THE PSEUDOMONAS FLORA AND TOBRAMYCIN PHARMACOKINETICS IN PATIENTS WITH CYSTIC FIBROSIS

DE PSEUDOMONASFLORA EN DE FARMAKOKINETIEK VAN TOBRAMYCINE BIJ PATIENTEN MET CYSTIC FIBROSIS

PROEFSCHRIFT

Ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.dr. C.J. Rijnvos
en volgens besluit van het college van dekanen.
De openbare verdediging zal plaatsvinden op
woensdag 27 september 1989
te 15.45 uur

door

ALPHONSUS MARIA HORREVORTS

geboren te Almelo.

PROMOTIECOMMISSIE:

Promotores:	Prof.dr. M.F. Michel
	Prof.dr. K.F. Kerrebijn
Overige leden:	Prof.dr. C. Hilvering
	Prof.dr. R.P. Mouton

To all patients who suffer from Cystic Fibrosis

CONTENTS

	Introduction to the studies	9
Chapter 1.	Pseudomonas aeruginosa in cystic fibrosis: Epidemiological investigations.	17
Chapter 2.	Ecology of Pseudomonas aeruginosa in patients with cystic fibrosis.	26
Chapter 3.	In vitro activity of new antimicrobial agents against Pseudomonas aeruginosa isolates from cystic fibrosis patients.	39
Chapter 4.	Chequerboard titrations: The influence of the composition of serial dilutions of antibiotics on the fractional inhibitory concentration index and fractional bactericidal concentration index.	44
Chapter 5.	Antibiotic interaction: Interpretation of fractional inhibitory and fractional bactericidal concentration indices.	56
Chapter 6.	Pharmacokinetics of tobramycin in patients with cystic fibrosis: Implications for the dosing interval.	61
Chapter 7.	Tobramycin in patients with cystic fibrosis: Adjustment in dosing interval for effective treatment.	76
Chapter 8.	Pharmacokinetics of antimicrobial drugs in cystic fibrosis: Aminoglycoside antibiotics.	92

Summary 113

Samenvatting 117

Nawoord 123

Curriculum vitae 126

INTRODUCTION TO THE STUDIES

Cystic Fibrosis (1-4) is the most common lethal genetic disease in Caucasians occuring in approximately 1:2500 live births, but is very rare among Orientals and native African negroes. The condition is transmitted as an autosomal recessive trait and heterozygotes do not express the disease. Since Cystic Fibrosis (CF) is an autosomal recessive trait and it may be assumed that virtually all patients will be identified as such, a heterozygous carrier frequency among whites is estimated to be between 1:20 to 1:30 (5). It is still impossible to detect the heterozygous state.

CF was first recognized as a distinct disease by Anderson in 1938 (6). Since then, the clinical manifestations of the disease have been well described. The disease affects the exocrine gland secretions throughout the body (4). These secretions show one or more abnormalities; an altered electrolyte content, a diminished water content and/or changed rheologic properties due to anomalous macromolecules. Clinically, the disease is characterized by exocrine pancreatic insufficiency and chronic obstructive pulmonary disease both secondary to obstruction by inspissated secretions of the mucous glands (4). The diagnosis CF is usually made by the detection of elevated concentrations of sodium and chloride in the sweat in the setting of gastro-intestinal and pulmonary disease. A family history of CF is of diagnostic assistance (7). Despite intensive research, however, the nature of the primary defect has still been incompletely elucidated.

Pulmonary infection is the major cause of morbidity and mortality in patients with CF (4). The lungs appear normal at birth. Eventually, the poor clearance of viscous mucus promotes colonization and infection by microorganisms. This initiates a chronic cycle of inflammation, mucous hypersecretion and bronchial obstruction finally leading to damage of the bronchial wall with abcess formation, bronchiectases and fibrotic foci. Complications of the pulmonary disease include pneumothorax, hemoptysis and pulmonary hypertension with cor pulmonale. As a result lung function deteriorates and most patients succumb to the

consequences of respiratory insufficiency. Since the first description of CF, median survival has increased to more than 20 years today and the quality of live has improved as a result of more complete understanding of the pathophysiology and advances in therapy, in particular antimicrobial treatment (1).

Bacteria are the most important microorganisms responsible for progression of lung pathology (8). Bacteria most frequently involved are Haemophilus influenzae, Staphylococcus aureus, Pseudomonas aeruginosa, and Pseudomonas cepacia. H. influenzae and S. aureus are often seen at an early stage while P. aeruginosa becomes more frequent with increasing age (9). The introduction of effective drugs against H. influenzae and S. aureus has improved the prognosis of CF remarkably since the 1950's. Since then P. aeruginosa has become the most frequent and most important pulmonary pathogen. In recent years, a progressive increase in the isolation rate of P. cepacia has been observed. Since P. cepacia is resistant to many antimicrobial drugs and also results in greater impairment of pulmonary function than does P. aeruginosa, in some centres, this microorganism has become a major clinical problem (10,11).
Viral infections are no more frequent in patients with CF than in the normal population, but it has been shown that they can induce acute bacterial exacerbations (12). Although colonization with Candida species is not uncommon and precipitins to Aspergillus are occasionally found, invasive fungal infections in CF are rare (8).

P. aeruginosa is by far the most important pulmonary pathogen in CF. Its prevalence is reported to be 30 to 90 percent (9). The original source, the main route of transmission and the factors which determine colonization are still unknown. Once acquired, this microorganism is seldom eradicated, although it may disappear temporarily from the sputum during treatment. Since P. aeruginosa is a well-known cause of nosocomial infections, the question of cross-colonization/infection has been discussed frequently (13-17). Studies monitoring sputum cultures from patients at summer camps showed that the risk of cross-colonization/infection was low or even undetectable. In contrast, siblings with CF generally harbour P. aeruginosa strains of the same types. This does not necessarily prove that cross-colonization/infection has occurred, rather that

siblings may have acquired the bacteria from a common source.

In the lungs of patients with CF, P. aeruginosa undergoes an environmental adaptation characterized by production of large amounts of mucoid exopolysaccharide (MEP) and by changes in somatic antigens and susceptibility to serum (18). The MEP in which microcolonies are embedded frustrates phagocytosis and contributes significantly to the secretion of high amounts of lysosomal enzymes by polymorphonuclear leukocytes (PMNs) (19-21). These enzymes are believed to cleave immunoglobulins (and immune complexes), complement components and complement receptors on PMNs, together resulting in an impaired phagocytosis. Moreover, in patients colonized by P. aeruginosa for a longer period of time most of the antibodies to outer membrane structures belong to nonopsonizing IgG subclasses which also negatively affect phagocytosis. Changes in somatic antigens during colonization are thought to be responsible for a shift from opsonic to nonopsonic antibody isotypes. The high levels of PMN-derived lysozomal enzymes in the lungs are in imbalance with their inhibitors resulting in proteolytic tissue damage. This is believed to be the primary cause of lung tissue destruction. Most patients with CF produce high concentrations of circulating antibodies to P. aeruginosa. Poor prognosis has been shown to be associated with a pronounced antibody response.
Susceptibility to human serum may explain why P. aeruginosa never becomes bacteraemic in CF patients.

The management of patients with CF is still based on the objectives and methods first described by Anderson (6) namely to ensure optimal nutrition and to prevent or control pulmonary infection. Although antimicrobial therapy has significantly improved the prognosis of CF, no consensus has yet been reached on the goals and rationale of antibiotic treatment (22). There are disagreements about the indications for therapy, the drug or drugs to be used, the route of administration, the dosage schedule, and the duration of treatment. In some centres, for instance, only acute exacerbations are treated while in others, patients are given antimicrobial therapy periodically or even continuously. Additionally, the parameters to monitor the effect of treatment appear to differ from centre to centre.

Many drugs, including antibiotics, are more rapidly eliminated from the circulation by patients with CF than by controls (23-25). These findings led to the suggestion that antimicrobial treatment could be more effective when it was based on the pharmacodynamics and pharmacokinetics of antibiotics in patients with CF rather than the normal dosage schedules. Exacerbations of chronic respiratory infections caused by P. aeruginosa are usually treated with an aminoglycoside in combination with an antipseudomonas ß-lactam antibiotic or a quinolone (26). Combination therapy is often given because it seems to offer better results than monotherapy, possibly due to synergistic interaction in relation to P. aeruginosa (27,28). Furthermore, resistance to combination therapy is believed to develop less rapidly (27). In addition to antimicrobial therapy, other supportive measures such as physiotherapy, coughing techniques and inhalation of mucolytic or bronchodilatating agents are used. Because of the inability to control pulmonary infection in CF with antibiotics, new therapies are being evaluated. These include: anti-inflammatory agents, immunomodulators, vaccines and monoclonal antibodies (29-32).

The ecology and susceptibility of P. aeruginosa and the pharmacokinetics and pharmacodynamics of tobramycin in patients with CF are subjects of study in this thesis.
Current knowledge of the typing of P. aeruginosa and difficulties in typing Pseudomonas isolates from patients with CF is discussed in chapter 1. The composition of the Pseudomonas flora in CF patients monitored over time by means of serotyping, active and passive pyocin typing and phage typing is described in chapter 2. The in-vitro activity of a number of antipseudomonas agents is presented in chapter 3, and in chapter 4 the in-vitro interactions between tobramycin and three antipseudomonas ß-lactam antibiotics are investigated by means of checkerboard titrations. In particular the effect of using various antibiotic dilution series was determined, the implications of which are discussed in chapter 5.
The results of a pharmacokinetic study of tobramycin in CF patients of different ages is reported in chapter 6. The data from this study were used to formulate guidelines for adequate and safe dosage of this antibiotic in CF. Whether or not application of these guidelines leads to better clinical results than those achieved

with conventional dosage schemes is explored in chapter 7. In chapter 8 the results obtained from our pharmacokinetic and pharmacodynamic studies are evaluated in relation to those reported in the literature.

REFERENCES

1. Wood RE, Boat TF, Doershuk CF. Cystic Fibrosis. Am Rev Respir Dis 1976;**113**:833-77.
2. di Sant'Agnese PA, Davis PB. Research in Cystic Fibrosis. N Engl J Med 1976;**295**:481-85, 534-39, 597-602.
3. Davis PB, di Sant'Agnese PA. A review. Cystic fibrosis at forty-quo vadis? Pediatr Res 1980;**14**:83-87.
4. Taussing LM ed. Cystic Fibrosis. New York: Thieme, 1984.
5. White R, Woodward S, Leppert M, O'Connell P, Hoff M et al. A closely linked genetic marker for cystic fibrosis. Nature 1985;**318**:382-84.
6. Andersen DH. Cystic Fibrosis of the pancreas and its relation to celiac disease. Am J Dis Child 1938;**56**:344-99.
7. Rosenstein BJ, Langbaum TS. Diagnosis. In Taussing LM ed. Cystic Fibrosis, New York: Thieme, 1984;85-114.
8. Høiby N. Microbiology of lung infections in cystic fibrosis patients. Acta Paediatr Scand 1982;(Suppl)**301**:33-54.
9. Cystic Fibrosis Foundation. Pulmonary infection in cystic fibrosis. "GAP" Conference Rep 1983;**7**:4.
10. Isles A, Maclusky I, Corey M, Gold R, Prober C, Fleming P, Levison H. Pseudomonas cepacia infection in cystic fibrosis: An emerging problem. J Pediatr 1984;**104**:206-10.
11. Prince A. Pseudomonas cepacia in Cystic Fibrosis Patients. Am Rev Respir Dis 1986;**134**:644-45.
12. Marks MI. Respiratory viruses in cystic fibrosis. N Engl J Med 1984;**311**:1695-96.
13. Hoogkamp-Korstanje JAA, van der Laag J. Incidence and risk of cross-colonization in cystic fibrosis holiday camps. Antonie van Leeuwenhoek 1980;**46**:100-01.
14. Kelly NM, Tempany E, Falkiner FR, Fitzgerald MX, O'Boyle C, Keane CT.

Does pseudomonas cross-infection occur between cystic fibrosis patients? Lancet 1982;**25**:688-89.

15. Zimakoff J, Høiby N, Rosendal K, Guilbert JP. Epidemiology of Pseudomonas aeruginosa infection and the role of contamination of the environment in a cystic fibrosis clinic. J Hosp Infect 1983;**4**:31-40.
16. Thomassen MJ, Demko CA, Doershuk CF, Root JM. Pseudomonas aeruginosa Isolates: Comparisons of Isolates from Campers and from Sibling Pairs with Cystic Fibrosis. Pediatr Pulmonol 1985;**1**:40-45.
17. Pedersen SS, Jensen T, Pressler T, Høiby N, Rosendal K. Does Centralized Treatment of Cystic Fibrosis Increase the Risk of Pseudomonas aeruginosa Infection? Acta Paediatr Scand 1986;**75**:840-45.
18. Penketh A, Pitt T, Roberts D, Hodson ME, Batten JC. The Relationship of Phenotype Changes in Pseudomonas aeruginosa to the Clinical Condition of Patients with Cystic Fibrosis. Am Rev Respir Dis 1983;**127**:605-08.
19. Høiby N, Döring G, Schiøtz P. The role of immune complexes in the pathogenesis of bacterial infections. Annu Rev Microbiol 1986;**40**:29-53.
20. Goldstein W, Döring G. Lysosomal enzymes from polymorphonuclear leukocytes and proteinase inhibitors in patients with cystic fibrosis. Am Rev Respir Dis 1986;**134**:49-56.
21. Döring G, Albus A, Høiby N. Immunological aspects of cystic fibrosis. Chest 1988;(Suppl)**94**:109S-14S.
22. Michel BC. Antibacterial Therapy in Cystic Fibrosis. A Review of the Literature Published Between 1980 and February 1987. Chest 1988;(Suppl)**94**:129S-40S.
23. Editorial. Antibiotic Dosage in Cystic Fibrosis. Lancet 1985;**4**:1020-21.
24. Groot de R, Smith AL. Antibiotic pharmacokinetics in cystic fibrosis: Differences and clinical significance. Clin Pharmacok 1987;**13**:228-53.
25. Prandota J. Clinical pharmacology of antibiotics and other drugs in cystic fibrosis. Drugs 1988;**35**:542-78.
26. Høiby N, Friis B, Jensen K, Koch C, Møller NE, Støvring S, et al. Antimicrobial chemotherapy in cystic fibrosis patients. Acta Paediatr Scand 1982;Suppl.**301**:75-100.

27. Michalsen H, Bergan T. Azlocillin with and without an aminoglycoside against respiratory tract infections in children with cystic fibrosis. Scan J Infect Dis 1981;(Suppl)**29**:92-97.
28. Scribner RK, Marks MI, Weber AH, Tarpay MM, Welch DF. Activities of various beta-lactams and aminoglycosides, alone and in combination, against isolates of Pseudomonas aeruginosa from patients with cystic fibrosis. Antimicrob Agents Chemother 1982;**21**:939-43.
29. Hancock REW, Mutharia LM, Monat ECA. Immunotherapeutic potential of monoclonal antibodies against Pseudomonas aeruginosa Protein F. Eur J Clin Microbiol 1985;**4**:224-27.
30. Thomassen MJ, Demko CA, Wood RE, Tandler B, Dearborn DG, Boxerbaum B, Kuchenbrod PJ. Ultrastructure and function of alveolar macrophages from cystic fibrosis patients. Pediatr Res 1980;**14**:715-21.
31. Auerbach HS, Williams M, Kirkpatrick JA, Colten HR. Alternate-day prednisone reduces morbidity and improves function in cystic fibrosis. Lancet 1985;**2**:686-88.
32. Pennington JE, Hickey WF, Blackwood LL. Active immunization with lipopolysaccharide Pseudomonas antigen for chronic pseudomonas bronchopneumonia in guinea pigs. J Clin Invest 1981;**68**:1140-48.

Chapter 1

PSEUDOMONAS AERUGINOSA IN CYSTIC FIBROSIS: EPIDEMIOLOGICAL INVESTIGATIONS

Horrevorts AM[1], Borst J[3], Kerrebijn KF[2] and Michel MF[1]

Departments of Clinical Microbiology and Antimicrobial Therapy[1] and Pulmonary Medicine in Children[2] (Sophia Children's Hospital), Erasmus University, Rotterdam; Special Department for Pseudomonas Research[3], National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.

SUMMARY

Pseudomonas aeruginosa is the most common pulmonary pathogen in patients with cystic fibrosis. Epidemiological studies using typing methods have failed to resolve the confusion about the main route of transmission, the prevalence of crossinfection, and the number of strains (types) which may colonize an individual patient. This may be due to the special properties frequently observed in isolates from these patients which affect the typing methods used. Several typing methods are evaluated for their potential as epidemiological tool for P. aeruginosa strains from patients with cystic fibrosis.

INTRODUCTION

P. aeruginosa has been identified as the most common and important pulmonary pathogen in patients with cystic fibrosis (CF) (1). The main route of transmission and the factors determining colonization are still unknown. Once acquired, this organism is seldom eradicated, although it may disappear temporarily from the sputum during treatment. Isolates of P. aeruginosa with differing morphological characteristics and antibiograms can be cultured from single sputum samples of

individual patients (2). These observations together with reported difficulties in typing CF isolates resulted in confusion about the number of types which might colonize an individual patient. Some reports have suggested that CF patients may be colonized by only a single type (2,3), while others reported colonization by two or more different types (4,5). Whether cross infection among patients with CF occurs or not remains also unclear (6-10). It is, however, conceivable that the type or types of P. aeruginosa present in the lungs of patients with CF in the course of time will be replaced by other type or types. Until now, few data are available. To gain an insight into these problems, epidemiological investigations are needed. Therefore, typing methods must be used in order to differentiate strains of P. aeruginosa from each patient and between patients. In this chapter several typing methods are evaluated for their potential as epidemiological tools for CF P. aeruginosa strains. Conventional techniques mostly performed are serotyping (O and H typing), pyocin typing and phage typing. Newer methods using cell membrane proteins and plasmid or chromosomal DNA fragments as an epidemiological marker are being introduced (11,12).

TYPING METHODS

Serological typing: O antigens

The peripheral part of the outer membrane of Gram negative bacilli consists of lipopolysaccharides (LPS), which comprise three parts; lipid A, the core region and a variable side chain (= O antigen) (13). Variations in this side chain form the basis of serological typing (14). Seventeen distinct types have been described, for which specific O antisera are available (15). Whereas 90% of clinical isolates of P. aeruginosa can be assigned to a specific O type by agglutination techniques (16), this is possible in only one third of P. aeruginosa isolates from patients with CF. Approximately one half of CF isolates are agglutinated by more than one O antiserum, and about one tenth do not react with any (17). The prevalence of polyagglutinating and nonagglutinating strains of P. aeruginosa in CF appears to increase with the duration of colonization (18). Strains exhibiting polyagglutinability possess less O-antigen-containing LPS than do monoagglutinating strains (19,20), due to the fact that these strains have partly lost the polysaccharide portion of the

LPS (17). Thereby, the central core of the LPS which is normally covered with these polysaccharides, becomes exposed to antibodies (17,19). Non agglutinability may be due to the loss of larger parts of the LPS structure (21). The sensitivity to fresh normal human serum and the loss of invasive virulence of both polyagglutinating and non agglutinating CF isolates may also be a consequence of deficiencies in the protective oligosaccharide specific side chains (17,22). The high frequency of polyagglutinating and nonagglutinating strains is a major problem in differentiating isolates of P. aeruginosa from patients with CF. To overcome the difficulties in serotyping, CF strains should be tested under different conditions (different growth temperatures in combination with different concentrations of the O antisera). The postulate underlying this procedure is that in non- or polyagglutinating strains the LPS shows deficiencies but that intact structures more or less exist under different conditions (21). Since phage infection can influence O serotype, it would be informative to examine whether or not the phages are involved in the shift from mono-to poly- and nonagglutinating strains (23,24).

An important phenotypic feature of the CF pseudomonas isolates is the production of mucoid exopolysaccharide. This mucoidy feature has been shown not to influence O typability or sensitivity to human serum (18,25).

Serological typing: H Antigens

The majority of P. aeruginosa strains are motile by means of a single polar flagellum. H typing has not been widely adopted for routine use perhaps because of the difficulties of preparing specific anti-flagellar sera. H typing can be useful in distinguishing between strains of the same O serotype. However, non flagellar strains are common in CF (26). Therefore this approach is not suitable.

Pyocin typing

Pyocins are antibacterial proteinaceous substances produced by P. aeruginosa. Three varieties of pyocins are produced by over 70 - 90% of clinical isolates, i.e. R pyocins, F pyocins and S pyocins (27-29). Production of and sensitivity to different pyocins vary considerably among strains and form the basis of pyocin

typing. Typability rates around 90% can be achieved (30). Two different methods are employed active and passive (30). In active pyocin typing, the pyocins produced by the test organism are tested for their antibacterial activity against a series of standard indicator strains. In the passive pyocin method, the procedure is reversed, so that the susceptibility of the test organism to a set of standard pyocins is determined. However results are influenced by the metabolic state of bacteria (31), and in CF isolates the production of mucus forms a barrier to pyocin activity (32). Modified typing techniques which are more efficient should then be performed (3,30).

Phage typing

A standard phage typing procedure for P. aeruginosa comprising 20 different phages was described by Asheshov (33). In general approximately 85% of all isolates are typable. However the technique is method dependent such that reproducibility can be as low as 60% (34). CF isolates have been shown to be less sensitive to lysis by the typing phages than are those from other patients (40% versus 85%) (35). In addition spontaneous loss of phage as well as phage infection may result in an altered susceptibility pattern (36). As with pyocin typing, production of mucus by the strains presents difficulties (34). Mucoid strains are less phage typable than non-mucoid strains (19% of mucoid CF isolates in contrast to 59% of non-mucoid CF strains) (35). Since phages and pyocins both attach to the bacterial surface, it is of interest to know to what extent changes in outer membrane structure (such changes in outer membrane structure do occur in isolates of P. aeruginosa from patients with CF) affect the specificity of receptor sites. In summary phage typing has not proved to be a suitable method for the typing of strains from patients with CF.

Plasmid or chromosomal DNA fragments and cell envelope proteins

Plasmid or chromosomal DNA fragments and cell envelope proteins are being introduced as a marker for epidemiological investigations (11,12,37), and are currently being evaluated although plasmid typing is limited only to isolates which possess these DNA fragments. Since changes in outer membrane structure have

been reported for P. aeruginosa isolates from patients with CF, the value of the use of cell envelope proteins as an epidemiological marker needs further investigation.

Epidemiological investigations

Several conventional typing systems have been used in an attempt to address epidemiological questions concerning CF patients, such as the route of transmission, the prevalence of cross-infection, and the number of strains (types) which may colonize an individual patient. Contradictory results might be explained by the limitations of the methods used as well as the peculiar properties of P. aeruginosa strains obtained from these patients. For instance, simultaneous infection with mono-, poly- and non agglutinating isolates of P. aeruginosa has been interpreted as either infection with multiple types or infection with phenotypic variants of a single type. Furthermore, isolates that possess the same O serotype are not necessarily related. Therefore, a combination of (conventional) methods in particular serotyping and pyocin typing may be more productive in helping to understand the natural history of P. aeruginosa infection in CF at least until the newer methods have been established.

REFERENCES

1. Cystic Fibrosis Foundation. Pulmonary infection in cystic fibrosis. "GAP" Conference Report 1983;**7**:4.
2. Thomassen MJ, Demko CA, Boxerbaum B, Stern RC, Kuchenbrod PJ. Multiple isolates of Pseudomonas aeruginosa with differing antimicrobial susceptibility patterns form patients with cystic fibrosis. J Infect Dis 1979;**140**:873-880.
3. Williams RJ, Govan JRW. Pyocine typing of mucoid strains of Pseudomonas aeruginosa isolated from children with cystic fibrosis. J Med Microbiol 1973;**6**:409-412.
4. Seale TW, Thirkill H, Tarpay M, Flux M, Rennert OM. Serotypes and antibiotic susceptibilities of Pseudomonas aeruginosa isolates from single sputa of cystic fibrosis patients. J Clin Microbiol 1979;**9**:72-78.

5. Diaz F, Mosovich LL, Neter E. Serogroups of Pseudomonas aeruginosa and the immune response of patients with cystic fibrosis. J Infect Dis 1970;**121**:269-274.
6. Kelly NM, Tempany E, Falkiner FR, Fitzgerald MX, O'Boyle C, Keane CT. Does Pseudomonas cross-infection occur between cystic fibrosis patients? Lancet 1982;**ii**:688-689.
7. Hoogkamp-Korstanje JAA, van der Laag J. Incidence and risk of cross-colonization in cystic fibrosis holiday camps. Antonie van Leeuwenhoek 1980;**46**:100-101.
8. Speert DP, Lawton D, Damm S. Communicability of Pseudomonas aeruginosa in a cystic fibrosis summer camp. J Pediatr 1982;**2**:227-229.
9. Zimakoff J, Høiby N, Rosendal K, Guilbert JP. Epidemiology of Pseudomonas aeruginosa infection and the role of contamination of the environment in a cystic fibrosis clinic. J Hosp Infect 1983;**4**:31-40.
10. Thomassen MJ, Demko CA, Doershuk CF, Root JM. Pseudomonas aeruginosa isolates: Comparison of isolates from campers and from sibling pairs with cystic fibrosis. Pediatr Pulmonol 1985;**1**:41-46.
11. Ogle JW, Janda JM, Woods DE, Vasil ML. Characterization and use of a DNA probe as an epidemiological marker for Pseudomonas aeruginosa. J Infect Dis 1987;**1**:119-126.
12. Walia S, Madhaven T, Williamson T, Kaiser A, Tewari R, Lewis W. Usefulness of protein patterns, serotyping and plasmid profiles in the epidemiologic finger printing of Pseudomonas aeruginosa. In: book of abstracts of the 1st International Meeting on Bacterial Epidemiological Markers, Brussel 1987:**88**.
13. Hammond SM, Lambert PA, Rycroft AN. The bacterial cell surface, Kapitan Szabo Publishers, Washington, DC 1984.
14. Chester IR, Meadow PM, Pitt TL. The relationship between the O-antigenic lipopolysaccharides and serological specificity in strains of Pseudomonas aeruginosa of different O-serotypes. J Gen Microbiol 1973;**78**:305-318.
15. Liu PV, Matsumoto H, Kusama H, Bergan T. Survey of heat-stable, major somatic antigens of Pseudomonas aeruginosa. Int J Syst Bacteriol 1983;**33**:256-264.

16. Brokopp CD, Gomez-Lus R, Farmer III JJ. Serological typing of Pseudomonas aeruginosa: use of commercial antisera and live antigens. J Clin Microbiol 1977;**5**:640-649.
17. Pitt TL, MacDougall J, Penketh ARL, Cooke EM. Polyagglutinating and nontypable strains of Pseudomonas aeruginosa in cystic fibrosis. J Med Microbiol 1986;**21**:179-186.
18. Penketh A, Pitt T, Roberts D, Hodson ME, Batten JC. The relationship of phenotype changes in Pseudomonas aeruginosa to the clinical condition of patients with cystic fibrosis. Am Rev Respir Dis 1983;**127**:605-608.
19. Ojeniyi B, Baek L, Høiby N. Polyagglutinability due to loss of O-antigenic determinants in Pseudomonas aeruginosa strains isolated from cystic fibrosis patients. Acta Path Microbiol Immunol Scand. Sect B 1985;**93**:7-13.
20. Meadow PM, Wells PL, Salkinoja-Salonen M, Nurmiaho EL. The effect of lipopolysaccharide composition on the ultrastructure of Pseudomonas aeruginosa. J Gen Microbiol 1978;**105**:23-28.
21. Hancock REW, Mutharia LM, Chan L, Darveau RP, Speert DP, Pier GB. Pseudomonas aeruginosa isolates from patients with cystic fibrosis: a class of serum-sensitive, nontypable strains deficient in lipopolysaccharide O side chains. Infect Immun 1983;**42**:170-177.
22. Luzar MA, Montie TC. Avirulence and altered physiological properties of cystic fibrosis strains of Pseudomonas aeruginosa. Infect Immun 1985;**50**:572-576.
23. Liu PV. Changes in somatic antigens of Pseudomonas aeruginosa induced by bacteriophages. J Infect Dis 1969;**119**:237-246.
24. Bergan T, Midtvedt T. Epidemiological markers for Pseudomonas aeruginosa. Acta Path Microbiol Scan. Sect B 1975;**83**:1-9.
25. Laniyi B, Bergan T. Serological characterisation of Pseudomonas aeruginosa. In: Methods in Microbiology, Vol 10, Bergan T, Norris JR, Eds, Academic Press. New York 1978:93-168.
26. Luzar MA, Thomassen MJ, Montie TC. Flagella and motility alterations in Pseudomonas aeruginosa strains from patients with cystic fibrosis. Infect Immun 1985;**50**:577-582.
27. Ishii S, Nishi Y, Egami F. The fine structure of a pyocin. J Molecul Biol 1965;**13**:428-431.

28. Govan JRW. Studies on the pyocins of Pseudomonas aeruginosa:production of contractile and flexuous pyocins in Pseudomonas aeruginosa. J Gen Microbiol 1974;**80**:17-30.
29. Ito S, Kageyama M, Egami F. Isolation and characterization of pyocins from several strains of Pseudomonas aeruginosa. J Gen Appl Microbiol 1970;**16**:205-214.
30. Govan JRW. Pyocin typing of Pseudomonas aeruginosa. In: Methods in Microbiology, Vol 10, Bergan T, Norris JR, Eds, Academic Press, New York 1978;61-91.
31. Bobo RA, Newton EJ, Jones LF, Farmer III LH, Farmer JJ. Nursery outbreak of Pseudomonas aeruginosa: epidemiological conclusions from five different typing methods. Appl Microbiol 1973;**25**:414-420.
32. Schwartzmann S, Boring III JR. Antiphagocytic effect of slime from a mucoid strain of Pseudomonas aeruginosa. Infect Immun 1971;**3**:762-767.
33. Asheshov EH. An assessment of the methods used for typing strains of Pseudomonas aeruginosa. In: Proceedings of the 6th International Congress of Bacteriology, Arseni A, Ed, Leontiadi Medical Editions, Athens 1974:9-22.
34. Bergan T. Phage typing of Pseudomonas aeruginosa. In: Methods in Microbiology, Vol 10, Bergan T, Norris JR, Eds, Academic Press, New York 1978:169-199.
35. Pitt TL. Epidemiological typing of Pseudomonas aeruginosa. Eur J Clin Microbiol Infect Dis 1988;**7**:238-247.
36. Zierdt CH, Schmidt PJ. Dissociation in Pseudomonas aeruginosa. J Bacteriol 1961;**87**:1003-1010.
37. Dijkshoorn L, Michel MF, Degener JE. Cell envelope protein profiles of Acinetobacter calcoaceticus strains isolated in hospitals. J Med Microbiol 1987;**23**:313-319.

Chapter 2

ECOLOGY OF PSEUDOMONAS AERUGINOSA IN PATIENTS WITH CYSTIC FIBROSIS.

Horrevorts AM[1], Borst J[2], Puyk RJT[2], de Ridder R[1], Dzoljic-Danilovic G[1], Degener JE[1], Kerrebijn KF[3], Michel MF[1]

Departments of Clinical Microbiology and Antimicrobial Therapy[1] and Pulmonary Medicine in Children[3] (Sophia Children's Hospital), Erasmus University, Rotterdam; Special Department for Pseudomonas Research[2], National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.

Accepted for publication in the Journal of Medical Microbiology.

SUMMARY

In this study, the composition of the Pseudomonas flora was monitored over time in 15 patients with cystic fibrosis. Sputum samples were obtained for culture over a period ranging from 2 to 60 months. Isolates of P. aeruginosa were typed with the aid of four different techniques: serotyping, active and passive pyocin typing, and phage typing. The maximum number of different serotypes found in the patients was three (one serotype in 9 patients; two serotypes in 5 patients; three serotypes in 1 patient). Pyocin and phage typing revealed no marked differences between strains of the same serotype in individual patients. An exacerbation of chronic respiratory infection was not associated with changes in the composition of the flora. These data show that the Pseudomonas flora in the lungs of patients with cystic fibrosis is constant over time.

INTRODUCTION

In 30-50% of the younger and 70-90% of the older patients with cystic fibrosis (CF) the lungs are chronically colonized with P. aeruginosa (2). Disagreement exists about the number of types which may colonize an individual CF patient. Some authors have reported that these patients may be colonized by only a single type (15,16), while others have suggested that two or more different types may be involved (3,13). It is conceivable that, in the course of time, changes in the flora will occur and that one type will be replaced by another. In this study, the composition of the Pseudomonas flora was monitored over time in 15 patients with CF using serotyping, active and passive pyocin typing and phage typing.

MATERIALS AND METHODS

Patients

Fifteen patients with CF comprised the study group. The diagnosis CF was based on increased sweat electrolyte levels (Na^+ > 60 mmol/l) and characteristic gastrointestinal and pulmonary disease. The patients suffered from chronic pulmonary P. aeruginosa infection. Each patient was followed during three consecutive exacerbations (A, B, and C) in order to determine fluctuations in the composition of the Pseudomonas flora in the sputum. An exacerbation was defined as follows; increased cough and production of sputum, tachypnoea, dyspnoea and weight loss. For exacerbation A isolates from a sputum sample obtained on the day of hospital discharge were investigated. For exacerbation B, isolates from samples obtained during hospital stay were typed (day of admission and twice weekly thereafter), while for C isolates from a sample obtained on the day of hospital admission were used. Samples were obtained after physiotherapy. All exacerbations had taken place between november 1981 and march 1986. Patients were hospitalised and given antipseudomonal therapy consisting of a ß-lactam antibiotic in combination with an aminoglycoside.

P. aeruginosa strains

Sputum was cultured such that different morphological types (14) could be isolated from the primary plate. Where possible, three colonies were selected from each morphological type and after identification (4) they were stored at -80° C until required.

Serotyping

Serotyping was performed by means of agglutination (7). Each clinical isolate was subcultured on two Tryptic Soy Agar (Oxoid) plates of which one was incubated at 30° C and the other at 37° C for 18 hours. After incubation the plates were handled separately as follows: the cell mass was removed with a cotton wool swab and suspended in saline. Both suspensions were steamed for 2½ hours, then centrifuged and resuspended in fresh saline to a density of about $4x10^{10}$ cells per ml giving two antigen suspensions (30° C Ag and 37° C Ag).
O-antisera had been produced in rabbits (8) and each serum had been tested for cross-reactivity with other O-antigens. If these occurred, the serum was absorbed. Two different concentrations of antisera were used; 4 and 8 times the homologous titre (4.Ab and 8.Ab). Thus, there were four test conditions (i.e. 30° C Ag x 4.Ab, 30° C Ag x 8.Ab, 37° C x 4.Ab, and 37° C x 8.Ab). 0.025 ml Ag suspension was added to 0.025 ml Ab in a microtitre tray (Dynatek, U-bottom), the plate was sealed with tape, carefully shaken for 10 seconds, then incubated at 37° C. After 18 hours, each plate was read visually and interpreted thus; - no agglutination, ± weak agglutination, + agglutination, + + strong agglutination. The four different combinations were compared and the strongest agglutination reaction was recorded. To illustrate the method, the 4 agglutination patterns of two strains chosen arbitrarily are shown in table I. Strain 138P2 (patient #4) agglutinated most strongly and most often with O-antiserum 9 in all of the four patterns. Strain 112P13 (patient #5) had serotype 10 with an agglutination strength + in each of the 4 patterns.

Table I: Sero-typing patterns of P. aeruginosa strains 138P2 (patient #4) and 112P13 (patient #5). The strains were typed under four different conditions (see materials and methods). Abbreviations: HT, homologous titre.

	Condition			
	I	II	III	IV
Incubation temperature of the strains	30° C	30° C	37° C	37° C
	+	+	+	+
Titre of anti-sera	4x HT	8x HT	4x HT	8x HT
Sero-typing results				
strain 138P2	$2^{+},6^{+},9^{++}$	$9^{+},10^{+},11^{+}$	9^{++}	$2^{+},9^{++}$
strain 112P13	10^{+}	10^{+}	$6^{\pm},10$	10^{+}

Table II: Codification of active pyocin typing results of P. aeruginosa strain 147P12 from patient #4 (see materials and methods).

Indicator strain no.	1	2	3	4	5	6	7	8
Growth inhibition of indicator strain by pyocins of 147P12	+	+	+	+	+	-	-	+
Code		1			2			d

Only 2 of the 4 patterns included serotype 6 with an agglutination strength ± and +, respectively.

Pyocin typing

Active pyocin typing was performed according to the method described by Govan (5) using 8 indicator strains and passive pyocin typing according to the method described by Osman (11) using 8 indicator pyocins. Thus, in both typing procedures each strain was subjected to 8 tests which were coded as + = growth inhibition, and - = no growth inhibition. The resultant patterns of test 1 to 3 and 4 to 6 were assigned a number; +++ =1, ++- =2, +-- =3, --- =4, -+- =5, -++ =6, --+ =7, +-+ =8, whereas the results of reactions 7 and 8 were assigned a letter; ++ =A, +- =B, -- =C, -+ =D. An example is given in table II. Thus, the pyocin type of a strain was indicated by two figures followed by one letter.

Phage typing

Phage typing was done with the aid of the Colindale Typing Set (which comprised 20 phages) using the method described by Asheshov (1).

RESULTS

The results obtained in this study are summarized in table III. The study group comprised 9 females and 6 males with CF (no siblings) aged between 7 and 18 years (column 1). The study period from exacerbation A to C ranged from 2 to 60 months (column II). The number of sputum samples ranged from 5 to 12 samples (column III) and the number of morphologically different colonies of P. aeruginosa per culture from 1 to 7 (column IV). The total number of P. aeruginosa strains isolated and examined by the typing techniques is given in column V and ranged from 18 to 86. In 9 of 15 patients (#1,#2,#4,#6-#11) only 1 serotype was

Table III: Typing results concerning isolates of P. aeruginosa from 15 patients with cystic fibrosis. The periods of bacteriological observation ranged from 2 - 60 months. #: At the beginning of the study period. Abbreviations: ST, Sero Typing; APT, Active Pyocin Typing; PPT, Passive Pyocin Typing; PhT, Phage Typing; -, type not found; . , no sample available.

I = Patient
II = Study period (mths)
III= Sputum samples (N)
IV = Morphological types per sample
V = Strains typed (N)

X: number of types present in sputum samples obtained during exacerbations A, B, and C

I	II	III	IV	V	VI ST	VII APT	VIII PPT	IX PhT	X A1	B1	02	03	04	05	06	07	08	09	10	C1
#1	2	6	1-3	36	1	- - - - + - - + = 45d	- + - + - + - - - = 58c	21	1	4	4	4	4	.	.	.	.	.	.	-
Female					1	= 45d	- + - - - - - - - = 54c	21	3	-	2	-	-	.	.	.	.	.	.	-
7 years#					1	= 45d	- - - - - - - - - = 44c	21	5	2	-	2	2	.	.	.	.	.	.	3
#2	2	7	2-4	47	2	+ + + + + - - + = 12d	- - - - - - - - - = 44c	-	1	3	5	1	2	2	.	.	.	.	.	2
Male					2	= 12d	= 44c	21/44/f8/119x/1214	-	-	1	-	2	-	.	.	.	.	.	1
14 years					2	= 12d	= 44c	44/f8/ 1214/	-	-	-	5	2	-	.	.	.	.	.	-
					2	= 12d	= 44c	21/ 119x	2	2	2	2	2	-	.	.	.	.	.	6
					2	= 12d	- - - + - + - + = 48d	21/ 119x	1	1	-	-	-	-	.	.	.	.	.	-
					2	= 12d	= 48d	-	-	-	-	-	-	1	.	.	.	.	.	1
#3	4	5	1-5	39	1	- - - - - - - - = 44c	- - - - - - - - - = 44c	21/119x/352	5	3	-	-	.	.	.	.	.	.	.	4
Male					1	= 44c	- + - - - - - - - = 54c	21/119x/352	1	-	-	-	.	.	.	.	.	.	.	7
16 years																				
					9	- + + - - - - - = 64c	- + - + - + - - - = 58c	16/21/f8/f10/109	7	5	-	2	.	.	.	.	.	.	.	1
					9	= 64c	- + - + - - - - - = 53c	16/21/f8/f10/109	-	-	3	1	.	.	.	.	.	.	.	-
					9	= 64c	- - - + - + - - - = 48c	16/21/f8/f10/109	-	1	-	-	.	.	.	.	.	.	.	-
#4	6	9	1-3	50	9	+ + + + + - - + = 12d	- + - - - - - - - = 54c	21	-	-	-	-	-	-	1	-	.	.	.	1
Female					9	= 12d	= 54c	-	1	1	-	1	-	1	-	-	.	.	.	1
17 years					9	= 12d	- + - - - - + - = 54b	21	1	-	-	-	1	-	1	-	.	.	.	1
					9	= 12d	= 54b	-	1	-	-	-	-	-	-	-	.	.	.	2
					9	= 12d	- + - + - + + - = 58b	21	-	-	-	-	-	2	-	-	.	.	.	-
					9	= 12d	= 58b	-	-	-	1	-	-	-	-	-	.	.	.	-
					9	- - - - - - - - = 44c	= 58b	21	-	-	-	1	-	2	-	-	.	.	.	1
					9	= 44c	= 58b	-	1	-	1	1	1	2	2	3	.	.	.	1
					9	= 44c	- + - + - + - - - = 58c	21	-	-	-	2	-	-	-	-	.	.	.	1
					9	= 44c	= 58c	-	-	-	-	-	1	-	-	-	.	.	.	-
					9	= 44c	- + - - - - + - = 54b	21	-	-	-	1	-	-	1	-	.	.	.	-
					9	= 44c	= 54b	-	-	-	1	-	-	-	-	-	.	.	.	-
					9	= 44c	- + - - - - - - - = 54c	21	-	1	-	-	-	-	1	-	.	.	.	-
					9	= 44c	= 54c	-	-	-	-	-	-	2	3	1	.	.	.	-
					9	= 44c	- - - - - - - - - = 44c	-	2	-	-	-	-	-	-	-	.	.	.	-
#5	9	6	1-3	36	6	- - - - - - - - = 44c	- - - - - - - - - = 44c	1214	-	-	-	-	-	.	.	.	.	.	.	4
Female					6	= 44c	= 44c	-	-	-	-	-	-	.	.	.	.	.	.	2
15 years																				

Column

I	II	III	IV	V	VI	VII	VIII	IX	X											
									number of types present in sputum samples obtained during exacerbations A, B, and C											
					ST	APT	PPT	PhT	A1	B1	02	03	04	05	06	07	08	09	10	C1
					10	+ + + + + - - + = 12d	- - - - - - - - = 44c	-	3	1	4	3	3	.	.	.	.	.	.	-
					10	= 12d	= 44c	21/1214	-	2	-	2	-	.	.	.	.	.	.	-
					10	= 12d	= 44c	21	-	2	-	1	-	.	.	.	.	.	.	-
					10	= 12d	= 44c	1214	-	3	-	3	-	.	.	.	.	.	.	-
					10	- + + + + - - + = 62d	= 44c	21	-	-	-	-	-	.	.	.	.	.	.	1
					10	= 62d	= 44c	-	-	-	-	-	-	.	.	.	.	.	.	2
#6	14	9	1-3	35	6	- - - - - - - - = 44c	+ + - + - + - + = 28d	31	3	3	6	3	5	1	-	-	.	.	.	-
Male					6	- + - - - + - - = 57c	+ + - + - + - + = 28d	31	-	-	-	-	-	-	3	3	.	.	.	1
13 years					6	+ + + - - + - + = 17d	= 28d	31	-	-	-	-	-	-	1	3	.	.	.	-
					6	= 44c	+ + + + + + + + = 11a	-	-	-	-	-	-	-	3	-	.	.	.	-
#7	15	5	1-2	20	9	- + - - - - - - = 54c	- + - + - + - - = 58c	16/21/44/f8/f10/109/352	1	-	-	-	.	.	.	.	.	.	.	2
Male					9	- - - - - - - - = 44c	= 58c	16/21/44/f8/f10/109/352	-	2	1	-	.	.	.	.	.	.	.	2
18 years					9	= 44c	= 58c	16/21/44/f8/f10/109	4	-	-	4	.	.	.	.	.	.	.	-
					9	= 44c	- - - - - - - - = 44c	16/21/44/f8/f10/109	1	-	1	-	.	.	.	.	.	.	.	2
#8	17	6	1-3	24	10	- - - - - - - - = 44c	- - - - - - - - = 44c	-	3	-	-	-	-	.	.	.	.	.	.	-
Female					10	= 44c	- - - + - + - - = 48d	21/24	1	-	-	-	-	.	.	.	.	.	.	-
12 years					10	+ + + + + + - - = 11c	= 48d	21/24	2	-	1	-	-	.	.	.	.	.	.	-
					10	- + + + + + - - = 61c	= 48d	21/24	3	4	2	3	-	.	.	.	.	.	.	-
					10	- + + + + + - + = 61d	= 48d	21/24	-	-	-	-	3	.	.	.	.	.	.	2
#9	17	6	1-5	50	6	+ + + - + - - - = 15c	- - - - - - - - = 44c	-	-	3	9	2	3	.	.	.	.	.	.	-
Female					6	- - - - + - - - = 45c	= 44c	-	-	-	3	3	3	.	.	.	.	.	.	-
11 years					6	= 45c	- + - + - + - - = 58c	31	1	-	3	3	-	.	.	.	.	.	.	-
					6	= 45c	= 58c	-	-	3	-	1	-	.	.	.	.	.	.	1
					6	= 45c	+ + - + - + - - = 28c	31	1	-	-	3	-	.	.	.	.	.	.	-
					6	= 45c	= 28c	-	-	5	-	-	-	.	.	.	.	.	.	-
					6	= 45c	+ + - - - - - - = 24c	-	-	1	-	-	-	.	.	.	.	.	.	2
#10	17	6	1-3	31	3	- - - - - - + - = 44b	+ + - - - - - - = 24c	21	3	1	-	-	3	.	.	.	.	.	.	3
Female					3	= 44b	= 24c	-	-	-	-	-	3	.	.	.	.	.	.	-
11 years					3	= 44b	- + - + - + - + = 58d	21	1	-	-	-	-	.	.	.	.	.	.	-
					3	= 44b	= 58d	-	1	-	3	-	-	.	.	.	.	.	.	-
					3	- - - - - + + - = 47b	+ + - - - - - - = 24c	-	-	-	-	3	-	.	.	.	.	.	.	-
					3	- + - - + + + - = 56b	= 24c	-	-	-	-	3	-	.	.	.	.	.	.	-
					3	- - - - - - - - = 44c	= 24c	-	1	-	3	-	-	.	.	.	.	.	.	-
					3	= 44c	- - - - - - - - = 44c	-	-	-	3	-	-	.	.	.	.	.	.	-
#11	18	5	1-3	18	2	- + - + + + - + = 51d	- + - + - + - + = 58d	21/24	2	3	-	-	.	.	.	.	.	.	.	-
Male					2	= 51d	= 58d	21/ 119x	-	-	3	-	.	.	.	.	.	.	.	-
15 years					2	= 51d	= 58d	21	-	-	-	3	.	.	.	.	.	.	.	-
					2	- + - + + + - - = 51c	= 58d	21/ 119x	-	-	-	-	.	.	.	.	.	.	.	2
					2	- + - - + - - + = 55d	= 58d	21/24	2	-	-	-	.	.	.	.	.	.	.	-
					2	- + + - - + - - = 67c	= 58d	21/ 119x	-	-	-	3	.	.	.	.	.	.	.	-

Column I	II	III	IV	V	VI	VII	VIII	IX	X number of types present in sputum samples obtained during exacerbations A, B, and C											
					ST	APT	PPT	PhT	A1	B1	02	03	04	05	06	07	08	09	10	C1
#12	19	12	1-7	86	2	+ + + + + - - + = 12d	- - - - - - - - = 44c	21/f7/119x	-	-	-	-	-	-	-	-	3	3	-	-
Female					2	= 12d	= 44c	-	-	-	-	-	-	-	-	-	1	1	-	-
8 years																				
					3	- - + - + - - - = 75c	- + - + - + - - = 58c	21/44/f7/119x	-	2	1	2	-	-	-	-	-	-	1	-
					3	= 75c	- + - - - - - - = 54c	21/44/f7/119x	1	-	-	-	-	-	-	-	2	-	-	-
					3	= 75c	- - - - - - - - = 44c	-	2	-	-	-	-	-	-	-	-	-	-	1
					8	+ + + + + - - + = 12d	- + - + - + - - = 58c	-	4	3	1	-	-	-	10	2	2	2	-	2
					8	= 12d	= 58c	31	-	2	-	-	-	3	4	-	3	4	-	-
					8	= 12d	= 58c	21/ f7/119x	-	2	1	-	-	-	-	-	-	-	-	-
					8	= 12d	- + - - - - - - = 54c	21/ f7/119x	1	2	-	-	-	-	-	-	-	-	-	1
					8	= 12d	= 54c	31	-	-	-	-	-	-	-	-	3	2	-	-
					8	= 12d	= 54c	-	1	1	1	-	1	-	-	1	3	2	-	2
#13	20	6	2-4	45	9	- - - - + - - - = 45c	- - - - - - - - = 44c	-	-	-	2	1	1	.	.	.	.	.	.	-
Female					9	= 45c	- + - - - - - - = 54c	-	-	-	4	4	1	.	.	.	.	.	.	-
8 years					9	= 45c	= 54c	44/f10/109	-	-	-	1	1	.	.	.	.	.	.	-
					9	= 45c	- + - - - - + - = 54b	-	2	1	-	2	3	.	.	.	.	.	.	-
					9	= 45c	- + - + - + + - = 58b	-	-	2	-	1	3	.	.	.	.	.	.	-
					11	+ + + + + + - + = 12d	- + - - - - - - = 54c	21/44/119x	1	2	-	-	1	.	.	.	.	.	.	2
					11	= 12d	= 54c	-	-	1	-	-	1	.	.	.	.	.	.	4
					11	= 12d	- - - - - - - - = 44c	21/44/119x	-	1	-	-	1	.	.	.	.	.	.	-
					11	= 12d	= 44c	-	-	-	2	-	-	.	.	.	.	.	.	-
#14	27	10	1-2	25	5	- - - - + - - - = 45c	- + - + - + - + = 58d	-	-	-	-	1	3	-	-	2	1	.	.	-
Female																				
9 years					6	+ + + - - - - - = 14c	+ + - - + - - + = 25d	21/31/73/119x	1	2	3	-	-	-	-	-	-	.	.	-
					6	+ + + + + + - - = 11c	+ + - - + - - + = 25d	21/31/73/119x	-	-	-	-	3	3	2	-	-	.	.	1
					6	+ + + - - - - - = 14c	- + - + - + - + = 58d	21/31	-	-	3	-	-	-	-	-	-	.	.	-
#15	60	5	1-2	18	2	+ + + + + - - + = 12d	- + - + - + - - = 58c	21/109	1	1	-	4	.	.	.	.	.	.	.	-
Male					2	= 12d	= 58c	-	1	-	-	.	.	.	.	.	.	.	.	-
17 years					2	= 12d	- + - - - - - - = 54c	21/109	-	-	3	1	.	.	.	.	.	.	.	-
					2	= 12d	= 54c	-	-	-	-	-	.	.	.	.	.	.	.	1
					2	= 12d	- - - - - - - - = 44c	21/109	-	-	1	-	.	.	.	.	.	.	.	-
					2	= 12d	= 44c	-	-	-	-	1	.	.	.	.	.	.	.	1
					6	+ + + - + - - - = 15c	- + - - - - - - = 54c	21/119x	-	-	-	-	.	.	.	.	.	.	.	1
					6	= 15c	- - - - - - - - = 44c	21/119x	-	-	-	-	.	.	.	.	.	.	.	2

detected (column VI). In 5 patients (#3,#5,#13-#15) 2 serotypes were found and in one patient (#12) 3 serotypes were present. In the patients whose sputum yielded more than one serotype, all serotypes were encountered in several samples, with the exception of strains with serotype 6 in patient #5 and again in patient #15 (column X).

Active pyocin typing (column VII) revealed similarities as well as differences between serotypically identical strains from the same patient. The differences in active pyocin type between serotypically identical strains were based on minor, consistent differences or on the non-typability of strains (code 44C). This applied also to passive pyocin typing (column VIII). The number of different passive pyocin types within a serotype, however, exceeded the number of active pyocin types (column VII vs VIII).

The phage type (column IX) of serotypically identical strains in the individual patients was either uniform (for instance patient #3 with serotype 1 with phage type pattern 21+119X+352, and serotype 9 with phage type pattern 16+21+F8+F10+109) or showed differences (for example the phage type patterns belonging to serotypes 2 and 8 found in patient #2 and #12, respectively) within a given pattern of phages. An exacerbation of the chronic respiratory infection did not affect the composition of the Pseudomonas flora (sputum samples from exacerbation B versus A and C, column X).

DISCUSSION

In 30-50% of the younger and 70-90% of the older CF patients the lungs are chronically colonized with P. aeruginosa (2). Reports on the number of different types of P. aeruginosa that may infect the airways of the individual CF patients do not concur (3,13,15,16). On the basis of differences in O-antigen, P. aeruginosa strains can be serologically classified into 17 different types (8). In more than 90% of clinical isolates of P. aeruginosa (non-CF strains), a type-specific O-antigen can be detected with type-specific O-antisera (7). Serotyping of strains from CF patients is impeded by the fact that about 60% of the isolates react with more

than one type-specific O-antiserum; only 30% show monoagglutination, and 10% agglutinate with none of the 17 antisera (12). Polyagglutinability is believed to be related to deficiencies in the lipopolysaccharide (LPS) (6). As a result, deeper structures of the LPS which are normally protected by the specific O-antigens, are involved in the agglutination (10). Nonagglutinability may be due to the loss of larger parts of the LPS structure (6). In order to enhance discrimination between the various isolates, active and passive pyocin typing and phage typing were used in addition to serotyping in this study. One of the difficulties in these three typing methods is the mucoid character of the P. aeruginosa strains isolated from CF patients. This problem can in part be avoided by repeated transfer of the strains to solid media, causing partial dissociation of the mucoid to non-mucoid colony forms or by the use of modified techniques (7,16).

The aim of this study was to establish whether in the course of time the composition of the P. aeruginosa flora in the lungs of individual CF patients is subject to changes or whether it remains constant. For this purpose sputum samples from 15 CF patients were cultured over a defined period of time. The four typing techniques described were then applied in an effort to compare P. aeruginosa strains isolated from the same patient at different times. In view of the large number of polyagglutinable P. aeruginosa strains found in CF patients, agglutination was studied by serotyping under four different conditions (different growth temperatures used in culturing the strains in combination with different strengths of the O-antisera). The postulate underlying this procedure was that in the polyagglutinable strains LPS shows deficiencies but that, in addition, there are also intact LPS structures in the wall, and that these can be detected with more or less evidence under different conditions (6). Of the strains tested, 20% proved to be monoagglutinable; 80% agglutinated with more than one O-antiserum. Nonagglutinable strains were not found. Of each polyagglutinable strain the agglutinations obtained under the four different conditions were compared. It was repeatedly found that one serotype predominated, either because this type prevailed and/or while it displayed the strongest agglutination. In addition, it was found that for a given patient strains of an identical serotype showed a

similar pattern of agglutinations. Also, the two pyocin typings and the phage typing revealed a largely homogeneous pattern, despite minor biological differences between strains. If these results are taken together with those obtained by serotyping, the conclusion is warranted that the composition of the P. aeruginosa flora in the lungs of patients with cystic fibrosis remains fairly constant over time. In a study using DNA restriction fragments as an epidemiological marker, a similar observation was made in three patients with CF (9).

REFERENCES

1. Asheshov EH 1974. An assessment of the methods used for typing strains of P. aeruginosa. In: Arseni A (Ed) Proceedings of the 6th International Congress of Bacteriolgy. Leontiadi Medical Editions, Athens, p 9-22.
2. Cystic fibrosis foundation. Pulmonary infection in cystic fibrosis. "GAP" Conference Report 1983;**7**:4.
3. Diaz F, Mosovich LL, Neter E. Serogroups of P. aeruginosa and the immune response of patients with cystic fibrosis. J Infect Dis 1970;**11**:269-274.
4. Doudoroff M, Palleroni NJ. Pseudomonas. In: Buchanan RE, Gibbons NE (Eds) Bergey's mannual of determinative bacteriology 8th edn. The Williams and Wilkins Company, Baltimore, 1974;p 217-243.
5. Govan JRW. Pyocin typing of P. aeruginosa. In: Bergan T, Norris JR (Eds) Methods in Microbiology. Academic Press, New York p, 1978;61-91.
6. Hancock REW, Mutharia LM, Chan L, Darveau RP, Speert DP, Pier GB. P. aeruginosa isolates from patients with cystic fibrosis: a class of serum-sensitive, non-typable strains deficient in lipopolysacharide O side chains. Infect Immun 1983;**42:**170-177.
7. Lanyi B, Bergan T. Serological characterisation of P. aeruginosa. In: Bergan T, Norris JR (Eds) Methods in Microbiology. Academic Press, New York 1978;p 93-168.
8. Liu PV, Matsumoto H, Kusama H, Bergan T. Survey of heat-stable, major somatic antigens of P. aeruginosa. Int J Sys Bacteriol 1983;**33**:256-264.

9. Ogle JW, Jada JM, Woods DE, Vasil ML. Characterization and use of a DNA probe as an epidemiological marker for P. aeruginosa. J Infect Dis 1987;**1**:119-126.
10. Ojeniyi B, Baek L, Ho/iby N,. Polyagglutinability due to loss of O-antigenic determinants in P. aeruginosa strains isolated from cystic fibrosis patients. Acta Path Microbiol Immunol Scand, Section B; 1985;**93**;7-13.
11. Osman MAM. Pyocin typing of P. aeruginosa. J Clin Pathobiol 1965;**18**: 200-202.
12. Pitt TL, MacDougall J, Penketh ARL, Cooke EM. Polyagglutinating and nontypable strains of P. aeruginosa in cystic fibrosis. J Med Microbiol 1986;**21**:179-186.
13. Seale TW, Thirkill H, Tarpay M, Flux M, Rennert OM. Serotypes and antibiotic susceptibilities of P. aeruginosa isolates from single sputa of cystic fibrosis patients. J Clin Microbiol 1979;**9**:72-78.
14. Sheehan DJ, Janda JM, Bottone EJ. P. aeruginosa: changes in antibiotic susceptibility, enzymatic activity, and antigenicity among colonial morphotypes. J Clin Microbiol 1982;**15**:926-930.
15. Thomassen MJ, Demko CA, Boxerbaum B, Stern RC, Kuchenbrod PJ. Multiple isolates of P. aeruginosa with differing antimicrobial susceptibility patterns from patients with cystic fibrosis. J Infect Dis 1979;**140**: 873-880.
16. Williams RJ, Govan JRW. Pyocin typing of mucoid strains of P. aeruginosa isolated from children with cystic fibrosis. J Med Microbiol 1973;**6**:409-412.

Chapter 3

IN VITRO ACTIVITY OF NEW ANTIMICROBIAL AGENTS AGAINST PSEUDOMONAS AERUGINOSA ISOLATES FROM CYSTIC FIBROSIS PATIENTS

Horrevorts AM[1], Heeres-Weststrate PL[1], Kerrebijn KF[2] and Michel MF[1]

Departments of Clinical Microbiology and Antimicrobial Therapy[1] and Pulmonary Medicine in Children[2] (Sophia Children's Hospital), Erasmus University Rotterdam, The Netherlands.

Published in the European Journal of Clinical Microbiology 1987,**6**:323-324.

INTRODUCTION

Chronic respiratory infections with Pseudomonas aeruginosa are encountered in 30-50% of younger and in 70-90% of older patients with cystic fibrosis. To minimize progression of pulmonary destruction, acute exacerbations of this type of infection demand antimicrobial chemotherapy. At present many antipseudomonas drugs are available or under development, and we tested the activity of these agents against P. aeruginosa isolates from patients with cystic fibrosis.

MATERIALS/METHODS AND RESULTS

Each agent was obtained as a standard laboratory powder from the manufacturer and stored and handled as recommended. The 51 isolates of P. aeruginosa were recovered from sputum of 51 patients with cystic fibrosis. Non had received

intravenous chemotherapy during the four weeks before the sputum sample was obtained. The isolates, including mucoid and non-mucoid colony types, were identified biochemically by means of the API 20 NE identification system and maintained on tryptic soy agar slants at room temperature until tested. MICs were determined by an agar dilution technique. Freshly prepared serial twofold dilutions of antibiotic were incorporated into Diagnostic Sensitivity Test agar (Oxoid), giving final concentrations of 0.016 to 2048 mg/l. Plates were inoculated with a multipoint inoculator, which delivered a final inoculum of 10^4 CFU per spot (1 microl of liquid) from overnight Mueller-Hinton broth cultures adjusted to 10^8 CFU/ml (using a barium sulfate standard) and diluted 10^{-1}. Inoculated plates were incubated at 35°C for 18 h. The MIC was the lowest concentration of antimicrobial agent which inhibited visible growth. One discrete colony or fine, barely visible haze was disregarded. P. aeruginosa ATCC 27853 was included as control organism in all sets of inoculations, and the MICs were consistent with established values within one twofold dilution.

The results are summarized in Table 1. On a weight basis ciprofloxacin was the most active of the agents tested, inhibiting 82% of the isolates at 0.5 mg/l. At clinically attainable serum concentrations tobramycin and amikacin had similar activity. Within the penicillin group piperacillin was the most active drug. The activity of ticarcillin alone and ticarcillin plus clavulanic acid was equivalent. Cefsulodin and BMY 28142 had the lowest MIC_{50} values in the cephalosporin group; the amount of drug required to inhibit 75% of the isolates was 8 mg/l for each of the six cephalosporins tested. The monobactams aztreonam and carumonam had similar MIC_{50} values; only 12% of the strains tested were not inhibited by 32 mg/l carumonam. For 92% of the isolates the imipenem MIC was 4 mg/l or less. No significant differences were seen in the activity of any drug against mucoid and non-mucoid strains. Cross-resistance between the penicillins, cephalosporins and monobactams was the rule. Although higher MICs of some drugs against isolates of P. aeruginosa from patients with cystic fibrosis have been reported (1-4) similar MIC ranges have also been documented (4-6).

Table 1: In vitro activity of various antimicrobial agents against 51 isolates of <u>P. aeruginosa</u> obtained from 51 patients with cystic fibrosis.

Antimicrobial agent	Cumulative percentage of isolates inhibited at given concentration (mg/l)															
	≤0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	≥1024
Ciprofloxacin	2	6	27	45	82	100										
Norfloxacin			4	12	41	59	82	100								
Tobramycin	2	4	6	8	41	78	94	98	100							
Amikacin			2	4	4	12	24	67	90	98	98	100				
Ticarcillin					6	10	12	18	31	49	75	84	92	96	100	
Ticarcillin + clavulanic acid					8	10	16	20	27	53	76	80	90	96	98	100
Azlocillin					4	10	16	39	67	78	80	82	90	98	100	
Piperacillin					8	18	43	67	82	92	94	98	100			
Cefsulodin			2	2	14	27	59	73	82	86	88	98	100			
Ceftazidime			2	2	6	10	40	75	78	88	96	100				
Cefpiramide				4	10	20	41	67	78	82	86	88	98	100		
BMY 28142				4	14	27	51	75	84	94	100					
Cefpirome				2	8	18	45	65	75	78	100					
Cefoperazone				2	6	16	37	63	76	80	86	98	100			
Aztreonam				6	14	20	24	55	78	88	94	96	98	100		
Carumonam				8	16	24	39	61	76	82	88	98	100			
Imipenem	2	2	4	14	24	51	76	92	98	100						

REFERENCES

1. Scribner RK, Marks MI, Weber AH, Tarpay MM, Welch DF. Activities of various ß-lactams and aminoglycosides, alone and in combination, against isolates of Pseudomonas aeruginosa from patients with cystic fibrosis. Antimicrob Agents and Chemother 1982;**21**:939-943.
2. Gordts B, Vandenborre C, Van der Auwera P, Butzler, JP. Comparison between the in vitro activity of new agents on Pseudomonas aeruginosa isolates from cystic fibrosis patients and other chronic infections. J Antimicrob Chemother 1984;**14**:25-29.
3. Conrad DA. Scribner RK, Weber AH, Marks MI. In vitro activity of BMY-28142 against pediatric pathogens, including isolates from cystic fibrosis sputum. Antimicrob Agents and Chemother 1985;**28**:58-63.
4. Klinger JD, Aronoff SC. In vitro activity of ciprofloxacin and other antibacterial agents against Pseudomonas aeruginosa and Pseudomonas cepacia from cystic fibrosis patients. J Antimicrob Chemother 1985; **15**:679-684.
5. Prince AS, Neu HC. Activities of new ß-lactam antibiotics against isolates of Pseudomonas aeruginosa from patients with cystic fibrosis. Antimicrob Agents Chemother 1981;**20**:545-546.
6. Aronoff SC, Klinger JD. In vitro activities of aztreonam, piperacillin, and ticarcillin combined with amikacin against amikacin-resistant Pseudomonas aeruginosa and Pseudomonas cepacia isolates from children with cystic fibrosis. Antimicrob Agents Chemother 1984;**25**:279-280.

Chapter 4

CHEQUERBOARD TITRATIONS: THE INFLUENCE OF THE COMPOSITION OF SERIALDILUTIONS OF ANTIBIOTICS ON THE FRACTIONAL INHIBITORY CONCENTRATION INDEX AND FRACTIONAL BACTERICIDAL CONCENTRATION INDEX

Horrevorts AM[1], de Ridder CM[1], Poot MC[1], de Jonge MJA[1], Degener JE[1], Dzoljic-Danilovic G[1], Michel MF[1] and Kerrebijn KF[2]

Departments of Clinical Microbiology and Antimicrobial Therapy[1] and Pulmonary Medicine in Children[2] (Sophia Children's Hospital), Erasmus University Rotterdam, The Netherlands.

Published in the Journal of Antimicrobial Chemotherapy 1987,**19**:119-125.

SUMMARY

Chequerboard titrations carried out with modified serial dilutions of antibiotics such that consecutive concentrations in these series were four times smaller than those in two-fold serial dilutions enable MICs and MBCs to be determined with greater accuracy. Interaction indices calculated by this method can differ markedly from those calculated on the basis of two-fold serial dilutions. The differences calculated in this study ranged from -0.30 to +1.06.

INTRODUCTION

Interactions between antibiotics can be studied in vitro using chequerboard

titrations with serial doubling dilutions. More accurate determination of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) will in turn produce better estimates of the parameters used to measure the interaction between antibiotics, namely fractional inhibitory concentration indices (FICs) and fractional bactericidal concentration indices (FBCIs) (1). In order to investigate the value of these more accurate determinations, we have studied three combinations of antibiotics against Pseudomonas aeruginosa, determining FICIs and FBCIs using both conventional doubling dilutions and a modified dilution series giving smaller incremental concentrations.

MATERIALS AND METHODS

Bacterial strains

Twenty-two strains of P. aeruginosa, isolated from sputa of 22 patients with cystic fibrosis were used. The strains were collected between October 1982 and October 1983, identified (2) and stored after freeze-drying. Serotyping and pyocin typing showed that the isolates were unrelated. Twelve strains (nos 1 to 12) were tested with each of three combinations of antibiotics; ten (nos 13 to 22) were tested only with the combination ticarcillin plus tobramycin. P. aeruginosa ATCC 27853 was used as reference strain.

Antibiotics

Ticarcillin (Beecham), cefsulodin (Ciba-Geigy), ceftazidime (Glaxo) and tobramycin (Eli Lilly & Co) were obtained in sterile, standardized powder form and stored at -20°C until use.

Medium

Isosensitest Broth (Oxoid) was used.

Serial dilutions of antibiotics

Serial dilutions were prepared from freshly prepared stock solutions in Isosensitest Broth of 2560 mg/l for ticarcillin, of 320 mg/l for cefsulodin and ceftazidime and of 40 mg/l for tobramycin. The stock solutions were further diluted as shown in Table I. Proceeding from a single stock solution, several two-fold serial dilutions (A, B, C and D) were obtained. The concentrations together form a modified serial dilution in which the intervals between consecutive concentrations were four times smaller than those in a two-fold serial dilution. The extreme concentrations of the series were 0.25 and 1792 mg/l for ticarcillin, 0.125 and 224 mg/l for cefsulodin and ceftazidime, and 0.03 and 28 mg/l for tobramycin. The serial dilutions were checked with the reference strain for which the range of MICs of ticarcillin is 16-32 mg/l (3), of cefsulodin and ceftazidime is 1.0-2.0 mg/l (4) and of tobramycin is 0.5-1.0 mg/l (3).

Inoculum

Shaken overnight cultures in Isosensitest Broth were adjusted to E600 nm=0.75 and diluted 1:1000 in broth. Such dilutions contained $5x10^5$ colony-forming units (CFU) per ml.

MIC and MBC determination

To 0.5 ml of each dilution of antibiotic 0.5 ml of the inoculum of a strain to be tested was added. After incubation of the cultures at 37°C for 18 h the MIC of the antibiotic for the strain was read. The MIC was the lowest concentration at which no macroscopically visible growth of the strain was observed. To determine the MBC, 0.1 ml of each culture without visible growth was transferred to a blood agar plate. After incubation at 37°C for 24 h the number of colonies was counted. The MBC was the lowest concentration at which 99.9% of the number of CFU originally present per ml in the cultures had been killed.

Chequerboard titrations

These were performed at the same time as the determinations of the MIC and MBC. In accordance with a chequerboard pattern 0.25 ml of each tobramycin concentration was combined with 0.25 ml of each concentration of a ß-lactam antibiotic. Next, 0.5 ml of the inoculum of a strain to be tested was added to each combination. After incubation of the cultures at 37°C for 18 h end points were read and MBCs determined.

Calculation of the degree of interaction between antibiotics in a chequerboard.

FICIs and FBCIs were calculated in the usual way (1,5).

Reproducibility of the chequerboard titrations

To establish the reproducibility of the method used, the effect of two combinations (ticarcillin + tobramycin and ceftazidime + tobramycin) on three random strains was studied five times. The titrations were carried out on different days, always with freshly prepared serial dilutions of the antibiotics.

Comparison of serial dilutions

The FICIs and FBCIs obtained with the serial dilutions used in this study, were compared with those obtained using two-fold serial dilutions as in row A alone (Table I).

Since several FICIs and FBCIs for a strain can be determined from a chequerboard, results were analyzed on the basis of both the lowest and the mean FICI and FBCI which could be calculated per titration.

Table I. Preparation of modified dilutions of tobramycin

Stock Broth solution[a] (volume units)	Concentrations (mg/l)	Row
4+6 =	16 → 8 → 4 etc.	A
5+5 =	20 → 10 → 5 etc.	B
6+4 =	24 → 12 → 6 etc.	C
7+3 =	28 → 14 → 7 etc.	D

[a]Tobramycin standard = 40 mg/l.

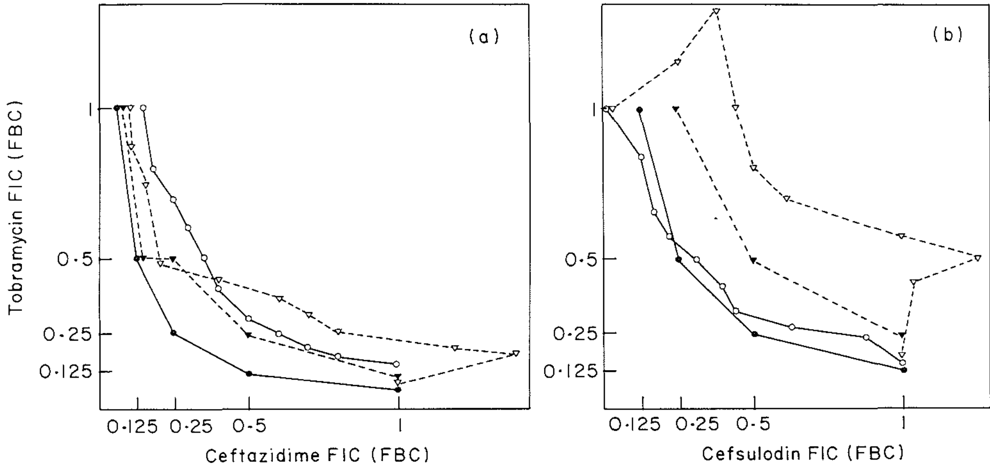


Figure 1. Isobolograms of ceftazidime+tobramycin against isolate no. 6 (a), and cefsulodin+tobramycin against isolate no. 2 (b). FICI: ●, two-fold dilutions; ○, modified dilutions. FBCI: ▼, two-fold dilutions; ▽, modified dilutions.

RESULTS

Table II lists the MICs and MBCs determined with modified serial dilutions of the antibiotics studied. Isolates nos 13 to 22, for which only ticarcillin and tobramycin MICs and MBCs were determined, had ticarcillin MICs >128 mg/l. To establish the reproducibility of chequerboard titrations using modified serial dilutions, the effect of ticarcillin + tobramycin and ceftazidime + tobramycin on three isolates (nos 2, 10 and 12) was studied five times. All titrations showed FICIs and FBCIs which never differed from the calculated mean by more than +0.15 or -0.15. This applied to the lowest as well as to the mean FICIs and FBCIs which could be calculated from the chequerboard titrations. Figure 1(a) shows the isobolograms of the combination ceftazidime + tobramycin against isolate no. 6, and Figure 1(b) those of the combination cefsulodin + tobramycin against isolate no. 2. In figure 1(a) a remarkable difference is demonstrated between the FICIs determined with modified serial dilutions and those determined with two-fold dilutions of the antibiotics. Notable differences between the FBCIs are shown in Figure 1(b).

For each of the isolates tested the differences were calculated between the FICIs and FBCIs found with the modified serial dilutions and those obtained with the two-fold serial dilutions. The calculated differences ranged from -0.30 to +1.06. Table III lists these differences in classes of 0.15 both for the lowest and for the mean FICIs and FBCIs calculable from the chequerboard titrations. In only 8% of the cases the difference was negative, and this indicates that the FICIs and FBCIs obtained with the modified serial dilutions often exceeded those found with the two-fold serial dilutions. This applies in particular to the FBCIs. Table III also shows that the distribution of the differences between the lowest values coincides with that of the differences between the mean values of the interaction indices. The differences between the strains 1 to 12 with ticarcillin MICs <128 mg/l and the strains 13 to 22 with ticarcillin MICs >128 mg/l are likewise evenly distributed.

Table II. Activity[a] of ticarcillin, cefsulodin, ceftazidime and tobramycin against 22 isolates of *P. aeruginosa* determined with modified dilutions of the antibiotics

	Ticarcillin			Cefsulodin			Ceftazidime			Tobramycin		
	MIC	MBC	MBC/MIC ratio	MIC	MBC	MBC/MIC ratio	MIC	MBC	MBC/MIC ratio	MIC	MBC	MBC/MIC ratio
Strain no. 1–12												
Range	1·75–48	3–96	1–4·6	0·75–8	1·25–16	1·2–21·3	0·75–28	0·75–80	1–4	0·25–1·25	0·50–2	1·5–2·7
Median	24	40	1·3	3	6	2	1·75	2·75	1·5	0·75	1·25	2
Strain no. 13–22												
Range	128–768	192–1280	1–5		n.d.			n.d.		0·50–6	1–12	1–4
Median	240	640	2							0·88	1·75	2

[a]Expressed as mg/l.
n.d., Not done.

Table III. Number of isolates for which a difference X (arranged in classes of 0·15) was found between FICIs or FBCIs determined with modified and two-fold serial dilutions of the antibiotics tested

Combination	$FICI_{md}{}^{a} - FICI_{td}{}^{a}$ / $FBCI_{md} - FBCI_{td}$: $-0{\cdot}30 < X \leqslant -0{\cdot}15$	$-0{\cdot}15 < X \leqslant 0{\cdot}00$	$0{\cdot}00 < X \leqslant +0{\cdot}15$	$+0{\cdot}15 < X \leqslant +0{\cdot}30$	$+0{\cdot}30 < X \leqslant +0{\cdot}45$	$+0{\cdot}45 < X \leqslant +0{\cdot}60$	$X \geqslant +0{\cdot}60$
Strain no. 1–12							
Ticarcillin +	1[b]	1 (3)[b]	9 (8)	1 (1)			
tobramycin	*2 (1)*	*1 (1)*	*2 (4)*	*6 (5)*	*1 (1)*		
Cefsulodin +		(2)	10 (10)	2			
tobramycin			*3*	*5 (4)*	*1 (6)*	*1 (2)*	*2*
Ceftazidime +		(3)	6 (5)	6 (4)			
tobramycin		*(1)*	*6 (2)*	*4 (6)*	*2 (2)*		*(1)*
Strain no. 13–22							
Ticarcillin +			7 (8)	2 (2)	1		
tobramycin			*6 (5)*	*1 (4)*	*3 (1)*		

[a]md, Modified dilutions; td, two-fold dilutions.
[b]The figures without brackets indicate the lowest FICIs or FBCIs, and those in brackets indicate the mean FICIs or FBCIs calculated per chequerboard.

DISCUSSION

FICIs and FBCIs are parameters which can be used to quantify the degree of interaction between antibiotics (1). According to Elion's equation a value below 1 is suggestive of synergism, a value of 1 indicates addition, and a value above 1 suggests antagonism (2,5). However, one seldom proceeds from these theoretical limits. The upper limit of synergism is generally an index of 0.5 in actual practice (6).

The accuracy of MIC and MBC determinations depends not only on day-to-day variations (6) but also on methodological factors (7-13) including the intervals between consecutive concentrations in the serial dilutions of antibiotics used (14). Accurate determination of MICs and MBCs will be reflected in parameters derived from them such as FICs, FBCs, FICIs en FBCIs.

In two-fold serial dilutions the sensitivity of the determination diminishes more with the increasing intervals between the higher concentrations than in the modified serial dilutions described here. On the other hand, the decreasing intervals between the lower concentrations more readily affect the reproducibility with modified serial dilutions. In view of these considerations chequerboard titrations would best be carried out with serial dilutions in which the intervals between consecutive concentrations would be constant (15). However, it is difficult to prepare such serial dilutions, and in addition, an unworkably high number of concentrations would be required to cover a sufficiently wide range. Serial dilutions containing concentrations as specified in the rows A and C (Tabel I) should meet the objection mentioned above. The findings presented here indicate that strains for which a given combination of antibiotics shows a similar interaction index in two-fold serial dilutions, may differ significantly in index when examined with the aid of modified serial dilutions. This should be borne in mind when such strains are used for further studies on the basis of a "similar" interaction index (e.g. in animal models). Moreover, in clinical practice it is advisable to apply certain margins to interaction indices determined with two-fold serial dilutions of antibiotics.

REFERENCES

1. Berenbaum MC. A method for testing for synergy with any number of agents. J Infect Dis 1978;**137**:122-30.
2. Doudoroff M, Palleroni NJ. Pseudomonas. In: Bergey's Manual of Determinative Bacteriology, 8th edn (Buchanan RE, Gibbons NE, Eds). The Williams and Wilkins Company, Baltimore, 1974:217-43.
3. Fass JF, Barnishan J. Minimal inhibitory concentrations of 34 antimicrobial agents for control strains Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853. Antimicrob Agents Chemother 1979;**16**:22-4.
4. Muitjens HL, van der Ros-van de Repe J. Comparative activities of 13 ß-lactam antibiotics. Antimicrob Agents Chemother 1982;**21**;925-34.
5. Elion GB, Singer S, Hitchings GH. Antagonists of nucleicacid derivatives. VIII. Synergism in combinations of biochemically related antimetabolites. J Biol Chem 1953;**208:**477-88.
6. Young LS. Significance of in vitro tests. In: Combination Antibiotic Therapy in the Compromised Host (Klastersky J, Staquet MJ. Eds). Raven Press, New York, 1982:1-15.
7. Gilbert DN, Kutscher E, Ireland P, Barnett, JA, Sanford JP. Effect of the concentrations of magnesium and calcium on the in vitro susceptibility of Pseudomonas aeruginosa to gentamicin. J Infect Dis 1971; suppl. **124**:37-45.
8. Weinstein RJ, Young LS, Hewitt WL. Comparison of methods for assessing in vitro antibiotic synergism against Pseudomonas and Serratia. J Lab Clin Med 1975;**86**:853-62.
9. Norden CW, Wentzel H, Keleti E. Comparison of techniques for measurement of in-vitro antibiotic synergism. J Infect Dis 1979;**140**;692-33.
10. Woolfrey BF, Fox JM, Quall CO. A comparison of minimum inhibitory concentration values determined by three dilution methods for Pseudomonas aeruginosa. Am J Clin Pathol 1981;**75**;39-44.

11. Hallander HO, Dornbusch K, Gezelius L, Jacobson K, Karlsson I. Synergism between aminoglycosides and cephalosporins with antipseudomonal activity: Interaction index and killing curve method. Antimicrob Agents Chemother 1982;**22**:743-52.
12. Brumfitt W, Hamilton-Miller JMT, Gooding A. Importance of methodology in determining bactericidal and bacteriostatic activities of azlocillin and ticarcillin against Pseudomonas aeruginosa. J Med Microbiol **1984;17**;37-44.
13. Eng RHK, Smith SM, Cherubin S. Inoculum effect of new ß-lactam antibiotics on Pseudomonas aeruginosa. Antimicrob Agents Chemother **1984;26**:42-7.
14. Woolfrey, B.F., Fox, J.M.K., Lally, R.T. & Quall, C.O. 81982). Broth microdilution testing of Pseudomonas aeruginosa and aminoglycosides: Need for employing dilutions differing by small arithmetic increments. J Clin Microb 1982;**16**;663-7.
15. Berenbaum MC. Correlations between methods for measurement of synergy. J Infect Dis 1980;**142**:476-8.

Chapter 5

ANTIBIOTIC INTERACTION: INTERPRETATION OF FRACTIONAL INHIBITORY AND FRACTIONAL BACTERICIDAL CONCENTRATION INDICES

Horrevorts AM[1], Michel MF[1] and Kerrebijn KF[2]

Departments of Clinical Microbiology and Antimicrobial Therapy[1] and Pulmonary Medicine in Children (Sophia Children's Hospital)[2], Erasmus University Rotterdam, The Netherlands.

Published in the European Journal of Clinical Microbiology 1987,4:502-503.

INTRODUCTION

Interactions between antimicrobial drugs can be studied in vitro using checkerboard titrations. This is commonly done with serial doubling dilutions of drugs. When serial dilutions with smaller intervals between consecutive concentrations are used, minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) can be determined with greater accuracy (1). More accurate determination of MICs and MBCs will be reflected in parameters derived from them, such as fractional inhibitory concentrations (FICs), fractional bactericidal concentrations (FBCs) and fractional inhibitory concentration indices (FICs) and fractional bactericidal concentration indices (FBCIs) (2).

FRACTIONAL CONCENTRATION INDICES

The statement mentioned in the introduction may be elucidated by means of a hypothetical example. Using two-fold serial dilutions of antibiotics X and Y, let 8 mg/l of X and 16 mg/l of Y be the lowest concentrations in combination inhibiting

Table 1: Preparation of a modified serial dilution (example). Proceeding from a single stock solution, several twofold serial dilutions (A, B, C and D) are obtained. The concentrations together form a modified serial dilution in which the intervals between consecutive concentrations are four times smaller than those in a twofold serial dilution.

Stock solution[#] (volume units)		Broth		Concentrations (mg/l)		Row
4	+	6	=	64 → 32 → 16 → 8	etc.	A
5	+	5	=	80 → 40 → 20 → 10	etc.	B
6	+	4	=	96 → 48 → 24 → 12	etc.	C
7	+	3	=	112 → 56 → 28 → 14	etc.	D

[#]Drug standard = 160 mg/l.

Table 2: Values of FICIs (FBCIs) from checkerboard titrations using twofold serial dilutions of drugs versus values (range) which might have been found if modified or semi-modified dilutions of drugs had been used.

FICI (FBCI) (twofold dilutions)[a]	FICI (FBCI) (modified dilutions)[b]	FICI (FBCI) (semi-modified dilutions)[c]
2.00	1.25 - 3.20	1.50 - 2.67
1.50	0.94 - 2.40	1.13 - 2.00
1.25	0.78 - 2.00	0.94 - 1.67
1.13	0.70 - 1.80	0.48 - 1.50
1.06	0.66 - 1.70	0.80 - 1.41
1.00	0.63 - 1.60	0.75 - 1.33
0.75	0.47 - 1.20	0.56 - 1.00
0.63	0.39 - 1.00	0.47 - 0.83
0.56	0.35 - 0.90	0.42 - 0.75
0.50	0.31 - 0.80	0.38 - 0.67
0.38	0.23 - 0.60	0.28 - 0.50
0.31	0.20 - 0.50	0.23 - 0.42
0.25	0.16 - 0.40	0.19 - 0.33
0.19	0.12 - 0.30	0.14 - 0.25
0.13	0.08 - 0.20	0.09 - 0.17

[a]The concentrations of row A alone, see Table 1.
[b]The concentrations of rows A + B + C + D together, see Table 1.
[c]The concentrations of rows A + C together, see Table 1.

the growth of a strain Z, where 32 and 64 mg/l are their respective MICs when used alone. FICs of X and Y deduced from these data will therefore equal 8/32=0.25 and 16/64=0.25, the sum of these fractions giving an FICI of 0.50. Using modified serial dilutions (Table 1), 8 mg/l of X and 16 mg/l of Y may again be the lowest concentrations of the antibiotics in combination inhibiting the growth of strain Z. However, if the individual MICs of X and Y for strain Z now turn out to be 20 and 40 mg/l, the FIC value of X becomes 8/20=0.40 and of Y 16/40=0.40, resulting in an FICI of 0.80. Thus, the use of modified serial dilutions of antibiotics X and Y caused an increase in FICI from 0.50 to 0.80.

Table 2 lists a number of FICIs (FBCIs) calculated from fictional checkerboard titrations using two-fold serial dilutions of antibiotics, followed by the extremes of the values which might have been found for the FICIs (FBCIs) if modified serial dilutions had been used. The table warrants three important conclusions. Firstly, using two-fold serial dilutions of antibiotics synergism cannot be assumed theoretically until the FICI (FBCI) is less than 0.63. Secondly, the use of modified serial dilutions could show that differences/similarities in activity between various combinations of antibiotics found with two-fold serial dilutions do not exist or could show that such differences/similarities remain unnoticed in two-fold serial dilutions. Thirdly, apart from the given limits (3,4), an additive and antagonistic effect are difficult to detect with two-fold serial dilutions because with these dilutions the next value which the FIC (FBC) can assume after 1 is 2.

In two-fold serial dilutions the sensitivity of the determination diminishes more with increasing intervals between the higher concentrations than in the modified serial dilutions. On the other hand, the decreasing intervals between the lower concentrations more easily affect reproducibility in modified serial dilutions. Serial dilutions containing concentrations as specified in the rows A and C (Table 1) should satisfy the objections mentioned above. The last column of Table 2 lists the extremes of the values which might have been found for the FICIs (FBCIs) given in the first column of Table 2 if these semi-modified dilutions had been

used.
In clinical practice it is thus advisable to apply certain margins to interaction indices determined using twofold serial dilutions of antibiotics (Table 2).

REFERENCES

1. Woolfrey BF, Fox JMK, Lally RT, Quall CO. Broth microdilution testing of Pseudomonas aeruginosa and aminoglycosides: need for employing dilutions differing by small arithmetic increments. J Clin Microbiol 1982;**16**:663-667.
2. Horrevorts AM, de Ridder CM, Poot MC, de Jonge MJA, Degener JE, Dzoljic-Danilovic G, Michel MF, Kerrebijn MF. Checkerboard titrations: the influence of the composition of serial dilutions of antibiotics on the fractional inhibitory concentration index and fractional bactericidal concentration index. J Antimicrob Chemother 1987;**19**:119-125.
3. Berenbaum MC. A method for testing for synergy with any number of agents. J Infect Dis 1978;**137**:122-130.
4. Hamilton-Miller JMT. Rationalization of terminology and methodology in the study of antibiotic interaction. J Antimicrob Chemother 1985; **15**:655-658.

Chapter 6

PHARMACOKINETICS OF TOBRAMYCIN IN PATIENTS WITH CYSTIC FIBROSIS: IMPLICATIONS FOR THE DOSING INTERVAL

Horrevorts AM[1], Degener JE[1], Dzoljic-Danilovic G[1], Michel MF[1], Kerrebijn KF[2], Driessen O[3] and Hermans J[4]

Departments of Clinical Microbiology and Antimicrobial Therapy[1] and Pulmonary Medicine in Children[2] (Sophia Children's Hospital), Erasmus University, Rotterdam; Departments of Pharmacology[3] and Medical Statistics[4], State University of Leiden, Leiden, The Netherlands.

Published in Chest 1985,**88**:260-264.

SUMMARY

The pharmacokinetics of tobramycin were evaluated in 15 patients (8 to 22 years of age) with cystic fibrosis (CF). A dose of 3.0 to 3.3 mg/kg of body weight was given intravenously over 20 minutes, and concentrations in serum were followed up to eight hours after initiation of the infusion. In the calculation of pharmacokinetic parameters, a two-compartment open model was used. The elimination half-life of the drug was highly inversely correlated with age ($p<0.0004$), and body weight ($p<0.00002$). Total Body Clearance (TBC), and Volume of distribution at steady state (Vdss) were directly correlated with age and body weight. However, when TBC and Vdss were corrected for Body Surface Area (BSA), no correlation could be demonstrated. The mean one-hour and eight-hour serum concentrations of tobramycin were 5.40 and 0.45 mg/l respectively. Between patients, considerable differences were found in the time after administration at which the serum concentration decreased below 1 mg/l. This interpa-

tient variation has clinical implications for tobramycin therapy in CF, in particular for the dosing interval.

INTRODUCTION

Chronic respiratory infections with Pseudomonas aeruginosa are encountered in 70 to 90 percent of patients with cystic fibrosis (CF)(1). Acute exacerbations are commonly treated with an aminoglycoside, often tobramycin, and an antipseudomonal ß-lactam antibiotic such as ticarcillin (2,3).
The Total Body Clearance (TBC) of a number of penicillin derivatives is unusually high in patients with CF. These penicillins have to be given more frequently or in a higher dosage than usual in order to ensure sufficiently protracted active serum concentrations (4-9). There are indications that the pharmacokinetics of aminoglycosides likewise deviate from the normal in CF. Frequent and high dosages are necessary to reach adequate serum concentrations (10-18). The amount of tobramycin required to control an exacerbation varies from patient to patient. This cannot be adequately explained by differences in severity of the disease or in the susceptibility of the bacterial flora to tobramycin. The therapeutic index of aminoglycosides is small in view of the possible ototoxic and nephrotoxic effects, and consequently safe and adequate administration of tobramycin is difficult, particularly in patients with CF.
This study presents data collected on the serum tobramycin concentration-time curve in 15 patients with CF. The results are used to formulate guidelines on tobramycin dosing in CF.

PATIENTS AND METHODS

Study Population

The study concerned 15 patients with CF, seven female and eight male patients ranging in age from 8 to 22 years. The diagnosis CF was based on increased

sweat electrolyte levels ($Na^+ \geq 60$ mmol/l) and characteristic gastrointestinal and pulmonary disease. All patients required tobramycin and ticarcillin therapy for an acute exacerbation of their chronic respiratory infection caused by P.aeruginosa. During therapy, renal and auditory functions were determined regularly, and always found to be normal. Before the study, informed consent was obtained from all subjects and their parents.

Serum Samples

The serum tobramycin concentration-time curve was measured on the eighth or ninth day of therapy. A dose of 3.0 to 3.3 mg/kg of body weight was given intravenously over 20 minutes. Blood samples (1 ml) were drawn at times, t being 0, 10, 15, 20, 25, 30, 35, 40, 50 minutes and 1, 1½, 2½, 4, 6 and 8 hours, from an indwelling plastic catheter in a forearm vein contralateral to the site of intravenous dosing. The samples were chilled to 4°C immediately. After centrifugation, the serum was stored at -80°C. Ticarcillin administration was discontinued 12 hours prior to the first sampling and then continued immediately after the last sampling.

Tobramycin Assays

Serum tobramycin concentrations were determined with an enzyme-immuno-assay. All samples were tested in duplicate. The accuracy (19) of the method was checked in advance with test sera containing a known amount of tobramycin. The amount of tobramycin measured in the test sera deviated from the true amount by a maximum of 17 percent. The lower limit of detection for this assay was 0.20 mg/l.

Pharmacokinetic and Statistical Analysis

A semilogarithmic plot of the concentrations of tobramycin vs time showed a

biexponential decay from the end of the infusion to the end of the dosing interval. Consequently, the data were analyzed according to a two-compartment open model.

The following equations were used (20):

for the infusion period: $C_t = \frac{A}{\tau \cdot \alpha}\left[1 - e^{-\alpha t}\right] + \frac{B}{\tau \cdot \beta}\left[1 - e^{-\beta t}\right]$

for the post-infusion period: $(t \geq \tau)$: $C_t = \frac{A}{\tau \cdot \alpha}\left[1 - e^{-\alpha \tau}\right] \cdot e^{-\alpha(t-\tau)} + \frac{B}{\tau \cdot \beta}\left[1 - e^{-\beta \tau}\right] \cdot e^{-\beta(t-\tau)}$

In this model, C_t is the serum concentration at time t (the mean serum concentration of tobramycin at t=0 was 0.21 mg/l;τ, the infusion period; α and β, rate constants; and A and B, intercept parameters. An estimate of the parameters A, α, B and β was obtained via a weighted least square adjustment, using the NLIN procedure from the SAS computer program package (21).

Of the derived parameters ($t_{1/2}$ = half-life; AUC = Area Under the Curve; TBC = Total Body Clearance; Vdss = Volume of Distribution at steady state); the TBC and Vdss were calculated independent of the model used (22). Pearson's test was applied to investigate the significance of correlations between results (23). The $p<0.05$ was chosen as the level of statistical significance.

RESULTS

The serum concentrations in patient 1, eight years of age, and in patient 15, 22 years of age, are plotted vs time in Figure 1. When studying all curves, the profile proves to change with age. With increasing age, the distribution (α) phase turns more quickly into the elimination (β) phase. In addition, elimination shows a faster decline.

Individual patient characteristics and pharmacokinetic parameters are listed in

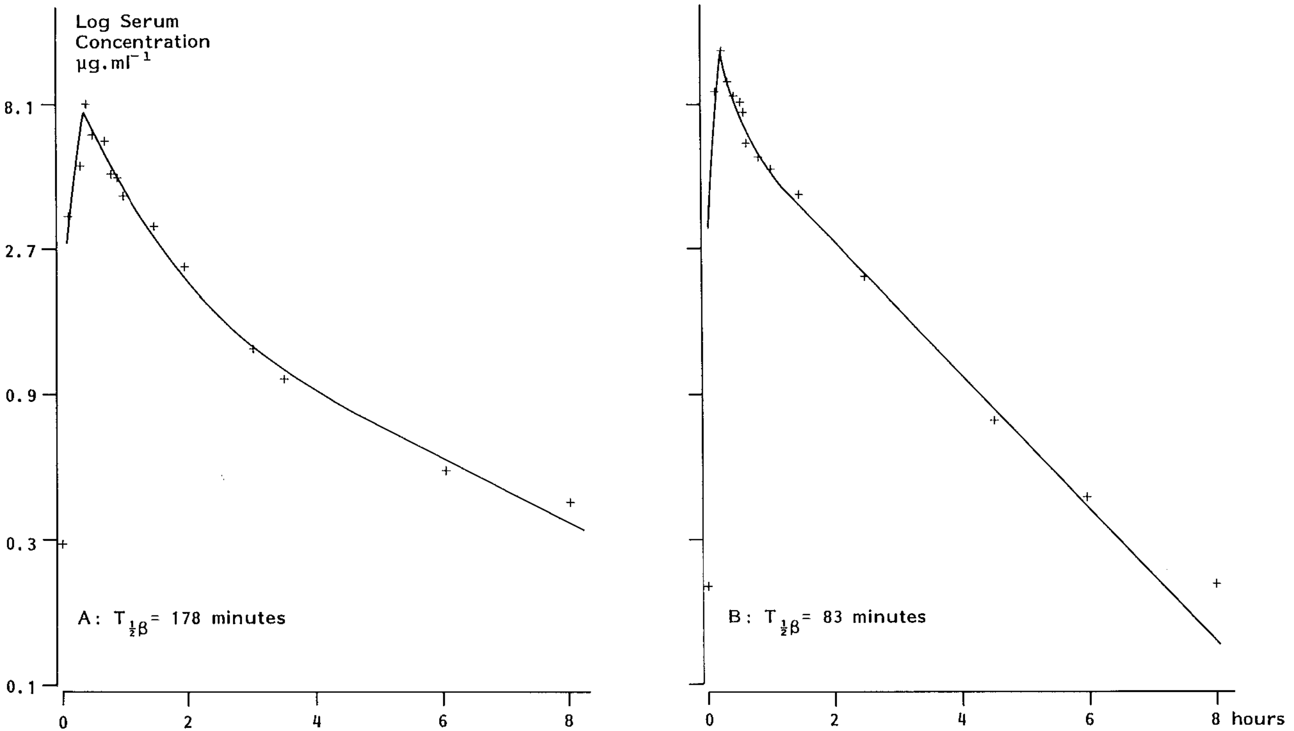


FIGURE 1. Semilogarithmic plot of tobramycin serum concentrations vs time in two patients with cystic fibrosis. A *(left)*, Patient No. 1, 8 years of age. B *(right)*, Patient No. 15, 22 years of age. The line through the observed concentrations is the computer curve of best fit.

Table 1—*Patients' Characteristics and Pharmacokinetic Parameters of Tobramycin From 15 Patients with CF*

Patient No.	Sex	Age, yr	Weight, kg	Height, m	BSA m^2	FVC* %	FEV_1* %	Dose, mg	$t_{1/2\alpha}$ min	$t_{1/2\beta}$ min	AUC/Dose $\mu g \cdot min \cdot ml^{-1} \cdot mg^{-1}$	TBC $ml \cdot min^{-1}$	TBC/BSA $ml \cdot 1.73m^2 \cdot min^{-1}$	VDSS L	VDSS/BSA $L \cdot 1.73m^{-2}$	Serum concentrations $\mu g \cdot ml^{-1}$ 1-hour	Serum concentrations $\mu g \cdot ml^{-1}$ 8-hour	Points of time between which serum concentration <1 $\mu g \cdot ml^{-1}$ hours
1	M	8	21.9	1.26	0.83	53	43	60	33.0	178	13.5	75	156	13.6	28.4	4.0	0.4	2½-4
2	F	10	22.4	1.31	0.86	34	21	75	28.9	188	10.3	97	194	16.1	32.3	4.5	0.3	2½-4
3	M	10	28.7	1.37	0.98	60	64	95	30.3	164	13.0	76	136	13.2	23.6	7.1	0.6	4 -6
4	F	11½	24.9	1.44	0.96	39	29	80	46.2	226	12.9	78	142	13.2	24.0	5.7	0.3	4 -6
5	F	12½	36.1	1.44	1.11	74	66	110	19.3	149	9.3	108	169	19.7	30.7	4.8	0.6	4 -6
6	F	14	35.8	1.54	1.17	60	54	120	16.0	103	8.9	111	166	15.4	23.1	6.0	0.4	4 -6
7	M	15	48.3	1.74	1.45	71	63	160	4.8	109	8.7	115	138	17.9	21.3	6.4	0.8	4 -6
8	F	15	37.3	1.62	1.24	54	35	115	13.1	149	10.8	93	131	19.0	26.7	5.1	0.6	6 -8
9	M	17½	47.7	1.65	1.39	75	63	145	9.9	101	7.1	141	176	23.0	28.8	5.5	0.5	4 -6
10	F	18	38.7	1.54	1.22	31	22	125	8.4	132	13.0	77	110	13.5	19.3	6.5	0.6	6 -8
11	M	18½	44.9	1.72	1.40	94	81	150	23.4	126	9.1	110	135	18.4	22.7	6.1	0.6	6 -8
12	M	19½	42.7	1.71	1.36	72	31	135	17.3	122	5.0	202	256	32.7	41.9	3.4	0.3	2½-4
13	M	19½	45.1	1.76	1.43	54	32	140	16.9	84	6.2	163	199	20.3	24.8	4.5	0.2	2½-4
14	F	20	48.3	1.68	1.41	83	46	150	14.3	103	8.0	117	144	16.4	20.3	6.1	0.4	4 -6
15	M	22	50.4	1.84	1.53	26	17	160	8.9	83	6.2	162	184	21.3	24.2	5.0	0.2	2½-4
mean		15.4	38.2	1.57	1.22	58.7	44.5	121.3	19.38	134.5	9.47	115.0	162.4	18.25	26.14	5.40	0.45	
sd		4.3	9.8	0.18	0.23	20.0	19.7	31.9	11.18	41.1	2.75	37.4	36.2	5.06	5.74	1.04	0.18	

* = Percent predicted values based on height. Abbreviations are as follows: CF, cystic fibrosis; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second; BSA, body surface area; $t_{1/2}$, half-life; AUC/Dose, area under the curve normalized for dose; TBC, total body clearance; TBC/BSA, TBC normalized for BSA; VDSS, volume of distribution at steady state; and VDSS/BSA, VDSS normalized for BSA.

Table 1. The mean one-hour and eight-hour serum tobramycin concentrations are 5.40 and 0.45 mg/l, respectively. All eight-hour (trough) concentrations are less than 2 mg/l. A 1 mg/l level is attained between 2½ and 4 hours after the start of the infusion in five patients, between four and six hours in seven patients, and between six and eight hours in three patients (last column, Table 1). These differences are not related to the height of the one-hour serum tobramycin concentrations.

The half-times of the distribution ($t_{½\alpha}$) and elimination ($t_{½\beta}$) phases are inversely correlated with age and body weight. A similar relationship is found for the AUC/Dose. The TBC and Vdss are directly correlated with age and body weight. However, correcting TBC and Vdss for BSA, no correlation could be demonstrated. Linear coefficients of correlation are shown in Table 2. None of the pharmacokinetic parameters correlate with pulmonary function, i.e., FVC and FEV_1.

DISCUSSION AND CONCLUSIONS

The serum concentration-time curve for tobramycin is characterized by a distribution (α) phase and a rapid (β) as well as a slow elimination (γ) phase (24). During the distribution phase, the tobramycin serum concentration is determined mainly by the flow of the drug into the tissues and to a lesser degree by elimination. When a pharmacokinetic equilibrium is attained between blood and tissue compartments, the serum concentration of tobramycin becomes fully dependent upon elimination. The rapid elimination (β) phase is determined by renal function, and if renal function is normal, the $t_{½}$ of this phase is about two hours (25). During the slow elimination (γ) phase, which becomes visible in a curve about 24 hours after discontinuation of tobramycin therapy, the elimination is dependent upon the amount of tobramycin presented to the kidneys. This involves the slow release of very low concentrations of tobramycin from the deep tissues, i.e., the inner ear and the renal cortex (25). The $t_{½}$ of this phase is a few days (25). The total amount of tobramycin administered can be recovered

Table 2—*Linear Coefficients of Correlation of Pharmacokinetic Parameters of Tobramycin vs Age and Body Weight (N=15 Patients with CF)*

	$t_{1/2\alpha}$	$t_{1/2\beta}$	AUC/Dose	TBC	TBC/BSA	V_{Dss}	V_{Dss}/BSA
Age	−0.67	−0.80	−0.74	+0.71	−0.10	+0.56	−0.11
p	<0.0063	<0.00032	<0.0015	<0.0033	ns	<0.033	ns
Body weight	−0.81	−0.88	−0.76	+0.66	−0.28	+0.53	−0.20
p	<0.0003	<0.000016	<0.0011	<0.0085	ns	<0.043	ns

unchanged from the urine (25).

In the CF patients described in this study, the serum concentrations of tobramycin were followed up to eight hours after a dose. A fresh dose of tobramycin was then given, and the interrupted administration of ticarcillin was resumed. Since the measured serum concentrations of tobramycin reflect the distribution (α) phase and the rapid elimination (β) phase, the data were analyzed according to a two-compartment open model. Of the derived pharmacokinetic parameters of tobramycin, half-lives and AUC/Dose are highly age and body weight dependent. For $t_{1/2\beta}$, a similar observation is made in neonates (26,27) and in non-CF children (28). The $t_{1/2\beta}$ is influenced by both Vdss and TBC ($t_{1/2\beta} \approx$ Vdss/TBC). In our patients, Vdss, as well as TBC, are increasing with age and body weight. If increasing Vdss does not offset increasing TBC, $t_{1/2\beta}$ will decrease.

Pharmacokinetic parameters of tobramycin have been collected in several studies of young patients with and without CF (13,15,18,26-30). After comparison of data, it would appear that both TBC and Vdss are greater in patients with CF than in those without. Because tobramycin is excreted primarily by the kidneys and is neither metabolized nor significantly protein bound (31), a possible explanation for an increased TBC in CF could be an enhanced renal clearance. However, renal tobramycin handling of the same order was found in patients with CF and in control subjects (18). This suggests in CF alternative routes of excretion. Potential extrarenal clearance pathways are the respiratory and gastrointestinal tract. Possible explanations for an increased Vdss include inflammation and inflammation-related pathology (32).

Since the therapeutic index of tobramycin is small, efforts must be made to ensure safe and effective serum concentrations. With 3.0 to 3.3 mg tobramycin/kg of body weight given intravenously over 20 minutes, one-hour serum concentrations (Table 1) are reached that may be regarded as safe and adequate (33). On the other hand, between patients, considerable differences are found in the time after administration at which the serum concentration of tobramycin decreases below 1 mg/l. These findings have consequences for tobramycin therapy in CF, in particular for the dosing interval. The risk of drug retention is least if the serum

concentration of tobramycin prior to each dose (trough level) is <2 mg/l (34). In vitro, a concentration of 1 mg/l is able to inhibit the growth of between 50 and 90 percent of isolates of P.aeruginosa (35). It may be assumed that given this concentration in the serum, the concentration of tobramycin in the bronchial tree will be considerably lower (36,37). In order to prevent toxic side effects of tobramycin, and simultaneously to ensure sufficiently protracted serum concentrations which may be expected to be therapeutically effective, in our CF patients, the dosing interval is adjusted to a trough level of 1 mg/l. Two days after initiation of therapy (3.3 mg/kg of body weight every eight hours), serum concentrations are measured at two, and again at six, hours after a dose. The values obtained are plotted semilogarithmically vs time. A straight line between the two points cuts the 1 mg/l level at the desired dosing interval. This procedure postulates that the serum concentration-time curve for tobramycin between two and six hours after a dose takes a linear course in a semilogarithmic plot. Due to the later transition from distribution (α) phase to elimination (β) phase, this is not the case in the youngest patients. So, the dosing interval estimated will be too long. On the other hand, in these patients, elimination takes a less steep course, and consequently, the risk of cumulation must be considered greater. In our opinion, this justifies the above procedure for the youngest patients as well. Adjustment in the dosing interval led in a number of our CF patients to a daily dose up to 26.4 mg/kg of body weight, i.e., 3.3 mg/kg of body weight every three hours. Although we have observed no toxic side effects of tobramycin to date, regular determination of serum concentrations (twice a week), as well as renal and auditory functions (once a week), remain necessary to monitor cumulation and toxicity of tobramycin.

REFERENCES

1. Cystic Fibrosis Foundation. Pulmonary infection in cystic fibrosis. "GAP" Conference Report 1983;7:4.

2. Parry MF, Neu HC. Tobramycin and ticarcillin therapy for exacerbations of pulmonary disease in patients with cystic fibrosis. J Infect Dis 1976;**134**(suppl):194-99.
3. Høiby N, Friis B, Jensen K, Koch C, Møller NE, Støvring S, et al. Antimicrobial chemotherapy in cystic fibrosis patients. Acta Paediatr Scand 1982;**301**(suppl):75-100.
4. Jusko WJ, Mosovich LL, Gerbracht LM, Mattar ME, Yaffe SJ. Enhanced renal excretion of dicloxacillin in patients with cystic fibrosis. Pediatrics 1975;**56**:1038-44.
5. Yaffe SJ, Gerbracht LM, Mosovich LL, Mattar ME, Danish M, Jusko WJ. Pharmacokinetics of methicillin in patients with cystic fibrosis. J Infect Dis 1977;**135**:828-31.
6. Bergan T, Michalsen H. Pharmacokinetics of azlocillin in children with cystic fibrosis. Arzneim-Forsch/Drug Res 1979;**29**:1955-57.
7. Michalsen H, Bergan T. Effect of cystic fibrosis on the pharmacokinetics of cefsulodin. In: Current Chemotherapy and Immunotherapy: Proceedings of the 12th International Congress of Chemotherapy. Florence, 1981:529-530.
8. Leeder JS, Spino M, Isles AF, Tesoro AM, Gold R, MacLeod SM. Ceftazidime disposition in acute and stable cystic fibrosis. Clin Pharmacol Ther 1984;**36**:355-62.
9. Spino M, Chai RP, Isles AF, Thiessen JJ, Tesoro AM, Gold R, et al. Cloxacillin absorption and disposition in cystic fibrosis. J Pediatr 1984;**105**:829-35.
10. Setia U, Gross PA. Administration of tobramycin and gentamicin by the intravenous route every 6 hours in patients with normal renal function. J Infect Dis 1976;**134**(suppl):125-29.
11. Vogelstein B, Kowarski A, Lietman PS. The pharmacokinetics of amikacin in children. J Pediatr 1977;**91**:333-39.
12. Finkelstein E, Hall K. Aminoglycoside clearance in patients with cystic fibrosis.. J Pediatr 1979;**94**:163-64.

13. Rabin HR, Harley FL, Bryan LE, Elfring GL. Evaluation of a high-dose tobramycin and ticarcillin treatment protocol in Cystic Fibrosis based on improved susceptibility criteria and antibiotic pharmacokinetics. In: Perspectives in cystic fibrosis: Proceedings of the 8th International Cystic Fibrosis Congress, Toronto: 1980:370-75.
14. Michalsen H, Bergan T. Pharmacokinetics of netilmicin in children with and without cystic fibrosis. Antimicrob Agents Chemother 1981; **19**:1029-31.
15. Kelly HB, Menendez R, Fan L, Murphy S. Pharmacokinetics of tobramycin in cystic fibrosis. J Pediatr 1982;**100**:318-21.
16. Kearns GL, Hilman B, Wilson JT. Dosing implications of altered gentamicin disposition in patients with cystic fibrosis. J Pediatr 1982;**100**:312-18.
17. MacDonald NE, Anas NG, Peterson GP, Schwartz RH, Brooks JG, Powell KR. Renal clearance of gentamicin in cystic fibrosis. J Pediatr 1983; **103**:985-90.
18. Levy J, Smith AL, Koup JR, Williams-Warren J, Ramsey B. Disposition of tobramycin in patients with cystic fibrosis: a prospective controlled study. J Pediatr 1984;**105**:117-24.
19. Reeves DS. Accuracy of gentamicin assays. Postgrad Med J 1974; **50**(suppl):20-23.
20. Breimer DD, Honhoff C, Zilly W, Richter E, Rossum van JM. Pharmacokinetics of hexobarbital in man after intravenous infusion. J Pharmaco Biopharm 1975;**3**:1-11.
21. SAS User's Guide. Cary, USA; SAS Inst Inc, 1982: ISBN 0-917382-32-3.
22. Chan KKH, Gibaldi M. Estimation of statistical moments and steady-state volume of distribution for a drug given by intravenous infusion. J Pharmaco Biopharm 1982;**10**:551-58.
23. Snedecor GW, Cochran WG. Statistical methods, 7th ed. Ames, Iowa: The Iowa State University Press, 1980.
24. Kahlmeter G, Jonsson S, Kamme C. Multiple-compartment pharmacokinetics of tobramycin. J Antimicrob Chemother 1978;**4**(suppl):5-11.

25. Schentag JJ, Lasezkay G, Cumbo TJ, Plaut ME, Jusko WJ. Accumulation pharmacokinetics of tobramycin. Antimicrob Agents Chemother 1978; **13**:649-56.
26. Kaplan JM, McCracken GH, Thomas ML, Horton LJ, Davis N. Clinical pharmacology of tobramycin in newborns. Am J Dis Child 1973; **125**:656-60.
27. Nahata MC, Powell DA, Durrell DE, Miller MA, Glazer JP. Intrapatient variation in tobramycin kinetics in low birth weight infants during first postnatal week. Eur J Clin Pharmacol 1984;**26**:647-49.
28. Hoecker JL, Pickering LK, Swaney J, Kramer WG, Eys van J, Kohl S. Clinical pharmacology of tobramycin in children. J Infect Dis 1978; **137**:592-96.
29. Levy J, Baran D, Klastersky J. Comparative study of the antibacterial activity of amikacin and tobramycin during pulmonary infection in patients with cystic fibrosis. J Antimicrob Chemother 1982; **10**:227-34.
30. Bauer LA, Piecoro JJ, Wilson HD, Blouin RA. Gentamicin and tobramycin pharmacokinetics in patients with cystic fibrosis. Clin Pharm 1983; **2**:262-64.
31. Pechere JC, Dugal R. Clinical pharmacokinetics of aminoglycoside antibiotics. Clin Pharmacok 1979;**4**:170-99.
32. Alexander MR, Schoell J, Hicklin G, Kasik JE, Coleman D. Bronchial secretion concentrations of tobramycin. Am Rev Respir Dis 1982; **125**:208-09.
33. Kucers A, Bennett NMcK. Tobramycin. In: Kucers A, Bennett NMcK, eds. The use of antibiotics, 3rd ed. London: William Heinemann Medical Books Ltd, 1979:359-76.
34. Jackson GG. Aminoglycoside antibiotics: resistance and toxicity-a summary. Rev Infect Dis 1983:**5**(suppl):314-16.
35. Zak O. Antibiotics and Pseudomonas aeruginosa. In: Sabath LD, ed. Pseudomonas aeruginosa. Bern: Hans Huber Publishers, 1980:133-59.
36. McCrae WM, Raeburn JA, Hanson EJ. Tobramycin therapy of infections due to Pseudomonas aeruginosa in patients with cystic fibrosis: effect of dosage and concentration of antibiotic in sputum. J Infect Dis 1976;**134**(suppl):191-93.

37. Smith BR, Lefrock JL. Bronchial tree penetration of antibiotics. Chest 1983;**83**:904-08.

Chapter 7

TOBRAMYCIN IN PATIENTS WITH CYSTIC FIBROSIS: ADJUSTMENT IN DOSING INTERVAL FOR EFFECTIVE TREATMENT

Horrevorts AM[1], de Witte J[2], Degener JE[1], Dzoljic-Danilovic G[1], Hop WCJ[3], Driessen O[4], Michel MF[1] and Kerrebijn KF[2]

Departments of Clinical Microbiology and Antimicrobial Therapy[1] and Pulmonary Medicine in Children[2] (Sophia Children's Hospital), Medical Statistics[3], Erasmus University, Rotterdam; Department of Pharmacology[4], State University of Leiden, Leiden, The Netherlands.

Published in Chest 1987,**92**:844-848.

SUMMARY

The efficacy of the dosing regimen of tobramycin was investigated in 28 patients with cystic fibrosis (CF) who had an acute exacerbation of chronic pulmonary infection with Pseudomonas aeruginosa. The initial dose of tobramycin was 3.3 mg/kg of body weight three times daily (i.e., 10 mg/kg/day). A highly significant relationship was found between the serum concentration of tobramycin immediately before a dose and the change in the Forced Expiratory Volume in one second (FEV_1), both measured on the tenth day of treatment ($r_s=0.75; p<0.001$). In nine of the 16 patients who had a six-hour serum concentration of 1 mg/l or less on the tenth day of treatment, the eight-hour dosing interval of tobramycin was shortened to achieve a serum concentration of tobramycin of about 1 mg/l prior to the next dose. In the other seven patients, the dosage of tobramycin was not changed. On the 20th day, seven of the nine patients in whom the dosing interval was shortened exhibited an increase in FEV_1

of 20 percent or more. Such an increase was observed only in one of the seven patients in whom the dosing interval was not reduced ($p<0.05$). We conclude that individualizing the dosage of tobramycin in patients with CF results in a better clinical outcome.

INTRODUCTION

Thirty to 50 percent of the younger patients and 70 to 90 percent of the older patients with cystic fibrosis (CF) have a chronic pulmonary infection with P. aeruginosa (1). Acute exacerbations are commonly treated with an aminoglycoside in combination with an antipseudomonas ß-lactam antibiotic (2). The Total Body Clearance of a number of antimicrobial agents, including aminoglycoside and ß-lactam antibiotics, is found to be increased in patients with CF, as compared with patients without CF (3-5). In order to ensure serum concentrations which may be expected to be therapeutically effective for patients with CF, the agents prescribed have to be given more frequently or in a higher dosage than usual (or both) (6,7). The therapeutic index of aminoglycosides is small in view of the possible ototoxic and nephrotoxic effects. The abnormal pharmacokinetics and the narrow therapeutic index make great demands on adequate and safe treatment with aminoglycosides in patients with CF.
Within populations of patients with CF, a wide interpatient variation in Total Body Clearance of tobramycin has been observed (8,9). We investigated whether this variation determined the outcome of treatment.

PATIENTS AND METHODS

Studiepopulation

Seventy-three patients with CF were admitted 246 times to the Sophia Children's Hospital in Rotterdam, the Netherlands, between January 1982 and July 1985. The diagnosis of CF was based on increased sweat electrolyte levels ($Na^{+} \geq 60$

mmol/l) and characteristic pulmonary and gastrointestinal disease. Criteria for eligibility in this study were the following: acute pulmonary exacerbation due to P.aeruginosa (the presence of other pathogens in the sputum in addition to P.aeruginosa was grounds for exclusion); chemotherapy consisting of tobramycin and ticarcillin intravenously based on the susceptibility pattern of the strains isolated from samples of sputum taken just before and during admission (Minimum Inhibitory Concentration [MIC] of tobramycin, $\leq$2 mg/l; MIC of ticarcillin, $\leq$64 mg/l); age at which pulmonary function can be measured reliably ($\geq$6 years); and no accompanying conditions such as heart failure, pneumothorax, and hemoptysis. In 46 (involving 28 separate patients) of the 246 admissions, these criteria of selection were fulfilled. For each of the 28 patients, only the first course of treatment after Jan 1, 1982 was involved in the study.

Antimicrobial Therapy

Treatment with antibiotics was started on the day of admission. A dose of 3.3 mg of tobramycin per kilogram of body weight was given intravenously over 20 minutes every eight hours. Additionally, ticarcillin was administered in a dose of 600 mg per kilogram of body weight per 24 hours; 300 mg/kg was given in a continuous infusion and 75 mg/kg every six hours. The duration of antimicrobial therapy ranged from 19 to 26 days (mean, 21 days).

Concomitant Therapy

All patients received routine chest physiotherapy twice per day, including bronchial drainage, percussion, and coughing techniques, after the administration of a mucolytic aerosol (Mycomist, 20 mg in 4 ml of saline solution), to which 0.2 mg of albuterol (salbutamol) was added. The patients' diet was adjusted, and they received supplementary vitamins and pancreatic enzymes.

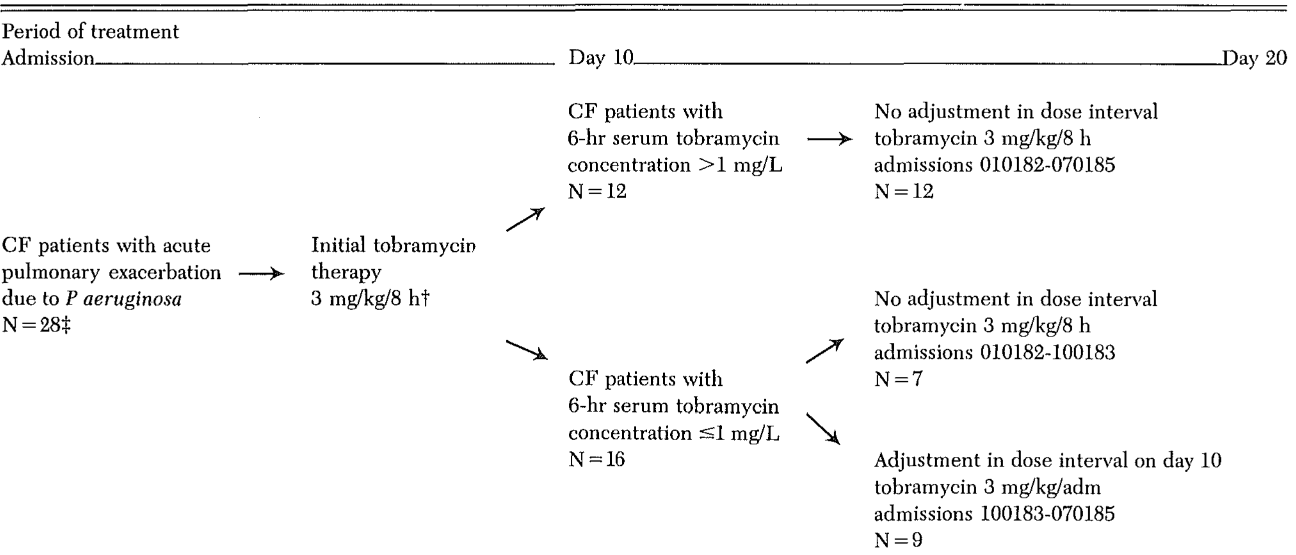
Serum Concentrations of Tobramycin

Serum concentrations were measured twice per week at one hour after the administration of a dose and prior to the administration of the next dose (predose serum tobramycin concentration). Concentrations were also measured two and six hours after administration on the 9th, 10th, or 11th day of treatment (referred to as the 10th day).
The concentrations of tobramycin in the samples of serum were determined with an enzyme-immunoassay. The lower limit of detection for this assay was 0.20 mg/l. The accuracy of the method was checked in advance with test sera containing a known concentration of tobramycin. The percentage of error of each result was calculated as follows: (true-reported/true) x 100. The accuracy (i.e., the mean percentage of error + 2 x standard deviation) was 9.6 percent, which can be graded as "good" by the criteria of Reeves and Bywater (10).

Adjustment in Dosing Interval of Tobramycin

An outline of the study is shown in Table 1. Adjustment in the dosing interval was dependent on the six-hour serum concentration of tobramycin measured on the tenth day of treatment. In patients in whom the six-hour serum concentration of tobramycin was more than 1 mg/l, initial dosage of tobramycin (i.e., 3,3 mg/kg every eight hours) was maintained. The dosage of tobramycin also was not altered in patients with a six-hour serum tobramycin concentration of 1 mg/l or less during admissions between Jan 1, 1982 and Oct 1, 1983; however, in such patients admitted between Oct 1, 1983 and July 1, 1985, on the tenth day of treatment, the dosing interval was shortened to achieve a predose serum tobramycin concentration of about 1 mg/l. This was done as follows (9): Serum concentrations at two and six hours after administration measured on the tenth day were plotted logarithmically against time. After a line was drawn through the two points, an estimate was made by extrapolation of how long after administration the concentration of tobramycin in the serum would reach the limit

Table 1—*Tobramycin in Patients with Cystic Fibrosis: Adjustment in Dosing Interval for Effective Treatment. Study Design**



Period of treatment

Admission		Day 10		Day 20
		CF patients with 6-hr serum tobramycin concentration >1 mg/L N = 12	→	No adjustment in dose interval tobramycin 3 mg/kg/8 h admissions 010182-070185 N = 12
CF patients with acute pulmonary exacerbation due to *P aeruginosa* N = 28‡ →	Initial tobramycin therapy 3 mg/kg/8 h† ↗ ↘			
		CF patients with 6-hr serum tobramycin concentration ≤1 mg/L N = 16	↗	No adjustment in dose interval tobramycin 3 mg/kg/8 h admissions 010182-100183 N = 7
			↘	Adjustment in dose interval on day 10 tobramycin 3 mg/kg/adm admissions 100183-070185 N = 9

*Study period: January 1st, 1982 till July 1st, 1985.
†Additionally, all patients received 600 mg/kg/day of ticarcillin.
‡Number of patients.

of 1 mg/l. This time was taken as the new dosing interval. The dose of tobramycin per administration was maintained at 3.3 mg/kg. Concomitant therapy and support of care were not altered during the whole period of study.

Pulmonary Function

The FEV_1 was taken as an indicator of the caliber of the airways. It was determined from a flow-volume measurement by integration of the maximal flows at the mouth. The highest value of three to five consecutive measurements was used. If the intrapatient variability of the consecutive measurements was 5 percent or less, FEV_1 was considered reliable. The FEV_1 was measured on admission and further twice per week during the time of treatment. Determination of FEV_1 was always within one hour after physiotherapy. The change in FEV_1 with respect to the FEV_1 measured on admission was calculated on the 9th, 10th, or 11th day of treatment (referred to as the tenth day). The change in FEV_1 after the 20th day with respect to the FEV_1 measured on the tenth day was determined on the 19th, 20th, or 21st day (referred to as the 20th day). In accordance with studies with bronchodilating or bronchoconstricting agents, a change in FEV_1 of 20 percent or more was considered significant (11,12).

Renal and Auditory Function

As a consequence of the narrow therapeutic index of tobramycin, creatinine clearance using the method of Schwartz et al (13) and auditory function were checked regularly. These were always within normal limits in the patients involved in the study.

Statistical Evaluation

The Mann-Whitney U-test was performed to detect statistically significant differences of variables between grouped populations. Percentages were

Table 2—*Efficacy of Tobramycin in Treating Acute Exacerbations of Chronic Pulmonary Infections due to* P aeruginosa *in 28 Patients with Cystic Fibrosis after Ten Days of Treatment**

Data	6-Hr Serum Concentration of Tobramycin†	
	≤1 mg/L	>1 mg/L
Patients' characteristics		
No. of patients (N = 28)	16 (9F + 7M)	12 (8F + 4M)
Age on admission, yr	14.8 ± 3.2	13.3 ± 4.4
Body weight on admission, kg	35.8 ± 10.0	35.4 ± 14.7
Height on admission, m	1.54 ± 0.15	1.51 ± 0.21
Creatinine clearance on admission, ml/min/1.73 sq m	124 ± 20	121 ± 21
Tobramycin serum concentrations		
1-hr on 10th day, mg/L	6.51 ± 0.64	6.86 ± 0.46
Predose on 10th day, mg/L	0.34 ± 0.11‡	0.89 ± 0.21‡
Pulmonary function		
FEV_1 on admission, percent of predicted	41.8 ± 15.4	42.5 ± 19.4
Increase in FEV_1 on 20th day, percent§	8.4 ± 8.2‡	42.5 ± 18.6‡

*Table values are means ± SD.
†Six hours after dose of 3.3 mg/kg of body weight measured on tenth day of treatment.
‡$p<0.001$ by Mann-Whitney U-test.
§Increase in FEV_1 is calculated as follows: (FEV_1 on 10th day − FEV_1 on admission)/(FEV_1 on admission) × 100 percent.

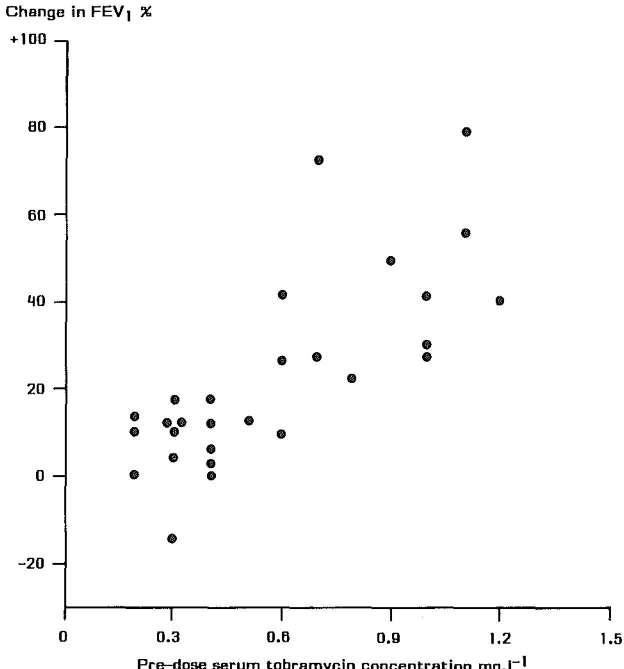


FIGURE 1. Correlation ($r_s = 0.75$; $p<0.001$) of predose serum tobramycin concentration vs change in FEV_1, both measured on tenth day of treatment, in 28 patients with cystic fibrosis. Change in FEV_1 is calculated as follows: (FEV_1 on tenth day − FEV_1 on admission)/(FEV_1 on admission) × 100 percent.

compared by Fisher's exact test. Coefficients of correlation given were Spearman's. A reference to all methods is Snedecor and Cochran (14).

RESULTS

Twenty-eight patients with CF (17 female and 11 male patients), ranging in age from 6.5 to 19.5 years, were involved in the study. The patients were divided into two groups according to their six-hour serum concentration of tobramycin on the tenth day of treatment (Table 2). In 16 patients, this concentration was 1 mg/l or less; in 12 patients, it was more than 1 mg/l. Both groups did not differ significantly in terms of sex, age, height, weight, creatinine clearance on admission, one-hour serum tobramycin concentration on the tenth day, and FEV_1 on admission. Highly significant differences were found in the predose serum tobramycin concentration ($p<0.001$) and the increase in FEV_1 ($p<0.001$), both on the tenth day. In the group of patients with a six-hour serum concentration of tobramycin greater than 1 mg/l, the mean predose serum tobramycin concentration and mean increase in FEV_1 were 0.89 ± 0.21 mg/l and 42.5 ± 18.6 percent, respectively, in contrast to 0.34 ± 0.11 mg/L and 8.4 ± 8.2 percent in the group of patients with a six-hour serum tobramycin concentration of less than 1 mg/l. The predose serum tobramycin concentration vs change in FEV_1, both on the tenth day, revealed a highly significant relationship (Fig 1;$r_s=0.75$;$p<0.001$). However, there was no such correlation between the one-hour serum concentration of tobramycin and the change in FEV_1, both on the tenth day was not found ($r_s=0.36$;$p>0.05$).

In nine of the 16 patients with a six-hour serum concentration of tobramycin of 1 mg/l or less on the tenth day, the dosing interval of tobramycin was shortened to achieve a predose serum tobramycin concentration of about 1 mg/l. The adjusted intervals for these patients varied between three and six hours. In the other seven patients a dosing interval of eight hours was maintained. The patients in whom the dosing interval was shortened compared to those in whom this was not done were similar in terms of sex, age, height, weight, creatinine clearance on

Table 3—*Efficacy of Tobramycin in Treating Acute Exacerbations of Chronic Pulmonary Infections due to* P aeruginosa *in 16 Patients with Cystic Fibrosis after 20 Days of Treatment**

Data	Adjustment in Dosing Interval on 10th Day of Treatment: No (8-hr Interval)	Yes (3-hr to 6-hr Interval)
Patients' characteristics		
No. of patients (N=16)	7 (4F+3M)	9 (5F+4M)
Age on admission, yr	14.6±4.1	15.1±2.6
Body weight on admission, kg	37.2±11.4	34.7±9.4
Height on admission, m	1.53±0.20	1.55±0.12
Creatinine clearance on 10th day, ml/min/1.73 sq m	124±22	122±20
Tobramycin serum concentrations, mg/L		
1-hr on 10th day	6.41±0.87	6.60±0.37
Predose on 10th day	0.31±0.07	0.37±0.13
1-hr on 20th day	7.03±1.03	6.63±0.97
Predose on 20th day	0.51±0.21†	1.34±0.46†
Pulmonary function		
FEV_1 on admission, percent of predicted	48.3±13.7	36.8±15.4
Increase in FEV_1 on 10th day, percent‡	10.6±5.9	6.6±9.6
Increase in FEV_1 on 20th day, percent§	8.1±10.3‖	34.0±26.9‖

*Table values are means±SD.
†p<0.001 by Mann-Whitney U-test.
‡Increase in FEV_1 is calculated as follows: (FEV_1 on 10th day − FEV_1 on admission)/(FEV_1 on admission) × 100 percent.
§Increase in FEV_1 is calculated as follows: (FEV_1 on 20th day − FEV_1 on 10th day)/(FEV_1 on 10th day) × 100 percent.
‖p<0.05 by Mann-Whitney U-test.

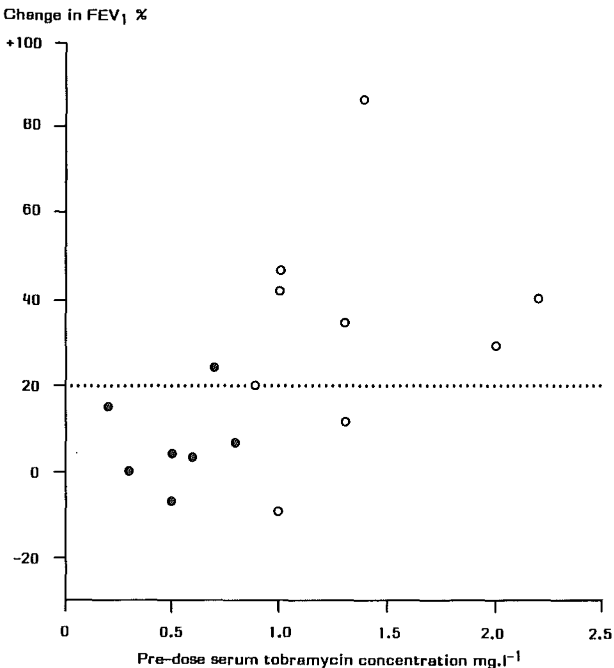


FIGURE 2. Correlation ($r_s=0.60$; $p=0.01$) of predose serum tobramycin concentration vs change in FEV_1, both measured on 20th day of treatment, in 16 patients with cystic fibrosis. Change in FEV_1 is calculated as follows: (FEV_1 on 20th day − FEV_1 on tenth day)/(FEV_1 on tenth day) × 100 percent. *Solid circles* indicate no adjustment in dosing interval on tenth day, *open circles* indicate adjustment in dosing interval on tenth day. Seven of nine patients in the adjusted group had increase in FEV_1 of 20 percent or more, compared to one of seven patients in unadjusted group ($p<0.05$; Fisher's exact test).

the tenth day, one-hour and predose serum tobramycin concentration on the tenth day, FEV_1 on admission, and increase in FEV_1 on the tenth day (Table 3). The mean increase in FEV_1 on the 20th day was significantly larger in the patients with a shortened dosing interval than in the patients with an unchanged dosing interval (34.0 ± 26.9 percent vs 8.1 ± 10.3 percent; $p < 0.05$). When the correlation of predose serum tobramycin concentration vs change in FEV_1, both on the 20th day, was performed, a significant relationship was found (Fig 2; $r_s = 0.60$; $p = 0.01$). A statistically significant correlation between one-hour serum tobramycin concentration and change in FEV_1, both on the 20th day, could not be demonstrated ($r_s = -0.31$; $p > 0.1$).

In seven of the nine patients in whom the dosing interval was adjusted, an increase in FEV_1 of 20 percent or more was observed. This was only the case in one of the seven patients in whom the dosing interval was kept at eight hours (Fig 2). This difference was statistically significant ($p < 0.05$).

DISCUSSION

In patients with CF, the pharmacokinetics of a number of drugs, including aminoglycoside and ß-lactam antibiotics, deviate form normal (15). Since patients with CF have a greater Total Body Clearance of aminoglycosides, the use of current dosing nomograms in these subjects may result in gross overdosing and especially underdosing (8,16). This study presents data which correlate predose serum tobramycin concentrations with clinical outcome in the routine treatment of acute pulmonary exacerbations due to P.aeruginosa in patients with CF. A significant direct relationship was found between predose serum tobramycin concentration and change in FEV_1. This indicates that the outcome of treatment with tobramycin in CF is highly dependent on the time during which effective serum and, hence, tissue concentrations are maintained. Our finding suggests a need for an altered dosing regimen in patients with low predose serum tobramycin concentrations and provides a simple means of improving the efficacy of antibiotic treatment in patients with cystic fibrosis. Adjustment can be either

achieved by increasing the dose per administration or by shortening the dosing interval. Increasing the dose implies a higher peak serum concentration of tobramycin (i.e., the serum concentration of tobramycin immediately after the infusion). In an animal model, a linearity was shown between concentrations of aminoglycosides in perilymph and the size of the doses injected (17). Division of doses in order to diminish high peak concentrations resulted in less ototoxicity (17). In order to prevent toxic side effects of tobramycin and in view of the achieved one-hour serum concentrations of tobramycin in our patients with CF (a one-hour serum tobramycin concentration between 6 and 8 mg/l may be regarded as safe and efficacious) (18), we decided not to increase the dose per administration but to shorten the dosing interval. The risk of drug retention is least if the serum concentration of tobramycin prior to each dose is less than 2 mg/l (19). A concentration of 1 mg/l is able to inhibit the in vitro growth of about 90 percent of the isolates of P. aeruginosa from patients with CF (20). It may be assumed that given this concentration in the serum, the bioactive concentration of tobramycin in the bronchial tree will be considerably lower (21). To avoid accumulation, as well as unnecessarily low serum concentrations for a considerable part of the dosing interval, we started to adjust the dosing regimen of tobramycin in patients with CF to a predose serum tobramycin concentration of approximately 1 mg/l. In this study, adjustment was carried out after ten days of treatment. Routinely, it will be more appropriate to have an evaluation of dosage after the fifth or sixth administration (steady-state phase). When pharmacokinetic data justify it, use of more frequent dosages should then be undertaken in order to rule out subtherapeutic serum concentrations of tobramycin as a cause for therapeutic failure.

The objective of antimicrobial treatment is to contain the infection. A reduction in infection results in a better patency of the airways and therefore leads to a better gas exchange (22). The FEV_1 is a reliable and reproducible measure of the caliber of the airways. In CF, the FEV_1 has proven to be an index that more closely reflects clinical improvement over a short period of time than other indices such as fever, white blood cell count, chest roentgenogram, or bacteriologic results

(23). This is in accordance with our own experience. The FEV_1 was therefore taken as an indicator for the effect of treatment. On the 20th day, seven of the nine patients who had been treated at shortened dosing intervals showed a significant increase in FEV_1 compared to the value measured on the tenth day. No such increase was observed for the other two patients. This could not be attributed to lower baseline pulmonary function or more severe disease state as assessed by the Schwachman scoring system (24).

Tobramycin was administered in combination with ticarcillin. The dosing regimen of ticarcillin was the same for all patients. The strains of P.aeruginosa isolated from the patients' samples of sputum were susceptible to both antibiotics (MIC of tobramycin, $\leq$2 mg/l; MIC of ticarcillin, $\leq$64 mg/l)(25). Initial susceptibility of the organisms to either antibiotic was not related to the improvement in FEV_1. In vitro interaction between tobramycin and ticarcillin against the strains was not investigated. The clinical relevance of in vitro interaction is still insufficiently defined (26). The reason for combined treatment was that resistance has been observed to emerge less often with combined therapy (27).

No side effects of tobramycin were observed in patients involved in the present study. Among other patients in whom the dosing interval was adjusted, accumulation of tobramycin was seen in one patient. Renal function improved after cessation of therapy. In a second patient, audiograms showed changes at high frequencies. This once again emphasizes the need to determine regularly the one-hour and trough serum concentrations of tobramycin (for instance, twice per week), as well as renal and auditory functions (for instance, once per week) in order to monitor accumulation and toxicity.

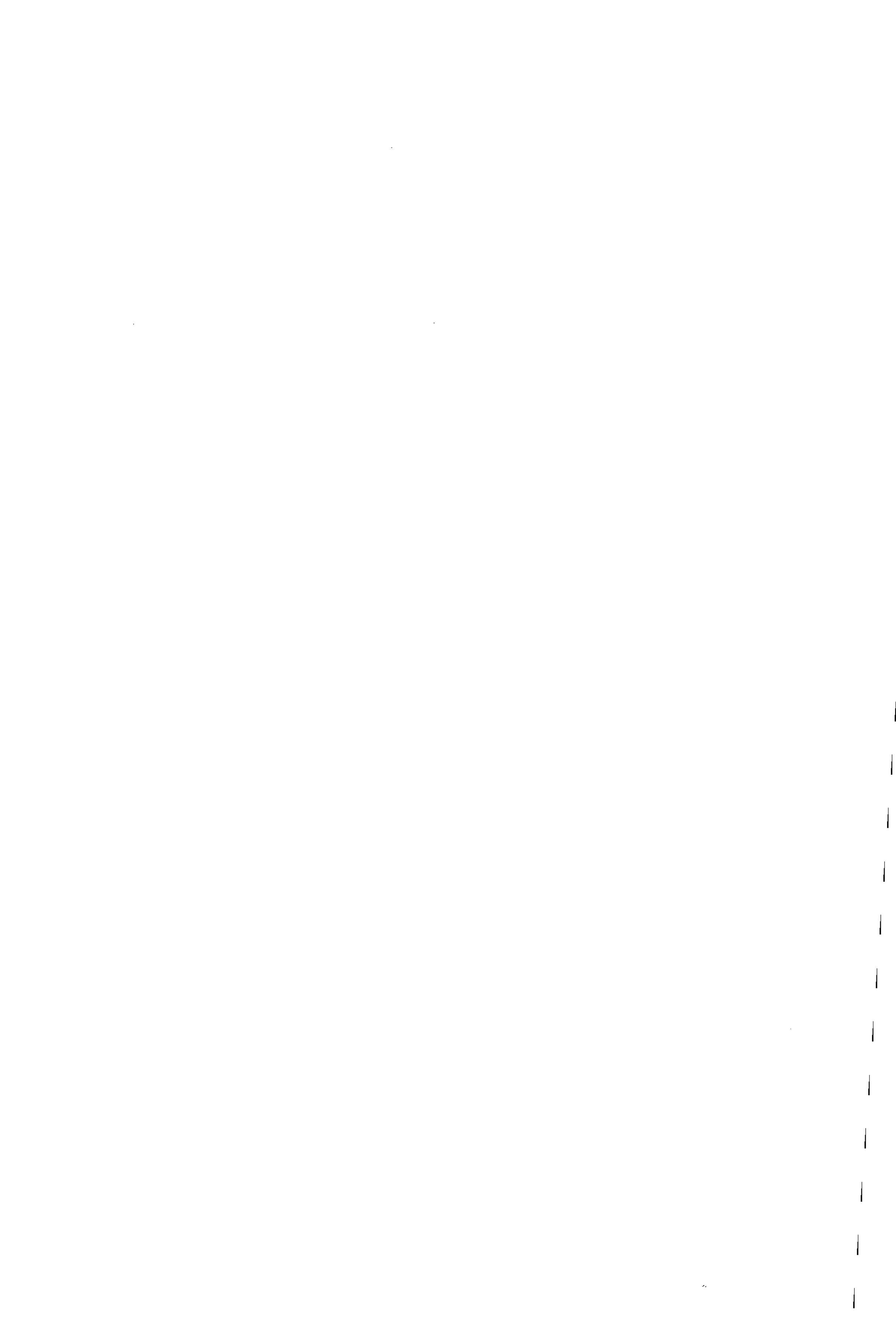
REFERENCES

1. Cystic Fibrosis Foundation. Pulmonary infection in cystic fibrosis. "GAP" Conference Rep 1983;7:4.
2. Davis PM, di Sant'Agnese PA. Diagnosis and treatment of cystic fibrosis. Chest 1984;**85**:802-09.

3. Levy J, Smith AL, Koup JR, Williams-Warren J, Ramsey B. Disposition of tobramycin in patients with cystic fibrosis: a prospective controlled study. J Pediatr 1984;**105**:117-24.
4. Leeder JS, Spino M, Isles AF, Tesoro AM, Gold R, MacLeod SM. Ceftazidime disposition in acute and stable cystic fibrosis. Clin Pharmacol Ther 1984;**36**:355-62.
5. Spino M, Chai RP, Isles AF, Thiessen JJ, Tesoro A, Gold R, et al. Cloxacillin absorption and disposition in cystic fibrosis. J Pediatr 1984;**105**:829-35.
6. Kelly HB, Menendez R, Fan L, Murphy S. Pharmacokinetics of tobramycin in cystic fibrosis. J Pediatr 1982;**100**:318-21.
7. Rabin HR, Harley FL, Bryan LE, Elfring GL. Evaluation of a high-dose tobramycin and ticarcillin treatment protocol in cystic fibrosis based on improved susceptibility criteria and antibiotic pharmacokinetics. In: Sturgess JM, ed. Perspectives in cystic fibrosis: proceedings of the 8th international cystic fibrosis congress. Mississauga, Ontario: Imperial Press, 1980:370-75.
8. Mann HJ, Canafax DM, Cipolle RJ, Daniels CE, Zaske DE, Warwick WJ. Increased dosage requirements of tobramycin and gentamicin for treating Pseudomonas pneumonia in patients with cystic fibrosis. Pediatr Pulmonol 1985;**1**:238-43.
9. Horrevorts AM, Degener JE, Dzoljic-Danilovic G, Michel MF, Kerrebijn KF, Driessen O, et al. Pharmacokinetics of tobramycin in patients with cystic fibrosis: implications for the dosing interval. Chest 1985;**88**:260-64.
10. Reeves DS, Bywater MJ. Quality control of serum gentamicin assays: experience of national surveys. J Antimicrob Chemother 1975;**1**:103-16.
11. Criteria for the assessment of reversibility in airways obstruction: report of the Committee on Emphysema, American College of Chest Physicians. Chest 1974;**65**:552-53.
12. Hargreave FE, Ryan G, Thomson NC, O'Byrne PM, Latimer K, Juniper EF, et al. Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. J Allergy Clin Immunol 1981;**68**:347-55.

13. Schwartz GJ, Haycock GB, Edelmann CM, Spitzer A. A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine. Pediatrics 1976;**58**:259-63.
14. Snedecor GW, Cochran WG. Statistical methods, 7th ed. Ames, Iowa: The Iowa State University Press, 1980.
15. Antibiotic dosage in cystic fibrosis (editorial). Lancet 1985; **1**:1020-21.
16. Kearns GL, Hilman BC, Wilson JT. Dosing implications of altered gentamicin disposition in patients with cystic fibrosis. J Pediatr 1982;**100**:312-18.
17. Federspil P, Schätzle W, Tiesler E. Pharmacokinetics and ototoxicity of gentamicin, tobramycin and amikacin. J Infect Dis 1976;**134**(suppl):200-05.
18. Moore RD, Smith CR, Lietman PS. Association of aminoglycoside plasma levels with therapeutic outcome in gram-negative pneumonia. Am J Med 1984;**77**:657-62.
19. Jackson GG. Aminoglycoside antibiotics: resistance and toxicity: a summary. Rev Infect Dis 1983;**5**(suppl):314-16.
20. Gordts B, Vandenborre C, VanderAuwera Ph, Butzler JP. Comparison between the in vitro activity of new agents on Pseudomonas aeruginosa isolates from cystic fibrosis patients and other chronic infections. J Antimicrob Chemother 1984;**14**:25-9.
21. Mendelman PM, Smith AL, Levy J, Weber A, Ramsey B, Davis RL. Aminoglycoside penetration, inactivation, and efficacy in cystic fibrosis sputum. Am Rev Respir Dis 1985;**132**:761-65.
22. Redding GJ. Restuccia R, Cotton EK, Brooks JG. Serial changes in pulmonary functions in children hospitalized with cystic fibrosis. Am Rev Respir Dis 1982;**126**:31-6.
23. Smith AL. Antibiotic therapy in cystic fibrosis: evaluation of clinical trials. J Pediatr 1986;**108**:866-70.
24. Schwachman H. Long term study of 105 patients with cystic fibrosis. Am J Dis Child 1958;**96**:6-15.
25. Barry AL, Thornsberry C. Susceptibility tests: diffusion procedures. In: Lenette

EH, Balows A, Hausler WJ, Shadomy H, eds. Manual of clinical microbiology, 4th ed. Washington, DC: American Society for Microbiology, 1985:978-79.
26. Yu PKW, Edson RS, Washington JA II, Hermans PE. Bactericidal and synergistic activity of moxalactam alone and in combination with gentamicin against Pseudomonas aeruginosa. Antimicrob Agents Chemother 1983;**23**:179-81.
27. Michalsen H, Bergan T. Azlocillin with and without an aminoglycoside against respiratory tract infections in children with cystic fibrosis. Scand J Infect Dis 1981;**29**(suppl):92-7.



Chapter 8

PHARMACOKINETICS OF ANTIMICROBIAL DRUGS IN CYSTIC FIBROSIS: AMINOGLYCOSIDE ANTIBIOTICS

Horrevorts AM[1], Driessen OMJ[2], Michel MF[1], Kerrebijn KF[3]

Department of Clinical Microbiology and Antimicrobial Therapy[1] and Pulmonary Medicine in Children[3] (Sophia Children's Hospital), Erasmus University, Rotterdam; Department of Pharmacology[2], State University of Leiden, Leiden, The Netherlands.

Published in Chest 1988,**94**:120S-125S.

SUMMARY

Patients with cystic fibrosis (CF) show abnormal aminoglycoside pharmacokinetics. After a conventional dose, the serum concentrations in CF patients are lower than those in non-CF patients. The lower serum concentrations in CF might be explained by increased total body clearance and/or a larger volume of distribution. The therapeutic range of aminoglycosides is narrow due to oto- and nephrotoxicity. The changed pharmacokinetics and the narrow therapeutic range make it difficult to ensure that patients with CF are adequately and safely treated with aminoglycosides. The mode of administration of aminoglycosides influences the antibacterial effect of these agents on Pseudomonas aeruginosa and the development of possible side effects. The therapeutic implications of these facts are discussed.

AMINOGLYCOSIDES

Structure

Aminoglycosides (1,2) consist of two or more aminosugars which, via a glycosidal bond, are bound to a central hexose or aminocyclitol molecule. Aminoglycosides are natural products isolated from cultures of Micromonospora and Streptomyces. Several derivatives have been synthesized from these products in order to enhance the antimicrobial range, reduce the toxicity and give the compounds better protection from enzymes able to inactivate aminoglycosides.

Antimicrobial activity

The antimicrobial activity of aminoglycosides (2) is based on the inhibition, on the ribosomal level, of bacterial protein synthesis. Aminoglycosides are bactericidal, their range of activity generally encompassing aerobic Gram-negative rods, staphylococci and some mycobacteria. Different aminoglycosides can differ in antimicrobial activity. In vitro studies have shown that tobramycin is more effective against P. aeruginosa than, in decreasing order, amikacin, gentamicin and netilmicin (2-4). The bactericidal effect of aminoglycosides on P. aeruginosa is greatly concentration-dependent (5,6): the higher the concentration, the quicker and more efficient the bacterial population is killed. In this respect, aminoglycosides differ from the ß-lactam antibiotics in that the bactericidal effect is related to the duration of the presence of an effective concentration (>MIC). Another difference between aminoglycosides and ß-lactam antibiotics used against P. aeruginosa is the so-called Post Antibiotic Effect (PAE) (5). After exposure of a P. aeruginosa population to an aminoglycoside during a given time, growth is not immediately resumed after discontinuation of the agent; it remains suppressed for some time. The duration of this continued suppression depends on the aminoglycoside concentration and the duration of the exposure. ß-Lactam antibiotics except imipenem do not show any PAE when used against P.

aeruginosa. Aminoglycoside concentrations lower than the Minimum Inhibitory Concentration (sub-MICs) may also have a morphological and quantitative effect on a bacterial population (5). P. aeruginosa growth has been demonstrated to be delayed by sub-MICs of aminoglycosides.

Aminoglycosides activity can be influenced by exogenous factors. The antimicrobial activity of aminoglycosides is diminished, for instance, in the presence of sputum (7-9). Moreover, inactive amide compounds can be formed with penicillins aimed against P. aeruginosa (10). The extent to which this happens differs from one aminoglycoside to the other (11). Tobramycin is inactivated more markedly than, in decreasing order, gentamicin, netilmicin or amikacin. In terms of laboratory technique, this means that an aminoglycoside concentration in samples from patients also receiving a penicillin should be analyzed soon after sampling (1-2 hours). If this is not feasible, then the sample should be stored at -70°C until analysis (11). The formation of amide compounds is believed to be of little clinical significance because the time required to form the compound exceeds the half-life time ($t_{½}$) of the individual agents (12). This would be of importance in the case of disturbed renal function, with increased half-life times.

Pharmacological properties

Aminoglycosides (1,13) are stable, weakly basic, water-soluble compounds. They are not absorbed from the intestines and can therefore only be given parenterally. After administration, they are distributed over the extracellular compartments. In the human body, aminoglycosides are not metabolized, show hardly any protein binding, and are excreted by the kidneys by glomerular filtration. A small percentage of the filtrated fraction is reabsorbed by the proximal tubules of the kidney. In healthy individuals, the creatinine clearance, therefore, exceeds that of the aminoglycoside.

Toxicity

The major side effects of aminoglycosides involve the kidney and the ear (14,15). Toxicity is based on accumulation. In the kidney, a fraction of the aminoglycoside dose in the ultrafiltrate is taken up by the proximal renal tubules via carrier-mediated pinocytosis (14,16,17). This implies that the actual pinocytosis is preceded by the binding of the aminoglycoside to a carrier: acid phospholipids localized in the brush border. This binding is a low-affinity large-capacity system. Since this system is saturable, low concentrations in the ultrafiltrate are reabsorbed more effectively than high concentrations. This means that of an aminoglycoside dose given by bolus injection, less is stored in the renal cortex than would be stored if the same dose was administered over the dose interval by continuous infusion. The mode of administration may also influence the accumulation in the labyrinth (18). The nephrotoxic effect is manifested by proteinuria, reduced glomerular filtration, and excretion of certain tubular enzymes (14). Renal function usually shows gradual recovery after discontinuation of therapy. Cochlear ototoxicity (15) is manifested by permanent degeneration of hair cells in the organ of Corti, starting in the high-pitch region of the basilar membrane. As a result of accumulation in the labyrinth, the degenerative process may continue for considerable length of time after stopping therapy. Vestibular ototoxicity (15) also occurs, but the patient is able to compensate for this disturbance so that vestibular lesions are less serious than cochlear damage.
The individual agents are thought to differ in toxicity (15,19,20). Netilmicin is believed to be the least ototoxic agent. It has been established empirically that the risk of toxic effects increases when (as a consequence of reduced renal function) the trough concentration starts to exceed a certain threshold value (21). The maximum trough value for gentamicin and tobramycin is believed to be 2 mg/l, for netilmicin 3 mg/l, and for amikacin 5 mg/l.

Pharmacokinetics

The pharmacokinetic behavior of aminoglycosides is best described on the basis of a 3-compartment model (22). The serum concentration-time curve is characterized by a phase of distribution (α) and a fast (β) as well as a slow (γ) phase of elimination. During the phase of distribution, the serum aminoglycoside concentration is determined largely by its flow into the tissues, and to a lesser extent by its renal excretion. Once a balance between blood and tissues is attained, the course of the serum aminoglycoside concentration is largely determined by renal function. Given normal renal function, the half-life of the fast phase of elimination is about 2 hours (23). During the slow phase of elimination, which manifests itself in a concentration-time curve about 24 hours after discontinuation of therapy, the excretion depends on the amount of aminoglycoside supplied to the kidneys. This involves the slow release of very low concentrations from the so-called deep compartments, including the labyrinth and the renal cortex. The half-life of the γ-phase is a few days (23).

Reports vary on the amount of aminoglycoside which penetrates into the bronchial secretions: the values found range from 10 to 70% of the serum concentration (13,24).

AMINOGLYCOSIDES AND CYSTIC FIBROSIS

Introduction

Thirty to 50% of the younger and 70-90% of the older patients with cystic fibrosis (CF) have a chronic pulmonary infection caused by P. aeruginosa (25). Acute exacerbations are commonly treated with an aminoglycoside in combination with an anti-pseudomonas ß-lactam antibiotic (26).

There are indications that the pharmacokinetics of some of these agents deviate from normal in CF (27). The drugs in question have to be given more frequently or in a higher than usual dose in order to ensure sufficiently protracted active

serum concentrations. The lower serum concentrations following a conventional dose could be explained, according to pharmacokinetic studies of aminoglycosides in CF patients, by an increased Total Body Clearance (TBC) and/or a larger Volume of distribution (Vd) (27). Most studies report an increased TBC of aminoglycosides in CF patients (Tables 1 and 2). Reports of an increased Vd in CF patients are less consistent (Tables 1 and 2). This may be due, on the one hand, to the fact that the Vd generally shows a greater interpatient variability (41), and, on the other hand, to the fact that the Vd value found depends on the state in which it is measured (non-steady state vs steady state) (42). The definition of the Vd also proves to have an influence. Levy et al (36) and Vogelstein et al (39) found increased Vd of tobramycin and amikacin, respectively, if it was expressed in l/kg. When calculated using body surface area, the Vd in CF patients was not significantly different from that in nonCF patients. This may have been due to an increased extracellular volume/body weight ratio in CF (whereas the extracellular volume/body surface area ratio is unchanged) (24). With regard to Table 1, the investigators compared their CF data with nonCF data from other studies. Consequently, differences in Vd (and TBC) may have resulted from differences in techniques used.

Total Body Clearance

The TBC can be calculated independent of the model by dividing the dose by the Area Under the concentration-time Curve (AUC) (43). The proportion of the dose which enters the system after intravenous administration can be estimated as virtually 100%. The accuracy with which the AUC can be determined increases with an increasing number of samples (blood samples in which the concentration is measured). In nonCF patients, 100% of a total dose of aminoglycoside administered is excreted in the urine, and release from the deep compartments continues for some considerable time after discontinuation of therapy (23). An increased TBC of aminoglycosides in CF patients could be the result of increased renal excretion due to increased filtration and/or diminished tubular reabsorption.

Table 1—*Reported Alterations in Aminoglycoside Pharmacokinetics in CF from Studies without Control Groups*

Drug	TBC*	Vd†	References
Gentamicin	↑‡	↑	Bauer et al[28]
	nd§	nd	MacDonald et al[29]
Tobramycin	↑	↑	Bauer et al[28]
	↑	↑	Horrevorts et al[30]
	↑	↑	Kelly et al[31]
Netilmicin	↑	↑	Bosso et al[32]

*Total body clearance
†Volume of distribution
‡Increased in CF
§Not different

Table 2—*Reported Alterations in Aminoglycoside Pharmacokinetics in CF from Studies with Control Groups*

	TBC*				Vd§		
Drug	ml/min/kg	ml/min/BSA#	RC/TBC†	t½‡	L/kg	L/BSA	References
Gentamicin	ND#	ND	. . .	ND	ND	ND	Hendeless et al¶[33]
	↑‖	↑	. . .	ND	↑	↑	Kearns et al[34]
	↑	. . .	. . .	↓**	ND	. . .	Mann et al[35]
Tobramycin	. . .	↑	ND	ND	↑	ND	Levy et al[36]
	↑	. . .	. . .	↓	ND	. . .	Mann et al[35]
Netilmicin	. . .	ND	. . .	↓	ND	ND	Michalsen et al[37]
Amikacin	ND	. . .	. . .	ND	ND	. . .	Autret et al[38]
	↑	↑	ND	ND	↑	ND	Vogelstein et al[39]
Sisomicin	↑	. . .	. . .	↓	. . .	ND	Marks et al[40]

*Total body clearance
†Ratio renal clearance-total body clearance
‡Half-life
§Volume of distribution
#Body surface area
ND = not different
. . . = not available
¶Stated in text, values for TBC and Vd not given
‖Increased in CF
**Decreased in CF

Levy et al (36) found an increased TBC of tobramycin in CF patients compared with controls, but this was not associated with increased renal clearance. This suggested that, in CF, there must be other than renal pathways for clearance of tobramycin (in bile or sputum). Metabolization of tobramycin in CF was also suggested. It should be borne in mind, however, that Levy et al (36) made their measurements after the third dose a situation in which a steady state of equilibrium is not yet attained. Unsaturated tissues attract a larger proportion of an aminoglycoside dose than saturated tissues. This implies that, as the tissues become more saturated, an increasing proportion of a dose is cleared in the urine per unit of time (42). The larger TBC in CF patients in the study of Levy et al (36) might therefore also be explained by differences in tissue saturation between CF patients and nonCF patients. Unlike Levy et al (36) with tobramycin, Vogelstein et al (39) found an increased renal clearance and a larger TBC of amikacin in CF patients (after the fourth dose). Michalsen et al (37) were unable to demonstrate differences in netilmicin renal clearance and TBC between CF patients and nonCF patients (after a single dose). The suggestion of Levy et al (36) that extrarenal clearance pathways could be responsible for the larger TBC of tobramycin in CF, was regarded as unlikely for gentamicin by MacDonald et al (29). They found more than 80% of the gentamicin administered in the urine within 4 hours after infusion of a single dose. We made a similar observation for tobramycin (Table 3). Renal function measured from glomerular filtration and tubular excretion has been thoroughly studied in CF and found not to differ from that in control subjects (44). The tubular resorption to which aminoglycosides are subject has not been adequately investigated so far. Should this be diminished in CF, then this implies that less of the drug is stored in the proximal tubules of the kidneys. An indication of this can be found in a study reported by Rabin et al (45) in which they found a shorter $t_{½\gamma}$ for tobramycin (63 hours in CF, vs 146 hours in nonCF patients), which suggests an altered exchange of aminoglycosides between blood and tissues in these patients.

Possible extrarenal clearance of an aminoglycoside in CF can be investigated simply by collecting all samples of urine during and for some time after therapy,

Table 3—*Urinary Elimination of a Single Dose of Tobramycin (2 mg/kg) in Patients with CF*

Patients N	Percentage of dose recovered within 3 to 6 hrs after infusion
1	78
2	90
3	79
4	80
5	85
6	77
7	85
8	78

Table 4—*Relationship of Aminoglycoside Pharmacokinetic Parameters vs Age, Body Weight and Severity of Disease in CF*

	Patients N	TBC*	Renal Clearance	Vd†	Reference
Gentamicin					
Age/BW	7	+‡	+	−‡	MacDonald et al[29]
	12	−	−	. . .	Mann et al[35]
Severity of disease	8	+	±∂	−	MacDonald et al[29]
Tobramycin					
Age/BW	15	+	. . .	+	Horrevorts et al[30]
	52	+	. . .	+	Hsu et al[47]
	11	−	. . .	−	MacDonald et al[49]
	17	−	. . .	−	Mann et al[35]
Severity of	15	−	. . .	−	Horrevorts et al[30]
disease	11	+	. . .	+	MacDonald et al[49]

*Total body clearance

†Volume of distribution

‡+:correlation; ±:weak correlation; −:no correlation

. . .Not available

and measuring the total recovery of the aminoglycoside in the urine. If there is extrarenal clearance, then this should be clearly less than complete.

Volume of Distribution

The Vd is the volume attained at a steady state condition. Some of the differences in Vd between different studies must be due to the fact that most were performed in a nonsteady state pharmacokinetics. In this state, the tissue compartments are either not or only partially loaded, and in any case studies differ in the degree of loading. With regard to aminoglycosides, a system is not completely loaded until therapy has been given for about 5 times the $t_{½}$ (43). Since the $t_{½}$ of aminoglycosides is several days, this means many days of therapy. Comparative pharmacokinetic steady state studies offer the only chance to discover whether the Vd of aminoglycosides in CF patients does indeed exceed that in control subjects. However, if in CF there is clearance of aminoglycosides which bypasses the central compartment (in sputum, for instance), then a pharmacokinetic balance could not be attained and the Vd at steady state of equilibrium could not be measured (46).

$T_{½}$ Elimination

The $t_{½}$ ($=t_{½\beta}$) elimination is a model-dependent parameter influenced by both the TBC and the Vd ($t_{½}$ elimination$\approx$Vd/TBC) (43). As pointed out, the pharmacokinetics of aminoglycosides can best be described on the basis of a 3-compartment model. With a 1-compartment model the $t_{½}$ elimination can be a mixture of distribution and elimination, and with a 2-compartment model it can be a mixture of elimination and return from the deep compartments. In the former, the value found for $t_{½}$ elimination can be too small, and in the latter, it can be too large. The method used, therefore, can influence the half-life. Since $t_{½}$ elimination$\approx$Vd/TBC, in studies disclosing both larger Vd and larger TBC values for CF, in reality the $t_{½}$ will not differ much from that of controls (Table 2). In studies in which the TBC is

increased but not the Vd, however, the $t_{½}$ elimination found in CF may be smaller (Table 2).

Interpatient Variability

The pharmacokinetic parameters of aminoglycosides would seem to show greater variability in CF patients than in nonCF patients. This may be due to inter-patient differences in age and in severity of disease. A number of studies have sought to find a correlation between TBC on the one hand, and age and severity of disease on the other. A summary of findings is presented in Table 4. Unlike Horrevorts et al(30), Hsu et al(47), and MacDonald et al (29), Mann et al (35), were unable to demonstrate a correlation between TBC and age; in their study, however, interpatient variability in the control group was at least as marked as in the CF group. Age dependence of the TBC of tobramycin in children with CF was found also in children without CF (Hoecker et al) (48). In the study of Hoecker et al (48) -as in those of Horrevorts et al (39), Hsu et al (47) and MacDonald et al (29)- the TBC of the aminoglycoside studied increased with increasing age. MacDonald et al (29) were the only investigators to suggest a correlation between de TBC of gentamicin and the severity of the disease (NHI score).
Correlations between Vd on the one hand and age or severity of disease on the other, are far less evident (29,49).

THERAPEUTIC IMPLICATIONS

Introduction

The preceding sections have discussed the antimicrobial action of aminoglycosides against P. aeruginosa, the side effects of these agents and their deviant pharmacokinetics in patients with CF. The implications of these findings with regard to adequate and safe aminoglycoside therapy for CF patients will now discussed focussing on the aminoglycoside tobramycin.

Points of importance in the treatment of infections include: a) the susceptibility of the microorganism to the antibiotic used; b) the concentration of the agent that can be attained at the site of infection. In our CF population, the MIC_{50} and MIC_{90} (Minimum Inhibitory Concentrations required to inhibit the growth of 50 and 90% respectively of the total number of strains tested) of tobramycin against P. aeruginosa are 1 mg/l and 2 mg/l respectively (50). In a previous study, we have investigated the pharmacokinetic behavior of tobramycin in 15 patients with CF; we followed the course of the serum tobramycin concentration after administration by iv infusion of 3.3 mg/kg over 8 hours (51). The study was performed after the patients had already received more than 25 such doses. The concentrations measured in the serum samples obtained immediately after the infusion (t=20 min) and 60 min, 4 hours and 8 hours after the start of the infusion, were (mean $\pm$ SD): 11.6$\pm$3.3 mg/l, 5.4$\pm$1.0 mg/l, 1.4$\pm$0,6 mg/l, and 0.5$\pm$0.2 mg/l. The serum tobramycin concentrations measured 20 and 60 minutes after the start of the infusion exceeded the MIC_{90} of tobramycin against the CF Pseudomonas isolates in all patients. Four hours after the start of the infusion a number of patients showed serum tobramycin concentrations lower than the MIC_{50}. This means that in some patients the serum tobramycin concentration is below the MIC of the aminoglycoside against CF Pseudomonas isolates during a large part of the dose interval. Bioactive tobramycin concentrations at the site of infection may be assumed to be lower than serum concentrations (9). Since the bactericidal activity of tobramycin against P. aeruginosa is highly concentration-dependent, intermittent administration is preferable to administration by continuous infusion. Following intermittent administration, serum concentrations are high for a short time after administration. Studies in CF patients have shown that the serum tobramycin concentration 1 hour after administration correlated with clinical and bacteriologic response (42,45). Attempts should be made to ensure 1-hour concentrations between 6 mg/l and 12 mg/l (53). Also with regard to side effects, intermittent administration is preferred above continuous infusion. It has been established empirically that trough concentrations of tobramycin exceeding 2 mg/l increase the risk of toxic side effects. This value gives an

indication of the length of the dose interval. This should be sufficiently long to ensure that the concentration decreases to below 2 mg/l after a dose. On the other hand, dose intervals during which the concentration is below 2 mg/l for a proportionally long time should be avoided. Low concentrations contribute little to effectiveness, but they do increase the risk of accumulation and therefore of toxicity. An earlier study has shown that a reduction of the dose interval based on a trough concentration of about 1 mg/l produced a better clinical response than a dose interval in which the concentration was below 1 mg/l for a considerable time. This may be due to the fact that by giving more frequent administrations, the benefit of high concentrations immediately after administration is enjoyed several times. Another explanation might be that during prolonged low concentrations, growth of the microorganisms nevertheless resumes. This can therefore be reduced by shortening the dose interval.

Treatment

In view of the marked interpatient variability, the dosage and the frequency of administration should be adjusted individually. Dosage should aim at 1-hour concentrations between 6 mg/l and 12 mg/l. Determination of the dose interval should aim at trough concentrations below 2 mg/l. The minimum trough concentration adhered to at the Sophia Children's Hospital is 1 mg/l. In view of the narrow therapeutic range of tobramycin, 1-hour and trough concentrations should be measured regularly, as should auditory and renal functions. The acute and chronic toxicity of repeated-dose tobramycin treatment in patients with cystic fibrosis seems to be very mild (54,55).

REFERENCES

1. Nagabhushan TL, Miller GH, Weinstein MJ. Structure-activity relationships in aminoglycoside-aminocyclitol antibiotics. In: Whelton A, Neu HC (eds). The Aminoglycosides; Microbiology, Clinical Use, and Toxicology. New York: Marcel Dekker, 1982:3-27.
2. Moellering RC. In vitro antibacterial activity of the aminoglycoside antibiotics. Rev Infect Dis 1983;**5**:S212-S32.
3. Scribner RK, Marks MJ, Weber AH, Tarpay MM, Welch DF. Activities of various ß-lactams and aminoglycosides, alone and in combination, against isolates of Pseudomonas aeruginosa from patients with cystic fibrosis. Antimicrob Agents Chemother 1982;**21**:939-43.
4. Moellering RC. Clinical Microbiology and the in vitro activity of aminoglycosides. In: Whelton A, Neu HC (eds). The Aminoglycosides; Microbiology, Clinical Use, and Toxicology. New York, Marcel Dekker, 1982:65-95.
5. Vogelman B, Craig WA. Kinetics of antimicrobial activity. J Pediatr 1986;**108**:835-40.
6. Gerber AU, Brugger HP, Feller C, Stritzko T, Stalder B. Antibiotic therapy of infections due to Pseudomonas aeruginosa in normal and granulocytopenic mice: comparison of murine and human pharmacokinetics. J Infect Dis 1986;**153**:90-7.
7. Bodem CR, Lampton LM, Miller DP, Tarka EF, Everett ED. Endobronchial pH. Relevance to aminoglycoside activity in Gram-negative bacillary pneumonia. Am Rev Resp Dis 1983;**127**:39-41.
8. Levy J, Smith AL, Kenny MA, Ramsey B, Schoenknecht FD. Bioactivity of gentamicin in purulent sputum from patients with2 cystic fibrosis or bronchiectasis: comparison with activity in serum. J Infect Dis 1983;**148**:1069-76.

9. Mendelman PM, Smith AL, Levy J, Weber A, Ramsey B, Davis RL. Aminoglycoside penetration, inactivation, and efficacy in cystic fibrosis sputum. Am Rev Resp Dis 1985;**132**:761-65.
10. Falkowski AJ, Creger RJ. Hydroxylamine technique for in vitro prevention of penicillin inactivation of tobramycine. Antimicrob Agents Chemother 1984;**26**:643-46.
11. Pickering L, Rutherford I. Effect of concentration and time upon inactivation of tobramycine, gentamicin, netilmicin and amikacin by azlocillin, carbenicillin, mecillinam, mezlocillin and piperacillin. J Pharmacol Exp Ther 1980;**217**:345-49.
12. Riff LJ, Jackson GG. Laboratory and clinical conditions for gentamicin inactivation by carbenicillin. Arch Inter Med 1972;**13**:887-91.
13. Pechere JC, Dugal R. Clinical pharmacokinetics of aminoglycoside antibiotics. Clin Pharm 1979;**4**:170-99.
14. Schentag JJ. Aminoglycoside pharmacokinetics as a guide to therapy and toxicilogy. In: Whelton E, Neu HC (eds). The Aminoglycosides; Microbiology, Clinical Use, and Toxicology. New York: Marcel Dekker, 1982:146-67.
15. Bendush CL. Ototoxicity: clinical considerations and comparative information. In: Whelton E, Neu HC (eds). The Aminoglycosides; Microbiology, Clinical Use, and Toxicology. New York: Marcel Dekker, 1982:453-86.
16. Guiliano RA, Verpooten GA, Verbist L, Wedeen RP, De Broe ME. In vivo uptake kinetics of aminoglycosides in the kidney cortex of rats. J Pharm Exp Ther 1986;**236**:470-75.
17. Guiliano RA, Pollet DE, Verpooten GA, Scharpé S, Paulus GJ, De Broe ME. Influence of dose regimen on renal accumulation of aminoglycosides. Arch Int Pharmacodyn 1982;**260**:277-79.
18. Federspil P, Schätzle W, Tiesler E. Pharmacokinetics and ototoxicity of gentamicin, tobramycin and amikacin. J Infect Dis 1976;**134**:S200-S05.
19. Kahlmeter G, Dahlager JI. Aminoglycoside toxicity; a review of clinical studies published between 1975 and 1982. J Antimicrob Chemother 1984;**13**(suppl):9-22.

20. Lerner AM, Cone LA, Jansen W, Reyes MP, Blair DC, Wright GE, Lorber RR. Randomised, controlled trial of the comparative efficacy, auditory toxicity, and nephrotoxicity of tobramycin and netilmicin. The Lancet 1983;1:1123-25.
21. Jackson GG. Aminoglycoside antibiotics: resistance and toxicity-a summary. Rev Infect Dis 1983;**5**:S314-S16.
22. Kahlmeter G, Jonsson S, Kamme C. Multiple-compartment pharmacokinetics of tobramycin. J Antimicrob Chemother 1978;**4**(suppl):5-11.
23. Schentag JJ, Lasezkay G, Cumbo TJ, Plaut ME, Jusko WJ. Accumulation pharmacokinetics of tobramycin. Antimicrob Agents Chemother 1978; **13**:649-56.
24. Kearns GL, Trang JM. Introduction to pharmacokinetics: Aminoglycosides in cystic fibrosis as a prototype. J Pediatr 1986;**108**:847-53.
25. cystic fibrosis Foundation. Pulmonary infection in cystic fibrosis. "GAP" Conference Rep 1983;**7**:4.
26. Davis PB, Di Sant' Agnese PA. Diagnosis and treatment of cystic fibrosis. Chest 1984;**85**:802-09.
27. De Groot R, Smith AL. Antibiotic pharmacokinetics in cystic fibrosis; differences and clinical significance. Clin Pharm 1987;**13**:228-53.
28. Bauer LA, Piecoro JJ, Wilson HD, Blouin R. Gentamicin and tobramycin pharmacokinetics in patients with cystic fibrosis. Clin Pharm 1983;**2:** 262-64.
29. MacDonald NE, Anas NG, Peterson RG, Schwartz RH, Brooks JG, Powell KR. Renal clearance of gentamicin in cystic fibrosis. J Pediatr 1983;**103**:985-90.
30. Horrevorts AM, Degener JE, Dzoljic-Danilovic G, Michel MF, Kerrebijn KF, Driessen O, Hermans J. Pharmacokinetics of tobramycin in patients with cystic fibrosis. Chest 1985;**88**:260-64.
31. Kelly HB, Menendez R, Fan L, Murphy S. Pharmacokinetics of tobramycin in cystic fibrosis. J Pediatr 1982;**100**:318-21.
32. Bosso JA, Townsend PL, Herbst JJ, Matsen JM. Pharmacokinetics and dosage requirements of netilmicin in cystic fibrosis patients. Antimicrob Agents Chemother 1985;**28**:829-31.

33. Hendeles L, Iafrate RP, Stillwell PC, Mangos JA. Individualizing gentamicin dosage in patients with cystic fibrosis: limitations to pharmacokinetic approach. J Pediatr 1987;**110**:303-10.
34. Kearns GL, Hilman BC, Wilson JT. Dosing implications of altered gentamicin disposition in patients with cystic fibrosis. J Pediatr 1982;**100**:312-18.
35. Mann HJ, Canafax DM, Cipolle RJ, Daniels CE, Zaske DE, Warwick WJ. Increased dosage requirements of tobramycin and gentamicin for treating Pseudomonas pneumonia in patients with cystic fibrosis. Pediatr Pulmonol 1985;**1**:238-43.
36. Levy J, Smith AL, Koup JR, Williams-Warren J, Ramsey B. Dispostion of tobramycin in patients with cystic fibrosis: a prospective controlled study. J Pediatr 1984;**105**:117-24.
37. Michalsen H, Bergan T. Pharmacokinetics of netilmicin in children with and without cystic fibrosis. Antimicrob Agents Chemother 1981;**19**:1029-31.
38. Autret E, Marchand S, Breteau M, Grenier B. Pharmacokinetics of amikacin in cystic fibrosis: a study of bronchial diffusion. Eur J Clin Pharm 1986;**31**:79-83.
39. Vogelstein B, Kowarski AA, Lietman PS. The pharmacokinetics of amikacin in children. J Pediatr 1977;**91**:333-39.
40. Marks MI, Vose A, Hammerberg S, Dugal R. Clinico-pharmacological studies of sisomicin in ill children. Antimicrob Agents Chemother 1978;**13**:753-58.
41. Winslade NE, Adelman MH, Evens EJ, Schentag JJ. Single-dose accumulation pharmacokinetics of tobramycin and netilmicin in normal volunteers. Antimicrob Agents Chemother 1987;**31**:605-09.
42. Laskin OL, Longstreth JA, Smith CR, Lietman PS. Netilmicin and gentamicin multidose kinetics in normal subjects. Clin Pharm Ther 1983;**34**:644-50.
43. Notari R.E. Biopharmaceutics and clinical pharmacokinetics; an introduction. 3rd ed. New York, Marcel Dekker, 1980.
44. Spino M, Chai RP, Isles AF, Williamson Balfe J, Brown RG, Thiessen JJ, MacLeod SM. Assessment of glomerular filtration rate and effective renal plasma flow in cystic fibrosis. J Pediatr 1985;**107**:64-70.

45. Rabin HR, Harley FL, Bryan LE, Elfring GL. Evaluation of a high dose tobramycin and ticarcillin treatment protocol in cystic fibrosis based on improved susceptibility criteria and antibiotic pharmacokinetics. In: Sturgess JM, ed, Perspectives in cystic fibrosis. Proceedings of the 8th International Cystic Fibrosis Congress, Toronto, 1980. Missisanga Ontario: Imperial Press, 1980:370-75.
46. Riggs DS. The mathematical approach to physiological problems. London, MIT Press, 1970;209-11.
47. Hsu MJ, Aguila HA, Schmidt VL, Munzenberger PJ, Kauffman RE, Polgar G. Individualization of tobramycin dosage in patients with cystic fibrosis. Pediatr Infect Dis 1984;**3**:526-29.
48. Hoecker JL, Pickering LK, Swaney J, Kramer WG, van Eys J, Feldman S, et al. Clinical pharmacology of tobramycin in children. J Infect Dis 1978;**137**:592-96.
49. MacDonald NE, Morris RF, Peterson RG. Disease severity as a factor in elimination of tobramycin in patients with cystic fibrosis. Pediatr Res 1987;**21**:238A.
50. Horrevorts AM. In vitro activity of new antimicrobial agents against Pseudomonas aeruginosa isolates from cystic fibrosis patients. Eur J Clin Microbiol 1987;**6**:323-24.
51. Horrevorts AM, de Witte J, Degener JE, Dzoljic-Danilovic G, Hop WCJ, Driessen O, Michel MF, Kerrebijn KF. Tobramycin in patients with cystic fibrosis. Adjustment in dosing interval for effective treatment. Chest 1987;**92**:844-48.
52. McCrae WM, Raeburn JA, Hanson EJ. Tobramycin therapy of infections due to Pseudomonas aeruginosa in patients with cystic fibrosis: effect of dosage and concentration of antibiotic in sputum. J Infect Dis 1976;**134**:S191-93.
53. Moore RD, Smith CR, Lietman PS. Association of aminoglycoside plasma levels with therapeutic outcome in gram-negative pneumonia. Am J Med 1984;**77**:657-62.

54. Powell SH, Thompson WL, Luthe MA, Stern RC, Grossniklaus DA, Bloxham DD, et al. Once-daily vs continuous aminoglycoside dosing: efficacy and toxicity in animal and clinical studies of gentamicin, netilmicin, and tobramycin. J Infect Dis 1983;**147**:918-32.
55. Pedersen SS, Jensen T, Osterhammel D, Osterhammel P. Cumulative and acute toxicity of repeated high-dose tobraycin treatment in cystic fibrosis. Antimicrob Agents Chemother 1987;**31**:594-99.

SUMMARY

Cystic fibrosis (CF) is the most common inherited disease among white Caucasians. The condition is transmitted as an autosomal recessive trait and heterozygotes do not express the disease. CF affects exocrine gland secretions throughout the body. Clinically, it is characterized by exocrine pancreatic insufficiency and chronic obstructive pulmonary disease. Pulmonary infection is the major cause of morbidity and mortality.

The microorganism most frequently isolated from CF sputum is P. aeruginosa. Once acquired, this organism is seldom eradicated, although it may disappear temporarily from the sputum during treatment. From single sputum samples, isolates of P. aeruginosa with differing morphological characteristics and antibiograms can be cultured. These observations together with reported difficulties in typing CF isolates resulted in confusion about the number of types which might colonize an affected individual. Some reports have suggested that CF patients may be colonized by a single type, while others reported colonization by two or more different types. These contradictory results might be explained by the limitations of the typing techniques per se as well as by the peculiar properties of P. aeruginosa which affect the methods. In particular the mucoid character and the poly- and nontypability in serotyping appear to account for most of the inconsistent observations. In chapter 1 several typing techniques were evaluated for their potential as epidemiological tools for isolates of P. aeruginosa from CF patients. Apart from the confusion about the number of types which might colonize an individual patient, it is conceivable, that the type or types present in the lungs during the course of time will be replaced by another type or types. In chapter 2 a study is described in which the composition of the pseudomonas flora was monitored over a period varying from 2 to 60 months in fifteen patients with CF aged between 7 and 18 years. Four conventional typing techniques were used, namely serotyping, active and passive pyocin typing, and phage typing. The

aim of the study was to establish whether the composition of the flora of P. aeruginosa in the lungs of individual patients is subject to changes or whether it remains constant over time. The number of different serotypes per patient ranged from one to three, and pyocin and phage typing showed no marked differences between strains of the same serotype. In general, patients whose sputum yielded more than one type harbored these in several samples. An exacerbation of the chronic respiratory infection did not affect the composition of the flora. Long term observations showed that a given type or types tended to recur supporting the conclusion that the composition of the pseudomonas flora in CF remains fairly constant over time.

During the last few decades antimicrobial therapy has significantly improved the prognosis of CF by reducing the progression of pulmonary destruction. In some centers only acute exacerbations are treated while in others, patients are given antimicrobial therapy periodically or even continuously. At present many antipseudomonas drugs are available for this purpose. In vitro assessment of these agents against P. aeruginosa recovered from sputum of patients with CF showed that on a weight basis ciprofloxacin was the most active agent tested (chapter 3). At clinically attainable serum concentrations tobramycin and amikacin had similar activity. If resistance to a ß-lactam agent occurred, cross resistance to other ß-lactams was the rule.
Exacerbations of chronic respiratory infections caused by P. aeruginosa are usually treated with an aminoglycoside in combination with an antipseudomonas ß-lactam antibiotic or a quinoline. Combination therapy is often given because it seems to offer better results than those obtained from monotherapy, possibly due to synergistic interaction in relation to P. aeruginosa. Furthermore, resistance to combination therapy is believed to develop less rapidly. The in vitro interaction between tobramycin on the one hand and three antipseudomonas ß-lactam antibiotics on the other was investigated by means of checkerboard titrations (chapter 4). Special attention was paid to the influence of the composition of the dilution series on the outcome by comparing twofold and modified serial dilutions

of the antibiotics. Consecutive concentrations in these modified serial dilutions were four times smaller than those in the twofold series permitting minimum inhibitory (MICs) and minimum bactericidal (MBCs) concentrations to be determined with greater accuracy. More accurate determination of MICs and MBCs will be reflected in parameters derived from them such as the fractional inhibitory (FICIs) and fractional bactericidal (FBCIs) concentrations indices which are a measure of drug interaction. It was shown that the indices (twofold versus modified serial dilutions) can differ markedly (from -0.30 to +1.06). Thus, the interpretation of these data depends also on the composition of the serial dilutions used. The theoretical background of this observation is discussed in chapter 5.

In CF patients, the pharmacokinetics of several antimicrobial agents, including aminoglycosides and ß-lactam antibiotics, differ from those observed in the normal individual. The agents in question are believed to be excreted more rapidly in this disease. Therefore, these drugs have to be given more frequently and in a larger dose in order to ensure that therapeutic concentrations are achieved and maintained for a sufficient duration at the infection site. In view of their oto- and nephrotoxicity aminoglycosides have a limited therapeutic range. To be adequate and safe, treatment of CF patients with these drugs has to fulfil strict criteria. The steady-state pharmacokinetics of tobramycin in fifteen patients with CF aged between 8 and 22 years are reported in chapter 6. In the calculation of pharmacokinetic parameters, a two-compartment open model was used. It was shown that the serum tobramycin concentration-time profile changes with age, in so far as with increasing age the distribution phase converts more rapidly to the elimination phase and in addition, elimination shows a faster decline. The half-lives of the distribution (α) and elimination (β) phases were inversely correlated with age and body weight ($t_{½\alpha}$ versus BW; r_s=-0.81, $p<0.0005$ and $t_{½\beta}$ versus BW; r_s=-0.88, $p<0.00005$). So was the ratio of the Area Under the Curve to the dose (AUC/dose versus BW; r_s=-0.76, $p<0.005$), while Total Body Clearance (TBC versus BW; r_s=+0.66, $p<0.01$) and Volume of distribution at steady state (Vdss versus BW; r_s= +0.53, $p<0.05$) were directly correlated. Between patients, no

marked differences were observed in the height of the one hour serum tobramycin concentrations, whereas considerable differences were found in the height of the serum tobramycin concentrations prior to the next dose (trough concentrations). This wide interpatient variation could have clinical implications for tobramycin therapy in CF, particularly for the dosing interval. In chapter 7 we investigated whether this variation determined the outcome of treatment. Twenty eight patients with an acute exacerbation of chronic pulmonary infection with P. aeruginosa were treated with tobramycin (3.3 mg/kg every 8 hours) in combination with ticarcillin (600 mg/kg/day). On the tenth day of treatment a significant correlation ($r_s = +0.75$, $p < 0.001$) was found between trough serum tobramycin concentration and increase in Forced Expiratory Volume in 1 second (FEV_1). In sixteen patients the increase in FEV_1 was less than twenty percent. To achieve a higher trough level, the dosing interval was shortened in nine of these sixteen patients; hence the 24 h dosage was increased. On the 20th day of treatment, FEV_1 had improved by at least twenty percent in seven of the subjects in whom the dosing interval was shortened, but in only one of those in whom the interval remained unchanged ($p < 0.05$). Therefore, individualization of tobramycin dosage in CF results in a better clinical outcome.

The implications of the pharmacological data described in the chapters 6 and 7 are discussed in chapter 8 in the light of current opinions on treatment with aminoglycosides of acute P. aeruginosa exacerbations in patients with cystic fibrosis.

SAMENVATTING

Cystic fibrosis (CF) is de meest voorkomende autosomale recessieve erfelijke ziekte onder het blanke ras. De pathogenese van CF is terug te voeren op afwijkingen in de secreten van exocriene klieren. De belangrijkste ziekteverschijnselen van CF vinden hun oorsprong in de tractus digestivus en de tractus respiratorius. De morbiditeit en mortaliteit van CF worden voor het merendeel bepaald door infekties van de luchtwegen.

Pseudomonas aeruginosa is het micro-organisme dat het meest frekwent uit sputa van patienten met CF wordt geïsoleerd. Wanneer dit micro-organisme eenmaal in het sputum van een patient is aangetroffen, verdwijnt het daaruit slechts zelden. In sputumkweken van CF patienten kunnen meerdere kolonievormen van P. aeruginosa worden aangetroffen. De gevoeligheid voor antimicrobiële middelen kan per kolonievorm verschillend zijn. Deze bevindingen tezamen met problemen rond het typeren van CF stammen hebben geleid tot verwarring over het aantal typen waarmee een CF patient gekoloniseerd kan zijn. Sommige studies maken melding van de aanwezigheid van slechts 1 type, andere van 2 of meer. Deze tegenstrijdige bevindingen zouden enerzijds kunnen samenhangen met de beperkingen van de typeringstechnieken op zich, terwijl anderzijds het typeren van P. aeruginosa stammen afkomstig van CF patienten wordt bemoeilijkt door speciale kenmerken welke deze stammen kunnen bezitten, zoals de produktie van een mukeus exopolysaccharide, en door veranderingen in het lipopolysaccharide van de buitenmembraan. In hoofdstuk 1 wordt de bruikbaarheid van een aantal typeringstechnieken voor het typeren van Pseudomonas-isolaten van patienten met CF besproken.
Afgezien van onduidelijkheden over het aantal P. aeruginosa typen dat in sputummonsters van CF patienten kan worden gevonden, is het denkbaar dat in de tijd gezien stammen van verschillend type elkaar opvolgen. In hoofdstuk 2 is de samenstelling van de Pseudomonasflora bij 15 patienten (leeftijden van 7-18

jaar) over perioden variërend van 2-60 maanden in kaart gebracht. Hierbij zijn de volgende typeringsmethoden gebruikt: de serotypering, de aktieve - en passieve pyocinetypering en de faagtypering. Het doel van de studie was na te gaan of de Pseudomonasflora in de longen van CF patienten in de tijd constant of wisselend van samenstelling is. Het aantal gevonden serotypen varieerde bij de patienten van 1 tot 3. Tussen stammen van hetzelfde serotype werden, per patient, geen grote verschillen in het pyocine- en het faagtype gevonden. Bij patienten met meer dan één serotype werden de verschillende typen in het algemeen in meerdere sputummonsters aangetroffen. Een exacerbatie bleek de samenstelling van de Pseudomonasflora niet te beïnvloeden. De typeringsresultaten tesamen laten zien dat de samenstelling van de Pseudomonasflora in de luchtwegen van patienten met CF in de tijd vrijwel constant is.

De prognose van CF is in de loop van de laatste decennia geleidelijk verbeterd. Daar de chronische ontsteking de voornaamste determinant van de prognose is, is de verbeterde prognose voor een belangrijk deel te danken aan de antimicrobiële therapie. In bepaalde centra worden uitsluitend akute exacerbaties behandeld. In andere daarentegen worden periodiek (wel of geen exacerbatie) of zelfs kontinu antimicrobiële middelen gegeven. Tegenwoordig zijn er vele preparaten met een antimicrobiële werking ten opzichte van P. aeruginosa op de markt. In hoofdstuk 3 is de in vitro gevoeligheid van P.aeruginosa stammen geïsoleerd uit sputa van patienten met CF bepaald voor een aantal van deze middelen. Ciprofloxacin bleek, op basis van het gewicht aan droge stof, het meest aktieve middel. Tobramycine en amikacine hadden, afgemeten aan de gebruikelijke serumconcentraties van elk, een overeenkomstige antibakteriële werking. Kruisresistentie tussen de onderzochte ß-lactam antibiotica kwam in de regel voor.

Exacerbaties van een chronische luchtweginfektie door P. aeruginosa worden bij CF meestal behandeld met een aminoglycoside in kombinatie met een tegen Pseudomonas aktief ß-lactam antibioticum of quinolon. Een dergelijke kombinatie

wordt vaak gegeven omdat deze (mogelijk door een synergistische interactie t.o.v. P. aeruginosa) tot betere resultaten leidt dan monotherapie. Bovendien zou door gekombineerde therapie minder snel resistentievorming optreden. In hoofdstuk 4 wordt ingegaan op de in vitro interaktie tussen het aminoglycoside tobramycine enerzijds en een drietal tegen P. aeruginosa werkzame ß-lactam antibiotica anderzijds. De interakties zijn bestudeerd met behulp van schaakbordtitraties. Onderzocht is met name of de samenstelling van antibiotikumverdunningsreeksen van invloed is op het uiteindelijke resultaat. Hiervoor zijn tweevoudig verdunde reeksen vergeleken met gemodificeerd verdunde reeksen. Van deze laatste zijn de stappen tussen elkaar opvolgende concentraties vier maal kleiner dan die van tweevoudig verdunde reeksen. Hierdoor is het mogelijk de minimaal remmende (MRCs) en de minimaal bactericide (MBCs) concentraties nauwkeuriger te bepalen. Dit moet dan ook gelden voor parameters (als de fraktionele remmende concentratie - en de fraktionele bactericide concentratie indices) die een maat zijn voor de interaktie tussen twee antibiotica aangezien deze aan de hand van respectievelijk de gevonden MRCs en MBCs berekend worden. Uit de studie bleek dat tussen de interactie indices van de tweevoudig verdunde reeksen en de gemodificeerd verdunde reeksen aanzienlijke verschillen kunnen bestaan (van -0.30 tot +1.06). Dit leidde tot de conclusie dat de interactie indices mede worden bepaald door de samenstelling van de gebruikte antibioticumverdunnings-reeksen. De theoretische achtergrond en praktische betekenis hiervan zijn besproken in hoofdstuk 5.

De farmacokinetiek van een aantal antimicrobiële middelen, waaronder aminoglycosiden en ß-lactam antibiotica, wijkt bij patienten met CF af van de norm. De betreffende middelen worden bij patienten met CF sneller uitgescheiden. Derhalve worden ze vaker en in een hogere dosis toegediend om te waarborgen dat gedurende een voldoende lange tijd op de plaats van infektie concentraties aanwezig zijn waarvan verwacht mag worden dat ze therapeutisch effektief zijn. Aminoglycosiden hebben in verband met oto- en nefrotoxiciteit een kleine therapeutische breedte. Aan een adekwate en veilige behandeling van CF

patienten met aminoglycosiden worden dientengevolge hoge eisen gesteld. In hoofdstuk 6 is een onderzoek beschreven naar de steady state farmacokinetiek van tobramycine bij 15 patienten met CF. De patienten variëerden in leeftijd van 8 tot 22 jaar. Bij de berekening van de farmacokinetische parameters is uitgegaan van een open twee-compartimenten model. Uit de studie bleek dat de concentratie-tijdcurve van tobramycine verandert met de leeftijd in die zin dat met oplopende leeftijd de verdelingsfase eerder overgaat in de eliminatiefase; bovendien gaat de eliminatie sneller verlopen.
De halfwaardetijden ($t_{½}$) van de verdelings- (α) en eliminatiefase (β) bleken omgekeerd evenredig te zijn met leeftijd en LichaamsGewicht ($t_{½\alpha}$ vs LG; r_s=-0.81, $p<0.0005$ en $t_{½\beta}$ vs LG; r_s=-0.88, $p<0.00005$). Dit was ook het geval met het Oppervlak Onder de Curve gedeeld door de Dosis (OOC/D vs LG; r_s=-0.76, $p<0.005$), terwijl de Totale Lichaams Klaring (TLK vs LG; r_s=+0.66, $p<0.01$) en het Verdelingsvolume tijdens steady state (Vdss vs LG; r_s=+0.53, $p<0.05$) rechtevenredig waren met leeftijd en gewicht.
De tobramycine-serumconcentratie 1 uur na de start van de infusie bleek tussen patienten niet veel te verschillen. Dit was anders met de concentratie vlak voor een volgende gift (dalspiegel). Dit verschil in dalspiegel tussen CF patienten zou voor de behandeling met tobramycine gevolgen kunnen hebben, met name voor het doseringsinterval. In hoofdstuk 7 is onderzocht in hoeverre de gevonden verschillen in dalspiegel van invloed zijn op het klinisch resultaat. Achtentwintig CF patienten met een acute exacerbatie van de chronische luchtweginfektie door *P. aeruginosa* werden behandeld met tobramycine (3 x dd 3,3 mg/kg) en ticarcilline (600 mg/kg/dg). Tussen de hoogte van de dalspiegel en de stijging van het FEV_1, beide gemeten op dag 10 van de behandeling, bleek een significante correlatie te bestaan (r_s=+0.75, $p<0.01$). Bij 16 patienten bedroeg de toeneming van het FEV_1 minder dan 20%. Bij 9 van deze 16 patienten is om een hogere dalspiegel te krijgen het doseringsinterval van tobramycine vanaf dag 10 verkort (deze patienten kregen tobramycine dus vaker per dag toegediend waardoor bij hen de totale dagdosis hoger werd). Bij de overige 7 patienten is niets aan de toediening van tobramycine veranderd. Op de 20e dag van de behandeling bleek bij 7 van

de 9 patienten bij wie het doseringsinterval van tobramycine op de 10^e dag was veranderd een toeneming van het FEV_1 van meer dan 20% te zijn opgetreden; dit was slechts bij 1 van de 7 patienten het geval bij wie het doseringsinterval op de 10^e dag van de behandeling niet was verkort. Dit verschil, dat significant bleek ($p<0.05$), betekent dat een klinisch beter resultaat bereikt wordt wanneer bij de therapie rekening wordt gehouden met de individuele farmacokinetiek van tobramycine.

De resultaten als beschreven in de hoofdstukken 6 en 7 worden in hoofdstuk 8 besproken en wel in het licht van heersende opvattingen over aminoglycoside-therapie bij CF patienten met acute exacerbaties veroorzaakt door P. aeruginosa.

NAWOORD

Graag wil ik mijn oprechte dank betuigen aan iedereen die een bijdrage heeft geleverd aan de totstandkoming van dit proefschrift, met name:

Mijn promotores Prof. Dr. M.F. Michel en Prof. Dr. K.F. Kerrebijn voor het begeleiden van het onderzoek, hun opbouwende kritiek, hun enthousiasme en bovenal hun eindeloos geduld.
Wijlen Dr. Jan Borst die op zeer persoonlijke wijze zijn specifieke kennis van Pseudomonas-bacteriën heeft uitgedragen. Hij heeft mij in de gelegenheid gesteld om onder de kundige leiding van Rob Puyk op zijn afdeling het typeren van Pseudomonas-stammen te leren.
Prof. Dr. C. Hilvering en Prof. Dr. R.P. Mouton, leden van de promotiecommissie, voor het beoordelen van het manuscript.
Dr. John Degener die de basis legde voor dit onderzoek. De dagelijkse leiding van het project was bij hem in vertrouwde handen. Hij was het die de betekenis van de farmacokinetiek voor het onderzoek heeft onderkend.
Dr. Gordana Dzoljic-Danilovic. Zij is als hoofd van het Bacteriologisch Laboratorium van het Sophia Kinderziekenhuis zeer nauw bij het onderzoek betrokken geweest. Haar hulp, suggesties en opmerkingen tijdens het onderzoek zijn zeer waardevol gebleken.
Dr. Oscar Driessen. Zijn vermogen voor mij moeilijke farmacokinetische vraagstukken op een inzichtelijke wijze uit te leggen was subliem.
De statistici Dr. J. Hermans en Drs. W. Hop op wier hulp altijd gerekend kon worden.
Dr. Irma Bakker-Woudenberg voor haar inbreng tijdens de werkbesprekingen. Zij volgde het onderzoek met grote interesse.
Ria van Amstel-de Ridder en Nelleke Heeres-Weststrate voor hun analytische hulp bij het onderzoek.

Ineke Vredenberg-Bom die jarenlang de te onderzoeken monsters doorsluisde van de afdeling Interne van het Sophia Kinderziekenhuis naar het Bacteriologisch Laboratorium.
De verpleegkundigen van de afdeling Interne van het Sophia Kinderziekenhuis (destijds aangevoerd door hoofdzuster Nel Kusters) die mij de naam "A. Tobra" bezorgden.
De physiotherapeuten van het Sophia Kinderziekenhuis voor het kundig afnemen van de benodigde sputummonsters.
Het analytisch personeel van het Bacteriologisch Laboratorium van het Sophia Kinderziekenhuis voor het verzamelen van de Pseudomonas-stammen en hun hulp bij allerlei bijkomende werkzaamheden.
Collegae, medewerksters en medewerkers van de afdeling Kinderpulmonologie van het Sophia Kinderziekenhuis.
Marion Poot, Maja de Jonge en Jaap de Witte. Zij hebben tijdens hun studie medicijnen in het kader van het keuzevak een bijdrage aan het onderzoek geleverd.
Ada Beukelman, Linda Harkes, Paula Jansen-Bogaars en Carolien van Bakel. Zij hebben het onderzoek secretarieel ondersteund.
Camille van de Zand en Margreet Boeren-den Boer voor het verzorgen van het manuscript van het proefschrift. Johan Mouton voor de keuze van het lettertype.
Peter Donnelly voor het doornemen van het manuscript op onvolkomenheden in de Engelse taal.
De medewerksters en medewekers van het Bacteriologisch Laboratorium van het Dijkzigt Ziekenhuis.
Het Nederlands Astma Fonds dat het onderzoek financieel mogelijk maakte.
Thea van Vlijmen voor het opschudden van mijn kussen zeer laat op de avond en Sophie (mijn oogappel) voor haar levenslust zeer vroeg in de ochtend.

CURRICULUM VITAE

De schrijver van dit proefschrift is geboren te Almelo op 7 november 1949.

1967
Eindexamen VWO aan het Pius X College te Almelo.

1980
Artsexamen aan de Katholieke Universiteit te Nijmegen.

1981-1983
In het kader van een door het Nederlands Astma Fonds gesubsidieerd projekt verbonden aan de afdeling Bakteriologie (hoofd Prof. Dr. M.F. Michel) van het Akademisch Ziekenhuis Dijkzigt te Rotterdam. In deze periode is gestart met het onderzoek dat in dit proefschrift wordt beschreven. Het onderzoek is in samenwerking met de afdeling Longziekten bij Kinderen (hoofd Prof. Dr. K.F. Kerrebijn) van het Sophia Kinder-ziekenhuis verricht.

1983-1986
Opleiding tot medisch-mikrobioloog op de afdeling Bakteriologie (opleider Prof. Dr. M.F. Michel) van het Akademisch Ziekenhuis Dijkzigt te Rotterdam.

1986-1988
Verbonden als medisch-mikrobioloog aan de afdeling Bakteriologie van het Akademisch Ziekenhuis Dijkzigt te Rotterdam.

1988-heden
Verbonden als medisch-mikrobioloog aan de afdeling Medische Mikrobiologie van het Akademisch Ziekenhuis Sint Radboud te Nijmegen.