# Chapter 12
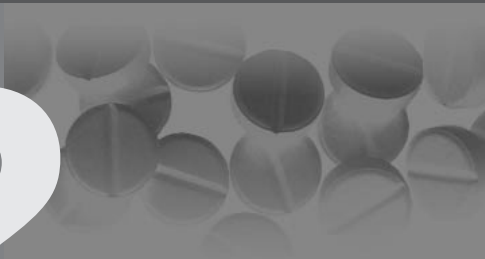
Summary, discussion and future directions

Maturation of organ systems and somatic growth during fetal life and throughout childhood exerts a profound effect on drug disposition. The maturation of drug-metabolizing enzymes is the predominant factor accounting for age-associated changes in nonrenal drug clearance.

The various studies described in this thesis were performed to study the impact of age on midazolam metabolism, as surrogate measure to assess the impact of development upon both CYP3A and UGT activity, and to examine the most well characterized polymorphism of the CYP3A4 gene with respect to its potential to influence CYP3A activity; a determinant of midazolam pharmacokinetics and pharmacodynamics.

## Summary

### Introduction
Chapter 1 describes the aims of the studies conducted as part of this thesis.

Chapter 2 reviews the current literature on the ontogeny of the cytochrome P450 3A subfamily of drug-metabolizing enzymes. The available data indicate that profound, often clinically important, changes occur in the activity of CYP3A isoforms during all stages of development. However, several critical information gaps exist with regard to the overall impact of ontogeny on CYP3A activity. The exact developmental pattern of CYP3A expression and its impact on the pharmacokinetics of CYP3A substrates has not been fully characterized during the first 3 to 5 years of life. The contribution of intestinal and renal CYP3A to the disposition of CYP3A substrates in children has not been well studied and thus, it is not known if development *per se* influences the functional expression of CYP3A activity in these organs. Finally, the genetic and possibly, neurohumoral, factors that govern the upregulation of CYP3A after birth are also still largely unknown.

Chapter 3 reviews the current literature on the developmental aspects of the UDP-glucuronosyltransferases (UGT) superfamily of drug-metabolizing enzymes. At least 10 different UGT isoforms have been identified, which all appear to display a different developmental pattern. Despite the presence of pharmacokinetic studies of UGT substrates in pediatric patients (e.g., lorazepam, morphine, acetaminophen), isoform specificity of enzyme action and its implications with respect to the disposition of pharmacologic substrates is generally not known. It is concluded that current knowledge of UGT ontogeny is insufficient and future investigations must consider the impact of development on isoform specific biotransformation of therapeutic drugs and xenobiotics.

### Pharmacokinetics
Chapter 4 describes the pharmacokinetics and metabolism of the CYP3A substrate midazolam, given as an intravenous bolus dose to 24 preterm infants, who were between 26 and 33 weeks of gestational age and between 3 and 11 days of postnatal age. Consequent to immature hepatic CYP3A4 activity, midazolam clearance and 1-OH-midazolam concentrations were markedly reduced in preterm infants as compared to previous reports from studies in older children and adults. Midazolam apparent volume of distribution and total plasma clearance were increased in those infants who

had postnatal indomethacin exposure as compared to those who did not. This finding suggests that indomethacin treatment of a patent ductus arteriosus produces a drug-treatment effect capable of altering the disposition of midazolam, an extensively metabolized drug, in premature neonates.

The pharmacokinetics, absolute oral bioavailability and metabolism of midazolam given as an oral bolus dose to 15 preterm infants are presented in Chapter 5. The oral bioavailability of midazolam is increased while the midazolam oral plasma clearance and 1-OH-midazolam concentrations are markedly reduced as compared to previous reports in older children and adults. These latter findings correspond to reduced functional activity of both intestinal and hepatic CYP3A4/5; developmental findings that characterize prematurity. These findings may also have important consequences for the oral availability of other CYP3A4 substrates as higher oral bioavailability may lead to higher plasma concentrations of CYP3A4 substrates which in turn, could increase the risk of toxicity in newborn infants should the dose of a drug not be modified *a priori* for developmental differences in the rate and extent of drug absorption.

Chapter 6 describes the population pharmacokinetics and biotransformation of midazolam administered as a continuous infusion in 21 pediatric intensive care patients who were between 2 days and 17 years of age. Midazolam plasma clearance in our subjects appeared to be lower as compared to data from other studies in pediatric intensive care patients; a potential consequence of reduced CYP3A4 activity associated with the lower mean age of patients in our study cohort. These studies demonstrated production and persistence of the glucuronide conjugate of 1-OH midazolam, the primary oxidative metabolite of midazolam catalyzed by CYP3A, in all infants. This finding suggests that the yet uncharacterized UGT isoforms responsible for the phase II metabolism of 1-OH midazolam are present at two days of postnatal age in preterm neonates. This study also identified the potential for important disease (e.g., renal and/or hepatic compromise) and treatment (e.g., concomitant administration of CYP3A4 inhibitors) co-variates to serve as predictors of altered midazolam pharmacokinetics and metabolism in pediatric intensive care patients. The derived population pharmacokinetic model may have clinical applicability for individualization and resultant optimization of midazolam therapy in children.

## *Pharmacogenetics*

Chapter 7 describes a new detection method for the CYP3A4*1B allele and its allelic frequency among 199 Dutch Caucasians. A simple and specific PCR-restriction fragment length polymorphism (RFLP) was developed. Allelic frequency for this mutation was found to be 5.3%. All identified individuals were heterozygous for this allele, giving a heterozygote frequency of 10.6%. It was concluded that this assay could greatly facilitate studies on the effect of this polymorphism in endogenous processes, environmental susceptibility to cancer and individual ability to metabolize drugs that are substrates for CYP3A4.

A new PCR-RFLP method for the detection of the CYP3A4*3 allele is described in Chapter 8. The frequency of this allele in 499 Dutch Caucasians and 66 pediatric patients was determined. Thirteen of 499 and 1 of 66 individuals were found to be heterozygous

for the allele, which renders an allelic frequency of approximately 1%. The results indicated that the CYP3A4*3 may be a true genetic polymorphism and thus, has the potential to influence the activity of this enzyme reflected by altered pharmacokinetics of CYP3A4 substrates. Future research was suggested to elucidate the effect of this polymorphism on CYP3A4 activity.

Chapter 9 describes an exploratory, pilot investigation that examined the possible association of the CYP3A4*1B allele with the pharmacokinetics of midazolam in 30 preterm infants. The data from this study demonstrated that the CYP3A4*1B allele was not apparently associated with changes in intravenous or oral midazolam clearance in preterm infants in the first 14 days of postnatal life. Recognizing the implicit limitations associated with sample size in this pilot pharmacogenetic study, it was concluded that further studies are needed to elucidate the potential impact of CYP3A*1B and other allelic variants of CYP3A4 and CYP3A5 on its activity in man.

### Pharmacodynamics

Chapter 10 describes the pharmacodynamics of midazolam given as a 30-minute intravenous infusion or oral bolus dose to 24 preterm infants. The results suggest that midazolam is effective in a majority of infants as reflected by reductions in COMFORT scores within 30 minutes after intravenous and one hour after oral midazolam administration. This evidence of a desired pharmacologic effect (i.e., midazolam was used to induce pre-procedural sedation) was observed without a clinically important change in hemodynamic or other safety parameters. However, a considerable proportion of patients did not appear to respond to midazolam. It was suggested that this lack of response may be due to the fact that patients truly experienced therapeutic failure consequent to developmental immaturity of drug-receptor interaction and/or was associated with the inability of the COMFORT score to adequately reflect sedation uniformly in preterm infants where the pharmacodynamic response may have been blunted by their physical instability.

Chapter 11 describes the pharmacodynamics of continuous midazolam infusion in 21 pediatric intensive care patients. The data indicate that midazolam dosing can be successfully titrated to achieve adequate sedation in pediatric intensive care patients. However, the need for different levels of sedation in different patient groups reduces the possibility of a single COMFORT score range to be used as indicator for adequate sedation. It is, therefore, suggested that individual COMFORT score ranges that are demonstrated to produce adequate sedation should be defined for each patient who receives midazolam by continuous intravenous infusion.

## Limitations

### Pharmacokinetics

The initial goal of this research project was to determine the ontogeny of CYP3A4 activity *in vivo*, in order to tailor drug therapy with CYP3A4 substrates according to age-related changes in CYP3A4 activity. When studying the impact of co-variates such as age on

drug metabolism *in vivo*, the need for a validated, safe and non-invasive pharmacologic probe sufficient to accurately profile the impact of development on enzyme activity is evident. At the time the studies described in this thesis were started (1997), midazolam clearance and the $^{14}$C-erythromycin breath test (EBMT) were the best validated probes to assess CYP3A activity *in vivo* (1). The latter probe has disadvantages, which render it less optimal for use in infants and young children. Since the EBMT uses radioactively labeled erythromycin, this method is unethical for use in newborn infants. We therefore decided to focus on midazolam plasma clearance as surrogate measure of CYP3A activity. Initially, we planned to investigate the possibility of an urinary metabolite:drug ratio as an alternative for plasma clearance, the latter requiring frequent blood sampling for accurate determination. However, during the first year of the studies, it became clear that CYP3A (i.e., CYP3A5) is also present in the kidney and is capable of biotransforming midazolam intrarenally. This, in turn, would be expected to affect the urinary metabolite:drug ratio and thereby, reduce the validity of this ratio as measure of hepatic (and intestinal) CYP3A activity (2). Consequently, we used midazolam plasma clearance as an *in vivo* pharmacologic probe to assess CYP3A activity. In order to reduce the number of samples taken for AUC calculations, we also investigated the possibility of a minimal sampling method (3). It was, however, not possible to derive such a minimal sampling schedule for use in preterm infants. The consequent need for repeated blood sampling restricted the recruitment of patients to those who already had an indwelling arterial catheter placed prior to the study for clinical purposes.

This restriction explains the gap in gestational ages studied (i.e. newborn infants between 33 and 42 weeks of gestational age). Preterm infants with higher gestational ages were not frequently admitted to our neonatal intensive care unit or were too unstable to receive midazolam, the latter being associated with hypotension in hemodynamically unstable patients (4).

Other important limitations of our studies are represented by the relatively small numbers of patients included and the need for an indwelling vascular catheter necessary for repeated blood sampling to accomplish the pharmacokinetic objectives of the study. This may explain why we did not find an age-related difference in midazolam disposition within our study populations in contrast to the differences observed (e.g., comparison of midazolam plasma clearance) between the neonatal and pediatric intensive care patients. However, other factors may also explain the lack of apparent developmental differences in midazolam disposition within the neonatal and pediatric patients groups.

First, midazolam is not only metabolized by CYP3A4 but also, by CYP3A5 and to an unknown extent by CYP3A7, the fetal CYP3A isoform (5). The contribution of CYP3A43 to the metabolism of midazolam is yet unknown (6). CYP3A4 expression is transcriptionally activated during the first week of life and is accompanied by a simultaneous decrease in the level of CYP3A7 expression (7). Additionally, CYP3A4 activity is low directly after birth and attains 30 to 40 percent of adult activity at 1 month of postnatal age. The ontogenic pattern of CYP3A5 and CYP3A43 remains to be elucidated. However, during the transition from fetal to neonatal life, total CYP3A content appears to be relatively stable (7). Hence, it is possible that the contribution of other CYP3A isoforms to the metabolism of midazolam may have obscured a postnatal increase in CYP3A4 activity early in life.

Second, these studies were performed in ill patients housed in intensive care units. In these patients, the disposition of midazolam is more likely to be influenced by both exogenous factors (e.g. drugs capable of altering CYP3A activity or the disposition of midazolam by other pathways of metabolism [glucuronidation] or elimination [renal compromise]) and endogenous factors (e.g. intrinsic hepatic disease or the effect of cytokines associated with systemic infection on CYP3A activity (8)). It was not possible to control for these co-variates consequent to the small number of patients included in the studies and also, their inherent medical conditions.

In contrast to cytochrome P450, knowledge concerning the impact of development on UGT activity is far from complete. The lack of specific probe drugs capable of assessing the activity of individual UGT isoforms and knowledge concerning isoform specificity for the biotransformation of important UGT substrates have contributed to this lack of knowledge. For example, our preliminary data suggest that glucuronidation of the CYP3A4/5 catalyzed primary metabolite of midazolam, 1-OH midazolam, occurs at levels comparable to those reported in adults at between 2 days and 17 years of postnatal life. This finding is in marked contrast to the observation that morphine glucuronidation is impaired at birth and increases to adult levels between 6 and 30 months of age (9). This discrepancy may be explained by the involvement of different UGT isoforms in the glucuronidation of midazolam and morphine. It is however unknown which UGT isoform(s) is/are involved in the formation of 1-OH-midazolam-glucuronide from 1-OH-midazolam. Therefore, our findings regarding midazolam can not be generalized with respect to their potential therapeutic implication to other UGT substrates used to treat infants and children.

## Pharmacogenetics

Individual differences in drug metabolism during childhood are only partially explained by the developmental changes involving drug-metabolizing enzymes. It is now recognized that genetic variation directs the expression of many drug-metabolizing enzymes and therefore also contributes to the interindividual differences in drug metabolism (10). At the time this research project started no genetic polymorphisms associated with altered CYP3A activity were known. Recently, many mutations in the CYP3A4, 3A5 and 3A7 genes have been described (11). We determined the allelic frequency of two CYP3A4 mutations in Dutch Caucasians and showed that based upon the frequency of both alleles studied, they may be considered as true genetic polymorphisms in this population. However, evidence is emerging that large interethnic differences exist in the frequency of CYP3A genetic polymorphisms (12). In the Netherlands, an increasing part of the populations is not Caucasian but rather, from Mediterranean, African and Middle-Eastern descent. Given that we did not study these distinct ethnic groups or a population that was the product of genetic admixture between Dutch Caucasians and the other populations, our data can not be extrapolated to the whole Western European population.

Next, we conducted a pilot study investigating a possible association between the CYP3A4*1B mutation and midazolam plasma clearance in preterm infants. The lack of such an association as observed in our patient cohort does, however, not rule out the existence of a genotype-phenotype relationship for CYP3A4*1B in preterm infants. The

number of patients in our study was small and the numbers of patients with and without the mutation was not balanced. However, *in vivo* data from adults suggest that the CYP3A4*1B mutation is not associated with clinically important differences in pharmacokinetics of CYP3A4 substrates (13) and thus, corroborates our experimental findings. Finally, due to the low allelic frequency of the CYP3A4*3 mutation, we were not able to investigate a possible genotype-phenotype relationship for this mutation consequent to the limited population of infants who received midazolam in our investigations.

### Pharmacodynamics

Our pharmacodynamic studies of midazolam illustrate the problems associated with the assessment of sedation in nonverbal children. To date, no validated sedation score for preterm infants is available, which leaves the distinction between therapeutic failure or 'sedation score' failure uncertain. Moreover, we encountered limitations of the use of the COMFORT score in pediatric intensive care patients. The COMFORT score that should be determined in situations when the patient is at rest does not adequately predict agitation associated with therapeutic nursing or medical procedures, or other "handling" (e.g., parental agitation) of young infants. Also, we experienced that the COMFORT score ranges defined to reflect adequate sedation in pediatric intensive care patients do not always reflect adequate sedation in all patient groups. For example, we found that deeper sedation appears to be needed in patients with pulmonary hypertension or malignant systemic hypertension. Finally, the heterogeneity (e.g., differences in disease state, organ function, concomitant medications, etc.) which characterizes the pediatric intensive care unit population may have confounded our ability to detect a pharmacokinetic-pharmacodynamic association for midazolam in neonatal and pediatric intensive care patients.

## Future directions

### Pharmacokinetics

Clearly, several information gaps exist with regard to the overall impact of ontogeny on CYP3A and UGT activity. *In vitro* and *in vivo* studies should together provide greater insights regarding the ontogeny of CYP3A and UGT activity and its impact on the pharmacokinetics of important substrates. First, *in vitro* studies should be conducted to elucidate isoform-specific developmental patterns for both the CYP3A and UGT, not only for hepatic tissue but also for intestinal and renal tissue. This is especially true given that co-regulation of hepatic and intestinal CYP3A4 is not apparent while the activity of the enzyme in both tissues demonstrates a clear developmental pattern(7) (14). Second, *in vitro* metabolism and isoform-specificity of CYP3A and UGT substrates frequently used in children (e.g. midazolam, cisapride, fentanyl, cyclosporin, morphine, ibuprofen) should be determined using relevant tissues (e.g., microsomes derived from liver, intestine, kidney) obtained not from adults but rather, from neonates, infants and children. In these experiments, relevant (i.e., therapeutic) drug concentrations and concentrations of enzyme should be used. Third, the pharmacokinetics and *in vivo* metabolism of these important CYP3A and UGT substrates should be completely

characterized from birth through the first 3 to 5 years of life. Also, other drugs frequently used in children should be investigated in order to clarify a possible 'compensatory' role of CYP3A and UGT activity, when the activity of other drug-metabolizing enzymes is developmentally impaired. Such studies are required to examine the developmental "breakpoints" of enzyme activity so that population extremes of sufficient magnitude to alter the dose vs. concentration vs. effect relationship for selected drugs and their active metabolites can be identified.

The information derived from these studies may aid the physician in designing truly age-appropriate dosing regimens for CYP3A and UGT substrates, which should improve both the safety and efficacy of drug treatment.

## Pharmacogenetics

Although many genetic polymorphisms of the CYP3A and UGT genes have been reported, the clinical importance of these polymorphisms with respect to the pharmacokinetics of frequently used drugs in children remains to be elucidated.

Recently, genetic polymorphisms underlying the large interindividual variation in CYP3A5 expression have been found (12). The investigators suggest that CYP3A5 may be the most important genetic contributor to interindividual and interethnic differences in CYP3A activity *in vivo*. Since interindividual differences in CYP3A activity, as reflected by midazolam clearance, appear to be even larger in preterm infants than in adults, this may reflect the interplay between genetic and developmental factors determining CYP3A5 activity. Therefore the investigation of the developmental expression of CYP3A5 and the effect of genetic polymorphisms on this expression should receive increased attention.

Also, recent reports suggest a role for nuclear receptors as PPAR, PXR and CAR in the regulation of drug metabolizing enzyme activity (15). The role of these and other factors in the developmental regulation of CYP3A and possibly, UGT activity should also be elucidated.

## Pharmacodynamics

Validated sedation scores for use in preterm infants are lacking. Although the COMFORT score is validated to assess sedation in pediatric intensive care patients and postoperative neonates (16), it is not specifically validated for use in preterm infants. A possible lack of response to stress, as often observed in preterm infants, may be due to an inability to respond resulting from physical instability consequent to prematurity related disease. Validation of the COMFORT score or other sedation scores for preterm infants should improve the quality of pharmacodynamic studies on sedative drugs in this patient population. Also, although the COMFORT score and its ranges associated with adequate sedation are validated for pediatric intensive care patients, it does not take into account circadian differences in sedation level, distress caused by nursing procedures and different needs of sedation for different groups of patients. In future studies of the pharmacodynamics of sedative drugs, these aspects should also be taken into account. As well, more work must be done to evaluate the impact of ontogeny and genetic polymorphisms on receptor expression as a possible basis for true developmental differences in pharmacodynamics.

In conclusion, this thesis illustrates that age is an important determinant of pharmacokinetics and pharmacodynamics of midazolam in preterm infants and children. Moreover, physiological changes consequent to development and disease such as patent ductus arteriosus, hepatic failure and renal failure all contribute to the variability in disposition and effect of midazolam in children. The dependence of many drugs on metabolic elimination underscores the need to obtain specific data in pediatric patients when developmental differences in drug metabolism serve as a primary determinant of drug disposition and/or action.

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