • Novel information methods for children, parents and caretakers including the initiation of separate outpatient consults by the HIV-nurse should be developed. • The efficacy of the education and support of HIV-1 infected children should be evaluated in a study setting.
National implementation of the developed protocol for interventions after treatment failure in HIV infected children will further improve the care for HIV-1 infected children.

Samenvatting van dit proefschrift

Chapter 22

Samenvatting van dit proefschrift

In de geïndustrialiseerde wereld zijn de morbiditeit en mortaliteit door HIV/aids sterk gedaald sinds het beschikbaar komen van krachtige antiretrovirale combinatie therapie, ook wel "highly active antiretroviral therapy" (HAART) genoemd. Deze daling wordt zowel bij volwassenen als bij kinderen gezien. Toch zijn de virologische respons percentages bij kinderen aanzienlijk lager dan die bij volwassenen (Hoofdstuk 3). De minder goede resultaten bij de behandeling van HIV-1 geïnfecteerde kinderen hangen mede samen met de voor deze leeftijdsgroep specifieke problematiek met betrekking tot de therapie trouw en de aanzienlijke variatie in de farmacokinetiek van antivirale middelen. Helaas is tot op heden weinig onderzoek gedaan naar de behandelresultaten van met HIV-1 geïnfecteerde kinderen. De beschikbare studies zijn daarnaast veelal ongecontroleerd en uitgevoerd in een kleine populatie. Dit proefschrift bevat een aantal studies over patiëntgebonden onderzoek bij HIV-1 geïnfecteerde kinderen. In de eerste hoofdstukken worden studies beschreven op het gebied van de klinische, virologische en immunologische aspecten van de behandeling met HAART. Het tweede deel van het proefschrift handelt over de farmacologie van antiretrovirale middelen bij kinderen. In het laatste deel worden psychosociale aspecten,samenhangend met de behandeling van HIV-1 geïnfecteerde kinderen besproken.

Klinische, virologische en immunologische aspecten van behandeling met HAART bij HIV-1 geïnfecteerde kinderen

Het merendeel van de studies in dit proefschrift zijn verricht in het Rotterdam cohort. Tevens werden een aantal studies uitgevoerd in samenwerking met de afdeling Kindergeneeskunde van de Heinrich Heine Universität in Düsseldorf.

Het Rotterdam cohort is een samenwerkingsverband tussen het Erasmus MC-Sophia Kinderziekenhuis in Rotterdam, het Universitair Medisch Centrum St. Radboud in Nijmegen en het VU Medisch centrum in Amsterdam. Tussen 1997 en 2004 werden in totaal 59 HIV-1 geïnfecteerde kinderen behandeld in het Rotterdam cohort. Het multidisciplinaire behandelteam bestaat uit een gespecialiseerde HIV-verpleegkundige, een maatschappelijk werkster, artsonderzoekers en kinderarts-infectiologen. De aan het Rotterdam cohort ten grondslag liggende filosofie is dat onderzoeksprojecten mede een bijdrage moeten leveren aan de verbetering van de zorg voor de geïnfecteerde kinderen. Daaruit vloeit voort dat dat de zorg voor HIV-1 geïnfecteerde kinderen georganiseerd moet worden op een geprotocolleerde manier.
Deze aanpak heeft geleid tot uitstekende resultaten. In Hoofdstuk 4 worden de klinische, virologische en immunologische resultaten van 4-jaars behandeling van HIV-1 geïnfecteerde kinderen met HAART beschreven. Van de 31 kinderen in deze groep is één patiënt overleden. De overige patiënten verkeren in een goede klinische conditie. Vier jaar na de start van de behandeling hadden 76% van de kinderen onmeetbaar lage hoeveelheden virus in het bloed bij een detectie grens van 50 HIV-1 RNA kopieën. Deze resultaten zijn vergelijkbaar met die verkregen in studies bij volwassenen met HIV/aids. De onderdrukking van het virus had tot

gevolg dat het mediane CD4+ T-cel getal blijvend herstelde. Bijwerkingen werden frequent geobserveerd en waren over het algemeen van gastro-intestinale aard en mild van karakter. Ondanks deze uitstekende resultaten werd therapie falen gedurende de vervolgperioden veelvuldig waargenomen. Dit hing samen met het toenemende aantal patiënten met therapie ontrouw. Het is dus van groot belang om HAART regimes te ontwikkelen die eenvoudiger van opzet zijn en waarbij minder frequent medicatie ingenomen behoeft te worden. Twee van deze regimes werden in het Rotterdam cohort bestudeerd (Hoofdstukken 5 en 6). In Hoofdstuk 5 wordt een combinatie van indinavir met laag gedoseerde ritonavir en 2 nucleoside reverse transcriptase remmers (NRTI) beschreven. Dit regime had een goede antiretrovirale werkzaamheid, maar gaf ook veel bijwerkingen. Hierdoor lijkt het niet geschikt voor toepassing in de Nederlandse situatie waar gemakkelijker in te nemen medicatie met minder bijwerkingen beschikbaar is. In Hoofdstuk 6 worden de resultaten van de behandeling met een combinatie van abacavir met 2 NRTIs besproken. Deze therapie werd toepast ter vereenvoudiging van het HAART regime van kinderen waarbij het HIV virus reeds volledig onderdrukt was. Dit 2 maal daagse (2dd) regime bleek gemakkelijk in gebruik. In het merendeel van de gevallen bleef het HIV virus onderdrukt. Echter bij één kind dat intensief was voorbehandeld met NRTIs faalde de therapie. Dit werd waarschijnlijk veroorzaakt door een in een eerder stadium verworven resistentie van het virus. Wij adviseren om voorzichtig te zijn met het gebruik van HAART gebaseerd op abacavir bij kinderen die reeds met NRTIs behandeld worden.

Nieuwe HAART combinaties zijn noodzakelijk om HIV-1 dat resistent geworden is te behandelen. Kinderen, geïnfecteerd met voor NRTI resistente HIV-1, werden behandeld met een combinatie van lopinavir/ritonavir en efavirenz. Dit zijn respectievelijk een protease remmer (PI) en een non-nucleoside reverse transcriptase remmer (NNRT). De gebruikelijke NRTIs werden bij deze combinatie niet toegepast. Het voordeel van de bovengenoemde combinatie is dat een krachtige antiretrovirale therapie gegeven kan worden, terwijl tegelijkertijd de eventuele bijwerkingen van NRTIs voorkomen worden. Deze pilot studie wordt beschreven in Hoofdstuk 7. Na de start van de medicatie werd het HIV-1 virus bij alle kinderen onderdrukt. De bijwerkingen van de medicatie waren over het algemeen mild en van voorbijgaande aard. Wel werd een stijging van de triglyceride en cholesterol waarden in het bloed bij respectievelijk 5 en 4 van de 7 kinderen gezien. Beide zullen zorgvuldig vervolgd moeten worden, aangezien verhoogde triglyceride en cholesterol waarden gerelateerd zijn aan het ontstaan van cardiovasculaire ziekten in de toekomst.

De zorg voor HIV-1 geïnfecteerde kinderen zal kunnen worden verbeterd door de ontwikkeling van parameters die het verloop van antiretrovirale therapie kunnen voorspellen. Bij volwassen wordt het aantal CD38+ CD8+ T-cellen als een dergelijke parameter beschouwd. Bij kinderen bestaan onduidelijkheden over het gebruik van CD38+ CD8+ T-cellen als voorspellende parameter. Verschillende factoren lijken hierbij een rol te spelen: Het CD38 molecuul wordt zowel tot expressie gebracht door geactiveerde cellen als door jonge (naïeve) cellen. Daarbij is de CD38 expressie heterogeen. Hierdoor is het moeilijk te definiëren welke cellen CD38-positief en welke cellen CD38-negatief zijn. Om geen gebruik te hoeven maken van een

arbitraire grenswaarde werd de mate van de CD38 expressie op CD8+ T-cellen bepaald. De expressie van CD38 werd bepaald voor het begin van de therapie en vervolgens iedere 3 maanden tijdens het gebruik van de medicatie (Hoofdstuk 9). Hierbij werd een daling gezien van de mediane CD38 expressie op CD8+ T-cellen bij alle patiënten na het starten van de medicatie. Er werd geen verschil gevonden in CD38 expressie tussen kinderen die wel en kinderen die niet op de therapie reageerden. Onze conclusie is dat de CD38 expressie op CD8+ T-cellen niet als (voorspellende) parameter voor de virale respons bij HIV-1 geïnfecteerde kinderen op HAART gebruikt kan worden.

De farmacologie van antiretrovirale middelen bij kinderen

De bloedspiegels van verschillende antiretrovirale middelen vertonen een grote variatie met name bij kinderen. Te lage bloedspiegels resulteren in het falen van de behandeling, terwijl overdosering bijwerkingen als gevolg heeft. Door het systematisch aanpassen van de dosering van de medicatie aan de hand van de medicatie spiegels in het bloed kan dit voorkomen worden. In Hoofdstuk 10 beschrijven we het gebruik van deze zogenaamde "therapeutic drug monitoring (TDM)". Het belang van TDM bij de behandeling van HIV-1 geïnfecteerde kinderen wordt nog eens extra onderstreept door de geringe hoeveelheid data die beschikbaar is over de juiste medicatie dosering van de antiretrovirale behandeling voor kinderen (Hoofdstuk 17).

TDM kan alleen toegepast worden voor PIs en NNRTIs. De NRTIs worden in de cel omgezet in de werkzame stof. Derhalve leveren de intracellulaire concentraties van deze stoffen de meest betrouwbare informatie op. De huidige methoden om intracellulaire spiegels te meten zijn echter complex en vereisen grote hoeveelheden bloed. In Hoofdstuk 11 beschrijven we een door ons ontwikkelde nieuwe methode om intracellulaire concentraties van NRTIs te meten. Met behulp van MALDI-TOF massa spectrometrie zijn we instaat zeer kleine hoeveelheden intracellulair omgezet AZT aan te tonen. Na optimalisatie van deze techniek zal het in de toekomst mogelijk zijn om met gebruikmaking van slechts geringe hoeveelheden bloed intracellulaire hoeveelheden van antiretrovirale medicatie bij patiënten te meten.

Farmacokinetische eigenschappen van verschillende protease remmers bij kinderen worden bestudeerd in de hoofdstukken 12 tot en met 14. In hoofdstuk 12 wordt de hoge interindividuele variabiliteit van de farmacokinetiek van nelfinavir bij HIV-1 geïnfecteerde kinderen beschreven. Een trend tot een verminderde blootstelling (oppervlakte onder de plasmaconcentratie tijd curve (AUC)) aan nelfinavir werd gevonden bij kinderen jonger dan 2 jaar en bij het gebruik van een dosering van 3 dd 20 mg/kg. Dit is klinisch van belang, omdat de nelfinavir spiegels daalden tot onder de eerder vastgestelde benodigde remmende concentratie. In Hoofdstuk 13 wordt de farmacokinetiek de combinatie van twee protease remmers, indinavir en ritonavir weergegeven. Ritonavir wordt in deze combinatie gegeven om de bloedspiegels van indinavir te verhogen (het zgn. "boosten") en niet om zijn antiretrovirale werking. Deze verhoging van de bloedspiegels van indinavir treedt op omdat ritonavir de werking van de leverenzymen die indinavir afbreken, remt. Dit heeft als gunstig gevolg dat de indinavir spiegels langer hoog blijven en de frequentie van de medicatie inname kan verminderen. In vergelijking met drie

maal daags (3dd) gebruikte indinavir bij volwassen en de drie maal daags (3dd) gebruikte indinavir bij kinderen werd met de combinatie indinavir/ritonavir een 2 maal hogere totale blootstelling en een 5 tot 8 maal verhoogde minimum concentratie gevonden. Géén van de kinderen in de studie had Cmin spiegels onder de benodigde remmende concentraties. Deze resultaten zijn gunstig te noemen voor toepassing bij HIV-1 geïnfecteerde kinderen. Echter, zoals beschreven in Hoofdstuk 5 ging dit medicatie regime gepaard met frequente bijwerkingen. In Hoofdstuk 14 worden de farmacokinetische data van lopinavir/ritonavir (PI) in combinatie met efavirenz (NNRTI) beschreven. Het is bekend dat NNRTIs de plasmaspiegels van PIs verlagen. Derhalve is het advies om de dosering van lopinavir 30% op te hogen indien dit middel gebruikt wordt in combinatie met efavirenz. Wij stellen vast dat de individuele lopinavir-spiegels sterk varieerden maar dat de mediane farmacokinetische parameters van efavirenz en lopinavir/ritonavir gelijk zijn aan eerder in de literatuur beschreven data. Gezien de grote variatie in de plasmaspiegels van met name lopinavir/ritonavir dient TDM te worden toegepast om patiënten met plasmaspiegels buiten het gewenste bereik tijdig te identificeren.

In de praktijk wordt zidovudine 2 maal daags (2dd) aan HIV-1 geïnfecteerde kinderen voorgeschreven. Hierover zijn tot op heden echter nooit farmacokinetische data gepubliceerd. In Hoofdstuk 15 beschrijven wij een retrospectieve analyse van de farmacokinetiek van zidovudine waarbij de doseerfrequentie van zidovudine terug gebracht werd van 3dd naar 2dd. De farmacokinetische profielen van beide doseerschema's waren niet significant verschillend, hetgeen duidt op bio-equivalentie.

De veranderingen van de farmacokinetische parameters na langdurig gebruik van indinavir worden bestudeerd in Hoofdstuk 16. In een kleine groep van 6 patiënten werd gevonden dat na meer dan 2 jaar indinavir gebruik bij 3 patiënten de farmacokinetische parameters onder de eerder vastgestelde benodigde remmende concentratie gedaald waren. In twee gevallen ging dit gepaard met het falen van de therapie.

Psychosociale aspecten samenhangend met de behandeling van HIV-1 geïnfecteerde kinderen

De kans dat de ouders/verzorgers zich niet goed aan het medicatie schema houden neemt toe bij aanwezigheid van psychosociale problematiek en bij afwezigheid van een sociaal netwerk bij de ouders/verzorgers. In Hoofdstuk 18 worden de sociale omstandigheden van gezinnen met HIV-geïnfecteerde kinderen besproken. Uit onze gegevens bleek dat de ouders/verzorgers van HIV-1 geïnfecteerde kinderen beschikken over een gering sociaal netwerk. Dit gaat gepaard met ernstige economische en psychosociale problematiek. Bij de behandeling van en de zorg voor HIV geïnfecteerde patiënten dient hiermee rekening gehouden te worden.
Bij het mislukken van de HIV-behandeling van een kind door onmacht of onwil van de ouders ziet het HIV-behandelteam zich geconfronteerd met complexe ethische en juridische vraagstellingen. Om de zorg voor HIV geïnfecteerde kinderen zo optimaal mogelijk te organiseren werd het Multidisciplinair HIV overleg opgericht waarin vertegenwoordigers van de Raad voor de Kinderbescherming, Jeugdzorg, het Arrondissementparket Rotterdam en het

HIV-behandelteam van het Erasmus MC-Sophia participeren. Tijdens de besprekingen werd de noodzaak onderschreven voor een gestandaardiseerde aanpak van therapie falen. In Hoofdstuk 20 wordt dit protocol "Werkwijze bij therapie ontrouw van HIV-geïnfecteerde kinderen" besproken. Toepassing van dit protocol leidde tot een verbeterde therapie trouw en daarmee tot onderdrukking van HIV in het bloed.

Dankwoord

Zo, het manuscript is af. Het ultieme moment is nu aangebroken, de laatste zinnen van mijn proefschrift kan ik gaan intikken. Tot het laatst bewaard, het snelst geschreven en waarschijnlijk het meest gelezen. Net als al de promovendi die mij reeds zijn voorgegaan beleef ik nogmaals het hele avontuur met al zijn ups en downs. Kortom de juiste melancholische stemming om eens een mooi dankwoord te schrijven:

Allereerst wil ik de kinderen en ouders die deelgenomen hebben aan de studies bedanken. Bloedprikken (niet altijd in één keer raak), onderzoeken en steeds maar weer dat gezeur over die pillen, het valt niet altijd mee bij ons op de poli. Toch bleven jullie ondanks alle problemen meestal goed gehumeurd, iets waar ik een voorbeeld aan kan nemen. Zonder jullie medewerking had ik dit proefschrift niet kunnen schrijven.

Prof.dr. R. de Groot, beste Ronald, de spreekwoordelijke Nederlandse middelmaat heeft op jou geen toepassing. Jouw motto's "you only fail when you stop trying" en "alle problemen zijn oplosbaar" zijn essentieel geweest voor dit proefschrift en mijn ontwikkeling. Je gedrevenheid is indrukwekkend, hoewel soms vermoeiend. Ik heb bewondering voor het feit dat je een groep hebt samengesteld waar een gezonde competitie heerst zonder dat dat een moment de onderlinge sfeer nadelig beïnvloed.

Dr. D.M. Burger, beste David, jij bent een onmisbare schakel in het HIV onderzoek gebleken. Jouw vroege inzicht in het belang van de farmacologie van HIV medicatie heeft niet alleen veel mogelijkheden tot onderzoek opgeleverd, maar ook daadwerkelijk bijgedragen tot een verbeterde behandeling van HIV geïnfecteerde patiënten. Dank voor de goede samenwerking en altijd relevante commentaren op de geschreven stukken.

Prof.dr. J.J.M. van Dongen en prof. dr. A.D.M.E. Osterhaus hartelijk dank voor de kritische beoordeling van mijn proefschrift. Prof.dr. Osterhaus, beste Ab, jouw rol in mijn promotie commissie is voor mij speciaal aangezien mijn interesse voor virussen op jouw lab is gewekt. Prof.dr. J.P. Mackenbach , prof.dr. I.D. de Beaufort, prof.dr. T.W. Kuijpers en prof.dr. H.A. Büller dank ik voor uw bereidheid plaats te willen nemen in de grote commissie. Dear dr. C. Giaquinto thank you for participating in my PhD-committee.

Dr. N.G. Hartwig, beste Nico. Hartelijk dank voor je bijdragen aan de manuscripten, de klinische lessen en het acuut regelen van een nieuwe computer voor mij toen dat heel erg nodig was.

Collega's infectio AIO's bedankt voor alle goede herinneringen en dat zijn er heel wat. Ester, Annemarie, Clementien, Debby, Edwin, Gwenda, Marieke, Linda, Jeroen, en Vishal. We hebben samen heel wat afgelachen en gemopperd. Clementien, dat bedrijf van ons Vermont Rietveld Fraaij & zonen/dochters wordt in ieder geval een aangenaam bedrijf om in te werken. Ik weet alleen niet of onze slogan (voor al uw menigokokken, RSV en HIV-problemen) echt marktwaarde heeft. Edwin en Jeroen, de Istanbul-kamer strikes again. Ik ben blij dat jullie mijn paranimfen willen zijn. Edwin, jou humor en raadgevingen hebben me er vaak door geholpen op momenten dat ik dat nodig had, geweldig! Jeroen, je kwam binnen als student en bent toen maar gebleven. Het samen op vrijdag om vijf uur 's middags bij andere afdelingen "lenen" van chemicaliën om onze proeven af te kunnen maken, blijft als een jongensboek voelen. Dat het uiteindelijk nog gelukt is ook, komt met name door jouw inspanningen. Als iemand de Maldi-methoden kan vervolmaken ben jij het, daar heb ik alle vertrouwen in. Het HIV-team: Gwenda, Linda en Juul. We waren het niet altijd eens maar dat was de kracht van het team. Ik denk dat we het goed gedaan hebben. Gwenda succes met het afronden van je promotie. Juul succes bij de oncologie, ik heb nog altijd veel aan je praktische lessen crisismanagement. Linda je bent een beetje de moeder aller kinder-HIV-verpleegkundigen geworden, ik weet zeker dat de groep bij jou in goede handen is. Annemarie, we hebben samen heel wat lief en leed gedeeld

tijdens onze gezamenlijke 'HIV-jaren'. Jouw proefschrift is nog altijd het naslagwerk over pediatrische HIV. Ik had het voorrecht daarop voort te mogen borduren. Het raamwerk dat jij hebt achter gelaten is essentieel voor goede HIV-zorg en onderzoek. Maar nu eerst gewoon roddelen en over van alles behalve HIV praten met een goed glas wijn.

Dan de "buren" José en Carin en de "onder-buren" Jolt, Ids, Mandy en Marinka dank voor de gezelligheid.

Conne, dank je voor al je hulp en natuurlijk de sappige roddels. Ik kom snel weer beppen en je koekjes op eten.

Alina, we hebben denk ik bewezen dat de apotheek en de kindergeneeskunde fantastisch kunnen samenwerken. Je rustige goed doordachte benadering van de problemen stond nogal eens in schril contrast met onze benadering, toch was dat nooit een probleem. Ik heb met veel plezier samengewerkt en hoop dat in de toekomst weer te doen.

Ellen en Katja, zonder jullie beiden was het immuno werk nooit van de grond gekomen. Beste Ellen veel succes in Arnhem, het HIV team zal je kennis enorm missen. Katja, dank voor de steun tijdens de werkzaamheden. Het blijft jammer dat we de samenwerking niet voort hebben kunnen zetten.

Georgette, de voorkant is fantastisch. Je vond het maar niets dat ik geen duidelijke opdracht gaf. Ik denk dat de voorkant niet beter uit had kunnen pakken.

Als AIO ben je in het Sophia zeker niet alleen: borrels, weekends en diners. Bedankt voor de leuke tijden.

The Düsseldorf group, Tim, Jennifer, Marie-Theres and Gaby. Thank you for your hospitality in Düsseldorf. It gave me the unique experience as a physician to work in an entirely different environment. Tim, you participated in this adventure which resulted in our two joined papers, thanks. Jennifer, mother, physician and researcher a though combination. All the best with the lipid study. Marie-Theres suddenly you were involved with this strange Dutch study and a doctor that did not even speak German properly. Despite all problems you went along with it. You organized the study and provided excellent care for the children. Without your efforts the whole thing would have been impossible.

Dan de familie die ik gruwelijk verwaarloosd heb: Pa, ma, Josine en Ludo altijd geïnteresseerd en bereid te helpen. Pa en ma, een civiel technisch ingenieur en een pedagoge, daar komen vreemde proefschriften van. Dank voor jullie steun door de jaren heen. Beste Pa, lang geleden ben ik ooit in het Stevin 3 lab begonnen als onderzoeker. Ik mocht toen allerlei betonblokken samen drukken of juist uit elkaar trekken voor jouw proefschrift. De interesse voor onderzoek is bij mij daarna nooit meer verdwenen. Helaas heb ik het sinds die tijd wel zonder vriesdroog kamers en megadrukpersen moeten stellen, iets wat ik nog atijd betreur. Familie we moeten vaker discussies voeren bij een goed glas wijn in een goed restaurant.

Dan de schoonfamilie, pa en ma Grotenhuis en Martijn, ook jullie bedankt voor jullie ondersteuning. Bathmen is altijd weer een oase van rust en gastronomie. Zonder jullie hulp was dit proefschrift nu half af en hadden alle huizen waar we in gewoond hebben waarschijnlijk geen behang gehad.

Dan de vrienden, er zijn veel mensen waar ik veel plezier mee heb kunnen maken, maar een paar wil ik speciaal bedanken:

Joost en Michiel, ik zit me te bedenken dat we elkaar al weer langer dan 10 jaar kennen! (Slik). Michiel samen met jou en Sintha hebben we heel Oost-Australie door gecruisd en nog steeds vrienden! Als dat geen vriendschap is. Mocht het dokteren niets voor je zijn kun je nog altijd reisgidsen gaan schrijven. Die voor mij en Kristel over New York was in ieder geval super. Joost, huisarts in het diepe zuiden, ik weet niet zeker of iedereen daar je liefde voor Spinoza deelt. Ik hoor er graag meer over en anders kun je bij mij altijd nog een vloot bouwen.

Sintha, Janneke, Robin, Arzu, Jan-Max, Menno, Eva, Friso, Walter, Ivan, Jeroen en Speedy hartelijk dan voor alle nachten in de kroeg, BBQs, bootreizen, pogingen tot muziek maken, bowlen, axis&allies-nachten, vrijgezellenavonden, trouwfeesten en andere momenten van intoxicaties en ook voor de momenten dat jullie naar mijn gezeur hebben geluisterd.

Lieve Kristel, de laatste en belangrijkste te bedanken persoon. Eigenlijk had jij ook als coauteur op de stukken moeten staan, zonder jou was het nooit gelukt. Dank voor je rotsvaste vertrouwen, steun en oneindige liefde. Laten we samen weer een nieuw avontuur gaan beginnen. Ik hou van je.

Curriculum vitae

Pieter Fraaij was born in Delft, The Netherlands, on April 27, 1975. He passed his secondary school exam in 1993 at the Hugo Grotius College in Delft. His medical training started in 1993 at the Erasmus MC in Rotterdam. In 1996 he participated in a research project on the development of an *in vitro* assay to detect enhancing antibodies in FIV-infected cats at the Department of Virology of the Erasmus MC, Rotterdam (head: prof.dr. A.D.M.E. Osterhaus). His Medical Degree was obtained in 1999. The research presented in this thesis was started in 2000 at the Department of Pediatrics, Division of Pediatric Infectious Diseases and Immunology, Erasmus MC- Sophia Children's Hospital in Rotterdam (head: prof.dr. R. de Groot). Studies were performed on the clinical, virological and immunologocal aspects of HAART in HIV-1 infected children; the clinical pharmacology of HAART in HIV-1 infected children; and the psychosocial aspects of the treatment of HIV-1 infected children. Additional clincal studies were performed at the Department of Pediatrics of the Heinrich Heine University Hospital, Düsseldorf, Germany (supervision: dr. T. Niehues). From 2001 to 2002 he was a member of the medical ethical board of the Erasmus MC and between 2003 and 2004 he participated in the postgraduate students committee of the molecular medicine postgraduate school of the Erasmus MC. In November 2004 he enrolled in the residency program in Pediatrics at the Erasmus MC- Sophia Children's Hospital, Rotterdam (head: prof. dr. H.A. Büller; prof. dr. A.J. van der Heijden).

Publications international

Fraaij PL, Rakhmanina N, Burger DM, de Groot R. Therapeutic drug monitoring in children with HIV/AIDS. Ther Drug Monit 2004;26(2):122-6.

Fraaij PL, Neubert J, Bergshoeff AS, van Rossum AM, Hartwig NG, Schroten H, et al. Safety and efficacy of a NRTI-sparing HAART regimen of efavirenz and lopinavir/ritonavir in HIV-1-infected children. Antivir Ther 2004;9(2):297-9.

Fraaij PL, Bergshoeff AS, van Rossum AM, Hartwig NG, Burger DM, de Groot R. Changes in indinavir exposure over time: a case study in six HIV-1-infected children. J Antimicrob Chemother 2003;52(4):727-30.

Fraaij PL, Van Rossum AM, Wolthers KC, Vossen AC, Jeucken YM, Kuijpers JH, van Lochem EG, Roos MTL, de Groot R., Hartwig NG. Initation of highly active antiretroviral therapy leads to an HIV-specific immune response in a seronegative infant. AIDS 2003;17(1):138-40

Fraaij PL, van Kampen JJ, Burger DM, de Groot R. Pharmacokinetics of antiretroviral therapy in HIV-1 infected children. Clin Pharmacokinetic: in press.

Fraaij PL, Verweel G, van Rossum AM, van Lochem EG, Schutten M, Weemaes CM, Hartwig NG, Burger DM, de Groot R. Sustained viral suppression and immune recovery after 4 years treatment of HIV-1 infected children wit HAART. Clin Infect Dis: in press.

Fraaij PL, Verweel G, van Rossum AM, Hartwig NG, Burger DM, de Groot R. Indinavir/low dose ritonavir containing HAART in HIV-1 infected children has potent antiretroviral activity, but is associated with side effects and frequent discontinuation of treatment. Submitted

Fraaij PL, Bergshoeff AS, Verweel G, van der Knaap L, van Rossum AM, Hartwig NG, Burger DM, de Groot R. Safety and efficacy of an abacavir based HAART regimen after pre-treatment with protease inhibitors in children with an undetectable HIV-1 viral load. Submitted.

Fraaij PL, van Rossum AM, Wolthers KC, Kuijpers JH, Weemaes CM, van Furth AM, Hartwig NG, Hooijkaas H, de Groot R, van Lochem EG. The CD38 expression level on CD8+ T-cells does not predict viral response to HAART in HIV-1 infected children. Submitted.

van Rossum AM, **Fraaij PL**, de Groot R. Efficacy of highly active antiretroviral therapy in HIV-1 infected children. Lancet Infect Dis 2002;2(2):93-102.

Bergshoeff AS, **Fraaij PL**, van Rossum AM, Verweel G, Wynne LH, Winchell GA, et al. Pharmacokinetics of indinavir combined with low-dose ritonavir in human immunodeficiency virus type 1-infected children. Antimicrob Agents Chemother 2004;48(5):1904-7.

van Kampen JJ, **Fraaij PL**, Hira V, van Rossum AM, Hartwig NG, de Groot R, et al. A new method for analysis of AZT-triphosphate and nucleotide-triphosphates. Biochem Biophys Res Commun 2004;315(1):151-9.

Bergshoeff AS, **Fraaij PL**, van Rossum AM, Wolfs TF, Geelen SP, de Groot R, et al. Pharmacokinetics of nelfinavir in children: influencing factors and dose implications. Antivir Ther 2003;8(3):215-22

Bergshoeff AS, **Fraaij PL**, Neubert J, Verweel G, Hartwig NG, Niehues T, de Groot R, Burger DM. Increased dose of lopinavir/ritonavir compensates for efavirenz induced drug-drug interaction in HIV-1 infected children. J Acquir Immune Defic Syndr: in press.

Bergshoeff AS, **Fraaij PL**, Verweij C, van Rossum AM, Verweel G, Hartwig NG, de Groot R, Burger DM. Plasma levels of zidovudine twice compared to thrice daily in six HIV-1 infected children. J Antimicrob Chemother: in press.

van Rossum AM, Dieleman JP, **Fraaij PL**, Cransberg K, Hartwig NG, Gyssens IC, et al. Indinavir-associated asymptomatic nephrolithiasis and renal cortex atrophy in two HIV-1 infected children. AIDS 2001;15(13):1745-7.

van Rossum AM, Bergshoeff AS, **Fraaij PL**, Hugen PW, Hartwig NG, Geelen SP, et al. Therapeutic drug monitoring of indinavir and nelfinavir to assess adherence to therapy in human immunodeficiency virus-infected children. Pediatr Infect Dis J 2002;21(8):743-7.

van Rossum AM, Dieleman JP, **Fraaij PL**, Cransberg K, Hartwig NG, Burger DM, et al. Persistent sterile leukocyturia is associated with impaired renal function in human immunodeficiency virus type 1-infected children treated with indinavir. Pediatrics 2002;110(2 Pt 1):e19.

Publications national

Fraaij PL, van Rossum AM, de Groot R. Pediatrische HIV-infectie. IATEC nieuws bulletin. 2001;17:19-20

Fraaij PL, van Rossum AM, de Groot R. Preventie van de overdracht van HIV van moeder naar kind. Infectieziekten Bulletin, RIVM. 2001;12(10);347-353

Fraaij PL, de Groot R, Rijkschroeff JB, van der Knaap L, Blondeau MJ, Segers T, van Es HL, Hartwig NG. Protocol gezamenlijke aanpak therapieontrouw. Medisch Contact 2004;59(45):1792-5

van Rossum A.M.C., **Fraaij P.L.A.**, de Groot R. Pediatrische HIV-infectie. Capita Selecta HIV/Aids. GlaxoSmithKline. 2001