# Maturation of Cytochrome P450 3A Mediated Drug Metabolism:

## Towards individualized dosing in children

Ibrahim Ince


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# Maturation of cytochrome P450 3A mediated drug metabolism: Towards individualized dosing in children

**Ontwikkeling van cytochroom P450 3A gemedieerd metabolisme van geneesmiddelen:**
**De weg naar individueel doseren bij kinderen**

**Proefschrift**

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Voor mijn zorgende moeder,
mijn wijze vader,
mijn geduldige zus,
voor mijn leven, mijn lieve Sermin

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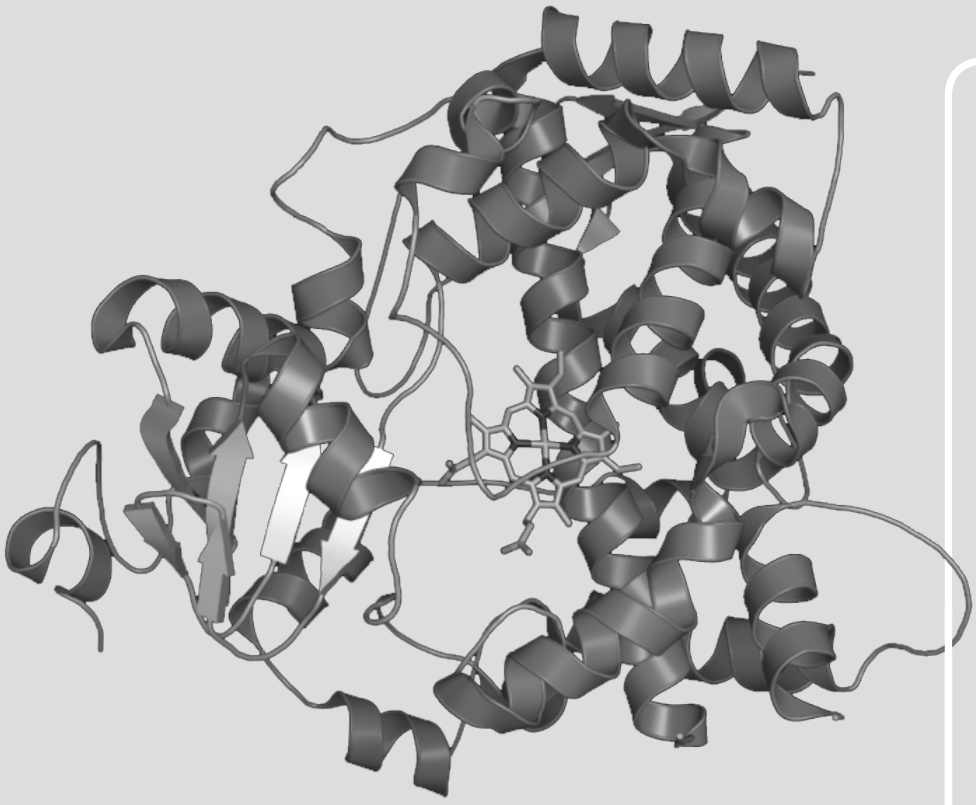

# 1 Maturation of Cytochrome P450 3A Mediated Drug Metabolism – General introduction

# MATURATION OF CYTOCHROME P450 3A MEDIATED DRUG METABOLISM

## GENERAL INTRODUCTION

Most drugs have not been adequately studied in children, leaving this population at an increased risk of underdosing or toxicity. Because of ethical, practical and financial constraints of pediatric clinical trials, [1-3] there is a high percentage of drugs that is used in an unlicensed or off-label manner, with percentages approximating 70% in children and more than 93% in critically ill neonates. [4-6] The main limitations in designing and conducting clinical studies in children are ethical, practical and scientific. Ethical issues are e.g. proxy consent, safety concerns and blood volume needed for pharmacokinetics. Practical issues pertain to the limited number of patients available and sample collection challenges. Scientific issues are related to the impact of growth and development on drug disposition, while increasingly attention is put on the long-term impact of early-life drug exposure on the developing brain. In the past the emphasis has been on the evaluation of the short-term direct effects of drugs, such as the increase in blood pressure upon the administration of vasoactive drugs. Fortunately, the current interest in primary outcome measures of drug treatment has led to an increase in the number of studies investigating long-term effects, i.e. neurodevelopmental outcome in children. An example is the recent study in which long-term cardiovascular effects of dobutamine and dopamine have been compared in preterm and low birth weight neonates. [7]

It is well known that differences exist in disposition and response to drugs between children and adults and between children of different ages. [3] These differences are not only due to changes in body and organ size, but also due to changes in the expression and function of drug metabolizing enzymes, and many other factors that can affect the absorption, distribution, metabolism, elimination (ADME), and effects of drugs in children. [3] Therefore, the extrapolation of dosing regimens from adults to children, based on body weight alone is far from optimal. Individualization of drug treatment on the basis of pertinent information on the pharmacokinetic and pharmacodynamic relation is needed in this vulnerable group of patients. This has led to international regulations, such as the Pediatric Regulation in Europe and the Best Pharmaceuticals for Children Act in the US to encourage the pharmaceutical industry to study new drugs also in children. Moreover, these regulations also encompass stimulatory efforts to study off-patent drugs.

A major hurdle the development of rational dosing regimens in children is the size of the research effort that is required. To study changes in the pharmacokinetics and the pharmacodynamics for every single drug across the whole pediatric age range and for every medical

indication, is not feasible when applying traditional approaches. In this regard application of the so-called "population approach" is an important alternative. Specifically, the population approach offers the advantage that pharmacokinetic parameter estimates can be obtained on the basis of a limited number of concentration measurements per child as all available data are analysed simultaneously while taking into account which observation originates from which child. [8, 9] Moreover covariate analysis can be applied to identify specific sources of variation. This enables the distinction between various sources of inter-individual variation (e.g. variation as a result of development *versus* disease related factors), and the development of guidelines for individualized dosing in the pediatric population by using simulation and modeling. [9, 10] Validation of pediatric pharmacokinetic and pharmacodynamic models is a crucial factor. Recently, a framework for the evaluation of pediatric population models has been proposed. [11]

Yet, even when applying the population approach, an immense effort is still required to describe variation in pharmacokinetics and pharmacodynamics of each and every drug in the pediatric population. In this regard there is an increasing interest in the application of a systems approach to the prediction of developmental changes. A key element on the systems approach is the distinction between "drug-specific" and "system-specific" parameters in pharmacokinetic models to describe variation on drug response. "System-specific parameters" relate to parameters describing physiological processes, such as organ perfusion and the expression/function of enzymes and transporters. In theory, knowledge on the changes in the system-specific parameters characterizing the absorption, distribution, metabolism, and excretion (ADME) of drugs, such as maturation in drug metabolizing enzyme, can be obtained by analyzing developmental changes in the pharmacokinetics of a paradigm compound that is metabolized by this specific pathway. This approach has been previously applied for the enzyme UGT2B7, in which information on maturation of UGTB7 was obtained by modeling the clearance of morphine as its biological marker, [12] which was followed by a successful prediction of zidovudine clearance, also a UGT2B7 substrate, in children of different ages using this biological system specific information derived from the morphine model. [13] Similarly, the maturation of glomerular filtration in preterm and term neonates, characterized using amikacin as paradigm compound, [14] proved predictive for other renally antibiotics in the neonatal age range. [15]

CYP3A is a Cytochrome P450 enzyme subfamily involved in the metabolism (oxidation) of many endogenous compounds, drugs and toxins. Even though it is known that CYP3A4 activity is low at birth and reaches adult values in the first years of life, there are still important gaps in our knowledge of the exact developmental patterns of individual CYP3A isoforms, CYP3A4, CYP3A5 and CYP3A7. In addition, most *in vitro* and *in vivo* clinical studies have failed to cover the whole pediatric age range. This information gap hampers the design and imple-

mentation of age-specific dosing guidelines of CYP3A substrate drugs, especially in neonates and young infants.

To characterize the developmental changes in the *in vivo* activity of CYP3A, changes in the pharmacokinetics of the paradigm compound midazolam can be used. The benzodiazepine midazolam is a well-known, and currently the best validated CYP3A substrate, which has been used extensively in children and adults for sedative purposes. [2, 16, 17] However, in many of the studies on the CYP3A-mediated pharmacokinetics of midazolam, either the age range studied was relatively small, or only few data were available for certain ages for identification or adequate description of the developmental changes of CYP3A across the entire human lifespan. A comprehensive dataset, composed of data from different studies including children of different ages, would allow for better understanding of the influence of age-related changes in CYP3A activity on the pharmacokinetics of drugs. An important factor that should be taken into account when combining data from different studies is, that other factors affecting the disposition, such as disease state or co-medication, must be accounted for. The derived CYP3A maturation function describing midazolam pharmacokinetics may then potentially be used as biological system specific information for the prediction of the pharmacokinetics of other CYP3A substrates. The aim of the investigations that are described in this thesis is to apply this semi-physiological approach to identify the system-specific maturation function of CYP3A mediated drug oxidation, using midazolam as *in vivo* paradigm compounds.

## SCOPE AND INTENT OF THE INVESTIGATIONS

**Part I** of this thesis describes our current understanding of CYP3A ontogeny (chapter 2) and presents a novel framework for the development of evidence-based dosing guidelines in children (chapter 3). In **chapter 2** an overview is presented of the current understanding on developmental changes of CYP3A activity, in the context of the study methods used. This insight provides a basis for future research. Although several methods exist to study the development of CYP3A activity at the molecular and the systems levels, there are still important gaps in our knowledge of the exact developmental patterns of individual CYP3A isoforms. This hampers the design of age-specific dosing guidelines of CYP3A substrate drugs, especially in neonates and infants. It is proposed that detailed insight in the developmental changes can be obtained by semi-physiological modeling of changes in the pharmacokinetics of paradigm compounds such as midazolam. In **chapter 3**, a multi-step approach for modeling and simulation, using nonlinear mixed effects modeling for the optimization of drug dosing in children, is proposed. This approach could be used to study the maturational behavior of CYP3A using CYP3A substrates as *in vivo* probes, as it allows sparse and infrequently obtained

data to be used, and may provide information of pathway-specific maturation that can be used to predict developmental changes in the disposition of drugs with similar disposition pathways.

**Part II** reports the development and application of a maturation model of CYP3A using midazolam and cisapride as paradigm compounds. In **chapter 4**, the interplay of critical illness and age in relation to the pharmacokinetics of the CYP3A substrate midazolam is investigated and quantified in children between 1 month and 17 of age upon intravenous administration of midazolam. In **chapter 5** a novel maturation function for the clearance of midazolam after intravenous administration from preterm neonates to adults is developed. The maturation of CYP3A mediated bioavailability after oral administration of midazolam is described in **chapter 6**. In **chapter 7** the maturation function for midazolam clearance and bioavailability is extrapolated to pediatric cisapride data obtained after oral administration, applying a semi-physiological approach for the development of pharmacokinetic models for CYP3A substrates in children.

**Part III** discusses the main findings and conclusions of the studies in this thesis, along with their implications, and presents recommendations in **chapter 8**. Critical illness is identified as the major determinant of midazolam clearance in children aged 1 month to 17 years of age. A biological system specific maturation function for CYP3A mediated clearance of midazolam after intravenous and oral administration is developed and applied to cisapride, which is another specific CYP3A substrate. This is done via a multi-step approach for modeling and simulation using nonlinear mixed effects modeling that allows sparse and infrequently obtained data from children to be used, providing the pathway-specific maturation functions. In **chapter 9** and **chapter 10** the overall summary of this thesis is given.

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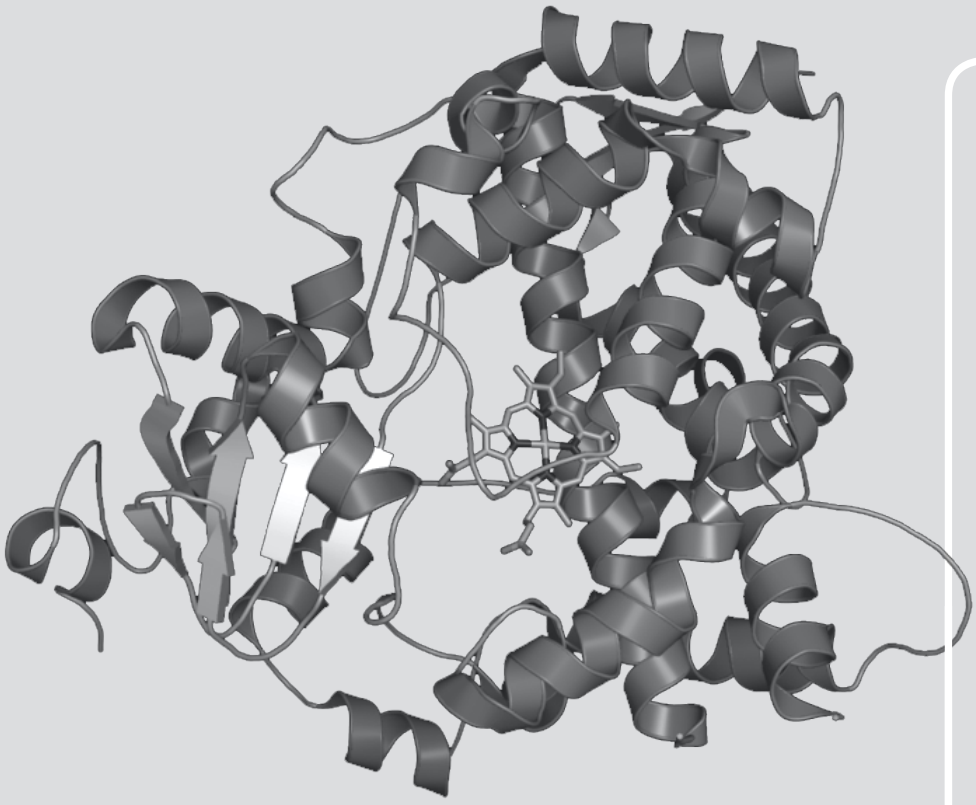
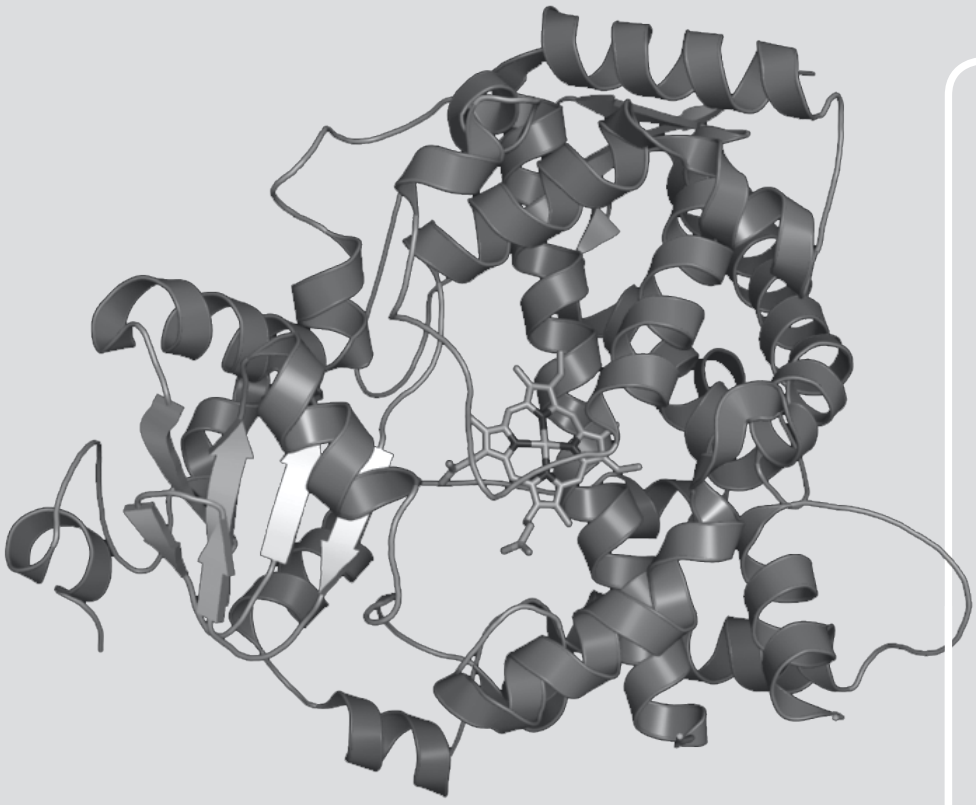


# Part I

## Mechanism-based modeling to develop rational dosing guidelines in children: CYP3A maturation



# 2 Tailor-made drug treatment for children: creation of an infrastructure for data-sharing and population PK-PD modeling

Ince I, de Wildt SN, Tibboel D, Danhof M, Knibbe CA,
Drug Discovery Today, 2009; 14(5-6):316-20

## ABSTRACT

Rational dosing guidelines for drugs in pediatrics are urgently needed. To develop these guidelines, we use population pharmacokinetic–pharmacodynamic (PK–PD) modeling and simulation by: (i) optimization of clinical trial designs based on preliminary data; (ii) development and internal validation of population PK–PD models using sparse data; (iii) external validation using independent data; and (iv) prospective clinical evaluation. Optimized dosing regimens for specific drugs may then serve as a basis to develop dosing guidelines for existing or newly developed drugs with similar disposition and/or effect. In addition to modeling of drug disposition (PK) pathways, we emphasize the need for modeling of effect (PD) pathways and the use of a multidisciplinary infrastructure for data-sharing.

## 1. PROBLEM DEFINITION FOR MEDICINES IN CHILDREN: ROLE OF THE DOSE

Well-known differences exist in disposition of, and response to, drugs between children and adults and between children of different ages [1]. Yet, because well-designed clinical studies in children are scarce, dosing schemes in children are usually derived in an empirical manner from clinical trials in healthy volunteers and/or restricted adult patient groups, and often based on a linear extrapolation on the basis of bodyweight [2]. To account for differences in drug disposition and/or drug response between children of different ages, higher or lower dosages per kilogram bodyweight are regularly recommended in different age groups. The scarcity of dedicated studies in children is explained by the ethical, practical and financial constraints of clinical trials in this patient population [1, 3, 4]

At present, approximately 70% of drugs prescribed to children in general, and more than 93% of drugs prescribed to critically ill neonates, are unlicensed or used in an off-label manner 5, 6 and 7. As a result, therapeutic failure, adverse events and sometimes, even fatalities may occur. Examples are chloramphenicol causing the grey baby syndrome [8] and the intravenous formulation of vitamin E causing the death of 38 neonates [9]. Neonates receiving a combination of penicillin and sulphisoxazole had a significantly higher mortality than those receiving oxytetracycline [10]. Also, the drug interactions of commonly used drugs in children may be unknown. Only recently, the concomitant administration of ceftriaxone and calcium has been reported to cause fatal reactions in the lungs and kidneys in neonates (see: http://www.fda.gov/cder/drug/InfoSheets/HCP/ceftriaxone.html; http://www.rocheusa.com/products/rocephin/rocephin-hcp-letter.pdf). These, and other, unknown reactions to drugs in children have important implications for the efficacy and/or safety of pharmacotherapy in this population. This lack of information is a problem not only for clinicians but also for the pharmaceutical industry. Given the new Paediatric Law, which has come into force in Europe in 2007, the information on drug disposition and effect in children is of great importance because, already at the end of Phase I of the development of a new drug, a pediatric investigational plan (PIP) has to be submitted to the European regulatory authorities (EMEA). With a final reward for this effort of a six-month patent extension and because the studies in children are now a prerequisite for labeling of drugs in Europe, more information and guidance to develop dosing guidelines for drugs used in children is of great value.

## 2. WHAT INFORMATION IN CHILDREN IS NEEDED?

Instead of an empiric-dosing regimen based on bodyweight alone, pediatric dosing regimens should be based on an understanding of the pharmacokinetic–pharmacodynamic (PK–PD) relationship of the drug in children. Therefore, to define effective and safe dosing regimens for

children of different ages, detailed information is needed on the PK (the drug-concentration *versus* time profile) and the PD (the drug-concentration *versus* effect relationship and the time-dependent transduction mechanism that governs the time course of the response) [11].

Both the PK and PD may change over the continuum of a child's life [1]. Age-related differences in PK may be caused by the differences in absorption, distribution, metabolism and/or excretion [1]. For example, profound developmental changes in the activity of the isoforms of drug metabolizing enzyme CYP3A have been shown to occur in the neonatal period and through infancy [12]. Also, the developmental changes in renal function can dramatically alter the plasma clearance of compounds with extensive renal elimination [1]. For example, ceftazidime, which is excreted primarily by the glomeruli, shows a plasma drug clearance that correlates with maturational changes in renal function [13]. The differences in PD can be caused by different pathogenesis in children compared to adults [14]. They can also result from age-related changes in receptor expression causing differences in target tissue sensitivity and age-related changes in the function of *in vivo* transduction and homeostatic feedback mechanisms governing the intensity of the response [1]. For example, children appear to respond differently to adults to antihypertensive drugs [15], despite similar PK in the age-range studied. Traditionally, the studies on PD have received less attention than PK studies, although it is generally accepted that the variability in PD is much larger than the variability in PK [16]. Although there is a lack of validated PD endpoints in children, it is of utmost importance to study the PD relation in children as well. Clinical trials in children should therefore consider age-related variability in PK and PD simultaneously, to be able to develop rational dosing schemes [1].

In practice, this age-related variability in PK and/or PD must be considered in the context of all other sources of intra- and interindividual variability resulting from genetic-, environmental- and disease-related factors and drug interactions [1]. For example, the role of pharmacogenetic (PGx) factors on treatment effect or PK should not be evaluated independently, but should be studied together with all other clinical covariates [17]. In a recent example on CYP2D6 polymorphisms in newborns, the influence of age, body weight and CYP2D6 polymorphisms classified as 2D6 activity score were simultaneously investigated. Each of the three variables was shown to affect the formation of tramadol to its main metabolite significantly 18 and 19, with size and age as major contributors to the variability (52.7%) while CYP2D6 activity score contributes 6.4% to this variability [18]. In our opinion, this study provides a sophisticated example on how to study different covariates such as age-related and genetic factors in a quantitative manner.

Recently, Alcorn *et al.* identified population PK, allometric scaling and physiologically based clearance scaling models as principal approaches to estimate pediatric systemic clearance in

the absence of comprehensive age-group-specific data [20]. In addition to modeling of drug disposition (PK) pathways, we emphasize that a similar approach for modeling of effect (PD) pathways should be undertaken to predict expected effects and safety over the developing age continuum.

## 3. HOW SHOULD THIS INFORMATION BE GATHERED?

Properly designed studies in children aiming at the development of PK–PD models are difficult to perform. Specific challenges are not only the availability of limited patient numbers but also ethical and practical restrictions to the volume and frequency of blood sampling and the number of PD observations, particularly in neonates. Modern technologies involving sampling and laboratory analyses have, however, been developed and can be used in pediatric populations. LC–MS/MS, for example, is highly sensitive and allows the use of very small volume samples for drug-concentration analyses [21]. Also, the use of saliva to monitor drug concentrations noninvasively [22], the use of dried blood spots from heel-prick samples [23] and the use of breath tests to study drug metabolism are examples of methods that may facilitate drug studies in children [24]. In addition to these novel approaches, the application of the so-called 'population approach' opens new avenues for drug studies in children. Population PK–PD modeling involves the application of concepts of 'non linear mixed effects modeling', where PK and/or PD parameters are simultaneously estimated in all individuals. The final results are population PK and/or PD parameters and estimates for the interindividual variability (variance) as well as intraindividual variability or residual error (variance). Following this characterization of the variability in PK and PD, the next step is the so-called covariate analysis, in which demographic and pathophysiologic (e.g. weight, age, liver and kidney function, disease severity and genetics) predictors of the variability are identified. If these predictors are associated with clinically significant shifts in the therapeutic index, they may serve as the basis for the design of individualized dosing schedules.

The most important advantage of the population approach is that it allows for the utilization of infrequently obtained samples and observations from actual patients at time points compatible with clinical care, rather than in a specific experimental setting. The approach allows for the analysis of relatively dense data, combinations of sparse and dense data or combinations of observations from experimental settings and clinical practice. Moreover, because the population approach is able to handle 'missing data' in individual patients, it greatly facilitates PK and/or PD studies in young children [23]. Finally, this approach ensures that the obtained information can indeed be directly applied in clinical practice and that the burden to the individual patient can be kept to a minimum [25].

## 4. ROLE OF MODELING AND SIMULATIONS IN PEDIATRIC CLINICAL DOSING STUDIES

The development of evidence-based dosing schedules using population PK–PD modeling involves several steps (Fig. 1). First, for the design of novel prospective clinical trials, the amount and quality of existing data from the literature or databases from industry or regulatory authorities need to be considered. These data are preferably obtained not only from children of the same age, but also from other age groups, from adults or even *in vitro* data (e.g. from the population-based PK modeling and simulation program SIMCYP™ that includes extensive *in vitro* libraries [26]) may be considered. These preliminary data are then used to simulate the concentration and effect *versus* time profiles and their variability in the (individual) patients in the trial. The simulations allow for the determination of an optimal sampling scheme, thereby minimizing the burden for the individual child and, at the same time, ensuring maximum information content. For example, a population PK model was developed by Rubino *et al.*[27] to describe the PK of gatifloxacin in children, for which data of an existing study were used [28]. The developed model can be used to estimate exposure in future studies and to perform simulation experiments for defining appropriate dosing regimens as well as to evaluate the comparative utility of anti-infective agents [27].

Second, on the basis of data of the prospective clinical trial alone, or in combination with data obtained retrospectively from clinical practice, the population PK–PD models are developed (Fig. 1). In general, when building a PK and/or PD model, the first step is the identification of a structural model (e.g. a two-compartment model for the PK or an E-max model to characterize the concentration–effect relationship). The next step is the development of a statistical model to describe the interindividual as well as residual variability (e.g. log-normal distribution of PK-parameters). The final step is the exploration of the influence of covariates on the values of the PK and/or PD parameter, such as body weight, age and renal function, where they are incorporated in the model as predictors with potential clinical relevance 29 and


**Figure 1.** Proposed multi-step approach for modeling and simulation using non-linear mixed effects modeling for the optimization of drug dosing in children. Legend Figure 1 The four steps that are proposed are 1) optimization of clinical trial designs based on simulations using preliminary data, 2) development and internal validation of population PK-PD models using sparse data, 3) external validation of the population PK-PD models using independent data and 4) prospective clinical evaluation of the PK-PD model based dosing regimen. *PK* pharmacokinetics, *PD* pharmacodynamics.

30. Because the three submodels are interrelated, the choice of the structural (and statistical) model may affect the choice of covariate model and vice versa. The process of finding a model that adequately describes the data is thus an elaborate task, where model refinement/checking is performed in several steps 11, 31 and 32. As an example for this step, we discuss a study where Peeters *et al.* used nonlinear mixed effects modeling to develop a model for dose estimations of propofol in nonventilated children after major surgery [29]. In that study, the choice of the structural model was made by a comparison of the objective function. In addition the diagnostic plots for examining bias and precision, the confidence interval of the parameter estimates, the correlation matrix and visual improvement of the individual plots were used to evaluate the model. This resulted in a two-compartment model for the PK and a simple E-max model for the PD relationship. The interindividual variability was best characterized by a log-normal model for the parameters. Thereafter, in the covariate model, covariates such as body weight, age, body surface area and body mass index were plotted independently against the individual post hoc parameter estimates and the weighted residuals to identify their influence. Body weight incorporated as a power function was found to be a significant covariate for elimination clearance, thereby reducing the interindividual variability (CV%) in clearance from 27% to 20%, which further refined the models.

An important issue is the validation of population PK–PD models. This concerns the internal validation, the external validation, the prospective validation and ultimately the cross-validation. Cross-validation tests the ability of the PK–PD model to predict the disposition and effect of drugs that share similar PK and/or PD pathways. Although it was recently reported that only 17% of published pediatric PK and PD models are validated [33], we believe that all models should be at least fully internally validated. This includes the presentation of not only standard diagnostic measures, such as model-predicted *versus* observed plots, but also the results of advanced internal modeling, such as bootstrap resampling, visual predictive checks or normalized prediction discrepancy errors (NPDE) [34] (Fig. 1). In our example, Peeters *et al.* used a bootstrap resampling method, which involves repeated random sampling to produce another dataset of the same size but with a different combination of individuals, to assess the stability of the parameter estimates and the robustness of the final model. Although not always feasible owing to a lack of pertinent data, external validation is important to address the accuracy of a model in patients from a different but plausibly-related population 35 and 36. The methodology of external validation is technically similar to internal validation [37]. The model-based individualized dosing regimens that result from the internally and also externally validated population PK–PD model should be evaluated in a prospective clinical trial. Although not performed in the study of Peeters *et al.* [30] the PK–PD model based dosing regimen can be tested in such a prospective clinical trial, to evaluate whether the proposed dosing regimen indeed leads to the expected concentrations and/or effects, which finally results in refining the dosing regimen for specific individuals in this population if needed.

After these prospective trials, individualized dosing schemes will lead to a predictable efficacy and safety profile for each child of a certain age, bodyweight and genetic background.

## 5. MECHANISM-BASED PK–PD MODELING IN CHILDREN

Obviously, if this approach needs to be applied to every single drug in pediatrics, large costs and significant time will be needed to develop evidence-based dosing schedules for each drug. An intriguing question is, to what extent does a mechanism-based PK–PD model constitute a basis for the development of dosing guidelines for drugs other than those that have actually been studied? A pertinent feature of mechanism-based PK–PD models is the strict distinction between drug-specific and biological system-specific parameters to characterize the time course of the drug effect [38]. In this respect, the kinetics of age-related changes in renal function, the functionality of drug metabolizing enzymes, drug transporters, as well as the expression function of pharmacological receptors, are patient-specific or biological system-specific properties. These system-specific properties, derived from one 'model' drug, could in principle serve as a basis for the prediction of age-related changes in the PK and PD of other drugs (the so-called cross-validation). Using simulations for drugs other than those used to generate biological system-specific information may significantly reduce the time and costs needed to develop drug-dosing guidelines for individual drugs. For example, because CYP3A is involved in the metabolism of many clinically prescribed drugs [12], the developmental pattern of CYP3A activity can be studied *in vivo* using surrogate marker drugs that are specific for these pathways [4]. Similarly, because the renal elimination of drugs in children is dependent on the maturation of physiological parameters such as renal blood flow, glomerular filtration and renal tubular function, an approach to model the age-dependent changes in renal clearance using a surrogate marker drug such as ceftazidim, which is almost completely renally eliminated [39], may provide a good basis for the estimation of renal drug clearance in pediatric populations [20]. Next, the information gained from these 'model' drugs, may be used for the development of evidence-based dosing regimens for other drugs sharing similar pathways [38]. Using this approach, however, different potential limitations should be considered. An example is the use of a population PK model for CYP3A activity that is developed using midazolam as a model drug, for cross-validation to the immunosuppressant tacrolimus, which is also a substrate for CYP3A. Because tacrolimus is not only a CYP3A substrate but also a substrate for the P-glycoprotein (Pgp), the use of this model can result in misleading estimations. Additionally to the use of accessory pathways of the new drug compared to the drug that was used to build the model, the differences in receptor affinity of the different drugs should be taken into account, when developing dosing or sampling schemes using models that have been developed using a similar acting drug. Despite these limitations, however, we believe that cross-validation should at least be further explored to

determine the exact value of this type of approach, because such an approach will significantly decrease the burden to the pediatric population.

## 6. OUR APPROACH IN PRACTICE: CURRENT PROJECT IN THE NETHERLANDS

In the Netherlands, a multidisciplinary research platform on mechanism-based population PK–PD modeling for the design of individualized dosing regimens in children has been established. Partners in this platform comprise four academic institutions and six leading international pharmaceutical industries (http://www.tipharma.com). The platform operates on the basis of a so-called 'matching fund' principle, which implies that the academic institutions and the industries each provide 25% of the budget; the Dutch government provides the other 50%.

The platform has a unique infrastructure for sharing anonymized data in a secure environment. There is restricted access for authorized investigators and there are extensive data checks and strict data management rules created for each research project (Table 1). This allows for data-sharing needed for initial analyses (step 1), and also for external validation or cross-validation of developed models. This data-sharing of existing data and potential data of newly developed drugs or studies does not only account for academia but also for the (competing) industry. In the platform, the supervision of the modeling is provided by academia and industry and the final population PK–PD models will be available for all partners.

**Table 1.** Main objectives for data management

| | | |
|---|---|---|
| **1** | Data Upload from academia and industry | a) proposal data handling (access restrictions)<br>b) anonimized raw dataset |
| **2** | Data Handling with tracking | check and validation of dataset (controlling and organizing data) |
| **3** | Data Merge with tracking | merge of validated anonimized raw datasets |
| **4** | Population PK-PD Modeling | model building<br>a) experimental model + parameters<br>b) finalized model + parameters |
| **5** | Data Extraction | a) model + parameters<br>b) report consisting of project proposal data handling, finalized models + parameters |

## 7. CONCLUDING REMARKS

In conclusion, we propose the use of population PK–PD modeling and simulation to develop evidence-based dosing schemes for children, with the ultimate goal to improve drug safety

and efficacy in this population. This approach will allow for sparse sampling in children and reduce the burden for the individual child. The PK–PD data analyses of our platform will result in novel paradigms not only for individualized dosing in children but also allow for the characterization of the biological system, by a distinction between drug-specific and system-specific determinants of drug effect. Ultimately, each 'model drug', reflecting specific developmental drug disposition and effect pathways, can then be used as a scientific basis to develop evidence-based dosing regimens for other existing or newly developed drugs, sharing the same pathways. In addition to modeling of drug disposition (PK) pathways, we emphasize the need for modeling of effect (PD) pathways and the use of a multidisciplinary infrastructure for data-sharing.

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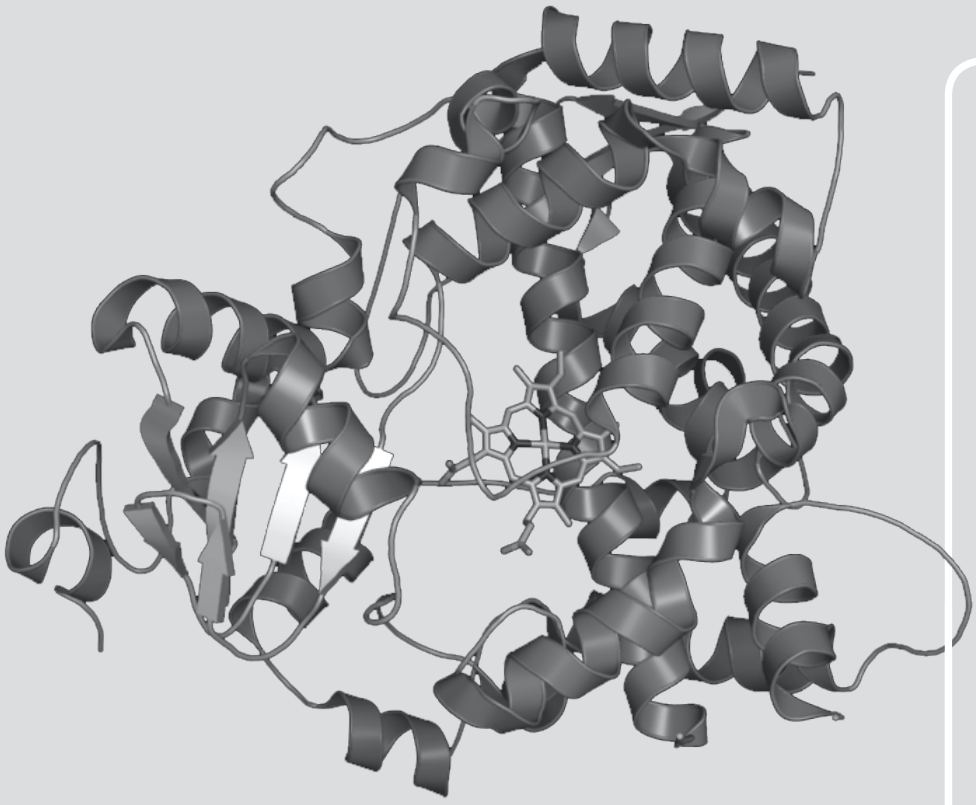

# 3 Developmental changes in the expression and function of cytochrome P450 3A isoforms: evidence from *in vitro* and *in vivo* investigations

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## ABSTRACT

The aim of this review is to discuss our current understanding of the developmental changes of the drug metabolizing enzyme Cytochrome P450 (CYP) 3A and its impact on drug therapy. In the last 10 years, several methods have been used to study the ontogeny of specific CYP3A isoforms *in vitro* and *in vivo*. Although most studies confirm previous findings that CYP3A4/5 activity is low at birth and reaches adult values in the first years of life, there are still important gaps in our knowledge of the exact developmental patterns of individual CYP3A isoforms, especially in this age range. Moreover, most *in vivo* clinical studies have also failed to cover the whole pediatric age range. To date, this information gap still hampers the design of age-specific dosing guidelines of CYP3A substrate drugs, especially in neonates and infants. Innovative study methods, including opportunistic sampling and sensitive analytical assays used in combination with physiologically based pharmacokinetics, and population pharmacokinetic model concepts may help to improve our understanding of the ontogeny of CYP3A and aid the application of this knowledge in clinical practice.

## 1. INTRODUCTION

The Cytochrome P450 (CYP) 3A drug-metabolizing enzymes are the most abundant of all CYP enzymes in the liver, and are involved in the metabolism of approximately half of the drugs metabolized in adults [1, 2]. Many drugs prescribed to children are also CYP3A substrates. New techniques and theoretical insights developed over the past decade have led to new perspectives on the ontogeny of CYP3A. The ontogeny of CYP3A in humans has been studied both *in vitro* and *in vivo*. While *in vitro* studies may yield detailed knowledge on enzyme activity, they are hampered by a scarcity of tissue from children of all ages, as well as by discrepancies between the clinical status of the tissue donor (i.e., mostly deceased or with underlying diseases) and that of the child who actually receives a CYP3A substrate in real life. On the other hand, *in vivo* studies in children who will actually receive the CYP3A substrate in question may be difficult to interpret, as the disposition of the CYP3A substrate is dependent on not only CYP3A activity but also other factors, such as protein binding and liver blood flow. The intrinsic clearance or metabolite/drug ratio of the optimal CYP3A probe should reflect only CYP3A activity [3]. The goal of this review is to provide an updated insight into current knowledge of the developmental aspects of CYP3A activity, in the context of the study methods used, including the ontogeny of hepatic and extrahepatic CYP3A activity *in vitro* and *in vivo*, and to provide guidance for future research aimed at developing evidence-based dosing schedules of CYP3A substrates in children.

## 2. CYTOCHROME P450 (CYP) 3A STRUCTURE, EXPRESSION, AND FUNCTION

The CYP3A subfamily, the most abundant subfamily of the CYP isoforms in the liver, consists of at least four isoforms: CYP3A4, 3A5, 3A7, and 3A43 [1, 2]. These four functional isoforms are located in tandem, and consist of a cluster spanning 231 kilobase pairs (kb) on chromosome 7 [1, 4]. The enzymes comprising theCYP3Asubfamily share at least 85% amino acid sequence homology, although there are substantial differences in substrate specificity expression [5, 6]. CYP3A enzymes are embedded in the endoplasmic reticulum in many organs, such as liver, intestine, kidney, lung, and brain, where they can catalyze a wide variety of biochemical reactions, including hydroxylation, N-demethylation, O-dealkylation, S-oxidation, deamination, or epoxidation of substrates [7]. CYP3A immunoreactivity was detected in midzonal and centrilobular regions of the adult human liver [8]; in the bowel, it was mainly detected in the enterocytes lining the lumen of the small intestine [9, 10]. CYP3A43, which is mainly expressed in the fetal liver and testis, but also in the kidney, pancreas, prostate, and skeletal muscle [2, 11], apparently contributes little to the overall rate of drug metabolism [11] and will therefore not be discussed in this review.

## 2.1 CYP3A4

CYP3A4 is the dominant form of CYP in the adult human liver and small intestine [12], but is also expressed in the esophagus [13], duodenum, and colon [10, 14]. CYP3A4 comprises 10–50% of the total adult hepatic CYP [14, 15]. Approximately 40% of the total CYP3A4 body content is thought to reside in the small intestine [16]. The large interindividual variation in CYP3A4 activity has been proposed to be highly heritable [17]. A recently discovered single-nucleotide polymorphism (SNP, CYP3A4*22) appears to be associated with decreased CYP3A4 expression and function [18, 19]. In contrast, most of the other CYP3A4 polymorphisms have very low frequencies, and the phenotypic effects are weak and often controversial [20, 21]. Also, genome-wide association studies in human liver failed to identify novel genetic markers for CYP3A4 [22, 23]. Interestingly, using a candidate-gene approach, Klein *et al.* [24] showed that genetic polymorphisms in genes involved in the regulation of CYP3A expression were associated with its activity. The strongest association was found for the peroxisome proliferator-activated receptor-a (PPAR-a) gene.

## 2.2 CYP3A5

In adults, CYP3A5 is 83% homologous to CYP3A4 and is also found in hepatic and intestinal tissue [25]. CYP3A5 appears to be the dominant CYP3A isoform in human kidney, lungs, blood, and pituitary gland [26–28]. Because of its polymorphic expression and the presence of the known defective CYP3A5*3 variant allele, CYP3A5 appears at this time to be the most important genetic contributor to interindividual differences in CYP3A-mediated drug clearance [27]. The nonfunctional form occurs very frequently in non-African populations (Caucasians, 91%; Japanese, 85%; Chinese, 65%; Mexicans, 67%; African Americans, 55%; American Indians, 40%) [21, 27, 29]. Functional CYP3A5 is encoded by the CYP3A5*1 allele [27]. The CYP3A5 genotype has been associated with the *in vivo* dispositions and/or effects of clinically important drugs such as tacrolimus [30, 31], saquinavir [32, 33], and statins [34, 35].

## 2.3 CYP3A7

The nucleotide sequence of CYP3A7 is nearly 90% homologous to that of CYP3A4 [36, 37]. CYP3A7 is the major CYP isoform detected in embryonic, fetal, and newborn liver and intestine [38–40]. During fetal life, CYP3A7 protein accounts for about 32% of the total CYP protein [15], and is involved in steroid metabolism (dehydroepiandrosterone, DHEA) [41] and retinoic acid metabolism, in which it appears to play a protective role [38, 41]. In adults, CYP3A7 is polymorphically expressed in the liver and intestine. In 90% of adults it is expressed in the liver at much lower levels than in fetuses, but a highexpression phenotype occurs in 10% of adults [13]. Two-thirds of the latter carry the CYP3A7*1C or (less frequently) the CYP3A7*1B

promoter allele. The CYP3A7*1C allele is the exclusive marker of high CYP3A7 expression in the intestine [13]. In fetal livers, CYP3A7*2, a common active CYP3A7 allele with an allele frequency ranging from 8 to 62% (dependent on ethnicity) [42], was associated with moderate DHEA metabolism in one study [43], while this could not be confirmed by others [42].

Substrate sharing between CYP3A7 and CYP3A4/3A5 is minimal. *In vitro* studies have shown that exogenous substrates shared between CYP3A7 and CYP3A4 are metabolized to a greater extent by CYP3A4 [44, 45].

## 3. DEVELOPMENTAL CHANGES IN CYP3A ACTIVITY

The impact of ontogeny on inter- and intraindividual variation in CYP3A activity has been investigated not only using *in vitro* techniques but also *in vivo*, with the use of pharmacological "probe" compounds [3, 46]. This section presents the relevant findings on the ontogeny of hepatic and extrahepatic CYP3A.

### 3.1 Ontogeny of hepatic CYP3A activity *in vitro*

Developmental changes in the expression and function of CYP3A enzymes can be measured at various levels *in vitro*: mRNA expression, enzyme expression, and enzyme activity, which will be separately discussed for CYP3A4, CYP3A5, and CYP3A7 in the following sections. Others have already discussed the benefits and problems of the different methods used to determine CYP3A mRNA expression, protein content, and activity *in vitro* [47–49]. A detailed discussion of these issues is outside the scope of this review.

#### *3.1.1 CYP3A4 mRNA expression*

There is uncertainty about the expression of CYP3A4 mRNA in embryonic and fetal liver. While Schuetz *et al.* did not find CYP3A4 mRNA in embryonic hepatic tissue (6–12 weeks gestational age) [50], others found CYP3A4 mRNA in fetal livers at a later gestational age (between 11 and 32 weeks) and at 0.1–10% of adult levels [38, 42, 51, 52]. This discrepancy may simply reflect the ontogenic pattern of CYP3A4, with CYP3A4 absent in embryonic life and very low expression of it during the fetal period. Alternatively, more recent techniques used in the later studies may have been more sensitive, allowing the detection of very low CYP3A4 expression levels. Lacroix *et al.* have shown that CYP3A4 mRNA levels rapidly increase immediately after birth, reaching approximately 50% of adult levels between 6 and 12 months of age [38].

### *3.1.2 CYP3A4 protein content*

As the CYP3A enzymes overlap greatly in their amino acid sequences, studies have been hampered by a lack of isoform-specific antibodies to determine protein levels for the individual enzymes. Using isoform-specific DHEA metabolite patterns for expressed CYP3A forms, Stevens *et al.* estimated CYP3A4 protein content in human liver microsomes from early gestation to 18 years of age [40]. Fetal levels of CYP3A4 were very low. In a combined set of liver microsomes, including samples 0–6 months of postnatal age, CYP3A4 content was approximately 2–20% of adult CYP3A4 content (ranging from 1 to 10 pmol/mg protein) [40]. Interestingly, a similarly low CYP3A4 content was estimated in 5- to 15-year-old children (mean 11 pmol/mg protein, n = 8 *versus* adults: 45.8 pmol/mg protein; n = 8) [40].

### *3.1.3 CYP3A4 enzyme activity*

Lacroix *et al.* showed very low activity of testosterone 6b-hydroxylase, a specific marker for CYP3A4 activity, in human fetal liver (n = 16, 14–40 weeks of gestational age), i.e., less than 10% of adult activity [38]. Similarly, Leeder *et al.* showed testosterone 6b-hydroxylation activity of 186.6–439.4 pmol/min/mg protein in 51 fetal liver microsomes (76 days to 32 weeks estimated gestational age) [42], which is approximately 3–23% of the reported adult activity [53]. As for the CYP3A4 enzyme activity after birth, Lacroix *et al.* [38], also using 6b-hydroxylation of testosterone, reported an increase in activity to 30–40% of adult levels in samples from 11 infants ranging in age from 8 days to 3 months. In two samples from children older than one year of age (exact ages not reported), CYP3A4 activity was similar to that in adults.

## 3.2 CYP3A5 mRNA expression

CYP3A5 is consistently demonstrated in embryonic liver [50]. Hakkola *et al.* found CYP3A5 mRNA to be 700-fold lower than that of CYP3A7 in fetal liver samples [54], whereas CYP3A5 mRNA expression was found to be 100-fold lower on average than that of CYP3A7 by Leeder *et al.* [42] when calculated per individual liver sample. This expression varied 123-fold [42].

## 3.3 CYP3A5 protein content

Wrighton *et al.* [55] detected CYP3A5 protein in no more than 10% of fetal livers. More recently, Stevens *et al.* [40] detected immunoreactive CYP3A5 in 54% of the fetal liver samples, with large interindividual variability (0–25 pmol/mg protein). Stevens *et al.* [40] also detected CYP3A5 protein in half of the liver samples (115 out of 212) from children up to 18 years of age. In general, the CYP3A5 protein level did not significantly change with age in the samples in which CYP3A5 was detected [40]. Unfortunately, CYP3A5 genotyping was not performed on these samples. Hence, an unbalanced representation of this genotype across the age groups cannot be ruled out. Although statistical comparisons between ethnic groups were

not made, the general trend was found for African Americans to have the highest mean CYP3A5 protein content [40].

### 3.4 CYP3A5 enzyme activity

As the substrate specificities of CYP3A4 and CYP3A5 show a large degree of overlap, until recently, no specific CYP3A5 probes were available to study CYP3A5 metabolism *in vitro*. For example, a similar clearance of midazolam to its metabolite 1-hydroxy midazolam was found for CYP3A4 and CYP3A5 [56]. In contrast, regioselectivity of CYP3A5 towards 1-hydroxy midazolam instead of 4-hydroxy midazolam was found, which may aid in differentiating between the activities of these CYP3A isoforms [27, 56]. A new specific inhibitor of CYP3A4 (CYP3cide) has been developed that is selective for this enzyme compared with CYP3A5, and may be useful for investigating the relative contributions of CYP3A4 and CYP3A5 to the metabolism of CYP3A substrates [57]. To our knowledge, the individual activities of CYP3A4 and CYP3A5 *in vivo* have not been studied to date.

### 3.5 CYP3A7 mRNA expression

In human fetal livers, CYP3A7 transcripts are the most abundant of the individual CYP3A isoforms, with an expression that varies 634-fold, ranging from 151 to 95,700 transcripts/ng of total RNA [42]. When 5 out of 54 liver samples with undetectable expression were removed, variability was significantly reduced to approximately 20-fold. The lack of detectable CYP3A7 mRNA levels could not be explained by known genetic variation in CYP3A7.

### 3.6 CYP3A7 protein content

CYP3A7 constitutes about 32% of the total CYP content in the human fetal liver [15], and it is not detected in other organs during embryogenesis (days 50–60) [58]. Stevens *et al.* estimated CYP3A7 levels in 77 fetal and pediatric liver microsome samples at 311 pmol/mg within the age group of 13.4–24 weeks of estimated gestational age (EGA). CYP3A7 levels substantially decreased with increasing EGA, down to 201 pmol/mg in the age group between 31 and 41 weeks EGA, and to 158 pmol/mg around full term [40]. In addition, Stevens *et al.* estimated high levels of CYP3A7 for children up to 6 months of age (exact values not reported). Between 1 and 15 years of age, however, CYP3A7 protein levels were extremely low (<5 pmol/mg protein) [40].

## 3.7 CYP3A7 enzyme activity

The 16a-hydroxylation of DHEA-sulfate (DHEA-S), which is mainly catalyzed by CYP3A7 and much less so by CYP3A4, was considerably higher in liver samples from 10–20 week fetuses than in a liver sample from an adult (maximum rate [Vmax] 990 *versus* 45 pmol/min/mg protein) [38, 41]. Lacroix *et al.* showed that 16a-DHEA-S hydroxylation more than doubled immediately after birth, peaked between postnatal days 1 and 7, and later dramatically decreased to only 10% of newborn levels between 3 and 12 months of age. The authors suggested that CYP3A7 is replaced by CYP3A4 after birth, irrespective of the gestational age at birth [38].

## 3.8 Ontogeny of extrahepatic CYP3A *in vitro*

### *3.8.1 Renal CYP3A*

In adults, CYP3A5 is the predominant isoform in the kidney, with genotype-based expression and activity [26]. Pediatric data on renal CYP3A are scarce. The ontogeny of renal CYP3A4 and CYP3A5 activities was studied in human fetal, pediatric, and adult kidney using ifosfamide metabolism to 2-dechloroethylifosfamide and 3-dechloroethylifosfamide as CYP3A4 and CYP3A5 probes, respectively [59]. Renal CYP3A4 and CYP3A5 activities were detected as early as 8 weeks of gestation, with a continuing presence up to adulthood [59].

### *3.8.2 Intestinal CYP3A*

Fetal CYP3A5 expression in duodenum was higher than that in liver, kidney, and brain, and increased from a gestational age of 15–42 weeks [60]. Intestinal CYP3A was also studied in 59 duodenal biopsies from Caucasian patients aged 1 month to 17 years [61]. The expression of CYP3A4 (expressed as the ratio of CYP3A4 mRNA to the number of villin mRNA copies) was high in the first year of life and then dropped. CYP3A4 expression accounted for 58.6 ± 20% of the total CYP3A mRNA [61]. This ratio varied more than 100-fold, implying a high interindividual variability in CYP3A4 expression [61]. CYP3A5 mRNA decreased with age, though not statistically significantly ($p = 0.061$). CYP3A7 mRNA was detected in 64% of the samples, but at much lower levels than for CYP3A4 and CYP3A5, and no significant change with age was found. Overall, intestinal CYP3A mRNA was higher in the first year of life than in older children up to 17 years of age. Johnson *et al.* [62] investigated intestinal biopsies or surgical resection tissue and found an age-dependent increase in CYP3A protein content ($p = 0.001$), with a significant difference (p\0.05) between fetuses and older children (including neonates), and between neonates and children over 5 years of age [62]. A possible explanation for the mismatch between mRNA and protein contents during the maturation process may be a post-transcriptional regulatory mechanism, as has been discussed for CYP2D6 by Treluyer *et al.* [63]. Intestinal CYP3A4 and CYP3A5 mRNA levels in pediatric liver recipients (age 0.1–15 years) at the time of transplant surgery were comparable to those in a similar

adult population [64]. Unfortunately, the authors did not study the relationship of age to expression levels within the pediatric study population. Interestingly, in both the children and the adult cohorts, intestinal CYP3A5 mRNA levels differed signficantly between CYP3A5*1 gene carriers and noncarriers. In CYP3A5*1 carriers, CYP3A5 mRNA accounted for 20–30% of all CYP3A mRNA detected [64, 65].

#### *3.8.3 Placenta and endometrium*

The placental and endometrial CYP3A7 protein content ppears to increase significantly from the first to the second trimester of pregnancy [28, 65]. CYP3A7 content in placenta and endometrium per gram of tissue is between 0.6 and 5.5% of the CYP3A7 content in fetal liver. During gestation, the contribution of CYP3A7 in endometrium and placenta to the metabolism of substrates for this isoforms should not be underestimated, since the placental weight is approximately five times more than the total fetal liver weight [28].

### 3.9 Ontogeny of CYP3A activity *in vivo*

An alternative to the *in vitro* approach for studying specific enzyme activity and its impact on the clearance of a drug is to use an *in vivo* enzyme-specific probe. The hepatic and/or intestinal enzyme activity is then determined by calculating the systemic clearance of an orally and/or intravenously administered drug. Comparing the *in vivo* results with *in vitro* findings will yield insight into the validity of extrapolating *in vitro* information on CYP3A activity to *in vivo* CYP3A activity. An ideal probe is enzyme-specific, which means that its systemic clearance (or a surrogate marker thereof, such as metabolite/drug concentration or pharmacodynamic outcome) will correlate with the enzyme activity without being affected by other physiological factors, such as plasma protein binding, hepatic blood flow, or clearance by other enzymes [67]. In 1994, Watkins [3] proposed criteria for the ideal CYP3A4 probe. For example, the suggested gold standard criterion is that the test measurement should correlate with the concentration of the target enzyme determined in liver biopsies obtained from the individuals undergoing the test. Another criterion that may be used is that the measurement should be reduced or increased when patients are treated with potent inhibitors or known inducers of the target enzyme, respectively [3]. In adults and children, CYP3A activity has been extensively studied *in vivo* using surrogate probes, including midazolam clearance, cisapride clearance, and dextromethorphan urinary metabolite ratio [3, 68, 69]. These and possibly other probes may help to further elucidate the ontogeny of CYP3A *in vivo* and its interplay with other covariates [70–75].

#### *3.9.1 Midazolam hepatic clearance*

Midazolam is extensively metabolized by CYP3A4 and CYP3A5 to 1-OH-midazolam, 4-OH-midazolam, and 1,4-OH-midazolam, and meets most of the criteria for an ideal *in vivo* CYP3A

probe for clinical studies [76]. The plasma clearance of a single intravenous midazolam bolus has been widely used to assess CYP3A activity *in vivo*. In 1994, Thummel *et al.* [77] showed that midazolam clearance was strongly correlated with total CYP3A content as well as with the 1-OH-midazolam formation ratio, so midazolam clearance can be used as surrogate for CYP3A activity. Alternatively, the clearance of a continuous midazolam infusion has been proposed. Both methods [67] have been applied to study the ontogeny of midazolam in young children [56, 78].

#### 3.9.1.1 Preterm and term neonates

Four studies reported similar and relatively low CYP3A-mediated clearance values ranging from 1.17 to 2.03 mL/kg/min in neonates after intravenous midazolam administration (Fig. 1). Ages ranged from 26 to 42 weeks of gestational age, and from 0 to 10 days of postnatal age [70, 79–81]. In one of these studies, the total CYP3A-mediated clearance was directly proportional to birth weight, while postnatal age had no apparent effect on the kinetics of midazolam. This may have been due to the small postnatal age range (0–13 days). The effect of weight was most probably related to gestational age, as children with an EGA of 39 weeks

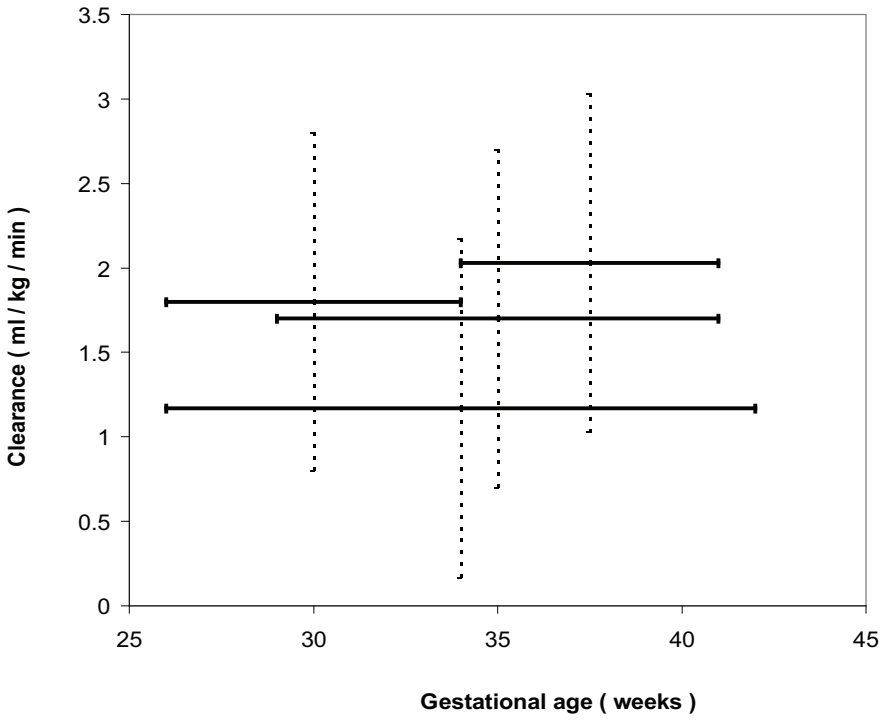


**Figure 1.** *In vivo* midazolam clearance values in (preterm) neonates (mean ± SD or range) from 26 to 41 weeks of gestational age [70, 79–81]. The average clearance value (±range, dotted vertical lines) over the investigated gestational age is plotted for each reported study.

had a 1.6 times higher clearance than younger children [79]. Although midazolam is also etabolized by CYP3A7 [56], only to a much smaller extent than by CYP3A4/5 [82], the reduced clearance of midazolam in the newborn may be explained by developmentally low CYP3A4/5 activity following birth [38].

### 3.9.1.2 Infants and children

In relatively healthy infants and older children, midazolam clearances were much higher (9.1–16.7 mL/kg/min) than in neonates (Fig. 2a, b) [83–88].

In the one study including an infant group (3 months to 2 years of age), this group showed the highest average clearance values expressed in mL/kg/min, suggesting either a possible peak in CYP3A activity [89] or a relative large liver (the liver size as a percentage of body weight is known to be higher in this age range than in older ages). None of these studies found an influence of age or body weight over the age ranges studied.

Unfortunately, for the age range where the largest surge in CYP3A would be expected (10 days to 3 months of age), data on midazolam clearance in relatively healthy children (e.g., ASA [American Society of Anesthesiologists] I surgical patients) are lacking. Interestingly, comparison of these clearance values of midazolam to those in adults (7.6–11 mL/kg/min) [90, 91] may suggest that the overall CYP3A activity does not change significantly after 2 years of age. This is also in agreement with the findings of Anderson and Larsson [92].

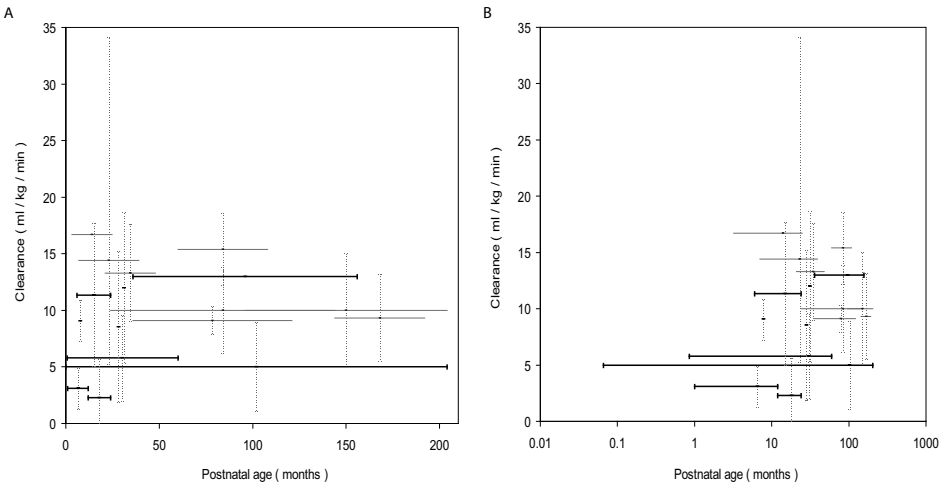


**Figure 2.** *In vivo* midazolam clearance values in children (mean ± SD or range) from 2 days to 17 years of age [71, 83–87, 89, 94, 95, 127, 139]: a normal age-scale, b logarithmic age-scale. Regular lines: relatively healthy children, thick lines: critically ill children. For each reported study, the average clearance value (±range, dotted vertical lines) is plotted over the investigated age range.

In contrast to the findings in relatively healthy children, two reports including critically ill children ranging in age from 2 days to 17 years [71] and from 26 days to 5 years [87], respectively, showed significantly lower midazolam clearances, with no reported influence of age-related changes (Fig. 2a, b). The reported median midazolam clearances were 5.0 and 5.8 mL/kg/min, respectively [71, 87]. In the first cohort, individual predicted midazolam clearances were related to the severity of organ failure, but not to C-reactive protein (CRP) as a marker of inflammation [93]. In addition, Hughes *et al.* [94] reported a low mean midazolam clearance value of 2.7 mL/kg/min in critically ill children between 1 months and 2 years of age, whereas that for critically ill children from 3 years up to 13 years of age was much higher at 13 mL/kg/min. In pediatric cardiopulmonary bypass patients (3 months to 8 years of age), midazolam clearance tended to be lower during bypass than at induction (average 8.7 *versus* 11.9 mL/kg/min) [95].

### *3.9.2 Midazolam oral clearance*

The apparent total oral clearance (CL/F) of orally administered midazolam has been proposed as a measure of combined hepatic and intestinal CYP3A activity in adults [3, 16]. In common with other substrates of CYP3A, such as cyclosporine, nifedipine, and verapamil, the oral bioavailability (F) of midazolam is significantly less than that predicted from the hepatic extraction ratio estimated from intravenous drug administration [96–100]. A substantial contribution of both gastrointestinal and liver metabolism to the first-pass elimination in humans has therefore been suggested [99].

Although the complementary DNA of hepatic CYP3A4 is similar to that of intestinal CYP3A4 [101], hepatic and intestinal activity do not appear to be regulated in concert. It is therefore unclear if intestinal CYP3A activity changes in parallel with hepatic CYP3A activity during ontogeny, but hepatic and intestinal ontogeny data suggest roughly the same pattern [62]. In adults, oral midazolam bioavailability is reported to be approximately 36% [102]. De Wildt *et al.* [75] reported high mean bioavailability of 49% in preterm infants 26–32 weeks gestational age and 3–10 days postnatal age), with a large range of 12–100%. Similar to the midazolam clearance after intravenous administration, midazolam oral clearance is markedly lower in preterm infants (0.04–0.93 L/kg/h) than in older children and adults 0.84–2.4 L/kg/h) [102, 103].

### *3.9.3 Cisapride*

Cisapride is a gastrointestinal prokinetic agent that stimulates the lower esophageal, gastric, small intestinal, and colonic motility. It was administered orally (unlicensed) children until withdrawn from the market in July 2000 [104]. No data on intravenous administration are available for this drug. The reported median cisapride oral plasma clearance rate was 538 mL/kg/h in 49 neonates between 24 and 42 weeks of gestational age, and 620 mL/kg/h in 35 children between 26 and 42 weeks of gestational age [105, 106]. More specifically, Kearns *et al.* divided the group 35 children into three postconceptional age groups. Cisapride clearance

increased with increasing post-conceptional age: from 542 mL/kg/h for an age range 28–36 weeks to 754 mL/kg/h for 36–42 weeks, and 846 mL/kg/h for 42–45 weeks [106], supporting the rapid increase in total CYP3A4 activity in the first weeks of life. In 17 healthy Caucasian adults aged between 22 and 48 years, the mean CL/F after oral administration 0.07 mg/kg cisapride was 510 mL/kg/h, meaning a higher clearance in mL/h due to the higher body weight in adults [69].

### *3.9.4 Dextromethorphan*

Using the urinary molar ratio of the CYP3A-catalyzed metabolite 3-hydroxymorphinan (3HM) to dextromethorphan (DX) (i.e., 3HM/DX), Blake *et al.* [107] studied CYP3A *in vivo* during consecutive healthy baby clinics from infants at age 2 weeks to 1 year; CYP3A activity slowly but steadily increased during this period. In response to this study [107], Johnson *et al.* pointed out that urinary drug/metabolite ratios are not pure markers of enzyme activity, being directly proportional to the unbound renal clearance of the parent drug and inversely proportional to partial clearance by the relevant enzyme [108], as shown in a previous investigation [109]. After correcting for renal function development, Johnson *et al.* established a more rapid increase in 3HM/DX ratio, reaching 72% of that of adults at the age of 1 year. In addition, Johnson *et al.* discuss that urinary drug/metabolite ratios as markers of enzyme activity may be sensitive to changes in urine pH [110]. At more acidic values, the lipidsoluble base dextromethorphan undergoes less tubular re-absorption and hence exhibits greater renal clearance [17]. Therefore, the urinary drug/metabolite ratio of DX is not an ideal marker of CYP3A activity.

### *3.9.5 Tacrolimus*

Several groups have studied the impact of age on the clearance of tacrolimus, an immunosuppressant drug mainly metabolized by CYP3A4/5 [64, 111–114]. This cannot be considered a true CYP3A probe as it is also a P-glycoprotein transporter (ABCB1) substrate. Nevertheless, considering its clearance is affected by genetic variation in CYP3A4/5, but not ABCB1, it is expected to largely reflect *in vivo* CYP3A activity [115]. Our group studied tacrolimus disposition in 48 kidney, 42 liver, and 39 cardiac transplant recipients aged 0.05–19 years [113]. In all transplant groups, the under-5 year olds needed significantly higher tacrolimus doses (expressed in mg/kg/12 h) than older children—on average twice as high. It is not clear why this should be so. It may be due to higher CYP3A activity, but may also be explained by allometric principles. Data on tacrolimus dosing in neonates are lacking, so the impact of CYP3A ontogeny on tacrolimus dosing requirements in the younger cohort is not known. As a tendency to perform transplantation in the neonatal period is apparent, dosing guidelines should account for possibly lower tacrolimus clearance.

Apart from age, tacrolimus disposition is also related to genetic variation in CYP3A5. Both kidney and heart transplant recipients who expressed CYP3A5 needed higher tacrolimus doses

than did non-expressors [113]. The importance of studying pharmacogenetic variation in the context of ontogeny is clearly illustrated by the interplay between age and CYP3A5 genotype in relation to tacrolimus disposition. While the sole impact of age (under/over 5 years) or CYP3A5 expressor status (yes/no) on tacrolimus dosing requirements is on average twofold, the combined impact (i.e., under 5 years of age and a CYP3A5 expressor *versus* over 5 years and a non-expressor) is on average fourfold [113]. Further research should aim to determine the impact of this variation on clinical outcome and establish individualized dosing guidelines.

## 4. CYP3A7 TO CYP3A4 TRANSITION

The presented *in vitro* and *in vivo* data unequivocally suggest a perinatal transition from CYP3A7 to CYP3A4 activity as well as a postnatal developmental pattern for CYP3A4 activity. In contrast, little is known about the factors that govern the transition from CYP3A7 to CYP3A4 activity after birth. The CYP3A4 gene expression is regulated by pregnane X receptor (PXR), constitutive androstane receptor (CAR), and steroid receptors, such as the glucocorticoid receptor (GR; NR3C1) and the vitamin D receptor, by its binding to the distal xenobiotic-responsive enhancer module (XREM) [116–119]. The hepatic nuclear factor-4a (HNF-4a), a protein mostly expressed in the liver, gut, kidney, and pancreatic beta cells, is critical for liver development [120] and increases the activity of the basal CYP3A4 reporter gene and a PXR/CAR-mediated reporter gene, which in turn mediates CYP3A4 gene expression [49, 121, 122]. Both CAR and PXR levels were relatively low but showed large interindividual variation in livers from both fetuses and infants up to 6 months of age. In contrast, retinoid X receptor (RXR) a levels were less variable and did not show an evident developmental pattern [12]. Fetal liver CYP3A7 and pediatric liver CYP3A4 expression levels were both related to receptor expression levels, but CYP3A4 more strongly than CYP3A7. The authors speculate that PXR and CAR expression levels are probably regulated by endogenous ligands (DHEA, DHEA-S) in line with CYP3A7's role in endogenous steroid biosynthesis, while the postnatal rise in PXR and CAR expression levels may reflect exposure to exogenous stimuli such as medication, diet, and xenobiotics. Interesting in this context is the observation by Blake *et al.* [73] that diet influences CYP3A4 activity in healthy infants. The *in vivo* CYP3A activity, as determined by the urinary 3-HM/DM ratio, rose faster in formula-fed infants than in breast-fed infants over the first 6 months of life.

DNA methylation may play a role in the differential expression of CYP3A7 and CYP3A4 before and after birth. A recent study showed much higher DNA methylation of important CYP3A4 regulatory sites in fetal compared to adult liver samples. Considering that DNA methylation can suppress gene expression, this hypermethylation in fetal livers may at least partially explain the absence of CYP3A4 before birth [123].

Unlike in CYP3A4 and CYP3A7, the distal XREM module is missing from the regulatory region of CYP3A5, which contains the ER6 motif and the DR3 motif that help induce PXR and

CAR [124, 125]. This may explain the lack of a correlation in pediatric livers between CYP3A5 expression and the nuclear factors PXR, CAR, RXRa, and HNFa [12].

## 5. *IN VITRO* TO *IN VIVO* EXTRAPOLATION

The delayed developmental expression pattern of CYP3A4 *in vitro* in neonates and infants [38, 42, 126] is largely consistent with the ontogeny of midazolam and cisapride clearance as well as dextromethorphan N-demethylation *in vivo* [79, 106, 107]. An exception is the low CYP3A4 protein estimations in children 5–15 years of age [40], which are not in agreement with the relatively high midazolam clearance in this age range [84–86, 127] as well as common experience with other CYP3A substrates. We can only speculate on this discrepancy. First, the disintegration of liver tissues after death and before storage may have contributed to lower than expected CYP3A protein levels. Furthermore, Stevens *et al.* [40] only had a limited number of samples from the 5–15 years old cohort available to them. Interestingly, lower midazolam clearance has been observed in critically ill children, which appears to be related to the severity of organ failure [128, 129]. Also the clearance of antipyrine, a nonspecific CYP probe, is significantly lower in children with sepsis and multi-organ failure when compared to children without sepsis [130].

This lower clearance is caused by downregulation of CYP3A activity by inflammation through interleukin-6 downregulation. Hence, the low CYP3A activity in children 5–15 years old may also be explained by their clinical characteristics [40]. The discrepancy between *in vitro* and *in vivo* results and the possible interplay with critical illness illustrates the limitations of extrapolating *in vitro* results to the real-life clinical situation. Moreover, the drug clearance mechanism is not similar in children and adults for all drugs. One of the classic examples of this is caffeine. This drug, which is a CYP1A2 substrate in older children and adults, is mainly cleared renally in neonates [67, 115]. Finally, age-related changes in protein binding, body composition, and liver size and flow may all contribute to age-related changes in pharmacokinetics, independent of drug metabolism, and should be taken into account when extrapolating *in vitro* data to the *in vivo* situation [131]. Notwithstanding the limitations of *in vitro* to *in vivo* extrapolation, many CYP3A-substrate drugs are being prescribed and developed for use in young children, so *in vitro* data from pediatric tissue could help develop age adjusted dosing guidelines. Physiologically based pharmacokinetic (PBPK) modeling may overcome many of the limitations involved in predicting the clearance of CYP3A substrates for groups of patients. Combining both drugrelated and physiology-related factors to model a drug's pharmacokinetics, this approach is theoretically appealing in children, as it allows physiological factors (such as size, liver blood flow, protein levels, body composition, drugmetabolizing enzyme activity) to be altered to reflect a "virtual" child of a certain age. However, the accuracy of the model-based

predictions depends on the quality and availability of *in vitro* studies presenting organ data and all other physiologic information for neonates up to adults. Several groups have used PBPK modeling to predict CYP3A clearance in children. Bjo¨rkman [132] developed a PBPK model and compared the results with aggregated published data on midazolam clearance. The model predicted midazolam clearance in neonates, older infants, and adults relatively well. This study is limited, however, in that only one neonatal study was available for validation, and that only (average) literature data were used to test the methods for their predictive performance. In another paper, reported *in vitro* data and *in vivo* clearance values for children were used to describe the ontogeny of CYP3A4 mediated clearance, showing that pediatric clinical trial development could greatly benefit from clearance scaling, particularly in guiding dosing regimens [133]. Anderson and Larsson [92] developed a PBPK model to describe the maturation of midazolam clearance from neonates to adulthood based on reported estimates at different ages. More recently, Johnson *et al.* [134] combined demographic, genetic, and physiological data from adults and children with *in vitro* data on human drug metabolism from children to predict the dispositions of 11 drugs over the whole pediatric age range, using the population-based simulator SimCYP. They were more successful than Bjorkman in predicting the drug disposition of midazolam when validated against available pediatric pharmacokinetic data. As PBPK models are based on postnatal age instead of body weight, it is not yet possible to predict drug disposition for preterm neonates, simply because a "negative age" cannot be set [134]. A possible solution to this problem is to implement information on gestational age, postnatal age and postmenstrual age into the model. More data than currently available would be needed to establish such a model. Also, more data on equivalent ages could be used; for example, a baby of age 10 weeks' postnatal age who was born at 30 weeks' gestational age is equivalent to a baby of age 40 weeks' gestational age at birth.

## 6. CONCLUSIONS AND FUTURE DIRECTIONS

It has been confirmed that CYP3A7 is the dominant isoforms before birth, but that its activity decreases to very low levels in the first week of life. *In vitro* studies show a slow rise in CYP3A4 activity after birth, but data from older children remain more ambiguous. CYP3A5 activity in livers expressing the gene appears to be relatively stable from fetal to adult life. The underlying mechanisms governing the transition from CYP3A7 to CYP3A4 still have not been elucidated. The majority of the *in vivo* studies, performed using specific substrate drugs, confirm that most of the developmental changes in CYP3A4 activity occur in the first months of life. Many *in vitro* and *in vivo* studies have included only a small number of patients, so results have been grouped over a large age range, which has often hidden the true ontogeny [56, 70, 79–81, 83, 84, 89]. In addition, other covariates such as severity of illness may have further obscured the ontogeny of CYP3A [128]. The influence of critical illness on CYP3A

activity is supported by *in vitro* and *in vivo* data on the impact of inflammation on CYP3A activity [129], and should be taken into account when using existing studies to predict CYP3A activity at a specific age. In addition to the influence of age-related changes on the variation in CYP3A activity in children, genetic variation should not be ignored. The most relevant of the known genetic polymorphisms is the CYP3A5*3 SNP, which is associated with reduced CYP3A5 activity. The interplay of CYP3A genotypes and age needs to be further elucidated, especially in neonates and infants, in whom the ontogeny has the greatest impact. More data are urgently required, especially in very young children. To resolve this issue, the so-called population approach could be used to study the maturational behavior of CYP3A using CYP3A substrates as *in vivo* probes, as it allows sparse and infrequently obtained data to be used [135]. These analyses may provide "pathway"-specific maturation functions that can be used to simulate the dispositions of drugs with similar disposition pathways using population pharmacokinetic analysis or in PBPK modeling. Importantly, these functions require careful probe drug selection and preferably intravenous administration to describe hepatic CYP3A ontogeny. As PBPK models cannot provide accurate predictions without proper model functions, it would be of great value to implement specific maturation functions in these models, based on original data obtained using the population approach. For example, just recently a semi-physiological glucuronidation function for young children was shown to predict the developmental clearance profile of UGT2B7 substrates [136]. In addition to a lack of proper model functions that describe enzyme maturation, poor predictions in adults as well as the selection of weak clinical studies can sometimes lead to incorrect predictions in children. Another major limitation of current PBPK models is the relative lack of reliable *in vitro* data. Efforts should also be made to collect pediatric tissues (including the clinical characteristics of the donor) across the whole pediatric age range, despite the inherent practical and ethical challenges. Improving PBPK models is an especially important task because of the interest of the European Medicines Agency and USFDA in this approach [137]. Innovative techniques such as liquid chromatography-mass spectrometry enable the simultaneous measurement of several drug-metabolizing enzymes and drug transporters, and could increase the yield of these studies in the context of the limited availability of tissue [138].

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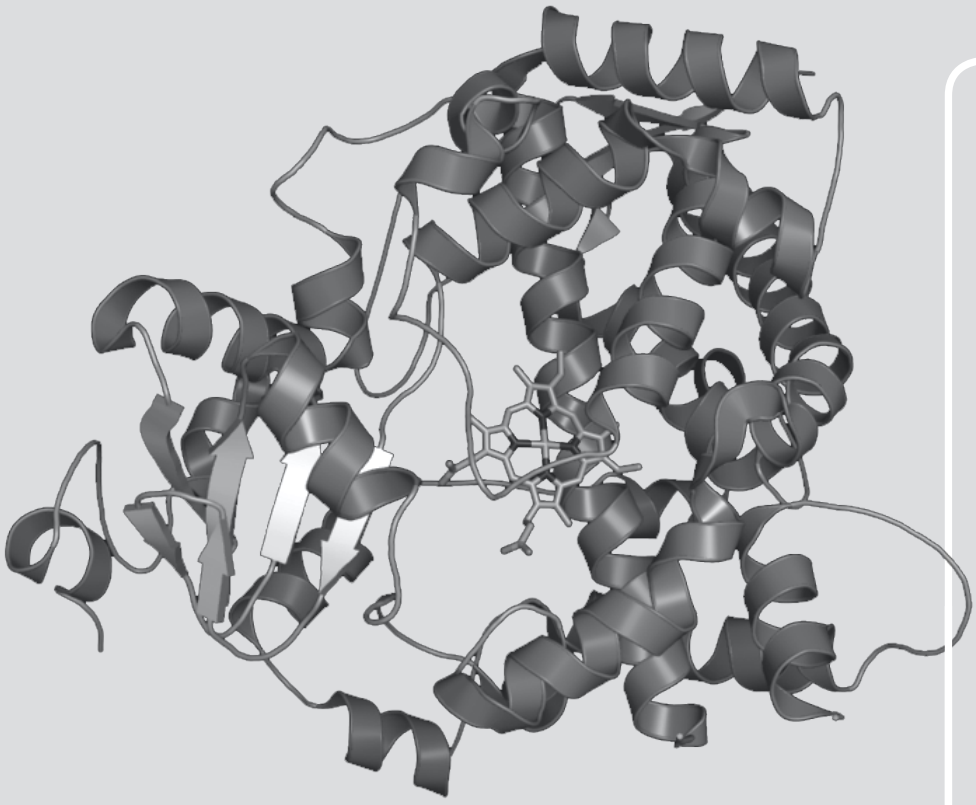
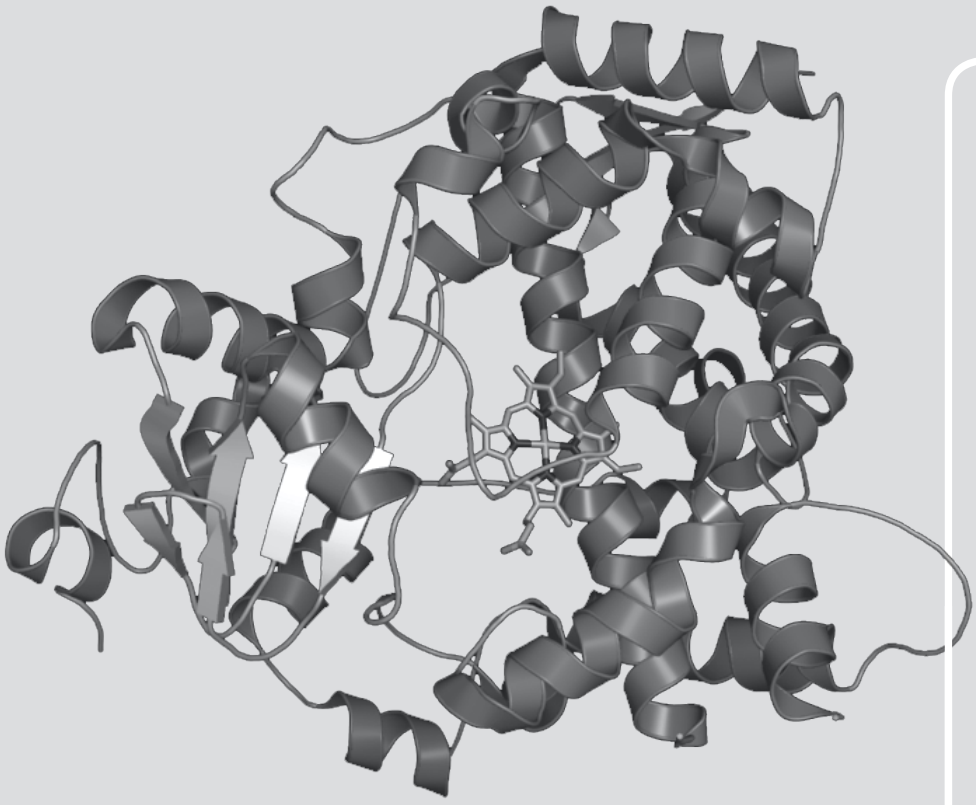


# Part II

## Development and application of a maturation function for CYP3A using midazolam as an *in vivo* probe



# 4 Critical illness is a major determinant of midazolam clearance in children aged 1 month to 17 years

Ince I, de Wildt SN, Peeters MY, Murry DJ, Tibboel D, Danhof M, Knibbe CA, Therapeutic Drug Monitoring, 2012 Aug;34(4):381-9

## ABSTRACT

Background: In children, a large variability in pharmacokinetics of midazolam, a cytochrome P450 3A4/5 (CYP3A4/5) enzyme substrate, has been described, which cannot be explained by agerelated changes alone. In this study, these age-related changes are studied in relation to other covariates to explain the variability in the pharmacokinetics of midazolam in children.
Methods: Population pharmacokinetic modeling was performed using a joint dataset of 3 studies conducted previously: study 1: pediatric intensive care patients requiring sedation in the intensive care unit; study 2: pediatric oncology patients undergoing an invasive procedure; study 3: otherwise healthy infants admitted for postoperative monitoring after elective major craniofacial surgery. Midazolam, 1-hydroxymidazolam, and 1-hydroxymidazolam glucuronide concentrations were considered to determine the pharmacokinetics of midazolam and metabolites using NONMEM 6.2. SimCYP pediatric simulator was used for simulation.
Results: Fifty-four children aged between 1 month and 17 years who received intravenous midazolam (bolus and/or continuous infusion) for sedation were included in this study. A reduction of 93% for CYP3A4/5 (midazolam to 1-hydroxymidazolam) and 86% for uridine diphosphate glucuronosyltransferase (1-hydroxymidazolam to 1-hydroxymidazolam glucuronide) mediated clearance was found in pediatric intensive care patients compared with the other 2 patient groups. We did not find a significant influence of age or bodyweight on CYP3A4/5-mediated total clearance. For uridine diphosphate glucuronosyltransferase–mediated clearance, bodyweight explained 41.5% of the variability.
Conclusions: From infancy to adolescence, critical illness seems to be a major determinant of midazolam clearance, which may result from reduced CYP3A4/5 activity due to inflammation. This may have important implications for dosing of midazolam and other CYP3A drug substrates in critically ill children.

## 1. BACKGROUND

Cytochrome P450 3A4 (CYP3A4) is the main active isoform of drug metabolizing enzymes in the human liver, which catalyzes the metabolism of about 50% of all drugs on the market. [1] In children, age-related changes in CYP3A4 activity are well recognized. CYP3A4 activity is low at birth with a surge of activity in the first months of life.[2] During infancy, overall CYP3A4 activity seems to exceed adult levels.[3]

Regarding the developmental pattern of CYP3A4, varying results exist when midazolam, which is a known and widely used model drug to investigate CYP3A4/5 activity, is studied.[4] A significant relationship between age or bodyweight and CYP3A4/5-mediated clearance of midazolam has been reported in pharmacokinetic studies in children between 1 month and 13 years of age and in those from 3 years to 10 years of age,[3, 5] whereas others did not find any such influence in children between 1 day and 17 years of age or between 3 months and 2 years of age.[6, 7] In addition, large interstudy and interindividual differences in reported midazolam clearances have been found, which cannot be explained by age-related changes alone.[6, 8–10] Typically, these studies have been performed in different patient cohorts. The study populations range from relatively healthy children undergoing procedural sedation to critically ill children who needed intensive care treatment.

To date, no comprehensive analysis has been performed on the basis of one combined dataset containing individual data from multiple subpopulations to investigate the relative contributions of age-related changes in CYP3A4/5 expression and function, compared with other factors, such as specific subpopulation or severity of disease (e.g., inflammation, infection, or organ failure [8, 9]) to the observed variation in midazolam clearance. The aim of this study was to analyze the interindividual variation in midazolam and metabolite pharmacokinetics in a heterogeneous group of children varying in age between 1 month and 17 years. In this analysis, both CYP3A4/5-mediated clearance (from midazolam to 1-OHmidazolam), uridine diphosphate glucuronosyltransferase (UGT)-mediated clearance (from 1-OH-midazolam to 1-OHmidazolam glucuronide), and renal clearance (elimination of 1-OH-midazolam glucuronide) were studied separately to identify the sources of variability for each step of metabolism.

## 2. METHODS

### 2.1 Patients and data

Children from 3 previous studies were included in the analysis. [6, 7, 10] Details of the 3 patient datasets are provided in Table 1.

**Table 1.** Overview of datasets used to develop the midazolam PK model.

| **Dataset** | | **Peeters, M.Y. *et al.* (2006)[7]** | **de Wildt SN *et al.* (2000)[10]** | **de Wildt SN *et al.* (2003)[6]** |
|---|---|---|---|---|
| **Patient population** | | Children after elective major craniofacial surgery | Pediatric oncology patients | PICU patients |
| **Indication for midazolam sedation** | | Postoperative sedation | Sedation for invasive procedure | Conscious sedation in intensive care |
| **Research Center** | | Erasmus MC – Sophia Children's hospital (Rotterdam, The Netherlands) | Purdue University (Indianapolis, USA) | Erasmus MC – Sophia Children's Hospital (Rotterdam, The Netherlands) |
| **Number of patients** | | 23 | 18 | 13 |
| **Number of samples** | | M: 198 1-OHM: 158 1-OHGMG: 175 | M: 82 1-OHM: 82 | M: 175 1-OHM: 129 1-OHMG: 171 |
| **Midazolam dose** | *median (range)* | 0.1 mg/kg iv loading dose, 0.05-0.2 mg/kg/hr infusion | 0.1 (0.03-0.53) mg/kg iv bolus dose | 0.1 mg/kg loading dose, 0.05-0.4 mg/kg/hr infusion |
| **Age (mos)** | *median (range)* | 11.5 (3.2 - 24.7) | 74 (39.0 – 194.0) | 68 (1.0 – 203.5) |
| **Number of patients in age-group** | *1-3 mos* | - | - | 3 |
| | *3-6 mos* | 4 | - | 1 |
| | *6-12 mos* | 11 | - | - |
| | *1-6 yrs* | 8 | 9 | 5 |
| | *6-12 yrs* | - | 4 | 2 |
| | *12-17 yrs* | - | 5 | 2 |
| **Bodyweight (kg)** | *median (range)* | 9.6 (5.1– 12) | 22.5 (12.6– 60.1) | 31 (3.6 – 60) |
| **Mechanically ventilated patients** | *Number of patients* | 2 | 0 | 10 |

*M* midazolam, *1-OHM* 1-hydroxy midazolam, *1-OHMG* 1-hydroxy midazolam glucuronide, *iv* intraveneus

## 2.2 Pharmacokinetic data analysis

Population pharmacokinetic analysis was performed using the first-order conditional estimation with η-ε interaction in NONMEM version 6.2, release 1.1 (GloboMax LLC, Hanover, MD).11 S-plus version 6.2.1 (Insightful software,Seattle, WA) with NM.SP.interface version 05.03.01 (by LAP&P, Leiden, The Netherlands) was used to visualize the data. According to routine procedures, model development was performed in 4 steps: (1) choice of the structural model, (2) choice of the error model, (3) covariate analysis, and (4) validation of the model. Discrimination between different models was made by comparison of the objective function. A decrease in the objective function of 3.84, corresponding to a value of $P < 0.05$, was considered statistically significant. Goodness-of-fit plots (observed *versus* individually predicted concentration, observed *versus* population predicted concentration, conditional weighted residuals *versus* time, and conditional weighted residuals *versus* population predictions) were used for diagnostic purposes. In addition, the confidence interval of the parameter estimates, the correlation matrix and visual improvement of the individual plots were used to evaluate the model. Furthermore, h-shrinkage as defined by Karlsson and Savic [12] was calculated for all model parameters for which interindividual variability was estimated, and overparameterization (ill conditioning) of the model was tested by calculating the condition number.[13]

## 2.3 Model development

The pharmacokinetic model used was a 2-compartment pharmacokinetic model for midazolam, parameterized in terms of central volume of distribution (V1), peripheral volume of distribution ($V_2$), intercompartmental clearance (Q), and CYP3A4/5-mediated clearance ($CL_1$), a 1-compartment model for 1-hydroxymidazolam, with central volume of distribution ($V_3$) modeled as a fraction of $V_1$, and UGT-mediated clearance ($CL_3$), and a 1-compartment model for 1-hydroxymidazolam glucuronide, with central volume of distribution ($V_4$), and renal clearance ($CL_4$). The individual value (post hoc value) of the parameters of the *i*th subject was modeled using equation: $Pi = P_{pop} \times e^{RV}$, where $P_i$ equals the individual or post hoc value of the parameters of the *i*th subject, and $P_{pop}$ is the population value of the parameter, and RV is the random variable for the interindividual variability of the parameter that is assumed to be a gaussian random variable, with mean zero and variance of $V_2$, assuming log-normal distribution. For midazolam and metabolites, the intraindividual variability was described with a proportional error model for all data, assuming a constant coefficient of variation over the entire concentration range. This means that, for the *j*th observed midazolam and metabolite concentration (Y) of the ith individual, $Y_{ij} = C_{pred,ij} \times (1 + RV_{1/2/3,ij})$, where $C_{pred}$ is the predicted midazolam or metabolite concentration and $RV_{1/2/3,ij}$ are random variables for midazolam ($_1$), 1-hydroxy-midazolam ($_2$), or 1-hydroxy-midazolam glucuronide ($_3$), with mean zero and variance $\sigma^2$.

## 2.4 Covariate analysis

Individual post hoc parameter estimates were plotted independently against the covariates to visualize potential covariate relationships. The following covariates were tested for all parameters: bodyweight, postnatal age, specific subpopulation, and mechanical ventilation (yes/no). Potential covariates were entered separately into the model using an allometric equation with an estimated exponent, an allometric equation with a fixed exponent, or a linear equation ($P_i = P_{pop} \times (COV_i/COV_{median})^K$, $P_{pop} \times (COV_i/ COV_{median})^{0.75}$, and $P_i = P_{pop} \times (COV_i/ COV_{median})$, respectively, where $P_i$ equals the individual or post hoc value of the parameters of the $_i$th subject, $P_{pop}$ is the population value of the parameter, COV is the concerned covariate, and K is the exponent. The influence of a covariate was tested for statistical significance by the use of the objective function. A P value < 0.005 was applied to evaluate the covariates in the forward inclusion, whereas on the other hand a more stringent P value of < 0.001 was used in the backward deletion. When ≥ 2 covariates were found to improve the model significantly, the covariate that showed the largest reduction of the objective function was included in the model. Additional covariates had to reduce this objective function further to be retained in the model. The choice of the model was evaluated further as discussed under 'pharmacokinetic data analysis' and 'model development.' This included individual and population parameter estimates *versus* the most predictive covariate in the model. [14]

## 2.5 Validation of the model

For the internal validation of the final model, first a bootstrap analysis was performed in S-plus, version 6.2.1 (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (by LAP&P, Leiden, The Netherlands). Two hundred datasets were resampled from the combined original datasets and refitted to the model.[15] Secondly, normalized prediction distribution errors (NPDE) were calculated [16] by using the NPDE package in R. [17, 18] For this method, the combined dataset that was used for building the model was simulated 1000 times, with inclusion of the interindividual variability and residual error.

## 2.6 Simulations

### *2.6.1 Simulation dosing regimen*

The parameter estimates from the final model were used for simulations to predict concentrations from the currently used traditional dosing schedule (iv bolus of 0.1 mg/kg, followed by an iv infusion of either 0.05 mg/kg/h for postoperative monitoring or 0.1 mg/kg/h for conscious sedation).[6, 7] The simulations were performed in S-plus, version 6.2.1 (Insightful software, Seattle, WA) with NONMEM SP.interface version 05.03.01 (by LAP&P, Leiden, The Netherlands), using the final parameter estimates in our model, which were the popula-

tion mean values. In addition, a new dosing schedule was designed, aiming for midazolam concentrations between 0.045 and 0.064 mg/mL for postoperative monitoring after major surgery and procedural sedation,7 and between 0.25 and 0.37 mg/mL for conscious sedation in pediatric intensive care (PICU) patients. [17] These concentrations correspond with previously reported adequate sedation levels of the respective population groups and are used for indicative purposes only, because no pharmacodynamic analysis was performed in our study. For the simulations, the population mean values of the parameters in the final population PK model were used.

*2.6.2 Simulations using SimCYP Pediatric Simulator*

The SimCYP Pediatric Simulator uses a physiologically based pharmacokinetic (PBPK) model, together with extensive libraries on demographics, developmental physiology, and ontogeny of drug elimination pathways. To explain the covariate relations for total midazolam clearance that were found in this study, 4 different scenarios that have been reported in the literature were simulated using the Pediatric simulator: a reduction in liver blood flow (50%), which may be a result of mechanical ventilation and/or illness, [18, 19] a reduction in total albumin content (90%), a combined reduction in CYP3A4 and CYP3A5 levels, in response to inflammation or infection (< 80%), [28] and a combination of reduction in liver blood flow (50%) and reduction in CYP3A4 and CYP3A5 levels (80%). These scenarios can be set manually in the program, for example, for the reduction in CYP3A4, the value that is available (137 pmole/mg of protein, representing a level in a healthy condition), is lowered by 80% to 27.4 pmole/mg of protein. One hundred time *versus* concentration profiles were simulated for 10 studies with 10 individuals. We compared the average simulated total midazolam clearance that was simulated for each scenario, with the average midazolam total clearance obtained without this scenario.

## 3. RESULTS

### 3.1 Patients

One thousand one hundred and seventy samples of midazolam and metabolite concentrations of 3 different datasets of 54 children, between 1 month and 17 years of age, were included for analysis. None of the patients received CYP3A inhibitors or inducers. A summary of the patient and data characteristics of the 3 studies is shown in Table 1.

### 3.2 Model development and covariate analysis

Using the pharmacokinetic model, it was found that the differences in all 3 clearance parameters between PICU patients, compared with the datasets of children after major cra-

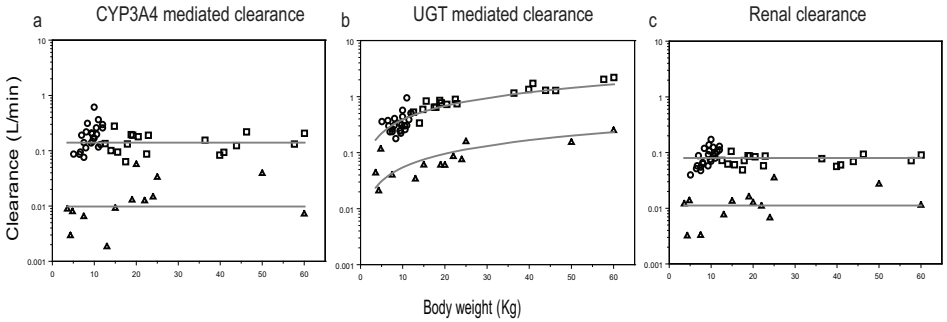



**Figure 1.** CYP3A4/5-mediated clearance of midazolam (M) to 1-hydroxymidazolam (1-OHM) *versus* bodyweight (BW) (A), UGT-mediated clearance of 1-OHM to 1-OHM glucuronide (1-OHMG) *versus* BW (B), and renal clearance of 1-OHMG *versus* BW, (C) in the final model. ○, major craniofacial surgery patients[7]; □, pediatric oncology patients [10]; △, PICU patients [6]; with a blackline as their population predicted values, respectively.

niofacial surgery and pediatric oncology patients together, were very large. This concerned the CYP3A4/5-mediated clearance step ($CL_1$), UGT-mediated clearance step ($CL_3$), and renal elimination step ($CL_4$) (Figs. 1A–C). As a result, the parameters of the model could not be estimated with appropriate significance when the data of all studies were analyzed together. To account for these differences, the influence of 2 covariates was tested: specific subpopulation (PICU patients *versus* children after elective major craniofacial surgery and pediatric oncology patients) and mechanical ventilation (yes/no).

First, for specific subpopulations, $CL_1$, $CL_3$, and $CL_4$ in PICU patients were estimated as a fraction of the corresponding clearance values in the dataset of children after elective major craniofacial surgery and pediatric oncology patients together (non-PICU patients). These additional parameters for specific subpopulations $fCl_1$, $fCl_3$, and $fCl_4$, significantly improved the model fit, resulting in a significant decrease in objective function of 129, 91, and 585, respectively ($P < 0.001$). After the addition of each additional parameter, the goodness-of-fit plots improved, and interindividual variability decreased to 73.4%, 82.6%, and 75.1% for $fCl_1$, $fCl_3$, and $fCl_4$, respectively. Secondly, implementation of mechanical ventilation (yes/no) did not result in significant improvement of the fit, except for implementation as a covariate for $Cl_3$, which resulted in a decrease in objective function of 14 points ($P < 0.001$).

After incorporation of specific subpopulation for $CL_1$, $Cl_3$, and $CL_4$, neither age nor bodyweight accounted for the remaining part of the observed interindividual variability of $CL_1$ and $CL_4$ ($P < 0.05$) (Figs. 1A, C). For $CL_3$, bodyweight in an exponential equation explained 41.5% of the remaining interindividual variability (Fig. 1B) and resulted in an additional reduction in objective function of 52 ($P < 0.001$). The estimated exponent for this bodyweight-based $CL_3$ equation was 0.81 (Table 2). For $V_1$, bodyweight was found to be linearly correlated as the exponent was not found to differ significantly from 1, with no differences between the 3 different datasets. For the other pharmacokinetic parameters, there were no significant

**Table 2.** Population parameter estimates obtained for the final midazolam PK model.

| Parameter | Model fit | | Bootstrap results | Explanation |
|---|---|---|---|---|
| | *Value (CV%)* | | *Value* | |
| $CL_{1,non\text{-}PICU}$ (L/min) | 0.16 | (9.1) | 0.15 | CYP3A4 mediated clearance of M in non-intensive care unit (non-PICU) patients |
| $fCL_{1,PICU}$ | 0.07 | (25.6) | 0.08 | Fraction of CYP3A4 mediated clearance of non-intensive care unit (non-PICU) patients |
| $V_{1,\ 11kg}$ (L) | 3.08 | (28.6) | 3.32 | Distribution volume of central compartment of M |
| $V_2$ (L) | 14.1 | (21.1) | 18.0 | Distribution volume of peripheral compartment of M, for a median individual of the population with 11 kg of bodyweight in $V_{1\ population}$ x (BW/11) |
| Q (L/min) | 0.30 | (19.1) | 0.30 | Intercompartmental clearance between central and peripheral compartment of M |
| $CL_{3,non\text{-}PICU,\ 11kg}$ (L/min) | 0.42 | (7.57) | 0.40 | UGT mediated clearance of 1-OH in non-intensive care unit (non-PICU) patients, for a median individual of the population with 11 kg of bodyweight in $CL_{3\ population}$ x $(BW/11)^K$ |
| $fCL_{3,PICU}$ | 0.14 | (18.5) | 0.14 | Fraction of UGT mediated clearance of non-intensive care unit (non-PICU) patients |
| *K* | 0.81 | (15.8) | 0.80 | Allometric exponent for UGT mediated clearance in $CL_{3i}$:$CL_3$ x $(BW/BW_{med})^k$) |
| $V_3$ (= fraction of $V_1$) | 0.07 | (46.2) | 0.09 | Distribution volume of 1-OHM compartment, as fraction central volume of M |
| $CL_{4,non\text{-}PICU}$ (L/min) | 0.08 | (25.6) | 0.07 | Renal clearance of 1-OHMG in non-intensive care unit (non-PICU) patients |
| $fCL_4 = fCL_{3,PICU}$ | 0.14 | (18.5) | 0.14 | Fraction of renal clearance of non-intensive care unit (non-PICU) patients |
| $V_4$ (L) | 2.95 | (23.8) | 2.93 | Distribution volume of 1-OHMG compartment |
| $\omega^2\ CL_1$ | 0.42 | (25.5) | 0.38 | Inter-individual variance in $CL_1$ |
| $\omega^2\ V_1 = V_4$ | 1.14 | (27.5) | 1.14 | Inter-individual variance in $V_1$ and $V_4$ |
| $\omega^2\ V_2$ | 1.88 | (21.6) | 2.3 | Inter-individual variance in $V_2$ |
| $\omega^2\ CL_3$ | 0.18 | (35.1) | 0.15 | Inter-individual variance in $CL_3$ |
| $\omega^2\ CL_4$ | 0.26 | (26.8) | 0.24 | Inter-individual variance in $CL_4$ |
| $\omega^2\ CL_1$-$CL_4$ interaction | 0.22 | (27.3) | 0.20 | Interaction between the inter-individual variance in $CL_1$ and $CL_4$ |
| $\omega^2\ V_{1/4}\ V_2$ interaction | 0.82 | (45.6) | 0.83 | Interaction between the inter-individual variance in $V_{1/4}$ and $V_2$ |
| $\sigma^2_{\ proportional}$ (M) | 0.18 | (18.6) | 0.17 | Residual variance for M |
| $\sigma^2_{\ proportional}$ (1-OHM) | 0.21 | (16) | 0.20 | Residual variance for 1-OHM |
| $\sigma^2_{\ proportional}$ (1-OHGM) | 0.14 | (23.9) | 0.13 | Residual variance for 1-OHMG |

M midazolam, *1-OHM* 1-hydroxy midazolam, *1-OHMG* 1-hydroxy midazolam glucuronide.

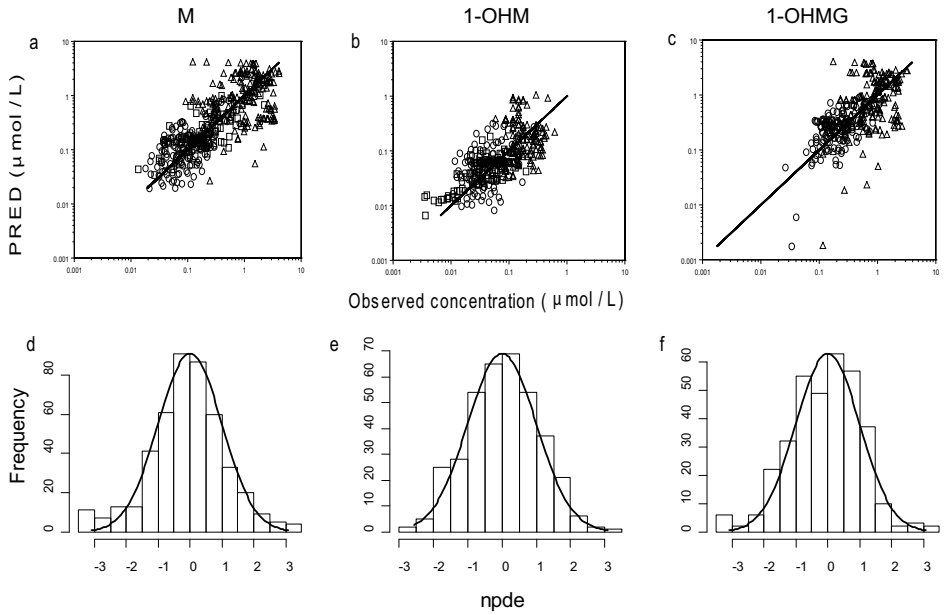



**Figure 2.** Visual diagnostics of the final model (upper panels; A–C) and internal validation (NPDE) of the final model (lower panels; D–F). *Upper panels*: Concentrations predicted by the model (PRED) *versus* the observed concentrations for midazolam (*M*) (A), 1-hydroxymidazolam (*1-OHM*) (B), and 1-OHM glucuronide (*1-OHMG*) (C). ○, major craniofacial surgery patients [7]; □, pediatric oncology patients [10]; △, PICU patients. [6] *Lower panels*: Histograms for the NPDE frequency distribution in the datasets for M (D), 1-OHM (E), and 1-OHMG (F). The *solid line* indicates a normal distribution.

differences between the 3 different populations, nor was there any influence of age or bodyweight. In Table 2, the pharmacokinetic parameter values along with their confidence intervals and interindividual variability of the final model are shown, together with the results of the bootstrap validation.

Figures 2A–C show that the population predicted concentrations are equally spread around the line of identity, as represented by the black solid line. For the interindividual variability, all values of η-shrinkage were <20%, which indicates that the individual parameter estimates are reliable. [12] The calculated condition number of 563 of the final model was well below the critical value for the indication of ill conditioning of 1000. [13]

The histograms of the NPDE for midazolam, 1-hydroxymidazolam, and 1-hydroxymidazolam glucuronide, as depicted in Figures. 2D–F, illustrate the distribution of the NPDEs, with means close to zero and variances slightly >1 (-0.07, 0.03, -0.08 for the means, and 1.40, 1.06, and 1.18 for the variances, respectively).

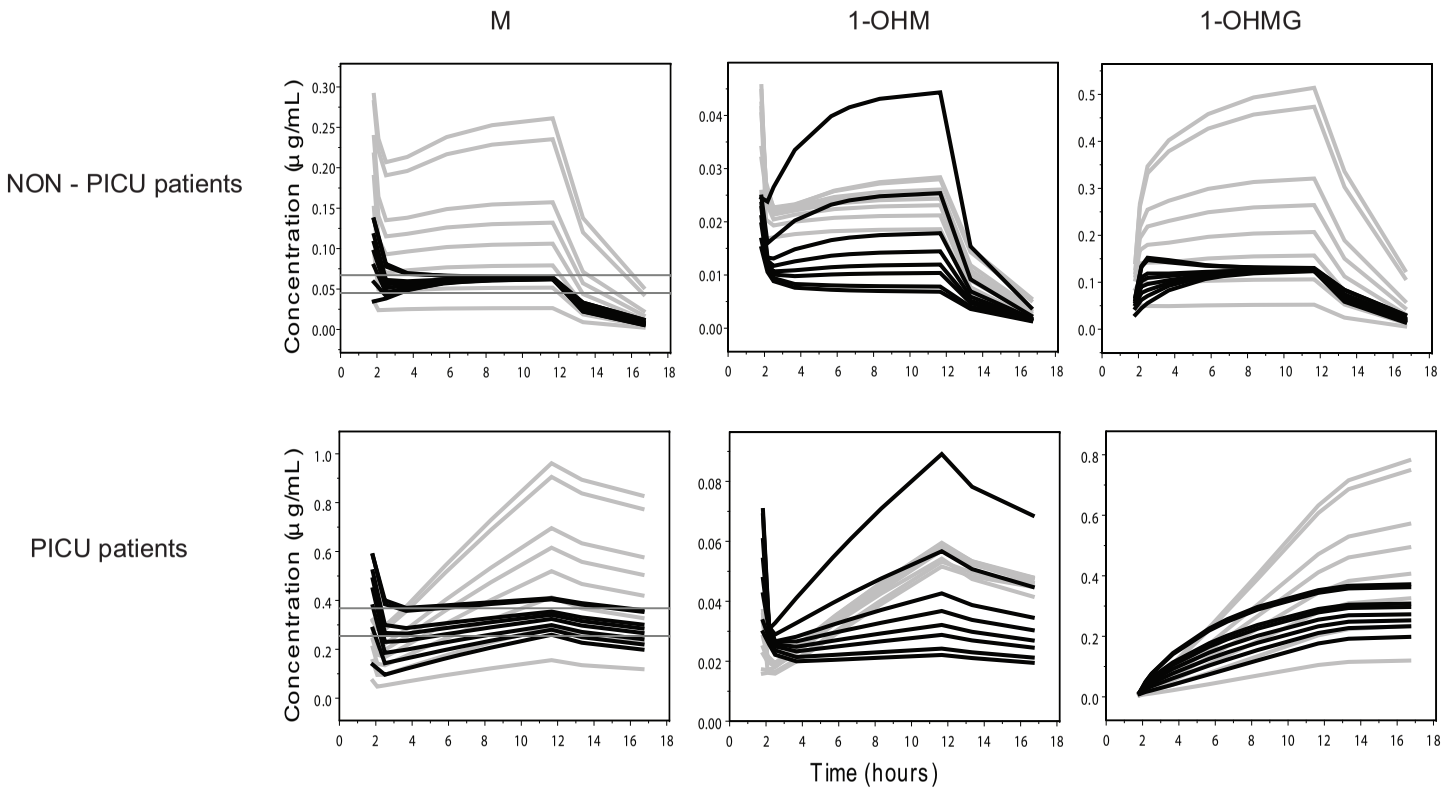



**Figure 3.** Simulated concentration *versus* time plots for midazolam (*M*), 1-hydroxymidazolam (*1-OHM*), and 1-OHM glucuronide (*1-OHMG*), using 2 different dosing schemes (bolus followed by infusion for 10 hours), in 8 children ranging from 5 to 60 kg of bodyweight. Upper panels: Concentrations of noncritically ill patients after 0.1 mg/kg bolus and 0.05 mg/kg/h (*gray lines*) and 0.05 mg/kg bolus and 0.6 mg/h (*black lines*). Gray horizontal reference lines: therapeutic M concentration range in the craniofacial surgery patients (3 months to 2 years of age). [7] Lower panels: Concentrations of PICU patients, after 0.1 mg/kg and 0.1 mg/kg/h (*gray lines*) and 0.2 mg/kg iv bolus and 0.5 mg/h (*black lines*). The gray horizontal reference lines: therapeutic M concentration range for PICU patients. [6]

No trends in time or concentration for midazolam and 1-hydroxymidazolam glucuronide concentrations were observed (figure not shown). A slight overprediction was observed (figure not shown) in both time and concentrations for 1-hydroxymidazolam concentrations.

## 3.3 Simulations

### *3.3.1 Simulation of new dosing strategy*

As illustrated in Figure 3, based on the traditional dosing regimen of 0.1 mg/kg, followed by an iv infusion of either 0.05 mg/kg/h for postoperative monitoring or 0.1 mg/kg/h for sedation, [6, 7] large differences in midazolam and metabolite concentrations between individuals can be anticipated in children between 5 and 60 kg. A considerably narrower range of concentrations of midazolam, 1-hydroxymidazolam and 1-hydroxymidazolam glucuronide can be expected, when a uniform dose of midazolam is administered. This dosing schedule consists of a loading dose of 0.2 mg/kg, followed by a uniform continuous iv infusion of 0.6 mg/h for infants after elective craniofacial surgery and pediatric oncology patients, and a loading dose of 0.2 mg/kg iv bolus, followed by a continuous iv infusion of 0.4 mg/h for the PICU patients.

### *3.3.2 Using SimCYP pediatric simulator*

Figure 4 shows the results from scenario simulations using the SimCYP Pediatric Simulator that reflect a physiological state that may occur upon admission to the PICU unit. A major impact was shown of reduced CYP3A4 and CYP3A5 abundance, and of reduced liver blood

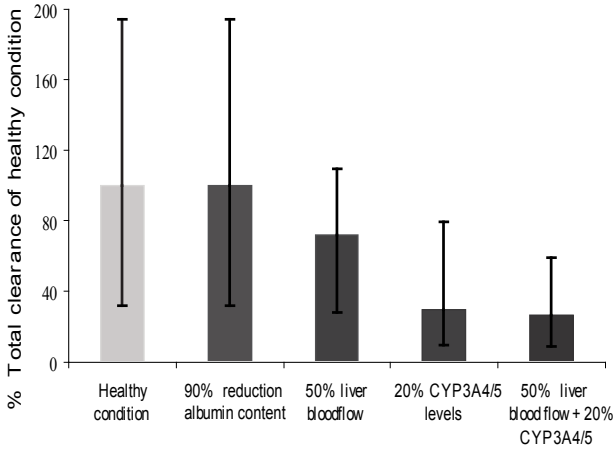



**Figure 4.** SimCYP scenario simulations with total midazolam clearance as a percentage (±SD) of typical healthy children (light gray), *versus* individuals with 90% reduction in total albumin content, 50% reduction in liver blood flow, 80% reduction in CYP3A4/5 levels and a combined reduction of 50% liver blood flow and CYP3A4/5 levels, respectively (*dark gray*).

flow, with a decrease in total midazolam clearance of 70% and 30%, respectively. We did not observe an effect of a reduction in albumin abundance on total midazolam clearance (Fig. 4).

## 4. DISCUSSION

In this study, we performed a comprehensive analysis based on a joint dataset of 3 different pharmacokinetics studies on midazolam and metabolites in pediatric patients. In this population pharmacokinetic study, we showed that critical illness is a major determinant for midazolam clearance in children between 1 month and 17 years of age.

Several other studies have reported lower clearance values in critically ill children. However, in contrast to our analysis, those studies comprised small age or weight range in the investigated population, so it remained unclear whether these lower clearances were due to differences in disease state or to a combination of disease state and age-related changes in CYP3A4 activity. [20–22] In our study, we were able to distinguish between these factors and found that all 3 clearance steps were lower in PICU patients than in children after elective major craniofacial surgery and the oncology patients. An explanation may be that CYP3A activity is regulated by inflammation, which has been reported in different animal and *in vitro* human studies. [23–25] Also, in patients with inflammatory states, drug metabolism is reported to be reduced, with consequent reduced drug clearances. [26, 27] In one study in critically ill children with sepsis and multiple organ failure, overall drug metabolism (using a nonspecific CYP450 substrate) was significantly decreased.[9] Our data are in line with these results, suggesting that critical illness and associated inflammation may result in highly significant downregulation of CYP3A activity.

We speculate that the discrepant findings between our PICU patients and the postoperative patient population can be explained as follows: In PICU patients, an ongoing inflammatory response may result in CYP3A4 gene repression. [8] PICU patients are seriously ill, even before entering the PICU, perhaps for several preceding days. [8] As the degradation half life of CYP3A4 is between 26 and 144 hours, and of CYP3A5 is around 36 hours, possibly the available CYP3A4 and CYP3A5 enzymes are depleted in these patients, maybe not at admission, but at least in the days after admission. [28, 29] Children who are admitted for postoperative sedation and monitoring after elective craniofacial surgery may also have CYP3A gene repression resulting from inflammation related to the surgical procedure. However, both the duration of their intensive care stay and inclusion in the study, that is, a maximum of 16 hours after surgery, is shorter than the degradation half life of the CYP3A4 and CYP3A5 enzymes. As a result, in these patients, sufficient levels of active CYP3A4 and CYP3A5 enzymes for oxidizing midazolam are still available. This is further supported by Kumar et al [30] who reported that

inducing a hemorrhagic shock *in vivo*, using a porcine model, did not significantly influence the CYP3A4/5-mediated clearance of intravenously administered midazolam. In their study, 40% of the available blood volume was removed from healthy pigs to induce hemorrhagic shock. Their finding supports the unchanged CYP3A4/5-mediated clearance values of midazolam in children undergoing craniofacial surgery that we found, [7] despite their blood loss during surgery. Blood loss during craniofacial surgery can reach average values of 24% of the total blood volume. [31] In addition, according to standard practice, red blood cells, platelets, and plasma are administered to the patients immediately upon surgical blood loss. As for the oncology patients, they were not in the immediate induction phase nor critically ill, for example, as a result of neutropenic fever. Therefore, in this population, CYP3A4/5 downregulation was also unlikely at the time of the study. It is a limitation of our study that we did not have data on inflammatory markers such as CRP and/or IL6. A prospective study in which these markers are measured could possibly support our hypothesis of downregulation of CYP3A activity as an important reason for the observed lower midazolam clearance.

To further explore the underlying mechanisms governing lower midazolam clearance in PICU patients, we used the SimCYP pediatric simulator. In addition to CYP3A4/5 downregulation, also a reduction in liver blood flow, influenced by mechanical ventilation and/or illness, [18, 19] and a reduction in total albumin has been reported in critically ill patients. [32] From these scenario simulations, as depicted in Figure 4, no influence of a reduction in total albumin content could be observed, even with a non-physiological decreased value to 10% of total available albumin levels. This may be explained by the fact that midazolam is a intermediate extraction ratio drug (0.3 – 0.7), which is hardly affected by its binding to serum albumin. [33] However, a 50% decrease in liver blood flow showed a substantial decrease in midazolam clearance compared with individuals without this reduction. Yet, the influence of reduced liver flow became less important when CYP3A4 and CYP3A5 abundance decreased to 20%, which by itself resulted in a major decrease in midazolam clearance. This average reduction of about 75% is close to what we observed in our analysis. Possibly a combination of these factors causes a maximal decrease in clearance in PICU patients.

In our cohort of patients between 1 month and 17 years of age, we could not identify any influence of age or bodyweight on CYP3A4/5-mediated clearance, using either allometric or linear functions. A possible explanation could be that the 3 individuals in the age range between 1 and 3 months, and 5 individuals between 3 and 6 months of age (Table 1), were not enough to decipher an impact of age-related changes on CYP3A4/5 activity in this age range. A lack of homogeneous distribution of age across the different patient cohorts may also have been a potential shortcoming to identifying a correlation with age. However, as even the youngest individuals had similar clearance values compared with individuals aged up to 17 years, this would suggest that the CYP3A4/5 activity has already reached adolescent

levels at the age of 1 month. The addition of more datasets, including children both older and younger than 1 month, could further delineate our findings. Recently, a maturation model for midazolam clearance has been reported, in which the maturational behavior of CYP3A4/5 across the pediatric age range was described using previously published pharmacokinetic parameters. [34] Johnson et al [35] predicted midazolam clearance using PBPK models. In contrast to our study, both studies showed an age-related increase of total midazolam clearance, using an allometric scaling function. The main difference between our study and theirs is that their models are based on PBPK assumptions and are validated using average reported midazolam clearance values. As our model is based on individual data points, and reflects true midazolam concentrations at different ages, we believe that our model may be a better reflection of the midazolam clearance across the pediatric age range. In addition, the literature studies that were used for their analysis contain both healthy and non-healthy patients, for which no correction was applied, which may also have influenced their results. Also, Björkman predicted midazolam clearance in children using different calculation methods, but showed that there are many uncertainties in the *in vitro* to *in vivo* extrapolation. [36] In contrast, for UGT-mediated clearance, we did find an age-related increase. This relationship may reflect the ontogenic pattern of a combination of UGT1A4, 2B4, and 2B7. An age-related increase in glucuronidation clearance is also found for morphine glucuronidation by UGT2B7, in preterm neonates, infants and children younger than 3 years of age. [37]

Simulations based on our final pharmacokinetic model showed that a uniform dosing schedule in milligrams per hour, with a consequently higher milligrams per kilogram dose in the younger compared with older children, results in less variation in midazolam and metabolite concentrations compared with the general practice of dosing continuous infusions on a milligrams per kilogram per hour basis. This finding may explain the observation of frequent failure to sedate PICU patients between 6 months and 2 years of age. [38] However, uniform dosing schedules are based on the assumption that the concentration–effect (PK-PD) relationship in children aged 1 month–17 years is not significantly different, which has not yet been investigated.

Because of the large weight range in our joint dataset, bodyweight was implemented in a linear equation on the central volume of distribution of midazolam and 1-hydroxymidazolam into the structural model. The high remaining interindividual variability on the volumes of distribution ($V_1$–$V_4$), could not be explained any further by bodyweight or age during the covariate analysis. Information on the lean bodyweight of the individuals may have explained more of the variability in these volumes between the individuals. [39] However, height was not available in the datasets. In addition, the estimated values for $V_3$ and $V_4$ may have been affected by the assumption that 100% of midazolam is metabolized to 1-hydroxy-midazolam, and must be exercised with caution, because accurate estimates can only be obtained by separate administration of the metabolite.

## 5. CONCLUSIONS

In conclusion, we found that in children between 1 month and 17 years of age, instead of age or bodyweight, critical illness seems to be the major determinant of the CYP3A4/5-mediated clearance of midazolam. For the UGT-mediated clearance of 1-hydroxymidazolam, we found an age-related increase. SimCYP simulations suggest that CYP3A4 and CYP3A5 enzyme abundance and/or liver blood flow, but not albumin concentration, are the most likely determinants for these findings.

## ACKNOWLEDGMENTS

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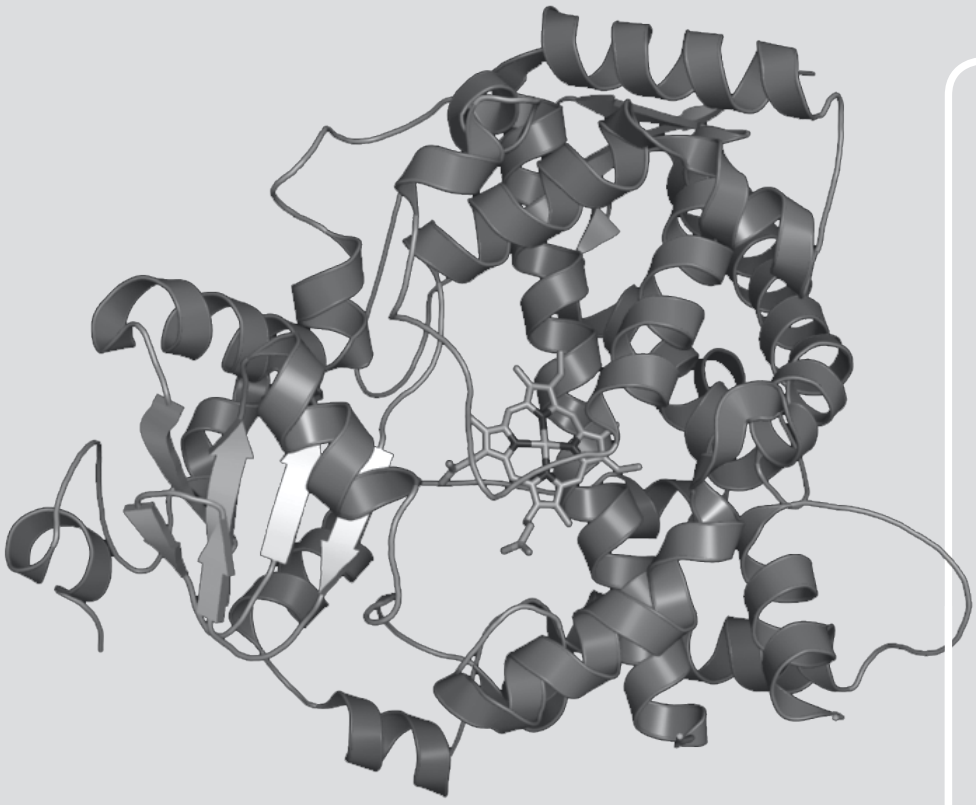

# 5 A novel maturation function for clearance of the cytochrome P450 3A substrate midazolam from preterm neonates to adults

Ince I, de Wildt SN, Wang C, Peeters MY, Burggraaf J, Jacqz-Aigrain E, van den Anker JN, Tibboel D, Danhof M, Knibbe CA, Clinical Pharmacokinetics, 2013 Jul;52(7):555-65

## ABSTRACT

Background and objective: Major changes in cytochrome P450 (CYP) 3A activity may be expected in the first few months of life with, later, relatively limited changes. In this analysis we studied the maturation of *in vivo* CYP3Amediated clearance of midazolam, as model drug, from preterm neonates of 26 weeks gestational age (GA) to adults.

Methods: Pharmacokinetic data after intravenous administration of midazolam were obtained from six previously reported studies. Subjects were premature neonates (n = 24; GA 26–33.5 weeks, postnatal age (PNA) 3–11 days, and n = 24;GA 26–37 weeks, PNA 0–1 days), 23 children after elective major craniofacial surgery (age 3–23 months), 18 pediatric intensive-care patients (age 2 days–17 years), 18 pediatric oncology patients (age 3–16 years), and 20 healthy male adults (age 20–31 years). Population pharmacokinetic modeling with systematic covariate analysis was performed by use of NONMEM v6.2.

Results: Across the entire lifespan from premature neonates to adults, bodyweight was a significant covariate for midazolam clearance. The effect of bodyweight was best described by use of an allometric equation with an exponent changing with bodyweight in an exponential manner from 0.84 for preterm neonates (0.77 kg) to 0.44 for adults (89 kg), showing that the most rapid maturation occurs during the youngest age range.

Conclusions: An *in vivo* maturation function for midazolam clearance from premature neonates to adults has been developed. This function can be used to derive evidencebased doses for children, and to simulate exposure to midazolam and possibly other CYP3A substrates across the pediatric age range in population pharmacokinetic models or physiologically based pharmacokinetic models.

## 1. BACKGROUND

Cytochrome P450 (CYP) 3A is the most abundant CYP enzyme in the human liver [1]. It is involved in the metabolism of over half of all metabolized drugs [2]. Large inter-individual and intra-individual variation has been shown for CYP3A, resulting in inter-individual differences in the clearance of CYP3A substrate drugs. An important factor explaining variation in CYP3A activity is age. However, the exact ontogenetic pattern of the CYP3A isoforms CYP3A4 and CYP3A5 *in vivo* is still unclear [3– 7]. This information gap hampers the development, for children, of individualized guidelines for dosing of CYP3A4/5 substrate drugs. To define the *in vivo* activity of CYP3A4/5, surrogate probes that correlate with actual enzyme activity can be used [8]. The best validated CYP3A4/5 probe is midazolam, which has been used extensively among adults and children [9, 10].

For CYP3A4/5-mediated clearance of midazolam in children between one month and 17 years of age, critical illness proved a more significant covariate than bodyweight [11]. However, particularly in the first days and weeks of life, CYP3A4/5 activity may be expected to be low on the basis of *in vitro* data and *in vivo* reports of reduced clearance of CYP3A substrates in neonates [6, 7, 12–15]. Therefore, we report here on the maturation of *in vivo* CYP3A-mediated clearance of midazolam across the entire human life span, using a dataset consisting of six midazolam studies in which the pharmacokinetics were studied in populations ranging in age from premature neonates to adults. The resulting maturation function may be used as a basis for evidence-based dosing of midazolam and, potentially, other CYP3A substrates in population pharmacokinetic or physiologically based pharmacokinetic (PBPK) models.

## 2. METHODS

### 2.1 Patients and data

Individual data from six previous studies were included in the analysis [3, 6, 16–19]. Individuals were premature neonates (two datasets: n = 32; gestational age (GA) 26–33.5 weeks, postnatal age (PNA) 3–11 days, and n = 24; GA 26–37 weeks, PNA 0–1 days), 23 children after elective major craniofacial surgery (age 3–23 months), 18 pediatric intensive care (PICU) patients (age 2 days–17 years), 18 pediatric oncology patients (age 3–17 years), and 20 healthy male adults (age 20–31 years). Details of the datasets are listed in Table 1.

**Table 1.** Overview of the datasets

| Dataset | de Wildt SN *et al.*[6] (2001) | Jacqz-Aigrain E. *et al.*[18] (1994) | Peeters, M.Y. *et al.*[3] (2006) | de Wildt SN *et al.*[16] (2003) | de Wildt SN *et al.*[17] (2000) | van Gerven J.M.A. *et al.*[19] (1997) |
|---|---|---|---|---|---|---|
| **Patient Population** | Preterm neonates | Preterm neonates with respiratory distress (RDS) syndrome | Children after elective major craniofacial surgery | Pediatric intensive care patients | Oncology patients | Male adults |
| **Indication for midazolam sedation** | Sedation for invasive procedure in Intensive Care | Mechanical ventilation in Intensive Care | Postoperative sedation | Conscious sedation in Intensive Care | Sedation for invasive procedure | Healthy volunteers |
| **Number of Patients** | 24 | 24 | 23 | 18 | 18 | 20 |
| **Postnatal Age** *median (range)* | 5 days (2.9-11) | 0 days (0-1) | 11.5 months (3.2-24.7) | 38.5 months (0.03-203.5) | 6.1 years (3.2–16.2) | 24 years (20-31) |
| **Gestational Age** *median (range)* | 28.3 weeks (26-33.6) | 32 weeks (26-37) | - | - | - | - |
| **Bodyweight** *median (range)* | 1.07 kg (0.77-1.6) | 1.64 kg (0.96-3.7) | 9.6 kg (5.1– 12) | 14 kg (2.8-60) | 22.5 kg (12.6– 60.1) | 72.5 kg (64-89) |
| **Mechanical ventilation** *N/* $N_{TOTAL}$ | 13 / 24 | 24 / 24 | 2 / 23 | 15 / 18 | 0 / 18 | 0 / 20 |
| **Midazolam Dose** *median (range)* | 0.1 mg/kg iv infusion in 30 minutes | 60 µg/kg/hr iv infusion If GA < 33w → after t > 24hr 30 µg/kg/hr | 0.1 mg/kg iv loading dose, 0.05-0.2 mg/kg/hr infusion | 0.1 mg/kg loading dose, 0.05-0.4 mg/kg/hr infusion | 0.1 (0.03-0.53) mg/kg iv bolus dose | 0.1 mg/kg iv infusion in 20 minutes |
| **Number of Samples** | 155 | 63 | 198 | 233 | 82 | 336 |

*iv* intravenous

## 2.2 Analysis of pharmacokinetic data

Population pharmacokinetic data analysis was performed using first-order conditional estimation (FOCE) with g–e interaction in NONMEM version 6.2, release 1.1 (Globo- Max LLC, Hanover, MD, USA) [20]. S-plus version 6.2.1 (Insightful Software, Seattle, WA, USA) with NM.SP.interface version 05.03.01 (_LAP&P, Leiden, The Netherlands) was used to visualize the data. Model development was performed in four steps: 1. choice of the structural model; 2. choice of the error model; 3. covariate analysis; and 4. validation of the model.

Discrimination between different models was by comparison of the objective function. A decrease in the objective function of 3.8, corresponding to a value of $p < 0.05$, was considered statistically significant. Goodness- of-fit plots (observed *versus* individually predicted

concentration, observed *versus* population predicted concentration, conditional weighted residuals *versus* time, and conditional weighted residuals *versus* population predictions) of all data, stratified per dataset, were used for diagnostic purposes. In addition, the confidence interval for the estimated parameters, the correlation matrix, and visual improvement of the individual plots were used to evaluate the model. Furthermore, η shrinkage, as defined by Karlsson *et al.* [21], was calculated for all model parameters for which inter-individual variability was estimated; overparameterization (ill-conditioning) of the model was tested by calculating the condition number [22].

## 2.3 Model development

For the structural model of midazolam, one, two, and three compartment models were tested. The previously reported effect of critical illness on CYP3A-mediated clearance of midazolam [11] was incorporated in the model, implying reduction of midazolam clearance in critically ill patients (datasets for premature neonates and PICU patients) compared with non-critically ill children and adults (datasets for children after major craniofacial surgery, pediatric oncology patients, and adults). The individual values (post-hoc values) of the parameters for the *i*th subject were modeled by use of Eq. 1, where P*i* equals the individual or post-hoc value of the parameter for the ith subject, and P*pop* is the population value of the parameter. The randomvariable (RV) is assumed to be a Gaussian randomvariable with a mean of zero and variance of ω2, assuming log-normal distribution (Eq. 1).

$$P_i = P_{pop} \times e^{RV} \quad (1)$$

$$Y_{ij} = C_{pred,ij} \times (1 + RV_{ij}) \quad (2)$$

The intra-individual variability was described by use of a proportional error model for all data, assuming a constant coefficient of variation over the entire concentration range, shown in Eq. 2, where j is the observed midazolam concentration (Y) for the ith individual, $C_{pred}$ is the predicted midazolam concentration, and $RV_{ij}$ is the random variable for midazolam, with a mean of zero and variance σ2.

## 2.4 Covariate analysis

Individual post-hoc parameters were plotted independently against the covariates to visualize potential covariate relationships. Covariates tested for all variables were: bodyweight, postnatal age, gestational age, study population, and mechanical ventilation (yes/no). Continuous covariates were separately entered into the model by use of a linear function (Eq. 3) or an allometric equation (Eq. 4), where P*i* equals the individual or post hoc value of

the parameter for the ith subject, P*pop* is the population value of the parameter, COV is the appropriate covariate, and K is the exponent, which may be estimated or fixed at 0.75.

(3) $P_i = P_{pop} \times (COV_i / COV_{median})$

(4) $P_i = P_{pop} \times (COV_i / COV_{median})^K$

(5) $P_{pop} \times (COV_i / COV_{median})^{BDE}$

(6) $BDE = coeff \times BW^{exp1}$

Alternatively, an allometric equation with an exponent that changes with bodyweight [23] was tested by Eq. 5, in which BDE represents the bodyweight-dependent exponent. For BDE, a sigmoidal function [23] and an exponential function (Eq. 6) with Coeff as a coefficient, BW as bodyweight, and exp1 as an exponent) [24] were tested.

(7) $P_i = P_{pop}$

(8) $IF(subgroup=y), P_i = P_{pop} \times (factor)$

Categorical covariates [e.g. mechanical ventilation (yes/ no) or study population (critically ill/healthy)] were tested by use of "IF" statements in which the parameter for one subgroup was estimated as a multiple of the parameter estimated for the other subgroup, as shown in Eqs. 7 and 8. In these equations, factor is the multiplication factor, and y is one of the two subgroups.

The effect of a covariate was tested for statistical significance by use of the objective function. P values <0.005 (decrease of objective function of 7.8 points) were used to evaluate the covariates in the forward inclusion whereas a more stringent p value of <0.001 (decrease of objective function of 10.83 points) was used in the backwards deletion step. When two or more covariates were found to significantly improve the model, the covariate that resulted in the largest reduction of the objective function was included in the model. Additional covariates had to reduce this objective function further to be retained in the model. The choice of the model was further evaluated as discussed in the sections "Analysis of Pharmacokinetic Data" and "Model Development". This also included estimates of individual and population parameters *versus* the most predictive covariate in the model [25].

### 2.5 Validation of the model

For internal validation of the final model, bootstrap analysis was performed in S-plus, version 6.2.1 with NM.SP.interface version 05.03.01. Two hundred datasets were resampled from the combined original datasets and refitted to the model [26]. Normalized prediction distribution errors (NPDE) were then calculated [27], by use of the NPDE package in R [28]. For this method, the dataset used for building the model was simulated 1.000 times with inclusion of inter-individual variability and residual error. 2.6 Simulation of Dosing Regimen Simulations were performed with S-plus, version 6.2.1 with NM.SP.interface version 05.03.01. The parameter estimates of the final pharmacokinetic model were used to simulate midazolam concentrations in critically ill children ranging from premature neonates to adolescents varying in bodyweight between 0.75 and 60 kg (0.75, 1, 2, 3, 5, 10, 20, 45, and 60 kg) upon bolus injection and an infusion duration of 24 h. Both dosing schedules used in clinical practice (traditional dosing schemes) and model-based dosing schedules were simulated. The traditional dosing scheme for premature neonates was an intravenous (iv) bolus of 0.05 mg/kg and infusion of 0.06 mg/kg/h [29–31]; for children and adolescents an iv bolus of 0.1 mg/kg and infusion of 0.1 mg/kg/h (conscious sedation for critically ill patients [32]) were chosen. The model-based dosing regimen was a dosing regimen that would result in therapeutic midazolam concentrations of 0.25–0.37 µg/mL for conscious sedation in children in the PICU [32]. Because therapeutic midazolam concentrations for premature neonates were unknown, these were set at 0.2 µg/mL, which was the lowest target concentration in a study of premature neonates [18].

## 3. RESULTS

### 3.1 Patients and data

1,105 midazolam concentrations from 136 individuals in six different datasets [3, 6, 16–19], with postnatal age varying between 0 days and 31 years and bodyweight between 0.77 and 89 kg, were included in the analysis. None of the patients received CYP3A inhibitors or inducers. A summary of the patient details and data characteristics for the six studies is given in Table 1.

### 3.2 Model development and covariate analysis

The pharmacokinetics of midazolam were best described by a two-compartment model with a proportional error model. In the model, lower midazolam clearance because of critical illness [13] was incorporated into datasets for premature neonates [6, 18] and the dataset

obtained for children admitted to intensive care [16]. Introducing interindividual variability for clearance (CL), intercompartmental clearance (Q), apparent volume of distribution of the central compartment ($V_1$), and apparent volume of distribution of the peripheral compartment ($V_2$), significantly improved model performance with a total decrease in objective function (-2LL) of 3276.7 ($p<0.001$).

In the covariate analysis, bodyweight was the most significant covariate for CL, resulting in a decrease in the objective function of 149.7 points ($p < 0.001$); it explained 65.4% of interindividual variability for CL (a decrease from 1.63 or 202.58% to 0.40 or 70.13%). The relationship between bodyweight and CL was best described by use of an allometric function with an exponent that varies with bodyweight (BDE [23]), as shown in Fig. 1a. The BDE was defined as

Coeff x $BW^{exp1}$, in which BW represents bodyweight, Coeff was 0.81 (coefficient of variation (CV) 8.2%), and exp1 was –0.135 (CV 31.4%) (Table 2), resulting in a BDE which decreased from 0.84 in a preterm neonate of 0.77 kg to 0.44 in an adult of 89 kg in an exponential manner. The covariate analysis also revealed that bodyweight had a significant influence on $V_1$ (delta - 2LL of - 52.8, $p<0.001$). The nature of this influence was best described by use of a linear function (Fig. 1b), because the exponent was found not to differ significantly from 1 when using an allometric function. $V_2$ in the postoperative craniofacial surgery patients [3] was 6.38-fold larger than for the five other datasets. A stable model for this covariate could be achieved only by including bodyweight in an allometric function for $V_2$ (Table 2). These two covariates resulted in a significantly improved model (-2LL of - 184.6, $p < 0.001$). Separate addition of each of these two covariates resulted in a significant decrease in the objective

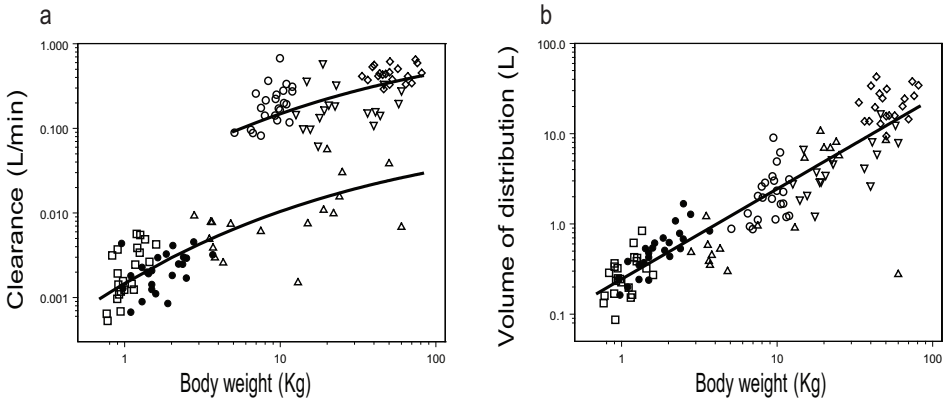


**Figure 1.** Post-hoc estimates of CYP3A4-mediated clearance of midazolam versus bodyweight (a) and central volume of distribution versus bodyweight (b) in the final model. *Open squares* preterm neonates, *filled circles* preterm neonates with RDS syndrome, *open circles* children after elective major craniofacial surgery, *open triangles* pediatric intensive care patients, *open inverted triangles* pediatric oncology patients, and *open diamonds* male adults, with a *black line* as their post-hoc population predicted values. *CYP* cytochrome P450, *RDS* respiratory distress syndrome

**Table 2.** Population parameter estimates obtained for the final midazolam pharmacokinetic model.

| Parameter | Model fit | | Bootstrap results | | Explanation |
|---|---|---|---|---|---|
| | *Value* | *(CV%)* | *Value* | *(CV%)* | |
| $\mathbf{CL_i = f \cdot Cl_{13kg} \cdot (BW/13)^{BDE}}$ | | | | | *CYP3A4/5 mediated clearance of midazolam* |
| $\boldsymbol{BDE = Coeff \cdot BW^{exp1}}$ | | | | | *BW dependent exponent of allometric exponent function on CL* |
| $Cl_{13kg}$ (L/min) | 0.17 | (8.4) | 0.17 | (8.2) | *CL for a median individual of the population with a BW of 13 kg* |
| Coeff | 0.81 | (8.2) | 0.81 | (8.0) | *Coefficient of the BDE function* |
| exp1 | -0.135 | (31.4) | -0.138 | (31.6) | *Exponent of the BDE function* |
| Q (L/min) | 0.69 | (18.1) | 0.71 | (20.4) | *Inter-compartmental clearance between central and peripheral compartment* |
| $\mathbf{V_{1i} = V_{1,\ 13kg} \cdot (BW/13)}$ | 2.7 | (18.3) | 2.7 | (18.4) | *Distribution volume of central compartment for a median individual of the* |
| $V_{1,\ 13kg}$ (L) | | | | | *population with a BW of 13 kg* |
| $\mathbf{V_{2i} = g \cdot V_{2,\ 13kg} \cdot (BW/13)^{exp2}}$ | | | | | *Distribution volume of peripheral compartment ($V_2$)* |
| $V_{2,\ 13kg}$ (L) | 6.38 | (5.9) | 6.38 | (6.2) | *$V_2$ for a median individual of the population with a BW of 13 kg* |
| exp2 | 0.76 | (7.6) | 0.76 | (6.1) | *Allometric exponent of $V_2$ in: $V_{2i}$:$V_2 \cdot (BW/13)^{exp2}$)* |
| g | 6.0 | (18) | 6.2 | (19.4) | *Multiple of $V_2$ for children after elective major craniofacial surgery[3]; g=6 children after elective major craniofacial surgery, g=1 all other patients* |
| $\omega^2$ CL | 0.37 | (17.6) | 0.35 | (16.8) | *Inter-individual variance in CL* |
| $\omega^2 V_1$ | 0.58 | (32.4) | 0.38 | (46.3) | *Inter-individual variance in $V_1$* |
| $\omega^2 V_2$ | 0.37 | (37.5) | 0.36 | (37.7) | *Inter-individual variance in $V_2$* |
| $\omega^2 (V_2\text{-}V_1)$ | 0.41 | (41.3) | 0.56 | (41.4) | *Correlation between the Inter-individual variance in $V_2$ and $V_1$* |
| $\sigma^2_{\ proportional}$ | 0.12 | (14.5) | 0.12 | (11.7) | *Residual variance (proportional error)* |

*BW* bodyweight, *BDE* bodyweight-dependent exponent, *CL* clearance, *Coeff* coefficient of the BDE function, *CYP* cytochrome P450, *f* fraction used to estimated CL for intensive-care patients compared with CL (*f* = 1 for healthy patients, *f* = 0.07 intensive care patients) [11]

function, albeit without a successful covariance step. For Q, there was no significant effect of age, bodyweight, or population. None of the other covariates tested had any effect on any of the pharmacokinetic parameters.

The pharmacokinetic parameters for the final model, with their confidence intervals, inter-individual variability, and the results of bootstrap validation are shown in Table 2. This table also shows that, after the covariate analysis, the inter-individual variance for Q could be fixed to 0 in the backward deletion step without deterioration of the model, and a successful covariance step could be achieved.

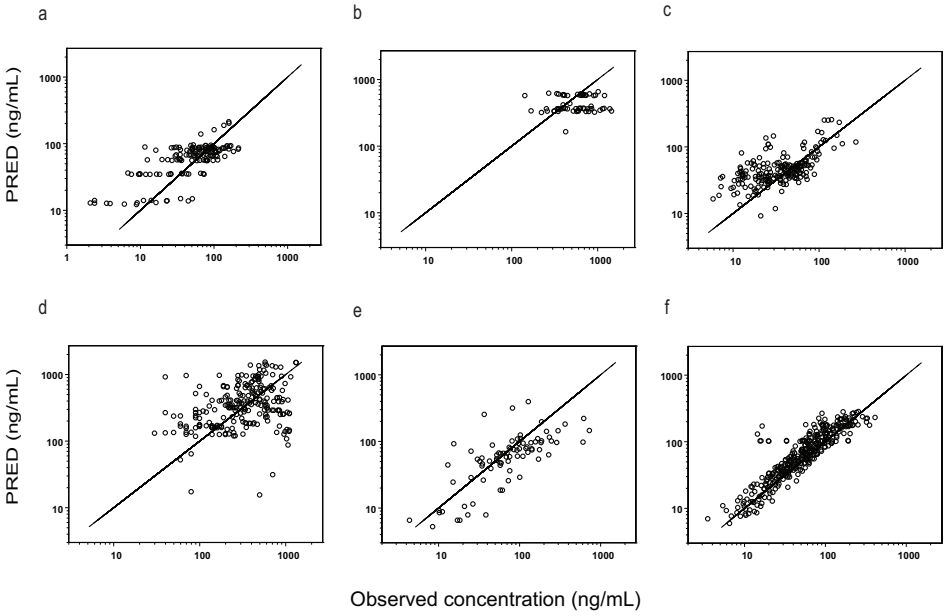


**Figure 2. a-f** Visual diagnostics of the final model per dataset. Population concentrations predicted by the model versus observed concentrations of midazolam. **a** de Wildt *et al.* [6]: preterm neonates; **b** Jacqz-Aigrain *et al.* [18]: preterm neonates with respiratory distress syndrome; **c** Peeters *et al.* [3]: children after elective major craniofacial surgery; **d** de Wildt *et al.* [16]: pediatric intensive care patients; **e** de Wildt *et al.* [17]: oncology patients; and **f** van Gerven *et al.* [19]: male adults. *PRED* predicted concentration. The *solid line* indicates the line of unity.

Figure 2a–f illustrates the population-predicted concentrations *versus* observed concentrations for each dataset separately. These diagnostic plots (log-scale) indicate that, overall, the concentrations predicted by the developed pharmacokinetic model are without bias, because the concentrations are evenly distributed around the line of unity, except for the lower concentrations from the craniofacial surgery patients (Fig. 2c), for which slight underprediction was observed. Validation of the final model using NPDE simulations (Fig. 3) illustrates

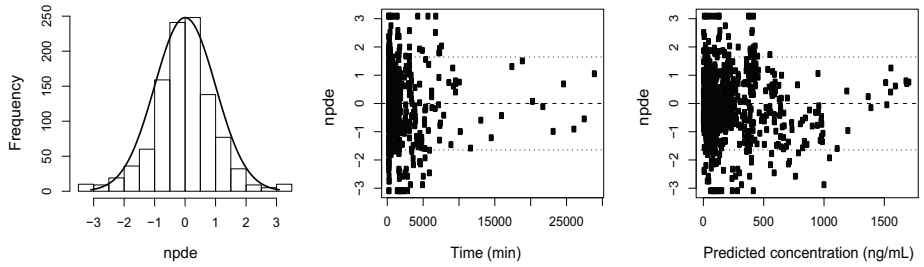


**Figure 3.** Internal validation and normalized prediction distribution errors (NPDE) of the final model. The histogram illustrates the distribution of the NPDEs for midazolam; the solid line represents a normal distribution. The distribution of NPDEs in time after first dose and against the predicted concentrations are also illustrated

the distribution of the NPDEs, with a mean close to zero (-0.04 for the mean and 0.98 for the variance) and with no observed trends in time or concentration. In the final model, all values of η-shrinkage were below 20% (CL 5.1%; $V_1$ 17.7%; $V_2$ 14.3%), which indicates that the individual estimates are reliable [21]. The calculated condition number of 195 for the final model was well below the critical value of 1.000 which is indicative of ill-conditioning [22].

### 3.3 Simulations to derive a model-based dosing regimen

Figure 4 shows the results of simulations performed using typical values of the parameters in the final model with the purpose of showing the exact influence of the covariates on the overall concentration–time relationships for different typical individuals. Figure 4 (a, c) illustrates that based on the traditional dosing scheme for conscious sedation in premature neonates and critically ill children large differences in midazolam concentrations between

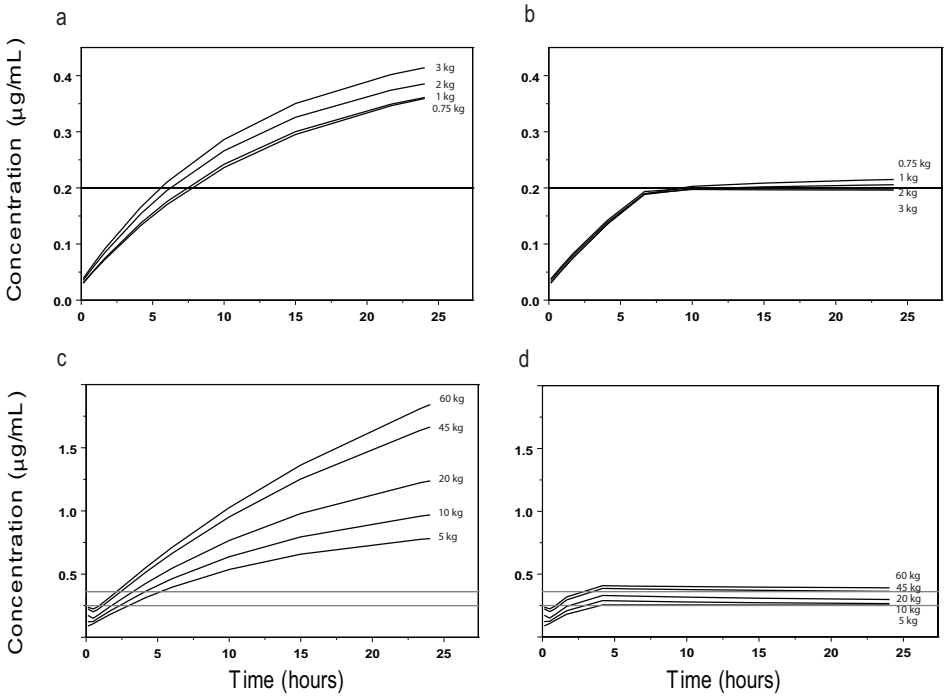


**Figure 4.** Simulated midazolam concentrations *versus* time for preterm neonates (a, b) and children in intensive care (c, d) with a traditional dosing regimen (a, c) and a model-based dosing regimen (b, d) aiming to achieve reported therapeutic midazolam concentrations (*horizontal gray lines*). a, b Midazolam concentrations for preterm neonates (0.75, 1, 2, 3 kg bodyweight) after traditional dosing regimen (bolus dose of 0.05 mg/kg followed by 0.06 mg/kg/h for 24 h) (a), and after the model-based dosing regimen as shown in Table 3 (b). c, d Midazolam concentrations for critically ill children (5, 10, 20, 45, and 60 kg bodyweight) after traditional dosing regimen (0.1 mg/kg bolus dose followed by 0.1 mg/kg/h for 24 h) (c), and after the model-based dosing regimen as shown in Table 3 (d).

**Table 3.** Traditional and model-based dosing schemes for conscious sedation with midazolam in preterm neonates and children in the intensive care

| Patient group | Weight *kg* | Infusion duration (h) *over 24 hrs* | Traditional dose[a] | | Model-based dose[a,b] | |
|---|---|---|---|---|---|---|
| | | | *mg/hr* | *mg/kg/hr* | *mg/hr* | *mg/kg/hr* |
| Preterm Neonates | 0.75 | 0 – 7 | 0.045 | 0.06 | 0.047 | 0.063 |
| | | 7 – 24 | 0.045 | 0.06 | 0.023 | 0.031 |
| | 1 | 0 – 7 | 0.06 | 0.06 | 0.06 | 0.06 |
| | | 7 – 24 | 0.06 | 0.06 | 0.03 | 0.03 |
| | 2 | 0 – 7 | 0.12 | 0.06 | 0.11 | 0.054 |
| | | 7 – 24 | 0.12 | 0.06 | 0.054 | 0.027 |
| | 3 | 0 – 7 | 0.18 | 0.06 | 0.15 | 0.051 |
| | | 7 – 24 | 0.18 | 0.06 | 0.076 | 0.025 |
| Children | 5 | 0 – 3 | 0.5 | 0.1 | 0.5 | 0.1 |
| | | 3 – 24 | 0.5 | 0.1 | 0.14 | 0.028 |
| | 10 | 0 – 3 | 1 | 0.1 | 1 | 0.1 |
| | | 3 – 24 | 1 | 0.1 | 0.22 | 0.022 |
| | 20 | 0 – 3 | 2 | 0.1 | 2 | 0.1 |
| | | 3 – 24 | 2 | 0.1 | 0.35 | 0.018 |
| | 45 | 0 – 3 | 4.5 | 0.1 | 4.5 | 0.1 |
| | | 3 – 24 | 4.5 | 0.1 | 0.59 | 0.013 |
| | 60 | 0 – 3 | 6 | 0.1 | 6 | 0.1 |
| | | 3 – 24 | 6 | 0.1 | 0.72 | 0.012 |

a Depicted doses are maintenance doses after a bolus dose of 0.05 mg/kg for preterm neonates and 0.1 mg/kg for intensive-care children, b Model-based doses were intended to achieve reported therapeutic midazolam concentrations of 0.25–0.37 µg/mL [32] for children and 0.2 µg/mL [29–31] for preterm neonates, and were 0.06 mg/kg$^{0.85}$/h for 7 h followed by 0.03 mg/kg$^{0.85}$/h for 17 h for preterm neonates and 0.1 mg/kg/h for 3 h followed by 0.05 mg/kg$^{0.65}$/h for 21 h for intensive care children.

individuals can be anticipated, particularly in the pediatric subpopulation. This is because the traditional dosing regimen is expressed in mg/kg/h whereas the results of this study show that clearance is non-linearly related to bodyweight.

For a model-based dosing regimen aiming for predefined midazolam concentration ranges over a 24 h period (Table 3) similar midazolam profiles are anticipated across the human life span (Fig. 4b, c). The model-based dosing regimen for preterm neonates that resulted from the simulations consists of 0.05 mg/kg bolus and 0.06 mg/kg$^{0.85}$/h for 7 h followed by 0.03 mg/kg$^{0.85}$/h for 17 h (Table 3). For children in intensive care (> 5 kg) requiring conscious sedation, the model-based dosing regimen consists of 0.1 mg/kg bolus and 0.1 mg/kg/h for 3 h followed by 0.05 mg/kg$^{0.65}$/h for 21 h (Table 3). In Table 3, both traditional and model-based guidelines are presented for conscious sedation with midazolam of preterm neonates and children in intensive care ranging in bodyweight between 0.75 and 60 kg.

## 4. DISCUSSION

We have developed a novel maturation function for midazolam clearance based on a dataset consisting of data obtained from six different clinical studies in (premature) neonates, infants, toddlers, children, adolescents, and adults. This model provides a quantitative insight in the developmental pattern of *in vivo* CYP3A activity across the pediatric age range, including premature neonates. This model may provide guidance to dosing of midazolam, and, potentially, other CYP3A substrates, for children across all age ranges, i.e. by applying this allometric function, which describes the relationship of clearance with bodyweight, in population pharmacokinetic and PBPK models, as a priori information.

Previously, we have shown for children between 1 month and 17 years of age that critical illness was a more important covariate than bodyweight for CYP3A-mediated clearance of midazolam [11]. Because CYP3A is known to mature, particularly in the first few days and weeks of life, we included in the current analysis midazolam clearance values spanning the age range from premature neonates to adults. This allowed the description of the change in CYP3A activity from birth onwards to adults. This analysis suggests that the major change in midazolam clearance occurs in the first year of life (Fig. 1). More specifically, in premature neonates between 0.5 and 4 kg, midazolam clearance was estimated to be only 2.6–21.8% of adult values. These results confirm very low CYP3A4/5 activity after birth [14], because midazolam is only slightly metabolized by CYP3A7 [33]. Our finding that the largest change in clearance occurs in the first few weeks of life is in agreement with CYP3A4/5 *in vitro* and *in vivo* phenotyping data. CYP3A4 mRNA levels in human fetal liver microsomes (gestational age 11–30 weeks) have been reported to be, on average, 10% of those in adults [14, 34]. Lacroix *et al.* [14] reported an increase in *in vitro* CYP3A4 activity between 1 and 3 months reaching 30–40% of adult levels, which approximates our *in vivo* findings of 25–35% of adult activity.

Data on CYP3A4 activity after the first year of life, as derived from *in vitro* and *in vivo* studies, are highly discrepant [35–38]. The finding by Stevens *et al.* [15] that invitro CYP3A4 protein content for ages from 5 to 15 years is only approximately 20% of adult levels is not in agreement with *in vivo* data on total clearance of midazolam. On the basis of average midazolam clearance data reported in the literature, Johnson *et al.* reported that CYP3A4/5-mediated midazolam clearance expressed in L/h reaches approximately 18 and 40% of clearance in adults at ages of 1 year and 5 years, respectively [39]. Interestingly, Anderson and Larsson [40], using average invivo clearance values from the literature, estimated midazolam clearance at birth in a term neonate to be 14% of that in adults, reaching 64% of that in adults at 1 year of age and 90% at 2 years of age. In addition, using the urinary ratio of dextromethorphan and its metabolite 3-hydroxymorphinan reported by Blake *et al.* [35], Johnson *et al.* [37] simulated

an increase in CYP3A4 activity that reaches 72% of that in adults at the age of 1 year. The large variation reported for CYP3A activity in *in vivo* and invitro studies may be related to the sub-optimal quality of the post-mortem tissue used for the *in vitro* studies, resulting in erroneously low estimates of CYP3A activity. In addition, the studies by Johnson *et al.* [39] and Anderson and Larsson [40] both used average clearance values instead of individual doses and concentrations to model the maturation of midazolam clearance. Furthermore, the amount of underlying individual data for children between 0 and 1 year of age in these pooled studies was very low, which could have resulted in erroneous estimates for this age range [40]. For our analysis we had access to raw data on demographics, doses, concentrations, and covariates for 136 individuals varying in age between premature neonates and adults. We therefore suggest that our analysis was less prone to the drawbacks mentioned above, which increases the validity of the value of the estimated maturation function for *in vivo* CYP3A4/5 activity. However, because the CYP3A4/5 abundance estimated by Johnson *et al.* [39] and Anderson and Larsson [40] was related to age, and not to bodyweight, the exact difference from our model based on bodyweight is difficult to derive; this should be kept in mind when comparing the results.

The maturation of CYP3A assessed on the basis of midazolam clearance was found to be highly non-linear (Fig. 1), and was best described by an allometric function with a BDE. This agrees with Wang *et al.* [23] who first reported that an exponent changing with bodyweight in an allometric equation best described changes in propofol clearance occurring between neonatal and adult age. In their model, the allometric exponent changed in a sigmoidal manner (Emax model) with bodyweight and had four parameters to be estimated [23]. For propofol clearance, this exponent was found to change from 1.43 for a hypothetical bodyweight of 0 kg to 0.55 for 10 kg upwards [23]. In our model, we used a simplified version of the model of Wang *et al.* [23], by allowing the exponent to change exponentially, thereby reducing the degrees of freedom from four to two, as previously used for busulphan in children [24]. This approach allowed us to adequately estimate all pharmacokinetic parameters without overparameterization of the model, and to describe midazolam clearance from premature neonates to adults without bias over the entire range of bodyweight. This contrasts with an allometric function with an estimated exponent or a fixed exponent of 0.75; these functions resulted in failure to minimize successfully or in inadequate description of clearance throughout the entire weight range of the population, without adding more age-related information in the model. Using those models, especially in the lowest weight range, midazolam clearances were overestimated. Although addition of an age-based function to capture maturation in the youngest age ranges, thus correcting this overestimation, could be considered, we decided to use a BDE model instead. With the BDE model, the use of two different functions for covariates that are correlated (i.e. age and bodyweight) which may potentially lead to inappropriate functions, is prevented [41]. Interestingly, by using the

BDE model in which the exponent was found to vary between 0.84 for neonates and 0.44 for adults, midazolam clearance was well estimated across the entire age range. The fact that maturation of CYP3A activity seems less steep than reported for propofol (highest exponents 0.91 and 1.44, respectively) may reflect the different pathways involved in metabolism of midazolam and propofol, CYP3A oxidation and glucuronidation, respectively [23]. It thus seems that pathway-specific functions must be developed across the age range for different common metabolic and elimination pathways that can be used to predict changes in drug metabolism and elimination in children. In particular, in view of the growing interest in pediatric PBPK models [42], it is important to quantify the *in vivo* maturational behavior of all metabolic enzyme systems that are clinically relevant.

In this analysis, results from the function for CYP3Amediated clearance of midazolam were used to derive a model-based dosing scheme for conscious sedation of premature neonates and children in intensive care. Table 3 shows that, instead of empirical dosing on the basis of mg/ kg/h, use of a non-linear maintenance dose is proposed. In accordance with the exponent found for changes in clearance within the pediatric range, an exponent of 0.85 is proposed for premature neonates whereas an exponent of 0.65 is used for older children. To prevent dosing errors, a table is provided to guide dosing according to these nonlinear functions. Figure 4 shows that, on the basis of this non-linear dosing scheme, similar concentrations can be expected across a wide range of bodyweight, whereas if mg/kg/h dosing schedules are used large variations may be expected because of the non-linear nature of the dependence of clearance on bodyweight. The target midazolam concentration chosen from literature [18, 32] may, however, be arbitrary, because it is conceivable that some children may require higher concentrations than others. Therefore the dosing table (Table 3) is only a guide for dose adjustment for children; the absolute value (e.g. the value 0.06 in 0.06 mg/kg0.85/h for premature neonates) may be adjusted at the discretion of the attending physician. A previous study by our group showed that it is, indeed, feasible to apply a non-linear dosing scheme in the context of a clinical trial [43]. In that study, morphine was dosed according to a function with an exponent of 1.44, implying that neonates require less and older children require more than the amount currently administered on the basis of a mg/kg/h dosing schedule.

No dosing implications for non-critically ill patients have been derived on the basis of the results of this study.We have previously reported that absolute midazolam clearance in critically ill patients ismuch lower (Fig. 1a) than in relatively healthy children [11].Wehypothesized this phenomenon was because of critical illness-related inflammation response causing CYP3A4 gene repression [44]. Because of the absence of midazolam data for non-critically ill neonates it was not possible to quantify the maturational behavior of CYP3A in healthy neonates by using midazolam clearance as surrogate. However, because healthy neonates and children most often receive bolus doses of midazolamfor diagnostic or therapeutic intervention,

changes in the volumeof distribution as a result of age may be more important than changes in clearance for this age group.While volume of distribution is found to scale linearly with bodyweight, there are no clear reasons to change the current practice of dosing a bolus in mg/kg across the pediatric age range when midazolam is given for procedural sedation. For V2, a 6.38-fold higher value was found for the postoperative craniofacial surgery patients [3] than for the five other types of patient. Because blood loss during craniofacial surgery may average 24% of the total volume of blood [45], blood transfusion is often required to maintain the blood cell, thrombocyte, and plasma levels at normal values. This may, eventually, have led to the greater V2 value of midazolam for these patients than for the other patients.

In this work we studied maturation of hepatic CYP3A activity using midazolam as an *in vivo* probe; the effect of ontogeny on intestinal CYP3A activity is still unknown. It has become clear over the years that CYP3A activity in the intestine is of considerable importance, necessitating investigation of maturation of CYP3A-mediated first passelimination of midazolam in children of different ages. Another limitation is that external validation of a pharmacokinetic model by use of independent data, and ultimately prospective clinical evaluation, which are important steps after internal validation of a model, must still be performed [46]. An important issue in the process of validation of pediatric modeling is the lack of sufficient data to perform external validation, and the ethical constraints on performing prospective clinical evaluation of a drug among children. However, although only 17% of published pediatric pharmacokinetic and pharmacodynamic models have been validated [47], we believe all models should, at least, be fully internally validated [46].

In addition, extrapolation of the maturation model for the CYP3A substrate midazolam will test the ability of the model to predict the system-specific properties of other drugs that share, at least partly, a similar pharmacokinetic pathway [48]. Another CYP3A substrate from the literature that has been well studied during the first months of life is cisapride [49]. Use of the maturation model for midazolam to describe cisapride pharmacokinetics in the youngest infants would be another step in the process of validation of the semi-physiological pharmacokinetic model.

## 5. CONCLUSION

We have developed an *in vivo* maturation function for CYP3A-mediated clearance of midazolam from premature neonates to adults. This maturation function can be used to derive evidence-based doses for premature neonates, infants, and children, and to simulate exposure to midazolam and possibly other CYP3A substrates across the pediatric age range in population pharmacokinetic or PBPK models.

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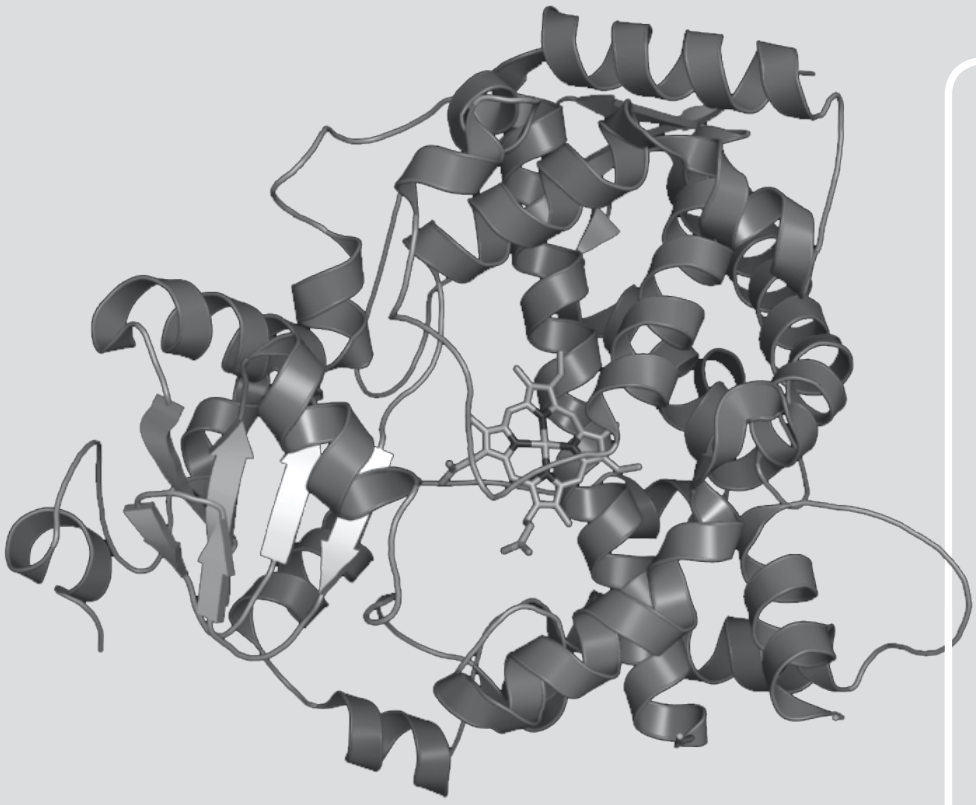

# 6 Population pharmacokinetic analysis on oral and intravenous midazolam across the human lifespan from preterm neonates to adults

Ince I, de Wildt SN, Barrett JS, Burggraaf J, Jacqz-Aigrain E, van den Anker JN, Danhof M, Tibboel D, Knibbe CA, Submitted

## ABSTRACT

Objectives: Many studies have focused on the maturation of clearance of the CYP3A substrate midazolam from preterm neonates to adults upon intravenous administration. The aim of this study was to investigate the pharmacokinetics of midazolam across the human lifespan with emphasis on maturation in oral bioavailability and absorption rate.

Methods: Pharmacokinetic data were obtained from a combined dataset of seven previously reported studies in 52 preterm infants (gestational age: 26-37 weeks, postnatal age 2-13 days), 324 children (2 days -18 years) and 20 adults, who received intravenous and/or oral midazolam. Population pharmacokinetic modeling and covariate analysis was performed using NONMEM v6.2.

Results: In the combined dataset, bodyweight proved a significant covariate for both clearance and oral bioavailability of midazolam, albeit at different functions. Midazolam clearance (population value of 0.20 L/min (CV 7.1%)) was influenced by bodyweight according to a power function with a bodyweight dependent exponent varying from 0.82 in preterm neonates to 0.28 in adults). Oral bioavailability of midazolam (population value of 24% (CV 7.5%)) was negatively influenced by bodyweight in a power function with an exponent of -0.24, resulting in a value of 67% for a preterm neonate of 0.77 kg to 17% in adults. No significant correlation of absorption rate (3 h-1) with age or bodyweight was found.

Conclusions: In the population pharmacokinetic model of midazolam, oral bioavailability and clearance each show a distinct developmental pattern. The results provide a basis for further mechanistic studies on maturation of physiological processes that determine the oral bioavailability and clearance of midazolam and other CYP3A substrates.

## 1. INTRODUCTION

Midazolam is a short-acting sedative commonly used orally or intravenously in both children and adults, which is mainly metabolised by CYP3A. CYP3A is the cytochrome P450 subfamily involved in the metabolism of almost half of all marketed drugs that are eliminated by metabolism. [1, 2, 3, 4, 5]. As CYP3A is expressed in the liver and the intestines, maturation of this enzyme system may contribute to developmental changes in both absorption and the hepatic clearance of CYP3A substrates across the human life span. Also for midazolam, it is reported that CYP3A activity is a major contributor to oral absorption. [6] While the ontogeny of hepatic CYP3A activity has been studied both *in vitro* and *in vivo,* [7, 8, 9, 10, 11, 12, 13] the impact of ontogeny on intestinal CYP3A activity is still largely unknown. *In vitro* studies on intestinal CYP3A show variable results, with a decrease in mRNA expression but an increase in protein levels in the first year of life. [14, 15] Although the cDNA of hepatic and intestinal CYP3A4 is similar, they do not appear to be regulated in concert. [16, 17]

Recently, the maturation of *in vivo* CYP3A mediated hepatic clearance of midazolam has been characterized from preterm neonates to adults upon intravenous administration. [18] As, among other factors, CYP3A activity may determine changes in the absorption of midazolam in children, [6] it is also of interest to study the pharmacokinetics of midazolam across the human life-span upon oral administration. Using oral bioavailability (F) as the parameter that captures different PK processes, such as absorption *per se*, intestinal and hepatic presystemic extraction, relevant information can be obtained on the sum of developmental changes in these processes. In adults, average reported midazolam oral bioavailability values range between 24-44%. [19, 20, 21, 22]. This value is much lower than the maximum estimated bioavailability on the basis of the hepatic extraction ratio, indicating that intestinal pre-systemic elimination is a significant factor contributing to the bioavailability. [20] Interestingly, similar low bioavailability values were reported in a study in healthy children from 2-12 years of age (average 27%) and in a study in children from 6 months to 12 years of age (average 36%, range 9-71%). [23, 24] In contrast, in preterm neonates (26-32 weeks of gestational age and 3-10 days postnatal age) in the pediatric intensive care, the median reported oral bioavailability was much higher at 49% (range 12%-100%). [25] Within the pediatric cohorts studied, however, so far no effect of age on oral bioavailability could be identified. This may be due to the relatively small sample sizes and/or small age ranges in these studies, which may lead to attribution of associated maturation in bioavailability to maturation in clearance/F. As a result, the exact developmental pattern of oral bioavailability has not been elucidated to date.

The aim of this study was to investigate the pharmacokinetics of the CYP3A substrate midazolam in a combined dataset of midazolam concentrations obtained from preterm neonates

and children to adults after oral and intravenous administration. This information may be used to design age-appropriate dosing schedules for oral midazolam that can be used in pharmacodynamic titration studies and/or provide a basis for further study to unravel the maturation in the different subprocesses contributing to absorption of CYP3A substrates.

## 2. METHODS

### 2.1 Patients and data

Individuals from seven studies that were available or shared within our group were included in the analysis except for four children receiving oral midazolam aged 15, 16, or 17 years, as they were considered obese according to the growth chart obtained from Centers for Disease Control and prevention [26] (bodyweight above 100 kg).

Subjects included 22 non-invasively ventilated preterm neonates of which 11 received oral (PO) and intravenous (IV) midazolam in a cross-over design (gestational age [GA], 26.3-32.1 weeks gestational age (GA) and postnatal age [PNA] 3-13 days, 6 received only PO (GA, 26-30.4 weeks; PNA, 4-8 days) and 11 received IV midazolam only (GA, 26-33.6 weeks; PNA, 2.9-11 days), [10, 25] 264 healthy children (1-18 years, PO), 24 preterm neonates with respiratory distress syndrome (GA, 26-37 weeks; PNA 0-1 days; IV), [27] 23 children after elective major craniofacial surgery (3-23 months, IV), [28] 18 paediatric intensive care patients (2 days to 17 years, IV), [9] 18 paediatric oncology patients (3-17 years, IV), [11] and 20 healthy male adults (20-31 years, IV) [29]. Details on the seven patient datasets are provided in table 1.

### 2.2 Pharmacokinetic data analysis

Population pharmacokinetic analysis was performed using the first-order conditional estimation (FOCE) with η-ε interaction in NONMEM version 6.2, release 1.1 (GloboMax LLC, Hanover, MD, USA). [30] S-plus version 6.2.1 (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P, Leiden, The Netherlands) was used to visualize the data. Model development was performed in four steps: 1) choice of the structural model, 2) choice of the error model, 3) covariate analysis and 4) validation of the model. Discrimination between different models was made by comparison numerical and visual diagnostics. A decrease in the objective function of 3.8, corresponding to a value of $p < 0.05$, was considered statistically significant. Goodness-of-fit plots (observed *versus* individually predicted concentration, observed *versus* population predicted concentration, conditional weighted residuals *versus* time, and conditional weighted residuals *versus* population predictions) were used for diagnostic purposes and model selection. In addition, the confidence interval of the parameter

**Table 1.** Overview of datasets used to develop the midazolam PK model.

| **Administration** | **ORAL AND INTRAVENOUS (PO + IV)** | **ORAL (PO)** | | **INTRAVENOUS (IV)** | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Study** | **de Wildt SN *et al.*** [10;25] | **de Wildt SN *et al.*** [25] | **J.S.Barrett** | **de Wildt SN *et al.*** [10] | **Jacqz-Aigrain E. *et al.*** [27] | **Peeters M.Y. *et al*** [28] | **de Wildt SN *et al*** [9] | **de Wildt SN *et al*** [11] | **van Gerven J.M.A. *et al*** [29] |
| **Patient Population** | Preterm neonates | Preterm neonates | Healthy children | Preterm neonates | Preterm neonates with Respiratory distress Syndrome (RDS) | Children after elective major craniofacial surgery | Pediatric intensive care patients | Oncology patients | Healthy male adults |
| **Indication for midazolam** | Sedation for invasive procedure in Intensive Care | Sedation for invasive procedure in Intensive Care | Sedation for invasive procedure | Sedation for invasive procedure in Intensive Care | Mechanical ventilation in intensive Care | Postoperative sedation | Conscious sedation in Intensive Care | Sedation for invasive procedure | Healthy volunteers |
| **N Patients** | 11 | 6 | 260 | 11 | 24 | 23 | 18 | 18 | 20 |
| **PNA** *median (range)* | 6 days (3-13) | 6 days (4-8) | 7 years (1–18) | 5 days (2.9-11) | 0 days (0-1) | 11.5 months (3.2-24.7) | 38.5 months (0.03-203.5) | 6.1 years (3.2–16.2) | 24 years (20-31) |
| **GA** *median (range)* | 28.3 weeks (26.3-32.1) | 29.4 weeks (26-30.4) | - | 28.3 weeks (26-33.6) | 32 weeks (26-37) | - | - | - | - |
| **Bodyweight** *median (range)* | 1.1 (0.8-1.94) | 1.1 (0.78-1.42) | 28.4 (9.1-92) | 1.07 (0.77-1.6) | 1.64 (0.96-3.7) | 9.6 (5.1–12) | 14 (2.8-60) | 22.5 (12.6–60.1) | 50.4 (64-89) |
| **Dose** *median (range)* | Crossover 0.1 mg/kg **PO** and **IV** with 72 hr washout between doses | 0.1 mg/kg only **PO** | 0.35 (0.07 -0.8) mg/kg **PO** | 0.1 mg/ kg only **IV** infusion in 30 minutes | 60 µg/kg/hr **IV** infusion If GA < 33w → after t > 24hr 30 µg/ kg/hr | 0.1 mg/kg **IV** loading dose, 0.05-0.2 mg/kg/ hr infusion | 0.1 mg/kg **IV** loading dose, 0.05-0.4 mg/kg/ hr infusion | 0.1 (0.03-0.53) mg/kg **IV** bolus dose | 0.1 mg/kg **IV** infusion in 20 minutes |
| **N Samples** | 112 | 46 | 810 | 134 | 63 | 198 | 233 | 82 | 336 |

estimates, the correlation matrix, and visual improvement of the individual plots were used to evaluate the model. Furthermore, η-shrinkage as defined by Karlsson *et al*. [31], was calculated for all model parameters for which inter-individual variability was estimated, and over-parameterisation (ill-conditioning) of the model was tested by calculating the condition number. [32]

## 2.3 Model development

The pharmacokinetic model used for oral midazolam disposition is a two-compartment pharmacokinetic model with a dose compartment, parameterized in terms of absorption rate constant ($k_a$), bioavailability (F), central volume of distribution ($V_2$), peripheral volume of distribution ($V_3$), inter-compartmental clearance (Q), and midazolam total clearance (CYP3A mediated clearance, CL). In the model, the previously reported influence of critical illness on CYP3A mediated clearance of midazolam [18] was incorporated, implying a reduction in midazolam clearance in critically ill patient datasets (preterm neonate and pediatric intensive care patients datasets) compared to non-critically ill children (datasets of children after major craniofacial surgery, pediatric oncology patients, and healthy adults). In addition, the previously reported 4.2 fold higher peripheral volume of distribution of midazolam ($V_3$) in the craniofacial surgery patients compared to all other patients was incorporated into the model. [18]

The individual value (*post hoc* value) of the parameters of the *i*th subject was modeled using equation: $P_i = P_{pop} \cdot e^{RV}$, where $P_i$ equals the individual or *post hoc* value of the parameters of the *i*th subject, and $P_{pop}$ is the population value of the parameter and RV is assumed to be a gaussian random variable with mean zero and variance of $\omega^2$, assuming log-normal distribution.

The intra-individual variability was described with a proportional error model for all data, assuming a constant coefficient of variation over the entire concentration range. This means for the *j*th observed midazolam concentration (Y) of the *i*th individual: $Y_{ij} = C_{pred,\,ij} \cdot (1 + RV_{ij})$, where $C_{pred}$ is the predicted midazolam concentration and $RV_{ij}$ is the random variable for midazolam, with mean zero and variance $\sigma^2$.

## 2.4 Covariate analysis

Individual *post hoc* estimates of the different structural pharmacokinetic parameters were plotted independently against the available covariates to visualize potential covariate relationships. The following covariates were tested for the structural parameters F, $k_a$, CL, Q, V2, and V3: bodyweight (BW), postnatal age (PNA), and study population. Potential covariates

were separately entered into the model using a linear or allometric equation ($P_i = P_{pop} \cdot (COV_i / COV_{median})$, or $P_i = P_{pop} \cdot (COV_i / COV_{median})^k$, respectively, where $P_i$ equals the individual or *post hoc* value of the parameters of the *i*th subject, $P_{pop}$ is the population value of the parameter, COV is the concerned covariate, and k is the exponent. Alternatively, an allometric equation with an exponent varying with bodyweight was tested: $P_i = P_{pop} \cdot (COV_i / COV_{median})^{BDE}$, in which BDE represents the bodyweight dependent exponent, BDE = Coeff x $BW^{exp2}$, with Coeff as a coefficient and BW as bodyweight with an exponent exp2. [33]

The influence of a covariate was statistically tested for significance by use of the objective function. A p value < 0.005 (decrease of objective function of 7.8 points) was applied to evaluate the covariates in the forward inclusion, while on the other hand a more stringent p value of <0.001 (decrease of objective function of 10.83 points) was used in the backward deletion. When two or more covariates were found to significantly improve the model, the covariate that showed the largest reduction of the objective function was included in the model. Additional covariates had to reduce this objective function further to be retained in the model. The choice of the model was further evaluated as discussed under 'pharmacokinetic data analysis'. Among others, this included also individual and population parameter estimates versus the most predictive covariate in the model. [34] Finally, the results of the model validation procedure was considered.

## 2.5 Validation of the model

For the internal validation of the final model, a bootstrap analysis was performed in S-plus, version 6.2.1 (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P, Leiden, The Netherlands). Two hundred datasets were resampled from the combined original datasets, and refitted to the model. [35]

Secondly, Normalized Prediction Distribution Errors (NPDE) were calculated, [36] by using the NPDE package in R. [37] For this method, the combined dataset that was used for building the model was simulated 1000 times with inclusion of the inter-individual variability and residual error.

## 2.6 Simulation dosing regimen

Simulations were performed using the parameter estimates from the final model to compare midazolam concentrations over time after oral and intravenous administration within different populations. In preterm neonates, an oral dose was explored which would lead to similar midazolam concentrations that would be obtained after a traditional IV bolus infusion of 0.1 mg/kg/30min. A similar approach was performed for obtaining a PO dose for a healthy

infant of 1 year (10 kg) and a healthy adolescent (60 kg) [38, 39, 40] that would lead to similar concentrations compared to a traditional IV bolus of 0.1 mg/kg. [11, 28] The simulations were performed in S-plus, version 6.2.1 (Insightful software, Seattle, WA) with NONMEM SP.interface version 05.03.01 (© by LAP&P, Leiden, The Netherlands), using the parameters estimates from the final model.

## 3. RESULTS

### 3.1 Patients

There were 2014 midazolam concentrations from seven different datasets consisting of 391 individuals aged between 0 days and 31 years of age. None of the patients received CYP3A inhibitors or inducers. A summary of the patient and data characteristics of the seven datasets is shown in table 1.

### 3.2 Model development and covariate analysis

Midazolam pharmacokinetics was adequately described with a two-compartment model with a dose compartment, with a proportional error model for all data upon oral or intravenous dose. The introduction of interindividual variability for CL, $V_3$, and F significantly improved the model performance with a total decrease in objective function (-2LL) of 5523.4 ($p < 0.001$). For the parameters $V_2$ and $k_a$, interindividual variability could not be identified with appropriate significance.

During the covariate analysis, bodyweight (BW) accounted for a substantial part (74.2% decrease in variance from 0.853 to 0.22) of the inter-individual variability on CL (decrease in -2LL of 357.1, $p < 0.001$). The relation between BW and CL was best described using an allometric function with a body weight dependent exponent (BDE), [33] whereby BDE was parameterized as reported before for midazolam, [41] resulting in the function $CL_i = CL_{21.6kg} \cdot (BW/21.6)^{BDE}$ with $BDE = Coeff \cdot BW^{-exp2}$. As such BDE was found to range from 0.82 in preterm neonates to 0.28 in adults (figure 1, table 2).

For oral bioavailability F, BW was found to negatively correlate with F, which was best parameterised using a negative allometric function,

$F = F_{21.6kg} \cdot (BW/21.6)^{-exp1}$, with an estimated exponent (exp1) of -0.24 (figure 1). The model improved significantly with this function explaining 17.8% of the inter-individual variability in F (decrease in variance from 0.364 to 0.299, decrease in -2LL of 28.3, $p<0.001$).

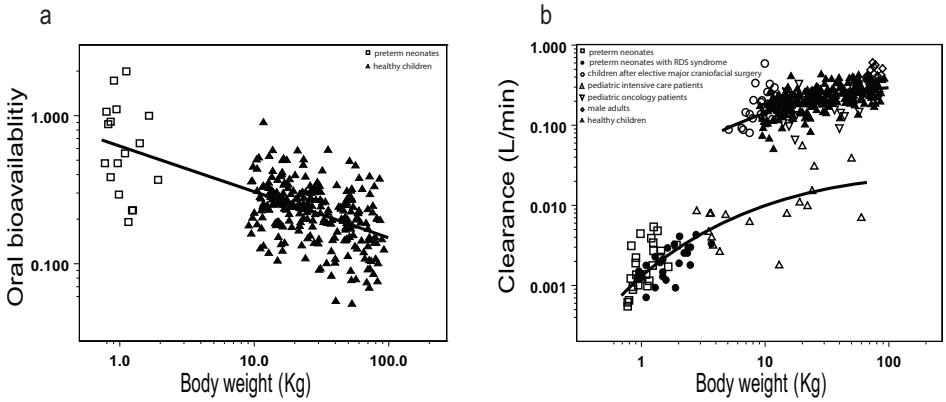


**Figure 1.** Posthoc estimates of oral bioavailability (a), and CYP3A mediated clearance of midazolam *versus* bodyweight (b); □ = preterm neonates, ▲ = healthy children, ● = preterm neonates with RDS, ○ = children after elective major craniofacial surgery, △ = pediatric intensive care patients, ▽ = pediatric oncology patients, ◊ = male adults, with a *black line* post-hoc population predicted values for oral bioavailability (a), and clearance of critically ill (b, lower line), and relatively healthy (b, upper line) patients.

V2 was found to correlate with BW in a linear manner, while using an allometric function the exponent was not found to differ significantly from 1. This function was successfully estimated together with an additional parameter for interindividual variance for $V_2$ which could not be estimated without adding BW as a covariate (decrease in -2LL of 452, delta degrees of freedom of 2, $p < 0.001$). In addition, bodyweight was added to $V_3$ in an allometric equation with an estimated exponent of 0.83, which in combination with the multiplication factor of $V_3$ for children after elective major craniofacial surgery (see methods section), improved the model significantly (delta degrees of freedom of 2, decrease in -2LL of 318.451, $p < 0.001$). For both Q and $k_a$, no influence of age or bodyweight could be determined.

In table 2, the pharmacokinetic parameter values along with their confidence intervals and inter-individual variability of the final model are shown, together with the results of the bootstrap validation.

Figure 2a illustrates that the overall population predicted concentrations (PRED) *versus* the observed concentrations, are equally spread around the line of identity, as represented by the black solid line. Figure 2b-h illustrates PRED *versus* observed concentrations for each of the seven datasets separately. For the inter-individual variability, some values of η-shrinkage were above the value of 30% (41.8, 35.8, 35, for $V_2$, and $V_3$, and F respectively), which indicates that conclusions should not only be based on visual diagnostics for these parameters. [31] The calculated condition number of 39 of the final model was well below the critical value for the indication of ill-conditioning of 1000. [32]

**Table 2.** Population parameter estimates obtained for the final midazolam PK model.

| Parameter | Model fit | | Bootstrap results | | Explanation |
|---|---|---|---|---|---|
| | *Value* | *(CV%)* | *Value* | *(CV%)* | |
| **Ka ($min^{-1}$)** | 0.054 | (9.0) | 0.055 | (8.6) | *Absorption coefficient of midazolam* |
| **$F = F_{21.6kg} \cdot (BW/21.6)^{-exp1}$** | | | | | *Oral bioavailability of midazolam (F)* |
| $F_{21.6\,kg}$ | 0.24 | (7.5) | 0.24 | (7.6) | *F for a median individual of the population with a BW of 21.6 kg* |
| exp1 | 0.31 | (6.3) | 0.31 | (19.2) | *Allometric exponent of F in: $F_i$:F • (BW/21.6) $^{-exp1}$* |
| **$CL_i = a \cdot CL_{21.6kg} \cdot (BW/21.6)^{BDE}$** | | | | | *CYP3A mediated clearance of midazolam* |
| $CL_{21.6kg}$ (L/min) | 0.2 | (7.1) | 0.2 | (6.6) | *CL for a median individual of the population with a BW of 21.6 kg* |
| **$BDE = Coeff \cdot BW^{-exp2}$** | | | | | *BDE of allometric exponent function for CL* |
| Coeff | 0.77 | (6.3) | 0.76 | (5.9) | *Coefficient of the BDE function* |
| exp2 | 0.24 | (7.5) | -0.23 | (18.3) | *Exponent of the BDE function* |
| **Q (L/min)** | 0.26 | (16.6) | 0.27 | (10.7) | *Inter-compartmental clearance between central and peripheral compartment* |
| **$V_{2i} = V_{2,\,21.6kg} \cdot (BW/21.6)$** | | | | | *Distribution volume of central compartment for a median individual of the population with a BW of 21.6 kg* |
| $V_{2,\,21.6kg}$ (L) | 7.4 | (0.1) | 7.5 | (10.1) | |
| **$V_{3i} = b \cdot V_{3,\,21.6kg} \cdot (BW/21.6)^{exp3}$** | | | | | *Distribution volume of peripheral compartment ($V_3$)* |
| $V_{3,\,21.6kg}$ (L) | 16 | (1.5) | 16.3 | (7.3) | *$V_3$ for a median individual of the population with a BW of 21.6 kg* |
| exp3 | 0.83 | (5.8) | 0.84 | (5.5) | *Allometric exponent for $V_3$ in $V_{3i} = b \cdot V_3 \cdot (BW/21.6)^{exp3}$)* |
| b | 4.2 | (30) | 4.3 | (20.8) | *Multiple to estimate $V_3$ in children after elective major craniofacial surgery* |
| **$\omega^2$ Cl** | 0.53 | (15.2) | 0.54 | (14.1) | *Inter-individual variability in CL* |
| **$\omega^2 V_2$** | 1.52 | (12.8) | 1.42 | (23.6) | *Inter-individual variability in $V_2$* |
| **$\omega^2 V_3$** | 0.88 | (26.7) | 0.87 | (14.6) | *Inter-individual variability in $V_3$* |
| **$\omega^2$ F** | 0.61 | (12.5) | 0.61 | (23.5) | *Inter-individual variability in F* |
| **$\sigma^2_{proportional}$** | 0.14 | (8.4) | 0.14 | (7.1) | *Residual variability (proportional error)* |

*BW* bodyweight, *BDE* bodyweight-dependent exponent, *CL* clearance, *Coeff* coefficient of the BDE function, *CYP* cytochrome P450, *a* fraction used to estimated CL for intensive-care patients compared with CL (*a* = 1 for healthy patients, *a* = 0.07 intensive care patients) [18]

The histograms of the NPDE for midazolam, as depicted in Figure 3, illustrate the distribution of the NPDEs for all data after IV administration and oral administration, with means close to zero (-0.04 for the mean, and 0.98 the variance), and with no observed trends in time or concentration.

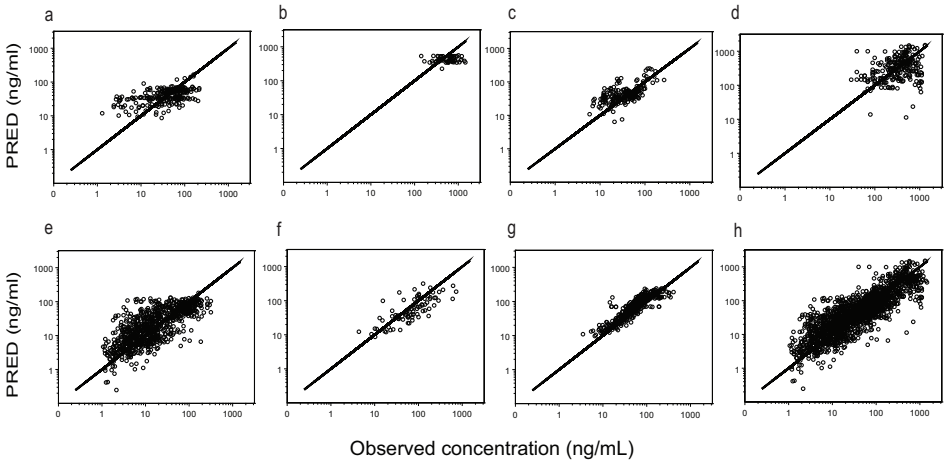


**Figure 2a-h.** Visual diagnostics of the final model per dataset. Population predicted concentrations by the model *versus* the observed concentrations of midazolam. Preterm neonates after IV and/or oral (PO) midazolam (a), preterm neonates with RDS syndrome (b), children after elective surgery (c), pediatric intensive care patients (d), healthy children (e), pediatric oncology patients (f), male adults (g), and all data (h). The *solid line* indicates the line of unity.

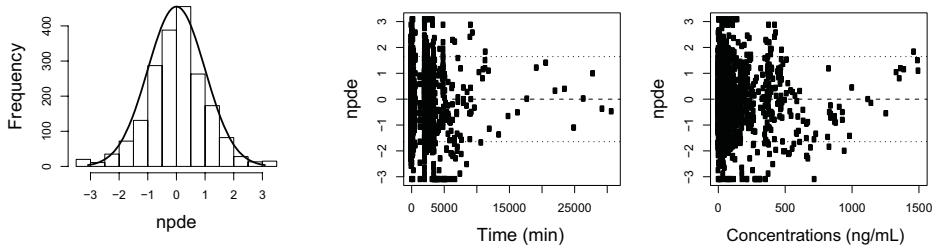


**Figure 3.** Internal validation (NPDE) of the final model. The histogram illustrates the distribution of the NPDE's for midazolam in which the solid line represents a normal distribution. In addition, the distribution of NPDE's in time after first dose and against the observed concentrations is illustrated.

## 3.3 Simulations

Figure 4 shows the results of the simulations on the basis of the final model in which a dose is selected that would result in similar exposure compared to commonly used intravenous bolus infusions. The simulations show that an oral dose for a preterm neonate to reach similar concentrations after a traditional intravenous bolus infusion of 0.1 mg/kg/30min is 0.16 mg/kg (figure 4a), an oral dose for a healthy infant to reach similar concentrations after traditional intravenous bolus of 0.1 mg/kg is 0.3 mg/kg (figure 4b), and an oral dose for a healthy adolescent to reach similar concentrations after traditional intravenous bolus of 0.1 mg/kg is 0.5 mg/kg (figure 4c). [25, 38, 39, 40]

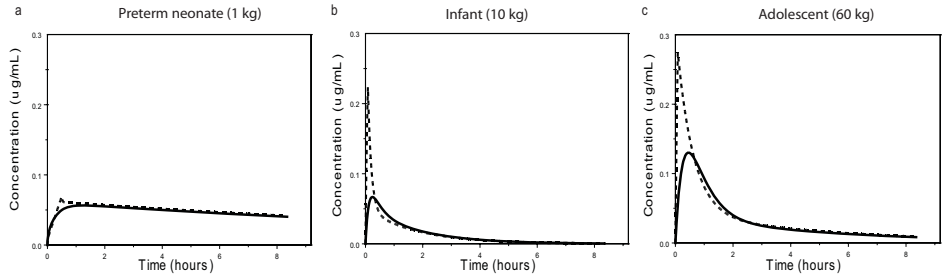


**Figure 4.** Comparison of simulated midazolam concentration *versus* time plots after PO *(solid lines)* and IV *(dotted lines)* administration within different populations. A PO dose for a preterm neonate to reach similar concentrations after traditional IV bolus infusion of 0.1 mg/kg/30min is 0.16 mg/kg (a), a PO dose for a healthy infant to reach similar concentrations after traditional IV bolus of 0.1 mg/kg is 0.3 mg/kg (b), a PO dose for a healthy adolescent to reach similar concentrations after traditional IV bolus of 0.1 mg/kg is 0.5 mg/kg (c).

## 4. DISCUSSION

In this pharmacokinetic analysis, we developed a maturation model for oral and intravenous midazolam pharmacokinetics from preterm neonates to adults, based on a combined dataset of seven different pharmacokinetic studies. Different developmental patterns for clearance and oral bioavailability of midazolam across the human life-span were identified that may guide dosing of midazolam for children of different ages for future pharmacodynamic studies. In addition, the results may be of value for future mechanistic studies in which the maturation of physiological sub processes contributing to oral bioavailability and clearance of CYP3A substrates are evaluated, such as liver bloodflow, intestinal and hepatic CYP3A enzyme activity.

In our dataset, we had access to concentrations upon both oral and intravenous midazolam. On the basis of simultaneous analysis of oral and intravenous data, we were able to estimate maturation in oral bioavailability F across the pediatric age range, while maturation in systemic clearance was already accounted for. In this respect, it is important that the results of the maturation function for clearance as identified in the current analysis are in good agreement with the function reported on maturation in clearance when iv data were modelled alone.[41] As shown in Figure 1, our findings do show an age-related change in oral bioavailability that has not yet been shown by other studies in children [24]. We think that this may be explained by the relatively small age-ranges included in those studies. As a result, maturation in oral bioavailability may erroneously have been attributed to maturation in CL/F. In our analysis, estimated oral bioavailability appears highest in neonates (67%), and decreases according to a power function resulting in a percentage of only 17% (7%-26%) of bioavailability in adults (Figure 1, Table 2). While the oral bioavailability in preterm neonates

could be estimated on the basis of observations in a cross-over study, the estimated oral bioavailability of midazolam in adults is, although overlapping in range, lower than estimated by others reporting values between 24% and 46% [19, 20, 21, 22]. Therefore, the sensitivity of the overall model to specific age and study populations should be further tested. In summary, this is the first analysis in which changes in oral bioavailability of midazolam from preterm neonates to adults in a combined dataset are quantified.

The oral absorption rate constant was estimated at $3hr^{-1}$ (Table 2), which was similar ($3.5\pm2.3$ $hr^{-1}$) to a study in children between 2 years and 12 years of age by Reed *et al*.. [24] Johnson *et al.* reported a two-fold higher absorption rate of approximately $6hr^{-1}$ (range 3.9-9.4hr-1) in children between 9 months and 12 years of age [42], where $k_a$ was estimated using population PK modeling techniques with two samples per patient in 45 patients, potentially limiting the exact estimation of $k_a$. In our analysis no relation of $k_a$ with either PNA or bodyweight could be identified. The variance of $k_a$ was fixed to 0, as there was no improvement of the model, both in terms of objective function as well as model stability, which could not be explained by overparameterization (condition number of 39 was well below 1000). While we emphasize that a potential limitation of our study is the sample density in the beginning of the PK time course, we conclude that on the basis of our dataset no age-related variability could be identified in $k_a$.

The estimated clearance for preterm neonates is much lower than in older children and adults, and is in line with low CYP3A4 activity at birth. Reed *et al.* [24] showed a CL/F for relatively healthy children with a mean bodyweight of 7.6, 25, and 55.7 kg to be 3.0, 2.5, and 1.5 L/kg/hr, respectively, which corresponds with our findings of 2.9, 2.2, and 1.6 L/kg/hr for the respective bodyweights. In another study by Payne *et al.* [23] in relatively healthy children from 3 to 10 years (mean of 17 kg), a CL/F of 3.5 (+/- 1.2) L/kg/hr was reported, which appears only slightly higher than our findings of 2.5 L/kg/hr.

Simulation of midazolam concentrations after oral and iv administration on the basis of the final model (Figure 4) show that the higher oral bioavailability in preterm infants necessitates downward dose-adjustments of oral midazolam doses in comparison to infants and adolescents to reach similar concentrations as when an intravenous dose is given (1.6, 3 and 5, respectively). As a higher midazolam oral bioavailability in this young population is most probably reflective of developmentally low intestinal and hepatic CYP3A activity, downward dose-adjustments of other CYP3A substrates when switched from intravenous to oral dosing may also be needed.

In our analysis we used oral bioavailability (F) as a parameter of interest to study oral absorption of midazolam. We emphasize that this parameter captures several PK processes such as

absorption *per se*, local intestinal CYP3A extraction and first pass hepatic CYP3A mediated extraction. Midazolam is a class 1 drug in the Biopharmaceutics Classification System (BCS) [43], meaning that it is highly soluble at a pH range of 1–7.5 at 37°C, and highly permeable (parent drug plus metabolites) with an absorption of 90% in comparison to an intravenous reference dose. This would imply that the stomach pH or gastric lavage would not influence the extent of midazolam absorption, neither would high-fat meals have a significant effect on oral bioavailability, [43] leaving fist-pass intestinal and hepatic metabolism as processes of interest for the much lower oral bioavailability reported in this study. In healthy adults, the contribution of intestinal midazolam extraction to oral bioavailability was estimated by distracting the hepatic midazolam extraction using systemic clearance, estimated hepatic blood flow and hematocrit from total clearance. [21] Hepatic and intestinal extraction appeared largely similar, supporting the hypothesis that intestinal CYP3A contributes significantly to midazolam first-pass metabolism. Unfortunately, liver blood flow reference values are not available for preterm infants. In older children, liver blood flow has been estimated using indocyanin green [44], which has inherent limitations as it also reflects liver transporter activity. [45] Consequently, we were not able to estimate with confidence the relative contribution of intestinal CYP3A activity to the first-pass metabolism of midazolam. Future research should therefore focus on unraveling the maturation in subprocesses that contribute to the *in vivo* derived oral bioavailability maturation function that we identified in this analysis.

It is a limitation of our study that oral data were lacking from children between two weeks and one year old. This precludes an estimation of the exact developmental pattern in this age range of combined hepatic and intestinal CYP3A activity *in vivo* at this time. However, it is clear that there are significant differences in midazolam clearance and oral absorption between children below two weeks and above one year of age.

## 5. CONCLUSIONS

In the population pharmacokinetic model of midazolam, oral bioavailability and clearance each show a distinct developmental pattern. This information may be used to design age-appropriate dosing schedules for oral midazolam that can be used in pharmacodynamic titration studies. The results may also provide a basis for further study on maturation of physiological subprocesses contributing to oral bioavailability and clearance of midazolam and other CYP3A substrates.

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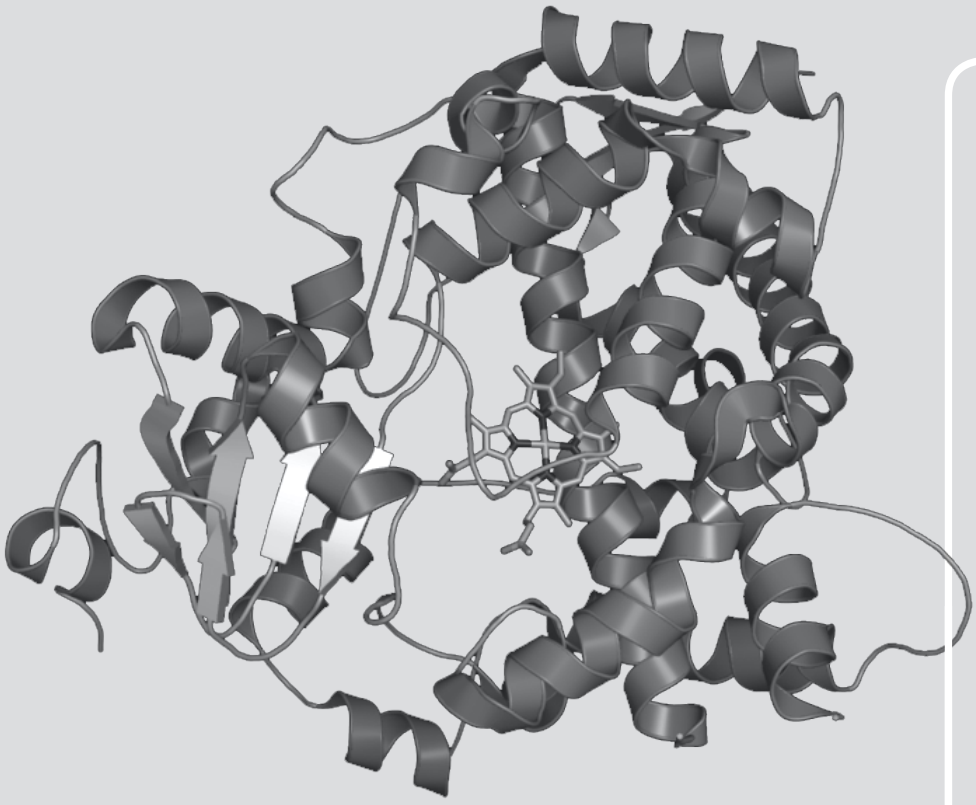

# 7 Extrapolation of the midazolam CYP3A maturation function to cisapride: towards a semi-physiological approach for pharmacokinetics modelling in children

Ince I, de Wildt SN, Vermeulen A, Tibboel D, Danhof M, Knibbe CA, Submitted

## ABSTRACT

Introduction: A maturation function for the CYP3A mediated clearance of midazolam has previously been established in a population of subjects ranging from preterm neonates up to adults. The aim of this study is to investigate whether this maturation function can be extrapolated to the pharmacokinetics of another CYP3A substrate, i.e. cisapride, in neonates and infants.

Methods: Cisapride plasma concentrations from 36 neonates and young infants (postnatal age: 4 - 102 days, postconceptional age: 28-54 weeks, BW: 0.98-6.55 kg) who received 0.2 mg/kg cisapride as a suspension were obtained from a previous study. A pharmacokinetic model was developed using the previously reported maturation function for midazolam clearance (CYP3A maturation model). In addition, a reference pharmacokinetic model was developed using a comprehensive covariate analysis.

Results: The descriptive and predictive properties of the CYP3A maturation model (with cisapride clearance described as $CL/F_i=CL/F_p*[(BW_i/BW_{median})^{BDE+0.31}]$ in which the bodyweight dependent exponent, $BDE = 0.768 * BW^{-0.237}$) were similar compared to the reference model on the basis of basic goodness-of-fit plots and NPDE. In the reference model, BW was also identified as the best predictor for cisapride clearance, albeit with a linear relationship.

Conclusions: The use of a maturation function for CYP3A mediated clearance as identified on the basis of the variation in midazolam clearance in subjects ranging from preterm neonates to adults yields an adequate description of the maturation of cisapride clearance in neonates and young infants. It is concluded that this semi-physiological modeling approach, using a system-specific maturation function for clearance, may be applied to other CYP3A oxidized drugs.

## 1. INTRODUCTION

Given the profound differences in both drug disposition and drug effect between children and adults, and between children of different ages, drug doses in children should be individualized on the basis of differences in pharmacokinetics and pharmacodynamics. Recently, major progress has been made in the identification of developmental changes in the clearance of drugs that are eliminated via different drug metabolizing enzymes and renal excretion (e.g. morphine, midazolam, propofol, and busulfan, aminoglycoside antibiotics). [2, 3, 4, 5, 6] These studies have shown that maturation of clearance across the human life-span is typically non-linear, and that there are substantial differences in maturation profiles between biotransformation routes and pharmacological targets. In these studies, the population approach has proven an essential tool in the identification of these maturation profiles as it allows for the identification of covariate functions describing maturation in clearance on the basis of sparse data. [7] An important property of the observed maturation profiles is that they are "system specific" in the sense that they reflect changes in the functioning of the biological system. [8, 9] As a result these maturation functions can be used as a basis to predict changes in the clearance of other drugs that are eliminated via the same elimination route. Recently this has been demonstrated for drugs that are eliminated through glucuronidation. [10, 11] Specifically, the maturation function that has been identified for the clearance of morphine, a UGT2B7 substrate, has been successfully applied to describe maturation in the clearance of zidovudine, also a UGT2B7 substrate, and potentially other UGT substrates. [10, 11]

The objective of the current investigation is to evaluate the predictive performance of the maturation function for CYP3A mediated oxidation that has recently been identified on the basis of changes in the clearance of midazolam following intravenous and oral administration, [1] for another CYP3A substrate. This maturation function for CYP3A mediated clearance of midazolam was successfully described by an allometric equation with an exponent varying with bodyweight, while the maturation of CYP3A mediated bioavailability was predicted with a negative allometric exponential function. [1] In assessing the predictive performance of this function for another CYP3A substrate, we used cisapride as a paradigm compound for CYP3A mediated oxidation. Although today the therapeutic use of cisapride has been largely restricted or even suspended, it was selected for this study, because data were available in neonates and young infants, and because it is mainly eliminated via CYP3A mediated metabolism. [12, 13]. The results of the model performance of the CYP3A maturation model are compared to the performance of a reference model that was developed using a comprehensive covariate analysis of the same cisapride pediatric dataset. [7]

## 2. METHODS

### 2.1 Patients and data

Neonates and young infants that received oral cisapride were included in the analysis. The data were received via the framework of Dutch Top Institute Pharma project in The Netherlands, number D2-104 (http://www.tipharma.com). Details on the patient dataset are provided in table 1.

**Table 1.** Overview of the dataset.

| Patient Population | *Neonates and young infants* |
|---|---|
| Number of patients | *N =36* |
| Male / Female | *N= 22 / 14* |
| Postnatal age *(days)*<br>*Median (range)* | *37 (4-102)* |
| Postconceptual age *(weeks)*<br>*Median (range)* | *37 (28-54)* |
| Bodyweight *(kg)*<br>*Median (range)* | *2.3 (0.98–6.55)* |
| Cisapride dose *(mg)*<br>*Median (range)* | *0.44 (0.196 – 1.31)* |
| N of samples per individual<br>*Median (range)* | *7 (4-7)* |

### 2.2 Pharmacokinetic data analysis

For both the maturation model and the reference model, population pharmacokinetic analysis was performed using the first-order conditional estimation (FOCE) with η-ε interaction in NONMEM version 6.2, release 1.1 (GloboMax LLC, Hanover, MD, USA). [14] S-plus version 6.2.1 (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P, Leiden, The Netherlands) was used to visualize the data.

Model development was performed in four steps: 1) choice of the structural model, 2) choice of the stochastic model, 3) covariate model and 4) validation of the model. The maturation model and the reference model differed in step 3 covariate model only (see under 'Covariate model').

Discrimination between different models in step 1 and 2 was made by comparison of the objective function. A decrease in the objective function of 3.8 points, corresponding to a value of $p < 0.05$, was considered statistically significant. Goodness-of-fit plots (observed *versus* individually predicted concentration, observed *versus* population predicted concentration, conditional weighted residuals *versus* time, and conditional weighted residuals *versus* popu-

lation predictions) were used for diagnostic purposes. In addition, the confidence interval of the parameter estimates, the correlation matrix, and visual improvement of the individual plots were used to evaluate the model. Furthermore, η-shrinkage as defined by Karlsson *et al*. [15], was calculated for all model parameters for which inter-individual variability was estimated, and over-parameterisation (ill-conditioning) of the model was tested by calculating the condition number. [16]

For discrimination between different models for step 3 and step 4 see under 'Covariate model' and 'Validation of the model', respectively.

## 2.3 Structural and stochastic model development

The structural pharmacokinetic model used for cisapride (step 1 as described in the 'Pharmacokinetic data analysis' section) is a one-compartment pharmacokinetic model with linear absorption and elimination, parameterized in terms of dose compartment (D), absorption rate constant (ka), volume of distribution (V/F), and cisapride total clearance (CL/F). This structural model was used for both the maturation model and the reference model.

For the stochastic model (step 2 as described in the 'Pharmacokinetic data analysis' section), the individual value (*post hoc* value) of the parameters of the *i*th subject was modeled using the equation: $P_i = P_{pop} \cdot e^{RV}$, where $P_i$ equals the individual or *post hoc* value of the parameters of the *i*th subject, and $P_{pop}$ is the population value of the parameter and RV is assumed to be a gaussian random variable with mean zero and variance of $\omega^2$, assuming log-normal distribution. The intra-individual variability was described with a proportional error model for all data, assuming a constant coefficient of variation over the entire concentration range. This means for the *j*th observed cisapride concentration (Y) of the *i*th individual: $Y_{ij} = C_{pred,\,ij} \cdot (1 + RV_{ij})$, where $C_{pred}$ is the predicted cisapride concentration and $RV_{ij}$ is the random variable for cisapride, with mean zero and variance $\sigma^2$. This stochastic model was used for both the maturation and the reference model.

## 2.4 Covariate model

For the covariate model (step 3 as described in the 'Pharmacokinetic data analysis' section) different approaches were used for the maturation and the reference model. For the maturation model, the system specific parameter values of the previously reported equation characterising age-related changes in midazolam clearance with an exponent varying with bodyweight [1] and in oral midazolam bioavailability with an allometric equation with a negative exponent [1] were incorporated to clearance (CL/F) and volume of distribution of the central compartment (V/F). For $(CL/F)_i = (CL/F)_{pop} \cdot (BW_i / BW_{median})^{BDE+B}$, BDE represents the

**Table 2.** Population parameter estimates of the maturation model and reference pharmacokinetic model for cisapride.

| Parameter | Maturation model | | Reference model | | Explanation |
|---|---|---|---|---|---|
| | *Value* | *(CV%)* | *Value* | *(CV%)* | |
| **$(CL/F)_i = (CL/F)_{2.3kg} \cdot (BW/2.3)^{BDE+B}$** | | | | | *CYP3A mediated oral clearance of cisapride (maturation model[1])* |
| $(CL/F)_{2.3kg}$ (L/h) | 1.38 | (11.5) | | | *CL/F for a median individual with a BW of 2.3 kg* |
| *BDE = Coeff x BW $^{exp1}$* | | | | | *BDE of power function on CL [1]* |
| *Coeff* | 0.768 | FIX | | | *Coefficient of the BDE function [1]* |
| *exp1* | -0.237 | FIX | | | *Exponent of the BDE function [1]* |
| *B* | 0.31 | FIX | | | *Exponent of power function on bioavailability [1]* |
| **$(CL/F)_i = (CL/F)_{2.3kg} \cdot (BW/2.3)$** | | | | | *Oral clearance of cisapride (reference model)* |
| $(CL/F)_{2.3kg}$ (L/h) | | | 1.33 | (10.8) | *CL for a median individual of the population with a BW of 2.3 kg* |
| **$(V/F)_i = (V/F)_{2.3kg} \cdot (BW/2.3)^B$** | | | | | *Distribution volume for cisapride(maturation model)* |
| $(V/F)_{2.3kg}$ (L) | 17.2 | (15) | | | *V for a median individual of the population with a BW of 2.3 kg* |
| *B* | 0.31 | FIX | | | *Exponent of the allometric function on bioavailability [1]* |
| **$(V/F)_i = (V/F)$** | | | | | *Distribution volume for cisapride(reference model)* |
| (V/F) (L) | | | 17.3 | (15.5) | *Population estimate of V (reference model)* |
| **Ka ($h^{-1}$)** | 0.36 | (24.1) | 0.36 | (23.2) | *Absorption rate constant for cisapride* |
| $\omega^2$ CL | 0.34 | (29.4) | 0.35 | (27.6) | *Inter-individual variance in CL* |
| $\omega^2$ V | 0.52 | (37) | 0.49 | (35.3) | *Inter-individual variance in V* |
| $\omega^2$ Ka | 0.66 | (33.6) | 0.63 | (33.3) | *Inter-individual variance in Ka* |
| $\sigma^2_{proportional}$ | 0.125 | (16.6) | 0.125 | (16.7) | *Residual variance (proportional error)* |
| OFV | 1077.404 | | 1075.882 | | *Objective function value* |

*BW* bodyweight, *BDE* bodyweight-dependent exponent, *CL* clearance, *Coeff* coefficient of the BDE function, *CYP* cytochrome P450, *B* Exponent of the allometric function on bioavailability,[1] Shrinkage: maturation model CL= 5.1%, V = 14.9%, Ka= 20%, reference model CL= 5.5%, V = 20.3%, Ka= 14.6%

body weight dependent exponent for clearance, i.e. BDE = Coeff x $BW^{exp1}$ with Coeff (0.786) as a coefficient and BW as body weight with an exponent exp1 (-0.237), and B (0.31) represents the exponent of the allometric function on bioavailability. [1] For $(V/F)_i = (V/F)_{pop} \cdot (BW_i / BW_{median})^B$, B (0.31) represents the exponent of the allometric function on bioavailability [1] (Table 2).

For the reference model, for step 3 covariate analysis, individual *post hoc* parameter estimates were plotted independently against the covariates to visualize potential covariate relation-

ships. [7] The following covariates were tested for all parameters: bodyweight (BW), postnatal age (PNA), and postconceptual age (PCA). Potential covariates were separately entered into the model using a linear or power equation ($P_i = P_{pop} \cdot (COV_i / COV_{median})$, $P_i = P_{pop} \cdot (COV_i / COV_{median})^K$, or $P_i = P_{pop} \cdot (COV_i / 70)^{0.75}$, respectively, where $P_i$ equals the individual or *post hoc* value of the parameters of the *i*th subject, $P_{pop}$ is the population value of the parameter, COV is the concerned covariate, K is the exponent, equals 1 in case of a linear function, or is estimated or fixed to 0.75 in case of a allometric (or bodyweight based) function. The influence of a covariate was statistically tested for significance by use of the objective function. A p value < 0.005 (decrease of objective function of 7.8 points) was applied to evaluate the covariates in the forward inclusion, while on the other hand a more stringent p value of <0.001 (decrease of objective function of 10.83 points) was used in the backward deletion. When two or more covariates were found to significantly improve the model, the covariate that showed the largest reduction of the objective function was included in the model. Additional covariates had to reduce this objective function further to be retained in the model. The choice of the model was further evaluated as discussed under 'data analysis' and 'model development'. This included also individual and population parameter estimates versus the most predictive covariate in the model. [17]

### 2.5 Validation of the model

For the internal validation of both maturation model and reference model (step 4 as described in the 'Pharmacokinetic data analysis' section), a bootstrap analysis was performed in S-plus, version 6.2.1 (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P, Leiden, The Netherlands). Two hundred datasets were resampled from the combined original datasets, and refitted to the model. [18]

Secondly, Normalized Prediction Distribution Errors (NPDE) were calculated, [19] by using the NPDE package in R. [20] For this method, the combined dataset that was used for building the model was simulated 1000 times with inclusion of the inter-individual variability and residual error.

## 3. RESULTS

### 3.1 Patients

Cisapride plasma concentrations of 36 individuals ranging from neonates to young infants were available for the analysis. Their postnatal age (PNA) ranged from 4 to 102 days while their postconceptional age (PCA) ranged between 28 to 54 weeks PCA, Body weights were

between 0.98 and 6.55 kg. None of the patients received CYP3A inhibitors or inducers. A summary of the patient and data characteristics is shown in table 1.

## 3.2 Model development and covariate analysis

Cisapride pharmacokinetics was well described with a one-compartment model with first-order absorption and elimination, and a proportional error model. Inter-individual variability was implemented on all parameters (CL/F, V/F and $k_a$), and significantly improved the model performance as reflected in a reduction of the objective function ($p<0.001$).

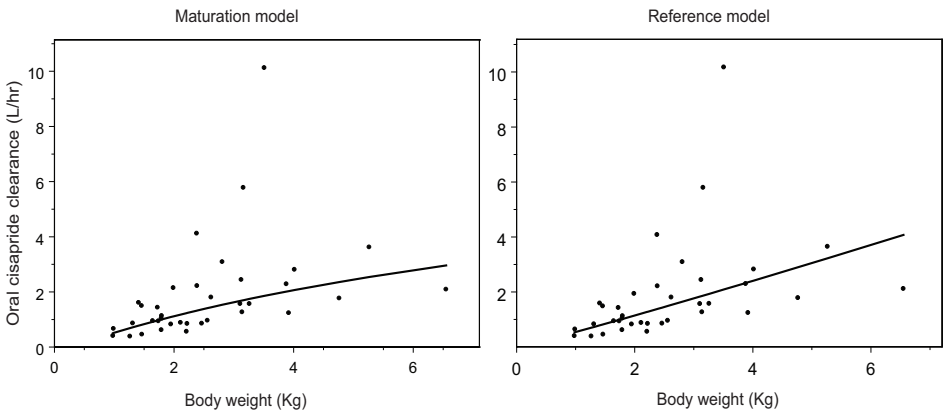


**Figure 1.** Posthoc individual estimates (solid circles) and population estimate (solid line) of CYP3A mediated oral clearance of cisapride *versus* bodyweight from the maturation model and the reference (covariate) model in neonates and young infants.

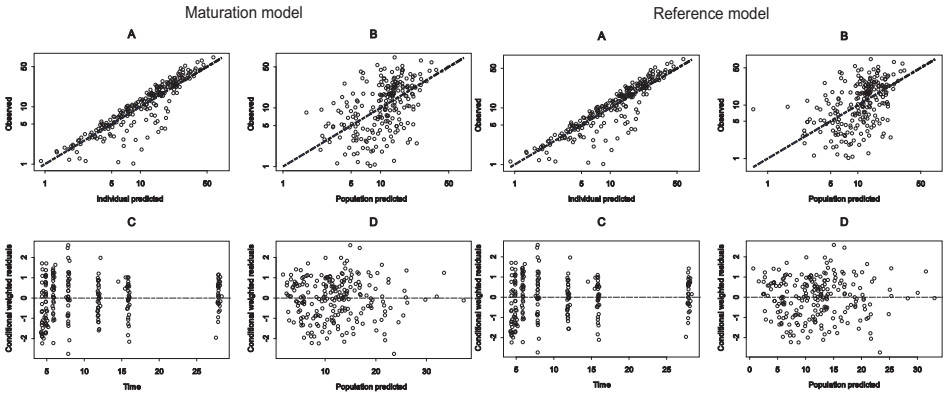


**Figure 2A-D.** Visual diagnostics of the maturation model (A-D, left), reference (covariate) model (A-D right). A & B = Individual and population and predicted concentrations *versus* the observed concentrations of cisapride, respectively. C & D = Conditional weighted residuals *versus* Time and Population predicted concentrations for cisapride, respectively. The *solid line* indicates the line of unity.

For the CYP3A maturation function model, the inclusion of the maturation function with fixed parameter values as previously identified for midazolam [1], on cisapride CL/F, and V/F, led to a significant improvement in the model fit as reflected in a reduction of the objective function value from 1084.336 to 1077.404 ($p < 0.01$) (figure 1). For the reference model, the comprehensive covariate analysis revealed that bodyweight explained a large part (74.7% decrease) of the inter-individual variability of CL/F and resulted in a much improved model fit as reflected in a decrease in -2LL of 8.45 points ($p < 0.005$). The correlation between body-weight and cisapride CL/F was best described using a linear function, as shown in figure 1. Table 2 summarizes the pharmacokinetic parameter values, their confidence intervals and the interindividual variation of both the CYP3A maturation function model and the reference model.

For both the maturation model and the reference model, figure 2 illustrates that the overall individual predicted concentrations (IPRED, a) and population predicted concentrations (PRED, b) *versus* the observed concentrations, are equally spread around the line of identity, as represented by the black solid line, and that there is no trend as a function of time (c) or PRED (d) *versus* conditional weighted residuals. For the inter-individual variability, η-shrinkage for cisapride clearance (CL/F) was below 6% for both models (maturation model 5.1% and

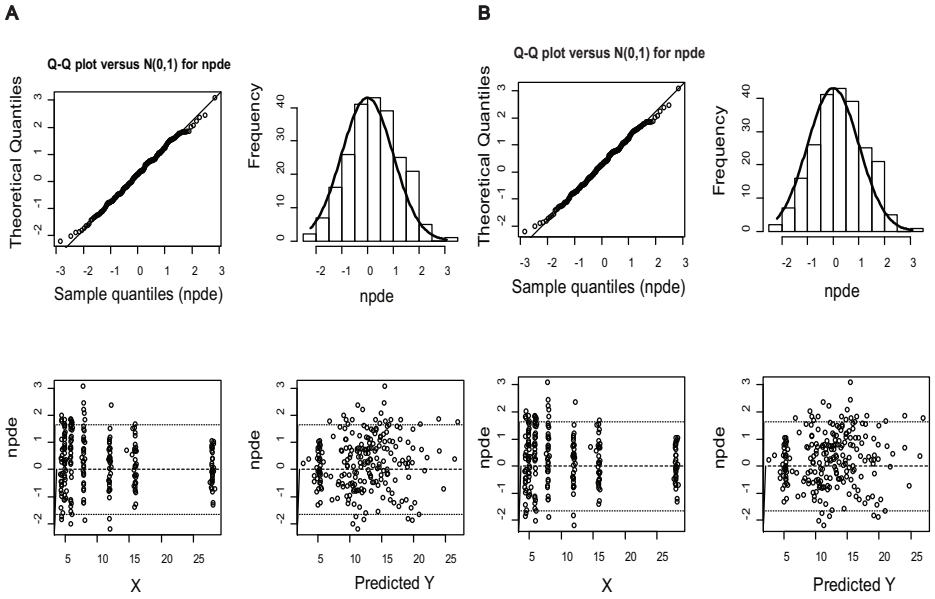


**Figure 3.** Internal validation (NPDE) of the maturation model (A, left), and reference model (B, right). The quantile-quantile (Q-Q) plot illustrates the NPDE's for cisapride *versus* the corresponding quantiles of a normal distribution, with the line y=x overlayed. The histogram illustrates the distribution of the NPDE in which the *solid line* represents a normal distribution. In addition, the distribution of NPDE's in time after first dose and against the observed concentrations is illustrated.

reference model 5.5%). For V/F and $k_a$, η-shrinkage was 14.9% and 20% for the maturation function model, and 14.6% and 20.3% for the reference model, respectively.

The histograms of the NPDE for cisapride, as depicted in Figure 3, illustrate the distribution of the NPDEs for all data resulting from the maturation function model (a), and the reference model (b), with means close to zero (0.25 for the mean, and 0.96 for the variance, for both the maturation function model and the reference model, respectively), and with no observed trends as a function of time or concentration.

## 3. DISCUSSION

In this study we investigated whether for drugs that are eliminated through a common enzyme (metabolic pathway), maturation functions for drug clearance and bioavailability can be extrapolated from one drug to another. To this end available data on the pharmacokinetics of cisapride in children were analysed and the performance of two models, a CYP3A maturation function model, which uses prior information on the maturation of midazolam clearance and bioavailability, and a reference model, which was developed on the basis of a comprehensive covariate analysis of the cisapride data *per se*, were compared. It was shown that the CYP3A maturation function model performed very comparable to the reference model for cisapride in young children. It is concluded that maturation in the clearance of cisapride can be predicted from the maturation in the clearance of midazolam, as both drugs are CYP3A substrates.

The prediction of the maturation in clearance across the human life-span presents a major challenge, because of the multifactorial nature of this process. Specifically, depending on the drug, multiple mechanisms with each their own maturation profile may contribute to variation in clearance: organ perfusion (i.e. hepatic blood flow), binding (i.e. to plasma proteins), intrinsic clearance (i.e. the expression and function of specific drug metabolizing enzymes and transporters) and route of administration. In the present investigation midazolam and cisapride were chosen as paradigm drugs, because they are both eliminated through CYP3A. Also, their plasma clearance has been shown to reflect CYP3A activity. Hence, the total plasma clearance was used as the parameter of interest. An important question is whether the same mechanisms determine the developmental changes in clearance for both drugs. The pharmacokinetics of midazolam are relatively complex. Following intravenous administration, midazolam is eliminated with a low to intermediate extraction in the liver. As a result the value of the systemic clearance is governed by both the degree of plasma protein binding and the intrinsic hepatic clearance. [21] With a value of around 95% the degree of plasma protein binding is relatively high. [22] Moreover, due to its binding to alpha-1-acid

glycoprotein the degree of plasma protein binding highly vary. Variation in systemic clearance can therefore be explained both by variation in the intrinsic clearance (the expression of hepatocellular CYP3A) and the degree of plasma protein binding. Moreover, upon oral administration, midazolam is subject to first-pass extraction, predominantly resulting from biotransformation in the gut wall, with a variable bio-availability ranging from 67% in preterm neonates to 16% in adults. [1] It cannot be excluded that maturation of CYP3A in the gut wall evolves differently over time as compared to its maturation in the liver. It is therefore still unclear if intestinal CYP3A activity changes in parallel with hepatic CYP3A activity during ontogeny, although hepatic and intestinal ontogeny data suggest roughly the same pattern. [23] In the previous investigation, the pharmacokinetics of midazolam following intravenous and oral administration were simultaneously analysed, yielding a single estimate of systemic clearance. [1]

The pharmacokinetics of cisapride bear similarities with midazolam. Specifically, upon oral administration the absolute bioavailability of cisapride is around 30%, [24] presumably as a result of a high first-pass effect due to CYP3A mediated intestinal and hepatic drug metabolism. Moreover with a value of 98% the degree of plasma protein binding is high. We can therefore not exclude that these similarities in oral bioavailability and plasma protein binding of midazolam and cisapride may explain the good performance of the maturation model based on information from midazolam for the cisapride pediatric dataset. In addition the range in age of the pediatric dataset of cisapride was small, even though the magnitude in bodyweight between the individuals was more than 6-fold. It is of great importance that the current maturation function for CYP3A that was derived on the basis of midazolam data across the human life-span is tested for other drugs mainly eliminated via the CYP3A4 pathway to further strengthen our hypothesis. These drugs may be newly developed drugs, or existing drugs currently used in children, such as tacrolimus, nifedipine, simvastatin and sirolimus.

In this respect, it needs to be emphasized that pharmacokinetic profiles are the result of the interaction between the drug and the biological system. The parameters used to describe pharmacokinetic profiles therefore reflect both the drug-specific and/or system-specific aspects of this interaction. The results from the current study indicate that developmental changes in CYP3A mediated clearance and bioavailability, as derived from midazolam data, represent system-specific changes in the functioning of CYP3A. The same has been shown for drugs which are cleared through glomerular filtration, [25] and glucuronidation (i.e. UGT2B7). [8, 10, 11] The distinction between drug-specific and system-specific parameters is a fundamental property of mechanism-based PK and PKPD models. [26] System-specific properties may therefore not only include static descriptors of the physiological system, but also temporal changes in the physiological system as a result of developmental changes in

the paediatric population. [10, 11, 26, 27] The cisapride model that was based on the CYP3A maturation model obtained with midazolam could therefore also be referred to as the 'system-specific model' for maturation of CYP3A mediated clearance.

To develop semi physiological functions that can be used to extrapolate system-specific information from one drug to another as we describe in this paper, it is emphasized that an extensive validation of the covariate model from which the system-specific information will be derived needs to be performed. [8, 28] The midazolam maturation model has been internally validated using bootstrap resampling and NPDE, of which the results confirmed the robustness of the model. [1] Although in the original analysis there was limited information on midazolam data after oral administration at the age between 1 month and 1 year of age, the CYP3A maturation function that was derived from the midazolam maturation model [1] was able to adequately predict the clearance of orally administered cisapride in individuals between 4 days up to 3 months of age. It should also be mentioned that extrapolation can only be performed to other populations with similar characteristics as the population from which the system-specific information was derived. In our analysis the age and weight range of the neonates and young infants receiving cisapride fell within the range of preterm neonates up to adults receiving midazolam and was an ideal population to test. It would be of great interest to also asses the extrapolation of the CYP3A maturation function based on midazolam to other populations, including the older age groups, which received cisapride or other CYP3A substrates.

## 4. CONCLUSION

The extrapolation of system specific information, i.e. the maturation in CYP3A mediated midazolam clearance from preterm neonates to adults, highly facilitated the descriptive and predictive modeling of cisapride concentrations in neonates and young infants. With this approach, in pharmacokinetic models a distinction is being made between system specific and drug specific information. Using this semi-physiological approach, age-related variation in clearance of drugs eliminated through the same pathway may be predicted, and may lead to optimization of sparse data analysis in children.

## ACKNOWLEDGEMENTS

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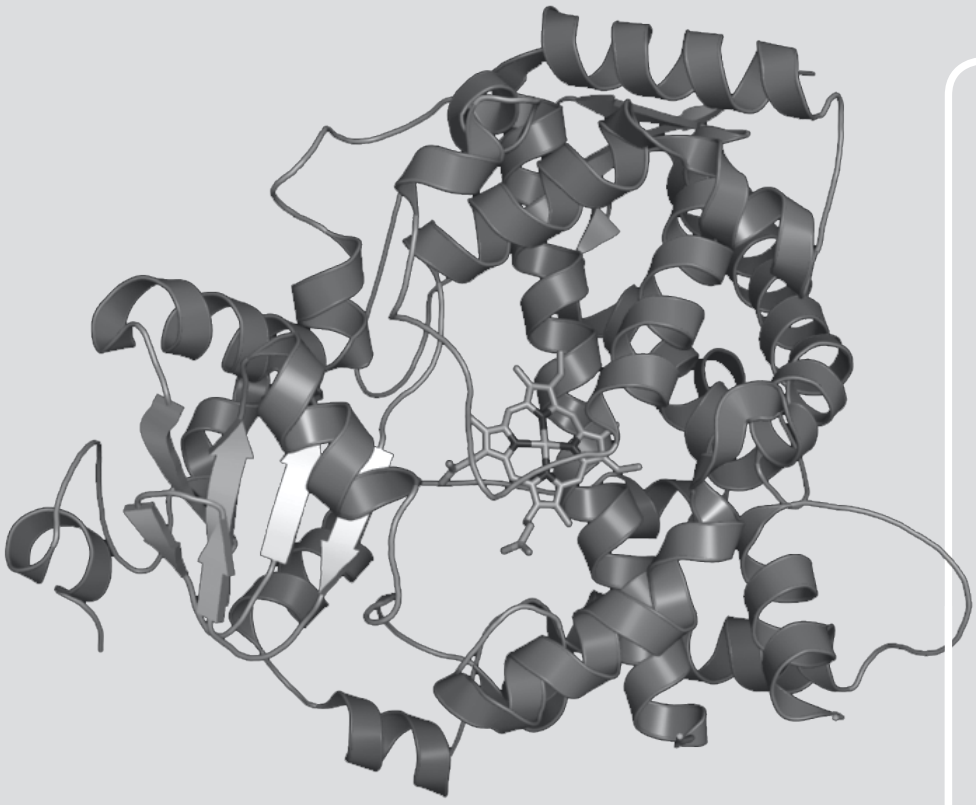
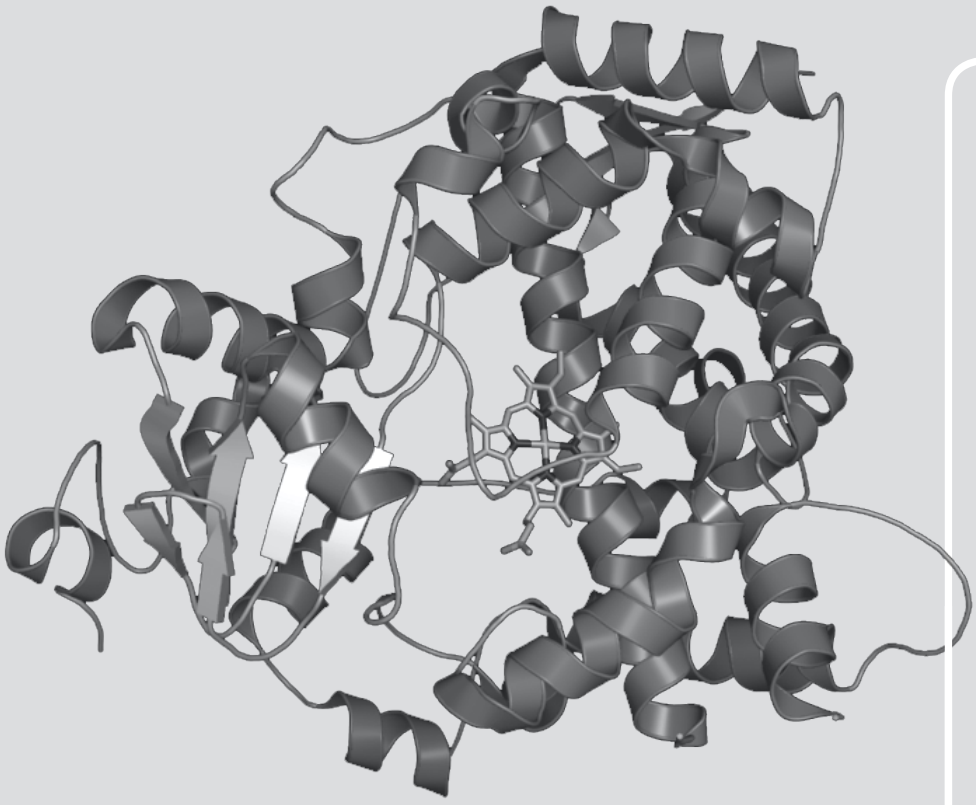


# Part III

## General discussion and summary



# 8 Maturation of cytochrome P450 3A Mediated Drug Metabolism – Summary, conclusions and perspectives

## MATURATION OF CYTOCHROME P450 3A MEDIATED DRUG METABOLISM

## GENERAL DISCUSSION

Most drugs in children are prescribed without having been adequately studied for this population. In many instances information on the optimal dose in children of various ages is lacking.[1] As a result the doses are most often extrapolated from adult doses with a simple bodyweight or body surface area correction. The relative lack of dedicated studies on the optimal use of drugs in children may be explained by the ethical, practical and financial constraints of clinical trials in this patient population.[2, 3] The number of pediatric patients that receive at least one off-label or unlicensed drug ranges between 80% to 93% in neonatal intensive care units, between 36% to 100% in paediatric hospital wards, and between 3.3% to 56% in nonhospital settings.[1]

It is well established that the response to drug treatment differs between adults and children as well as between children of different ages. It has also been shown that this may be explained, at least in part, by differences in the pharmacokinetics, as these changes are known to be particularly large when the entire human life-span is considered. All the pharmacokinetic processes, absorption, distribution, metabolism and elimination (ADME) have been shown to vary with age.[2] Therefore, to prevent underdosing or toxicity, it is essential to have quantitative information on the developmental changes in pharmacokinetic processes, in the context of all other sources of variation.

In recent years, important progress has been made in the characterization of variation in pharmacokinetics in children, by application of the population approach. Briefly, with this approach, information on the variation in pharmacokinetics can be obtained on the basis of sparse data and with incomplete data sets.[4] Moreover, in a systematic covariate analysis the sources of the interindividual variation can be identified. This constitutes a rational basis for the individualized dosing of a specific drug in clinical practice.[5] The development of population pharmacokinetic models for individual drugs requires a major research effort. It is therefore practically impossible to develop descriptive population pharmacokinetic models for all drugs that are used in pediatric clinical practice. Therefore a more systematic approach is needed to adequately predict variation in pharmacokinetics in children.[6]

In mechanistic terms, variation in pharmacokinetics may be caused by variation in size (of the various tissues and organs), variation in binding (i.e. to plasma proteins, tissues), variation in distribution (i.e. due to differences in perfusion or barrier function) and variation in systemic clearance (due to variation in the perfusion of the eliminating organs) and the intrinsic

clearance (due to variation in the expression and function of drug metabolizing enzymes and transporters). In theory, variation in the pharmacokinetics of drugs in children can be predicted on the basis of pertinent quantitative information on the changes in biological function. This constitutes a scientific basis for a "systems approach" to the optimization of drug treatment in children.

Information on the variation in pharmacokinetic mechanisms can be obtained by studying changes in the pharmacokinetics of paradigm drugs. For example information on variation in the activity of a drug metabolizing enzyme can be obtained by studying variation in the intrinsic clearance of paradigm drug. In theory, this "system-specific" information can be used to predict variation in the elimination of another drug that is metabolized by the same enzyme.[6]

The aim of the research described in this thesis was twofold. The first objective was to characterize developmental changes in the activity of Cytochrome P450 3A in children on the basis of changes in the pharmacokinetics of midazolam as a paradigm drug, following intravenous and oral administration. The second objective was to explore the prediction of developmental changes in the pharmacokinetics of a second drug (cisapride) by using the system-specific information on the maturation obtained with midazolam

## 1. POPULATION PK MODELING AS A TOOL FOR INDIVIDUALIZED DRUG DOSING IN CHILDREN

In chapter 2 we propose the use of population PK-PD modeling and simulation to develop evidence-based dosing schemes for children, with the ultimate goal to improve drug safety and efficacy in this population. The most important advantage of this population approach is that it allows for the use of infrequently and routinely obtained samples and observations from actual patients at time points compatible with clinical care, rather than in a specific experimental setting. The population approach allows for the analysis of relatively dense data, combinations of sparse and dense data or combinations of observations from experimental settings and clinical practice. As the population approach is able to handle 'missing data' in individual patients, it greatly facilitates pharmacokinetic studies in young children,[7] ensures that the obtained information can indeed be directly applied in clinical practice and that the burden to the individual patient can be kept to a minimum.[4] In addition, the population approach allows the analysis of so-called covariates, such as the influence of age-related variability on PK and/or PD parameters, and must be considered in the context of all other sources of intra- and inter-individual variability resulting from genetic-, environmental- and disease related factors and drug interactions. Population PK-PD modeling and simulation should be used as a tool to develop evidence-based dosing schemes for children. In addition

to the development of PK-PD models, an internal validation and external validation using independent data should be performed, followed by a prospective clinical evaluation. These validations and evaluation are important as they would demonstrate the reliability of the model predictions for specific drugs,[8] and may then serve as a basis to develop dosing guidelines for existing or newly developed drugs with similar disposition and/or effect.

### Recommendations

Population PK-PD modeling and simulation for children should be applied as follows:

1. Clinical trial designs based on prediction of the values of pharmacokinetic parameters of drugs in children, resulting from a population approach.
2. Development and internal validation of population PK-PD models using sparse data from these clinical trials.
3. External validation using independent data.
4. Prospective clinical evaluation of the simulated dosing guidelines.
5. Optimization of future clinical trial designs based on prediction of the values of pharmacokinetic parameters of drugs in children, resulting from a population approach.

## 2. CYP3A ONTOGENY AND THE CLEARANCE OF CYP3A SUBSTRATES

In chapter 3 we provide an updated overview of current knowledge on the developmental changes in CYP3A activity. A variety of *in vitro* and *in vivo* methods have been used to study the ontogeny of hepatic and extra-hepatic CYP3A activity.

It was confirmed that CYP3A7 is the dominant isoform before birth, but that its activity decreases to very low levels in the first week of life. The underlying mechanisms governing the transition from CYP3A7 to CYP3A4 are still largely unknown. One of the factors that may play a role in the differential expression of CYP3A7 and CYP3A4 is age-related variation in DNA methylation. This variation may be governed by exposure to endogenous (e.g hormones) and exogenous (e.g. feeding) substrates during the transition from fetal through neonatal life. *In vitro* studies show a slow rise in CYP3A4 activity after birth but data from older children remain more ambiguous. The available data show information gaps in the first year after birth, which makes it difficult to describe the exact changes that occur for specific ages in children. Although CYP3A5 activity in livers expressing the gene appears to be relatively stable from fetal to adult life, it is important is that genetic variation is taken into account as CYP3A5 is polymorphically expressed. The interplay of CYP3A genotypes and age needs to be further investigated, especially in neonates and infants, in whom the ontogeny has the greatest impact.

The lack of original studies in children is still high. Nevertheless, the majority of the existing *in vivo* studies performed using specific substrate drugs, report that most of the developmental changes in CYP3A4 activity occur in the first months of life. It is important that more original studies in children should be performed to describe changes in both hepatic and intestinal activity of CYP3A, and quantify the exact changes that occur, so that this information can be used to develop dosing schemes for the pediatric population.

### Conclusions and Recommendations

1. Based on *in vitro* studies, it is impossible to describe a full CYP3A maturation profile for the entire age-range.

2. *In vivo* studies using paradigm drugs are a valuable addition to describe the maturation profile for CYP3A for the entire age-range.

3. In addition to the influence of age-related changes on the variation in CYP3A activity in children, critical illness, genetic variation, and feeding are other important factors causing interindividual variability of CYP3A substrate disposition in children.

4. Efforts should be made to collect pediatric intestinal and liver tissues (including the clinical characteristics of the donor) across the whole pediatric age range to further characterize CYP3A ontogeny. Despite the inherent practical and ethical challenges, as large information gaps still remain, especially in the first year after birth.

5. A specific maturation function for CYP3A based on original data obtained using the population approach should be implemented in population PK and/or PBPK models including both *in vitro* and *in vivo* information, for describing and predicting more accurate drug concentrations for CYP3A specific drugs at specific ages and to developing new individualized dosing schemes.

## 3. THE INFLUENCE OF CRITICAL ILLNESS ON CYP3A ENZYME ACTIVITY

Chapter 4 describes a comprehensive population pharmacokinetic analysis of three different pharmacokinetic studies on midazolam and metabolites in children between 1 month and 17 years of age. In this study, critical illness is identified as a major determinant of midazolam clearance. In critically ill patients a 93% decrease in midazolam clearance is observed compared to non-critically ill patients. We speculated that this large difference can be explained by an ongoing inflammatory response leading to CYP3A4 gene repression.[9] To provide a

basis for this finding, the SimCYP Pediatric Simulator that includes a physiologically based pharmacokinetic (PBPK) model together with extensive libraries on demographics, developmental physiology, and ontogeny of drug elimination pathways, was applied. The results showed that a reduction in CYP3A4 and CYP3A5 levels, as opposed to reduced albumin levels, and hepatic blood flow, had the highest impact on midazolam clearance. For Pediatric Intensive Care Unit (PICU) patients that are seriously ill, the duration of their stay in the PICU is typically longer than the degradation half life of CYP3A4 (26-144 hours) and CYP3A5 (36 hours) in the human body.[10, 11] This could lead to a substantial reduction in the amount of available CYP3A4 and CYP3A5 enzymes in these patients at least in the days after admission.[10, 11] A limitation of our study was that we did not have data on plasma proteins as albumin and Alpha-1-acid glycoprotein (AGP) and/or inflammatory markers such as CRP and/or IL6 to support our hypothesis of inflammation-reduced CYP3A activity. Also, our study was not designed to show a causal relationship between inflammation, CYP3A down-regulation, and reduced midazolam clearance.

Furthermore, we did not study the impact of inflammation on the PK-PD relationship. Receptor expression may also be altered due to inflammation, which may result in a need for higher instead of lower drug doses to reach the desired therapeutic effect[12], even though in adult intensive care patients lower doses of the anesthetic propofol have been reported in case of more severely ill patients.[13]

### Recommendation

1. Data on proteins involved in protein binding, such as albumin and AGP, and inflammatory markers such as CRP and/or IL6 should be collected in further studies in pediatric critically ill patients, to support the hypothesis of their correlation with a decrease in CYP3A activity, and with midazolam clearance.

2. The effect of inflammation in relation to the PK-PD relationship of CYP3A substrates should be elucidated.

## 4. SEMI-PHYSIOLOGICAL MODELING

### 4.1 Maturation of CYP3A mediated clearance of drugs

In chapter 5 we developed a novel maturation function for midazolam clearance, based on a dataset consisting of data obtained from six different clinical studies in (premature) neonates, infants, toddlers, children, adolescents, and adults. This model provides a quantitative insight in the developmental pattern of *in vivo* CYP3A activity across the pediatric age range,

including premature neonates. We proposed that this model may provide guidance to dosing of midazolam, and, potentially, other CYP3A substrates, for children across all age ranges, i.e. by applying this maturation function, which describes the relationship of clearance with bodyweight, in population pharmacokinetic and PBPK models, as a priori information.

In premature neonates between 0.5 and 4 kg, we showed that midazolam clearance was estimated to be only 2.6–21.8% of adult values. These results confirm very low CYP3A4/5 activity after birth,[14] because midazolam is only slightly metabolized by CYP3A7. [15] Our finding that the largest change in clearance occurs in the first few weeks of life is in agreement with CYP3A4/5 *in vitro* and *in vivo* phenotyping data.[14, 16] Data on CYP3A4 activity after the first year of life, as derived from *in vitro* and *in vivo* studies, are highly discrepant. [17-20] The large variation reported for CYP3A activity in *in vivo* and *in vitro* studies may be related to the sub-optimal quality of the post-mortem tissue used for the *in vitro* studies, resulting in erroneously low estimates of CYP3A activity.[21, 22] In addition, the use of average clearance values instead of individual doses and concentrations to model the maturation of midazolam clearance may be another reason for the high variation. Furthermore, the amount of underlying individual data for children between 0 and 1 year of age in these pooled studies was very low, which could have resulted in erroneous estimates for this age range.[21] For our analysis we had access to raw data on demographics, doses, concentrations, and covariates for 136 individuals varying in age between premature neonates and adults. We therefore suggest that our analysis is less prone to the drawbacks mentioned above, which increases the validity of the estimated maturation function for *in vivo* CYP3A4/5 activity. The maturation of CYP3A assessed on the basis of midazolam clearance was found to be highly non-linear, and was best described by a bodyweight based function with an exponent that depends on body weight as well (bodyweight dependent exponent, BDE). This is in line with observations by Wang *et al.*[23] who first reported that an exponent changing with bodyweight in an allometric equation best described changes in propofol clearance occurring between neonatal and adult age. By using the BDE model in which the exponent was found to vary between 0.84 for neonates and 0.44 for adults, midazolam clearance was well estimated across the entire age range. In our model, we used a simplified version of the model of Wang *et al.*[23], by allowing the exponent to change exponentially instead of in a sigmoidal manner (Emax model), as was previously also applied for busulphan in children.[24] The use of a different function to describe midazolam compared to propofol clearance across the entire age range is likely dependent on differences in drug properties. For example, propofol is a marker for UGT1A9 activity and being a high extraction ratio drug, its clearance is mainly influenced by hepatic blood flow.[23] As the maturation of CYP3A activity seems less steep than reported for propofol (highest exponent 0.91 *versus* 1.44, respectively), we suggest that pathway-specific functions must be developed across the age range for different common metabolic and elimination pathways that can be used to predict changes in drug metabolism and elimination in children. In particular, in view of the growing interest in pediatric PBPK models,[25] it is

important to quantify the *in vivo* maturational behavior of all metabolic enzyme systems that are clinically relevant. Maturation functions on enzyme activity derived from *in vitro* studies that are required by PBPK models may be lacking for many enzymes.

From the function for CYP3A mediated clearance of midazolam, we derived a model-based dosing scheme for midazolam administration in premature neonates and children in intensive care. We proposed that instead of empirical dosing on the basis of mg/kg/h, the use of a non-linear maintenance dose should be used, to reach similar midazolam concentrations across age groups. In accordance with the exponent found for changes in clearance within the pediatric range, an exponent of 0.84 is proposed for premature neonates whereas an exponent of 0.65 is used for older children. A previous study by our group on morphine glucuronidation clearance, showed that it is indeed feasible to apply such a non-linear dosing scheme in the context of a clinical trial.[26] In that study, it is shown that neonates indeed require less morphine and older children require more morphine than the amount currently administered on the basis of a mg/kg/h dosing schedule, thereby confirming the finding that dosing in pediatrics should be nonlinear. The specific nonlinear dosing guideline was derived on the basis of an internally validated pharmacokinetic model of morphine,[27] which was externally validated by use of independent data,[28] which are important steps after internal validation of a model.[29] In addition to modeling of drug disposition (PK) pathways, we emphasize the need for modeling of effect (PD) pathways, as the effects of drugs can also be affected by age-related changes and health status. Based on the results on the PK and/or PD of a 'model drug', that is representative for a specific developmental drug disposition and/or effect pathway, a scientific basis is provided to develop evidence-based dosing regimens for existing or newly developed drugs that are eliminated through the same pathway.

## Recommendations

1. All population PK models should be fully internally and externally validated.[8]

2. Extrapolation of the maturation model for the CYP3A substrate midazolam to other CYP3A substrates will test the ability of the model to predict the system-specific properties of other drugs that share, at least partly, a similar pharmacokinetic pathway.

3. In addition to modeling and predicting the disposition of drugs in children, modeling the PK-PD relationship of drugs is highly needed as both disposition and effect of drugs may reflect specific developmental drug disposition and effect pathways.

## 4.2 Maturation of CYP3A mediated bio-availability of drugs

In chapter 6 we describe in our population pharmacokinetic analysis a maturation model for oral and intravenous midazolam pharmacokinetics from preterm neonates to adults. Our analysis is the first in which changes in oral bioavailability of midazolam from preterm neonates to adults in a combined dataset is quantified with a single maturation function, and may provide insight in the maturation of its disposition and may therefore guide evidence-based dosing of oral midazolam for children of different ages. We discussed that our findings do show an age-related change in oral bioavailability that has not been reported by other studies in children. An important feature of the research in this thesis is that data were obtained over the entire pediatric age range from premature neonates to adults, and may explain why other studies that included relatively small age-ranges could not find a change in oral bioavailability in children.While in our analysis no relation of ka with either age or bodyweight could be identified, the estimated oral bioavailability appears highest in the neonates (67%), decreasing in an exponential manner to lower values in children at 1 year of age, resulting in a percentage of only 16% (7%-26%) of bioavailability in adults.

While in adults the contribution of intestinal midazolam extraction to oral bioavailability can be estimated calculating the hepatic midazolam extraction using systemic clearance, estimated hepatic blood flow and hematocrit from total clearance,[31] liver blood flow reference values are not available for preterm infants. In older children, liver blood flow has been previously estimated using indocyanin green,[21] which has inherent limitations as it also reflects liver transporter activity. Consequently, we were not able to estimate with confidence the relative contribution of intestinal CYP3A activity to the first-pass metabolism of midazolam.

In our analysis, we showed that higher oral bioavailability in preterm infants necessitates downward dose-adjustments of oral midazolam doses in comparison to older children to reach similar concentrations as when an intravenous dose is given. As a higher midazolam oral bioavailability in this young population is most probably reflective of developmentally low intestinal and hepatic CYP3A activity, downward dose-adjustments of other CYP3A substrates may also be needed.

It is a limitation of our study that oral data were lacking for children between two weeks and one year old. This precludes an estimation of the exact developmental pattern in this age range of combined hepatic and intestinal CYP3A activity *in vivo* at this time, and therefore necessitates the need for more data in this age-range.

### Recommendations

1. Higher oral bioavailability in preterm infants (67%) necessitates downward dose-adjustments of oral midazolam doses in comparison to older children to reach similar

concentrations as when an intravenous dose is given, as the oral bioavailablity decraeases further to only 16% of bioavailability in adults.

2. As a higher midazolam oral bioavailability in this young population is most probably reflective of developmentally low intestinal and hepatic CYP3A activity, downward dose-adjustments of other CYP3A substrates may also be needed.

3. Data from children between two weeks and one year old are needed to estimate the exact developmental pattern in this age range of combined hepatic and intestinal CYP3A activity *in vivo*.

## 5. EXTRAPOLATION OF A CYP3A MATURATION FUNCTION TO OTHER SUBSTRATES

In chapter 7 we showed that maturation functions for drug clearance and bioavailability that describe changes in the functioning of the underlying physiological system can be extrapolated from one drug to another drug eliminated through the same pathway. The CYP3A maturation function model on the clearance and bioavailability of midazolam showed similar performance compared to the reference model for cisapride that was built on cisapride data in children.

Observed pharmacokinetic profiles are the result of the interaction between a drug and the physiological system. Therefore the parameters used to describe pharmacokinetic profiles represent drug-specific and/or system-specific aspects of this interaction. It should be noted that extrapolation can only be performed to other populations with similar characteristics as the population from which the system-specific information was derived. The age and weight range of the children in our analysis in chapter 7 receiving cisapride fell within the range of preterm neonates and infants receiving midazolam and was an ideal population to test. It is of great interest to also extrapolate the CYP3A maturation function based on midazolam to other populations (older children, critically ill) that received cisapride and/or to other CYP3A substrates, such as tacrolimus, cyclosporine, and nifedipine. In our opinion, this semi-physiological approach may ultimately lead to the facilitation and optimization of sparse data analysis in children, not only for existing, but also for newly developed drugs.

### Recommendations

1. A semi-physiological approach differentiating between different drug disposition pathways should be applied to facilitate optimization of sparse data analysis in children.

2. Maturation functions for drug clearance and bioavailability that describe changes in the functioning of the underlying physiological system, can be extrapolated from one drug to another drug that is eliminated through the same pathway

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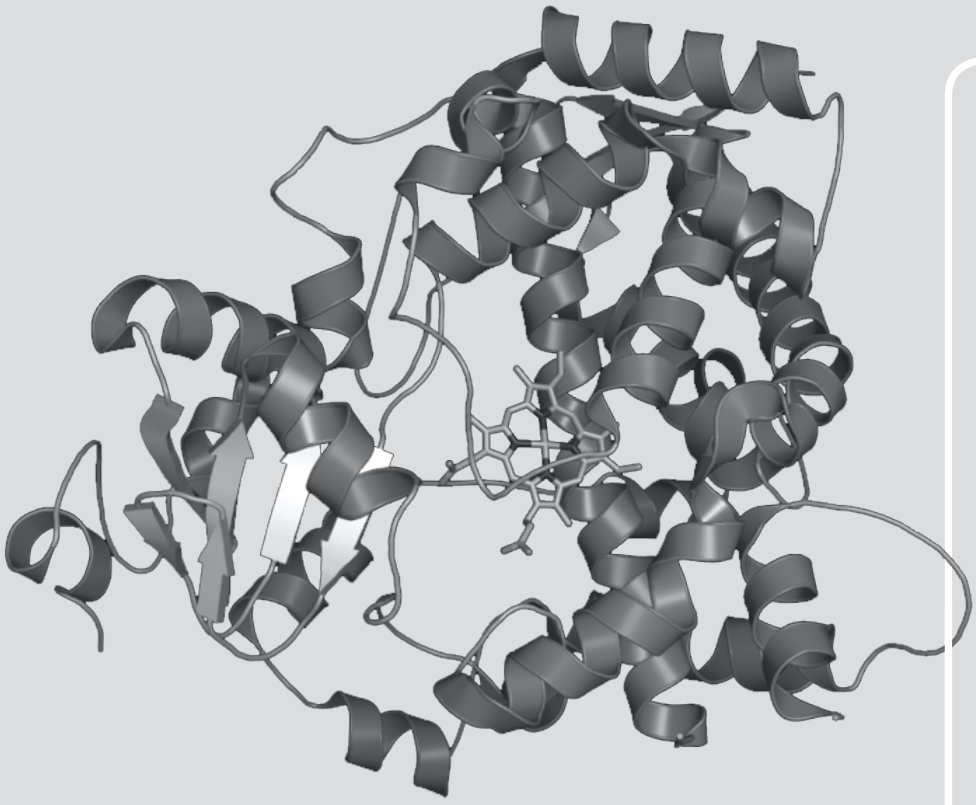


# 9 English Summary

## ENGLISH SUMMARY

As a result of ethical, practical and financial constraints, pediatric clinical trials are difficult to perform, and therefore most drugs in children are used in an unregistered or off-label manner with risk of underdosing or toxicity. It is well known that the absorption, distribution, metabolism, elimination, and the effect of drugs differ between children and adults, and between children of different ages. This is not only due to different body and organ size, but also due to changes in the expression and function of drug metabolizing enzymes, and many other factors.

CYP3A is a Cytochrome P450 enzyme subfamily involved in the metabolism of a large group of administered drugs. At least three clinically important isoforms are distinguished, of which CYP3A4 is the dominant isoform present in adult liver and small intestine, and CYP3A7 is mainly detected in embryonic, fetal, and newborn liver and intestine. The third, CYP3A5, undergoes polymorphic expression and seems the most important genetic contributor to interindividual variation in CYP3A-mediated drug clearance.

Importantly, both *in vitro* and *in vivo* data show developmental changes for CYP3A activity, which largely impact the disposition of CYP3A drug substrates across the pediatric age span. This finding may have important dose implications for these drugs. Still, determining age-appropriate dosing for every single CYP3A substrate is not feasible for practical and financial reasons.

Midazolam is a benzodiazepine frequently used in children for sedation during procedures or ICU treatment, and it is mainly metabolized by CYP3A4 and CYP3A5. The clearance of midazolam has been validated as surrogate marker of CYP3A4/5 activity *in vivo*. This may then provide a tool to develop dosing guidelines for other CYP3A substrates in a mechanism-based approach.

In this thesis, we used a model-based approach to describe CYP3A maturation *in vivo*, to be used for the development of rational drug dosing guidelines of CYP3A substrate drugs in the pediatric population.

## PART I. MECHANISM-BASED MODELING TO DEVELOP RATIONAL DOSING GUIDELINES IN CHILDREN: CYP3A MATURATION

The traditional approach to determining dosing regimens in children is quite costly and time-consuming. It entails testing of every individual drug, for which multiple blood draws are needed, and different age groups and clinical conditions should be included, among other

things. A good alternative is the so-called "population approach" for studying age-related changes in the pharmacokinetics and the pharmacodynamics of drugs when only sparse and unbalanced data are available. In addition, 'model drugs' that reflect specific developmental drug disposition and effect pathways may serve to establish evidence-based dosing regimens for other existing or newly developed drugs sharing the same pathways.

In **chapter 2** we present the following approach to develop evidence-based dosing guidelines for children: (i) optimization of clinical trial designs based on preliminary data; (ii) development and internal validation of population PK–PD models using sparse data; (iii) external validation using independent data; and (iv) prospective clinical evaluation.

In **chapter 3** we present current knowledge and our perspective on the ontogeny of the CYP3A subfamily, a drug metabolizing enzyme subfamily involved in the metabolism of almost half of all metabolized drugs currently on the market. The literature review makes clear that CYP3A7 is the dominant isoform before birth, but that its activity decreases to very low levels in the first week after birth. *In vitro* studies show a slow rise in CYP3A4 activity after birth, but data from older children remain more ambiguous. CYP3A5 activity in livers expressing the gene appears to be relatively stable from fetal to adult life. The mechanisms underlying the transition from CYP3A7 to CYP3A4 have not been elucidated in great detail. As tissue collection from children of all ages is incomplete and insufficient, it is not yet possible to describe a full CYP3A maturation profile for the entire age-range based only on *in vitro* data. In addition, there may be discrepancies between the clinical status of the tissue donor, and that of the children receiving a CYP3A substrate in real life. *In vivo* studies using paradigm drugs confirm that most of the developmental changes in CYP3A4 activity occur in the first months of life. However, there are still important gaps in our knowledge of the exact developmental patterns of individual CYP3A isoforms, especially in the first year of life.

## PART II. DEVELOPMENT AND APPLICATION OF A MATURATION FUNCTION FOR CYP3A USING MIDAZOLAM AS AN *IN VIVO* PROBE

In **chapter 4**, we studied age-related changes in relation to other covariates to explain the variability in the pharmacokinetics of midazolam. The study population was a group of fifty-four children aged 1 month to 17 years who received intravenous midazolam (bolus and/or continuous infusion) for sedation. Critical illness proved to be a major determinant of midazolam clearance from infancy to adolescence, probably due to inflammation, which reduces CYP3A4/5 activity. Disease severity may therefore have important implications for dosing of midazolam and other CYP3A drug substrates in children.

In a next step we investigated, in **chapter 5**, the influence of age-related changes on CYP3A mediated clearance of midazolam from preterm neonates to adults. To this aim we analyzed pharmacokinetic data described in six previously reported studies, and which were obtained after intravenous administration of midazolam. Subjects were premature neonates (n = 24; gestational age (GA) 26–33.5 weeks, postnatal age (PNA) 3–11 days, and n = 24;GA 26–37 weeks, PNA 0–1 days), 23 children after elective major craniofacial surgery (age 3–23 months), 18 pediatric intensive-care patients (age 2 days–17 years), 18 pediatric oncology patients (age 3–16 years), and 20 healthy male adults (age 20–31 years). We developed an *in-vivo* maturation function for midazolam clearance, which is an allometric equation with an exponent changing with bodyweight in an exponential manner. The exponent changed from 0.84 for preterm neonates (0.77 kg) to 0.44 for adults (89 kg), which implies that the most rapid maturation occurs during the youngest age range. This function may be used to derive evidence-based doses for children and to simulate exposure to midazolam, and possibly other CYP3A substrates, across the pediatric age range in population pharmacokinetic models or physiologically based pharmacokinetic models.

In **chapter 6** we investigated the pharmacokinetics of midazolam across the human lifespan with emphasis on maturation in oral bioavailability. We used pharmacokinetic data from a combined dataset of seven previously reported studies in 52 preterm infants (GA: 26-37 weeks, postnatal age 2-13 days), 324 children (2 days -18 years) and 20 adults, who received intravenous and/or oral midazolam. We found that midazolam oral bioavailability and clearance each show a distinct developmental pattern. Oral bioavailability of midazolam was negatively influenced by bodyweight in a power function, as shown from a value of 67% in a 0.77 kg preterm neonate to 17% in a 70 kg adult. Midazolam clearance was influenced by bodyweight according to a power function with a bodyweight dependent exponent, which varied from 0.82 to 0.28. Our simulations show that when switching from an intravenous to an oral dose, dosing should be increased by a factor of 1.6, 3 and 5, respectively, in preterm neonates, older children and adults to result in similar plasma exposure.

The semi-physiological model described in **chapter 6** contains both drug and system specific information. The latter comprises information on CYP3A maturation, growth of organs, liver-blood flow, *etcetera*, which in theory could be used to predict the pharmacokinetics of other *in vivo* CYP3A substrates, such as cisapride. As a next step, in **chapter 7**, we therefore applied the CYP3A maturation function for midazolam to cisapride. The dataset consisted of cisapride plasma concentrations from 36 neonates and young infants (postnatal age: 4 - 102 days, postconceptional age: 28-54 weeks, bodyweight: 0.98-6.55 kg) who received 0.2 mg/kg cisapride as a suspension. The descriptive and predictive performance of the model including the CYP3A maturation function was similar to a comprehensive covariate analysis. This semi-physiological approach enables to separate system specific and drug specific information in

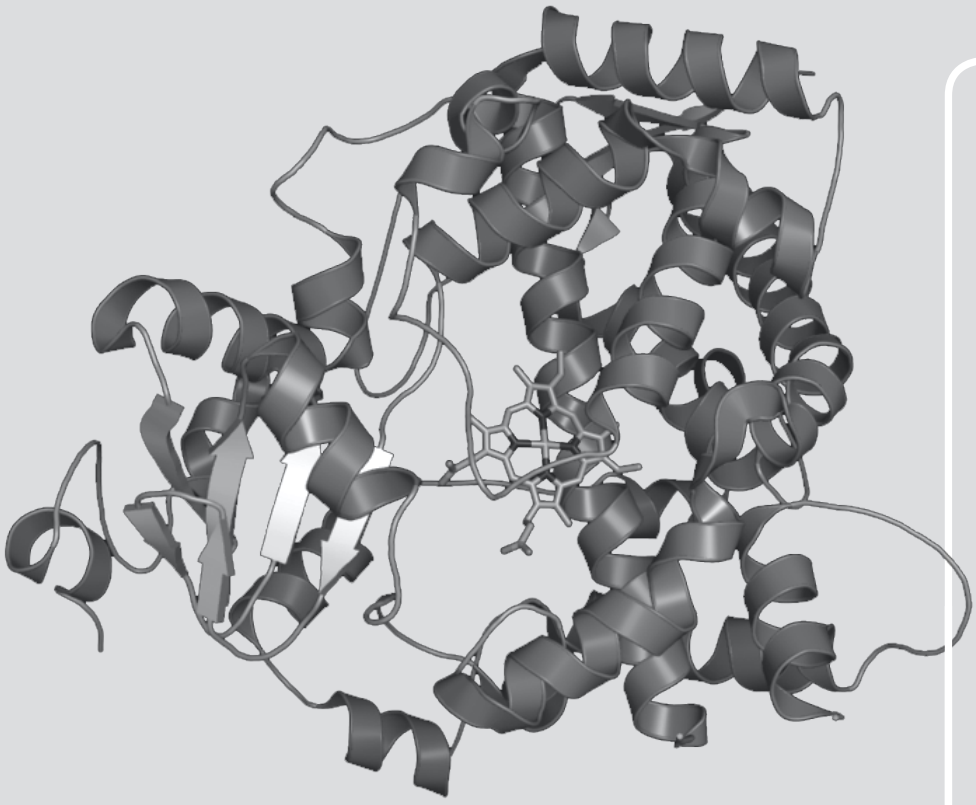
pharmacokinetic models, thereby reducing the amount of data needed to develop dosing guidelines in children.

## PART III. GENERAL DISCUSSION AND SUMMARY

In **chapter 8**, the main findings and conclusions of the studies in this thesis are discussed, along with their implications, and recommendations are presented. These can be summarized as follows:

1. Population PK-PD modeling and simulation for drugs administered to children should be applied along the following steps:
    a. Clinical trial designs based on prediction of the values of preliminary pharmacokinetic parameters of drugs in children.
    b. Development and internal validation of population PK-PD models using sparse data from these clinical trials.
    c. External validation using independent data.
    d. Prospective clinical evaluation of the simulated dosing guidelines.
    e. Optimization of future clinical trial designs based on prediction of the values of pharmacokinetic parameters of drugs in children, resulting from a population approach.
2. Data from *in vitro* studies are not sufficient to describe a full CYP3A maturation profile for the entire age range. *In vivo* studies using paradigm drugs could provide additional data.
3. Efforts should be made to collect pediatric tissues (including the clinical characteristics of the donor) across the whole pediatric age range, but especially in the first year after birth.
4. Apart from age, critical illness and genetic variation may cause interindividual variability of CYP3A substrate disposition in children.
5. A specific maturation function for CYP3A based on original data obtained using the population approach should be implemented in population PK and/or PBPK models, including both *in vitro* and *in vivo* information.
6. Data on albumin and AGP, and inflammatory markers such as CRP and/or IL6, should be collected in further studies in critically ill children, to support the hypothesis that these proteins and markers are correlated with a decrease in CYP3A activity, and with midazolam clearance.
7. The effect of inflammation in relation to the PK-PD relationship of CYP3A substrates should be elucidated.
8. Extrapolation of the maturation model for midazolam to other CYP3A substrates will test the ability of the model to predict the system-specific properties of other drugs that share, at least partly, the similar pharmacokinetic pathway.

9. Modeling the PK-PD relationship of drugs is highly needed as both disposition and effect of drugs may reflect specific developmental drug disposition and effect pathways.
10. In view of the high oral bioavailability of midazolam in preterm infants (67%) they should receive lower oral midazolam doses in to reach similar concentrations as when an intravenous dose is given. This may hold true for other CYP3A substrates as well.
11. Data from children between two weeks and one year old are needed to estimate the exact developmental pattern in this age range of combined hepatic and intestinal CYP3A activity *in vivo*.
12. A semi-physiological approach differentiating between different drug disposition pathways could reduce the amount of data needed to develop dosing guidelines in children.
13. Maturation functions for drug clearance and bioavailability that describe changes in the functioning of the underlying physiological system can be extrapolated from one drug to other drugs eliminated through the same pathway.



# 10 Nederlandse Samenvatting

## NEDERLANDSE SAMENVATTING

Wegens ethische, praktische en financiële beperkingen, is er weinig klinische onderzoek verricht naar de werking van geneesmiddelen bij kinderen. Dit heeft geleid tot de toepassing van ongeregistreerde en off-label medicatie, met verhoogd risico op onderdosering of toxiciteit. Het is bekend dat de absorptie, distributie, metabolisme, eliminatie en het effect van geneesmiddelen verschillen tussen kinderen en volwassenen en tussen kinderen van verschillende leeftijden. Dit is niet alleen het gevolg van verschillen in lichaamsomvang en de grootte van organen, maar ook door vele andere factoren, zoals veranderingen in de expressie en functie van enzymen die betrokken zijn bij geneesmiddelmetabolisme.

Een subfamilie van het enzym Cytochroom P450, te weten CYP3A, is betrokken bij de oxidatie van een groot deel van toegediende geneesmiddelen. CYP3A bestaat uit minstens drie klinisch relevante isoformen, waaronder CYP3A4 als de dominante isoform in de volwassen lever en dunne darm. CYP3A7 komt voornamelijk voor in de lever en darmen in de embryonale en foetale stadia en bij pasgeboren. De derde isoform, het genetisch polymorfe CYP3A5, lijkt de belangrijkste genetische bijdrage te leveren aan de interindividuele variatie in CYP3A-gemedieerde klaring.

Zowel *in vitro* en *in vivo* is aangetoond dat CYP3A ontwikkelingsveranderingen ondergaat. Dit heeft grote invloed op de farmacokinetiek (PK) van aan kinderen toegediende geneesmiddelen die door CYP3A gemetaboliseerd worden. Deze veranderingen kunnen daarom ook belangrijke gevolgen hebben voor de dosering van deze geneesmiddelen. Toch is het door praktische en financiële beperkingen niet haalbaar om individuele doseerschema's te ontwikkelen voor elk CYP3A-substraat.

Midazolam is een benzodiazepine dat vaak wordt toegepast bij kinderen als kalmeermiddel tijdens een (ICU) behandeling, en die voornamelijk wordt gemetaboliseerd door CYP3A4 en CYP3A5. De klaring van midazolam is gevalideerd als surrogaat voor het bepalen van de *in vivo* CYP3A4/5-activiteit. Dit surrogaat zou toegepast kunnen worden om doseringsrichtlijnen voor andere CYP3A-substraten te bepalen, via een mechanistische aanpak.

In dit proefschrift hebben we gebruik gemaakt van de zogenaamde 'mechanism-based' benadering voor het beschrijven van de *in vivo* maturatie van CYP3A4, die kan dienen om rationele doseringsrichtlijnen van CYP3A-substraten in de kinderpopulatie te ontwikkelen.

## DEEL I. 'MECHANISM-BASED' MODELEREN VOOR DE ONTWIKKELING VAN RATIONELE DOSERINGSRICHTLIJNEN BIJ KINDEREN: CYP3A MATURATIE

De traditionele methode om doseerrichtlijnen bij kinderen te bepalen is duur en tijdrovend. Elk geneesmiddel zou onderzocht moeten worden, waarvoor veel patiënten uit alle leeftijdsgroepen nodig zijn. Ook moet rekening worden gehouden met verschillende ziektebeelden. Een goed alternatief is de zogenaamde "populatiebenadering". Met deze benadering kunnen leeftijdsgerelateerde veranderingen in de PK en de farmacodynamiek (PD) van geneesmiddelen bestudeerd worden, ook als er weinig of eenzijdige informatie beschikbaar is. Daarnaast zijn er geneesmiddelen die kunnen dienen als surrogaat en kunnen gebruikt worden om de maturatie van specifieke PK en PD processen in kaart te brengen. Via deze surrogaten kunnen er op een mechanistische manier doseringsschema's vastgesteld worden voor andere bestaande, of nieuw ontwikkelde geneesmiddelen die op dezelfde manier door het lichaam worden uitgescheiden of hun werking hebben.

In **hoofdstuk 2** stellen we daarom de volgende benadering voor: ( i ) optimalisatie van klinische studies op basis van aanwezige gegevens; (ii ) ontwikkelen en uitvoeren van interne validaties van populatie PK-PD modellen op basis van schaarse informatie; ( iii ) uitvoeren van externe validaties met behulp van onafhankelijke data; en ( iv ) uitvoeren van prospectieve klinische evaluaties.

In **hoofdstuk 3** presenteren we de huidige kennis en ons perspectief op de maturatie van de CYP3A enzym subfamilie, die is betrokken bij de afbraak van bijna de helft van alle huidig gebruikte geneesmiddelen. Het literatuuronderzoek maakt duidelijk dat CYP3A7 de dominante isovorm is vóór de geboorte, maar dat de activiteit afneemt tot een zeer laag niveau in de eerste week na de geboorte. *In vitro* studies tonen een langzame stijging van de CYP3A4 activiteit na de geboorte, maar de gegevens van de oudere kinderen blijven meer ambigu. De CYP3A5-activiteit in levers die het actieve CYP3A5-gen produceren lijkt relatief stabiel te zijn gedurende het leven. De mechanismen die ten grondslag liggen aan de overgang van CYP3A7 tot CYP3A4 zijn niet in detail opgehelderd. Wegens onvoldoende beschikbaarheid van weefsels van kinderen van alle leeftijden, is het nog niet mogelijk gebleken om alleen op basis van *in vitro* data een volledig CYP3A-maturatieprofiel voor de gehele leeftijdsreeks te beschrijven. Bovendien kunnen er verschillen zijn tussen de klinische toestand van de weefseldonor en van het kind die het CYP3A-substraat daadwerkelijk ontvangt. *In vivo* studies, waarbij surrogaten voor CYP3A4 gebruikt werden, bevestigen dat de CYP3A4-activiteit het snelst stijgt gedurende de eerste levensmaanden. We missen echter nog belangrijke informatie over het exacte ontwikkelingspatroon van de individuele CYP3A-isovormen, met name in het eerste levensjaar.

## DEEL II. ONTWIKKELING EN TOEPASSING VAN EEN MATURATIEFUNCTIE VOOR CYP3A MET MIDAZOLAM ALS *IN VIVO* SURROGAAT

In hoofdstuk 4 bestudeerden we de leeftijdsgerelateerde veranderingen in relatie tot andere zogenaamde covariaten om de variabiliteit te verklaren in de PK van midazolam. De onderzoekspopulatie was een groep van vierenvijftig kinderen van 1 maand tot 17 jaar oud die intraveneus midazolam (bolus en / of continue infusie) ontvingen voor sedatie. Kritisch ziek zijn bleek op alle leeftijden een belangrijke determinant voor de klaring van midazolam, waarschijnlijk als gevolg van ontsteking die de CYP3A4/5 activiteit kan verlagen. Voorzichtigheid is dus geboden bij het doseren van midazolam en andere CYP3A substraten bij kritisch zieke kinderen, waarbij onbedoeld hoge geneesmiddelspiegels in het bloed kunnen optreden. Of dit ook betekent dat kritisch zieke kinderen lager gedoseerd moeten worden is nog onduidelijk, omdat niet bekend is wat kritisch ziekte doet op het effect van de meeste geneesmiddelen.

In een volgende stap onderzochten we, in **hoofdstuk 5**, de invloed van leeftijdgerelateerde veranderingen op de CYP3A-gemedieerde klaring van midazolam van premature pasgeborenen tot volwassenen. Voor dit doel gebruikten we PK-gegevens uit een gecombineerde dataset van zes eerder gerapporteerde studies van 24 premature neonaten, 23 kinderen na electieve craniofaciale chirurgie, 18 kinderen op een intensive care afdeling, 18 kankerpatiëntjes en 20 gezonde mannen. We ontwikkelden een *in vivo* maturatiefunctie voor midazolamklaring. Deze functie bestaat uit een machtsvergelijking met een exponent die exponentieel verandert met het lichaamsgewicht, van 0,84 voor prematuren (0,77 kg) tot 0,44 voor volwassenen (89 kg), wat impliceert dat de snelle maturatie optreedt in de jongste leeftijdsperiode. Deze functie kan worden gebruikt in populatie PK modellen of zogenaamde 'physiologically based' PK (PBPK) modellen om 'mechanism-based' doseerschema's te bepalen en de blootstelling aan midazolam en mogelijk andere CYP3A-substraten te simuleren in kinderen van verschillende leeftijden.

In **hoofdstuk 6** onderzochten we de PK van midazolam van prematuur tot volwassen, met de nadruk op de maturatie in orale biologische beschikbaarheid. We gebruikten PK-gegevens uit een gecombineerde dataset van zeven eerder gerapporteerde studies bij 52 prematuren, 324 kinderen en 20 volwassenen, na intraveneuze en / of orale toediening van midazolam. De orale biologische beschikbaarheid en de klaring van midazolam lieten elk een ander maturatiepatroon zien. De orale biologische beschikbaarheid van midazolam werd negatief beïnvloed door lichaamsgewicht, in een machtsfunctie, met een waarde van 67% bij een premature pasgeborene van 0,77 kg tot 17% bij een volwassene van 70 kg. De midazolamklaring werd beïnvloed door lichaamsgewicht volgens een machtsfunctie met een lichaamsgewichtafhankelijke exponent die varieerde van 0,82-0,28. Onze simulaties tonen aan dat bij het overschakelen van intraveneuze naar orale toediening, de dosering zou moeten worden

verhoogd met een factor van respectievelijk, 1.6, 3 en 5, bij premature pasgeborenen, oudere kinderen en volwassenen, om toch een vergelijkbaar plasma blootstelling te bereiken.

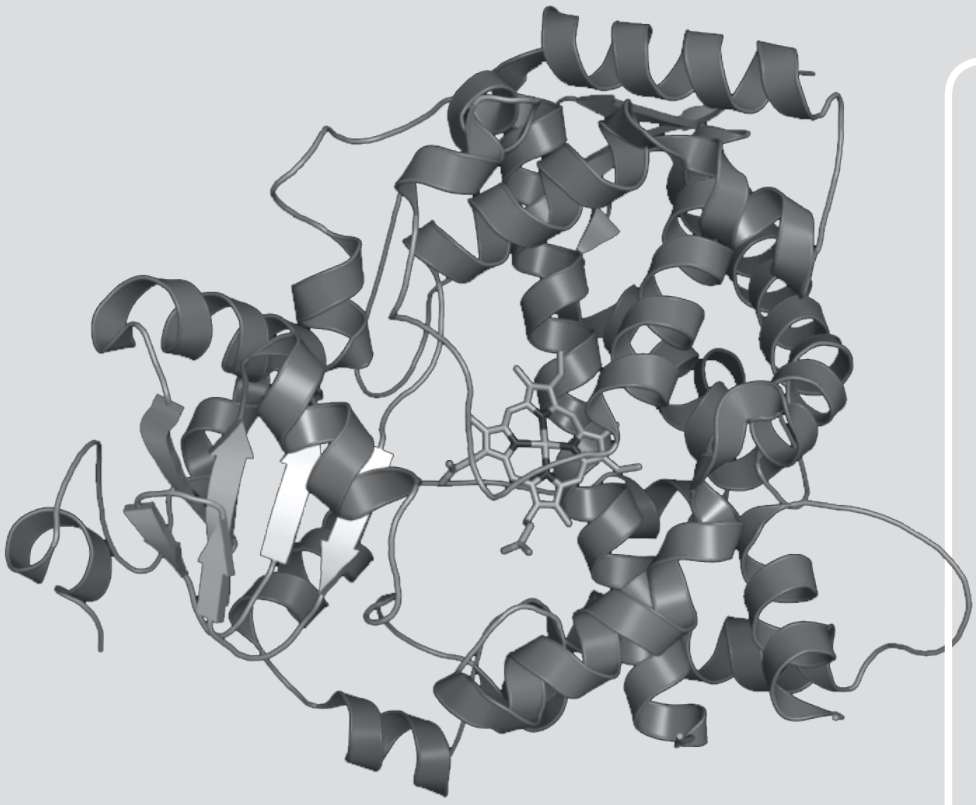
Het in **hoofdstuk 6** beschreven semifysiologisch model bevat zowel geneesmiddel- als systeemspecifieke informatie. De laatstgenoemde omvat informatie over de maturatie van CYP3A, de groei van organen, leverdoorbloeding, *etcetera*. Deze informatie kan in theorie worden gebruikt om de PK van andere *in vivo* CYP3A-substraten, zoals cisapride, te voorspellen. Als volgende stap, in **hoofdstuk 7**, hebben we dan ook de CYP3A-maturatiefunctie voor midazolam toegepast op cisapride. De dataset bestond uit cisapride plasmaconcentraties van 36 pasgeborenen en jonge kinderen die 0,2 mg/kg cisapride toegediend kregen als suspensie. De beschrijvende en voorspellende prestaties van het model met de CYP3A-maturatiefunctie waren vergelijkbaar met die van een nieuw model voor cisapride waarbij een uitgebreide covariaatanalyse was uitgevoerd. Met deze semifysiologische benadering kunnen we de systeem-specifieke en geneesmiddel-specifieke informatie in PK modellen scheiden, waardoor er minder gegevens nodig zijn om doseringsrichtlijnen bij kinderen te ontwikkelen.

## DEEL III. ALGEMENE DISCUSSIE EN SAMENVATTING

In hoofdstuk 8 worden de belangrijkste bevindingen, conclusies en implicaties van de studies in dit proefschrift besproken en worden aanbevelingen gedaan. Deze kunnen als volgt worden samengevat:

1. Populatie PK-PD modellering en simulatie voor geneesmiddelen die toegediend worden bij kinderen zou als volgt moeten worden uitgevoerd:
   a. Klinische studie ontwerpen op basis van de voorspelling van de waarden van de beschikbare PK parameters van geneesmiddelen bij kinderen.
   b. Ontwikkeling en de interne validatie van populatie PK-PD modellen met behulp van verkregen informatie uit deze klinische studies.
   c. Externe validatie met behulp van onafhankelijke gegevens.
   d. Prospectieve klinische evaluatie van de gesimuleerde doseerschema's.
   e. Optimalisatie van toekomstige klinische studies gebaseerd op de voorspelling van de waarden van de PK parameters van geneesmiddelen bij kinderen, die volgden uit de populatiebenadering.
2. Gegevens uit *in vitro* studies zijn niet voldoende om een volledige CYP3A maturatieprofiel voor de gehele levensduur beschrijven. *In vivo* studies met surrogaatgeneesmiddelen zouden aanvullende informatie kunnen verstrekken.

3. Het verdient aandacht om weefsels van kinderen (met inbegrip van de klinische kenmerken van de donor) te verzamelen over de gehele kinderleeftijd, met name in het eerste jaar na de geboorte .
4. Naast leeftijd kunnen kritische ziekte en genetische variatie interindividuele variabiliteit van de PK van CYP3A-substraten bij kinderen veroorzaken.
5. Een specifieke maturatiefunctie voor CYP3A die is ontwikkeld met de populatiebenadering op basis van originele *in vitro* en *in vivo* gegevens, zou geïmplementeerd moeten worden in populatie PK en/of PBPK modellen.
6. Gegevens over albumine en AGP en ontstekingsmarkers zoals CRP en / of IL6, zouden moeten worden verzameld bij studies met kritisch zieke kinderen, om de hypothese te ondersteunen dat deze eiwitten en ontstekingsmarkers zijn gecorreleerd met een afname van de CYP3A-activiteit en met midazolamklaring.
7. Het effect van ontsteking op de PK-PD relatie van CYP3A-substraten zou uitgezocht moeten worden.
8. Extrapolatie van het maturatiemodel voor midazolam naar andere CYP3A-substraten zal het voorspellende vermogen kunnen testen van het model om de systeemspecifieke eigenschappen van andere geneesmiddelen die op zijn minst gedeeltelijk dezelfde PK route volgen.
9. Het modelleren van de PK-PD relatie van geneesmiddelen is dringend nodig, omdat zowel de PK als de PD specifieke maturatie in de PK- en PD-routes zou kunnen weerspiegelen.
10. Hogere orale biobeschikbaarheid bij prematuren (67 %) vereist een lagere orale midazolamdosering vergeleken met oudere kinderen om vergelijkbare concentraties te bereiken als wanneer een intraveneuze dosis wordt gegeven. Dit zou ook kunnen gelden voor andere CYP3A-substraten.
11. Gegevens van kinderen tussen twee weken en een jaar oud zijn nodig om de exacte maturatie van gecombineerde lever- en darm CYP3A-activiteit *in vivo* te beschrijven in deze leeftijdscategorie.
12. Een semifysiologische benadering die onderscheid maakt tussen de verschillende PK-routes van geneesmiddelen kan de hoeveelheid gegevens verminderen die nodig is om doseringsrichtlijnen bij kinderen te ontwikkelen.
13. Maturatiefuncties voor de klaring en biologische beschikbaarheid van geneesmiddelen die de veranderingen van de werking van het onderliggende fysiologisch systeem beschrijven, kunnen worden geëxtrapoleerd van het ene naar het andere geneesmiddel dat via dezelfde route wordt gemetaboliseerd.



# Appendices

# PhD Portfolio

**Name PhD student:** Ibrahim Ince
**Erasmus MC department:** Intensive Care and Pediatric Surgery
**PhD Period:** October 2007 – October 2012
**Promotors:** Prof. Dr. D. Tibboel, Prof. Dr. C.A.J. Knibbe
**Copromotor:** Dr. S.N. de Wildt

| | Year | Workload (ECTS) |
|---|---|---|
| **General academic skills** | | |
| Business & Entrepreneurial Skills, Zeist | 2012 | 1 |
| Introductory Drugs discovery & Development Cycle course, Zeist | 2009 | 1 |
| **Specific courses** | | |
| PK/PD modeling and Simulation in R, Leiden | 2010 | 1 |
| Advanced R course, Leiden | 2010 | 4 |
| Time-to-Event course, Leiden | 2010 | 0.5 |
| Binary and categorical data analysis, Leiden | 2009 | 2 |
| Hands-on Experience with Automated *In vitro-In vivo* Extrapolation-Applications, Leiden | 2009 | 1 |
| Hands-on Experience with Automated *In vitro-In vivo* Extrapolation-Concepts, Leiden | 2009 | 1 |
| Introductory course on drug research, Noordwijkerhout, | 2008 | 3 |
| Introduction to NONMEM for population PK and PKPD modeling and Simulation, Leiden | 2008 | 3 |
| Introduction to pharmacoepidemiology, Leiden | 2008 | 3 |
| Pharmacokinetic&Pharmacodynamic modeling – basis concepts, Leiden | 2008 | 3 |
| **Presentations** | | |
| NVKFB: oral | 2011 | 0.4 |
| PAGE: poster | 2011 | 0.3 |
| LACDR Spring Symposium: poster | 2011 | 0.3 |
| SimCYP: oral | 2010 | 0.4 |
| NVKFB: oral | 2010 | 0.4 |
| 6th International Symposium on Measurement and Kinetics of *In vivo* Drug Effecta: poster | 2010 | 0.3 |
| Tipharma Spring Meeting: poster | 2010 | 0.3 |
| LACDR spring symposium: poster | 2010 | 0.3 |
| NVKFB: poster | 2010 | 0.3 |
| PAGE: poster | 2010 | 0.3 |
| PAGE: poster | 2009 | 0.3 |
| LACDR spring symposium: poster | 2009 | 0.3 |

| | | |
|---|---|---|
| Tipharma Spring Meeting: poster | 2009 | 0.3 |
| ESDP: oral | 2008 | 0.4 |
| TI Pharma Pring meeting: oral | 2008 | 0.4 |
| LACDR spring symposium: poster | 2008 | 0.3 |
| PAGE: poster | 2008 | 0.3 |

| **International conferences** | | |
|---|---|---|
| PAGE, Athens, Greece | 2011 | 1 |
| PAGE, Berlin, Germany | 2010 | 1 |
| PAGE, Sint Petersburg, Russia | 2009 | 1 |
| PAGE, Marseille, France | 2008 | 1 |
| ASCPT, Orlando, USA | 2008 | 1 |

| **Teaching activities** | | |
|---|---|---|
| Supervision student Biopharmaceutical Sciences (2x) | 2011 | 3 |
| Assistant teacher Biochemistry 2, Leiden University, Leiden | 2011 | 1 |
| Pharmacology 3-day workshop, Erasmus Medical Centre Sophia Childrens Hospital, Rotterdam | 2010 | 1.2 |
| Asisstent teacher annual Pharmacology course, Leiden University, Leiden (4x) | 2008-11 | 6 |

# List of publications

**Ince I**, de Wildt SN, Vermeulen A, Tibboel D, Danhof M, Knibbe CA. *Extrapolation of the midazolam CYP3A maturation function to cisapride: towards a semi-physiological approach for pharmacokinetics modeling in children.*
Submitted.

**Ince I**, de Wildt SN, Barrett JS, Burggraaf J, Jacqz-Aigrain E, van den Anker JN, Danhof M, Tibboel D, Knibbe CAJ. *Population pharmacokinetic analysis on oral and intravenous midazolam from preterm neonates to adults.*
Submitted.

**Ince I**, Knibbe CA, Danhof M, de Wildt SN. *Developmental changes in the expression and function of cytochrome P450 3A isoforms: evidence from in vitro and in vivo investigations.*
Clinical Pharmacokinetics, 2013 May;52(5):333-45

**Ince I**, de Wildt SN, Wang C, Peeters MY, Burggraaf J, Jacqz-Aigrain E, van den Anker JN, Tibboel D, Danhof M, Knibbe CA. A Novel Maturation Function for *Clearance of the Cytochrome P450 3A Substrate Midazolam from Preterm Neonates to Adults.*
Clinical Pharmacokinetics, 2013 Jul;52(7):555-65

**Ince I**, de Wildt SN, Peeters MY, Murry DJ, Tibboel D, Danhof M, Knibbe CA. *Critical illness is a major determinant of midazolam clearance in children aged 1 month to 17 years.*
Therapeutic Drug Monitoring, 2012 Aug;34(4):381-9

**Ince I**, de Wildt SN, Tibboel D, Danhof M, Knibbe CA. *Tailor-made drug treatment for children: creation of an infrastructure for data-sharing and population PK-PD modeling*. Drug Discovery Today, 2009 Mar;14(5-6):316-20

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