Towards a System-Based Pharmacology Approach to Predict Developmental Changes in Renal Drug Clearance in Children

Roosmarijn F. W. De Cock

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Roosmarijn De Cock

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Promotoren:

Prof. dr. C.A.J. Knibbe

Prof. dr. M. Danhof

Prof. dr. K. Allegaert (University Hospitals, Leuven, Belgium)

Promotiecommissie:

Prof. dr. J.N. van den Anker (Children's National Medical Center, Washington D.C., United States and Sophia's Children Hospital & Erasmus Medical Center, Rotterdam, The Netherlands)

Prof. dr. A.F. Cohen

Prof. dr. P.H. van der Graaf

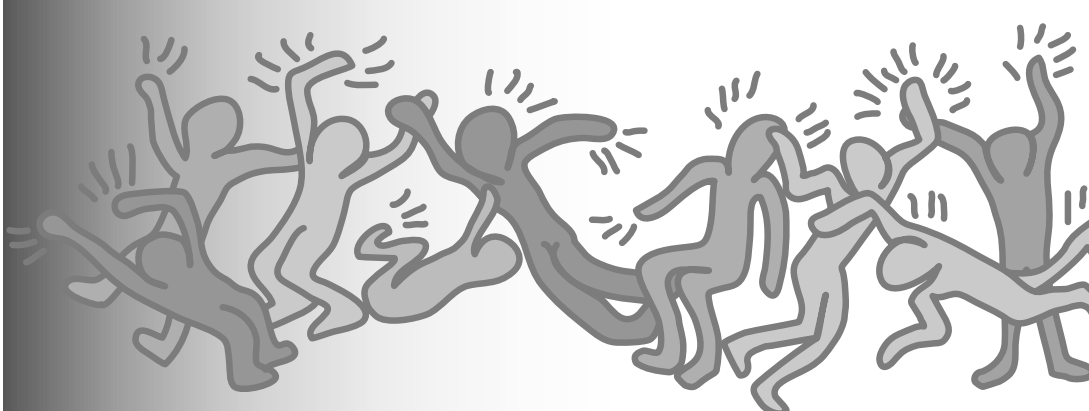
Prof. dr. A.P. IJzerman

Prof. dr. B. van de Water

Contents

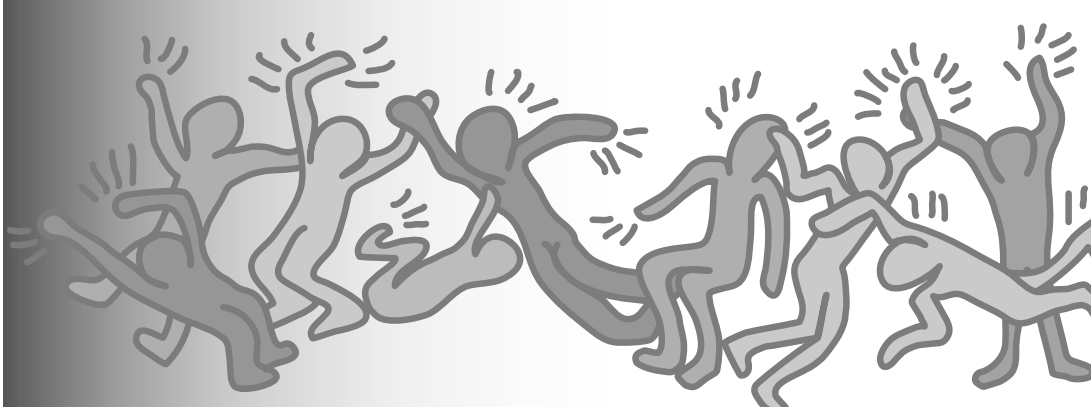
Section I

Background and Introduction



Chapter 1

Scope and Outline



1.1. Introduction: Towards a system-based pharmacology approach to predict developmental changes in renal drug clearance in children

The response to drugs may be different in children compared to adults. Notwithstanding these differences, to date, evidence-based drug dosing algorithms are often lacking in the pediatric age range [1-4]. This can be explained by ethical, practical and economical issues which may seriously impede the design and implementation of pediatric clinical trials [2, 4, 5]. Consequently, drug doses for children are often empirically derived from adult dosing guidelines based on bodyweight or age, irrespective the physiological differences due to developmental changes [1, 3, 6-8]. Despite the shortcomings of empirically scaling from adult dosing regimen to children, it is still the most commonly used approach which may result in therapeutic failure [9] or occurrence of toxic effects [10-13].

These differences in drug response between children and adults may be caused by differences in pharmacokinetics (PK), pharmacodynamics (PD) or both. While a child grows, differences are seen in body composition, maturation of drug metabolizing enzymes, cardiac output, blood flow and functionality of the drug eliminating organs liver and kidneys. All these differences are seen as potential sources influencing the pharmacokinetics of drugs [14]. Furthermore, developmental changes in the functionality and expression of receptors and differences in disease status may alter the pharmacodynamics and therefore the pharmacological response to drugs [14, 15]. In order to develop rational evidence-based dosing schemes in children, these developmental changes in pharmacokinetics and pharmacodynamics need to be characterized.

To counterbalance the lack of information on PK and PD of drugs in children, the Pediatric Regulation (EMA) came into force in Europe [16]. The main aim of this regulation was to facilitate the development and availability of drugs for children. Furthermore, funding by the European Union became available to promote research of off-patent drugs in children. As a result a large increase was seen in the number of PK and/or PD studies in children, potentially leading to evidence-based and individualized dosing schemes in children. However, despite the efforts of the industry and academia to perform research in children, most of the drugs in pediatrics are still used in off-label or unlicensed manner [1, 3, 4].

Performing PK/PD studies in the pediatric age range is very challenging. Besides

the fact that only a limited number of children is available, since studies are only performed in children suffering from a particular disease, also ethical, practical and economical issues occur (e.g. limited number and volume of blood samples). In addition to the use of very sensitive analysis techniques which require only a very small blood volume, advanced statistical tools are needed so that the burden for each child is kept to a minimum while still addressing the study objective: the development of rational, evidence-based and individualized dosing regimens in children. Consequently, the population approach using non-linear mixed effect modeling is the preferred approach since all data of all patients are simultaneously analyzed while still taking into account that different observations originate from different patients. An important advantage of this approach is that it allows the analysis of dense but also sparse and unbalanced data, which is often encountered in clinical practice. Furthermore by using this approach both the inter- and intra-individual variability can be estimated separately [17, 18]. Finally specific predictors of variability also called covariates, in PK or PD can be identified which subsequently can be used to determine new evidence-based and individualized dosing regimens [19-22].

However, if for each drug, models needs to be developed and validated over the entire pediatric age range to obtain rational, evidence-based and individualized dosing guidelines, a tremendous amount of time and costs will be involved. Therefore the objective of the research described in this thesis was to describe the developmental changes in renal function by using a more system-based pharmacology approach [23]. This means that the developmental changes of the various subprocesses that contribute to renal clearance (glomerular filtration, tubular secretion and tubular reabsorption) need to be characterized.

Renal clearance is responsible for the elimination of a large number of water-soluble drugs and their metabolites. Various mechanisms contribute to the renal clearance: glomerular filtration, tubular secretion and tubular reabsorption. Each of these processes exhibit different rates of maturation in an independent way [24]. Although renal clearance is well defined in adults, limited information is available on the developmental changes in renal function in the pediatric age range. It is known that nephrogenesis starts at week 5-6 of gestation and continues until 36 weeks of gestation [14, 25, 26]. Development of tubular processes starts from 36 weeks of gestation and continues during childhood. During the first weeks of life, a rapid increase is seen in glomerular filtration and tubular functions due to haemodynamic changes [24]. Moreover, development in tubular processes seems to be delayed in comparison with glomerular filtration. For the glomerular filtration rate, it is known that adult levels are reached at approximately 6-12 months of age when corrected for body surface area. Meanwhile the development of tubular processes is more gradual since adult

levels are not reached until 1-5 years of age [24]. Since the renal elimination of most of the drugs is covered by glomerular filtration and only a limited number of drugs is undergoing tubular secretion or reabsorption, the primary focus in this thesis was to describe the developmental changes in glomerular filtration. Moreover, once the developmental changes in GFR are characterized, this can be used to describe the maturation of tubular processes.

To estimate GFR, different methods can be used. First, GFR can be measured by determination of the inulin clearance which is considered to be the gold standard because inulin is an exogenous substance that is completely filtered by the glomerulus and not secreted or reabsorbed by the renal tubules [27]. Although simplified methods have been proposed to determine GFR from the decrease of the inulin concentration in plasma rather than from 24 hour urine collection, still some limitations are linked to this technique. These can make the routine application cumbersome in paediatric and certainly in neonatal clinical practice. Some of the constraints are the limited commercial availability of inulin, the burden caused by collection of additional blood samples and the advanced assay methodology that is required to measure inulin concentration in blood [28-30]. A second method to assess GFR is by measuring creatinine clearance. There is however a number of factors that must be taken into account when using creatinine clearance as a substitute for glomerular filtration. First of all creatinine is not only filtered by the glomerular filtration but also in part secreted by the tubular secretion [24, 31]. Moreover, the formation of creatinine is determined by age, muscle mass and gender and this complicates the estimation of creatinine clearance in children on the basis of plasma concentrations. In addition, in the first days of life, serum creatinine concentrations reflect maternal creatinine concentrations [25, 32, 33]. Furthermore, serum creatinine concentrations are known to increase with a peak in the second part of the first week of life with a subsequent progressive decrease throughout neonatal life making it difficult to interpret the renal function based on creatinine in the first week of life. The peak creatinine concentration is most pronounced in the most immature neonates and is due to passive back leak of creatinine through renal tubular leaky cells [33]. Although the Schwartz formula based on creatinine concentrations and body length is often used to estimate GFR, it often leads to overprediction of GFR [34]. Due to the reasons mentioned above, it is preferred not to use creatinine as a marker to measure GFR in children and certainly not in neonates. A third method to measure GFR is by injection of radioisotopes [28, 29]. This method is also not recommended in children for the same reasons mentioned as with inulin.

Therefore, the most pragmatic method, which has been proposed before, is to assess GFR in the pediatric age range by determining the clearance of a drug which

is exclusively eliminated by GFR [35-37]. Moreover, when evaluating GFR by describing the clearance of renally excreted drugs, information can directly be obtained during clinical practice. Finally, in the context of the system-based pharmacology approach, it will also be evaluated whether information on the developmental changes in the clearance of one drug can be used to describe clearance of other drugs eliminated by the same route. This implicates that a distinction is made between system-specific and drug-specific properties to evaluate whether the system-specific properties derived from one drug describing the underlying physiological changes, can be extrapolated to other drugs, eliminated through the same route.

1.2. Developmental changes in glomerular filtration in preterm and term neonates by describing the pharmacokinetics of renally excreted antibiotics

In *Section II* of this thesis a system-based pharmacology approach is used to describe the developmental changes in GFR in preterm and term neonates. Using this approach, the pharmacokinetics of one specific aminoglycoside, i.c. amikacin, a drug which is almost entirely eliminated by GFR, are first described in preterm and term neonates. To characterize these developmental changes, a systematic covariate analysis is performed in which all potential covariates are tested for significance and included in the model when they are sufficiently predictive of the variability in amikacin clearance. Based on the model, including the model covariates, a new model-based dosing algorithm can be developed for amikacin in neonates. Secondly, to extrapolate information from one drug to another drug, a distinction is made between system-specific and drug-specific information in the derived model. In this respect, the pediatric covariate model can be considered to contain system-specific information on the developmental changes in GFR and therefore the covariate model can be extrapolated to the other renally excreted drugs [23, 38, 39].

In **chapter 3**, the developmental changes in GFR were quantified in 874 preterm and term neonates aged between 1-30 days by describing maturation in clearance of amikacin. In a systematic covariate analysis the influence of birth bodyweight, current bodyweight, postmenstrual age, gestational age, postnatal age, co-administration of ibuprofen and creatinine was studied. To ascertain that the model was able to describe the data without bias, the model was both internally and externally validated [40, 41]. The internal validation was based on two different methods: a bootstrap analysis and a normalized prediction distribution error (NPDE) analysis. For the external validation two different datasets were used. Finally simulations were performed to obtain a

new optimized and individualized dosing algorithm for amikacin in preterm and term neonates which would result in achieving target peak and trough concentrations in each individual neonate.

In **chapter 4** the new-model based dosing regimen for amikacin in neonates was tested in a prospective clinical trial including 579 preterm and term neonates. In this study it is assessed whether the proposed dosing regimen for amikacin resulted in the expected concentrations and whether further dose adjustments need to me made.

In **chapter 5** the system-based pharmacology approach is applied for between drug extrapolations. In this chapter it is illustrated how information of one drug is extrapolated to other drugs eliminated through the same route. Using this approach, it is hypothesized that covariate models contain quantitative information on the developmental changes in the underlying physiological pathways [23]. To test this hypothesis, the covariate model of amikacin, describing the developmental changes in GFR in preterm and term neonates, was directly extrapolated to four other renally excreted drugs: netilmicin, tobramycin, vancomycin and gentamicin. Meanwhile, the population values describing the absolute values of parameters like clearance and volume of distribution are still estimated in the population analysis as they are considered to be drug-specific. The descriptive and predictive performance of the models using the amikacin covariate model was compared to the independent reference models which were developed for each dataset based on a systematic covariate analysis. This approach in which information of one drug is extrapolated to another drug can be considered a semi-physiological approach which may lead to optimization of sparse data analysis in children. Furthermore another not unimportant advantage is the large reduction in both time and costs when models can be developed using information of one drug to another drug.

1.3. Developmental changes in renal function (GFR and tubular processes) in preterm and term neonates by describing the pharmacokinetics of cefazolin

Renal function consists of glomerular filtration, active and passive tubular secretion and reabsorption. Cefazolin is a drug which is eliminated by both GFR and active tubular secretion [42, 43]. In **chapter 6**, the pharmacokinetics of cefazolin are described in preterm and term neonates. On the basis of both total and free cefazolin concentrations in 36 neonates, a one compartment pharmacokinetic model

was developed in which non-linear protein binding was taken into account. In a systematic covariate analysis, all potential covariates (age and weight related covariates, albumin, creatinine, free fatty acids, bilirubin, gender) were tested for significance. In addition, based on the final model, Monte Carlo simulations were performed to illustrate the exposure to cefazolin following currently used dosing regimens and to derive an optimal dosing regimen in preterm and term neonates.

To be able to quantify the developmental changes in tubular secretion in neonates, it was hypothesized that when clearance of cefazolin was higher than the clearance through GFR, it is due to clearance by tubular processes. In the previous section, the developmental changes in GFR were quantified by describing the pharmacokinetics of amikacin in neonates. Consequently, this semi-physiological GFR model based on amikacin clearance was directly incorporated on cefazolin clearance. The remaining part was then considered as describing the clearance by active tubular secretion. To quantify these developmental changes in active tubular secretion, a systematic covariate analysis was performed. These results are discussed in the section "Summary, conclusions and perspectives" of this thesis.

1.4. Renal and hepatic elimination of propylene glycol in preterm and term neonates

Besides the active substance(s), drug formulations often contain excipients. One of the frequently used excipients to increase the solubility and/or stability is propylene glycol. In general, excipients are considered to be safe, however toxic effects were reported in adults, children and neonates due to the administration of propylene glycol [44-48]. This may be explained by the limited knowledge on the pharmacokinetics of propylene glycol. In adults, it is known that 45% of propylene glycol is eliminated through the renal route and 55% is metabolized in the liver by alcohol dehydrogenase to lactate and pyruvate [49, 50]. However, renal clearance of propylene glycol is expected to be lower in neonates compared to adults, due to immaturity of the renal function. Therefore, the aim of this section was to characterize renal and hepatic elimination of propylene glycol in preterm and term neonates.

In a first analysis (**chapter 7**) the pharmacokinetics of propylene glycol were quantified based on 372 plasma samples of propylene glycol co-administered with intravenous paracetamol or phenobarbital, available in 62 preterm and term neonates. Based on a systematic covariate analysis different covariates were tested. The final model was subsequently used to simulate exposure to propylene glycol upon ad-

ministration of paracetamol or phenobarbital in neonates using the dosing regimens applied in the study.

In **chapter 8** renal and hepatic clearance of propylene glycol was characterized based on both plasma and urine samples of propylene glycol in 69 preterm and term neonates using a one compartment model parameterized in renal clearance, hepatic clearance and volume of distribution. Based on this model, the percentage of propylene glycol eliminated through the renal route and hepatic route was quantified in preterm and term neonates.

1.5. Developmental changes in GFR from neonates until adults

Glomerular filtration is supposed to reach adult levels between 6 months – 1 year of age when expressed per m^2 body surface area. However an exact quantification of the maturation of GFR throughout the pediatric age range is missing. Therefore, the aim in this section (*Section V* – **chapter 9**) was to describe the developmental changes in GFR across the entire pediatric age range from preterm neonates to adults. To perform this analysis, a system-based pharmacology approach was used since the developmental changes in GFR from neonates until adults were characterized by describing the pharmacokinetics of three renally excreted drugs: gentamicin, tobramycin and vancomycin in one analysis whereby again the distinction was made between system-specific and drug-specific properties (see *Section II*). Using this approach the covariate model on clearance for the three drugs is not tested separately but the same covariate model on clearance was implemented for the three drugs as this part of the model is considered to contain system-specific information. Finally the model describing the developmental changes in GFR across the pediatric range was validated by performance of an NPDE analysis [41].

1.6. Conclusion and Perspectives

Section VI of this thesis provides a summary of the results and conclusions of the different chapters of this investigation. Furthermore the results are discussed as well as the applicability of the different covariate models to other drugs. Finally, an overview is given on future perspectives.

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Chapter 2

The Role of Population PK-PD Modeling in Pediatric Clinical Research

Roosmarijn F. W. De Cock*, Chiara Piana*, Elke H.J. Krekels, Meindert Danhof, Karel Allegaert, Catherijne A.J. Knibbe

*RDC and CP equally contributed to this paper

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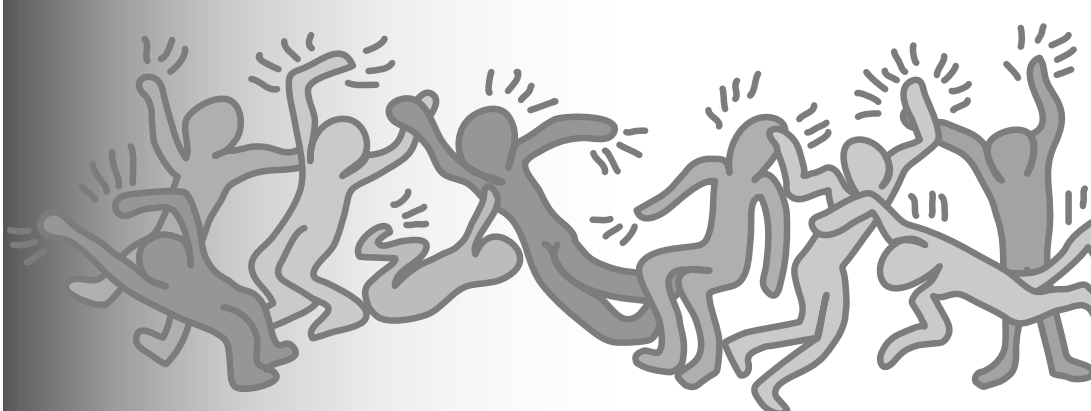

Abstract

Children differ from adults in their response to drugs. While this may be the result of changes in dose-exposure (pharmacokinetics (PK)) and/or exposure-response (pharmacodynamics (PD)) relationships, the magnitude of these changes may not be solely reflected by differences in bodyweight. As a consequence, dosing recommendations empirically derived from adults dosing regimens using linear extrapolations based on bodyweight, can result in therapeutic failure, occurrence of adverse effect or even fatalities. In order to define rational, patient tailored dosing schemes, population PK-PD studies in children are needed. For the analysis of the data, population modeling using non-linear mixed effect modeling is the preferred tool since this approach allows for the analysis of sparse and unbalanced datasets. Additionally it permits the exploration of the influence of different covariates such as bodyweight, age and other covariates, to explain the variability in drug response. Finally, using this approach, these PK-PD studies can be designed in the most efficient manner in order to obtain the maximum information on the PK-PD parameters with the highest precision. Once a population PK-PD model is developed, internal and external validations should be performed. If the model performs well in these validation procedures, model simulations can be used to define a dosing regimen which in turn needs to be tested and challenged in a prospective clinical trial. This methodology will improve the efficacy/safety balance of dosing guidelines which will be of benefit to the individual child.

2.1. Introduction

Children differ from adults in their response to drugs. These differences may be caused by changes in the pharmacokinetics (PK) and/or pharmacodynamics (PD) between children and adults and may also vary between children of different ages. The PK of a drug includes processes of absorption, distribution, metabolism and elimination of a drug whereas the PD comprises the physiological and biological response to the administered drug and therefore may represent both efficacy and safety measures. While a child grows, enzyme pathways (involved in the PK), function and expression of receptors and proteins (involved in the PD) mature, which can be referred to as 'developmental changes' in childhood. The maturation rates of these developmental changes vary however between the pathways and receptors and often do not correlate solely with the increase in bodyweight of the child. The question is

therefore how to obtain data in children that allow for the study of these developmental changes ultimately resulting in evidence based dosing regimens for drugs in children.

To date, only a small number of drugs used in children is licensed for use in this specific group. Up to 70% of the drugs in pediatric intensive care, and 90% of the drugs in neonatal intensive care, are prescribed in an off-label or unlicensed manner [1-4]. Pediatric dosing regimens are usually empirically derived from adult regimens using linear extrapolations based on bodyweight. Since these developmental changes are non-linear dynamic processes, this dosing paradigm may result in under or over-dosing particularly in specific age groups. This may cause therapeutic failure, occurrence of severe adverse effects or even fatalities such as fatalities occurring after long-term sedation with high doses of propofol [5, 6] and occurrence of the grey baby syndrome in neonates after treatment with chloramphenicol [7, 8]. As a result, dose adjustments in the younger age groups are often proposed. For vancomycin for example lower doses are administered in neonates younger than 1 week (20 mg/kg/day) compared to 1-4 week-old neonates (30 mg/kg/day) and children between 1 month and 18 years (40 mg/kg/day) [9].

Instead of the *a priori* use of bodyweight for dosing guidelines in children, detailed information on PK and potentially also the PD needs to be considered in order to define effective and safe dosing regimens throughout the pediatric age range. The lack of PK and PD information on drugs in children has lead to the European Regulation which entered into force in 2007. This law imposes pharmaceutical companies to perform research in the whole pediatric age-range for all drugs that are developed for the European market, by requiring the submission of a pediatric investigational plan (PIP) in the early stages of the development of a new drug. In this PIP, a full description has to be given of the studies and of drug formulation in the pediatric population. In case little information is available about efficacy and safety of a drug, studies in children are only performed after more information is obtained in the adult population to increase the safety of the pediatric study [10-12]. The main targets of introducing the Pediatric Regulation were to facilitate development and availability of medicines in children between 0 and 17 years, to improve the availability of information about medicines used in children, to ensure that the medicines are of high quality, can be administered in a safe and effective way and that pediatric studies are performed in an ethically correct way [10]. The reward for this effort is a six month supplementary production certificate for the pharmaceutical company.

Both for industry and for academic researchers, performing (PK-PD) studies in children in order to develop rational dosing schemes is very challenging because

of ethical and practical issues. Unlike studies in healthy adults, research in healthy children is considered to be unethical, so all pediatric studies are performed in the vulnerable group of children suffering from a disease. In all clinical trials, an informed consent has to be signed by the patient before he or she can be enrolled into a trial. In pediatric trials, this informed consent can not be obtained by the patient that participates in the trial, and is therefore replaced by the consent of the parents or guardians. In older age groups, in addition to this consent, an assent is used in which the aim of the study is explained in an age-appropriate language so that children can understand [1, 4, 13].

Apart from ethical issues, practical challenges also occur when performing studies in children. There are limitations to the number and volume of samples that can be obtained, resulting in infrequent sampling possibilities and the need for advanced drug assay techniques with improved sensitivity. Another complicating factor is the limited available number of subjects that suffers from the same disease. Finally, pharmacodynamic endpoints that measure the efficacy of the drug, and which are validated for children may be lacking. All these factors call for highly advanced study designs and analysis techniques so that the burden for each child can be kept to a minimum while still addressing all the study objectives.

This paper aims to inform clinical pharmacologists, pediatricians and pharmacists about population PK-PD modeling in pediatric drug research. Advanced statistical tools are discussed that can be used to develop rational dosing schemes based on the PK and PD of a drug in children, despite practical and ethical restrictions. Using these tools, covariates can be identified in order to define appropriate doses and dosing intervals based on individual characteristics of each child with minimal burden to each patient. The paper also describes how to evaluate the predictive performance of the models by different validation methods including a prospective clinical trial. Ultimately, the efforts result in an individualized dosing regimen based on the PK-PD relation through the pediatric age-range.

2.2. PK-PD in children

Developmental changes in childhood can affect all PK processes from absorption until elimination as well as the pharmacodynamic effects. For example, in neonates intra-gastric pH is elevated (>4) which may increase the bioavailability of acid-labile compounds (penicillin G) and decrease the bioavailability of weak acids (phenobarbital) when given orally [14]. Additionally, gastric emptying in neonates is delayed, which

means that also the absorption of drugs e.g. paracetamol is slower in neonates [15, 16]. Other examples are changes in metabolizing enzyme capacity in children. Although most uridine 5'-diphosphate (UDP)-glucuronosyltransferases (UGTs) and P-450 cytochromes (CYPs) are expressed during the first week of life, the activity at birth in comparison with adults is often low, e.g. UGT2B7 activity at birth is around 10% of the adult level and maturation rates of different enzyme systems are known to mature at different rates [14, 17-20].

In addition, renal function and liver flow are influenced by physiological changes depending on age, e.g. the glomerular filtration rate in mL/min/70kg in full term neonates is 35% of the adult value, while mL/min/70kg adult values are reached at approximately 1 year old [21]. When using units of mL/min/70kg however, it should be realized that actual values of GFR in children are still very low compared to adult values because of correction for differences in total body weight between adults and infants.

Furthermore the body composition of children changes continuously resulting in an age-dependent proportion of body water and fat, which influences the distribution of drugs. For example, the total amount of body water (80-90 % of the bodyweight) is higher in neonates compared to adults (55-60%). Hydrophilic drugs like aminoglycosides have a larger volume of distribution in neonates which can be explained by larger extra-cellular fluid (45% of the bodyweight) compared to adults (20%) [14, 22].

In order to characterize the specific influence of developmental changes in childhood on the PK of a drug, concentration-time profiles are necessary, which require measurements of drug concentrations. For ethical reasons, in pediatric studies, discomfort, like pain and anxiety associated with venipuncture, must be restricted and practical issues limit the volume and amount of blood samples that can be obtained. Therefore, sensitive analysis techniques requiring only small blood samples should be used. While HPLC methods have reported to require only 50 µL of blood [23], more recently LC-MS methods can measure up to ten different drugs in volumes as low as 50-100 µL [24]. Additionally, also alternative matrices such as saliva should be explored as a non-invasive, more child-friendly alternative to measure a drug concentration. An example in this respect is a LC-MS/MS method which was developed and validated for the measurement of busulphan in saliva [24]. Also the use of a dried blood spot method e.g. for tacrolimus can facilitate the measurement of drugs in children [25]. Another method is capillary electrophoresis which requires only a low sample volume for the quantification of drugs in biological fluids [26].

Changes between children and adults may also result from differences in the

pharmacodynamics of a drug in children, e.g. by changes in the relative number and function of receptors. These age-related PD differences are until present rarely reported in literature, but one of the few examples is the increased sensitivity to d-tubocurarine, an antagonist of nicotinic neuromuscular acetylcholine receptors, in neonates and infants compared to children and adults [27]. Other examples are the observed lower minimum alveolar concentration (MAC) of isoflurane in preterm neonates compared to full-term neonates and older children [28, 29] and the different sensitivity to bronchodilators because of the lack of smooth muscles in the airways in neonates [30].

To study the PD of a drug in children, the use of a PD endpoint which is validated for use in children is a prerequisite. An illustrative example is the measurement of pain in young children. Since they are not able to report their pain using a visual analogue scale, an observational scale has been developed. This comfort behavioral (COMFORT-B) scale was developed and validated for use in children under the age of three years [31]. The scale assesses six behavioral items: alertness, calmness, muscle tone, body movement, facial tension, and crying (non-ventilated children) or respiratory response (ventilated children). All items range from 1 (no distress) to 5 (severe distress), resulting in a total score of varying from 6 to 30. This validated scale can then be used as a PD endpoint for the development of PD models for pain and/or sedation in children of different ages [32-34].

The influence of covariates such as the developmental changes, disease status and genetics on the PK and PD of drugs in children is depicted in Figure 1.

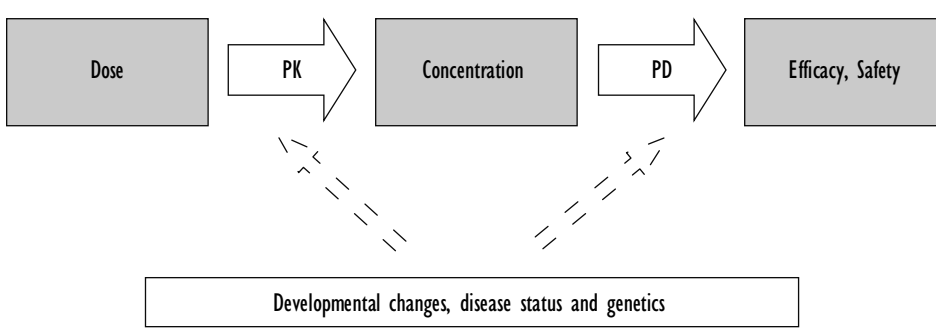

Figure 1: Schematic representation of the relationship between dose and concentration (pharmacokinetics, PK) and between concentration and a pharmacological (side) effect (pharmacodynamics, PD). Important covariates which may affect both the PK and/or PD are bodyweight, age, disease status (e.g. critically ill *versus* healthy children) and genetics.

When both the PK and PD of a drug in children are characterized, the developed models can be used to derive rational dosing regimens with predictable efficacy and concentration profiles. An example of such a PK-PD model with a derived dosing regimen is an article published by Peeters *et al*. In this paper both the PK and the PD were characterized in children, the latter with the use of the COMFORT-B scale as pharmacodynamic endpoint [33]. Based on the model it was found that propofol clearance is two times higher in non-ventilated children compared to ventilated children and adults. For the PD, a model was derived in which an effect of propofol was characterized within a naturally occurring sleep pattern of children in the ICU. Both models (PK as well as PD) were used to simulate concentrations as well as the effects that could be expected using different dosing schemes (Figure 2). As a result, based on this PK-PD model, a propofol dose of 30mg/h was recommended for a child of 10 kg which will result in adequate COMFORT-B scales in the night following craniofacial surgery.

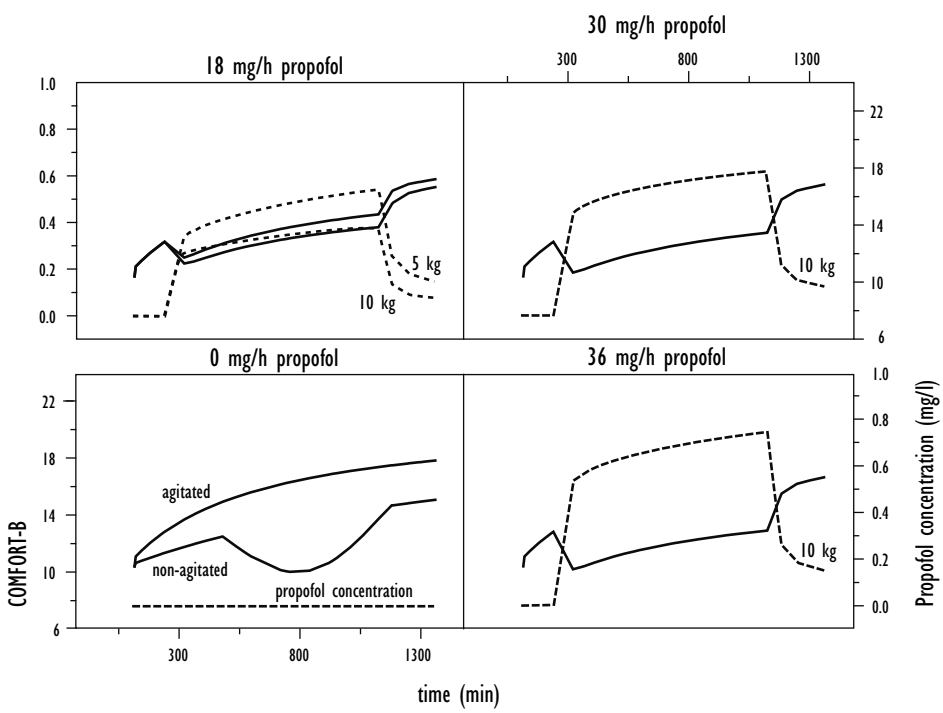

Figure 2: Simulation of propofol concentrations and response using COMFORT-B score *versus* time based on developed PK and PD models, after administration of different doses of propofol (0, 18, 30, and 36 mg/h) for a 10 kg and a 5 kg non-ventilated infant in the first night at the Intensive Care following craniofacial surgery. Target COMFORT-B scores are between 12 and 14 preferably. Reproduced from [Peeters MY, Prins SA, Knibbe CA, DeJongh J, van Schaik RH, van Dijk M, *et al*. Propofol pharmacokinetics and pharmacodynamics for depth of sedation in nonventilated infants after major craniofacial surgery. Anesthesiology 2006 Mar;104(3):466-74.]

2.3. Methods to analyse data: standard two-stage or population approach

When concentration-time and concentration-effect datasets obtained in children are considered for analysis, two different methods can be applied: the standard two-stage approach and the population approach using non-linear mixed effect models [35-38]. When using the standard two-stage approach or classical approach, in a first step parameters are estimated in each individual based on individual concentration-time profiles (figure 3A). In a second step, these parameters are summarized by calculating the mean or median of the parameters and the variability between subjects (SE or IQR). A major drawback of this methodology is that this approach requires a relatively high number of samples in each individual patient (Figure 3A) while each patient has to contribute roughly the same number of samples. Moreover it is very difficult to distinguish between inter-individual (variability between subjects), intra-individual and residual variability (variability within one subject, measurement error, and model misspecification) and as a result inter-individual variability is often overestimated [39].

Since usually only a limited number of observations can be obtained in pediatric subjects, the population approach using non-linear mixed effect modeling to obtain PK and PD parameters, is the preferred approach [37]. The population approach differs from the standard two stage-approach in the fact that the analysis is based on simultaneous analysis of all data of the entire population while still taking into account that different observations come from different patients (Figure 3B). Additionally the population approach allows not only for the analysis of dense data but also for sparse (limited number of observations per individual) and unbalanced data (unequal distribution of observations in various parts of the concentration-time profile in the individuals) or a combination of both. Finally both the interindividual and intra-individual variability are separately estimated in the dataset using this approach.

As a result of this methodology, when designing a pediatric study of which the data will be analyzed using the population approach, it is advisable to collect samples at different times (or time-windows) or to s*et a*lternating sampling schemes in subgroups of patients. This also means that (part of the) samples can be collected during routine clinical sampling. Consequently, the burden for the child that participates in the trial is reduced and the statistical power to develop a model describing the concentration-time or concentration-effect profile is not affected or improved.

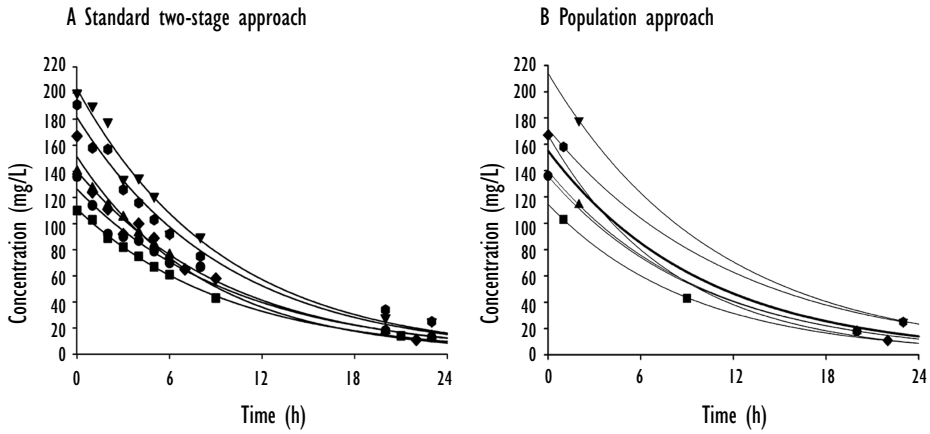

Figure 3: Concentration-time profiles of the same study using two different approaches. In figure 3A the standard two-stage approach is applied to a rich dataset. 3B shows the population approach with mixed effect modeling applied to the same dataset using only two datapoints for each individual so a sparse dataset is created. In 3A, in each of the six individuals 10 samples are available. The different symbols correspond to different individuals. Each black line corresponds to a separate fit to the 10 data points of each individual.
In 3B, which uses the mixed effect modeling approach, two samples of the 10 per subject in 2A are used. The different symbols correspond to the six different individuals. The black line illustrates the concentration time plot based on the population mean values of the PK parameters (PRED). The grey lines show the plots of the individual patients, which are based on the population mean values together with the measured concentrations of the specific individual (IPRED).

The term 'mixed' in non-linear mixed effects modeling stands for a mixture of fixed and random effects. For the fixed effects, a structural model describing the PK or PD is chosen (e.g. a two-compartment model for PK or an E_{max} model for PD). The random effects quantify the variability that is not explained by the fixed effects. These random effects include inter-subject and intra-subject and random variability (Figure 4), which are both simultaneously and separately estimated. It is often assumed that the variability between subjects follows a normal distribution with a mean of zero and variance ω^2. Equation 1 is used to describe the relationship between individual and population parameter estimates.

$$\theta_i = \theta_{mean} \bullet e^{\eta i} \qquad \text{(Equation 1)}$$

where θ_i represents the parameter of the ith subject, θ_{mean} the population mean, and ηi the variability between subjects. The residual error is in generally described

using a proportional error (error is dependent on the concentration, which means a higher absolute error at higher concentrations (equation 2)) or additive error (constant for all observations (equation 3)) or a combination of both. This means for the *j*th observed concentration of the *i*th individual the relation (Y_{ij}):

$$Y_{ij} = C_{pred,ij} \bullet (1+\varepsilon_{ij}) \qquad \text{(Equation 2)}$$

$$Y_{ij} = C_{pred,ij} + \varepsilon_{ij} \qquad \text{(Equation 3)}$$

where c_{pred} is predicted concentration and ε_{ij} is a random variable with mean zero and variance σ^2.

In general, model building requires three different steps. First a structural model (fixed effects) has to be designed, then a statistical sub-model (random effects) has to be developed and in the final step a covariate sub-model is identified.

The structural model describes the overall trend in the data. The choice of structural model (e.g. one, two or three-compartment model for PK and an Emax model for PD) is to be based upon the best *a priori* information about the drug to be studied [40]. The structural model uses fixed effects parameters such as clearance and volume of distribution for PK or E_{max} and EC_{50} for PD. The population values for these parameters are called typical values (TV).

After selecting the structural model, the statistical sub-model which accounts for the inter-individual as well as the residual variability is chosen and tested. Information on both inter- and intra- and residual variability is of clinical value, because it describes differences in clinical response between and within patients and may therefore provide guidance to rational dose adjustments. With the population approach, both these random effects are obtained, apart from estimates of both the population values (TV) and the individual values of PK and PD parameters (so called *post hoc* parameter estimates).

In the final step the covariate sub-model is determined which expresses relationships between covariates and parameters of the structural model (e.g. influence of bodyweight on volume of distribution or clearance). Covariates can be individual-specific (age, bodyweight, genetic profile, etc) or time-varying (renal

function, hemodynamic parameters, body temperature etc). The covariate analysis will be explained more in details in the following section.

As these three models are interrelated, the choice of the structural (and statistical) model may affect the choice of the covariate model and vice versa. The process of finding a model that adequately describes the data is thus an elaborate task, where model checking/refining is performed in several steps. To assess model fit in relation to the observed concentrations or effect measures, scatter plots or the so called goodness-of-fit plots are created (see Validation of the PK-PD models). Free software packages (Xpose, PSN etc.) are available to generate these plots.

The most commonly used software package for model building, which is also supported by the European Medicines Agency (EMEA) is the nonlinear mixed-effect modeling program NONMEM (GloboMax/ ICON, Ellicott City, MD) [4, 41-43]. NONMEM estimates parameters (e.g. clearance, volume of distribution or EC_{50}) via a maximum likelihood approach. This means that with the given data, the estimations of the parameters are the estimations which occur with the highest probability. Alternative software packages that can be used are for example Monolix, WinNonMix, USC*PAC which uses nonparametric maximum likelihood methods [44] or ADAPT using maximum *a posteriori* (MAP) methods [45].

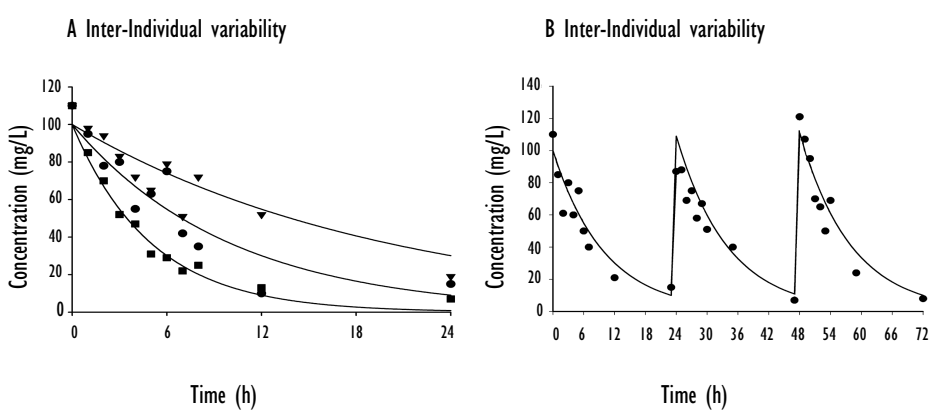

Figure 4: In 4A, the inter-individual variability is shown between three individuals who received the same dose. 4B presents the intra- or residual variability by showing the concentration-time profile after repeated administration. Both these random variables are assumed to be normally distributed with a mean of zero and a variance of ω^2 or σ^2 respectively.

2.4. Covariate analysis

To determine the optimal dose based on the individual characteristics of a patient, a covariate analysis has to be performed [40, 46, 47]. The aim of the covariate analysis is to identify specific predictors (covariates) of PK and PD variability and can typically be studied in population models. Covariate analysis involves the modeling of the distribution of the individual parameter estimates as a function of covariates which can be of demographic (e.g. age, bodyweight, gender), patho-physiological (e.g. renal or hepatic function), and genetic/environmental origin and/or be the result of the concomitant use of other drugs, which may influence the PK and/or PD. The identification of predictive covariates for variability provides the scientific basis for rational and individualized, patient tailored dosing schemes.

The influence of developmental changes in childhood can be explored primarily by using size and/or age as covariates. Size (bodyweight) can be incorporated into the model using two different approaches. The first approach or 'allometric size approach' includes size *a priori* by using a bodyweight based exponential equation with a fixed exponent of 0.75 for clearance and 1 for volume of distribution [48-52]. Once size is incorporated in the model using this fixed manner, the influence of age is investigated, being the difference between actual value of the PK parameter and the 0.75 allometric equation. When incorporating age as a covariate, different age descriptors may be used like postmenstrual age (PMA), gestational age (GA) or postnatal age (PNA) [53]. The choice for any of these age descriptors is based on the results of the systematic covariate analysis as described below [50, 54]. In the second approach or 'systematic covariate analysis', bodyweight is regarded as a covariate as any other which means that the descriptive properties on the PK parameters are evaluated in a systematic covariate analysis as described below [55-57].

In a systematic analysis, when studying the influence of covariates, scatter plots and summary plots of individual parameter estimates and/or weighted residuals *versus* covariates are used to screen for appropriate covariates to include in the covariate sub-model. Additionally these plots are used to explore the nature of the influence of the covariate (linear, exponential, allometric, subpopulations etc). Likely candidate covariates are then added to the model (forward inclusion). The influence of each covariate on the parameters is examined separately and compared to the simple model (no covariates). To assess whether the model with covariate statistically improved the fit to the data, the difference between their objective function value, referred to as log-likelihood ratio, is calculated. This ratio is assumed to be Chi-

square distributed, which means that a reduction in objective function of 3.84 is considered to be significant ($P<0.05$) [43, 58]. Beside the reduction in objective function, goodness-of-fit plots of the simple model and covariate model are explored for diagnostic purposes. Furthermore, the confidence interval of the parameter estimates, the correlation matrix (indicates the relationship between two structural parameters) and visual improvement of the individual plots are used to evaluate the model. Finally, a superior model is expected to reduce the inter-subject variance and/or the residual error terms. This procedure of covariate modeling implies that each covariate is only implemented if this can be fully justified by the data and the results of the statistic evaluations.

When two or more covariates are found to significantly improve the model the covariate that reduced the objective function most is included in the model after which the other covariates are tested again for their significance. After all covariates that significantly improved the objective function are added to the simple model, a backward deletion is performed, which means that each covariate is removed from the full model, one at a time (the one which causes the smallest increase in objective function first). Retaining or removing the covariate is statistically tested by the use of the objective function (Chi-square test) until each covariate has been tested.

In datasets containing sparse data, there may not be enough information to accurately estimate inter- and intra-individual variability. This causes the values of these parameters to shrink to 0, resulting in individual parameter estimates that are closer to the population parameter estimates than they really are. This phenomenon is called shrinkage [59]. Shrinkage may cause individual predictions, individual parameter estimates and diagnostics based on them to be less reliable. It can also hide, falsely introduce or distort the shape of covariate relationships.

Shrinkage is the result of properties of the data and is therefore difficult to avoid. One can only be aware of the presence of shrinkage, realize the influence it may have on the covariate analysis and use diagnostics other than those based on individual predictions or individual weighted residuals in the model building and model evaluation procedures.

2.5. Validation of PK-PD models

The objective of a PK or PK-PD modeling exercise is usually not just to describe the dataset of the sample of individuals that were studied. Generally, models are used

to simulate which concentrations and/or effects and their variability can be expected when different doses are given to future patients. These simulations may therefore lead to optimized dosing recommendations or to optimization of new studies for the entire population where the sample of individuals belongs to. It is often said that 'all models are wrong, but some are useful' [60]. In order to define whether a model is useful and valid for clinical and trial simulations, thorough evaluation and validation of the model is necessary. Although validations of PK models are only performed in 17% of the published pediatric studies [4] and in 28% of the adults studies [61], proper model validations are an essential step in model building. For this purpose, different evaluation and validation methods are available. As described before [62], a proper validation and evaluation procedure includes an internal model evaluation followed by an external evaluation and a prospective clinical study.

The first evaluation method is the basic internal model validation used to assess whether the model is able to describe the learning dataset (dataset used to develop the model) accurately and without bias. This evaluation should actually be considered the final stage of the model building procedure. Subsequently, in the external evaluation it is assessed whether the model is able to describe one or more external datasets (datasets other than the one used to develop the model) adequately. Alternatively if a dataset is sufficiently large the original dataset may be split in two so that the model is developed using one part (about two thirds) of the dataset and evaluated externally using the other part (one third) of the dataset. In pediatric studies, it is then especially important to stratify the data correctly and ascertain that all age groups are represented in equal proportions in both datasets.

Various techniques are available for the validation and evaluation of population PK and PK-PD models (both for internal and external validation procedures).

- Basic goodness-of-fit plots ((1) individual predicted *versus* observed concentrations, 2) population predicted *versus* observed, 3) (conditional) weighted residuals *versus* time and 4) (conditional) weighted residuals *versus* dependent variable plots). $WRES$ and $CWRES$ are calculated as the following:

$$WRES = \frac{y_i - E_{FO}(y_i)}{\sqrt{Cov_{FO}(y_i)}} \qquad \text{(Equation 4)}$$

$$CWRES = \frac{y_i - E_{FOCE}(y_i)}{\sqrt{Cov_{FOCE}(y_i)}} \qquad \text{(Equation 5)}$$

- Where y_i is the vector of measurements, $E(y_i)$ is the expectation of the data and $Cov(y_i)$ is the covariance matrix of the data [63].

- These plots are used in model building, but can also be used to ascertain that there is no trend or bias in the model predictions of the final model. Furthermore, these plots can also be used for both the internal and external evaluation of the model.

- In a bootstrap analysis new datasets are generated by resampling from the original dataset and is therefore an internal validation of the model. The new datasets are subsequently refitted to the original model, yielding mean values and standard errors for every model parameter.

- A bootstrap analysis provides information on the stability of the model and its dependence on specific individuals in the learning dataset. With the freely available PSN or Wings for NONMEM software packages an automated bootstrap analysis can be performed.

- In a visual predictive check (VPC) [64] a PK or PD profile is simulated a 100 to 1000 times and lines for the median values and their 90% prediction interval are plotted in a graph. The observed values in the internal or external dataset are subsequently plotted on top of this. It can then be visually checked whether 90% of the observations are within the indicated prediction interval and whether there is no bias in the observations compared to prediction interval. In figure 5, two examples of a VPC are given, showing when a model does not work and when a model does work on the same data.

- The VPC is a simulation-based diagnostic that can be used when the PK or PD profiles for all individuals in the dataset are similar and it allows for easy interpretation of the result. For this diagnostic tool, there are not statistical tests and all evaluations are based on visual evaluations. When the individual profiles are expected to deviate largely from one another because there is for instance a large variability in the time and amount of dose administrated, or when there are many covariates, the use of this diagnostic becomes more difficult.

- Another simulation-based diagnostic which can be used for both internal and external validations is the normalized prediction distribution error (NPDE) [65]. An example of an NPDE published before is shown in figure 6 [55]. This method yields information on how accurate the model predicts the median

value of the observations and the variability within them. The interpretation of this diagnostic is less straightforward than for the VPC, but the advantage of this method is that it can be used when the variability in dosing regimen (both in time, amounts and rates) is high or when there is a large number of covariates in the model. This can for instance be the case for data obtained during routine pediatric clinical practice. Software (e.g. NPDE add-on package for R) [66] to perform this analysis is freely available. For the NPDE, beside visual evaluation of the plots, statistical tests are available. These statistical tests are however reported to be highly sensitive and powerful, so that decisions for the model should primarily be based on visual assessments. An example is the statistically significant deviation of zero of the mean value because of the large number of data, while the actual deviation is small (e.g. 0.074) and not of clinical relevance.

If the model performed well in both the evaluation procedures, the dosing algorithm that results from the PK-PD model needs to be tested and challenged in a prospective (clinical) trial. If the predictive performance of the model is corroborated by the trial it can be used with confidence in clinical practice.

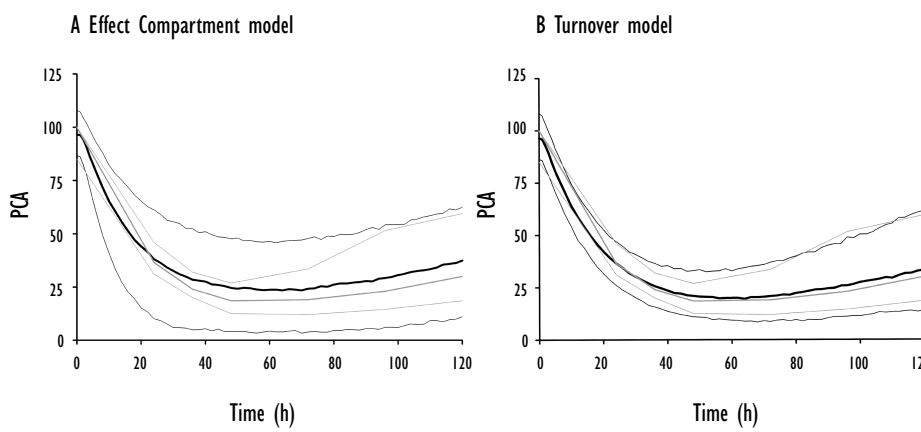

Figure 5: Two examples of a visual predictive check (VPC) are illustrated based on the same dataset (warfarin concentrations and prothrombin complex activity (PCA)) using two different models. In 5A the VPC of the effect compartment model is shown, while in 5B the VPC of the turnover model is demonstrated. The median (black thick line) and the 90% intervals (black thin lines) together with the observed data (PCA) (dots) are shown. Based on both graphics, the turn over model is the most appropriate model since 90% of the observations are lying within the prediction interval. Furthermore, unlike the effect compartment model, no bias is seen in the observations. Reproduced from [Holford N, 2005. The visual predictive check - Superiority to Standard Diagnostic (Rorschach) Plots. PAGE 14, Abstr 738. (http://www.page-meeting.org/?abstract=738)].

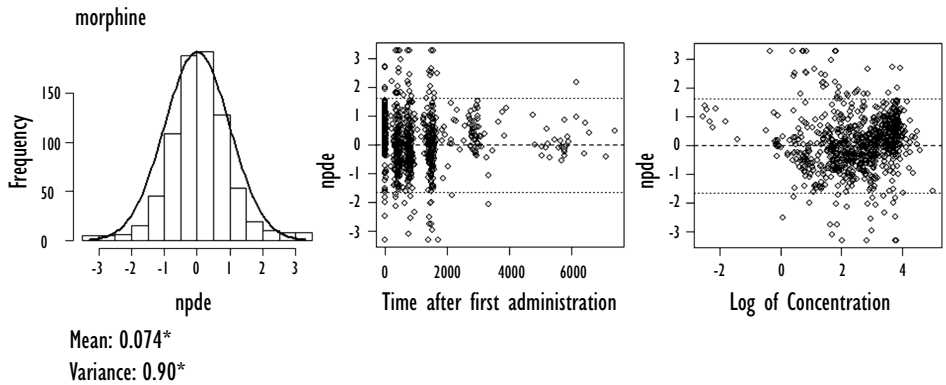

Figure 6: Example of a normalized prediction distribution error (NPDE) analysis, which show the NPDE distributions for morphine. The normal distribution is presented by the solid line. The values for the mean and standard deviation of the observed NPDE distribution are given below the histogram, with * indicating a significant difference of a mean of 0 and a variance of 1 at the $p<0.05$ level, as determined by the Wilcoxon signed rank test and Fisher test for variance. Additionally the distribution of NPDE vs time after the first dose and NPDE vs the log of the concentrations are also shown. The dotted lines represent the 90% distribution of the NPDE. Reproduced from [Knibbe CA, Krekels EH, van den Anker JN, DeJongh J, Santen GW, van Dijk M, *et al.* Morphine glucuronidation in preterm neonates, infants and children younger than 3 years. Clin Pharmacokinet2009;48(6):371-85] with permission from Wolters Kluwer Health | Adis (© Adis Data Information BV [2006]. All rights reserved.)

2.6. Optimal design of pediatric studies

When new population PK-PD studies are performed, it is important to design these studies in the most efficient manner possible to obtain maximum information about the PK and PD parameters so that they can be determined with the highest precision [51, 67]. When designing PK-PD studies in pediatrics certain factors need to be taken into account e.g. age-range of the pediatric group, therapeutic index, possibility to collect blood samples, availability of validated PD endpoints for children, and the availability of sensitive analytical methods.

When optimizing a PK or PK-PD study design, using literature data from adults or children of different age-ranges or possible *in vitro* or pre-clinical data, a concentration-time or effect-time profile for a study can be simulated. This can help to identify possible shortcomings in the design or to perform a power-analysis. Alternatively software packages are available (WINPOPT [68], PopED [67] and PFIM [69]) that can help to identify the optimal number and time points of observations in a study based on the prior information on a drug [70]. To determine the appropriate sample size certain

factors, which are summarized in Table 1, need to taken into account. Each of these factors can influence the required number of patients and/or samples in a positive or negative way. In a study of Peeters *et al.* [32] only 24 patients (aged between 3 and 24 months) were required to determine both the PK and PD since rich sampling was performed (median of 11 samples per child) and no covariates were found in the relatively homogenous population. This in contrast to a study performed by Knibbe *et al.* [55] in which 250 children were included. This higher number was required because in addition to the large dispersion in age from (preterm) neonates up to toddlers of 3 years of age, only 1 to 4 samples were available for each subject. Moreover infusion rates and additional bolus doses varied for each child during the study to obtain the desired analgesic effect. In another example [71], only 6 patients (aged between 1 and 5 years) were required in which 7 samples per patient were collected. This lower number of patients (N=6) compared to the study of Peeters *et al.* (n=24) can be explained because there often exist a lower variability in PK than in PD which results in a lower required number of patients (Table 1).

Table 1: Factors influencing the required number of patients and/or samples per patient.

Factor	Number of patients/samples
Study of PK only	relatively small number of patients/samples
Study of PK-PD relationship	relatively high number of patients/samples
Even distribution of covariates (age, bodyweight)	↓ number of patients/samples
↑ Number of changes in dose	↑/- number of patients/samples (depending on other aspects of the study design)
↑ Number of samples/child	↓ number of patients
Use of optimal sampling strategies	↓ number of patients/samples
Different sampling windows(e.g. two or three sampling schemes)	↓ number of patients/sample

2.7. Conclusions and perspectives

In view of the European Regulation which came into force in 2007, it seems now time to use the progress that has been made in the field of integrated PK-PD modeling [72] to develop rational and individualized dosing schemes for children. Because of the possibility to analyse sparse and unbalanced datasets thereby minimizing the burden for each child, population PK-PD modeling and simulation using non-linear mixed effect modeling has become the preferred tool to develop effective and safe dosing regimens for children. Specifically in pediatrics where the developmental changes have to be taken into account, which may influence the PK and/or the PD of the drugs, this advanced statistical tool is of critical value.

Before dosing regimens can be tested in clinical practice, proper validations of the models should be performed, for which recently adequate tools have been developed. Beside internal and external validations, prospective clinical trials, which allow for the evaluation of the model based dosing regimens, are needed, not only to adjust the proposed dosing regimen but also to convince pediatricians to use the information that has been generated using these modeling exercises.

Furthermore, one of the future goals may be to explore possibilities for cross-validation of the models, in which the reported influences of developmental changes on a certain PK or PD parameter of one drug are evaluated for use in another drug that go through the same metabolic route or share the same mechanism of action. In this respect, physiologically-based pharmacokinetic (PBPK) models are needed. PBPK models consider the physiological and biochemical processes by using *in vitro* data to describe the PK of drugs [73, 74]. The combination of these two approaches may use the information that is already available in a more optimal way in defining effective and safe dosing regimens for every individual patient.

In conclusion, analyses of pediatric data using population PK-PD modeling and covariate analysis will result in individualized dosing regimens for children of different age, bodyweight and genetic background. Thus population PK-PD modeling constitutes an innovative approach to the study of drug effects in this very special patient population, which is otherwise difficult to study.

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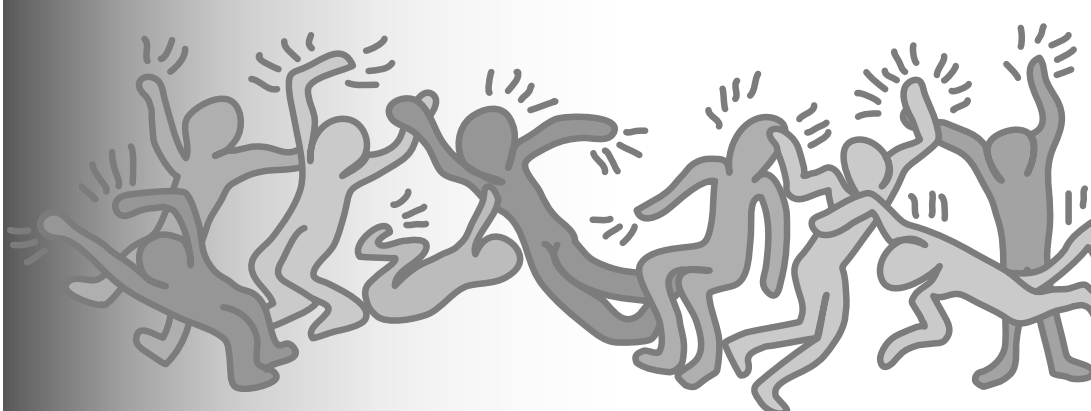
Section II

Developmental Changes in Glomerular Filtration in Preterm and Term Neonates by Describing the Pharmacokinetics of Renally Excreted Antibiotics



Chapter 3

Maturation of glomerular filtration rate in neonates as reflected by amikacin clearance

Roosmarijn F.W. De Cock, Karel Allegaert, Michiel F. Schreuder, Catherine M.T. Sherwin, Matthijs de Hoog, Johannes N. van den Anker, Meindert Danhof, Catherijne A.J. Knibbe

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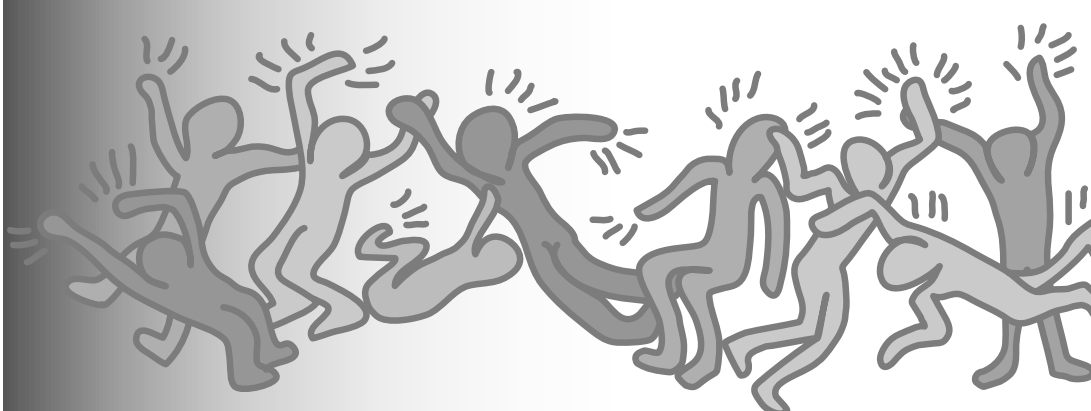

Abstract

Background and Objectives

During the newborn period and early infancy, renal function matures resulting in changes in glomerular filtration rate (GFR). This study was performed to quantify developmental changes in GFR in (pre)term neonates by use of amikacin clearance as proof of concept. The model was used to derive a rational dosing regimen in comparison to currently used dosing regimens for amikacin.

Methods

Population pharmacokinetic modeling was performed in NONMEM 6.2. using data of 874 neonates obtained from two previously published datasets (gestational age 24-43 weeks; postnatal age 1-30 days; birth weight 385-4650 g). The influence of different age and weight related and other covariates was investigated. The model was validated both internally and externally.

Results

Postmenstrual age was identified as the most significant covariate on clearance. However the combination of birth weight and postnatal age proved to be superior over postmenstrual age alone. Birth weight was best described using an allometric function with an exponent of 1.34. Postnatal age was identified using a linear function with a slope of 0.2 while co-administration of ibuprofen proved to be a third covariate. Current weight was the most important covariate for volume of distribution using an allometric function. The external evaluation supported the prediction of the final pharmacokinetic model. This analysis illustrated clearly that the currently used dosing regimens for amikacin in reference handbooks may possibly increase the risk of toxicities and should be revised. Consequently a new model-based dosing regimen based on current bodyweight and postnatal age was derived.

Conclusions

Amikacin clearance, reflecting GFR in neonates, can be predicted by birth weight representing the antenatal state of maturation of the kidney, postnatal age representing postnatal maturation and co-administration of ibuprofen. Finally the model reflects maturation of GFR allowing for adjustments of dosing regimens of other renally excreted drugs in preterm and term neonates.

3.1. Introduction

During the newborn period and early infancy renal function matures, resulting in differences in glomerular filtration rate (GFR) at different stages of development. Although GFR is well defined in adults and many efforts were undertaken in the past to describe the maturation of GFR in the pediatric age range [1-6], the description of GFR is still limited in pediatrics, particularly in neonates. Nephrogenesis starts in the embryo at week 5-6 of gestation and is completed at week 36 [7-9]. Birth causes hemodynamic changes leading to an increase in renal blood flow and a decrease in renal vascular resistance resulting in rapidly rising GFR during the first weeks of life [4, 7-9] approaching adult GFR levels at approximately 6-12 months of age. To define safe and effective dosing regimens for renally excreted drugs throughout the pediatric age range GFR, particularly in the first year of life, needs to be quantified.

Different methods have been described to calculate GFR in neonates based on either determination of clearance of endogenous (creatinine [10, 11]) or exogenous compounds (inulin [11-14], radio-isotopes [11]). However several limitations are linked to each of these methods making routine application cumbersome in pediatric and certainly in neonatal clinical practice [10, 12, 15-17]. The most pragmatic method, which has been proposed before [18, 19] is to assess GFR in the pediatric age range by determination of the clearance of a drug that is exclusively eliminated by GFR.

Therefore the aim of this study was to describe the pharmacokinetics of amikacin in preterm and term neonates with specific emphasis on clearance, since this parameter reflects GFR. A full covariate analysis was performed in which the influence of all bodyweight and age related, and other covariates on amikacin clearance were tested. The results based on the present amikacin datasets may ultimately serve to predict the maturation of both GFR and clearance of other renally excreted compounds in preterm and term neonates.

3.2. Methods

3.2.1. Patients

Model building was based on data from 874 neonates, obtained after combining two published datasets [1, 2]. Both studies were conducted at the University Hospital Leuven Belgium. More details on the studies can be found in the original articles [1,

[2]. Patients were enrolled in any of the two studies when at least two samples (peak and trough) were available for each patient. In the first study, 205 preterm neonates were considered. Forty-three patients were excluded since they received acetylsalicylic acid while other patients received ibuprofen (n=71), or no nonsteroidal anti-inflammatory drugs (n=91). In the second study, data from 715 patients were collected of which 71 patients received ibuprofen while the other patients (n=668) did not receive any nonsteroidal anti-inflammatory drugs. Three patients were considered as outliers due to administrative errors and were excluded from the second dataset. Data on prenatal use of betamethasone were also collected. More details on patient characteristics are shown in table I.

3.2.2. Drug administration, bloodsampling and assay

Before 2002, an amikacin dose of 20mg/kg/36hours was administered to neonates with a postmenstrual age below 30 weeks and 20mg/kg/24hours to neonates with a postmenstrual age of ≥30 weeks. After 2002, dosing was based on Langhendries *et al.*[20].

Amikacin (Amukin®; Bristol Myers Squibb, Braine-l'Alleud, Belgium) was administered by an intravenous infusion over 20 minutes. Blood samples were collected just before (trough) and 1 hour after initiation of administration (peak) of the second dose. In some of the individuals, more than two samples were available. Amikacin concentrations were measured with fluorescence polarization immunoassay using an Abbott TDx kit (Abbott Laboratories, Diagnostics Division, Abbott Park, IL, USA). The lower limit of quantification was 0.8 mg/L. The coefficient of variation (CV) was <3.5% (assessed at 5,15 and 30 mg/L).

3.2.3. Pharmacokinetic analysis and model evaluation

The pharmacokinetic analysis was performed using the non-linear mixed effects modeling software NONMEM version 6.2. (Globomax LLC, Hanover, MD, USA). Tools like S-Plus, PsN, XPose and R were used to visualize and evaluate the models. Model building was performed in four different steps: (i) selection of structural model, whereby a one- as well as a two-compartment model was tested, (ii) choice of statistical sub-model, (iii) covariate analysis, (iv) model evaluation. To discriminate between different pediatric (covariate) models the framework proposed by Krekels *et al.* [21] to systematically evaluate the descriptive and predictive performance of pediatric models was used as a guide. This framework was used because evaluation tools that are routinely used in the adult population may not suffice in the pediatric population due to the scarcity of the data, the increased variability in dosing and

Table I: Clinical characteristics of the patients in both model building datasets and external datasets, presented as median (range).

	Model building dataset 1 (15)	Model building dataset 2 (16)	External dataset 1 (17)	External dataset 2 (18)
Number of patients	162	712	80	159
Gestational age (weeks)	28 (24-30)	33 (24-43)	26 (24-41)	31 (25-42)
Postnatal age (days)	2 (1-3)	2 (1-30)	16 (3-30)	1 (1-3)
Birth weight (g)	1052 (475-1910)	1990 (385-4650)	880 (440-4430)	1740 (526-5420)
Current bodyweight (g)	1052 (475-1910)	1990 (385-4780)	1060 (450-4430)	1665 (526-5420)
Co-administration of ibuprofen	71 (43%)	47 (6%)	0	0

Birth weight = weight at day of birth, current bodyweight = weight at day of blood sampling

sampling schemes or the heterogeneity in the population. Therefore advanced and additional diagnostics next to standard tools may be required in the pediatric population compared to the adult population. This includes the following standard tools: discrimination between models by comparison of the objective function (OFV) and total number of parameters. A decrease in OFV of more than 7.8 points was considered as statistically significant ($p<0.005$ based on X^2 distribution). Furthermore, the goodness-of-fit plots (both observed *versus* individual and population predicted concentrations, time as well as population predictions *versus* conditional weighted residuals) were evaluated with specific emphasis on observed *versus* population predicted concentrations [21]. Moreover improvement of individual plots, confidence intervals of the parameter estimates and correlation matrix were assessed. Over-parameterization (ill-conditioning) was tested by calculating the condition number by dividing the largest eigen value to the smallest eigen value [22]. Finally in pediatric datasets there is often not enough information to accurately estimate the inter- and intra-individual variability. Therefore shrinkage was considered [23]. Other pediatric specific evaluation tools are mentioned in the section Covariate analysis and Internal evaluation procedure.

3.2.4. Covariate analysis

Covariates were plotted independently against the individual *post hoc* parameter estimates and the weighted residuals to visualize potential relationships. The following covariates were evaluated for inclusion: gestational age, postnatal age, postmenstrual age (sum of gestational and postnatal age), birth weight (weight at day of birth), current bodyweight (weight at day of blood sampling), co-administration of

ibuprofen, prenatal exposure to betamethasone and creatinine concentrations. Information on co-administration of dopamine, ventilation and positive blood culture was not available for all patients. Consequently they could not be evaluated as possible covariates. However in the past it was shown that the impact of these covariates was not significant [1, 6, 24].

Potential covariates were separately implemented into the model using a linear or allometric equation (equation 1).

$$P_i = P_p \bullet (\frac{Cov}{Cov_{Median}})^k \qquad \text{(Equation 1)}$$

In this equation Pi represents the individual parameter estimate of the ith subject, Pp equals the population parameter estimate, Cov is the covariate and k is the exponent which was fixed to 1 for a linear function or estimated for an allometric function. The significance of a covariate was statistically tested by use of the objective function. A p value <0.005 was applied to evaluate the covariates in the forward inclusion (decrease of OFV of at least 7.8 points) while a more stringent p value of <0.001 was used in the backward deletion (decrease of OFV of at least 10.83 points). When two or more covariates were found to significantly improve the model, the covariate causing the largest reduction in OFV was left in the model. Additional covariates had to reduce this OFV further to be retained in the model. In order to select the final covariate model, as suggested by Krekels *et al.* [21], individual and population parameter estimates were plotted against the most predictive covariate to evaluate whether the individual predicted parameters were equally distributed around the population predicted parameters. The choice of the covariate model was further evaluated as discussed in the previous paragraph whereby the results of the internal evaluation were also considered.

3.2.5. Internal evaluation procedure

The final pharmacokinetic model was validated using two methods [21]: (i) the bootstrap resampling method, and (ii) the normalized prediction distribution error (NPDE) method. The bootstrap analysis to evaluate the stability was performed in S-plus, version 6.2.1 (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P Consultants BV, Leiden, The Netherlands). The model building datasets were resampled 1000 times to produce a new dataset of the same size containing a different combination of individuals. The parameter estimates were summarized in terms of mean values and standard errors and were compared with the

estimates obtained from the model building datasets.

The accuracy of the model was evaluated with the NPDE method [25, 26] in which the observed and simulated concentrations are compared using the NPDE software package in R. In this study each observation was simulated 1000 times after which the software assembled the predictions in a cumulative distribution and determined the value of the cumulative distribution at the observed concentration. The normalized prediction distribution errors were then obtained after applying the inverse function of the normal cumulative density function [25, 26]. The NPDE as simulation-based diagnostic is preferred over the visual predictive check (VPC) since it easier to interpret when data are obtained during routine clinical practice causing a high variability in both dosing and sampling schemes. Consequently a NPDE is often preferred over a VPC in the analysis of pediatric datasets. The results of NPDE method are visualized in different graphs: (1) quantile-quantile plot (2) histogram showing the distribution of the normalized prediction distribution errors which are expected to follow a normal distribution, (3) scatterplot NPDE *versus* time and (4) scatterplot NPDE *versus* predicted concentrations.

3.2.6. External evaluation procedure

External evaluation was performed by using two published external datasets [6, 27]. In total 517 concentrations were available obtained from 80 neonates in the first [6] and 159 neonates in the second external dataset [27]. In external dataset 1, peak (taken 60 minutes after start of infusion) and trough concentrations (measured at 24 hours) were available. In external dataset 2, only 1 ample was available, which was collected between the first and second dose. More details on the studies (including information on co-medication and prenatal drug treatment) can be found in the original articles [6, 27]. In neither one of these two external datasets, ibuprofen was administered. Patient characteristics of the external datasets are given in table 1.

The final pharmacokinetic model (with all parameters fixed to final values with maxeval=0 and without covariance step) was used to simulate concentrations for each data point of the two external datasets. Additionally, the final pharmacokinetic model was used to compute the NPDE [25, 26] for each of the external datasets. Each concentration was simulated 1000 times.

Finally parameters of the final model were re-estimated on the basis of the two model building datasets and external dataset 1. In a second step both external datasets combined with both model building datasets were analyzed.

3.2.7. Simulations of currently used dosing regimens

The parameter estimates from the final pharmacokinetic model were used to simulate concentration time profiles upon different dosing regimens currently used or suggested in reference textbooks [6, 20, 28-30]. Simulations were performed in three patients (gestational age 24, 32, 40 weeks) selected from the two model building datasets. The three patients were selected to cover the entire study population in terms of bodyweight and gestational age. For the first preterm patient (gestational age 24 weeks) the lowest birth weight was chosen. In the second preterm (gestational age 32 weeks) and third term patient (gestational age 40 weeks) a median birth weight of 1730 g and 3520 g, was chosen respectively. In the simulations, five consecutive doses were administered starting from day at birth. The simulations were performed excluding the interindividual and residual variability. Based on the results a new dosing schedule was designed, aiming to achieve Cmax values in the range of 24-35 mg/L [6] and trough values below or between 1.5-3 mg/L [31]. Since the dose was given over 20 minutes in the model building datasets while in most reference textbooks (Neofax® [28], Red Book® [29], BNFc [30], Sherwin *et al.* [6]) an infusion time of 30 minutes is applied, both infusion rates were used to simulate the concentration-time profiles of the model-based dosing regimen.

3.3. Results

3.3.1. Patients and data

The pharmacokinetic analysis was based on 2186 observations from 874 neonates obtained from two studies performed by Allegaert *et al.* [1, 2]. The external evaluation was executed using two previously published datasets [6, 27] containing data of 80 and 159 neonates respectively. A summary of all patient characteristics is presented in table I.

3.3.2. Pharmacokinetic model building

A two compartment model parameterized in terms of clearance (CL), inter-compartmental clearance (Q), volume of distribution of central compartment (V1) and peripheral compartment (V2) was preferred over a one compartment model since it was able to describe the model building datasets more accurately. The objective function of the final two compartment model (OFV=7738) was significantly lower ($p<0.001$) compared to the corresponding one compartment model (OFV=7946).

Furthermore the goodness-of-fit plots improved. In particular samples taken later than 48 hours after dosing were more accurately described using the two compartment model. However, when estimating Q and V2 independently of CL and V1, no covariance step could be given, probably due to over parameterization of the model. As a result the model was simplified by estimating Q and V2 as fraction of CL and V1, respectively, resulting in no increase in OFV and even better diagnostics plots. Because of failure of the bootstrap, V2 was equalized to V1 in the final model which only resulted in an increase in OFV of 7 points and similar diagnostic plots. The residual variability was best described using a combined additive and proportional error model.

Table II: Population parameter estimates of the final pharmacokinetic model based on two model building datasets, the values obtained after bootstrap of the final pharmacokinetic model and the model parameter estimates after combining the model building datasets together with the external datasets.

Parameter	Simple model	Final pharmacokinetic model (Model building datasets)	Bootstrap final pharmacokinetic model	Model building datasets and external dataset 1	Model building datasets, external dataset 1 and external dataset 2
	Value (CV%)	Value (CV%)	Value (CV%)	Value (CV%)	Value (CV%)
Fixed Effects					
CL (L/h)	0.0743 (3.11)				
CL(L/h/kg BWb)		0.0493 (2.21)	0.0495 (2.68)	0.0496 (2.3)	0.0485 (2.06)
Q (fraction of CL)	0.681 (6.3)	0.415 (12.3)	0.446 (13.94)	0.422 (12.3)	0.36 (10.9)
CL*((BWb/median)$^{\theta}$)	-	1.34 (2.04)	1.34 (2.22)	1.34 (2.02)	1.33 (1.84)
CL*(1+θ*(PNA/median))	-	0.213 (9.81)	0.217 (10.33)	0.211 (9.1)	0.202 (10.6)
CL*θ(IBU)	-	0.838 (3.88)	0.836 (4.13)	0.838 (3.96)	0.851 (3.43)
V1=V2 (L/kg cBW)	0.716 (2.19)	0.833 (1.34)	0.827 (1.47)	0.836 (1.34)	0.845 (1.18)
V1* ((cBW/median)$^{\theta}$)	-	0.919 (2.46)	0.915 (2.52)	0.909 (2.22)	0.91 (2.14)
Interindividual variability					
ω^2 (CL)	0.677 (6.51)	0.0899 (14.9)	0.0917 (15.36)	0.097 (13.9)	0.0822 (13.3)
Residual Error					
σ^2 (proportional)	0.184 (6.25)	0.0614 (8.19)	0.0580 (8.47)	0.0614 (8.26)	0.0592 (8.07)
σ^2 (additive)	1.15 (13.6)	0.267 (27.2)	0.489 (36.73)	0.3 (25.9)	0.297 (23.7)

CL = Clearance, Q = Intercompartmental clearance, V1 = volume of distribution of central compartment, V2 = Volume of distribution of peripheral compartment, BWb = bodyweight at birth, cBW = current bodyweight, PNA = postnatal age, θ(IBU)=1 : no co-administration of ibuprofen, θ(IBU)=0.838 : co-administration of ibuprofen

3.3.3. Systematic covariate analysis

The systematic covariate analysis identified current bodyweight as most important covariate implemented on volume of distribution using an allometric function (table II) causing a drop in the OFV of 1488 points. For clearance postmenstrual age was identified as most important covariate causing a drop in OFV of 1160 points. However, birth weight and postnatal age together proved to be superior (ΔOFV 1598 points) to postmenstrual age alone. The model using postmenstrual age as covariate on clearance was not able to describe the data as well as the final model with birth

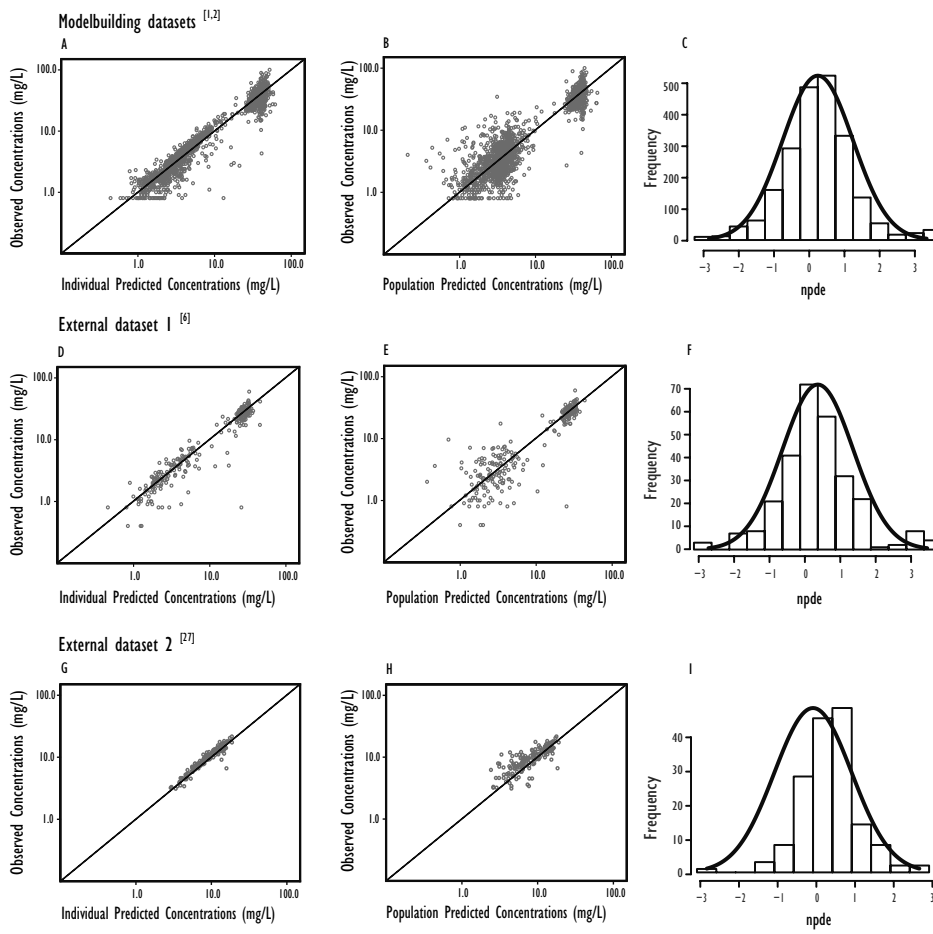

Figure 1: Observed *versus* individual predicted concentrations and observed *versus* population predicted concentrations of (a-b) the model building datasets [1,2], (d-e) external dataset 1 [6] and (g-h) external dataset 2 [27]. The histograms show the distribution of the NPDE method of (c) the model building datasets, (f) external dataset 1 and (i) external dataset 2. The solid line represents a normal distribution.

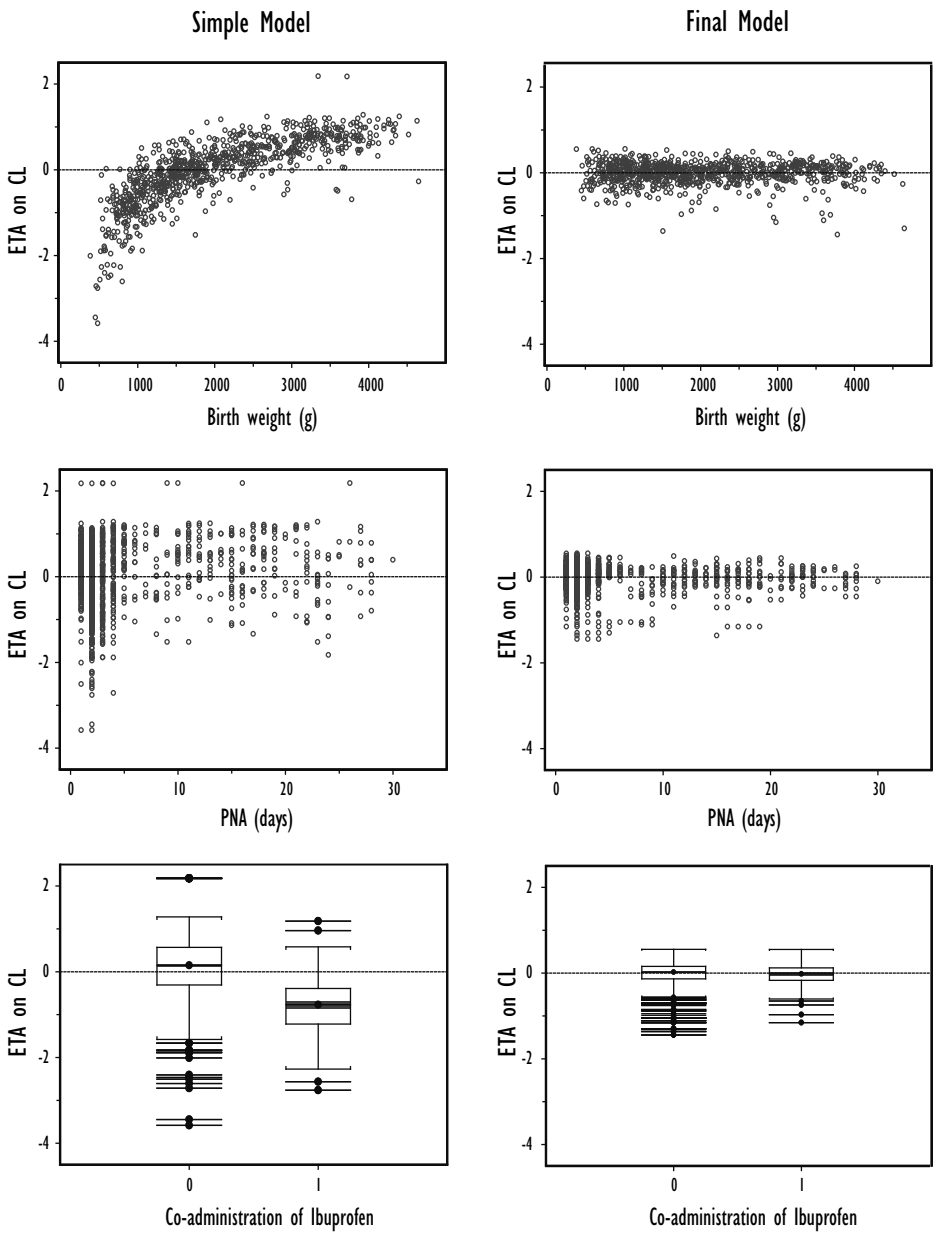

Figure 2: Interindividual variability for clearance (Eta on CL) *versus* birth weight, postnatal age (PNA) and co-administration of ibuprofen for the simple (left) and the final model (right).

weight and postnatal age unless additionally the covariates birth weight and/or postnatal age also were introduced. Consequently birth weight was implemented as first covariate on clearance using an allometric function with an estimated exponent of 1.34 (ΔOFV 940 points). A further decrease in OFV of 659 points was achieved by implementing postnatal age linearly on clearance. The model further improved by introducing co-administration of ibuprofen (ΔOFV 26 points) as third covariate on clearance. A correlation between creatinine concentrations and clearance, as seen in adults, was not identified in this population.

3.3.4. Final pharmacokinetic model and internal evaluation

Table II gives an overview of the parameter estimates of the simple and final pharmacokinetic model together with the values obtained from the bootstrap analysis. In figure 1a-b, observed *versus* individual and population predicted concentrations are given for the final pharmacokinetic model, while in figure 1c the histogram of the NPDE is shown. The histogram follows the normal distribution expected by the solid line indicating the accuracy of the final pharmacokinetic model. No trend was seen in the NPDE *versus* time or *versus* predicted concentrations (data not shown). In figure 2 interindividual variability in clearance is plotted against birth weight, postnatal age and co-administration of ibuprofen for the simple and the final pharmacokinetic model to illustrate that by introducing these three covariates into the model, a significant part of the interindividual variability (68%) is explained. This is also reflected by the estimate of interindividual variability in clearance which was reduced from 0.677 to 0.0899 when the three covariates were introduced (table II). Plotting the population and individual predicted values for clearance *versus* birth weight (data not shown) as proposed by Krekels *et al.* [21] illustrated that the individual predicted values are equally scattered around the population predicted values. No ill-conditioning was detected since the condition number (value of 43) for the final pharmacokinetic model was far below the critical value of 1000.

The model-based predicted clearance values of the final pharmacokinetic model *versus* birth weight for PNA 0, 14, 28, with and without co-administration of ibuprofen are illustrated in figure 3. The figure shows horizontally the influence of birth weight representing the antenatal maturation and vertically postnatal age representing postnatal maturation.

3.3.5. External evaluation of the final model

The predictive performance of the final pharmacokinetic model was evaluated using two previously published external datasets [6, 27] (table I). In figure 1 observed

Table III: Amikacin dosing recommendations in preterm and term neonates according to 5 dosing regimens currently used or suggested in reference text books.

Guideline	Currently used dosing guidelines					
	Gestational age (weeks)	Postnatal age (days)	Current body-weight (g)	Duration IV infusion (minutes)	Dose (mg/kg)	Interval (hours)
Langhendries *et al.* [21]*	< 28	-	-	20	20	42
	28-30	-	-		20	36
	31-33	-	-		18.5	30
	34-37	-	-		17	30
	> 37	-	-		15.5	24
Sherwin *et al.*[17]	< 29	-	-	30	15	36
	29-36	-	-		14	24
	> 36	-	-		15	24
Neofax® (2009) [32]	< 30 or **	0-7	-	30	18	48
		8-28	-		15	36
		> 28	-		15	24
	30-34	0-7	-		18	36
		> 7	-		15	24
	> 34	-	-		15	24
RedBook® (2009) [38]	-	1-30	< 1200	30	7.5	18-24
		0-7	1200-2000		7.5	12
			> 2000		7.5-10	12
		>7	1200-2000		7.5-10	8-12
			> 2000		10	8
BNFc (2009) [39]	-	-	-	30	15	24

* 6 hour prolongation of dosing interval when ibuprofen is co-administered

** Neonates suffering from asphyxia, having a patent ductus arteriosus or co-administration of indomethacin

versus individual (d,g) and population predicted concentrations (f,i) are given for both the external datasets. Additionally, the histograms of the NPDE are shown in figure 1f and 1i respectively. While the final pharmacokinetic model is able to predict the data of external dataset 1 with adequate precision and without bias, a slight bias is seen for external dataset 2 in which sampling was not performed at peak and trough timepoints but in between these two moments. This small bias is observed in figure 1h showing observed *versus* predicted concentrations as well as in figure 1i in which the normal distribution is shifted from the solid line. Furthermore a trend was seen in the NPDE *versus* time and the npde *versus* predicted concentrations (data not shown).

However combined analysis of the two model building datasets and the external dataset 1 as well as the model building datasets and both external datasets, revealed that fairly similar parameter values were obtained (table II), indicating the stability of the final pharmacokinetic model.

3.3.6. Simulations of currently used dosing regimens

Concentration-time profiles for amikacin for three different individuals (gestational age 24, 32 and 40 weeks and birth weight 480, 1730 and 3520g, respectively) following five different dosing regimens currently used or proposed in reference textbooks (table III) were predicted on the basis of the final pharmacokinetic model (figure 4). Peak concentrations below the target range of 24-35 mg/L [6] and concentrations above the aimed trough concentration range of 1.5-3 mg/L [31] are represented by a black dot while predicted peak and trough concentrations within the target range are indicated by open circles. The dosing guidelines suggested by the Red Book® and the British National Formulary for children (BNFc) are potentially inducing toxicity in preterm and even term neonates since target trough values are not reached which may be associated with a higher risk for nefro- or ototoxicity [32, 33] due to aminoglycoside accumulation. Although the dosing guidelines according to Langhendries *et al.* [20], Sherwin *et al.* [6] and Neofax® approach the target trough concentrations more closely, adjustments are needed for all of them since target

Table IV: A model-based dosing regimen for preterm and term neonates based on target Cmax concentrations of 24-35 mg/L and trough concentrations below 2-5 mg/L.

Model-based dosing regimen						
Guideline	Gestational age (weeks)	Postnatal age (days)	Current bodyweight (g)	Duration IV infusion (minutes)	Dose (mg/kg)	Interval (hours)
Model-based dosing regimen *	-	< 14	0-800	20/30	16	48
	-		800-1200		16	42
	-		1200 - 2000		15	36
	-		2000 - 2800		13	30
	-		≥ 2800		12	24
	-	≥ 14	0-800		20	42
	-		800 - 1200		20	36
	-		1200 - 2000		19	30
	-		2000 - 2800		18	24
	-		≥ 2800		17	20

*10 hours prolongation of dosing interval when ibuprofen is co-administered

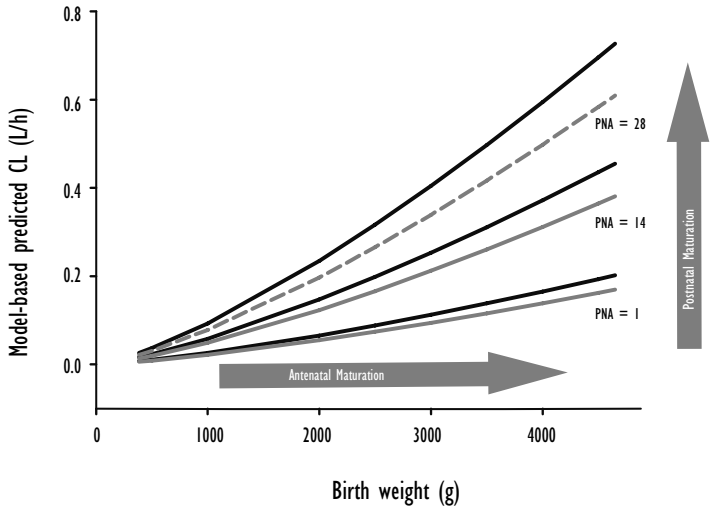

Figure 3: Model-based predicted amikacin clearance values *versus* birth weight for postnatal age (PNA) of 0, 14 or 28 days with (black line) and without (grey line) co-administration of ibuprofen.

trough concentrations below 1.5-3 mg/L are only reached in patient 2 by Neofax®. Target peak and trough values are only reached in all three patients using the model-based new dosing regimen (table IV) which is based on current bodyweight (covariate for volume of distribution, determining the peak concentration), postnatal age and co-administration of ibuprofen (covariates for clearance determining the dosing interval).

3.4. Discussion

During infancy renal function matures resulting in changes in GFR, which is most pronounced in neonates. Since amikacin is almost entirely eliminated by GFR, we aimed to quantify developmental changes in GFR in (pre)term neonates by describing the maturation of amikacin clearance.

The pharmacokinetic model developed in this study was based on 2186 trough and peak concentrations from 874 (pre)term neonates obtained from two datasets [1, 2] covering an extensive variation in gestational age, postnatal age and birth weight. An internal and external evaluation was performed to demonstrate descriptive and predictive properties. Birth weight, representing maturation of GFR until birth [34], proved to be the most important covariate for clearance. Birth weight, which

ranged between 385g and 4650g, was found to influence clearance on the basis of an allometric function as shown in figure 3. Maturation after birth was quantified using postnatal age (range 1-30 days) as covariate for clearance (figure 3). In previous studies [1, 2, 4, 6, 35-38], which were often based on a more restricted number and range in patients and data, these two processes were merged together into one covariate, postmenstrual age, which is a combination of gestational and postnatal age. The model with postmenstrual age was inferior compared to the final pharmacokinetic model with both birth weight and postnatal age unless birth weight and postnatal age were added as second and third covariate to postmenstrual age. We consider our approach using birth weight and postnatal age superior because the combination of postmenstrual age, birth weight and postnatal age repeatedly uses the same information. More specifically, birth weight and postnatal age as morphometric surrogates for organ function are distinctly different and independent covariates since birth weight reflects the antenatal maturation and postnatal age is representing postnatal maturation. The limited predictive value of postmenstrual age in this analysis may be explained by the large variation in gestational and postnatal age, as postmenstrual age does not distinguish between pre- and postnatal maturation. Meanwhile birth weight also proved to be a superior covariate compared to gestational age. For the same gestational age, a large range in birth weight was observed in our datasets, showing that birth weight represents more accurately the antenatal maturation thereby reflecting (dys)maturity or (dys)function of the neonate. The specific influence of birth weight, postnatal age and co-administration of ibuprofen is given in figure 3. This figure illustrates clearly how clearance of amikacin increases with birth weight (antenatal maturation) and postnatal age (postnatal maturation). Large differences in clearance values (4.4 fold) are observed when comparing an individual with a birth weight of 1 kg (0.026 L/h) and 3 kg (0.112 L/h) at day 1. This difference in clearance is still present at day 28 between an individual of 1 kg (0.092 L/h) and 3 kg (0.404 L/h) indicating that catch-up growth of prematurely born neonates (e.g. normalizing in height, weight and clearance values) does not appear in the first month. Furthermore it also illustrates the reduction in GFR following administration of ibuprofen [1, 39-42] causing a decrease in amikacin clearance of 16.2%. This decrease in clearance by ibuprofen was seen before and is caused by inhibition of the cyclo-oxygenase cascade inducing a downregulation in the formation of prostaglandins. This results in a reduction of the vasodilative effects which normally help to support the glomerular filtration rate and glomerular perfusion leading to a decrease in clearance.

In some previous trials, GFR was determined by measuring the clearance of inulin, which is considered as the gold standard[13]. In most of these publications gestational age was found as most important covariate for clearance in (pre)term neonates [43-45], followed by an increase in GFR due to postnatal age [44, 45]. However these findings are

based on a smaller number of patients and more narrow age range compared to our analysis in which birth weight and postnatal age were identified as most important covariates. Although the use of amikacin as a marker for GFR may also have some restrictions - amikacin itself may influence the renal function after repetitive dosing as well as the fact that amikacin is often given to treat neonatal sepsis, a disease state that also may influence renal function - our findings are based on a very large dataset of preterm as well as term neonates with a postnatal age up to 30 days. Furthermore as discussed previously birth weight was found to represent more accurately the antenatal maturation of the kidney in our analysis compared to gestational age. Moreover practical and ethical constraints make a prospective evaluation of maturation of GFR by measuring clearance of inulin not possible in this age group.

Model-based concentration-time profiles were simulated for three different patients using 5 different dosing guidelines (table III, table IV) (figure 4). According to our simulations the dosing guidelines suggested by Langhendries *et al.* [20], Sherwin *et al.* [6] and Neofax® approach the target values closely even though target trough values between 1.5-3 mg/L [31] are not reached, except in patient 2 by Neofax® (figure 4). A possible explanation might be that target trough values between 2-5 mg/L [20, 28] instead of 1.5-3 mg/L [31] were aimed for in the past. Regarding the dosing guidelines suggested by Langhendries *et al.* [20] target trough values may also not be reached since this dosing regimen was only validated for neonates directly after birth, implying that postnatal age was not taken into account. However, all dosing regimens for amikacin currently used or suggested in reference handbooks in both preterm and term neonates up to 30 days possibly increase the risk of toxicities and therefore need to be updated. Especially the dosing regimens proposed in the Red Book® and BNFc may potentially induce nefro- and ototoxicity in preterm and even term neonates since target trough values are not reached [32, 33]. Moreover potential risk of oto- and nefrotoxicity is not only related to higher trough concentrations but is also linked to treatment duration. Therefore future studies should be considered on treatment duration to even further reduce amikacin toxicity. Finally, exposure to aminoglycosides in neonates with mutations in the MT-RNR1 gene that are associated with aminoglycoside-induced hearing loss [46] should be avoided. A prenatal screening in which mothers are tested for these variants would prevent the exposure of aminoglycosides to babies at risk.

Based on the final pharmacokinetic model a new dosing regimen was developed by adjusting the dose to current bodyweight (covariate for volume of distribution, determining the peak concentration), postnatal age and co-administration of ibuprofen (covariates of clearance, determining the dosing interval). When ibuprofen is co-administered it is suggested to extend the dosing interval to 10 hours since

trough concentrations between 1.5-3 mg/L are not yet reached upon a prolongation by 6 hours. For the model-based dosing regimen concentration-time profiles were simulated using an infusion time of 20 and 30 minutes. Both infusion times resulted in the same trough concentrations and only slightly higher peak concentrations when the dose was administered over 20 minutes which are of no clinical relevance. This limited influence of infusion rate was expected since the final pharmacokinetic model based on data using an infusion time of 20 minutes was able to describe accurately the data of external dataset 1 in which an infusion time of 30 min was applied.

Even though the final model described the data of all age and weight ranges most adequately a slight bias was observed in the plot 1b. When re-evaluating retrospectively the medical records it seemed that these individuals were suspect for perinatal asphyxia. Although Langhendries *et al.* [20] proposed before that the time interval for amikacin dosing needs to be adapted following perinatal asphyxia, we were not able to identify in our datasets which patients were suffering from asphyxia since no robust indicators have been identified in practice. Therefore we could not study asphyxia as a covariate. In order to better determine the impact of perinatal asphyxia in future models, we suggest to prospectively report potential indicators (Apgar score, lactate, Thompson score) [47].

Although the stability of the final pharmacokinetic model was indicated by the bootstrap and the NPDE as well as the ability to predict external dataset 1 accurately, the predictive performance was slightly biased for external dataset 2. This could not be explained by differences in age or bodyweight or any other covariate between external dataset 2 and the other datasets. The only observed discrepancy was the time at which samples were taken. Unlike the model building datasets and external dataset 1, no peak and trough but only midterm samples, taken between 3.5 - 33 hours, were available in external dataset 2. While this result indicates that the final pharmacokinetic model is not entirely able to describe the midterm samples which are concentrations measured in a different phase of the distribution, the results of external dataset 1 show that the model very well predicts peak and trough concentrations which are used as surrogate markers for respectively efficacy and safety of amikacin. In this respect it can be emphasized that in clinical practice only trough samples are of interest in terms of aminoglycoside accumulation monitoring meaning that midterm samples should be avoided.

The clinical response was not investigated in the new dosing regimen and may be considered as one of the limitations of this study. However amikacin was given in this population when an infection was suspected. Another remark can be made on the adequate use of antibiotics given in association with the aminoglycosides. Aminogly-

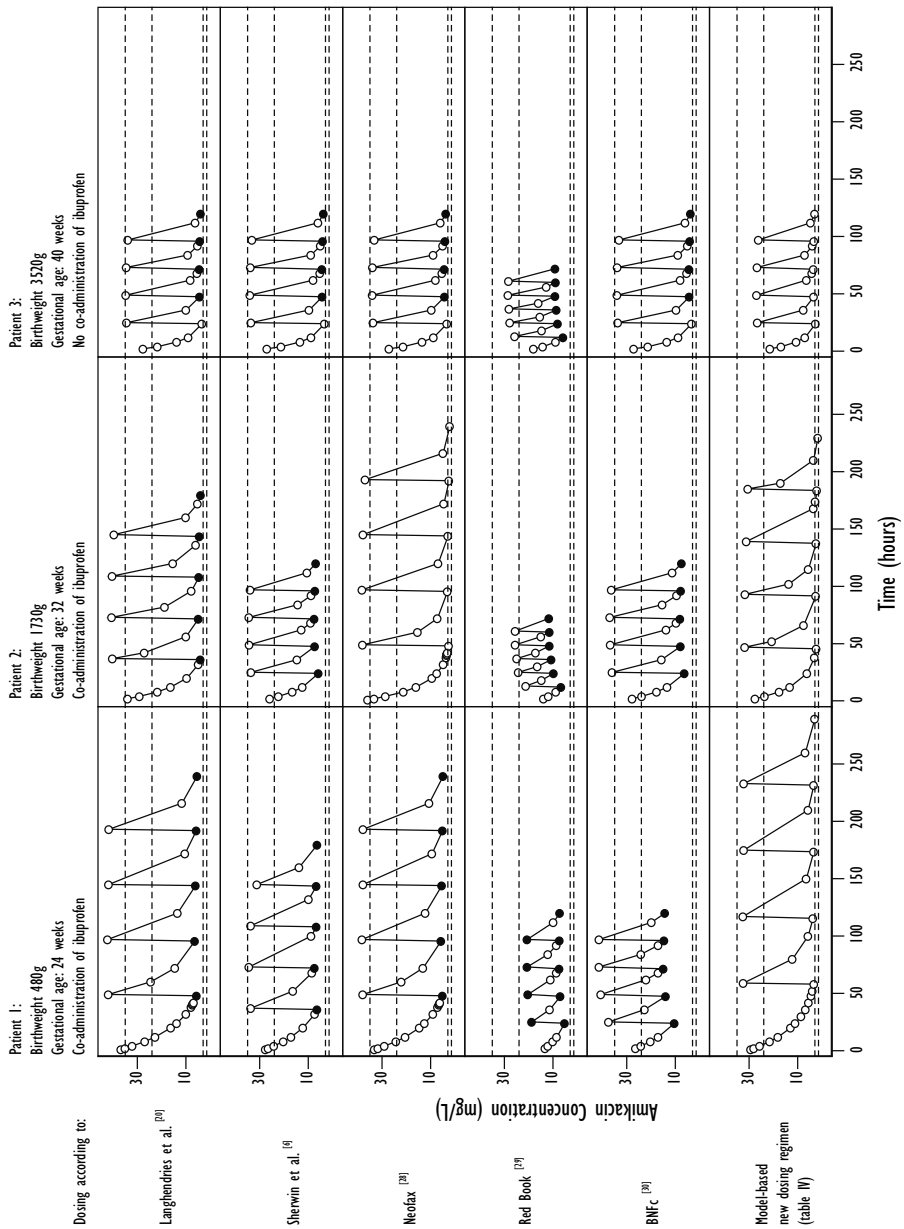

Figure 4: Model-based predicted concentration-time profiles for three individuals using five different dosing guidelines (Langhendries *et al.* [20], Sherwin *et al.* [6], Neofax® [28], Red Book® [29] and British National Formulary for children (BNFc) [30]) (table III) and according to the model-based new dosing regimen (table IV). GA = gestational age, BWb = birth weight. The dotted lines indicate the target peak (24-35 mg/L) and trough (1.5-3 mg/L) amikacin concentrations aimed for. Peak concentrations below and trough concentration above the target range are indicated by a black dot.

cosides are often administered in association with beta-lactam antibiotics. Although it is well known that aminoglycosides exhibit a postantibiotic effect, the acceptable duration between two administrations in terms of this postantibiotic effect remains still unclear. Future studies are needed to develop rational dosing schemes for the antibiotics given in association with amikacin based on the new dosing regimen.

Finally creatinine concentrations could not be identified as a significant covariate in this study. To a certain extent, this was anticipated since creatinemia in the first 3 days of postnatal life reflects maternal renal function. In addition, creatinemia trends throughout neonatal life display an initial progressive increase with peak concentration in the second part of the first week of life due to passive back leaking of creatinine through the renal tubular cells, with a subsequent decrease throughout neonatal life [15, 16].

3.5. Conclusions

Amikacin clearance in neonates can be predicted by combination of the morphometric surrogates for organ function: birth weight representing the antenatal state of maturation of the kidney, postnatal age representing postnatal maturation and co-administration of ibuprofen. Postmenstrual age proved to be less predictive compared to the contribution of birth weight and postnatal age together. This study shows notably that the dosing regimens for amikacin suggested in reference handbooks for both preterm and term neonates up to 30 days need to be updated. Finally the model reflects maturation of GFR allowing for adjustments of dosing regimens of other renally cleared drugs.

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Chapter 4

Prospective Validation of a Model-based Dosing Regimen for Amikacin in Preterm and Term Neonates

Roosmarijn F.W. De Cock*, Anne Smits*, Karel Allegaert, Sophie Vanhaesebrouck, Meindert Danhof, Catherijne A.J. Knibbe

* Both authors contributed equally to the manuscript

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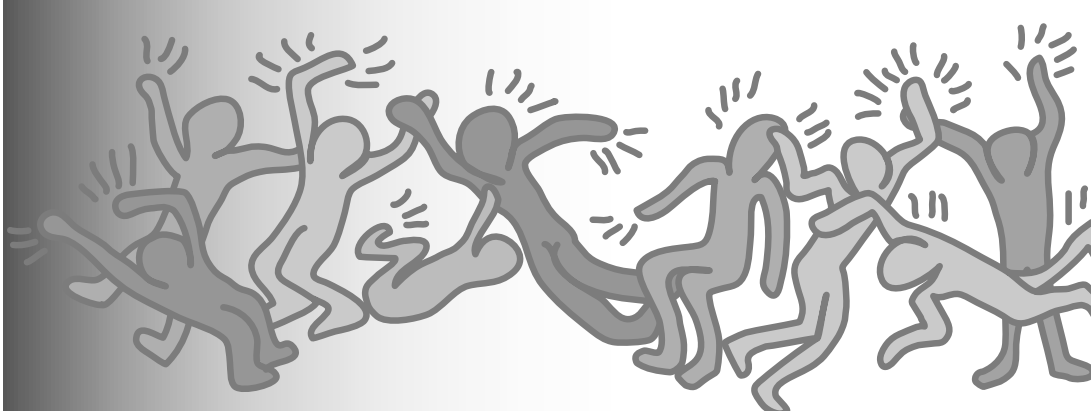

Abstract

Introduction

The dosing regimens for amikacin in neonates in reference handbooks may potentially lead to lack of efficacy and/or toxic effects. Therefore, a novel dosing regimen with bodyweight, postnatal age and co-administration of ibuprofen as covariates was proposed based on a recently derived population pharmacokinetic model. The aim of the current study was to prospectively evaluate the accuracy and precision of this novel model-based dosing regimen for amikacin in preterm and term neonates aged between 1-30 days.

Methods

The model-based dosing algorithm for amikacin was prospectively evaluated in 579 (pre)term neonates (median birth bodyweight 2285g (range 420-4850g), postnatal age 2 days (range 1-30 days), gestational age 34 (range 24-41 weeks)). Observed peak and trough concentrations (n=1195), obtained upon application of the novel dosing algorithm in these 579 individuals, were compared with the concentrations predicted by the model. Additionally, a NPDE (normalized prediction distribution error) was performed for all observed concentrations of the prospective dataset. Finally, Monte Carlo simulations were performed to evaluate amikacin exposure in (pre)term neonates of different bodyweight and age.

Results

Across the entire neonatal population, observed amikacin concentrations were accurately predicted by the final pharmacokinetic model without bias. Moreover the accuracy of the model was confirmed by the NPDE. Based on the Monte Carlo simulations, it was shown that peak concentrations above 24 mg/L were reached in almost all patients with different bodyweight, postnatal age and use of ibuprofen. Depending on bodyweight and age, trough concentrations below 3 mg/L were found for 78-100% of the individuals when ibuprofen was co-administered and for 45-96% of the individuals when ibuprofen was not co-administered.

Conclusion

A novel model-based dosing algorithm for amikacin leads to optimized peak and trough concentrations in preterm and term neonates with varying birth bodyweight,

current bodyweight, postnatal age and ibuprofen co-administration. The model-based approach for dosing drugs in the highly variable population of neonates, as applied here for amikacin, substantially contributes to the individualization of dosing drugs in neonates.

4.1. Introduction

Even to date, drugs in children are often used in an off-label or unlicensed manner [1, 2]. Moreover, despite profound differences between children and adults, most dosing regimens are derived from adult dosing regimens using linear extrapolations based on bodyweight, which may lead to under- or overdosing [3, 4]. This, in turn, may result in therapeutic failure or occurrence of adverse or even toxic effects. In order to establish rational and evidence-based dosing regimens, detailed information is needed on the variation in pharmacokinetics (PK) and pharmacodynamics (PD) of drugs in children [5]. However, the execution of PK-PD studies in children faces significant challenges resulting from ethical considerations and practical issues such as the limited number and volume of blood samples. These limitations can be partly overcome by the application of population PK and/or PD analysis techniques using non-linear mixed effect modeling [3, 4, 6]. The advantage of the population approach is that pharmacokinetic and pharmacodynamic parameters can be derived from dense data but also from sparse and unbalanced data, which is often the case when analyzing data from routine pediatric or neonatal clinical practice. Furthermore, by using this approach both the inter- and intra-individual variability can be estimated separately. Finally, specific predictors of variability also called covariates, can be identified which subsequently can be used as the basis to develop new evidence-based and individualized dosing regimens [4]. When applying this approach it is crucial to establish in a prospective clinical trial whether the proposed dosing regimen indeed leads to the expected concentrations and/or effects [5].

Although amikacin is commonly used in pediatric clinical practice, it has been shown (figure 1) that the currently used dosing regimens for amikacin in reference handbooks may potentially lead to lack of efficacy and/or toxic effects and need to be updated [7]. Therefore on the basis of a population pharmacokinetic model for amikacin which was recently developed, a novel model-based dosing regimen was developed in which the dose is individualized on the basis of current bodyweight (a covariate found for volume of distribution), birth bodyweight, postnatal age and co-administration of ibuprofen (covariates for clearance) [7]. As model-based dosing regimens in which identified covariates are used as a basis for dosing may result

in more effective and safe use of drugs in the pediatric population, the aim of the current study was to prospectively evaluate this novel model-based dosing regimen for amikacin in preterm and term neonates aged between 1-30 days, on the basis of a comparison of the observed *versus* the model-based predicted concentrations, an NPDE analysis and Monte Carlo simulations.

4.2. Methods

4.2.1. Patients

All neonates admitted to the Neonatal Intensive care unit of the University Hospitals Leuven from whom routine amikacin therapeutic drug monitoring (TDM) samples were available between July 2011 and December 2012 were considered for inclusion in this study. During this period a simplified version of the model-based dosing regimen [7], was applied (Table I). Patients were excluded from this analysis if initiation of amikacin treatment was based on a previously used dosing regimen, when data were missing or if patients had a postnatal age above 30 days.

4.2.2. Drug administration and TDM sampling

Amikacin (Amukin, Bristol Myers Squibb, Braine-L'Alleud, Belgium) was administered as an intravenous infusion over 20 minutes. As part of routine clinical care, blood samples for amikacin TDM were collected just before (trough sample) and 1 hour after administration of the second dose (peak sample).

4.2.3. Amikacin assay

Up to May 31th 2012, amikacin concentrations were measured with a fluorescence polarization immunoassay using an Abbott TDx kit (Abbott Laboratories, Diagnostics Division, Abbott Park, IL 60064 USA). The lower limit of quantification (LLOQ) was 0.8 mg/L. According to the insert, the coefficient of variation was < 5% (assessed at 5, 15 and 30 mg/L). From May 31th 2012, amikacin quantification occurred with an immunoassay based on a kinetic interaction of microparticles in solution (KIMS) on Roche/Hitachi Cobas c systems (Roche Diagnostics GmbH, Mannheim, Germany). Also in this assay, the LLOQ was 0.8 mg/L. According to the insert, the coefficient of variation was < 4%. To avoid censoring of data below the LLOQ, these concentrations were replaced by LLOQ/2 (i.e. 0.4 mg/L) as suggested in literature [8].

Table I: Simplified model-based dosing regimen used in the current study and original model-based dosing regimen [7] of amikacin for preterm and term neonates. The differences between both dosing regimens are highlighted in a grey field.

	Simplified model-based dosing regimen		Original model-based dosing regimen [7]	
Current bodyweight (g)	PNA <14 days	PNA ≥ 14 days	PNA <14 days	PNA ≥ 14 days
0-800	16 mg/kg/48h (Gr. 1)	20 mg/kg/42h (Gr. 2)	16 mg/kg/48h	20 mg/kg/42h
800-1200	16 mg/kg/42h (Gr. 3)	20 mg/kg/36h (Gr. 4)	16 mg/kg/42h	20 mg/kg/36h
1200-2000	15 mg/kg/36h (Gr. 5)	18 mg/kg/30h (Gr. 6)	15 mg/kg/36h	19 mg/kg/30h
2000-2800	15 mg/kg/30h (Gr. 7)	18 mg/kg/24h (Gr. 8)	13 mg/kg/30h	18 mg/kg/24h
≥ 2800	15 mg/kg/24h (Gr. 9)	18 mg/kg/20h (Gr. 10)	12 mg/kg/24h	17 mg/kg/20h

The dosing interval was prolonged 10 hours, when ibuprofen was co-administered or when asphyxia was diagnosed/ considered by the treating physician. Duration of the intravenous infusion was 20 minutes. PNA = postnatal age. Gr = dosing group.

4.2.4. Pharmacokinetic analysis

To evaluate the predictive performance of the recently developed pharmacokinetic model [7], a pharmacokinetic analysis was performed using NONMEM VI in which model-based individual and population predicted concentrations were simulated for each observation in the prospective dataset. These simulated individual and population predicted concentrations were obtained by use of the recently developed pharmacokinetic model [7] in which all parameters were fixed to the final values with MAXEVAL = 0 and without covariance step. Subsequently the individual and population predicted concentrations predicted by the population pharmacokinetic model were visually compared to the observed, measured concentrations. Additionally, to evaluate the accuracy, the recently developed pharmacokinetic model [7] was used to compute an NPDE (normalized prediction distribution error method) [9, 10] for each of the observations of the prospective dataset. A histogram of the NPDE distribution and scatterplots showing the NPDE *versus* time and *versus* predicted concentration were used as evaluation tools [9, 10]. Finally the parameters of the recently developed pharmacokinetic model [7] were re-estimated on the basis of the data of the prospective dataset.

4.2.5. Monte Carlo simulations

Monte Carlo simulations were performed to evaluate whether target peak (above 24 mg/L) [11] and trough concentrations (below 3.0 mg/L) [12] of amikacin in preterm and term neonates were attained following the simplified model-based dosing regimen (Table I), the original model-based dosing regimen (Table I) [7], the dosing regimen

proposed by Neofax® [13] and the dosing regimen proposed by the British National Formulary for Children (BNFc) [14]. For the peak concentrations, concentrations below 24 mg/L, between 24 – 35 mg/L [11] and above 35 mg/L were evaluated. For the trough concentrations, concentrations between 1.5 - 3.0 mg/L were evaluated because this was the primary target of the model based dosing regimen [7]. In addition, the percentage of trough concentrations below 1.5, between 3.0 – 5.0 and above 5.0 mg/L was evaluated, the latter because this concentration is associated with toxicity. The results of the Monte Carlo simulations were compared among the different neonatal dosing groups as defined in Table I.

For the Monte Carlo simulations, the covariates identified in this recently developed final pharmacokinetic model [7] - birth bodyweight and PNA (covariates found on clearance) and current bodyweight (covariate found on volume of distribution) - were sampled from the prospective dataset taking into account their correlation. The Monte Carlo simulations were performed twice in 5000 individuals following the model-based dosing regimen, one in which ibuprofen was not co-administered and one in which ibuprofen was co-administered because co-administration of ibuprofen was found to result in a 16% reduction in neonatal clearance [7]. For the simulations, 5 consecutive doses of amikacin were administered over 20 or 30 minutes depending on the dosing regimen (Table I).

4.3. Results

4.3.1. Patients

During July 2011 and December 2012 a total of 701 preterm and term neonates were evaluable for the pharmacokinetic analysis of this prospective evaluation of the model-based dosing regimen. In total a 122 patients were excluded from this analysis: 32 because the dosing regimen was based on a previous dosing regimen, 76 neonates because there were missing data and 14 patients because of a postnatal age above 30 days. A summary of the patient characteristics (N=579) evaluated in this study are presented in Table II together with the patient characteristics of the recently developed model for amikacin [7].

4.3.2. Pharmacokinetic analysis

Figure 2 shows the individual and population predicted concentrations *versus* concentrations observed in this prospective study for the different dosing groups based on current bodyweight and postnatal age as described in Table I. Both panels indicate

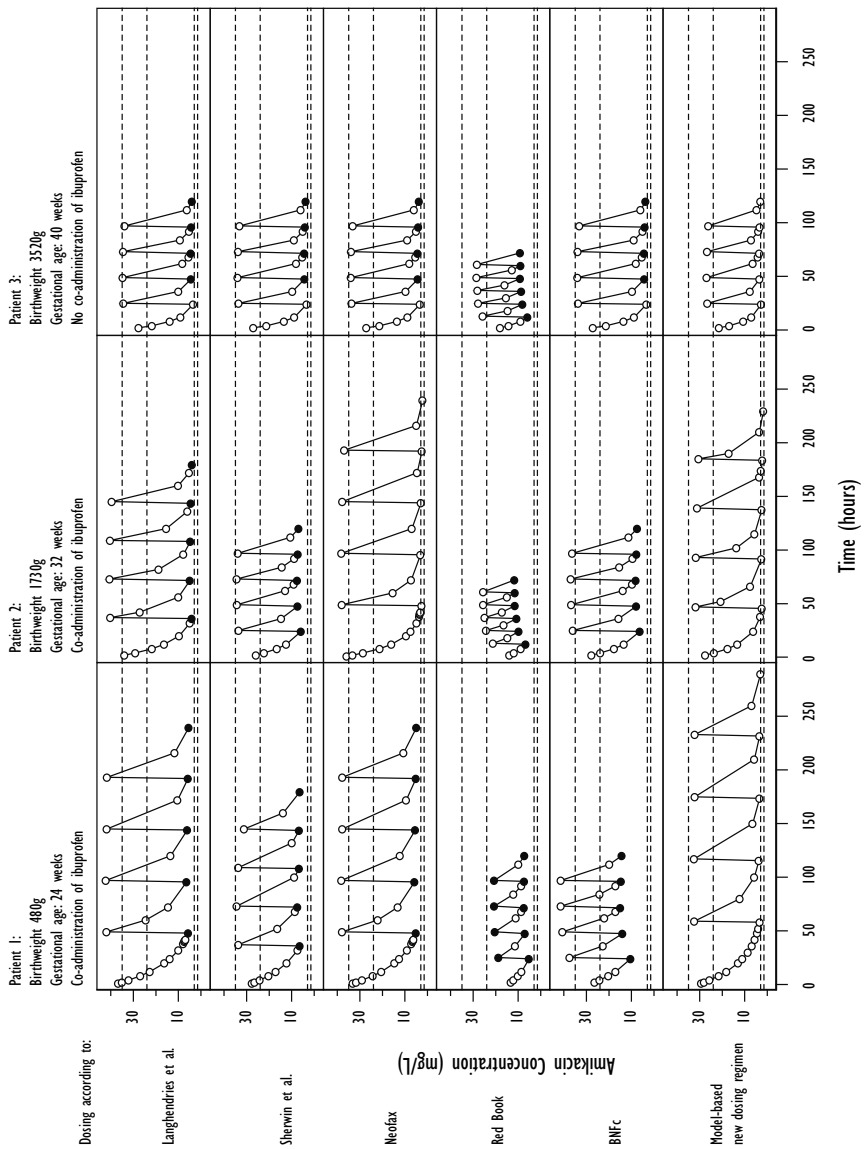

Figure 1: Model-based predicted concentration-time profiles for three typical neonates (480g, 1730g and 3520g) at postnatal age of 1 day on the basis of five different dosing guidelines (Langhendries *et al.*, Sherwin *et al.*, Neofax®, Red Book® and the British National Formulary for Children (BNFc)) and according to the new model-based dosing regimen.

The dotted lines indicate the target peak (24-35 mg/L) and trough (1.5-3 mg/L) amikacin concentrations aimed for. Peak concentrations below and trough concentration above the target range are indicated by a black dot.

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a lack of bias and an adequate prediction of the observed concentrations across the different bodyweight and age groups. Moreover, the distribution of the data points around the line of unity of the observed *versus* predicted plot indicates that the interindividual variability is both acceptable and similar across the entire neonatal population. This is also reflected in figure 3 in which the interindividual variability on clearance is plotted against birth bodyweight, postnatal age and co-administration of ibuprofen. Figure 3 illustrates that the final pharmacokinetic model of amikacin is able to describe the prospective dataset accurately across the different covariates as no trend is seen in the interindividual variability on clearance *versus* these covariates. This result was obtained such despite the fact that there were small differences between the dataset that was used to build the model [7] compared to the current prospective dataset (Table II). Table III gives an overview of the parameter estimates of the recently published final pharmacokinetic model [7] together with the parameter estimates obtained on the basis of the current prospective dataset in 579 individuals. Based on the values in Table III, it can be seen that fairly similar parameter values are obtained when evaluating the prospective dataset compared to the previously obtained parameters, which indicates the stability of the model. The population value for clearance and volume of distribution was slightly higher in the current prospective dataset (0.066 L/H and 1.03 L) compared to the values of the recently developed amikacin model (0.049 L/H and 0.833 L). This can be explained by the fact that in the current prospective dataset, slightly more mature neonates are included as reflected by the small differences seen in gestational age, postmenstrual age, birth bodyweight and current bodyweight (Table II). Figure 4 shows the results of the NPDE analysis. The histogram follows the normal distribution indicated by the black solid line. Additionally, no trend is seen in the NPDE *versus* time and the NPDE *versus* predicted concentrations indicating the accuracy of the model.

Table II: Clinical characteristics of the patients included in the recently published analysis on amikacin [7] and in the current prospective analysis (median, range, or absolute number and incidence).

Characteristics	Recently developed amikacin model [7] N= 874	Current prospective amikacin analysis N=579
Gestational age (weeks)	32 (24-43)	34 (24-41)
Postmenstrual age (weeks)	33 (24-43)	34 (24-45)
Postnatal age (days)	2 (1-30)	2 (1-30)
Birth bodyweight (g)	1750 (385-4650)	2285 (420-4850)
Current bodyweight (g)	1760 (385-4760)	2100 (420-5040)
Co-administration of ibuprofen (n (%))	118 (13.5)	29 (5)

Table III: Final parameter estimates and coefficients of variation (CV%) of the pharmacokinetic model that was recently developed on the basis of the original dataset (n=874) [7] and on the basis of the current prospective dataset (n=579)

Parameter	Recently developed amikacin model [7] n=879 patients	Current prospective amikacin analysis n=579 patients
Fixed effects		
CLp in CL = CLp x (bBW/median)^m x (1+ n x (PNA/median)) x o (ibuprofen)	0.049 (2.21)	0.066 (3.2)
m	1.34 (2.04)	1.30 (2.95)
n	0.213 (9.81)	0.302 (9.34)
o	0.838 (3.88)	0.846 (6.55)
Vp in V1 = Vp x (cBW/median)^p	0.833 (1.34)	1.03 (1.47)
p	0.919 (2.46)	0.863 (4.03)
Q = r x CL	0.415 (12.3)	0.480 (13.5)
V2=V1	V2=V1	V2=V1
Interindividual Variability		
ω^2 (CL)	0.0899 (14.9)	0.0921 (19.3)
Residual Variability		
σ^2 (proportional)	0.0614 (8.2)	0.0448 (22.3)
σ^2 (additive)	0.267 (27.2)	0.315 (16.3)

CLp = population value for clearance (L/h), Vp = population value for volume of distribution of the central compartment (L), bBW = bodyweight at birth (g), cBW = current bodyweight (g), PNA = postnatal age (days), Q = intercompartmental clearance (L/h), V2 = Volume of distribution of the peripheral compartment (L), median values for the recently developed model for amikacin: bBW = 1750g, PNA = 2 days, cBW=1760g; median values for the current prospective study: bBW = 2285g, PNA = 2 days, cBW = 2100g

4.3.3. Monte Carlo simulations

Monte Carlo simulations were performed to illustrate the exposure to amikacin in two times 5000 preterm and term neonates (with and without ibuprofen administration) following the simplified model-based dosing regimen, the original model-based dosing regimen [7], the dosing regimen proposed by Neofax®[13] and the British National Formulary for Children [14]. In Table IV the percentages of the individuals with trough concentrations after 5 doses below 1.5 mg/L, between 1.5-3.0 mg/L, between 3.0-5.0 mg/L and above 5.0 mg/L and peak concentrations after 5 doses below 24 mg/L, between 24-35 mg/L and above 35 mg/L following the Monte Carlo simulations in 5000 individuals according to the different dosing regimens

are given when ibuprofen is not co-administered. In Table V, these percentages are shown when ibuprofen is co-administered. Both tables are graphically presented in figure 5 and figure 6 in which ibuprofen is not co-administered and co-administered, respectively. In both figures the upper panels (A) represent the trough concentrations while the lower panels (B) represent the peak concentrations. Based on these tables and figures, it can be seen that for the model based dosing regimens (with and without ibuprofen) the percentages for trough concentrations between 1.5 and 3.0 mg/L are relatively constant across the ten different age and weight groups. This confirms the predictions that were performed in the previous analysis [7] as the model based dosing guideline was designed to aim for trough concentrations between 1.5 and 3.0 mg/L across the entire neonatal age range. Overall, the figures and tables show that trough concentrations below 3.0 mg/L [12] are reached in most individuals of the different dosing groups upon the simplified model-based (78-100% and 45-96%) and original model-based dosing regimen (86-100% and 62-92%) whereas these percentages were much lower upon Neofax® (25-96% and 40-100%) and BNFc (2-100% and 3-100%), when ibuprofen is co-administered or not, respectively. From these results, it seems that particularly the dosing guidelines suggested by BNFc may potentially induce toxicity since target trough values below 5 mg/L are not reached in many individuals and specifically in neonates with a postnatal age < 14 days. More specifically, trough concentrations above 5 mg/L which are associated with oto- and nephrotoxicity, were observed in 0-20%, 0-9%, 0-43% and 0-93% of the individuals of the different dosing groups as defined in Table I, for the simplified dosing regimen, original dosing regimen [7], Neofax and BNFc, respectively (Table IV and V). Considering the peak concentrations, it can be seen that for the simplified model-based dosing regimen peak concentrations above 24mg/L are reached in almost all individuals while for the original model-based dosing regimen [7], Neofax® and BNFc target peak concentrations are not reached in all individuals of one or more dosing subgroups. Finally, it can be seen that higher peak concentrations are reached in the different dosing groups upon the dosing regimens of Neofax® or the BNFc compared to the model-based dosing regimens [7].

4.4. Discussion

Recently a population pharmacokinetic model was developed for amikacin in 874 preterm and term neonates [7]. Based on the final model which was both internally and externally validated, a model-based dosing regimen was developed for amikacin in preterm and term neonates aged between 1-30 days [7]. The main aim of this dosing regimen was to obtain trough concentrations of 1.5-3.0 mg/L and peak

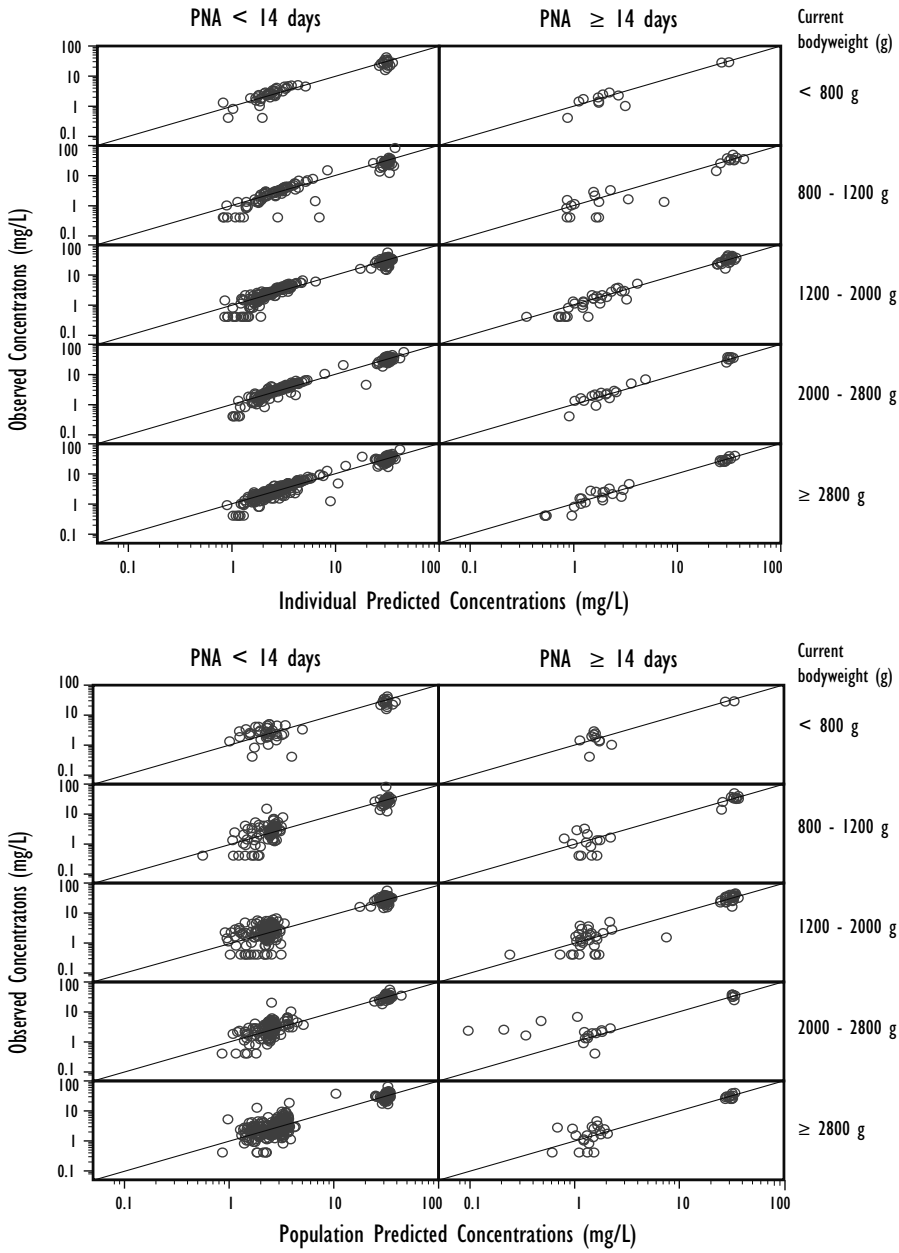

Figure 2: Observed *versus* model-based individual and population predicted concentrations for the different dosing groups based on current bodyweight and postnatal age (Table I) for the current prospective dataset.

concentrations above 24 mg/L across the entire neonatal population for which ten dosing groups were defined [7] (Table I). To reach this aim, the model-based dosing regimen is based on current bodyweight, a covariate found on volume of distribution which determines the peak concentrations, postnatal age and co-administration of ibuprofen, covariates found on clearance which determine the maintenance dose and the dosing interval, respectively (Table I). In order to evaluate whether the model-based dosing guideline for amikacin indeed leads to the expected concentrations, a prospective clinical trial is imperative [4, 5]. In the current prospective clinical study we first evaluated the predictive value of the previously derived model [7] for observed concentrations in a newly collected dataset consisting of 579 preterm and term neonates (Table II) in which neonates were dosed on previously derived covariates (Table I). Furthermore, we evaluated the target attainment of the trough concentrations between 1.5 and 3.0 mg/L [12] and peak concentrations between 24 and 35 mg/L [11] across the entire preterm and term neonatal age range as targets of the model-based dosing algorithm in the original study [7]. For this purpose, Monte Carlo simulations were performed to simulate peak and through concentrations following the model-based dosing guideline and currently used guidelines to evaluate their performance across the different age groups that can be identified in neonates.

Based on the results presented in this analysis, it can be concluded that the final pharmacokinetic model is able to predict the observed concentrations in the current study without bias across the entire neonatal range (Figure 2). Moreover, in figure 3, it is shown that the covariates birth bodyweight, postnatal age and co-administration of ibuprofen are correctly implemented on clearance as no trend is seen when plotting the interindividual variability on clearance *versus* the different covariates. This means that the conclusion that 10 different neonatal dosing groups (Table I) are needed to cover the large differences seen in clearance values between preterm and term neonates is justified. Finally when evaluating figure 2 and figure 3, it can be concluded that only random variability is remained in the model while variability allocated to covariates is explained. This latter is of course of major importance when developing and evaluating new model-based dosing regimens on the basis of these covariates, which we have done in the current analysis.

Another important aim of the current prospective analysis was to evaluate whether the aimed target peak (24-35 mg/L) [11] and trough concentrations (1.5-3 mg/L) [12], as defined in the recently published study [7], were reached in preterm and term neonates. When evaluating the results of the Monte Carlo simulations (Table IV and V and Figure 5 and 6), peak and trough concentrations were found in the target range in most individuals of the different dosing groups in case of the model-based dosing regimens. Moreover, the number of individuals with trough con-

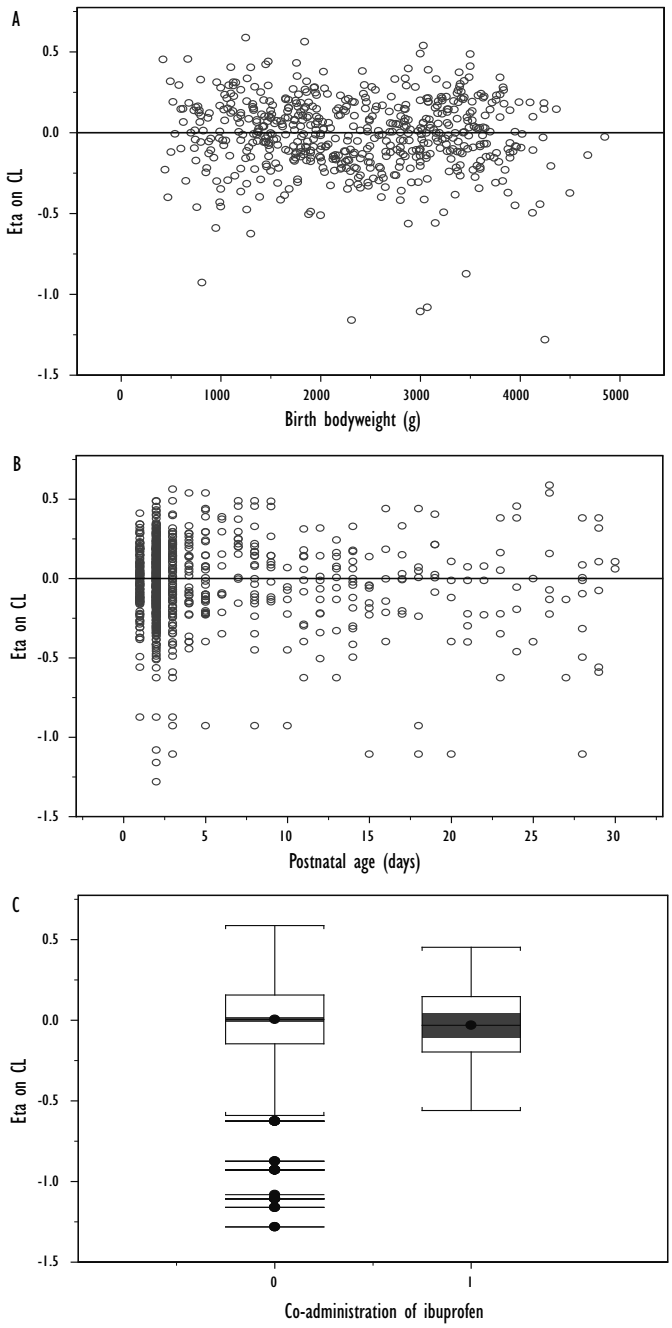

Figure 3: Interindividual variability (eta) on clearance (CL) *versus* birth bodyweight (a), postnatal age (b) en co-administration of ibuprofen (c) for the current prospective dataset using the recently developed pharmacokinetic model [7].

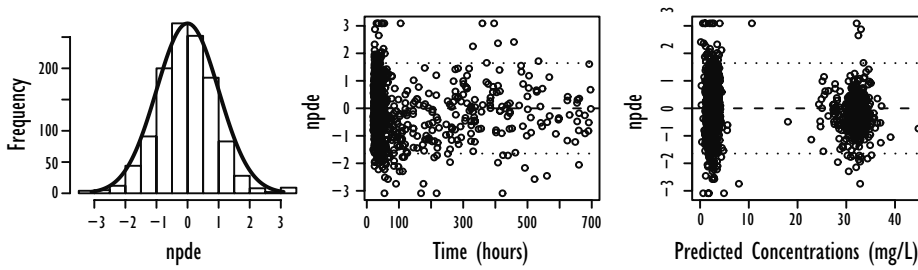

Figure 4: Results of the NPDE analysis performed for the prospective dataset using the recently developed pharmacokinetic model [7]. Left panel: Histograms of the NPDE distribution with the solid line representing a normal distribution as a reference, Middle panel: NPDE *versus* time (hours); Right panel: NPDE *versus* observed concentrations (mg/L).

centrations above 5 mg/L, which are generally related to occurence of oto- and/or nephrotoxicity, are notably lower in the model-based dosing regimens compared to the dosing regimen proposed by Neofax® and BNFc, in particular when ibuprofen is given (Table IV and V). However, 11% and 20% of the individuals of group 7 (current weight 2000-2800g, PNA<14 days) and group 9 (current weight >2800g, PNA<14 days) of the simplified model-based dosing regimen, respectively, had trough concentrations above 5 mg/L when ibuprofen was not co-administered. In addition, as shown in tables IV and V, a higher percentage of individuals with trough concentrations above 3 mg/L (38% and 55%) compared to the original model-based dosing regimen (28% and 38%) can be expected upon this simplification of the model-based algorithm. Although less neonates in group 7 and group 9 are observed with trough concentrations above 3 mg/L upon the original model-based dosing regimen [7], the original model-based dosing regimen does not offer an alternative since a notable number of patients (between 8-33%) does not reach peak concentrations above >24 mg/l needed for efficacy. This deviation may be partly explained by the fact that in the original study, less data of neonates were available in group 7 and 9 compared to the current prospective study. Consequently this indicates the importance of a prospective validation as more information can be obtained in certain age and weight groups. As a result, new Monte Carlo simulations need to be performed in both these groups to evaluate whether lower trough concentrations are obtained when the dosing interval is prolonged.

The Monte Carlo simulations emphasize that the dosing regimen proposed by Neofax® and BNFc for amikacin in preterm and term neonates may potentially lead to toxicity. This applies in particular to the dosing regimen of BNFc, which does not make any distinction between weight or age groups, but proposes a similar dose and

Table IV: The percentage of the individuals of the subgroups 1-10 as defined in Table I with trough concentrations after 5 amikacin doses below 1.5 mg/L, between 1.5-3 mg/L, between 3-5 mg/L and above 5 mg/L and peak concentrations (grey background) after 5 doses below 24 mg/L, between 24-35 mg/L and above 35 mg/L following the Monte Carlo simulations in 5000 individuals according to the different dosing regimens (simplified model-based dosing regimen, original model-based dosing regimen[7], Neofax® [13] and BNFc [14]) when ibuprofen was not co-administered.

Concentration (mg/L)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group10
Simplified model-based dosing regimen										
< 1.5	34%	55%	30%	65%	27%	58%	17%	53%	8%	58%
1.5-3	45%	30%	45%	30%	45%	38%	45%	36%	37%	36%
3-5	16%	15%	21%	5%	23%	4%	27%	11%	35%	6%
> 5	5%	0%	4%	0%	5%	0%	11%	0%	20%	0%
< 24	0%	0%	0%	0%	0%	0%	0%	3%	0%	0%
24-35	95%	50%	94%	71%	97%	83%	90%	97%	81%	94%
>35	5%	50%	6%	29%	3%	17%	10%	0%	19%	6%
Original model-based dosing regimen [7]										
< 1.5	33%	55%	29%	43%	25%	56%	25%	60%	16%	61%
1.5-3	41%	30%	48%	36%	47%	36%	47%	32%	46%	25%
3-5	21%	15%	18%	14%	22%	8%	23%	5%	29%	11%
> 5	5%	0%	5%	7%	6%	0%	5%	3%	9%	3%
< 24	0%	0%	0%	0%	0%	0%	9%	3%	24%	9%
24-35	96%	50%	93%	64%	97%	70%	91%	89%	76%	84%
>35	4%	50%	7%	36%	3%	30%	0%	8%	0%	7%
Neofax® [13]										
< 1.5	12%	50%	14%	71%	19%	64%	14%	56%	20%	84%
1.5-3	28%	35%	34%	19%	41%	33%	37%	33%	41%	16%
3-5	36%	15%	25%	10%	24%	3%	30%	11%	24%	0%
> 5	24%	0%	27%	0%	16%	0%	19%	0%	15%	0%
< 24	0%	0%	0%	10%	0%	0%	0%	14%	0%	30%
24-35	31%	100%	44%	90%	50%	100%	49%	86%	52%	70%
>35	69%	0%	56%	0%	50%	0%	51%	0%	48%	0%
BNFc [14]										
< 1.5	0%	0%	0%	31%	1%	50%	3%	79%	9%	76%
1.5-3	3%	50%	5%	38%	12%	40%	24%	18%	34%	24%
3-5	13%	25%	19%	31%	32%	10%	39%	3%	37%	0%
>5	84%	25%	76%	0%	55%	0%	34%	0%	20%	0%
< 24	0%	0%	0%	0%	0%	0%	0%	28%	0%	26%
24-35	43%	100%	48%	100%	63%	100%	73%	72%	78%	74%
>35	57%	0%	52%	0%	37%	0%	27%	0%	22%	0%

Table V: The percentage of the individuals of the subgroups 1-10 as defined in Table I with trough concentrations after 5 amikacin doses below 1.5 mg/L, between 1.5-3 mg/L, between 3-5 mg/L and above 5 mg/L and peak concentrations (grey background) after 5 doses below 24 mg/L, between 24-35 mg/L and above 35 mg/L following the Monte Carlo simulations in 5000 individuals according to the different dosing regimens (simplified model-based dosing regimen, original model-based dosing regimen[7], Neofax® [13] and BNFc [14]) when ibuprofen was co-administered.

Concentration (mg/L)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9	Group 10
Simplified model-based dosing regimen										
< 1.5	45%	60%	46%	72%	50%	72%	42%	81%	33%	82%
1.5-3	39%	40%	43%	21%	40%	28%	43%	14%	45%	14%
3-5	13%	0%	8%	7%	9%	0%	12%	5%	18%	4%
> 5	3%	0%	3%	0%	1%	0%	3%	0%	4%	0%
< 24	0%	0%	0%	0%	0%	0%	0%	0%	0%	2%
24-35	97%	50%	95%	50%	99%	85%	96%	92%	93%	80%
>35	3%	50%	5%	50%	1%	15%	4%	8%	7%	18%
Original model-based dosing regimen [7]										
< 1.5	48%	55%	50%	88%	47%	48%	48%	84%	49%	81%
1.5-3	38%	45%	38%	12%	42%	40%	42%	14%	41%	19%
3-5	11%	0%	11%	0%	9%	12%	9%	2%	9%	0%
> 5	3%	0%	1%	0%	2%	0%	1%	0%	1%	0%
< 24	0%	0%	0%	0%	0%	0%	8%	0%	33%	8%
24-35	99%	50%	97%	52%	98%	44%	92%	89%	67%	90%
>35	1%	50%	3%	48%	2%	56%	0%	11%	0%	2%
Neofax® [13]										
< 1.5	7%	35%	7%	52%	13%	37%	7%	44%	10%	63%
1.5-3	18%	40%	22%	38%	30%	48%	26%	33%	40%	33%
3-5	35%	25%	28%	5%	28%	15%	34%	20%	23%	4%
> 5	40%	0%	43%	5%	29%	0%	33%	3%	27%	0%
< 24	0%	0%	0%	10%	0%	0%	0%	5%	0%	12%
24-35	16%	100%	26%	90%	32%	100%	32%	95%	37%	88%
>35	84%	0%	74%	0%	68%	0%	68%	0%	63%	0%
BNFc [14]]										
< 1.5	0%	0%	0%	0%	0%	23%	1%	62%	4%	69%
1.5-3	0%	15%	2%	54%	4%	50%	11%	31%	21%	31%
3-5	7%	40%	9%	38%	20%	23%	32%	7%	37%	0%
>5	93%	45%	89%	8%	76%	4%	56%	0%	38%	0%
< 24	0%	0%	0%	0%	0%	0%	0%	10%	0%	7%
24-35	28%	85%	28%	100%	40%	100%	51%	90%	58%	93.%
>35	72%	15%	72%	0%	60%	0%	49%	0%	42%	0%

dosing interval for all neonates, independent of age or weight. As a result, neonates with a PNA<14 days are particularly at risk for toxic effects as the concentrations remain above the target trough concentrations, when ibuprofen is not given. When ibuprofen is given, even more individuals are at risk. Therefore on the basis of the results of this study, it can be concluded that the currently used dosing regimens in reference handbooks need to be updated, certainly when ibuprofen is co-administered. Moreover it should also be considered to lower the dose in neonates with a PNA<14 days, certainly in the lower weight groups (800-2000g). Considering the administration of ibuprofen, it should however be noted that in the Monte Carlo simulations ibuprofen is given to all weight groups, while in the recently developed amikacin model [7], ibuprofen was only given to preterm neonates in case of open ductus. Since ibuprofen can also be given to these neonates as anti-inflammatory drug, we chose to perform the Monte Carlo simulations also in term neonates when ibuprofen is co-administered to illustrate amikacin exposure. However this means that caution is needed when interpreting the results.

In this analysis we show that amikacin dosing in neonates can be optimized on the basis of a previously developed model resulting in a dosing algorithm that is evaluated in a prospective clinical study. However, when for each drug such an approach should be followed much time and resources would be needed. Therefore, advanced approaches to use information from one drug for another drug are needed [17]. Recently, it was shown by two different groups that the pharmacokinetic covariate model of amikacin contains system-specific information on the developmental changes in glomerular filtration in preterm and term neonates [15]. Consequently the covariate model on amikacin clearance with birth bodyweight, postnatal age and co-administration of ibuprofen as most important covariates could be used to predict the dosage regimens of other renally excreted drugs (netilmicin, tobramycin, gentamicin and vancomycin) in neonates [15, 16]. This semi-physiological approach may be used to optimize sparse data analysis and may facilitate development of pharmacokinetic models and evidence-based dosing regimens in the pediatric population [17]. As a result, in future analyses it should be evaluated whether the different dosing groups used in this prospective analysis, which are based on the recently developed model for amikacin [7], can be applied to the other renally excreted drugs. In this context, it should also be noted that the dosing regimen for amikacin in neonates according to BNFc [14], as illustrated in this analysis, is rather simple while the dosing regimen according to BNFc for gentamicin is much more complicated. Based on the fact that covariate models are considered to contain system-specific information, this is rather remarkable. Therefore, as stated above, the dosing regimens of other renally excreted drugs such as gentamicin, tobramycin, vancomycin and netilmicin in neonates may need to be revised accordingly.

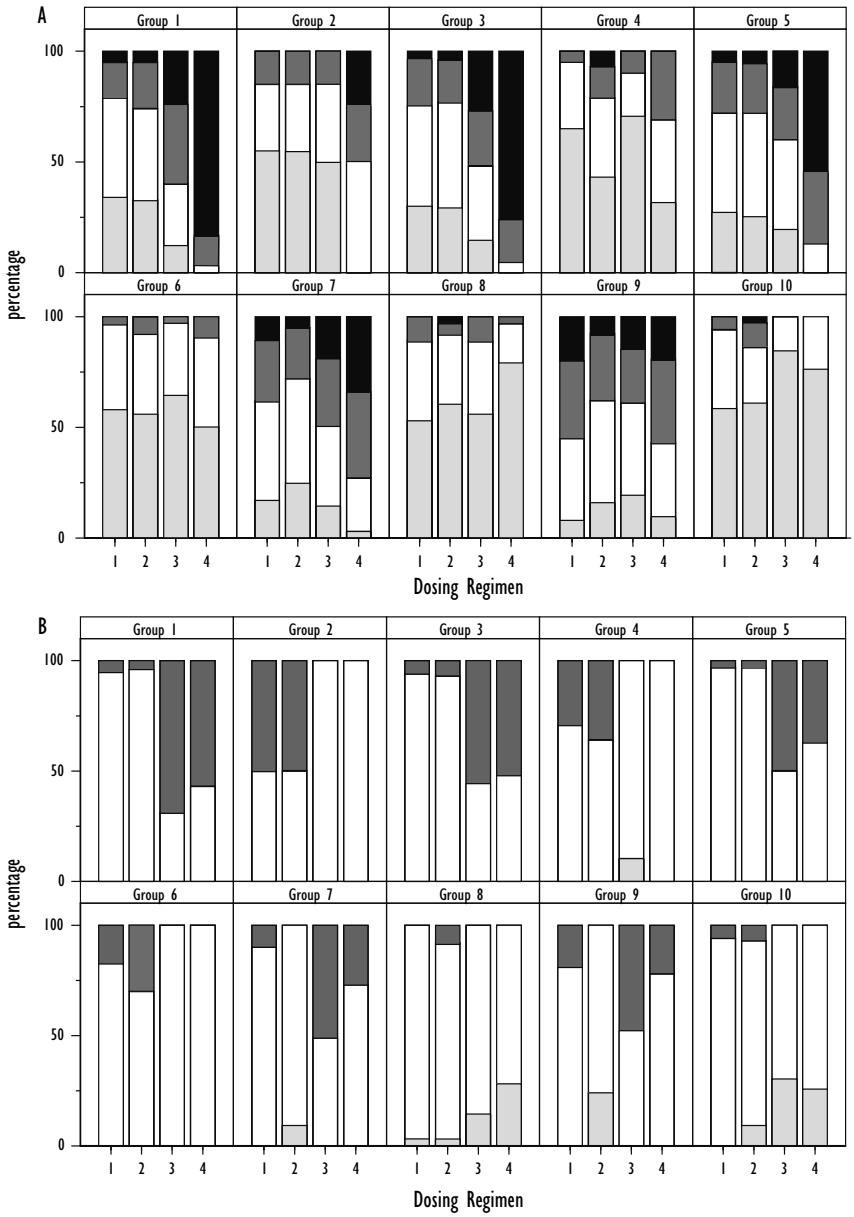

Figure 5: Bar graphs to illustrate the percentages of individuals of the different dosing groups as defined in Table I with A/ trough concentrations after 5 doses below 1.5 mg/L (light grey), between 1.5-3 mg/L (white), between 3-5 mg/L (dark grey) and above 5 mg/L (black) and B/ peak concentrations after 4 doses below 24 mg/L (light grey), between 24-35 mg/L (white) and above 35 mg/L (dark grey) following the Monte Carlo simulations in 5000 individuals according to the different dosing regimen (1= simplified model-based dosing regimen, 2 = original model-based dosing regimen [7], 3 = Neofax® [13], 4 = BNFc [14]) when ibuprofen was not co-administered.

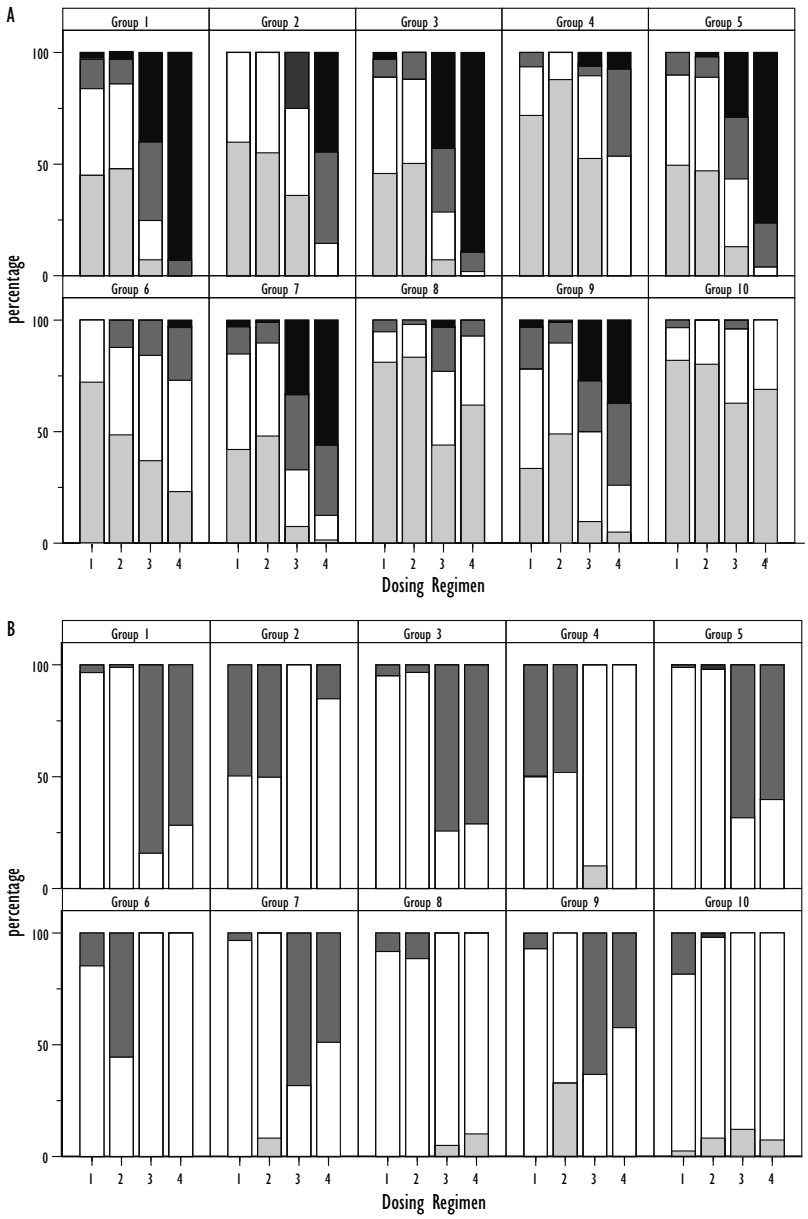

Figure 6: Bar graphs to illustrate the percentages of individuals of the different dosing groups as defined in Table I with A/ trough concentrations after 5 doses below 1.5 mg/L (light grey), between 1.5-3 mg/L (white), between 3-5 mg/L (dark grey) and above 5 mg/L (black) and B/ peak concentrations after 4 doses below 24 mg/L (light grey), between 24-35 mg/L (white) and above 35 mg/L (dark grey) following the Monte Carlo simulations in 5000 individuals according to the different dosing regimen (1= simplified model-based dosing regimen, 2 = original model-based dosing regimen [7], 3 = Neofax® [13], 4 = BNFc [14]) when ibuprofen was co-administered.

Finally it can be concluded that evidence-based dosing regimens are often lacking in the pediatric population due to practical and ethical constraints. Based on the analysis in this study, it can be determined that population pharmacokinetic modeling facilitates the development of drug dosing regimens in children. First of all, the use of this methodology makes it possible to derive pharmacokinetic and/or pharmacodynamic parameters from sparse and unbalanced data. Secondly covariates which explain the interindividual variability can be identified and serve as a guide for individualized dosing. Moreover the pharmacokinetic model used in this study was extensively validated before testing it in a prospective study. Based on this prospective study, it can be concluded that the model-based dosing regimen for amikacin with current bodyweight, postnatal age and co-administration of ibuprofen as covariates, results in target peak and trough concentrations in a very high percentage of individuals. Particularly the consistency of the predictions across the different weight categories are emphasized (figure 5 and 6) as across the different subgroups similar percentages in the target trough concentration range were found (Table IV and V). These predictions are in large contrast with predictions that were obtained upon currently used guidelines such as from Neofax® or BNFc for which the dosing regimens need to be reevaluated, particularly in neonates <14 days and the lower weight groups (800-2000g) or when ibuprofen is given. Consequently model-based dosing regimens in which developmental changes are taken into account may result in more effective and safe use of drugs in the pediatric population.

4.5. Conclusions

A novel model-based dosing algorithm for amikacin yields plasma concentrations that are close to the targeted concentrations in preterm and term neonates with varying birth bodyweight, current bodyweight, postnatal age and ibuprofen co-administration. The model-based approach for dosing drugs in the highly variable population of neonates, as applied here for amikacin, substantially contributes to the individualization of dosing drugs in neonates.

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Chapter 5

A Neonatal Amikacin Covariate Model Can Be Used to Predict Ontogeny of Other Drugs Eliminated through Glomerular Filtration in Neonates

Roosmarijn F.W. De Cock, Karel Allegaert, Catherine M.T. Sherwin, Elisabet I. Nielsen, Matthijs de Hoog, Johannes N. van den Anker, Meindert Danhof, Catherijne A.J. Knibbe

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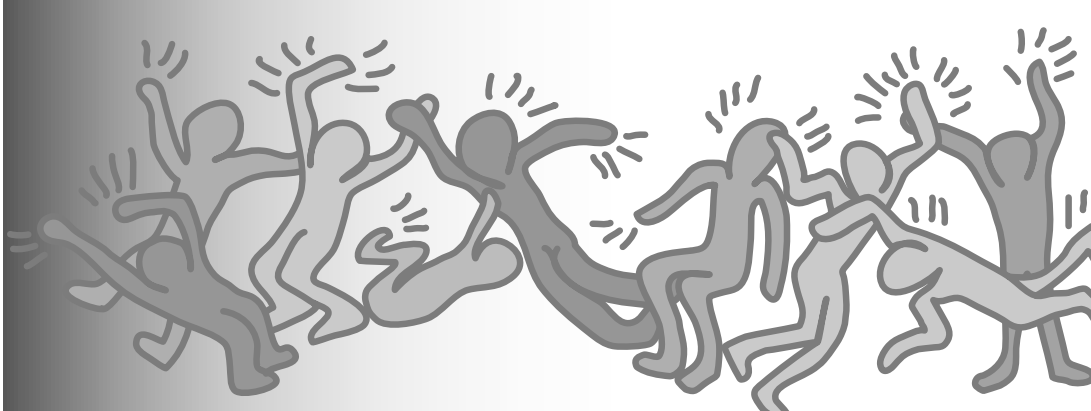


Abstract

Purpose

Recently, a covariate model characterizing developmental changes in clearance of amikacin in neonates has been developed using birth bodyweight and postnatal age. The aim of this study was to evaluate whether this covariate model can be used to predict maturation in clearance of other renally excreted drugs.

Methods

Five different neonatal datasets were available on netilmicin, vancomycin, tobramycin and gentamicin. The extensively validated covariate model for amikacin clearance was used to predict clearance of these drugs. In addition, independent reference models were developed based on a systematic covariate analysis.

Results

The descriptive and predictive properties of the models developed using the amikacin covariate model were good, and fairly similar to the independent reference models (goodness-of-fit plots, NPDE). Moreover, similar clearance values were obtained for both approaches. Finally, the same covariates as in the covariate model of amikacin, i.e. birth bodyweight and postnatal age, were identified on clearance in the independent reference models.

Conclusions

This study shows that pediatric covariate models may contain physiological information since information derived from one drug can be used to describe other drugs. This semi-physiological approach may be used to optimize sparse data analysis and to derive individualized dosing algorithms for drugs in children.

5.1. Introduction

Although regulations like the Pediatric Rule (FDA) and the Pediatric Regulation (EMA), encourage pharmaceutical companies to perform research in the pediatric age range when new drugs are developed, to date, drugs in pediatrics are often administered in an off-label or unlicensed manner [1-3]. Because of practical, ethical and economical reasons, it remains very challenging to perform pharmacokinetic and pharmacodynamic studies in the pediatric population with the ultimate aim to develop rational dosing regimens [4, 5]. One of the preferred approaches to facilitate the knowledge on the pharmacokinetics and pharmacodynamics in pediatrics is by applying population pharmacokinetic/pharmacodynamic (PK/PD) modeling [4, 6-8]. This approach is based on a simultaneous analysis of all data of the entire population while still taking into account that different observations are derived from different patients. Consequently, this population approach allows for the analysis of sparse and unbalanced data, which often applies to pediatric clinical studies. Moreover, the application of the population approach may lead, besides a reduction in invasiveness and burden for the patients, to considerably reduced costs.

However, to avoid that for each new or existing drug a systematic and time-consuming pharmacokinetic and/or pharmacodynamic analysis needs to be conducted [9, 10], new approaches are required. One approach, which is gaining more attention in industry, academia and regulatory agencies, is to develop evidence-based dosing regimens in children by PK/PD modeling and simulation in which extrapolations are performed between populations that vary in age [10-12] (bridging). Another recently proposed approach is the use of information obtained from one drug for extrapolation to other drugs that are eliminated through the same route [13]. This implicates that pediatric covariate models also contain biological system-specific information reflecting underlying physiological changes that can be used between drugs [13-15].

In a previous analysis, the developmental changes in amikacin clearance were characterized in more than 800 (pre)term neonates with varying gestational ages, birth bodyweights and postnatal ages, on the basis of birth bodyweight and postnatal age as covariates representing antenatal and postnatal maturation of the kidney, respectively [16]. The aim of this study was to evaluate whether this internally and externally validated covariate model of amikacin in (pre)term neonates contains system-specific information on the developmental changes in glomerular filtration and that therefore the covariate model can be extrapolated to other drugs eliminated through glomerular filtration. In this study the amikacin covariate model was primarily

extrapolated to netilmicin, tobramycin, vancomycin and gentamicin, drugs which were used as paradigm compounds as they are all almost entirely eliminated through GFR and with similar physicochemical properties compared to amikacin.

5.2. Methods

5.2.1. Patients and data

For this analysis, data of renally excreted antibiotics in neonates were obtained from 5 different (in part) previously published studies [17-21]. Since the amikacin covariate model [16] which was based on data from 874 neonates varying in postnatal age between 1-30 days, was used to describe the data of the other renally excreted drugs, only neonates with a postnatal age until 30 days were included from these datasets. Besides trough and peak samples taken before and at 1 hour after initiation of the dose, respectively, samples at varying time points were available in all datasets [17, 19-21], except for the tobramycin dataset [18]. An overview of the patient characteristics of the different datasets is given in table 1. The different datasets are discussed briefly here, while more details on the studies can be found in the original articles [17-21].

Amikacin [16]

A dataset of amikacin containing 2186 concentrations from 874 (pre)term neonates (birth bodyweight (bBW) 385-4650g, postnatal age (PNA) 1-30 days) was used to obtain the amikacin covariate model. Patients were enrolled in the study when at least one peak and trough concentration was available for each patient.

Netilmicin [17]

This dataset contained 267 netilmicin concentrations, collected in 88 (pre)term neonates (bBW 470-3000g, PNA 3-30 days). Concentrations were taken at the administration of the third dose or after a change in dose or dosing interval.

Tobramycin [18]

Four-hundred and seventy (pre)term neonates (bBW 485-5245g, PNA 1-4 days) were included in this dataset of which only paired peak and trough concentrations were available (taken after and before the fourth dose) resulting in 940 tobramycin concentrations.

Vancomycin [19]

This dataset contained 689 vancomycin concentrations collected in 273 preterm neonates (bBW 385-2550g, PNA 1-28 days). Concentrations were taken around the second or third dose infusion of vancomycin.

Gentamicin [20, 21]

For this drug two different datasets were available.

The first dataset (Gentamicin A), was obtained after combining previously published data [20] with more recently obtained data, resulting in a total of 1531 concentrations from 673 (pre)term neonates (bBW 440-5240g, PNA 1-30 days).

In the second dataset (Gentamicin B) [21], 796 gentamicin concentrations were available of 59 (pre)term neonates (bBW 520-4950g, PNA 1-30). In this study several concentrations taken at different time points (e.g. 15 min or 4-8h after the end of the infusion), besides peak and trough, were available.

5.2.2. Pharmacokinetic Modeling

Model development

Non-linear mixed effect modeling was used to analyze the pharmacokinetic data. The first-order conditional estimation method with interaction option was used in NONMEM 6.2. (ICON Development solutions, Hanover, MD, USA). The following tools were used to visualize and evaluate the model: S-Plus version 6.2.1 (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P Consultants BV, Leiden, The Netherlands), PsN and R (version 2.10.1). To test the hypothesis of between-drug extrapolation of covariate models, two different population pharmacokinetic models were developed for each dataset [14]: 1) Models using the amikacin covariate model [16] and 2) Independent reference models based on a systematic covariate analysis. More information on both approaches can be found below under Covariate model. Model development was performed in four different steps: (i) choice of the structural model, (ii) choice of the statistical sub-model, (iii) choice of the covariate model, (iv) model evaluation. Discrimination between models was based on different diagnostic tools [22]. A difference in objective function value (OFV) of 3.9 points or more was considered as statistically significant ($p<0.05$ based on X^2 distribution). Finally, the goodness-of-fit plots, the total number of parameters, visual improvement of individual plots, correlation matrix, confidence intervals of

Table I: Overview of the patient characteristics of the model developed for amikacin applied in the models using the amikacin covariate model and of the different datasets used in the current analysis as basis for the models using the amikacin covariate model and the independent reference models. Values are expressed as median (range).

	Amikacin dataset [16]	Datasets used in this analysis				
Dataset	Amikacin[16]	Netilmicin[17]	Tobramycin[18]	Vancomycin[19]	Gentamicin A[20]	Gentamicin B[21]
Number of patients	874	88	470	273	673	59
Gestational age (weeks)	32 (24-43)	28 (23-41)	32 (24-43)	29 (23-34)	34 (23-43)	29 (23-42)
Postmenstrual age (weeks)	33 (24-43)	30 (23-44)	32 (24-43)	30 (24-38)	36 (23-44)	30 (23-42)
Postnatal age (days)	2 (1-30)	15 (3-30)	2 (1-4)	14 (1-28)	3 (1-30)	6 (1-30)
Birth bodyweight (g)	1750 (385-4650)	1000 (470-3000)	1530 (485-5245)	1140 (385-2550)	2350 (440-5240)	1279 (520-4950)
Current bodyweight (g)	1760 (385-4760)	1115 (470-3592)	-	1170 (415-2630)	2550 (440-5420)	1009 (480-5315)
Co-administration of ibuprofen or indomethacin (n(%))	118 (13.5)	-	45 (9.6)	23 (8.4)	70 (10.4)	6 (10.2)

parameter estimates, ill-conditioning [23] and shrinkage [24] were assessed. The ill-conditioning was assessed by taking the ratio of the largest and smallest eigenvalue of the covariance matrix of the estimate from the NONMEM output.

Structural model

For the structural model, both one-, two and three-compartment models were tested. A two compartment model parameterized in terms of clearance (CL), inter-compartmental clearance (Q), volume of distribution of the central compartment (V1) and the peripheral compartment (V2) was found to best describe the different datasets for both the models using the amikacin covariate models as the reference models. Only for the reference model of tobramycin, a two compartment model could not be supported as only peak and trough samples were available. Therefore, a one compartment model was preferred for the tobramycin reference model. For some of the models no covariance step could be given or the bootstrap failed meaning that some of the models were possibly overparameterized. As a result these models were simplified by equalizing V2 to V1 or Q to CL or by estimating Q as a fraction of clearance. These assumptions did not influence the estimate of the parameters of primary focus (CL and V1) with changes in parameter estimates being less than 5%.

Statistical submodel

The interindividual variability was tested assuming a log-normal distribution in an individual i (*post hoc* value) and is given by the following equation:

$$\theta_i = \theta_{TV} \bullet e^{\eta i} \qquad \text{(Equation 1)}$$

in which θ_{TV} is the typical value of the parameter and η_i is assumed to be a random variable with mean value zero and variance ω^2. For the intra-individual variability and residual error (statistical submodel), proportional, additive and combination error models were tested. In this analysis, the interindividual variability was only estimated on clearance since the interindividual variability on the other parameters (V1, V2 and Q) could not be estimated and was therefore fixed to zero for all models. For the intra-individual variability and residual error a proportional error model (equation 2) was chosen for all the models:

$$Y_{ij} = C_{pred,ij} \bullet (1+\varepsilon_{ij}) \qquad \text{(Equation 2)}$$

where Y_{ij} is the *j*th observation in the *i*th individual, $C_{pred,ij}$ is the predicted concentration and ε_{ij} is a random variable from a normal distribution with a mean of zero and estimated variance of σ^2.

Covariate model

For each dataset two population pharmacokinetic models were developed as proposed in the analysis of Krekels *et al.* [14]

1/ Models using the amikacin covariate model [16]: In these models, the internally and externally validated covariate model for amikacin [16] (figure 1), was directly incorporated into the pharmacokinetic model that was developed for each dataset. This implicates that birth bodyweight was implemented as a covariate on clearance using a power function with an exponent of 1.34 as well as postnatal age using a linear function with a slope of 0.213. In the original covariate model of amikacin, co-administration of ibuprofen was identified as a third covariate on clearance, causing a 16.2% decrease in clearance of amikacin. This decrease in clearance was also implemented in the current analysis when ibuprofen or indomethacin was co-

administered. Although the decrease in glomerular filtration was reported to be more pronounced after the administration of indomethacin compared to ibuprofen [25], in this analysis the 16.2% decrease in clearance seen for ibuprofen was also applied for indomethacin.

Current bodyweight was implemented on volume of distribution using a power function with 0.919 as exponent. While the pediatric covariate model is considered to describe the developmental changes in clearance and volume of distribution, the population values of these parameters were still estimated by NONMEM since they are considered drug specific properties [14] (equation 3):

$$CL_i = CL_p \bullet \left(\frac{bBW}{bBW_{Median}}\right)^{1.34} \bullet \left(1+\left(0.213 \bullet \frac{PNA}{PNA_{Median}}\right)\right) \bullet 0.838_{ibuprofen}$$

Drug specific property

Amikacin covariate model

(Equation 3)

where CL_i represents the clearance in the ith individual, CL_p represents the population value of clearance and is estimated separately for each drug since it is considered to be a drug specific property, and the amikacin covariate model with birth bodyweight (bBW), postnatal age (PNA) and co-administration of ibuprofen is considered to describe the developmental changes in clearance through glomerular filtration.

2/ Independent reference models [14]: For these models a systematic covariate analysis [22] was performed in which the following covariates were tested for significance: birth bodyweight (weight at day of birth), current bodyweight (weight at day of blood sampling), gestational age, postmenstrual age, postnatal age, serum creatinine, co-administration of ibuprofen or indomethacin. Covariates were tested using a linear or power function. For serum creatinine, linear or power functions were tested in the denominator since a negative relationship was seen between serum creatinine concentrations and clearance. Previously, it has been shown that serum creatinine values in the first days of life are derived from the mother reflecting maternal renal function instead of neonatal renal function [26, 27]. Additionally, a progressive increase in serum creatinine concentrations has been reported with maximum serum creatinine concentrations at day 3-4 after birth followed by a subsequent decrease. This trend may be caused by differences in duration and extent of passive tubular back leak [28]. As a consequence, serum creatinine values in the first five days of life were not taken into account in this analysis.

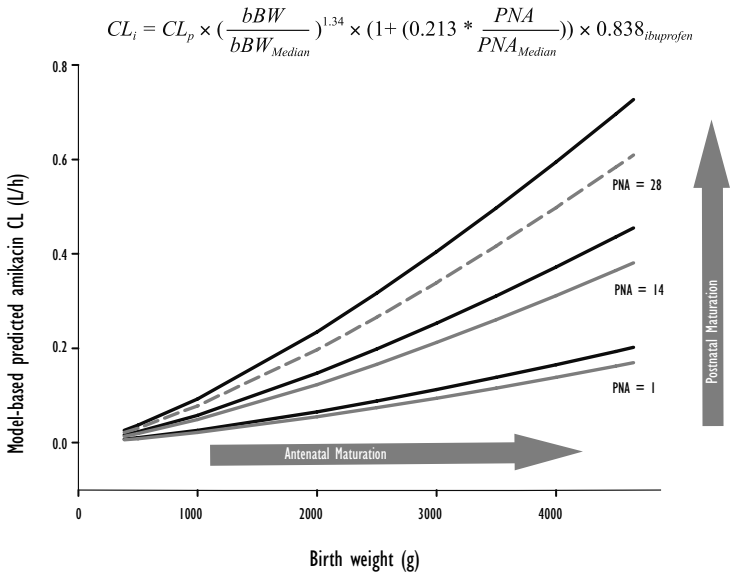


Figure 1: Covariate model of amikacin[16] which was applied to the other renally excreted drugs. The figure illustrates the model-based predicted amikacin clearance (CL) values *versus* birth bodyweight (bBW) for postnatal age of 0, 14 and 28 days with (grey) and without (black) co-administration of ibuprofen. Birth bodyweight reflects the antenatal maturation of the kidney, postnatal age is reflecting the postnatal maturation. Reproduced from [De Cock RF, Allegaert K, Schreuder MF, *et al.* Maturation of the glomerular filtration rate in neonates, as reflected by amikacin clearance. Clin Pharmacokinet 2012 Feb 1;51 (2): 105-17] with permission from Adis (© Springer International Publishing AG [2012]. All rights reserved.)

The significance of a covariate was statistically evaluated by the use of the objective function value. In the forward inclusion a p value <0.005 was considered as statistically significant while a more stringent p value <0.001 was used in the backward deletion. When two or more covariates were found to significantly improve the model, the covariate that reduces the objective function value the most was retained into the model and served as a basis for subsequent inclusion of additional covariates. In addition, the individual and population predicted parameters were plotted against the most predictive covariate to evaluate whether the individual predicted parameters were equally distributed around the population predicted parameters [22]. Finally the covariate model was evaluated as mentioned previously under Model development, whereby the results of the Model validation were also considered.

5.2.3. Model validation

The models using the amikacin covariate model as well as the independent reference models were internally validated using two different methods [22].

To evaluate parameter precision and stability a non stratified bootstrap analysis was performed in which 1000 replicate datasets of the same size as the original data analysis but with a different combination of individuals were generated. The parameter estimates obtained with the bootstrap were compared to the parameter estimates of the final models.

To evaluate the predictive properties of the models using the amikacin covariate model and reference models, the normalized prediction distribution error method (NPDE) was used, which is a Monte-Carlo simulation-based diagnostic in which the random effects were included [29, 30] The dataset was simulated 1000 times in NONMEM, each observed concentration was subsequently compared to the simulated reference distribution using the NPDE add-on package in R. A histogram of the NPDE distribution in the total dataset and plots of NPDE *versus* individual predicted concentrations and *versus* time were used to evaluate the final model.

5.2.4. Comparison of the models using the amikacin covariate model and independent reference models

The descriptive and predictive performance of the models using the amikacin covariate model and the independent reference models was compared by different diagnostic tools [14, 22]. The goodness-of-fit plots were compared to visually evaluate the descriptive performance. Secondly, individual and population clearance values obtained in the models using the amikacin covariate model were compared with the values obtained in the independent reference models [14]. To evaluate the difference in clearance values more closely between both models, the population clearance values were plotted for both approaches *versus* birth bodyweight for PNA 1, 14 and 28 days. Furthermore, the individual and population predicted parameters were plotted against the most predictive covariate for both approaches to evaluate whether the individual predicted parameters were equally distributed around the population parameters [22]. Additionally, the objective function values were evaluated as the models developed using both approaches are based on the same datasets. Finally, the results of the model validation (bootstrap analysis) as well as ill-conditioning and shrinkage were assessed. The predictive performance of the models using the amikacin covariate model and reference models was evaluated by comparison of the NPDE-results.

Table II: Final parameter estimates and their corresponding coefficients of variation (CV%) of the model developed for amikacin applied in the models using the amikacin covariate model and of the models derived in the current study using the amikacin covariate model for netilmicin, tobramycin, vancomycin, gentamicin dataset A and gentamicin dataset B.

Parameter	Amikacin[16]	Netilmicin[17]	Tobramycin[18]	Vancomycin[19]	Gentamicin A[20]	Gentamicin B[21]
Objective function value	7738.145	278.771	970.81	2763.631	1824.456	570.064
Fixed effects						
CLp in CL = CLp x (bBW/median)^{m} x (1+ n x (PNA/median)) x o (ibuprofen)	0.049 (2.21)	0.051 (5.22)	0.062 (2.06)	0.053 (2.74)	0.049 (1.47)	0.047 (3.12)
m	1.34 (2.04)	1.34	1.34	1.34	1.34	1.34
n	0.213 (9.81)	0.213	0.213	0.213	0.213	0.213
o	0.838 (3.88)	-	0.838	0.838	0.838	0.838
Vp in Vl = Vp x (cBW/median)^{p}	0.833 (1.34)	0.995 (7.64)	1.03 (1.42)	0.913 (2.69)	0.762 (1.9)	0.731 (3.35)
p	0.919 (2.46)	0.919	0.919	0.919	0.919	0.919
Q = r x CL	0.415 (12.3)	-	-	0.904 (10.4)	-	1.47 (14.3)
Q=CL	-	Q=CL	Q=CL	-	Q=CL	-
V2=Vl	V2=Vl	V2=Vl	V2=Vl	V2=Vl	V2=Vl	V2=Vl
Interindividual Variability						
ω^2 (CL)	0.0899 (14.9)	0.186 (32.4)	0.15 (10.7)	0.11 (12.1)	0.106 (13.4)	0.0536 (21.8)
Residual Variability						
σ^2 (proportional)	0.0614 (8.2)	0.117 (18.2)	0.044 (9.62)	0.095 (7.89)	0.0804 (9.37)	0.0483 (12.1)
σ^2 (additive)	0.267 (27.2)	-	-	-	-	-

CLp = population value for clearance, Vp = population value for volume of distribution of the central compartment, bBW = bodyweight at birth, cBW = current bodyweight, PNA = postnatal age, Q = intercompartmental clearance, V2 = Volume of distribution of the peripheral compartment

5.3. Results

5.3.1. Pharmacokinetic Modeling

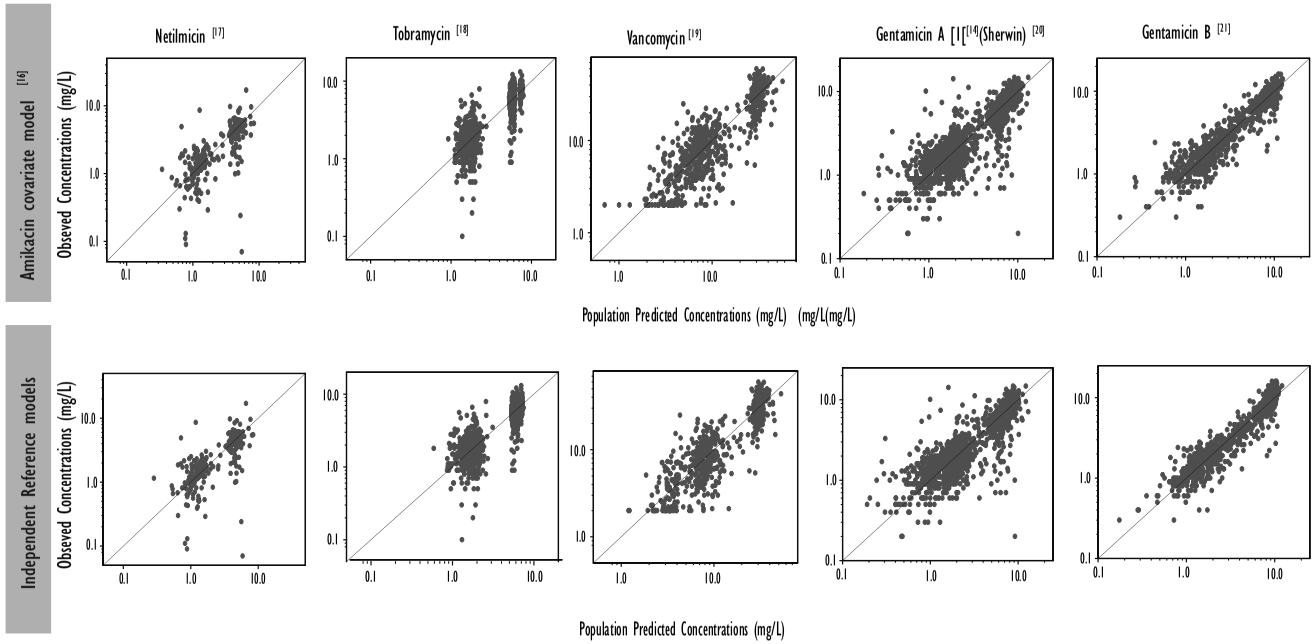
1/ Models using the amikacin covariate model [16]:

In these models, the previously published amikacin covariate model (Figure 1) was directly incorporated in the pharmacokinetic models of the different drugs. The parameter estimates obtained for the models using the amikacin covariate model are shown in table II together with the parameter estimates obtained in the final model for amikacin [16]. As illustrated in figure 2 (top panels), the models using the amikacin covariate model described the observed concentrations without bias. The individual *post hoc* clearances and population predicted clearances *versus* the most predictive covariate (birth bodyweight) are given in figure 3, showing that the population predicted clearance values are describing the individual *post hoc* clearances without bias. Furthermore, the results of the NPDE analysis in figure 4 show that the models can predict the median concentrations in the different datasets accurately. Finally, no trend was seen in the plots of the NPDE *versus* time and predicted concentrations (figure 4).

2/ Independent reference models:

In the independent reference models of netilmicin, vancomycin and gentamicin datasets A and B, birth bodyweight and postnatal age were identified as the most important covariates to describe clearance. Current bodyweight was found as most important covariate to describe volume of distribution. Birth bodyweight and current bodyweight were implemented on clearance and volume of distribution of the central compartment, respectively, using a power function. Postnatal age was implemented using a power function (netilmicin, gentamicin A and gentamicin B datasets) or linear function (vancomycin dataset) depending on the dataset. For tobramycin, birth bodyweight was implemented on both clearance and volume of distribution using a power function. Based on the statistical criteria, postnatal age was not identified as a covariate on clearance. This may be explained by the fact that data of tobramycin were only available for the first four days after birth. In figure 2 (bottom panels) the observed *versus* population predicted concentrations are illustrated for the independent reference models. In table III, the different parameter estimates are given for the reference models of the 5 different neonatal datasets. In the various independent reference models, serum creatinine was not found as a covariate to describe clearance. Furthermore when plotting the individual

Figure 2: Observed *versus* population predicted concentrations for the models using the amikacin covariate model (top) and the independent reference models (bottom) of netilmicin, vancomycin, tobramycin, gentamicin A and gentamicin B.



and population predicted clearance values *versus* birth bodyweight, it was seen that the individual *post hoc* clearances were randomly scattered around the population predicted clearances (figures not shown). Finally the results of the NPDE analyzes showed that the independent reference models were able to adequately predict the median concentrations of the different datasets (figures not shown).

5.3.2. Comparison of the models using the amikacin covariate model and independent reference models

In figure 2, observed *versus* population predicted concentrations are shown for the models using the amikacin covariate model as well as the independent reference models. Visual examination of the plots shows that both the models using the amikacin covariate model as well as the independent reference models are able to predict the observed concentrations and that the difference in performance of the two approaches is negligible. In figure 5 the individual and population clearance values for the models using the amikacin covariate model are plotted *versus* those of the independent reference models of the different datasets. While both approaches estimate similar individual and population clearance values for netilmicin, tobramycin, gentamicin A and gentamicin B, a slight difference in population clearance values is seen for vancomycin, a drug with slightly different physicochemical and pharmacokinetic drug properties compared to amikacin and the other drugs. In figure 6, the population clearance values obtained using both approaches are plotted *versus* birth bodyweight for PNA 1, 14 and 28 days. To obtain the clearance values for the models using the amikacin covariate model, the full study range of the amikacin dataset was used while for the independent reference models, the study range available for that particular dataset was applied, explaining the differences seen in the length of both lines illustrating the population clearance values using both approaches. Based on this figure, it was concluded that at day of birth (day 1) and 14 days similar clearance values are obtained for both approaches for all drugs, while at day 28, a slight difference is seen for vancomycin and gentamicin B. For tobramycin it should be noted that no population clearance values are illustrated following the independent reference model for day 14 and 28 since this model is based on the original dataset which only included data during the first four days after birth. When plotting the individual and population predicted parameters against the most predictive covariate for both approaches, it was observed that the individual predicted parameters were equally distributed around the population parameters. Finally, when considering the differences in objective function values between the models using the amikacin covariate model and the reference models (table II and table III), it was seen that the reference models of netilmicin, vancomycin, and gentamicin A and B had a lower objective function value (Δ objective function value: netilmicin 5 points, vancomycin

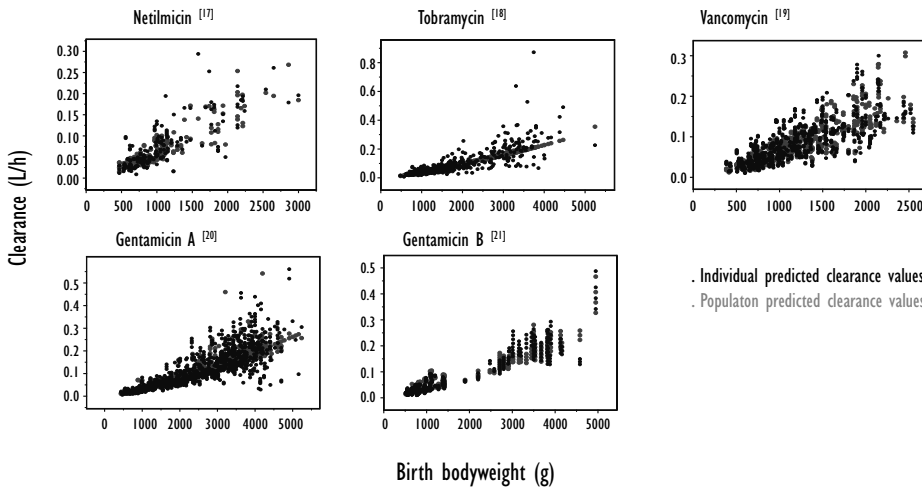


Figure 3: Individual *post hoc* (black) and population predicted (grey) clearance values (l/h) *versus* the most predictive covariate, birth bodyweight (g), for the models using the amikacin covariate model.

23 points, gentamicin A 67 points, gentamicin B 59 points) as compared to the models using the amikacin covariate model. For tobramycin, the objective function value of the reference model was 43 points higher compared to the model using the amikacin covariate model, which can be explained by the use of a one compartment reference model *versus* a two compartment model using the amikacin covariate model. Furthermore, table II and table III show that the coefficients of variation of both fixed and random effects are well below 50% indicating that both approaches are able to estimate the parameters with high precision. Moreover, no ill-conditioning was detected in the models using both approaches since the condition number of the final pharmacokinetic models (range 2.23-64.44) was far below the critical value of 1000. Finally, η-shrinkage expressed as a percentage was identified to be below 20% for all final pharmacokinetic models using both approaches.

Results of the bootstrap analysis showed that the median estimated values based on re-sampled data were close (<20%) to the estimated values of the final models using the amikacin covariate model and independent reference models. This suggests that the final models using the amikacin covariate model and the independent reference models are stable and that the estimated parameter values are precise.

Considering the predictive performance, both the models using the amikacin covariate model as well as the independent reference models perform similar since both approaches can accurately predict the overall median concentrations.

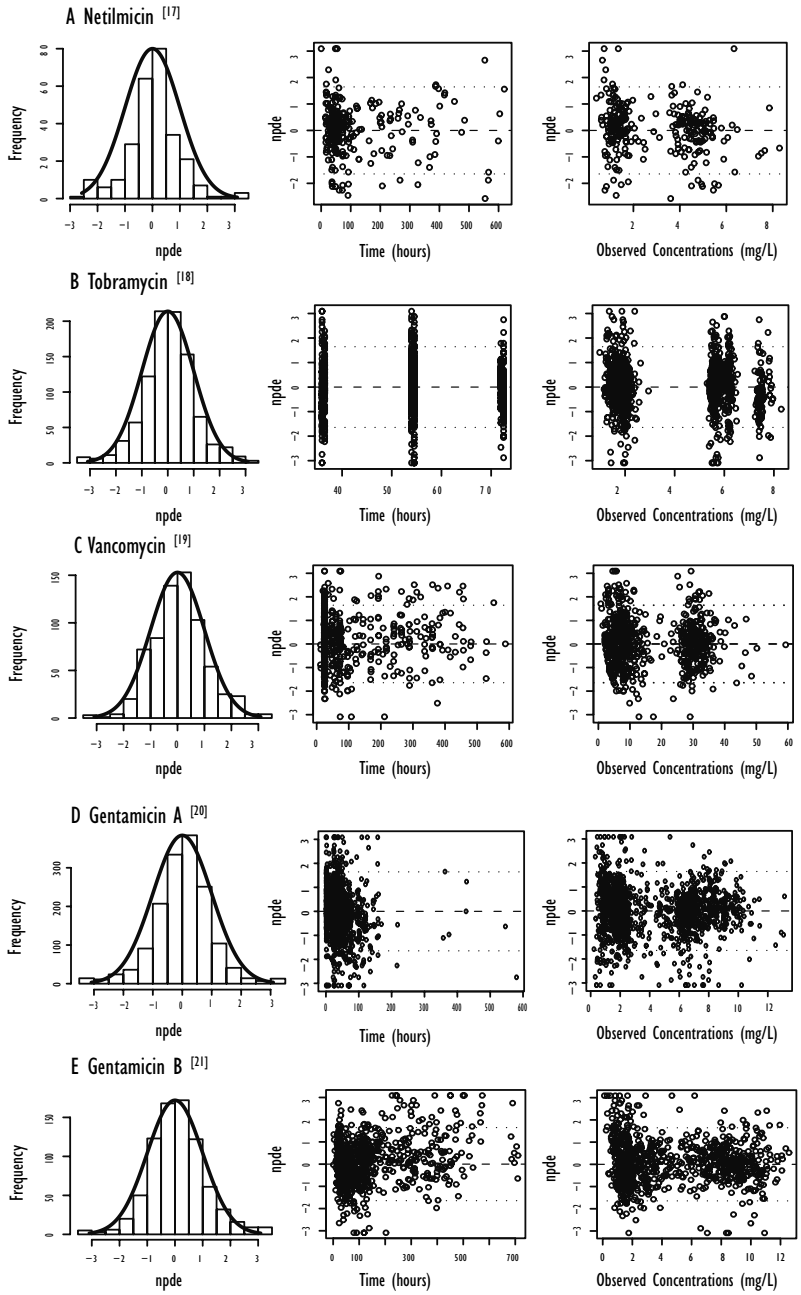


Figure 4: Results of the NPDE analysis for the models using the amikacin covariate model of a) netilmicin, b) tobramycin, c) vancomycin, d) gentamicin A and e) gentamicin B. Left panel: Histograms of the NPDE distribution with the solid line representing a normal distribution as a reference, Middle panel: NPDE *versus* time (hours); Right panel: NPDE *versus* observed concentrations (mg/L).

In addition, there was no bias in the normalized prediction distribution errors over time, nor over the predicted concentration range.

Table III: Final parameter estimates and their corresponding coefficients of variation (CV%) of the independent reference models derived in the current study for netilmicin, tobramycin, vancomycin, gentamicin dataset A and gentamicin dataset B.

Parameter	Netilmicin[17]	Tobramycin[18]	Vancomycin[19]	Gentamicin A[20]	Gentamicin B[21]
Objective function value	273.659	1013.684	2740.457	1757.241	511.263
Fixed effects					
CLp in CL = CLp x (bBW/median)^m x (1+ n x (PNA/median))	-	0.067 (1.43)	0.038 (9.24)	-	-
m	-	1.31 (2.43)	1.1 (5.72)	-	-
n	-	-	0.955 (19.7)	-	-
CLp in CL = CLp x (bBW/median)^o x (PNA/median)^p	0.063 (5.92)	-	-	0.097 (1.5)	0.046 (4.04)
o	1.44 (7.57)	-	-	1.36 (2.12)	1.41 (4.42)
p	0.481 (18.5)	-	-	0.458 (8.78)	0.371 (8.36)
Vp in Vl = Vp x (cBW/median)^q	0.65 (7.14)	0.926 (1.45)	0.618 (2.85)	1.07 (2.3)	0.508 (1.74))
q	1 (13.4)	0.859 (3.41)	0.952 (8.21)	0.807 (5.29)	0.848 (3.27))
Q = r x CL	-	-	-	-	0.688 (12.1)
Q = CL	Q=CL	-	Q=CL	Q=CL	-
V2 = s	-	-	-	-	0.846 (25.9)
V2 = Vl	V2=Vl	-	V2=Vl	V2=Vl	-
Interindividual Variability					
ω^2 (CL)	0.188 (32.1)	0.065(10.4)	0.103 (12.5)	0.102 (13.4)	0.0357 (22.1)
Residual Variability					
σ^2 (proportional)	0.114 (17.9)	0.0439 (9.16)	0.0938 (7.64)	0.0776 (9.27)	0.0465 (8.65)

CLp = population value for clearance, Vp = population value for volume of distribution of the central compartment, bBW = bodyweight at birth, cBW = current bodyweight, PNA = postnatal age, Q = intercompartmental clearance, V2 = Volume of distribution of the peripheral compartment

5.4. Discussion

To facilitate the development and availability of drugs in children and to avoid the development and validation of PK/PD models for each new or existing drug, new approaches are needed. Therefore the aim of the current study was to evaluate whether the internally and externally validated covariate model of amikacin in (pre) term neonates [16] can be extrapolated to other drugs eliminated through glomerular filtration in neonates. This implicates that pediatric covariate models also contain biological system-specific information reflecting underlying physiological changes [13-15]. To test this hypothesis the covariate model of amikacin was directly incorporated in the pharmacokinetic model for netilmicin, vancomycin, tobramycin, gentamicin A and gentamicin B, drugs that, like amikacin are almost entirely eliminated through glomerular filtration. Using this approach a distinction is being made between drug-specific and system-specific information as explained in the methods section in which the pediatric covariate model is considered system-specific while the population values are considered to be drug-specific. Subsequently the descriptive and predictive performance of models using the amikacin covariate model was compared to the independent reference models in which the covariate model was identified using a systematic covariate analysis [14, 22].

To extrapolate information from one drug to another a few requirements need to be met [14]. First of all it is a prerequisite that the covariate models, which are assumed to contain system-specific information, are extensively validated. In this analysis the covariate model, developed to describe the pharmacokinetics of amikacin, was based on the analysis of 2186 amikacin samples in 874 (pre)term neonates. The covariate model was both internally and externally validated [16]. Furthermore, it is important that the covariate models which are extrapolated to other drugs, are based on a considerable number of samples from a large patient cohort with varying characteristics such as gestational age, birth bodyweight and postnatal age. In addition, it should be emphasized that the covariate models can only be extrapolated to populations with clinical characteristics that are within the studied range of the applied covariate model. In this analysis the amikacin covariate model developed for (pre)term neonates between 1 and 30 days was extrapolated to five other datasets in which the clinical characteristics are similar compared to the amikacin dataset considering bodyweight and age range (table I). Finally, a similar disease status was seen between the patients used for development of amikacin covariate model and the patients collected for the analysis of the unstudied drugs since all patients were admitted to the neonatal intensive care unit. When all the mentioned requirements are fulfilled,

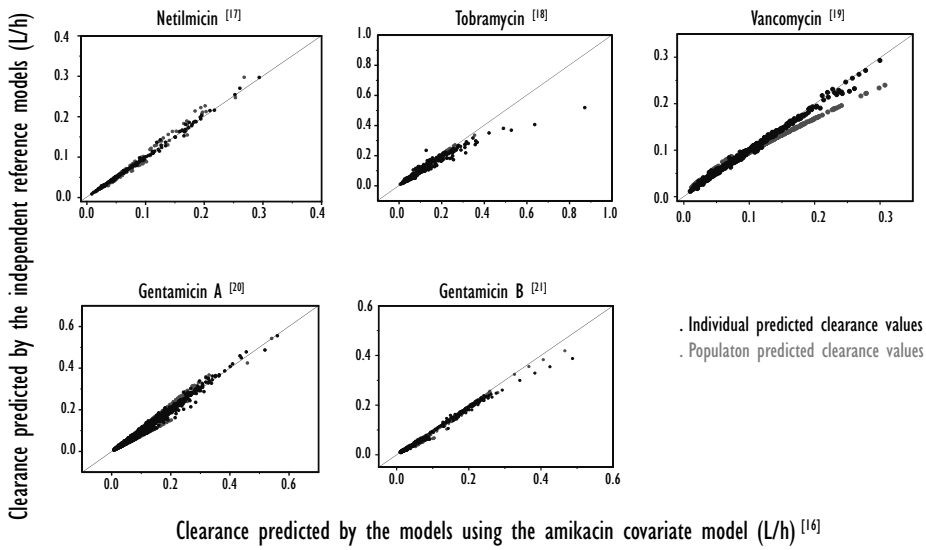


Figure 5: Individual and population predicted clearances for the models using the amikacin covariate model *versus* the independent reference models for netilmicin, vancomycin, tobramycin, gentamicin dataset A and gentamicin dataset B.

model development of the unstudied drugs (netilmicin, vancomycin, tobramycin and gentamicin) may be based on an even more limited number of data which is by all means a major advantage in the design and sampling strategy of (pediatric) clinical trials since the number of patients and the burden for patients participating in the trial can be reduced. However a limited amount of data still needs to be available to estimate the population parameter values for each drug as these are considered to be drug-specific parameters (see methods section - covariate model). When all the above mentioned requirements are fulfilled, an advantage of utmost importance is seen in the time required to develop and validate models using a covariate model which already has been extensively validated (weeks) compared to reference models (months).

The descriptive and predictive performance of the models using the amikacin covariate model was confirmed by figure 2, 3 and 4. This suggests that the covariate model of amikacin may contain system-specific information on the developmental changes in glomerular filtration. In an analysis of Krekels *et al.* [14], the same concept was applicable since it was illustrated that the covariate model for the glucuronidation of morphine in (pre)term neonates to children up to 3 years of age was able to describe the developmental changes in the glucuronidation of zidovudine in term

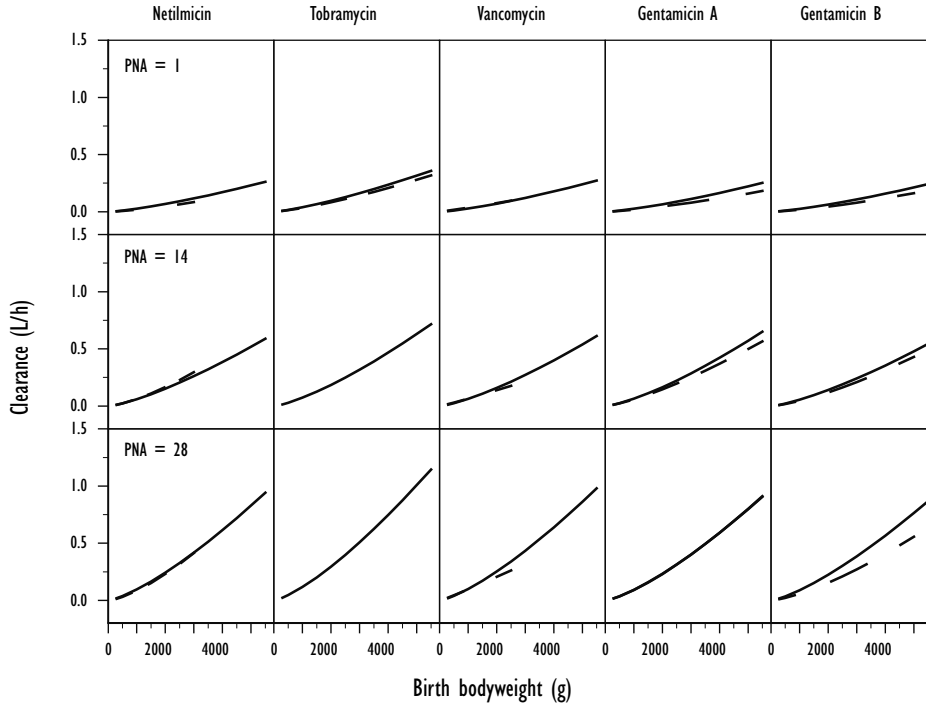


Figure 6: Population clearance values for the models using the amikacin covariate model (black) and the independent reference models (dotted line) *versus* birth bodyweight for PNA 1 (above), 14 (middle) and 28 days (below) for netilmicin, vancomycin, tobramycin, gentamicin A and gentamicin B. For the clearance values obtained with the amikacin covariate model, the full study range of the amikacin dataset is used while for the independent reference models, the study range available for that particular dataset is used causing the differences seen in the length of both lines illustrating the population clearance values using both approaches. Moreover for tobramycin, no clearance values for to the independent reference model could be illustrated for PNA 14 and 28 days since no data were available.

neonates and infants. Although similar individual and population clearance values are predicted by both approaches as seen in figure 5, a slight difference is seen in population clearance values of vancomycin and gentamicin B between the reference model and the model using the amikacin covariate model for the higher population values for clearance. This is also reflected in figure 6 in which this slight difference was seen between the clearance values for vancomycin and gentamicin B following both approaches at day 28. It is however unknown which of the two approaches best reflects the true clearance of these drugs. For gentamicin B, it should be noted that this difference seen in the higher clearance values of both approaches is only based on a limited number of data. Considering vancomycin, it is possible that the population clearance values predicted by the reference model are slightly different

because this model is only based on vancomycin data from preterm neonates (Table I). This limitation in information is less important in the model using the amikacin covariate model in which missing information is supplemented by information gained from the amikacin covariate model. The slight difference in population clearance values between the two approaches can also indicate that although vancomycin is mainly eliminated by glomerular filtration, the elimination of vancomycin may be modified by the presence of tubular processes (secretion or reabsorption) [31], which is not captured by the amikacin model. Finally, the difference may also be due to the different physicochemical properties of vancomycin compared to the other drugs, because in contrast to netilmicin, tobramycin and gentamicin, drugs that belong to the same class as amikacin, namely the aminoglycosides, vancomycin is a tricyclic glycopeptide. Besides the large difference in molecular mass of vancomycin (1449.3 g/mol) compared to amikacin (585.603 g/mol), netilmicin (475.58 g/mol), tobramycin (467.515 g/mol) and gentamicin (477.596 g/mol), the difference between the two drugs classes is also reflected in the protein binding. For the aminoglycosides the protein binding is below 10% in adults while this is much higher (approximately 55%) for the glycopeptide vancomycin. For antibiotics with a higher protein binding a lower renal clearance is often seen since only free drug is eliminated through the renal function [32].

In this analysis, the amikacin covariate model was in a first step extrapolated to drugs which are also almost entirely eliminated through GFR and with similar physicochemical properties compared to amikacin. However the majority of the drugs is eliminated by different elimination routes (hepatic and renal elimination). Therefore in a future analysis, the extension of the amikacin covariate model will be evaluated as well as the exact influence of differences in physicochemical and pharmacokinetic drug properties on the extrapolation of the amikacin covariate model to other drugs which are eliminated by different routes. To analyze this, a future analysis needs to be performed as done by Krekels *et al.* [15]. In that analysis the exact influence of differences in physiochemical properties on the extrapolation potential of the glucuronidation function was examined by using a physiological based (PBPK) modeling approach. Finally it will also be evaluated whether it is possible to characterize developmental changes in tubular processes in preterm and term based on the amikacin covariate model describing the developmental changes in GFR. A combination of all these different strategies (extrapolation to other drugs, adult data or non-clinical data) [33-35] will result in an approach focusing on the underlying system instead of focusing on the drugs and may facilitate development of pharmacokinetic models and evidence-based dosing regimens in the pediatric population.

5.5. Conclusions

In this study it was demonstrated that the descriptive and predictive performance of the models using the amikacin covariate model was similar to the independent reference models. This indicates that the use of system-specific information from one drug to other drugs may lead to optimization of sparse data analysis in children and that the covariate model, which in this case is describing the developmental changes in GFR, can be used to evaluate and optimize study and sampling design. As a consequence, the covariate model may play an important role to determine first-in-child dosing strategies and evidence-based dosing regimens of new and existing drugs.

Supplement Table I: Bootstrap results and their corresponding coefficients of variation (CV%) of the models derived in the current study using the amikacin covariate model for netilmicin, tobramycin, vancomycin, gentamicin dataset A and gentamicin dataset B.

Parameter	Netilmicin[17]	Tobramycin[18]	Vancomycin[19]	Gentamicin A[20]	Gentamicin B[21]
Fixed effects					
CLp in CL = CLp x (bBW/median)^{m} x (1+ n x (PNA/median)) x o (ibuprofen)	0.051 (5.08)	0.062 (3.36)	0.053 (2.81)	0.049 (1.52)	0.047 (3.15)
m	1.34	1.34	1.34	1.34	1.34
n	0.213	0.213	0.213	0.213	0.213
o	-	0.838	0.838	0.838	0.838
Vp in V1 = Vp x (cBW/median)^{p}	1.01 (7.18)	1.04 (8.07)	0.914 (2.74)	0.763 (1.9)	0.731 (3.37)
p	0.919	0.919	0.919	0.919	0.919
Q = r x CL	-	-	0.907 (10.5)	-	1.47 (13.85)
Q=CL	Q=CL	Q=CL	-	Q=CL	-
V2=V1	V2=V1	V2=V1	V2=V1	V2=V1	V2=V1
Interindividual Variability					
ω^2 (CL)	0.188 (35.2)	0.15 (11.3)	0.11 (12.6)	0.106 (13.6)	0.0530 (21.7)
Residual Variability					
σ^2 (proportional)	0.116 (18.0)	0.045 (17.6)	0.095 (7.94)	0.081 (9.39)	0.0477 (12.1)

CLp = population value for clearance, Vp = population value for volume of distribution of the central compartment, bBW = bodyweight at birth, cBW = current bodyweight, PNA = postnatal age, Q = intercompartmental clearance, V2 = Volume of distribution of the peripheral compartment

Supplement table II: Bootstrap results and their corresponding coefficients of variation (CV%) of the independent reference models derived in the current study for netilmicin, tobramycin, vancomycin, gentamicin dataset A and gentamicin dataset B.

Parameter	Netilmicin[17]	Tobramycin[18]	Vancomycin[19]	Gentamicin A[20]	Gentamicin B[21]
Fixed effects					
CLp in CL = CLp x (bBW/median)^{m} x (1+ n x (PNA/median))	-	0.066 (1.60)	0.034 (10.38)	-	-
m	-	1.31 (3.18)	1.07 (18.57)	-	-
n	-	-	1.09 (25.13)	-	-
CLp in CL = CLp x (bBW/median)^{o} x (PNA/median)^{p}	0.062 (6.79)	-	-	0.096 (1.5)	0.046 (4.37)
o	1.43 (8.34)	-	-	1.36 (2.14)	1.40 (4.97)
p	0.410 (50.18)	-	-	0.460 (10.67)	0.372 (9.05)
Vp in VI = Vp x (cBW/median)^{q}	0.66 (6.78)	0.929 (2.22)	0.613 (2.96)	1.07 (2.30)	0.504 (2.14))
q	0.97 (14.75)	0.839 (16.21)	1.00 (7.75)	0.808 (5.17)	0.851 (3.54))
Q = r x CL	-	-	-	-	0.733 (17.25)
Q = CL	Q=CL	-	Q=CL	Q=CL	-
V2 = s	-	-	-	-	0.851 (25.99)
V2 = VI	V2=VI	-	V2=VI	V2=VI	-
Interindividual Variability					
ω^2 (CL)	0.196 (34.4)	0.063(12.85)	0.18 (21.13)	0.101 (13.63)	0.035 (22.1)
Residual Variability					
σ^2 (proportional)	0.114 (18.76)	0.0458 (25.54)	0.0975 (7.97)	0.078 (9.38)	0.0453 (8.46)

CLp = population value for clearance, Vp = population value for volume of distribution of the central compartment, bBW = bodyweight at birth, cBW = current bodyweight, PNA = postnatal age, Q = intercompartmental clearance, V2 = Volume of distribution of the peripheral compartment

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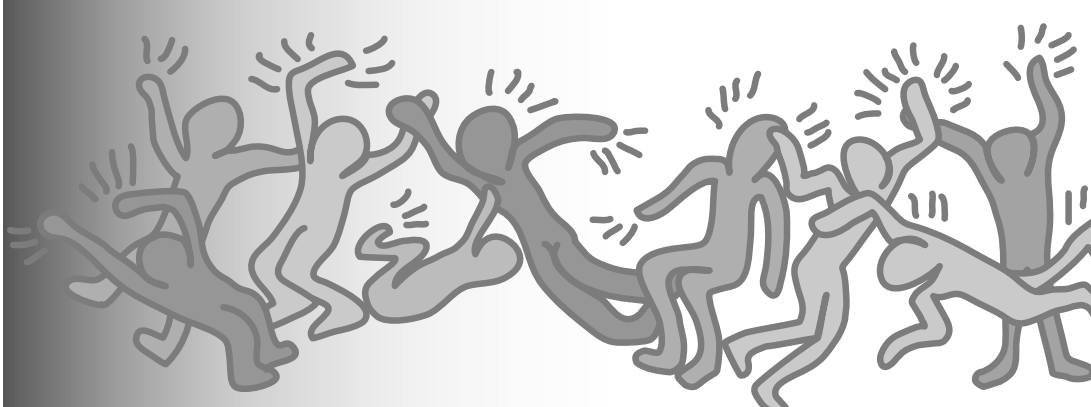
Section III

Developmental Changes in Renal Function (GFR and Tubular Processes) in Preterm and Term Neonates by Describing the Pharmacokinetics of Cefazolin



Chapter 6

Population Pharmacokinetic Modeling of Total and Unbound Cefazolin Plasma Concentrations as a Guide for Dosing in Preterm and Term Neonates

Roosmarijn F.W. De Cock[#], Anne Smits[#], Karel Allegaert, Jan de Hoon, Veroniek Saegeman, Meindert Danhof, Catherijne A.J. Knibbe

[#] Both authors contributed equally to the manuscript

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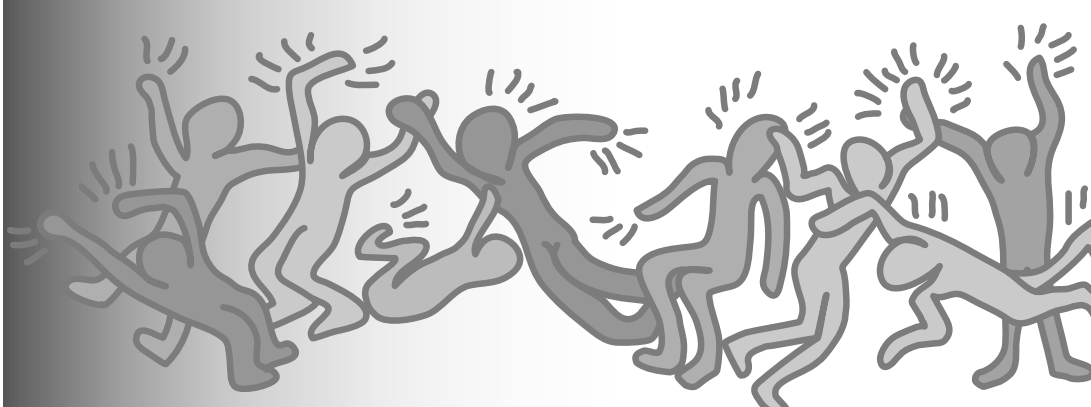

Abstract

Objectives

Cefazolin is frequently administered for antimicrobial prophylaxis and treatment of infections. In neonates, pharmacokinetic observations are limited and dosing regimens variable. The aim of this study was to describe the pharmacokinetics of cefazolin in neonates based on total and unbound concentrations to optimize cefazolin dosing.

Methods

Thirty-six neonates [median birth bodyweight (bBW) 2720 (range 540-4200)g, current bodyweight (cBW) 2755 (830-4200)g, postnatal age (PNA) 9 (1-30) days] receiving intravenous cefazolin (50mg/kg/8h) were included. Based on 119 total and unbound plasma concentrations, a population pharmacokinetic analysis with a covariate analysis was performed. Monte Carlo simulations were performed aiming for unbound concentrations above a minimal inhibitory concentration of 8 mg/L (>60% of time) in all patients.

Results

A one-compartment pharmacokinetic model was developed in which total and unbound concentrations were linked by a maximal binding capacity B_{max} of 136 mg/L and a dissociation constant for cefazolin protein binding K_D of 46.5 mg/L. Current bodyweight was identified as covariate for volume of distribution (Vd), bBW and PNA for clearance (Cl) and albumin plasma concentration for B_{max}, explaining 50%, 58% and 41% of interindividual variability in Vd, Cl and B_{max}, respectively. Based on Monte Carlo simulations, a bodyweight and PNA adapted dosing regimen was proposed resulting in similar exposure across different weight and age groups.

Conclusions

A neonatal pharmacokinetic model taking into account total and unbound cefazolin concentrations with saturable plasma protein binding was identified. As current bodyweight and PNA were the most important covariates, these may be used for individualized dosing in neonates.

6.1. Introduction

Based on a European survey, 15% of antimicrobial use for surgical prophylaxis in children is covered by first generation cephalosporins [1]. In a United States point prevalence survey in pediatric (PICU) and neonatal intensive care unit (NICU) patients, cefazolin was used in respectively 17.6% and 1.2% of patients on the day of survey [2]. Indications for cefazolin administration in neonates are mainly prophylactic (72%), to a lesser extent therapeutic (17%) (e.g. coagulase-negative staphylococcal sepsis) [3] or empiric (11%) [2]. While the pharmacokinetics (PK) of cefazolin have been described in adults, information on cefazolin PK in early life is limited [4-6]. Cefazolin is highly bound to human serum albumin and this binding displays saturation [7-9]. Only the unbound cefazolin distributes to the extravascular compartments and undergoes renal elimination. Neonates have a proportionally large total body water volume, immature renal function and low albumin level [10-12]. This population specific physiology likely affects cefazolin disposition.

Efficacy of cefazolin relates to the time *unbound* cefazolin concentrations exceed the minimal inhibitory concentration (MIC) for a given pathogen ($T_{>MIC}$) [13]. In neonates, often regarded as vulnerable and even immunocompromised patients, effective cefazolin therapy requires at least 60% of $T_{>MIC}$ [14].

Up to now, neonatal cefazolin clearance values described in literature are based on total cefazolin concentrations only, necessitating a cefazolin PK analysis integrating both total and unbound drug concentrations in neonates. Moreover, currently used cefazolin dosing regimens for neonates are variable (Table S1) [15-21].

Therefore, the aim of this study was to describe the pharmacokinetics of cefazolin in preterm and term neonates on the basis of both total and unbound cefazolin concentrations. Based on the final pharmacokinetic model, Monte Carlo simulations were performed to illustrate exposure to cefazolin in (pre)term neonates following currently used dosing regimens. Subsequently, a model-based dosing regimen was developed for preterm and term neonates.

6.2. Methods

6.2.1. Ethics, study population and drug dosing

The patients included in this study are based on a previously published cohort of 39 neonates and young infants, all admitted to the Neonatal Intensive Care Unit of the University Hospitals Leuven Belgium [8]. The study was approved by the ethical board of the hospital, registered at ClinicalTrials.gov (NCT01295606) and parental written informed consent was obtained. Inclusion was feasible if cefazolin (Cefazolin Sandoz®, Sandoz, Vilvoorde, Belgium) was administered intravenously as routine surgical prophylaxis. At induction of surgery, a cefazolin 50 mg/kg dose was administered over 30 minutes. According to the local standard of care (depending on foreign body implantation or contamination risk of the procedure), additional 50 mg/kg cefazolin dose(s) could be administered every 8 hours up to a maximum of 48 hours. As in the present analysis only neonates with postnatal age (PNA) 1-30 days were included, three patients (PNA 48, 51 and 108 days) were excluded from the original dataset [8]. Clinical characteristics were extracted from the medical files. Albuminaemia (g/L), indirect serum bilirubin concentrations (mg/dL) and serum creatinine (mg/dL) registered in a time interval of 24 h before or after the first cefazolin administration were collected. Plasma free fatty acids concentrations were determined in samples at the end of the study. Clinical characteristics of the study population are presented in Table 1.

6.2.2. Blood sampling

Blood samples were collected in lithium-heparin tubes at fixed time points, i.e. at 0.5, 2, 4 and 8 h after the first cefazolin administration and subsequently at 8 h intervals prior to each scheduled cefazolin administration, to determine total and unbound cefazolin concentrations. However, the number of samples collected from each patient was limited since the predefined total volume of blood available for sampling per patient was maximized to 1 mL/kg bodyweight. Blood samples (0.6 mL/sample) were immediately centrifuged (5 minutes, 4500 rpm at 4 °C) and the resulting 0.3 mL plasma was stored at -20°C in two aliquots of 0.15 mL.

6.2.3. Drug assay

Total and unbound cefazolin concentrations were determined by High Performance Liquid Chromatography after solid-phase column extraction. The initial method was developed in our laboratory [22] and adapted for measurement of cefazolin in small

Table 1: Clinical characteristics of the patients included in the study. Data are presented as median (range) or incidence.

Patient characteristics	Median (range)
Number of patients	36
Number of samples	119
Birth bodyweight (g)	2720 (540-4200)
Current bodyweight (g)	2755 (830-4200)
Postnatal age (PNA, days)	9 (1-30)
Gestational age (weeks)	37 (24-40)
Postmenstrual age (PMA, weeks)	38 (25-41)
Albumin (g/L)	34.5 (28.2-43.7)
Creatinine (mg/dL)	0.46 (0.26-1.03)
Free fatty acids (mmol/L)	0.08 (0-0.84)
Indirect bilirubin (mg/dL)	2.91 (0.1-11.13)
Gender (male/female)	22 / 14

volume plasma samples [8]. The lower limit of quantification for cefazolin was 0.1 μg/mL, with a coefficient of variation lower than 20%. Intra-assay precision and accuracy averaged 3.9 and 5.5% respectively. Inter-assay precision and accuracy averaged 5.7 and 6.8%, respectively, which is in line with FDA analytical recommendations [23, 24].

6.2.4. Biochemical assays

Albumin, indirect bilirubin and creatinine (enzymatic) were quantified on Roche Modular P (Roche Diagnostics, Basel, Switzerland). Free fatty acids were determined with a kit from DiaSys (DiaSys, Diagnostic Systems, Holzheim, Germany).

6.2.5. Population pharmacokinetic analysis

Model development

The population pharmacokinetic analysis was performed using the non-linear mixed effect modeling software NONMEM version 6.2 (Globomax LLC, Hanover, MD, USA) using the first-order conditional estimation method with the interaction option (FOCE-I). Tools like S-Plus version 6.2.1 (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P Consultants BV, Leiden, The

Netherlands), PsN and R (version 2.10.1) were used to visualize and evaluate the model.

The model building process was performed in a stepwise manner: (i) choice of the structural model, (ii) choice of the statistical sub-model, (iii) choice of the covariate model, (iv) model evaluation. Different diagnostic tools were used to discriminate between the different models [25]. A decrease in objective function (OFV) of 3.9 points or more was considered statistically significant ($p<0.05$ based on X2 distribution, for nested models). Furthermore, the goodness-of-fit plots were evaluated. Finally the total number of parameters, visual improvement of individual plots, correlation matrix, confidence intervals of parameter estimates, ill-conditioning [26] and shrinkage [27] were assessed.

Structural and statistical sub-model

A one and two compartment pharmacokinetic model was fitted to both total and unbound cefazolin concentrations using NONMEM VI, subroutine ADVAN6, TOL=3. Unbound cefazolin concentrations were related to total cefazolin concentrations by the following equation, taking into account non-linear protein binding [28].

$$C_{unbound} = \frac{1}{2} \bullet (C_{total} - B_{max} - K_D) + \sqrt{(C_{total} - B_{max} - K_D)^2 + 4 \bullet K_D \bullet C_{total}}$$

(Equation 1)

In this equation $C_{unbound}$ represents the unbound cefazolin concentrations, C_{total} the total cefazolin concentrations, B_{max} the maximum protein binding and K_D the dissociation constant.

For the statistical sub-model, the inter-individual variability was assumed to follow a log-normal distribution. For the intra-individual variability and residual error, a proportional, additive and a combined error model were tested.

Covariate analysis

The following covariates were evaluated in the covariate analysis: birth bodyweight (bodyweight at day of birth, bBW, gram), current bodyweight (bodyweight at day of blood sampling, cBW, gram), postnatal age (PNA, days), gestational age (GA, weeks), postmenstrual age (PMA, weeks, combination of GA and PNA in weeks), al-

buminaemia (g/l), creatininaemia (mg/dL), free fatty acids (mmol/L), indirect bilirubin (mg/dL) and gender. Potential covariates were separately implemented into the model using a linear or power equation (equation 2):

$$P_i = P_p \bullet (\frac{Cov}{Cov_{Median}})^k \quad \text{(Equation 2)}$$

In this equation P_i represents the individual parameter estimate of the *ith* subject, P_p equals the population parameter estimate, Cov is the covariate and k is the exponent which was fixed to 1 for a linear function or was estimated for a power function. Covariates were considered statistically significant if the objective function decreased 7.8 points (p-value <0.005) or more. The covariate causing the largest reduction in objective function was chosen as a basis to sequentially explore the influence of additional covariates. The choice of the covariate models was further evaluated as discussed under Model development, whereby the results of the Model validation were also considered.

Model validation

The stability of the final pharmacokinetic model was evaluated by a bootstrap analysis, in which the model building dataset was resampled 1000 times, in S-plus, version 6.2.1. (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P Consultants BV, Leiden, The Netherlands). To evaluate the accuracy of the model the normalized prediction distribution error (NPDE) method was performed. To perform this analysis the dataset was simulated 1000 times after which each observed concentration was compare to the simulated concentrations using the NPDE package in R [29, 30].

6.2.6. Monte Carlo simulations

To evaluate $T_{>MIC}$, the Clinical and Laboratory Standards Institute (CLSI) 2012 [31] MIC interpretative criteria for susceptibility to cefazolin corresponding with the 5 bacterial species isolated most frequently from neonatal blood cultures from our department were used. Therefore, all positive blood culture results (n=137) from our unit, for the period January - October 2012, were retrospectively collected. Identification of bacterial isolates was done by use of MALDI Biotyper (Bruker Daltonics, Bremen, Germany). *Staphylococcus* species contributed for 94.4% of the top 5 isolates. Consequently, the CLSI MIC interpretative criterion for susceptibility to cefazolin of *Staphylococcus* species (8 mg/L) was used as target MIC (Table 2) [31].

As effective cefazolin therapy is reported to require at least 60% of $T_{>MIC}$ [15], the probability of attaining unbound cefazolin concentrations during 60% of the dosing interval [14] above 8 mg/L was evaluated on the basis of Monte Carlo simulations using the final pharmacokinetic model. These Monte Carlo simulations were performed in 1000 individuals to evaluate the exposure to cefazolin in (pre)term neonates following the currently used dosing regimen in this study and the dosing regimen proposed by the Dutch Children's Formulary [15]. The covariates identified in the final pharmacokinetic model were sampled from the original dataset taking into account their correlation. Albumin was randomly generated according to the observed distribution in these 36 neonates. For the simulations, cefazolin doses were administered over 30 minutes every 8 hours until 48 hours after the first dose. To evaluate the results of the Monte Carlo simulations, 4 different groups (Group 1: PNA ≤ 7 days, cBW ≤ 2000g, Group 2: PNA ≤ 7 days, cBW > 2000g, Group 3: PNA > 7 days, cBW ≤ 2000g, Group 4: PNA > 7 days, cBW > 2000g) were created. Based on these results, a new model-based dosing regimen was proposed.

Table 2: The 5 bacterial species isolated most frequently from neonatal blood cultures (n=137) in the Leuven neonatal intensive care unit for the period January 2012 until October 2012. Corresponding CLSI MIC values are reported.

Isolate	Contribution to all positive blood cultures (%)	Contribution to top-5 isolates (%)	CLSI MIC values (mg/L)		
			Susceptible	Intermediate	Resistant
1) S. epidermidis	51.82	65.74			
2) S. hominis	9.49	12.04			
3) S. aureus	6.57	8.33	≤ 8	16	≥ 32
4) S. capitis	6.57	8.33			
5) E. coli	4.38	5.56	≤ 2	4	≥ 8

S.: Staphylococcus, E.: Escherichia, CLSI: Clinical and Laboratory Standards Institute, MIC: Minimal Inhibitory Concentration.

6.3. Results

6.3.1. Patients

The pharmacokinetic analysis was based on 119 plasma concentrations of cefazolin obtained in 36 (pre)term neonates with PNA 1-30 days. Median total and unbound cefazolin plasma concentrations, were respectively 101.09 (range 17.44-404.22) mg/L and 41.15 (range 5.34-261.38) mg/L. Median unbound fraction was 0.40 (range 0.14-0.73). Clinical characteristics are presented in Table 1.

6.3.2. Population pharmacokinetic analysis

Structural and statistical sub-model

A one compartment model was selected as structural model because a two compartment model was not superior over a one compartment model. The final one compartment pharmacokinetic model, taking into account total and unbound

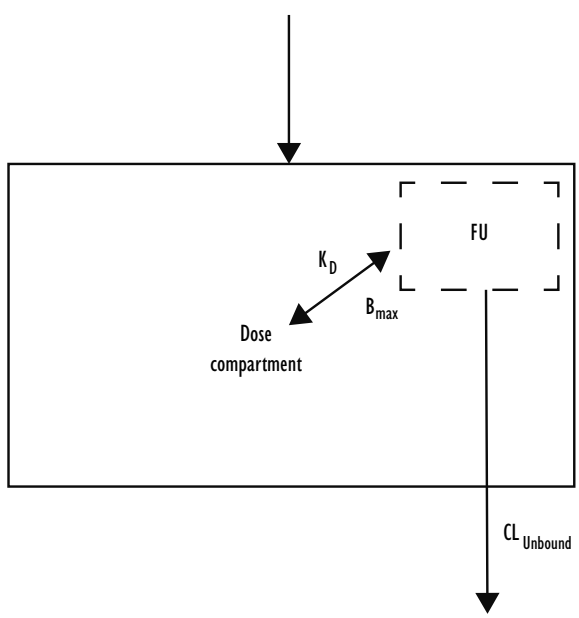

Figure 1: Schematic representation of the pharmacokinetic model using both total and unbound concentrations of cefazolin. K_D = Dissociation constant, B_{max} = Maximum protein binding, FU = unbound fraction of cefazolin, $CL_{unbound}$ = Clearance of unbound cefazolin.

cefazolin concentrations, was parameterized in terms of clearance, volume of distribution (Vd), maximum protein binding B_{max} and the dissociation constant K_D (Figure 1). By the determination of B_{max} and K_D, unbound cefazolin concentrations could be calculated from total concentrations (equation 1). Initially, a separate proportional error was estimated for total and unbound cefazolin concentrations. Since these errors were not significantly different ($p>0.05$), the model was simplified by estimating one proportional error for both total and unbound concentrations.

Table 3: Model-based population pharmacokinetic parameter estimates and the values obtained after the bootstrap analysis.

Parameter	Simple model without covariates	Final pharmacokinetic covariate model	Bootstrap final pharmacokinetic model
	Value (CV%)	Value (CV%)	Value (CV%)
Fixed effects			
CL (L/h) = CLp	0.229 (11.7)	-	-
CLp in CL = CLp x (bBW/median)^m x (1+(PNA/median) x n)	-	0.185 (12.8)	0.187 (13.3)
m	-	1.37 (16.4)	1.41 (17.3)
n	-	0.496 (38.5)	0.524 (44.5)
V (L) = Vp	0.812 (3.0)	-	-
Vp in V = Vp x (cBW/median)	-	0.863 (3.55)	0.860 (3.63)
B_{max} (mg/L) = $B_{max}p$	143 (14.5)	-	-
$B_{max}p$ in B_{max} = $B_{max}p$ x (ALB/median)	-	136 (12.6)	141 (14.5)
Kd (mg/L) =Kdp	53.2 (22.9)	46.5 (20.9)	49.5 (24.1)
Interindividual variability (ω^2)			
ω^2 CL	0.535 (33.6)	0.163 (35.1)	0.149 (38.0)
ω^2 V	0.14 (29.1)	0.0259 (38.6)	0.0258 (43.2)
ω^2 Bmax	0.102 (41.0)	0.0367 (54.0)	0.0368 (56.7)
Residual variability (σ^2)			
σ^2 (proportional)	0.0332 (22.1)	0.0351 (21.5)	0.0342 (22.5)

CL= clearance, CLp = population value for clearance for an individual with birth bodyweight of 2720g and postnatal age of 9 days, V = Volume of distribution, Vp = population value for volume of distribution for an individual with a current bodyweight of 2755g, B_{max} = maximum protein binding, $B_{max}p$ = population value for maximum protein concentration for an individual with an albumin concentration of 34.5 g/L, Kd = Dissociation constant of the drug, Kdp = population value of dissociation constant of the drug, bBW = birth bodyweight, cBW = current bodyweight, PNA = postnatal age, ALB = concentration of albumin

Covariate Model

Current bodyweight was found as most important covariate on Vd. Initially, current bodyweight was implemented on Vd using a power function with an estimated exponent of 0.94. However since the 95% confidence interval of this parameter included 1, a linear relationship between current bodyweight and Vd was used ($p>0.05$). Implementation of current bodyweight on Vd caused a significant drop in objective function (OFV) of 46 points ($p<0.005$). Although for clearance, PMA was identified as most important covariate, a combination of the covariates birth bodyweight and PNA was preferred over PMA alone. First of all, both analyses resulted in a comparable improvement of the model (i.e. same reduction in objective function (ΔOFV 32 points, $P< 0.005$). Secondly, the combination of birth bodyweight and PNA allows to make a distinction between the antenatal (birth bodyweight) and postnatal (PNA) maturation component of cefazolin clearance. Birth bodyweight was implemented on clearance using a power function with an estimated exponent of 1.37, while PNA was implemented using a linear function with an estimated slope of 0.496 (Table 3). The model was further improved (ΔOFV 12 points, $P< 0.005$) by introducing albumin on B_{max} using a linear function (Table 3).

The parameter estimates of the simple and the final pharmacokinetic model and the values obtained from the bootstrap analysis are provided in Table 3. In Figure 2, the observed *versus* predicted concentrations are plotted for the total and unbound concentrations showing that the model adequately describes the data. In Figure S1, the inter-individual variability in clearance, Vd and B_{max} is plotted against the relevant covariates for the simple and the final pharmacokinetic model. A significant part of the interindividual variability is explained (Figure S1). This is also reflected by the decrease in the estimates of the interindividual variability when comparing the simple and the final pharmacokinetic model which results in a decrease of 50% of the inter-individual variability on Vd, 58% on clearance and 41% on B_{max} (Table 3). In Figure 3 the observed and population predicted bound and unbound cefazolin concentrations are plotted from which B_{max} and the value for the unbound concentration for which the binding was half-maximal (K_D) can be derived. Variation in population predicted bound and unbound cefazolin concentrations are explained by differences in current bodyweight, birth bodyweight and PNA of the subjects (Figure 3).

The number of binding sites on the albumin molecule was derived from $B_{max,}$ which was corrected for molecular weight of albumin (67000 g/mol) and cefazolin (454.5 g/mol) (Equation 3), and the median albumin concentration (34.5 g/L) (Equation 4) and proved 0.6.

$$B_{max} = 0.136g/L \bullet (\frac{67000g/mol}{454.5g/mol}) = 20g/L \quad \text{(Equation 3)}$$

$$Number\ of\ binding\ sites = (\frac{20g/mol}{34.5g/mol}) = 0.6 \quad \text{(Equation 4)}$$

Model Validation

The results of the bootstrap analysis (Table 3) show that the median estimated values based on the resampled dataset are within 10% of the values obtained in the final model. The NPDE histograms are following the normal distribution, indicating the accuracy of the final pharmacokinetic model (Figure 2). Furthermore no trend was seen between the NPDE *versus* time or *versus* predicted concentrations (figures not shown). The number of ill-conditioning (74.6) was far below the critical number of 1000 indicating that the final pharmacokinetic model was not overparameterized. Finally, η-shrinkage expressed as a percentage was identified to be 9.8% for clearance, 21.2% for Vd and 30% for B_{max}.

6.3.3. Monte Carlo simulations

Concentration-time profiles following the currently used dosing regimen, the dosing regimen proposed by the Dutch Children's Formulary and the new model based-dosing regimen (Table 4) were predicted based on Monte Carlo simulations using the final pharmacokinetic model (Figure 4). In Figure S2, box plots illustrate the median and interquartile ranges (5% and 95%) of the individual predicted concentrations at 60% of the dosing interval after the first dose and after the fourth or sixth dose. This illustrates that less than 10% of the individual predicted concentrations at 60% of the dosing interval are below a MIC of 8 mg/L. Relatively high cefazolin peak concentrations are reached, particularly in neonates in group 1, 2 and 3 following the dosing regimen used in the current study and in group 3 following the dosing regimen proposed by the Dutch Children's Formulary (Figure 4, S2). Therefore, a new dosing regimen was advised based on the dosing regimen proposed by the Dutch Children's Formulary but including a lower dose for group 3 (Table 4). Using this dosing regimen, 0%, 1.2%, 0.7% and 1.0% of the individuals of group 1, 2, 3 and 4, respectively, would be exposed to concentrations below 8 mg/L at 60% of the dosing interval (Figure S2B).

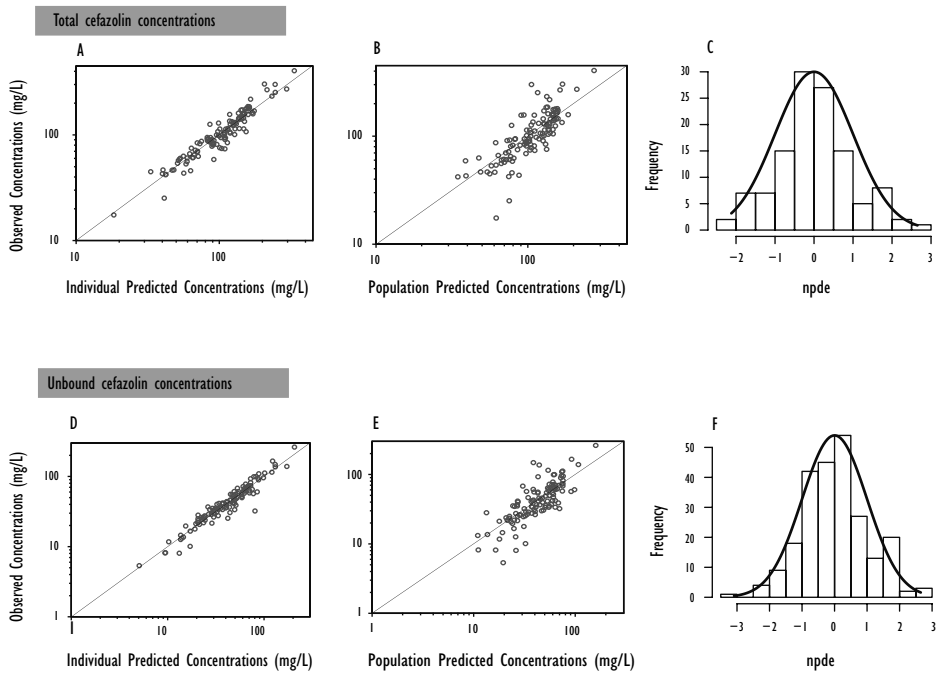

Figure 2: Observed *versus* individual predicted concentrations (a,d) and population predicted concentrations (b,e) for total (upper panels) and unbound (lower panels) cefazolin concentrations. The histograms show the distribution of the normalized prediction distribution error (NPDE) methods for total (c) and unbound (f) cefazolin concentrations.

6.4. Discussion

Neonatal cefazolin PK data are outdated since they are mainly based on total drug concentrations collected in a limited number of subjects. We aimed to characterize cefazolin pharmacokinetics and its covariates based on both total and unbound drug concentrations. In our study, the median cefazolin clearance value (coefficient of variation, %) for a neonate with a birth bodyweight of 2720 g and PNA 9 days was 0.185 (12.8) L/h (i.e. 0.068 L/kg/h). This is slightly higher than the earlier reported values of 0.53-1.10 mL/kg/min (i.e. 0.032-0.066 L/kg/h) in 11 neonates receiving 30 mg/kg cefazolin intravenously. Since only the unbound cefazolin is pharmacologically active and total drug concentrations only partially reflect unbound concentrations (Figure 3), we would like to emphasize that unbound concentrations need to be measured instead of using estimated unbound concentrations based on a fixed protein binding percentage. Especially in highly protein bound drugs this is of relevance.

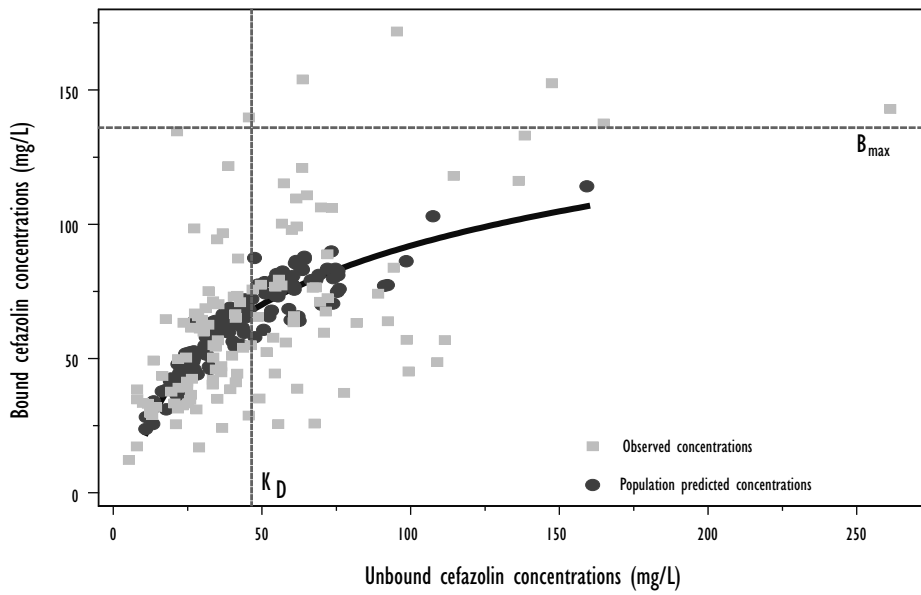

Figure 3: The relationship between the observed (*square*) and model-based predicted (*circle*) bound and unbound cefazolin concentrations (mg/L) in 36 (pre)term neonates. B_{max} (protein binding defined as the maximum estimated concentration bound to albumin) and K_D (dissociation constant defined as the unbound concentration which corresponds to 50% of the maximum binding capacity) are illustrated.

Postnatal age and birth bodyweight were the most important covariates of neonatal cefazolin clearance. This is in line with expectations, taking into account the elimination of cefazolin by renal route. Renal clearance displays maturation during early life and covariates birth bodyweight and PNA can hereby respectively reflect the prenatal and postnatal maturation [32]. Furthermore, age and bodyweight were earlier documented as clearance predictors of other beta-lactams in neonates [33-36]. We can only hypothesize on factors affecting the remaining unexplained cefazolin clearance variability within the neonatal population. Possibly, maturation of the renal tubular activity is a contributing factor. Also for other beta-lactams (e.g. amoxicillin, flucloxacillin) the presence of other elimination pathways, in addition to glomerular filtration rate (GFR), such as tubular secretion or non-renal clearance routes was suggested earlier [33, 37]. Since only the unbound drug can be eliminated and since compound specific clearance depends on compound specific protein binding, we hereby want to stress that the mean (± standard deviation) protein binding of flucloxacillin (74.5±3.1%) and in particular amoxicillin (11.7±2.7%) is lower compared to cefazolin [34, 38] Therefore, results of amoxicillin and flucloxacillin may not be directly applied to cefazolin.

Table 4: Dosing recommendations for cefazolin in preterm and term neonates according to dosing regimens used in the current study, the Dutch Children's Formulary and a new model-based proposed dosing regimen. For concentration-time profiles of these dosing regimens for neonates with different clinical characteristics we refer to Figure 4.

Guideline	PNA (days)	cBW (g)	Dose (mg/kg)	Interval (h)
Used in the current study	-	-	50	8
Dutch Children's Formulary	≤ 7 days	≤ 2000g	25	12
	≤ 7 days	> 2000g	50	12
	7-28 days		50	8
Proposed dosing regimen	≤ 7 days	≤ 2000g	25	12
	≤ 7 days	> 2000g	50	12
	7-28 days	≤ 2000g	25	8
	7-28 days	> 2000g	50	8

PNA = postnatal age, cBW = current bodyweight

The number of binding sites for cefazolin on the albumin molecule based on this analysis was calculated to be 0.6 (equation 3 and 4), which corresponds well with the number of binding sites for cefazolin on albumin previously found in literature (0.7) [7, 39, 40].

We documented relatively high cefazolin plasma concentrations based on a 50 mg/kg/8h cefazolin dosing regimen, administered to all study patients. This is likely due to the absence of any bodyweight and/or age- adapted dosing. Simulation of the dosing regimen proposed by the Dutch Children's Formulary resulted in lower cefazolin concentrations. However, based on Figure 4 and S2, the dose administered to neonates in group 3 when using the Dutch Children's Formulary, still needs further reduction. A new bodyweight- and age-based dosing regimen is suggested, derived from the dosing regimen proposed by the Dutch Children's Formulary, but with a dose reduction for group 3 in order to reach similar exposure in all four groups (Table 4). With this new model-based dosing regimen the target of 8 mg/L for 60% of the dosing interval was reached for >90% of the patients (i.e. 100%, 98.8%, 99.3% and 99% of the individuals of group 1, 2, 3 and 4, respectively).

When compared to the dosing regimen used in this study, a total daily dose reduction of 67%, 33% and 50% for patients in respectively group 1, 2 and 3 is proposed resulting in similar exposure in all groups. The proposed dosing regimen is hereby more in line with some of the recommendations presented in Table S1. As a consequence of cefazolin dose reduction, albumin binding places become available

Table S1: Overview of cefazolin dosing regimens for neonates and young infants. The dosing regimen used in the current study as well as the dosing regimen provided by the Dutch Children's Formulary and different handbooks are presented. Data are adapted to mg/kg/dose.

Reference	Age		Weight	Cefazolin dose and interval
NICU UZ Leuven				50 mg/kg/dose, q8h
Dutch Children's Formulary[15]	< 1 week PNA		< 2000 g	25 mg/kg/dose, q12h
			> 2000 g	50 mg/kg/dose, q12h
	1-4 weeks PNA			50 mg/kg/dose, q8h
Neonatal and pediatric pharmacology, Yaffe and Aranda 2011[16]	0-4 weeks PNA		< 1200 g	20 mg/kg/dose, q12h
	<1 week PNA		1200-2000 g	20 mg/kg/dose, q12h
			> 2000 g	20 mg/kg/dose, q12h
	≥ 1 week PNA		1200-2000 g	20 mg/kg/dose, q12h
			> 2000 g	20 mg/kg/dose, q8h
The Harriet Lane Handbook 2012[18]	≤ 1 week PNA			20 mg/kg/dose, q12h
	> 1 week PNA		≤ 2000 g	20 mg/kg/dose, q12h
			> 2000 g	20 mg/kg/dose, q8h
Neofax 2011[19]	≤ 29 weeks PMA,	0-4 weeks PNA		25 mg/kg/dose, q12h
		> 4 weeks PNA		25 mg/kg/dose, q8 h
	30-36 weeks PMA,	0-2 weeks PNA		25 mg/kg/dose, q12h
		> 2 weeks PNA		25 mg/kg/dose, q8 h
	37-44 weeks PMA,	0-1 week PNA		25 mg/kg/dose, q12h
		> 1 week PNA		25 mg/kg/dose, q8h
	≥ 45 weeks PMA, all			25 mg/kg/dose, q6h
Nelson's Textbook of Pediatrics 2007[17]	< 1 week PNA			20 mg/kg/dose, q12h
	> 1 week PNA			13-20 mg/kg/dose, q8h
The Sanford guide to antimicrobial therapy 2012-2013[20]	≤ 29 weeks PMA,	0-4 weeks PNA		50 mg/kg/dose, q12h
		> 4 weeks PNA		50 mg/kg/dose, q8h
	30-36 weeks PMA,	0-2 weeks PNA		50 mg/kg/dose, q12h
		> 2 weeks PNA		50 mg/kg/dose, q8h
	37-44 weeks PMA,	0-1 week PNA		50 mg/kg/dose, q12h
		> 1 week PNA		50 mg/kg/dose, q8h
	≥ 45 weeks PMA, all			50 mg/kg/dose, q6h
Redbook 2012[21]	≤ 1 week PNA		≤ 2000 g	25 mg/kg/dose, q12h
			> 2000 g	25 mg/kg/dose, q12h
	> 1-4 weeks PNA		≤ 2000 g	25 mg/kg/dose, q12h
			> 2000 g	25 mg/kg/dose, q8h

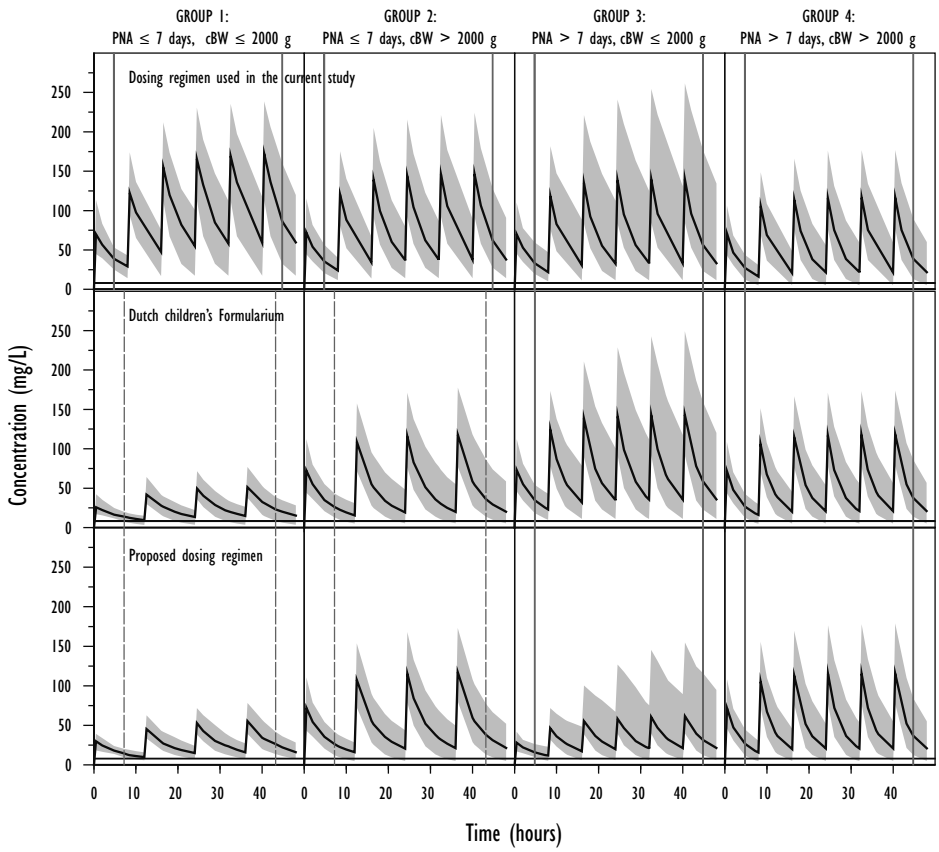

Figure 4: Concentration-time profiles based on 1000 Monte Carlo simulations using the final pharmacokinetic model following the dosing regimen used in this study (upper row), the dosing regimen proposed by the Dutch Children's Formulary (middle row) and the new model-based proposed dosing regimen (bottom row) in 4 different groups based on current bodyweight and postnatal age. The black line represents the median of the simulated profiles and the grey area represents the 90% confidence interval of the simulated values. The black horizontal line corresponds to the minimal inhibitory concentration of 8 mg/L. The grey vertical lines indicate the time at which 60% of the dosing interval is reached (4.8 and 44.8 hours) for a dosing interval of 8 hours. The grey vertical dotted lines indicate the time at which 60% of the dosing interval is reached (7.2 and 43.2 hours) for a dosing interval of 12 hours. PNA = Postnatal age, cBW = Current bodyweight.

for other endogenous (e.g. bilirubin) or exogenous compounds competing for the same albumin binding places. In neonates, frequently showing hyperbilirubinaemia (increased bilirubin production and decreased glucuronidation) and/or receiving multi drug therapies, this is a relevant and population specific advantage. Recent PK reports of other beta-lactam antibiotics commonly used in neonatal intensive care units also suggested dose adaptations compared to previously used regimens. To further illustrate this, a reduction in drug dose and interval for amoxicillin [33] and an increase of initial dose with subsequent dose reduction depending on the micro-biological isolate, for flucloxacillin [37] were suggested in neonates. This emphasizes the need for population specific PK studies in neonates. Since study methodologies can differ, a correct definition of the aimed PK target is required to achieve reliable dosing evaluations in this specific population [14, 41]. In general, we have to be aware that total daily dose reduction of an antimicrobial may lead to increased bacterial resistance and ineffectiveness [42]. Prospective validation of the new dosing regimen is therefore necessary, but this was not the intention of the present study.

The strength of our analysis is the measurement of both total and unbound cefazolin concentrations in a relevant neonatal cohort. Additionally, the final pharmacokinetic model can be used to optimize dosing regimens for other pathogens in different settings by changing the target MIC value and/or the $T_{>MIC}$. However, there are some limitations. First, the MIC values used were not prospectively determined. Secondly, the success of antibiotic prophylaxis depends not only on selection of the antimicrobial drug and drug dosing but also on the correct, well-timed drug administration and subsequent tissue distribution. Direct measurement of drug concentrations in the surgical site tissues [43, 44] may provide additional information to include in PK models, but is very challenging in this population [45].

We conclude that total and unbound cefazolin concentrations in neonates could be described by a one compartment PK model which includes saturable protein binding. Birth bodyweight and PNA were defined as the most important covariates contributing to cefazolin clearance variability. A new model-based neonatal cefazolin dosing regimen was proposed, however prospective validation of this dosing regimen is needed.

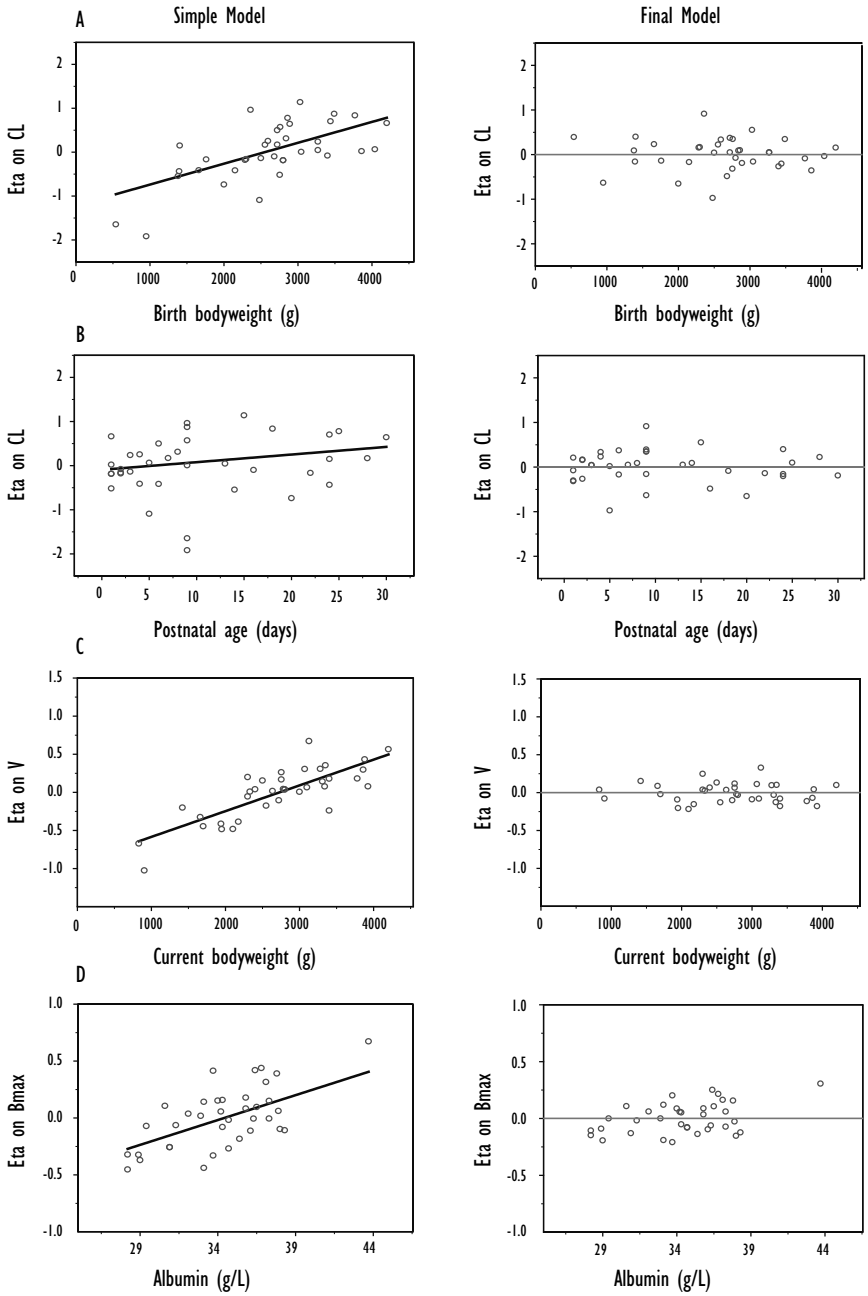

Figure SI: Interindividual variability (ETA) in a) clearance *versus* birth bodyweight, b) clearance (Cl) *versus* postnatal age, c) volume of distribution (V) *versus* current bodyweight, d) Maximum protein binding (B_{max}) *versus* albumin for the simple (left) and final covariate model (right).

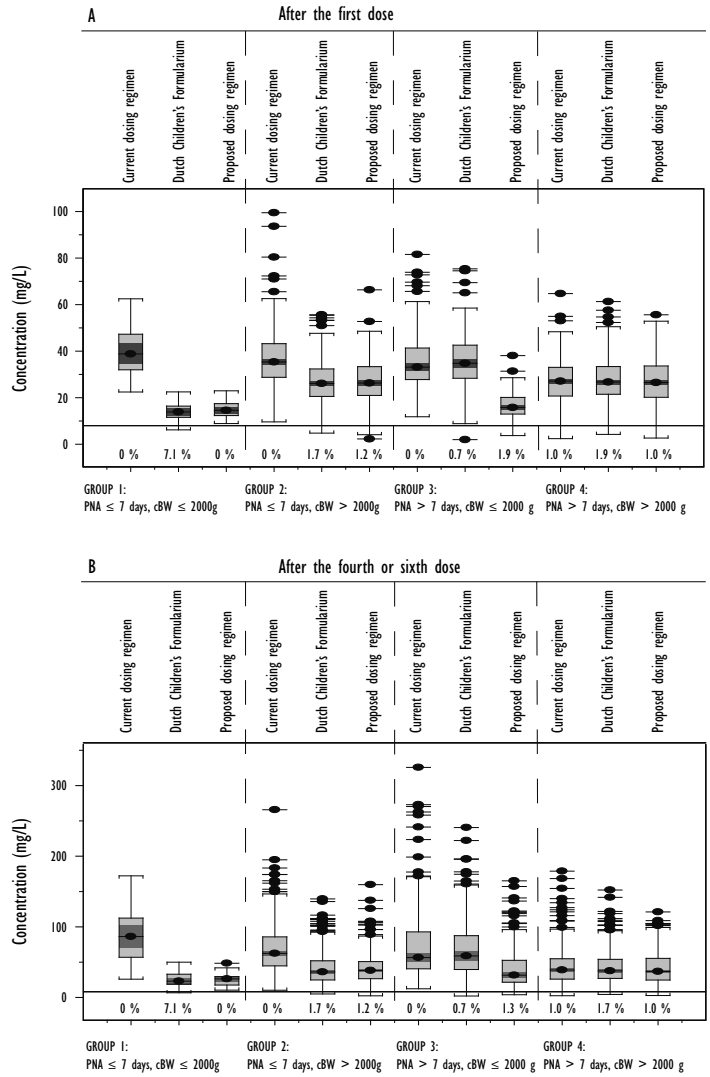

Figure S2: Individual predicted concentrations based on Monte Carlo simulations in 1000 individuals *versus* 4 different groups based on current bodyweight (cBW) and postnatal age (PNA). Plot A represents the individual predicted concentrations at 60% of the dosing interval after the first dose which corresponds to 4.8 or 7.2 hours after the first dose for a dosing interval of 8 or 12 hours respectively. Plot B represents the individual predicted concentrations at 60% of the dosing interval after 4 or 6 doses which corresponds to 44.8 or 43.2 hours based on a dosing interval of 8 or 12 hours, respectively. The black horizontal line corresponds to the minimal inhibitory concentration of 8 mg/L. For each group 3 boxplots are shown following the dosing regimen applied in this study (left), the dosing regimen suggested by the Dutch Children's Formulary (middle) and the new model-based proposed dosing regimen (right). Box plots illustrate median, interquartile range (5-95%) and outliers. The percentage of individuals with a concentration below 8 mg/L at 60% of the dosing interval is indicated for each dosing regimen per group.

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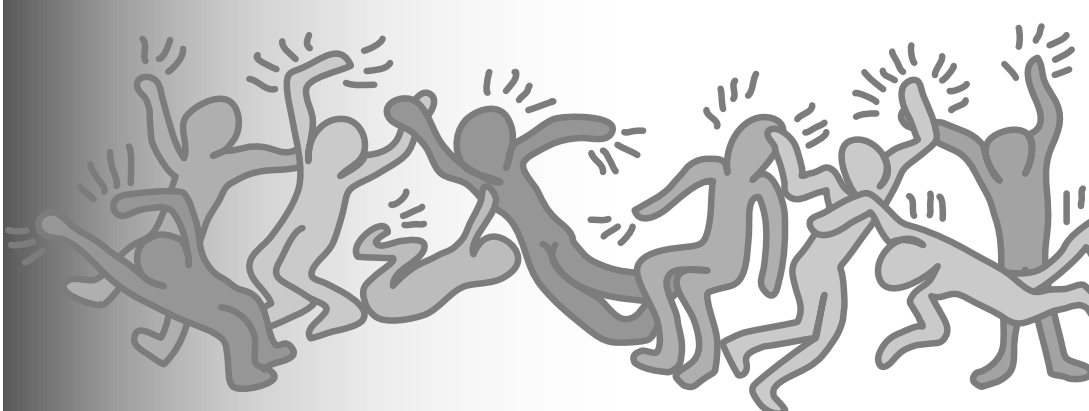
Section IV

Renal and Hepatic Elimination of Propylene Glycol in Preterm and Term Neonates



Chapter 7

Developmental Pharmacokinetics of Propylene Glycol in Preterm and Term Neonates

Roosmarijn F.W. De Cock, Catherijne A.J. Knibbe, Aida Kulo, Jan de Hoon, Rene Verbesselt, Meindert Danhof, Karel Allegaert

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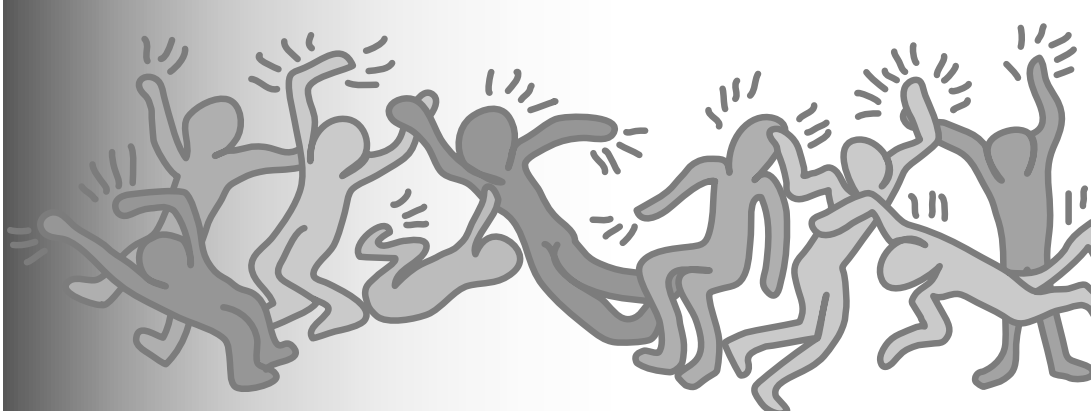

Abstract

Aim

Propylene glycol (PG) is often applied as an excipient in drug formulations. As these formulations may also be used in neonates, the aim of this study was to characterize the pharmacokinetics of propylene glycol, co-administered intravenously with paracetamol (800mgPG/1000mg paracetamol) or phenobarbital (700mgPG/200mg phenobarbital) in preterm and term neonates.

Methods

A population pharmacokinetic analysis was performed based on 372 PG plasma concentrations from 62 (pre)term neonates (birth weight (bBW) 630-3980g, postnatal age (PNA) 1-30days) using NONMEM 6.2. The model was subsequently used to simulate PG exposure upon administration of paracetamol or phenobarbital in neonates (gestational age 24-40 weeks).

Results

In a one compartment model, birth weight and PNA were both identified as covariates for PG clearance using an allometric function ($CL_i=0.0849x\{(BWb/2720)^{1.69}x(PNA/3)^{0.201}\}$). Volume of distribution scaled allometrically with current bodyweight ($V_i=0.967x\{(BW/2720)^{1.45}\}$), and was estimated 1.77 times higher when co-administered with phenobarbital compared to paracetamol. By introducing these covariates a large part of the interindividual variability on clearance (65%) as well as on volume of distribution (53%) was explained. The final model shows that for commonly used dosing regimens, the population mean PG peak and trough concentrations ranges between 33-144 and 28-218 mg/L (peak) and 19-109 and 6-112 mg/L (trough) depending on birth weight and age of the neonates for paracetamol and phenobarbital formulations, respectively.

Conclusion

A pharmacokinetic model was developed for PG co-administered with paracetamol or phenobarbital in neonates. As such, large variability in PG exposure may be expected in neonates which are dependent on birth weight and postnatal age.

"What is already known about this subject"

Propylene glycol is commonly used as an excipient in dose forms and is ingested by neonates when administering different drugs. While propylene glycol is generally considered to be safe, toxic effects like bradycardia, lactic acidosis and convulsions have been reported. Information on the pharmacokinetics of propylene glycol in neonates is lacking to provide insights on the possible risk of toxicity.

"What this paper adds"

This study describes the pharmacokinetics of propylene glycol in preterm and term neonates co-administered with paracetamol and phenobarbital. A pharmacokinetic model was developed which identified birth weight and postnatal age as important covariates for clearance. The model was used to simulate exposure to propylene glycol co-administered with both drugs.

7.1. Introduction

Since a substantial number of drugs have poor solubility or stability, excipients are often needed. Propylene glycol (PG) is a frequently applied cosolvent to increase the solubility and/or stability of several drugs like e.g. phenobarbital, paracetamol, lopinavir, ritonavir or lorazepam, compounds which are also often administered in neonates [1]. Although propylene glycol is generally regarded as safe, concentration related toxicity has been reported in the adult, pediatric and neonatal population and may involve bradycardia, depression of the central nervous system, increase in anion gap, lactic acidosis, hepatic dysfunction or kidney injury [1-4].

Little is known on the pharmacokinetics of propylene glycol in children. In adults, it has been described that approximately 45% of the administered dose of propylene glycol is eliminated through the kidney. The other 55% is metabolized through alcohol dehydrogenase in the liver to lactate and pyruvate and eventually to carbon dioxide and water [5-7]. While the elimination half-life of propylene glycol is estimated to be 2-5 hours in adults [2, 8], prolonged elimination half-lives of 10.8-30.5 hours have been reported in preterm neonates (< 1.5 kg) [5, 9]. In particular neonates and infants are therefore potentially at increased risk for toxic effects due to a more pronounced propylene glycol exposure [10]. In spite of this, current guidelines on the use of propylene glycol in drugs or food are limited and conflicting. Although the Food

and Drug Administration (FDA) as well as the European Medicine Agency (EMA) have developed guidelines concerning the safe use of propylene glycol, these guidelines vary largely between these agencies. The FDA established an acceptable daily intake of propylene glycol of 25 mg/kg bodyweight. EMA proposed a maximum daily dose of 400 mg/kg for adults and 200 mg/kg for children [11]. This discordance in the different guidelines reflects the lack of information on the safe use of propylene glycol in general, and of specific advices for the pediatric and neonatal age ranges in particular.

To date, to our best knowledge, no pharmacokinetic studies on propylene glycol have been performed in children nor in the full spectrum of neonates. Only a limited number of pediatric reports, exploring possible toxic effects of propylene glycol, are available [3, 12-15]. In this perspective, it is of relevance that the FDA recently warned on serious health problems in premature neonates receiving Kaletra®, which contains a combination of lopinavir and ritonavir dissolved in ethanol (356.3 mg ethanol/mL) and propylene glycol (152.7 mg/mL). Adverse events as cardiac, renal and respiratory problems were reported in premature neonates, likely due to a decreased ability to eliminate either ethanol, propylene glycol or both [16, 17].

Because of the conflicting guidelines and observations on the (in)tolerabililty to PG in neonates, the aim of this study was to characterize the pharmacokinetics of propylene glycol, when co-administered with intravenous paracetamol or phenobarbital in preterm and term neonates.

7.2. Methods

7.2.1. Patients

This pharmacokinetic analysis was based on observations collected in 68 (pre) term neonates from a previously published study [1] evaluating short-term clinical and biochemical tolerability to propylene glycol co-administered with intravenous paracetamol (Paracetamol Sintetica, Mendrisio, Italy) containing 800 mg propylene glycol per 1000 mg paracetamol solution or intravenous phenobarbital (Luminal Injektionlösung, Desitin Arzneimittel, Hamburg, Germany) containing 700 mg propylene glycol per 200 mg of phenobarbital. The study was conducted at the University Hospitals Leuven (Belgium) at the neonatal intensive care unit following approval by the local ethical board (B-32220084836) and study registration (PARANEO, EUdraCT 2009-011243-39, www.clinicaltrials.gov). Neonates were included after informed written parental consent. The decision to prescribe a source of intravenous PG, either paracetamol-PG or phenobarbital-PG, was made by the

attending physician and based on the clinical needs. For paracetamol, a loading dose of 20 mg/kg was given, followed by a maintenance dose of 5-10mg/kg every 6 hours, depending on postmenstrual age [1]. For phenobarbital, a loading dose of 20 mg/kg phenobarbital was given, followed by a maintenance dose of 5 mg/kg/day [18]. The number of samples in every individual neonate ranged from 1 to 11 collected between 20 minutes until 20.5 hours after dose administration. Six patients were considered as outliers due to unexplainably high concentrations of propylene glycol, likely caused by analytical interferences after visual inspection of the individual chromatographies. The clinical characteristics of the included patients (N=62) are summarized in table 1.

7.2.2. Analytical assay

Propylene glycol concentrations were determined by high performance liquid chromatography with photodiode array detection described by Kulo *et al.* [19]. The developed accurate, specific, sensitive and rapid method was validated for quantification of propylene glycol in low volume neonatal plasma (15-46 mg/L) and urine (20-175 mg/L). Samples with concentrations higher than this were re-analysed after dilution until they fell within the calibration range. The inter-assay and intra-assay precision was between 8.1 -14.1% and 2.3 -12.7% respectively while the lower limit of quantification was 0.25 mg/L.

7.2.3. Population pharmacokinetic analysis and Model evaluation

The population pharmacokinetic analysis was performed using the non-linear mixed effect modeling software NONMEM version 6.2. (Globomax LLC, Hanover, MD, USA). S-Plus, PsN and R were used for visualization and evaluation of the models. Development of the model was performed in four different steps: (i) choice

Table 1: Clinical characteristics of the patients, receiving propylene glycol co-administered with paracetamol, phenobarbital or both, presented as median (range).

Characteristics	Paracetamol	Phenobarbital	Paracetamol + Phenobarbital
Number of patients	34	25	3
Gestational age (weeks)	38 (24-41)	34 (27-40)	36 (35-37)
Postmenstrual age (weeks)	38 (25-41)	34 (28-46)	36 (35-37)
Postnatal age (days)	3 (1-28)	2 (1-82)	3 (2-5)
Birth weight (g)	2990 (630-3820)	1965 (815-3980)	2490 (2245-2514)
Current bodyweight (g)	2990 (700-4100)	1965 (780-3980)	2435 (2145-2490)

Birth weight = weight at day of birth, current bodyweight = weight at day of blood sampling

of the structural model, (ii) choice of the statistical sub-model, (iii) covariate analysis, (iv) model evaluation. The descriptive and predictive performance between different models was evaluated by different diagnostic tools [20]. A decrease in objective function (OFV) of 3.9 points or more was considered as a statistically significant difference ($p<0.05$ based on X2 distribution) for structural and statistical models while a more stringent p value of 0.005 was used for the evaluation of covariate models. In addition, goodness-of-fit plots, including observed *versus* individual predicted, observed *versus* population predicted, conditional weighted residuals *versus* time and conditional weighted residuals *versus* population predicted, were used for diagnostic purposes. Furthermore, the total number of parameters, visual improvement of individual plots, confidence intervals of parameter estimates, and correlation matrix were assessed as diagnostic criteria during model development. Finally, ill-conditioning [21] and shrinkage [22], which may occur in pediatric analyses [20], were determined.

7.2.4. Structural model

A one and two compartment model was fitted to the data. The interindividual variability in the pharmacokinetic parameters was assumed to follow a log normal distribution. The value of a particular parameter in an individual i (*post hoc* value) is given by the following equation:

$$\theta_i = \theta_{TV} \bullet e^{\eta i} \qquad \text{(Equation 1)}$$

in which θ_{TV} is the typical value of the parameter and η_i is assumed to be a random variable with mean value zero and variance ω^2. The residual variability was best described by a proportional error model. This means for the jth observed concentration of the ith individual the relation (Y_{ij}):

$$Y_{ij} = C_{pred,ij} \bullet (1+\varepsilon_{ij}) \qquad \text{(Equation 2)}$$

where C_{pred} is the predicted concentration and ε_{ij} is a random variable with a mean of zero and a variance of σ^2.

7.2.5. Covariate analysis

To visualize potential relationships between covariates and parameter estimates, plots of the individual *post hoc* parameter estimates and weighted residuals *versus* covariates were generated. The following covariates were evaluated: gestational age, postmenstrual age, postnatal age, birth weight (weight at day of birth) and current bodyweight (weight at day of blood sampling). Potential covariates were implemented into the model using a linear or allometric equation (equation 3).

$$P_i = P_p \bullet (\frac{Cov}{Cov_{Median}})^k \qquad \text{(Equation 3)}$$

In this equation Pi represents the individual parameter estimate of the ith subject, Pp equals the population parameter estimate, Cov is the covariate and k is the exponent which was fixed to 1 for a linear function or estimated for an allometric function.

Covariates were separately implemented into the model and considered statistically significant when the OFV decreased with at least 7.8 points (p value <0.005). When more than one covariate significantly reduced the OFV, the covariate causing the largest drop in OFV was left into the model. Additional covariates had to reduce this OFV further to be retained in the model. Subsequently, the contribution of each covariate was re-evaluated in the backward deletion for which a more stringent p value <0.001 (OFV 10.83 points) was used. To select the final covariate model, the individual and population predicted values were plotted against the most predictive covariate to evaluate whether the individual predicted parameters were equally distributed around the population predicted parameters [20]. The covariate model was further evaluated as discussed previously in the section Population Pharmacokinetic analysis. Finally, the results of the model validation procedure (see below) were also considered.

7.2.6. Internal validation

For the internal validation of the final pharmacokinetic model, two different evaluation tools were used. The first method was the bootstrap resampling method to evaluate model precision and stability. The 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 Consultants BV, Leiden, The Netherlands) in which 1000 replicates were generated. Parameter estimates obtained in the bootstrap analysis were compared to the parameter estimates of the original dataset.

For the second internal evaluation method, the normalized prediction distribution error method (NPDE) was used, which is a simulation-based diagnostic to determine the accuracy of the model [23, 24]. The observed and simulated concentrations were compared using the NPDE package in R. A histogram of the NPDE distribution and scatterplots showing the NPDE *versus* time and *versus* predicted concentration were used to evaluate the final model.

7.2.7. Model-based simulations for propylene glycol co-administered with paracetamol or phenobarbital

Using the final PK model, simulations were performed in three different patients (birth weight 630g, 1500g and 3500g and gestational age 24, 32, 40 weeks) with a postnatal age of 1 and 28 days. The current bodyweight at a postnatal age of 28 days was 950g, 1950g and 4100 g, respectively. These three patients were selected to cover the entire population of the current study in terms of gestational age and bodyweight. The parameter estimates obtained in the final pharmacokinetic model were used to simulate concentrations of propylene glycol after administration of intravenous paracetamol (Paracetamol Sintetica, Mendrisio, Italy: 800mg PG/ 1000mg paracetamol) or intravenous phenobarbital (Luminal Injektionlösung, Desitin Arzneimittel, Hamburg, Germany: 700mg PG/ 200mg phenobarbital) in the dosing regimens applied in this study. For paracetamol, a loading dose of 20 mg/kg was given, followed by a maintenance dose of 10mg/kg every 6 hours [1]. For phenobarbital, a loading dose of 20 mg/kg phenobarbital was given, followed by a maintenance dose of 5 mg/kg/day [18].

7.2.8. Maximally acceptable levels of propylene glycol in neonates

Different approaches were applied to provide a basis for maximally acceptable concentrations of propylene glycol in neonates. First, the exposure to propylene glycol upon administration of propylene glycol as a result of paracetamol or phenobarbital was compared to levels observed in a previously published study in 68 preterm and term neonates in which tolerability of propylene glycol was evaluated and no toxic effects were reported [1]. In a second approach, a maximum concentration was defined on basis of the toxic effects related to the osmolar changes. The increase in osmolar gap can directly be linked to propylene glycol concentrations by the following relationship [2]: [osmolar gap = concentration of propylene glycol (mg/

dL) / 7.6] while osmolar gap is considered the first indicator of propylene glycol accumulation before propylene glycol toxicity appears related to other metabolic disturbances or clinical symptoms [6]. In a study of Yahwak *et al.* [25] in adults, an increase in osmolar gap of 10 mOsm/L was linked to elevated propylene glycol concentrations and an increase of 12 mOsm/L resulted in clinical changes suggestive of propylene glycol toxicity. Furthermore, in studies by Feldman *et al.* [26] and Giacoia *et al.* [27], a standard deviation of 8 mOsm/L in serum osmolality has been described in neonates. Based on these observations, we considered the maximum allowed propylene glycol plasma concentration to remain below 608 mg/L, which corresponds to a maximum change in osmolar gap of 8 mOsm/L. The proposed maximum concentration of 608 mg/L is in close agreement with previously published results by Wilson *et al.* [6] in which metabolic abnormalities were reported for concentrations ranging between 580 and 1270 mg/L [6]. However our proposed maximum concentration of propylene glycol of 608 mg/L should be viewed with caution since it is only based on findings reported in literature, for adult patients. It is therefore not validated in neonates. Finally, a third possible maximum safe concentration was identified by performing

Table II: Model-based population pharmacokinetic parameter estimates and the values obtained after the bootstrap analysis.

Parameter	Simple model without covariates Value (CV%)	Final pharmacokinetic covariate model Value (CV%)	Bootstrap final pharmacokinetic model Value (CV%)
Fixed effects			
CL (L/h) = CLp	0.060 (11.8)	-	-
CLp in CL= CLp x (bBW/median)^m x (PNA/median)^n	-	0.085 (4.9)	0.085 (5.24)
m	-	1.69 (10.2)	1.68 (11.44)
n	-	0.20 (31.9)	0.20 (37.62)
V (L) = Vp	0.90 (10.2)	-	-
Vp in V = Vp x (cBW/median)^o x p	-	0.97 (6.58)	0.97 (7.05)
o	-	1.45 (10.4)	1.45 (11.28)
p (phenobarbital)	-	1.77 (12.1)	1.79 (13.10)
Interindividual variability (ω^2)			
ω^2 (CL)	0.69 (23.9)	0.12 (26.3)	0.11 (30.91)
ω^2 (V)	0.64 (23.9)	0.18 (25.6)	0.17 (27.99)
Residual Variability (σ^2)			
σ^2 (proportional)	0.036 (12.1)	0.036 (11.8)	0.036 (11.40)

CL = Clearance, CLp = population value for clearance, V = Volume of distribution, Vp = population value for volume, bBW = bodyweight at birth, cBW = current bodyweight, PNA = postnatal age

simulations based on the guidelines for propylene glycol administration in children established by the EMA (200 mg/kg/day) and the FDA (25 mg/kg/day). To the very best of our knowledge, these guidelines are neither supported by observational data. In these simulations 100 mg or 12.5 mg of propylene glycol depending on the guidelines by the EMA or FDA, respectively was administered in three different neonates (bBW 630 g, 1500 g and 3500g) every 12 hours since drugs containing propylene glycol are often given in this manner in clinical practice in neonates. It was simulated to be given by a bolus injection over 15 min to illustrate the highest potential exposure to propylene glycol.

7.3. Results

7.3.1. Patients

The pharmacokinetic analysis was based on 372 observations obtained from 62 neonates. The number of samples taken per neonate ranged between 1-11. Thirty-four neonates received propylene glycol by intravenous administration of paracetamol compared to twenty-five neonates who received phenobarbital while three neonates receiving a combination of both paracetamol and phenobarbital. Patient characteristics are summarized in table 1.

7.3.2. Structural pharmacokinetic model

A one compartment model parameterized in terms of clearance and volume of distribution with a proportional error model best described the plasma concentrations of propylene glycol.

7.3.3. Covariate analysis

In the systematic covariate analysis, birth weight was found the most important covariate for clearance causing a drop in OFV of 82 points ($p<0.001$). Birth weight was best implemented on clearance using an allometric function in which a value of 1.69 was estimated for the exponent. When evaluating other covariates, current weight was found the most important covariate for volume of distribution using an allometric function with an estimated exponent of 1.48 (ΔOFV 48 points, $p<0.001$). Furthermore, a significant difference in volume of distribution was seen between neonates receiving phenobarbital and paracetamol. The volume of distribution was estimated to be 1.77 times higher (95% confidence interval: 1.35-2.19) for neonates receiving phenobarbital (ΔOFV 18 points, $p<0.001$). Finally, further improvement

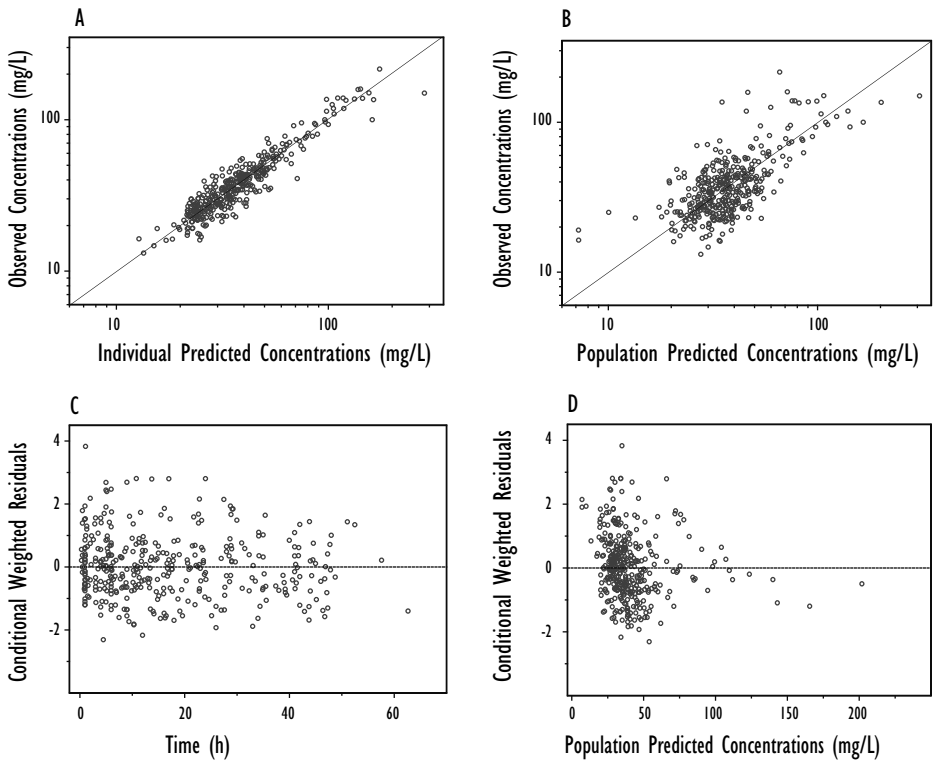

Figure 1: Diagnostic plots for the final pharmacokinetic model: (a) Observed *versus* individual predicted concentrations, (b) Observed *versus* population predicted concentrations, (c) Conditional weighted residuals *versus* time, (d) Conditional weighted residuals *versus* population predicted concentrations.

of the model fit was seen when postnatal age was introduced on clearance using an allometric function with an estimated exponent of 0.201. This last covariate was responsible for the smallest but still significant drop in the objective function (Δ OFV = 15 points, $p<0.001$). All parameter estimates of the final pharmacokinetic model are summarized in table II. The diagnostic plots are represented in figure 1. By introducing these covariates a large part of the interindividual variability on clearance (65%) as well as on volume of distribution (53%) is explained (table II). This is reflected by the estimates of interindividual variability in clearance and volume of distribution which were reduced from 0.69 to 0.12 and 0.64 to 0.18, respectively.

7.3.4. Model validation

The values for the parameter estimates obtained during the bootstrap procedure

are shown in table II. The parameter estimates obtained after bootstrapping were within 8% of the values obtained in the final pharmacokinetic model. Of the total number of runs (N=1000), 100% was successful, only 34 runs did not have a covariance step.

The results of the NPDE analysis are depicted in figure 2. The histogram follows the normal distribution indicated by the black solid line (figure 2a). No trend is seen in the NPDE *versus* time (figure 2b) and the NPDE *versus* predicted concentrations (figure2c). The plot with the individual predicted parameter estimates and population parameter estimates for clearance and volume of distribution *versus* the most predictive covariate, birth weight and current bodyweight respectively, showed that the individual predicted parameter estimates are randomly scattered around the population parameter estimates (figures not provided). The number of ill-conditioning (8.28) was far below the critical value of 1000 meaning that the final pharmacokinetic model is not over-parameterized. Finally, η-shrinkage expressed as a percentage was identified to be below 20% for clearance (14.8%) and volume of distribution (6.2%).

The model-based predicted clearance values for the final pharmacokinetic model *versus* birth weight for PNA 1, 7, 14, 21 and 28 days are shown in figure 3.

7.3.5. Model-based simulations for propylene glycol co-administered with paracetamol or phenobarbital

Concentration-time profiles of propylene glycol after standard dosing regimens of intravenous paracetamol (800mg PG/1000mg paracetamol) or phenobarbital (700mg PG/200mg phenobarbital) that were used in this study, were simulated in three different neonates (bBW 630g, 1500g and 3500g, respectively) at a postnatal

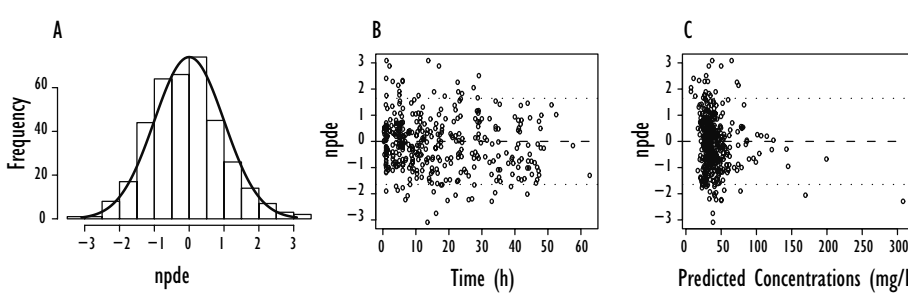

Figure 2: Results of the NPDE analysis: (a) the histogram shows the NPDE distribution, the solid line indicates a normal distribution, (b) NPDE *versus* time after first dose, (c) NPDE *versus* predicted concentrations.

Table III: Propylene glycol (PG) dosages when co-administered with paracetamol or phenobarbital in currently used dosages.

Drug	Propylene glycol content	Dosing guideline for drug	Drug-associated daily dose propylene glycol (mg/kg/day)	Ref.
IV Paracetamol 10mg/mL	800 mg PG/1000 mg paracetamol	Loading dose: 20 mg/kg	40	(1)
		Maintenance dose: 10 mg/kg every 6 hours	16-32	
IV Phenobarbital 200 mg/mL	700 mg PG/200 mg phenobarbital	Loading dose: 20 mg/kg/day	70	(18)
		Maintenance dose: 5 mg/kg/day	17.5	

age of 1 and 28 days (figure 4). The administered dose of paracetamol, phenobarbital and the corresponding dose of propylene glycol are given in table III. Figure 4 shows that population mean value for trough and peak concentration of propylene glycol co-administered with paracetamol for a neonate of 630 g at day 1 was estimated to be 109 and 144 mg/L, respectively, and for a neonates of 3500g at day 28 trough and peak concentration of propylene was estimated to be 19 and 33 mg/L, respectively.

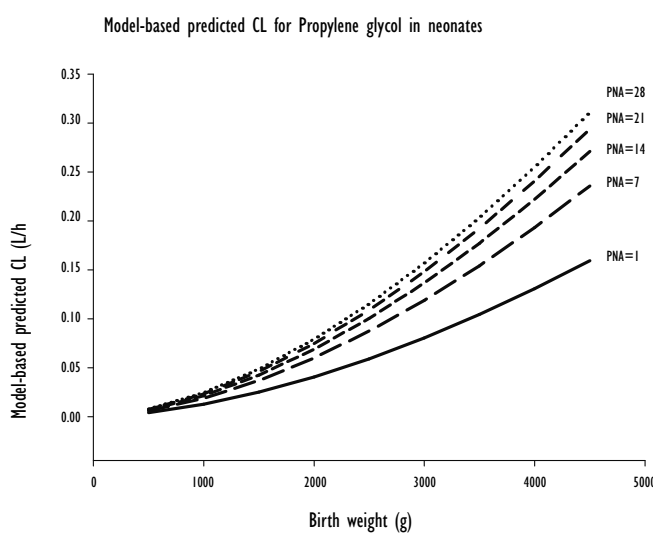

Figure 3: Model-based predicted clearance values of propylene glycol *versus* birth weight for postnatal age (PNA) of 0, 7, 14, 21 and 28 days.

The expected population mean peak and trough propylene glycol concentrations after administration of phenobarbital varied between 28-218 and 6-112 mg/L, respectively, depending on birth weight (630g-3500g) and postnatal age (1-28 days) of the neonate (table III, figure 4).

7.4. Discussion

While propylene glycol is considered to be safe and inactive, upon high concentrations toxic effects like lactic acidosis, bradycardia and convulsions may occur. The risk of propylene glycol toxicity is higher in infants and neonates compared to adults since they have a lower metabolic capacity as well as an immature renal function resulting in a lower elimination capacity. The aim of this study was to characterize the pharmacokinetics of propylene glycol and its covariates in neonates following intravenous administration.

The pharmacokinetic model developed in this study was based on 372 propylene glycol plasma concentrations obtained in 62 preterm and term neonates after administration of paracetamol, phenobarbital or both. Birth weight was found the most important covariate for clearance while an increase in clearance was seen with postnatal age. The population value for clearance of 0.0849 L/h reported here in neonates is very low compared to the clearance value reported in adults which was found to vary between 144-390 mL/min/1.73m2 (8.64-23.4 L/h/1.73m2) [5]. This may indicate that either the alcohol dehydrogenase enzyme pathway or primary renal elimination, or most likely both, are immature during the first month of life. For renal function this has been described before by studying amikacin clearance in neonates, which likely reflects glomerular filtration in neonates [28] . The model-based predicted clearance values of propylene glycol *versus* birth weight for postnatal age 1, 7, 14, 21 and 28 are shown in figure 3. Large differences in clearance values are seen between neonates of 1 kg (0.013 L/h) and neonates of 4 kg (0.13 L/h) at day of birth. This 10-fold difference in clearance is still seen one month after birth. Furthermore this figure illustrates that during the first two weeks of life the largest increase in clearance is observed. These results correspond well with the advice of the FDA to avoid Kaletra®, a propylene glycol containing oral solution in premature babies until 14 days after due date, or in full-term babies younger than 14 days postnatal age [16, 17]. Volume of distribution scaled with current weight and was estimated 1.77 times higher in neonates receiving phenobarbital compared to neonates receiving paracetamol. The volume of distribution of a neonate of 1 kg (0.23L or 0.40L) (co-administered with paracetamol or phenobarbital, respectively) was very different

compared to a neonate of 4 kg (1.69 L or 3L). This difference may possibly be explained by the fact that phenobarbital is often given to neonates after perinatal asphyxia which may lead to a change in the pharmacokinetic parameters e.g. higher volume of distribution. Unfortunately asphyxia could not be investigated as a covariate since no potential indicators (e.g. Apgar score, serum lactate concentration) were identified. The large variability in clearance and volume of distribution as a result of birth weight, PNA and current weight is reflected by the large range in expected peak and trough concentrations that can be expected upon commonly used doses of paracetamol and phenobarbital in neonates varying in birth weight between 630g and 3500g and between a PNA of 1-28 days (figure 4). The stability and predictability of the final pharmacokinetic model was demonstrated by the bootstrap (table II) as well as the NPDE (figure 2), which are both advanced validation methods for paediatric pharmacokinetic models.

Although dose-related toxic effects have been reported upon administration of propylene glycol, only a limited number of pediatric reports are available in literature. Glasgow *et al.* [13] and MacDonald *et al.* [14] described hyperosmolality and clinical symptoms of propylene glycol toxicity in small infants (< 1500 g birth weight) due

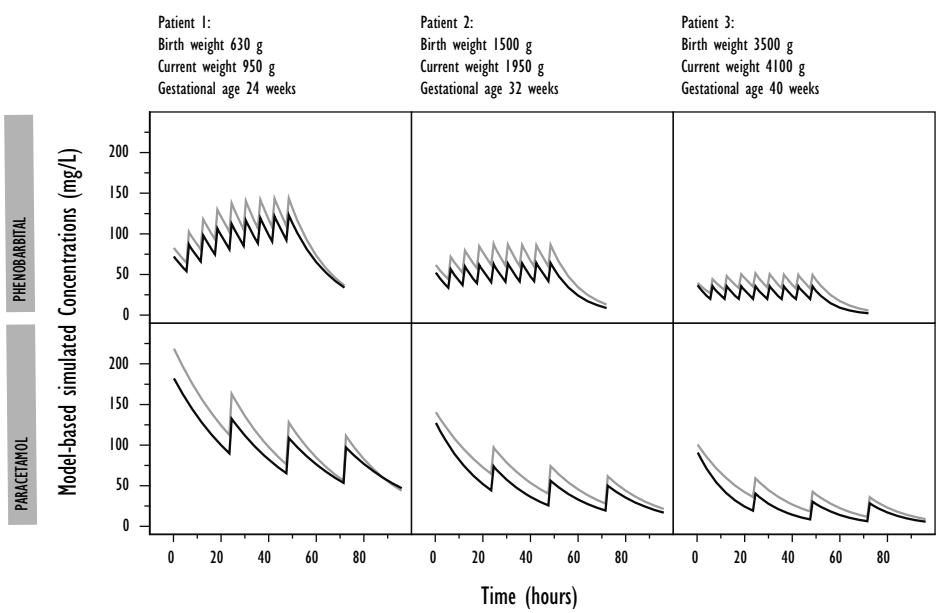

Figure 4: Model-based simulated concentration-time profiles of propylene glycol for three neonates (birth weight 630g, 1500g and 3500 g) after administration of paracetamol (800mg propylene glycol/1000mg paracetamol, upper panel) and phenobarbital (700mg propylene glycol/200mg phenobarbital, lower panel) in doses according to table III. The grey lines illustrate the concentration-time profiles for the neonates at birth. The black lines represent the concentration-time profiles at a postnatal age of 28 days (current weight 950g, 1950g and 4100g).

to very high propylene glycol exposure (3000mg/kg) in multivitamins injections. In retrospective studies of Shehab *et al.* [3] and Whittaker *et al.* [15] it was concluded that neonates at the neonatal intensive care unit are indeed exposed to potentially toxic doses of propylene glycol due to administration of commonly used drugs (e.g. phenobarbital, lorazepam, phenytoin, paracetamol) cosolved in propylene glycol but data on toxicity were not reported. In a study of Chicella *et al.* [12] a propylene glycol containing lorazepam formulation was administered to 11 infants between 1-15 months of age. In this study, there were neither clinical nor laboratory abnormalities observed, but accumulation of propylene glycol occurred during continuous infusion of lorazepam. Consequently, propylene glycol containing formulations should be used with caution in the pediatric and certainly in the neonatal age range especially when this results in high PG exposure. Based on literature, the first indicator of a risk for subsequent propylene toxicity is propylene glycol accumulation and changes in osmolar gap. Accumulation may subsequently result in biochemical changes and eventually toxic effects like e.g. bradycardia, hepatic or renal injury, depression of the central nervous system.

To provide a basis to interpret the simulated concentrations of PG co-administered with paracetamol or phenobarbital in neonates, different approaches were provided in the methods section. However to identify maximum safe concentrations, more pharmacokinetic and pharmacodynamic studies are needed in neonates, particularly with drugs containing high concentrations of propylene glycol. To illustrate this concept, simulations were performed to illustrate the potential exposure of propylene glycol co-administered with lorazepam (828mg PG/2mg lorazepam). Based on the final pharmacokinetic model of propylene glycol co-administered with paracetamol, substantially higher concentrations of PG are obtained depending on the dose of lorazepam. Simulated propylene glycol concentrations upon lorazepam in a dose of 0.015 mg/kg/h [18] (daily dose of 149 mg/kg/day of propylene glycol) varied between 540 mg/L for a neonate of 630g at day 1 and 123 mg/L for a neonate of 3500g at day 28. Upon a dose of lorazepam of 0.1 mg/kg/day as described by Chicella *et al.* [12], concentrations of propylene glycol varying between 798-3563 mg/L were obtained, depending on birth weight and postnatal age. It should be noted that these concentrations are generated under the assumption of linear pharmacokinetics of propylene glycol, while higher daily doses of propylene glycol were administered to the neonates (149 mg/kg/day or 996 mg /kg/day) with lorazepam compared to paracetamol or phenobarbital. As a result of the assumption of linear pharmacokinetics, the estimates of the exposure to propylene glycol must be considered conservative.

In case of non-linearity in pharmacokinetics, even higher exposures are expected. At least, PG accumulation upon the lorazepam dosing in neonates is in line with PG accumulation and toxicity described in adults [2, 6].

7.5. Conclusion

A pharmacokinetic model with birth weight and postnatal age as covariates for clearance was developed for propylene glycol co-administered with paracetamol or phenobarbital in preterm and term neonates. As such, large variability in exposure of propylene glycol may be expected in neonates which are dependent on birth weight and postnatal age. The model can be used to simulate concentrations of propylene glycol co-administered with paracetamol and phenobarbital in neonates. As the exact safe concentrations are still undefined, more studies are needed to characterize the pharmacokinetics of propylene glycol in neonates and children.

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Chapter 8

Low but Inducible Contribution of Renal Elimination to Clearance of Propylene Glycol in Preterm and Term Neonates

Roosmarijn F.W. De Cock, Karel Allegaert, Sophie Vanhaesebrouck, Jan de Hoon, Rene Verbesselt, Meindert Danhof, Catherijne A.J. Knibbe

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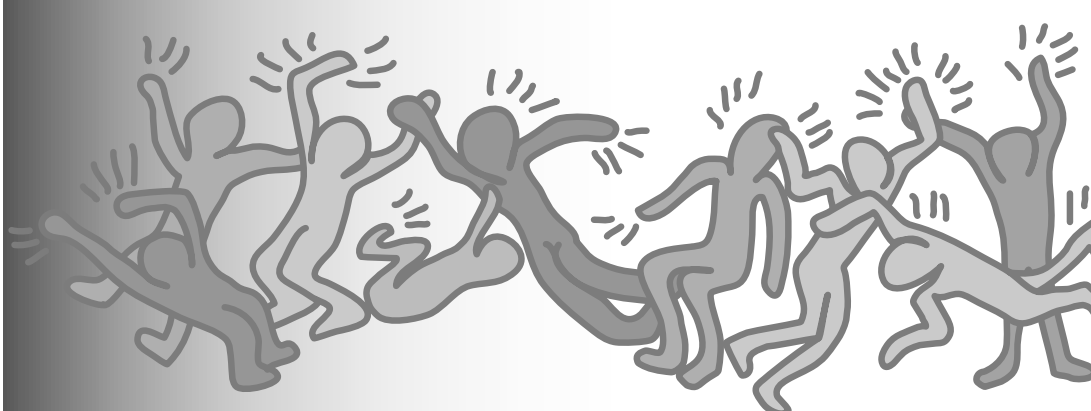


Abstract

Background

Despite limited information on the pharmacokinetics of excipients, propylene glycol (PG) is often used as a excipient both in adults and children. The aim of this study is to characterize renal and hepatic elimination of propylene glycol in preterm and term neonates.

Methods

The pharmacokinetic analysis of PG was performed in NONMEM 6.2. on the basis of PG concentrations in plasma and/or urine samples for a total of 69 (pre)term neonates (birth weight 630-3980g, gestational age 24-41 weeks, postnatal age 1-29 days) who received PG co-administered with IV paracetamol (5-10 mg/kg/6 hours), phenobarbital (5 mg/kg/day) or both. To capture the time dependent trend in renal excretion of PG, different models based on time after first dose, urine volume and creatinine amount in urine were tested.

Results

A one compartment model parameterized in terms of renal clearance, hepatic clearance and volume of distribution was found to adequately describe the observations in both plasma and urine. After the first dose, renal elimination of propylene glycol was 15% of total clearance, which increased over time to 25% at 24 hours after the first dose of PG. This increase was best described using a hyperbolic function based on time after the first dose.

Conclusions

Renal elimination of PG in (pre)term neonates is low, particularly compared to the reported percentage of 45% in adults, but may increase with time after first dose of PG. To study whether this increase is caused by an auto-induced increase in renal secretion or a reduction of tubular reabsorption of PG, further research is needed.

8.1. Introduction

Propylene glycol is a frequently used excipient in many drug formulations to increase solubility and/or stability of drugs. Although excipients may be expected to be inactive compounds, toxic effects related to propylene glycol such as bradycardia, lactic acidosis, hepatic and renal dysfunction, increase in anion gap, have been described in adults, children and neonates [1-5]. As a consequence, both the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) have established guidelines considering maximum daily doses of propylene glycol. However, no conformity is seen between the guidelines of both agencies [6]. While this may be explained by a lack of specific information relating to the safe use of propylene glycol, it is emphasized that the available knowledge on the pharmacokinetics of propylene glycol mainly focuses on adults. However children and certainly neonates are at increased risk for the toxic effects of propylene glycol due to accumulation of propylene glycol [7-9], which is reflected by the prolonged elimination half-life in preterm neonates (10.8-30.5 hours) [10] compared to adults (2-5 hours) [1, 11].

In adults, it is known that 45% of propylene glycol is eliminated through the renal route and 55% is metabolized in the liver by alcohol dehydrogenase (ADH) to lactate and pyruvate [8, 12, 13]. Previously it has been reported that the activity of alcohol dehydrogenase is reduced in neonates reaching adult levels at about 5 years of age [14]. Due to immaturity of the renal function, renal clearance of propylene glycol may also be expected to be lower in neonates compared to adults. Assuming that maturation processes in the liver and kidneys may differ in rate and nature, one pathway or the other may be more relevant for a specific age group resulting in for instance age-specific drug-drug interactions for the pathway concerned. Even though based on plasma observations the pharmacokinetics of propylene glycol have recently been characterized in preterm and term neonates [15], no information is available on the contribution of renal elimination to the overall clearance of propylene glycol in neonates. The aim of this study was therefore to characterize the pharmacokinetics of propylene glycol differentiating between renal elimination and hepatic metabolic pathways based on propylene glycol observations in both plasma and urine in preterm and term neonates.

8.2. Methods

8.2.1. Patients and Data

The analysis was based on data of 69 preterm and term neonates consisting of previously published observations of propylene glycol in plasma [3, 15] and observations of propylene glycol in urine. Urine samples could only be collected in neonates with a urinary bladder catheter already in place for clinical indications. An overview of the clinical characteristics is given in table 1. Propylene glycol was co-administered with either intravenous paracetamol (Paracetamol Sintetica, Mendrisio, Switzerland) containing 800 mg propylene glycol per 1000 mg paracetamol solution or intravenous phenobarbital (Luminal Injektionlösung, Desitin Arzneimittel, Hamburg, Germany) containing 700 mg propylene glycol per 200 mg of phenobarbital or both. For paracetamol, a loading dose of 20 mg/kg was given followed by a maintenance dose of 5-10 mg/kg every 6 hours. This corresponds to a dose of propylene glycol varying between 16-40 mg/kg/day. For phenobarbital, a loading dose of 20 mg/kg was given followed by a maintenance dose of 5 mg/kg/day. This corresponds to a dose of 70 mg/kg/day of propylene glycol when a loading dose is given and 17.5 mg/kg/day when a maintenance dose is given. In 46 neonates, plasma samples of propylene glycol which was co-administered with paracetamol, phenobarbital or both, was available while in 16 neonates both urine and plasma samples of propylene glycol co-administered with paracetamol were available. In 7 neonates, only urine samples of propylene glycol co-administered with paracetamol were available. Urine samples were available in 6 hour fractions collected over 24 hours. In one patient, urine was collected every 6 hours with a maximum of 48 hours instead of 24 hours after administration of the first dose. The study was conducted in Leuven NICU following approval by the local ethical board of the University Hospitals Leuven (B-32220084836).

8.2.2. Analytical method

Propylene glycol was determined in low volume neonate plasma (15-46 mg/L) and urine (20-175 mg/L) by high performance liquid chromatography with photodiode array detection as described by Kulo *et al* [16]. Samples with concentrations higher than the validation range were re-analyzed after appropriate dilution until they fell into the validation range. Plasma samples were diluted with drug-free human plasma and urine samples with water.

8.2.3. Pharmacokinetic analysis: Model development

The non-linear mixed effect modeling software (NONMEM 6.2) (Globomax LLC, Hanover, MD, USA) using the first-order conditional estimation method with the interaction option (FOCE-I) was used to perform the population pharmacokinetic analysis. Tools like S-Plus version 6.2.1 (Insightful software, Seattle, WA) with NM.SP. interface version 05.03.01 (© by LAP&P Consultants BV, Leiden, The Netherlands), PsN and R (version 2.10.1) were used to visualize and evaluate the model.

The model was developed in four different steps: (i) choice of the structural model, (ii) choice of the statistical sub-model, (iii) choice of the covariate model, (iv) model evaluation. A decrease in objective function (OFV) of 3.9 points or more was considered statistically significant ($p<0.05$ based on X2 distribution, for nested models). In addition, the following goodness-of-fit plots were evaluated for diagnostic purposes: (i) observed *versus* individual predicted concentrations, (ii) observed *versus* population predicted concentrations, (iii) conditional weighted residuals *versus* time, (iv)conditional weighted residuals *versus* population predicted concentrations. Finally, the total number of parameters, visual improvement of individual plots, correlation matrix, confidence intervals of parameter estimates, ill-conditioning [17] and shrinkage [18] were assessed.

Table 1: Clinical characteristics (median (range)) of the studied patient population receiving propylene glycol (PG) co-administered with intravenous paracetamol, phenobarbital or both.

Characteristics	All patients	Patients with plasma data only	Patients with both plasma and urine data	Patients with urine data only
Number of patients	69	46	16	7
Number of samples per patient	1-18	1-11	5-18	4
Co-administered drug	paracetamol, phenobarbital or both	paracetamol, phenobarbital or both	paracetamol	paracetamol
Gestational age (weeks)	37 (24-41)	35 (25-40)	37.5 (24-41)	37 (30-39)
Postmenstrual age (weeks)	37 (25-46)	34 (25-46)	38 (26-41)	38 (30-39)
Postnatal age (days)	3 (1-82)	1 (1-82)	3 (1-28)	2 (2-11)
Birth weight (g)	2720 (630-3980)	2200 (700-3980)	3050(630-3600)	2650 (1160-3315)
Current bodyweight (g)	2720 (700-4100)	2490 (700-3980)	3030 (630-4100)	2560 (1160-3315)
Urine Volume (mL/6 hours)	48 (14-115)	-	48 (14-0.115)	5 (17-113)

Birth weight = bodyweight at day of birth. Current bodyweight = bodyweight at day of blood sampling. PG = propylene glycol

Structural model

For the structural model, a one compartment model was developed using NONMEM VI, subroutine ADVAN6, TOL=3. The concentrations of propylene glycol in plasma were expressed as mg per L. The excretion of propylene glycol in urine was expressed as a cumulative amount in mg and calculated by multiplying the urinary concentration (mg/L) with urine volume.

Statistical submodel

For the statistical submodel, the interindividual variability was tested assuming a log-normal distribution in an individual *i* (*post hoc* value) and is given by the following equation:

$$\theta_i = \theta_{TV} \bullet e^{\eta i} \qquad \text{(Equation 1)}$$

in which θ_{TV} is the typical value of the parameter and η_i is assumed to be a random variable with mean value zero and variance ω^2. For the intra-individual variability and residual error, proportional (equation 2), additive (equation 3) and combination (equation 4) error models were tested:

$$Y_{ij} = C_{pred,ij} \bullet (1+\varepsilon_{ij}) \qquad \text{(Equation 2)}$$

$$Y_{ij} = C_{pred,ij} + \varepsilon_{ij} \qquad \text{(Equation 3)}$$

$$Y_{ij} = C_{pred,ij} \bullet (1+\varepsilon_{1,ij}) + \varepsilon_{2,ij} \qquad \text{(Equation 4)}$$

where Y_{ij} is the *j*th observation in the *i*th individual, $C_{pred,ij}$ is the predicted concentration and ε_{ij} is a random variable from a normal distribution with a mean of zero and estimated variance of σ^2.

Covariate analysis

A comprehensive covariate analysis was performed in which the following covariates were evaluated: gestational age, postmenstrual age, postnatal age, birth weight (weight at day of birth), current bodyweight (weight at day of blood or urine sampling), time after first dose, urine volume, urine volume/kg, amount of creatinine in urine (mg). Covariates were implemented into the model using a linear or power equation (equation 5):

$$P_i = P_p \bullet (\frac{Cov}{Cov_{Median}})^k$$ (Equation 5)

In this equation P_i represents the individual parameter estimate of the *i*th subject, Pp equals the population parameter estimate, Cov is the covariate and k is the exponent, which was fixed to 1 for a linear function or estimated for a power function. Specific covariate models that were also tested to capture the time-dependent trend in renal elimination of PG are described below under Characterization of changes in renal clearance of propylene glycol over time. A covariate was considered to be statistically significant when causing a decrease in objective function of 7.8 points (p-value <0.005). When more than 1 covariate significantly reduced the OFV, the most significant covariate was retained into the model. This model was subsequently considered as the basis for the inclusion of additional covariates. The contribution of each covariate was then re-evaluated in the backward deletion for which a more stringent p-value <0.001 (ΔOFV 10.8 points) was used. In addition, the individual and population predicted parameters were plotted against the most predictive covariate to evaluate whether the individual predicted parameters were equally distributed around the population predicted parameters [19]. Finally the covariate model was evaluated as mentioned previously under Pharmacokinetic analysis: model development, whereby the results of the Model validation were also considered.

Characterization of changes in renal clearance of propylene glycol over time

As a systematic trend in propylene glycol amounts in urine over time after first dose was observed, different models were tested.

Model I. Implementation of an (intermediate) Michaelis-Menten model [20] for renal clearance of propylene glycol.

Model II. Implementation of urine volume normalized to kilograms current bodyweight as a covariate on renal clearance (equation 5).

Model III. Implementation of both time after first dose and current bodyweight as covariates on renal clearance of propylene glycol (equation 5).

Model IV: Implementation of a hyperbolic model based on time after first dose on renal clearance (equation 6):

$$CL_{Ri} = CL_{max} \bullet \left(\frac{Time^{k}}{Time_{50}^{k} + Time^{k}} \right)$$

(Equation 6)

In equation 6, CL_{Ri} equals the individual renal clearance of the ith subject, CL_{max} represents the maximum renal clearance of propylene glycol, Time represents time after first dose, Time50 is the time after first dose at which half of the maximum clearance is reached and k is the estimated exponent. Based on observations in adults [8, 12, 13], CL_{max} was fixed at 0.45/0.55 of the hepatic clearance, which indicates that renal elimination also increases in correspondence with changes in hepatic clearance.

Model V. Implementation of amounts of creatinine, determined in each of the 6-hourly urine fractions, as a covariate on renal clearance of propylene glycol.

8.2.4. Model Validation

A bootstrap was performed in S-plus, version 6.2.1. (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P Consultants BV, Leiden, The Netherlands) in which 1000 replicates were generated. The parameter estimates obtained in the bootstrap were subsequently compared to the parameter estimates of the original dataset.

In addition, due to the small number of subjects in whom urine data were available, a n-1 jackknife was performed to evaluate the stability of the model when one subject with urine data at a time was removed from the dataset.

Finally the normalized prediction distribution error method (NPDE) was used as a simulation-based diagnostic. The dataset was again simulated 1000 times, after which the observed and simulated concentrations were compared using the NPDE package in R [21, 22].

8.2.5. Simulations

Simulations were performed in NONMEM 6.2 based on the final pharmacokinetic model to simulate renal and hepatic clearance of propylene glycol across the individuals of the current study (birth weight 630g, 1500g, 2500g and 3500g and with a postnatal age of 1 and 28 days). In the simulations, four consecutive doses of propylene glycol co-administered with paracetamol were given. Simulations were performed with the exclusion of the interindividual and residual variability in order to demonstrate the exact influence of the covariates identified in this study.

8.3. Results

8.3.1. Patients and data

Patient and data characteristics are summarized in table 1. The pharmacokinetic analysis was based on 372 plasma concentrations of propylene glycol co-administered with intravenous paracetamol, phenobarbital or both and 79 urine samples of propylene glycol co-administered with paracetamol (table 1). Postnatal age for all patients varied between 1 and 29 days, except for one patient with a postnatal age of 82 days.

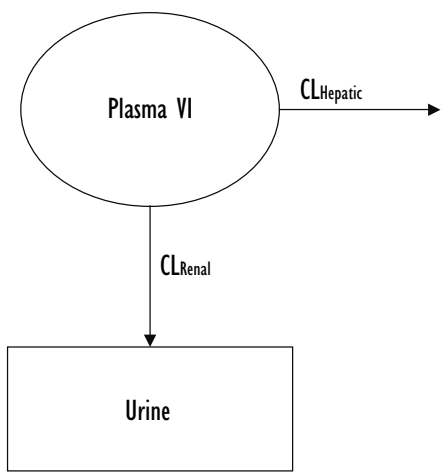


Figure 1: Schematic representation of the pharmacokinetic model of propylene glycol.

8.3.2. Pharmacokinetic analysis: Model development

Structural Model

Based on propylene glycol samples in both plasma and urine, a one compartment model was used, parameterized in terms of hepatic clearance (CL_H), renal clearance (CL_R) and volume of distribution (Vl). A schematic representation of the model is given in figure 1. A different proportional error was estimated for plasma and urine concentrations.

Covariate Model

A/ Hepatic clearance of propylene glycol

For hepatic clearance, birth weight was found as the most important covariate causing a drop in OFV of 81.5 points ($p<0.001$), when implemented using a power function. Subsequently, current bodyweight was identified as most important covariate on volume of distribution also using a power function, causing a drop in OFV of 50 points ($p<0.001$). Furthermore, the volume of distribution was estimated to be 1.71 times higher between neonates receiving phenobarbital compared to neonates receiving paracetamol, as was reported before [15]. This may possibly be explained by the fact that phenobarbital is often given to neonates after perinatal asphyxia which may cause differences in pharmacokinetic parameters like an increase in volume of distribution [15]. Finally postnatal age (PNA) was found as significant covariate on hepatic clearance. In this analysis, PNA was best implemented using a linear function, causing a drop in OFV of 20 points ($p<0.001$). After introducing these covariates, the observed plasma concentrations were well described by the model (figure 2, upper panels).

B/ Renal clearance of propylene glycol

When evaluating the amounts of propylene glycol in urine, a systematic trend in conditional weighted residuals *versus* time (figure 3) was seen, which could not be explained by bodyweight or PNA. To elucidate this time-dependent trend, which indicates that the excretion of propylene glycol in urine increases over time after first dose, five different models were tested as explained in the methods section.

As a first approach, a non-linear pharmacokinetic model was tested by implementation of an (intermediate) Michaelis-Menten model on renal clearance (Model I). Even though the time-dependent trend could indicate non-linear pharmacokinetic behavior of renal excretion of propylene glycol, this model did not result in an improved fit. As a second approach, urine volume (normalized to current bodyweight) was added as a covariate. This linear implementation of urine volume on renal clearance showed a significant improvement of the model illustrated by the decrease in objective function (ΔOFV 26 points, $p<0.001$) and improvement of the diagnostic plots. In model III, both time after first dose and current bodyweight were implemented as covariates on renal clearance using power functions. Compared to model II, a higher drop in objective function was seen in model III (ΔOFV 69 points, $p<0.001$), which was also reflected by visual improvement of the diagnostic plots. In model IV, implementation of the hyperbolic model based on time after first dose in

model IV, resulted in a drop in objective function of 67 points ($p<0.001$). In model V, no correlation was found between the amount of propylene glycol and creatinine in urine. Therefore implementation of creatinine did not result in an improved fit.

Based on the results, model III and model IV were identified as the best models to describe the time-dependent trend in the excretion of propylene glycol in urine. Although model IV had a slightly higher objective function compared to model III (Δ OFV = 2 points, $p>0.05$)), model IV was chosen as the best model since model IV was able to describe the data evenly well with one parameter less compared to model III. This hyperbolic function included an exponent and a parameter to describe the time after the first dose when 50% of the maximum renal clearance is reached, which were estimated to be 0.69 and 42.2 hours, respectively (table 2).

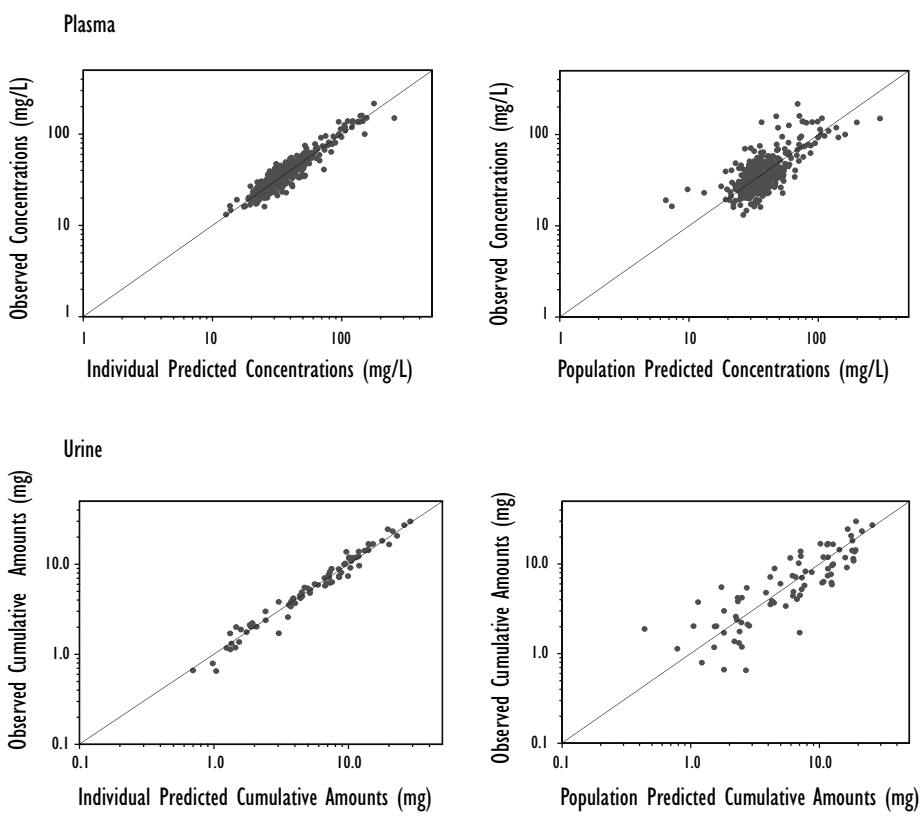


Figure 2: Observed *versus* individual predicted concentrations/amounts (left panels) and population predicted concentrations/amounts (right panels) of propylene glycol for plasma (upper panels) and urine (lower panels) observations for the final model.

Table II: Population pharmacokinetic parameter estimates of the simple model without covariates, the final pharmacokinetic model, the bootstrap analyis and n-1 jackknife analysis.

Parameter	Simple model without covariates	Final pharmacokinetic model	Bootstrap final pharmacokinetic model	n-1 Jackknife final pharmacokinetic model
	Value (CV%)	Value (CV%)	Value (CV%)	Value (CV%)
Objective function	2464	2230	-	-
Fixed effects				
CL_H (L/h) = CL_H p	0.045 (15.0)	-	-	-
CL_H p in CL = CL_H p x (bBW/median)^m x (1+(PNA/median)xn)	-	0.056 (8.1)	0.055 (9.4)	0.056 (8.1)
m	-	1.68 (10.5)	1.69 (11.7)	1.68 (10.5)
n	-	0.12 (41.9)	0.13 (46.0)	0.12 (41.9)
V (L) = Vp	0.92 (9.6)	-	-	
Vp in V = Vp x (cBW/median)^o x p	-	1.01 (6.5)	1.01 (6.5)	1.01 (6.5)
o	-	1.46 (10.2)	1.46 (10.6)	1.46 (10.1)
p	-	1.71 (11.8)	1.71 (13.0)	1.71 (11.8)
CL_R (L/h) = CL_R p	0.0098 (11.8)	-	-	-
CL_R = CLmax x ((time)^q/ ((time50)^q +(time)^q))	-	-	-	-
CLmax		0.45/0.55 x CL_H	0.45/0.55 x CL_H	0.45/0.55 x CL_H
Time50	-	42.2 (49.8)	51.4 (75.7)	42.1 (50.9)
q	-	0.69 (17.3)	0.71 (21.7)	0.66 (17.7)
Interindividual variability (ω^2)				
ω^2 CL	1.01 (25.9)	0.16 (31.4)	0.15 (36.3)	0.16 (31.5)
ω^2 V	0.61 (23.4)	0.17 (24.2)	0.16 (24.9)	0.17 (24.3)
ω^2 CLU	0.35 (32)	0.33 (32.5)	0.31 (37.6)	0.34 (33.1)
Residual variability (σ^2)				
σ^2 plasma (proportional)	0.037 (12.1)	0.034 (12.5)	0.034 (12.8)	0.034 (12.6)
σ^2 urine (proportional)	0.069 (25.5)	0.032 (33.0)	0.031 (36.6)	0.032 (33.3)

CL_H= hepatic clearance, CL_H p = population value for hepatic clearance, V = Volume of distribution, Vp = population value for volume of distribution, CL_R = renal clearance, CL_R p = population value for renal clearance, CLmax = maximum renal clearance = 0.45/0.55 x CL, bBW = bodyweight at birth, cBW = current bodyweight, PNA = postnatal age, time = time in hours after administration of the first dose of propylene glycol, time50 = time at which 50% of the maximum clearance is reached.

The parameter estimates of the final pharmacokinetic model are summarized in table 2. Figure 2 depicts (a) the observed *versus* the individual predicted concentrations/amounts and (b) the observed *versus* the population predicted concentrations/amounts for plasma and urine observations. In figure 3, the conditional weighted residuals *versus* time for the urine samples are illustrated for the simple without covariates and the final model. By introducing these covariates, 69% of the interindividual variability in hepatic clearance, 53% in volume of distribution and 3% in renal clearance was explained (table 2). In figure 4, the population estimates of renal and hepatic clearance *versus* birth weight for a postnatal age of 1 and 28 days are illustrated. Renal excretion of propylene glycol in neonates proved 15, 20, 23 and 25% of total clearance at 6, 12, 18 and 24 hours after the administration of the first dose respectively. At 30, 36, 42 and 48 hours after administration of the first dose, these percentages would be 27, 28, 29 and 30%, respectively. In figure 5, total clearance, hepatic clearance and renal clearance of propylene glycol is presented in four different neonates (birth weight 630g, 1500g, 2500g and 3500g) at a postnatal age of 1 and 28 days.

8.3.3. Model Validation

The results of the bootstrap analysis (N=1000) showed that the median estimated values based on re-sampled data were within 9% of the estimated values of the final pharmacokinetic model except for the estimated value of time at which 50% of the maximum renal clearance is reached, which was within 18%. All CV percentages were below 50%, except for the estimated value of time at which 50% of the maximum renal clearance was reached for which the CV percentage amounted 75%. As this may be explained by the small number of individuals in which urine data

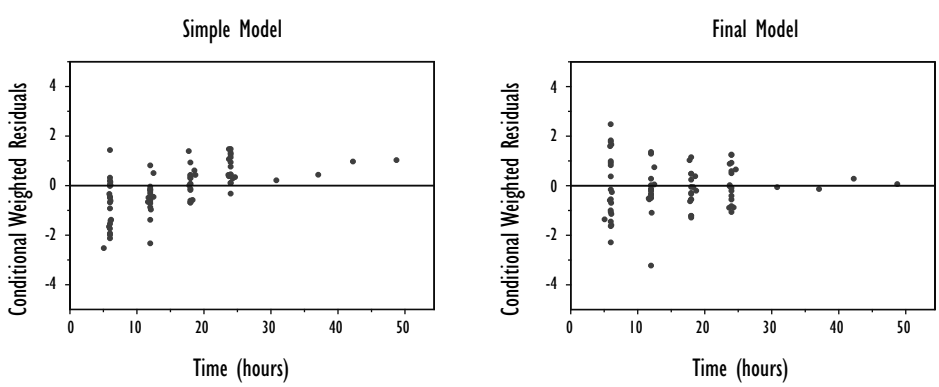


Figure 3: Conditional weighted residuals *versus* time for amounts of propylene glycol in urine for the simple (left panel) and the final (right panel) model.

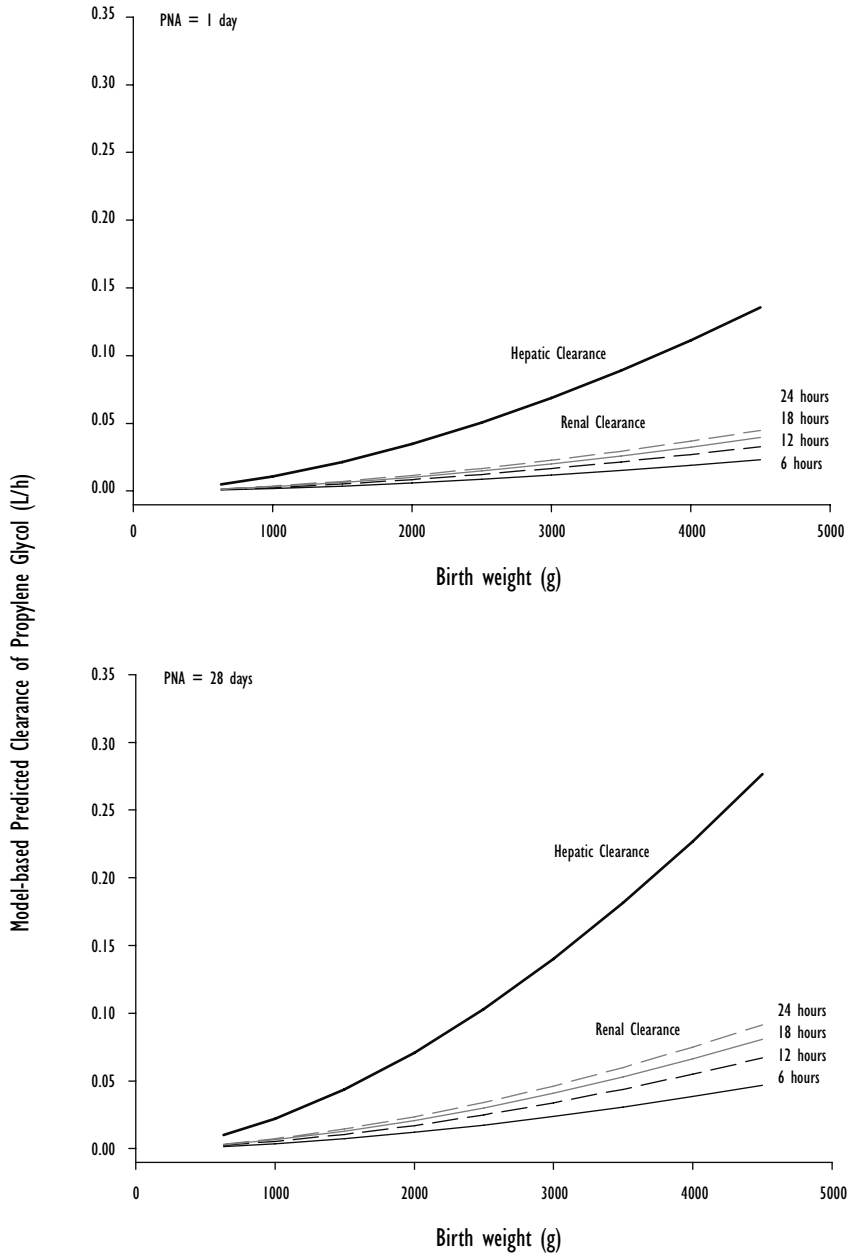


Figure 4: Model-based predicted hepatic (black line) and renal (grey lines) clearance of propylene glycol *versus* birth weight for typical neonates with a post-natal age of 1 day (upper panel) and of 28 days (lower panel). The different grey lines for renal clearance show how renal clearance changes with time after first dose of propylene glycol.

were available, a n-1 jackknife was performed, in which parameter estimates are recomputed leaving out the urine data of 1 patient at a time. In a total of 23 datasets, the values for the parameter estimates obtained were within 3% of the estimates of the final pharmacokinetic model. The CV percentage of the estimated value of time at which 50% of the maximum clearance was reached was 50.9% while the other CV percentages of the estimated parameters were all below 50%.

The results of the normalized prediction distribution error (NPDE) method analysis for the plasma and urine concentrations showed that the model can predict the median concentrations in plasma and urine since the histograms follow the normal distribution. Furthermore no trend was seen in the NPDE *versus* time and NPDE *versus* predicted concentrations (figure 6).

On the basis of the condition number of 32.9, it was concluded that the model was not over-parameterized. The percentage of η-shrinkage was identified to be 21% on hepatic clearance, 9% on volume of distribution and 37% on renal clearance. The plots illustrating the most predicted covariate, birth weight, current bodyweight and time after first dose *versus* the individual and population predicted parameter estimates for hepatic clearance, volume of distribution and renal clearance, respectively, showed that the individual parameter estimates were randomly scattered around the population parameter estimates (Supplement figure 1).

8.4. Discussion

Although propylene glycol is often used as an excipient in drug formulations and is regarded to be safe, toxic effects (e.g. bradycardia, lactic acidosis, convulsions) have been reported in the adult and pediatric age range upon administration of propylene glycol. In adults it is known that about 45% of propylene glycol is eliminated through the renal function and 55% of the administered dose of propylene glycol is metabolized in the liver to lactate and pyruvate [8, 12, 13]. Due to immaturity of the renal function, renal clearance of propylene glycol may be expected to be lower in neonates compared to adults. Therefore the aim of this study was to characterize the contribution of renal clearance *versus* hepatic clearance of propylene glycol in neonates based on excreted amounts of PG in urine.

Based on the final pharmacokinetic model, renal elimination of propylene glycol in neonates was low compared to hepatic elimination and increased over time after first dose of PG. The latter was best described using a hyperbolic function based on time

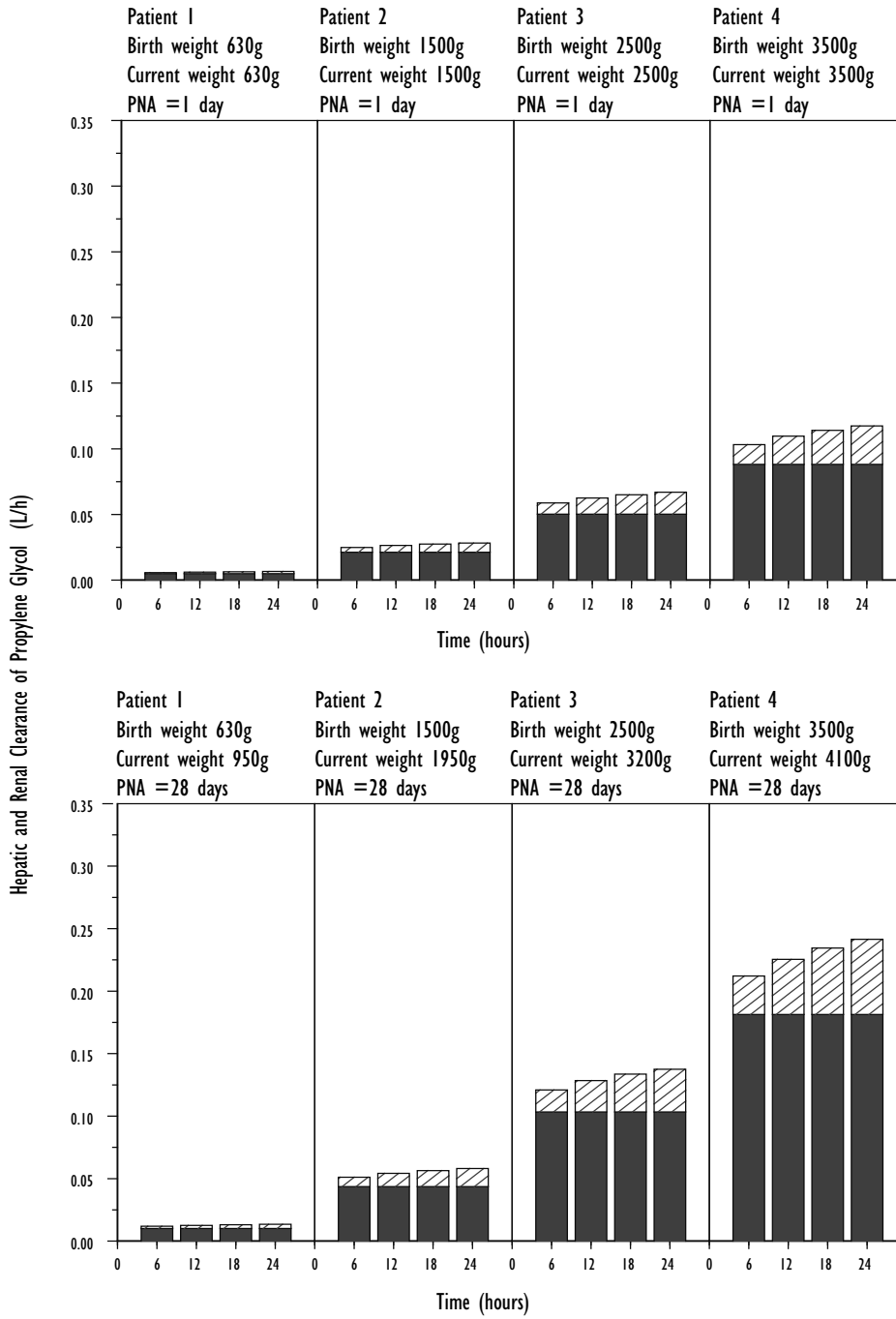


Figure 5: Hepatic (grey) and renal (striped) clearance of propylene glycol for four typical neonates with birth weight 630g, 1500g, 2500g and 3500g and a postnatal age of 1 day (upper panels) and of 28 days (lower panels).

after first dose. Based on this hyperbolic model, renal elimination of propylene glycol was estimated to be 15, 20, 23 and 25% of total clearance at 6, 12, 18 and 24 hours after the administration of the first dose respectively (figure 4 and 5). At 48 hours after the first dose, this percentage of renal elimination of propylene glycol increased even further to 30%. Despite this increase over time after dose, renal elimination of propylene glycol in neonates still remains substantially lower compared to adults, for which renal clearance of propylene glycol was reported to be 45%. The consequence of this finding is that maturational changes in the ratio between renal and metabolic clearance may influence the magnitude of drug-drug interactions. As in neonates hepatic clearance of propylene glycol proves the most important elimination route, drug-drug interactions for the alcohol dehydrogenase enzyme will become more important in neonates compared to adults. In this perspective, the advice of the FDA to avoid Kaletra®, a propylene glycol containing solution, in premature babies until 14 days after their due data or in full-term neonates younger than 14 days of postnatal age is of relevance. Kaletra® is a solution, which contains a combination of lopinavir and ritonavir solved in ethanol (356.3 mg ethanol/mL Kaletra®) and propylene glycol (152.7 mg/mL Kaletra®). Adverse events as heart, kidney and breathing problems were reported in premature neonates, which were likely due to a decreased ability to eliminate either ethanol or propylene glycol or both [23, 24]. Since both alcohols are metabolized by ADH in the liver, the high concentration of ethanol may possibly interfere with the metabolism of propylene glycol since ethanol has the highest affinity for ADH. While in adults this probably will be compensated by the renal route, in neonates this route is still immature which may result in accumulation of propylene glycol. As such, the results of this study may indicate that due to maturational changes, some drug interactions are of more relevance for specific age categories.

In the current analysis, it was found that the elimination of propylene glycol increased with time after first dose, independently of bodyweight or postnatal age of the neonate. To describe this increase in renal elimination of propylene glycol different models were tested: I) Model describing non-linear pharmacokinetics using an (intermediate) Michaelis-Menten model, II) Model with urine volume normalized to current bodyweight implemented as covariate on renal clearance, III) Model with both time after first dose and current bodyweight, implemented as covariates on renal clearance, IV) Model using a hyperbolic model based on time after first dose, V) Model with amount of creatinine as covariate on renal clearance of propylene glycol. Implementation of urine volume in the second model (Model II) caused a significant decrease in objective function which can be explained by the fact that similar to excretion of PG, urine volume increased over time as well. This time dependent increase in urine volume may potentially be induced by the osmotic

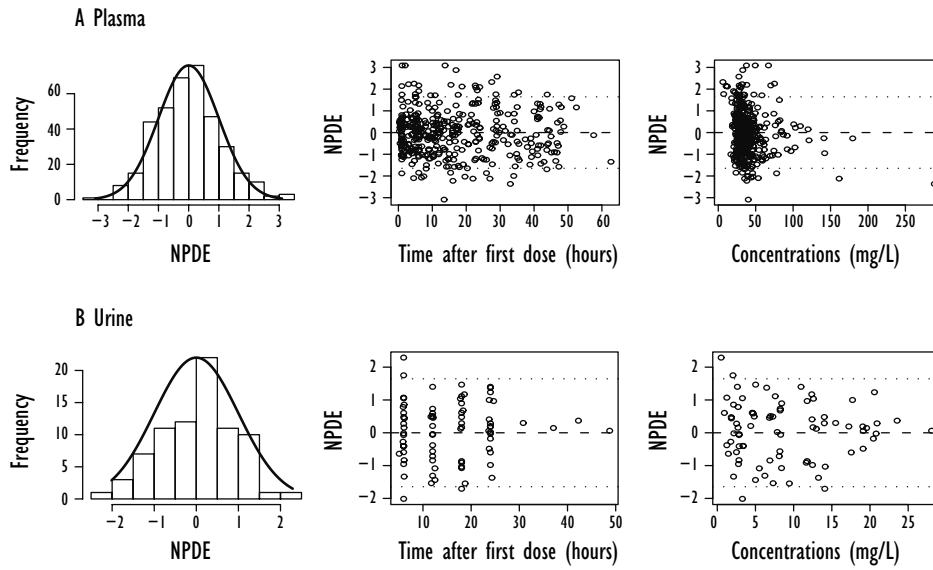
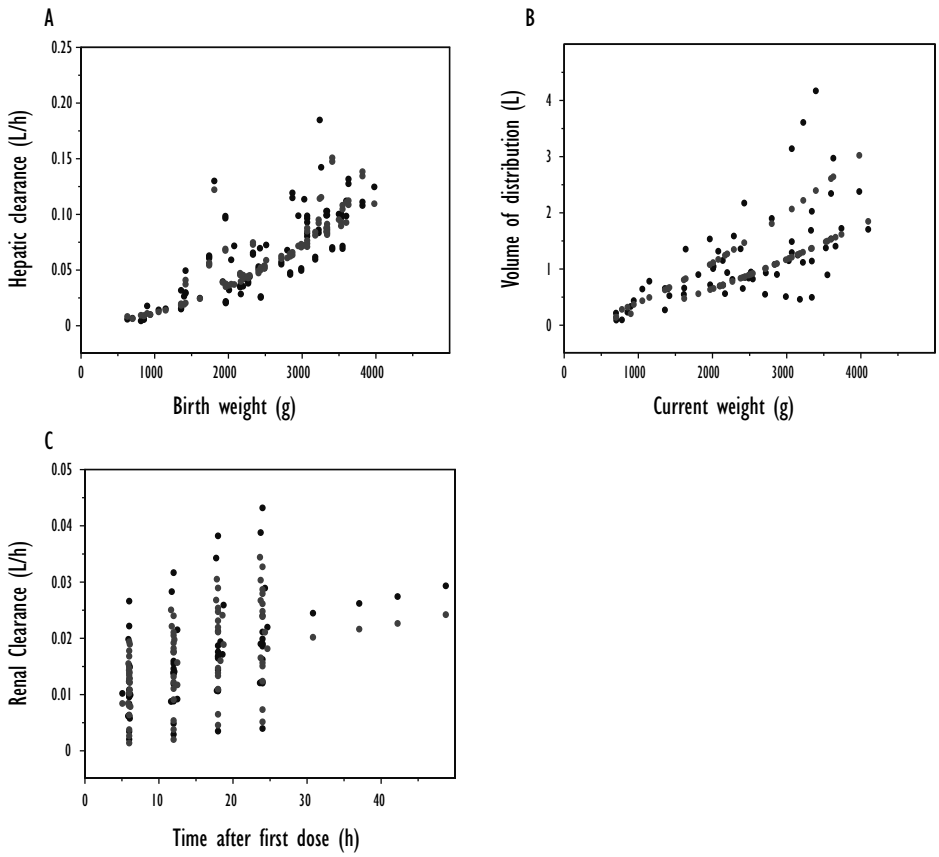


Figure 6: NPDE results for plasma and urine observations of the final model for propylene glycol. The histograms show the NPDE distribution for propylene glycol in plasma (upper panel) and urine (lower panel) with solid lines indicating a normal distribution. The distribution of the NPDE *versus* time after the first dose of propylene glycol and the NPDE *versus* the propylene glycol concentrations are also illustrated.

effects of propylene glycol leading to higher volumes of urine when more propylene glycol is excreted by the renal route. Subsequently, to evaluate whether this time dependent increase in renal elimination of propylene glycol is due to renal maturation or specifically caused by propylene glycol, the amount of creatinine was determined in urine (model V). No increase in renal elimination of creatinine was seen over time implicating that other factors are causing this trend. A potential explanation for these findings is an auto-induced increase in renal clearance of propylene glycol, including either glomerular filtration, active tubular secretion or both. Another explanation may be found in failure of the tubular reabsorption upon propylene glycol use, causing this time-dependent increase of propylene glycol in urine. Finally, the model using a hyperbolic function based on time after first dose (model IV) was identified as the best model. In this model, it was taken into account that the increase in renal clearance of propylene glycol will level of at a certain bodyweight or age as seen in adults for which renal clearance of propylene glycol is estimated to be around 45% compared to hepatic clearance which was estimated to be 55% [8, 12, 13]. As a result, in model IV renal elimination of propylene glycol was estimated as a fraction of hepatic elimination, which also indicates that besides the increase over time after the first dose, renal elimination of propylene glycol increases in a similar manner



Supplement figure 1: Individual predicted (black dot) and population predicted (grey dot) parameter estimates for hepatic clearance (a), volume of distribution (b) and renal clearance (c) *versus* the most predictive covariate birth weight, current weight and time after first dose.

as hepatic clearance with birth weight and postnatal age. The time at which 50% of the maximum renal clearance is reached, was estimated to be 42.2 hours. This value should however be considered with caution since it is based on limited data. To evaluate this time-dependent increase in renal elimination of propylene glycol, which has not been described before in any age group, further research is needed, particularly in preterm neonates who are exposed for several days to drugs containing high concentrations of propylene glycol (e.g. lorazepam).

The hepatic clearance of propylene glycol in preterm and term neonates was best described using birth weight and postnatal age as covariates. Birth weight,

representing antenatal maturation of hepatic clearance was implemented using a power function. Maturation after birth of hepatic clearance was quantified by postnatal age, implemented using a linear function. The identification of these covariates confirmed the results of a previous analysis where only plasma data of propylene glycol were available [15]. The influence of both covariates on hepatic clearance is illustrated in figure 4 and figure 5. Both figures illustrate clearly how hepatic clearance of propylene glycol is increasing with birth weight and postnatal age and indicate that caution is needed when propylene glycol is administered to preterm neonates at the first days of life. The sum of hepatic and renal clearance of propylene glycol as illustrated in figure 5 correspond well with the total clearance of propylene glycol previously found in preterm and term neonates using plasma samples [15].

Finally, the amount of propylene glycol given in this study following the administration of paracetamol or phenobarbital varied between 16 and 70 mg/kg/day. Although for some of the patients the daily dose of propylene glycol was higher than the maximum daily dose suggested by the FDA (25 mg/kg/day), it was much lower than the maximum daily intake proposed by the EMA. These guidelines should however be considered with caution since the guideline proposed by the FDA is based on the administration of propylene glycol as a food additive and it has not been revised since 1974. Moreover both guidelines are to our knowledge not based on observational data.

8.5. Conclusion

Based on the current analysis of propylene glycol data in plasma and urine in preterm and term neonates, renal and hepatic elimination rates of propylene glycol were determined. Hepatic elimination of propylene glycol proved to be the most important elimination route in (pre)term neonates. Renal elimination of propylene glycol increased over time after first dose and proved to be 15, 20, 23 and 25% of the total clearance at 6, 12, 18 and 24 hours after administration of the first dose, respectively. To evaluate whether this increase indicates an auto-induced increase in renal secretion or failure of tubular reabsorption of propylene glycol, further studies are needed.

Acknowledgements

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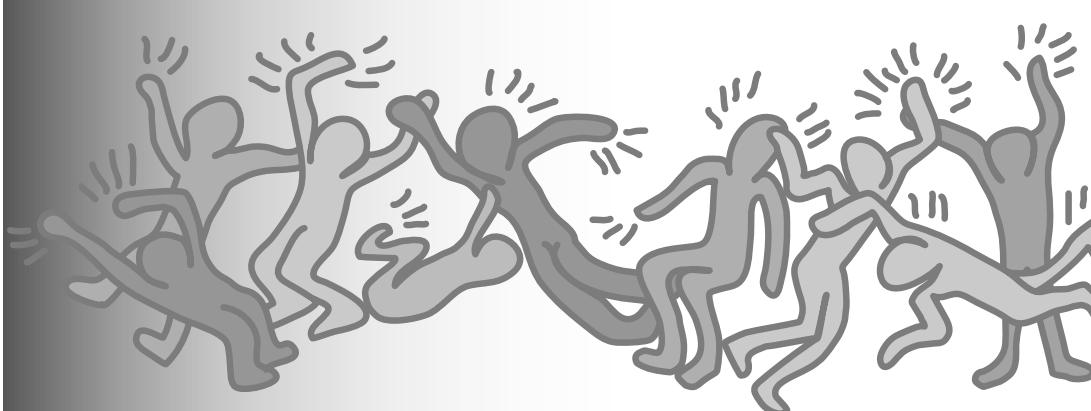
Section V

Developmental Changes in Glomerular Filtration Rate from Neonates until Adults



Chapter 9

Simultaneous Pharmacokinetic Modeling of Gentamicin, Tobramycin and Vancomycin Clearance from Neonates to Adults: towards a Semi-physiological Function for Maturation in Glomerular Filtration

Roosmarijn F.W. De Cock, Karel Allegaert, Janneke M. Brussee, Catherine M.T. Sherwin, Hussain Mulla, Matthijs de Hoog, Johannes N. van den Anker, Meindert Danhof, Catherijne A.J. Knibbe

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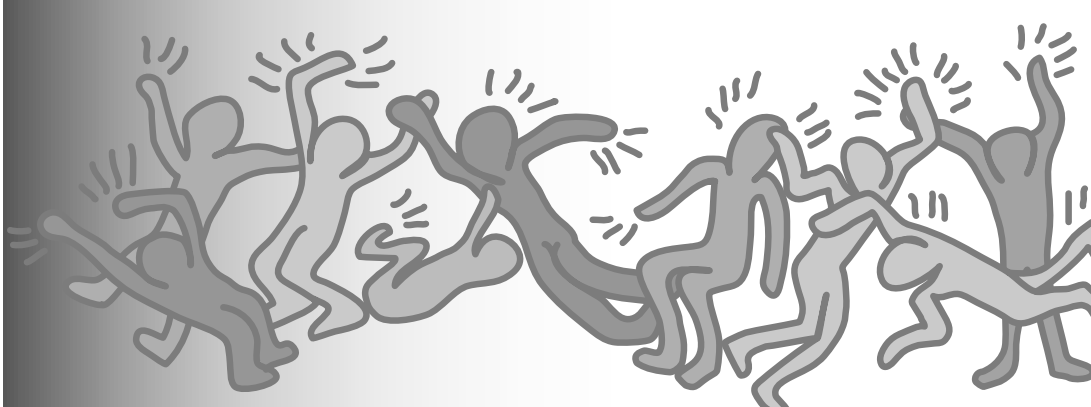

Abstract

Purpose

Since glomerular filtration rate (GFR) is responsible for the elimination of a large number of water-soluble drugs, the aim of this study was to develop a semi-physiological function for GFR maturation from neonates to adults.

Methods

In the pharmacokinetic analysis (NONMEM VI) based on data of gentamicin, tobramycin and vancomycin collected in 1760 patients (age 1 day-18 years, bodyweight 415g-85kg), a distinction was made between drug-specific and system-specific information. Since the maturational model for clearance is considered to contain system-specific information on the developmental changes in GFR, one GFR maturational function was derived for all three drugs.

Results

Simultaneous analysis of these three drugs showed that maturation of GFR mediated clearance from preterm neonates to adults was best described by a bodyweight-dependent exponent (BDE) function with an exponent varying from 1.4 in neonates to 1.0 in adults ($Cl_{GFR}= Cl_{drug}*(BW/4kg)^{BDE}$ with $BDE=2.23*BW^{-0.065}$). Population clearance values (Cl_{drug}) for gentamicin, tobramycin and vancomycin were 0.21L/h, 0.28L/h and 0.39L/h for a full term neonate of 4kg, respectively.

Discussion

Based on an integrated analysis of gentamicin, tobramycin and vancomycin, a semi-physiological function for GFR mediated clearance was derived that can potentially be used to establish evidence based dosing regimens of renally excreted drugs in children.

9.1. Introduction

Children may differ from adults in their response to drugs due to differences in pharmacokinetic (PK) and/or pharmacodynamic (PD) relationships [1-3]. A pre-requisite to developing rational dosing schemes for the pediatric age range (from neonates to adults), is to understand how developmental changes influence this PK and PD relationship [4]. Given the large number of drugs used and the wide range in age and bodyweight in the pediatric population, a major effort would be needed to obtain this information for all drugs used in children. Therefore novel approaches to support pediatric data analysis, to develop predictive pharmacokinetic models and to develop rational dosing schemes in children are required. A promising approach would be the characterization of maturation in important metabolic and excretion routes across the pediatric life-span from preterm neonates to adults [4, 5]. On the basis of model drugs, these maturation functions can be derived and subsequently be used to predict the PK for other drugs that are metabolized or excreted through the same pathway [6, 7].

Glomerular filtration rate (GFR) is responsible for the elimination of a large number of water-soluble drugs and drug metabolites. In adults, GFR is well defined with a value of around 120 ml/min [8]. Concerning the pediatric age range, it is known that nephrogenesis starts at week 5-6 of gestation and continues until 36 weeks of gestation [8-11]. Furthermore, during the first weeks of life, a rapid increase is seen in GFR which is mainly due to hemodynamic changes [8]. Adult levels, as expressed per body surface area, are reached at approximately 6-12 months of age [8]. However, partly due to the expression of GFR per body surface area, the application of these functions in the analysis of renally excreted drugs in different age categories is complicated underlining the need for novel functions quantifying GFR across the pediatric life-span. GFR can be determined on the basis of the concentrations of endogenous (creatinine) or exogenous compounds (inulin, radio-isotopes). Nevertheless, several limitations are linked with each of these methods in the pediatric age range. Therefore the most pragmatic method to assess maturation in GFR is the determination of the clearance of a (model) drug that is almost entirely eliminated through GFR and that is widely used in clinical practice across the pediatric age range [12-14]. The advantage of the use of clearance of renally excreted drugs as a measure to determine GFR, is that this information can be gathered in daily clinical practice. The latter is of course of major importance in the pediatric and neonatal age range to keep the burden for each patient to a minimum.

The aim of this analysis was to develop a semi-physiological function to describe maturation in GFR on the basis of simultaneous population pharmacokinetic modeling of gentamicin, tobramycin and vancomycin, which are almost entirely eliminated through GFR. Since this analysis is based on three different drugs, a novel system-based pharmacology approach was applied [5]. More specifically, within the model a distinction was made between drug-specific and system-specific properties [5]. Consequently, the pediatric covariate model on clearance was considered to contain system-specific information on the developmental changes in GFR and therefore the same covariate model on clearance was implemented for all three drugs. The population values for clearance and volume of distribution and the covariate model on volume of distribution were considered as drug-specific values and estimated for each drug separately.

9.2. Methods

9.2.1. Patients and Data

Data of gentamicin, tobramycin and vancomycin were included in this analysis, which were available from previously published studies [15-18] and from retrospective data collection at the intensive care units of the Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands. In total, data from 1812 subjects were available, which were divided into 4 different age categories according to FDA guidelines [19]: 1) neonates (0-1 month), 2) children 1 month-2 years, 3) children 2-12 years, 4) children 12-18 years. Fifty-two patients (N=14 neonates, N=22 patients aged between 1-23 months, N=15 patients aged between 2-11 years, N=1 patients aged between 12-18 years) with creatinine values three times higher than the age-related reference values [20-24], were excluded from the analysis as they were considered to be patients with severe renal dysfunction. Beside peak and trough samples taken before and 1 hour after initiation or completion of the dose, there were often samples available at other time points. Available data are briefly discussed below while more details on the studies can be found in the original articles [15-18]. An overview of the different datasets is given in table 1.

Gentamicin [15, 16]

For gentamicin, data of two different studies were combined into one dataset resulting in a total of 1705 samples available from 717 patients (682 neonates, 26 infants 1-24 months, 5 children 2-12 years, 4 children 12-18 years, with a bodyweight range between 440g-80 kg).

Table I: Overview of the study and patient characteristics (median (range))

Drug	Gentamicin	Tobramycin	Vancomycin
Number of subjects	717	614	429
Number of blood samples	1705	1273	1168
Age	2 days (1 day-15 yrs)	3 days (2 days-18 yrs)	16 days (1 day-17 yrs)
Subjects (n) per age group (range)			
1 (1-28 days)	682 (GA 23-43)	463 (GA 23-43)	283 (GA 23-34)
2 (1-23 months)	26	67	87
3 (2-11 years)	5	48	42
4 (12-18 years)	4	36	17
Bodyweight	2600g (440g-80kg)	2010g (485g-85kg)	1800g (415g-85kg)
Serum creatinine (µmol/L)	72 (12-104)	72 (5-130)	51 (7-144.1)

GA = Gestational age (weeks)

Tobramycin [17]

A total of 1273 tobramycin concentrations available from 614 patients were included in this analysis (463 neonates, 67 infants 1-24 months, 48 children 2-12 years, 36 children 12-18 years, with a bodyweight range between 485g – 85kg). This tobramycin dataset consisted of data of preterm and term neonates aged up to 4 days of age obtained from a study performed by de Hoog *et al.*[17] and data of patients ranging between a postnatal age of 9 days and 18 years of age obtained from a retrospective analysis performed at the intensive care units of the Erasmus MC-Sophia's Children Hospital, Rotterdam, the Netherlands. Patients were included in the retrospective data analysis when they were younger than 18 years and when bodyweight, age and serum creatinine concentration (not exceeding three times the age-related reference value as explained above) was available.

Vancomycin [18]

For vancomycin 1168 concentrations were available from a total of 429 patients (283 neonates, 87 infants 1-24 months, 42 children 2-12 years, 17 children 12-18 years, with a bodyweight range between 415g – 85kg). Two hundred and sixty nine preterm neonates between 1 and 30 days of age were included from a study performed by Allegaert *et al.* [18] and 160 patients ranging between 4 days and 17 years of age were obtained from a retrospective analysis performed at the intensive care units of the Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands. For the retrospective data analysis, the same criteria as explained under tobramycin were used.

9.2.2. Pharmacokinetic modeling

The population pharmacokinetic analysis was performed with the non-linear mixed effect modeling software NONMEM 6.2. (Globomax LLC, Hanover, MD, USA) using the first-order conditional estimation method with the interaction option (FOCEI). Tools like S-Plus version 6.2.1 (Insightful software, Seattle, WA) with NM.SP.interface version 05.03.01 (© by LAP&P Consultants BV, Leiden, The Netherlands), PsN and R (version 2.10.1) were used to visualize and evaluate the model. Four different steps were used to develop the model: (i) choice of the structural model, (ii) choice of the statistical sub-model, (iii) choice of the covariate model, (iv) model validation.

Structural and statistical model

For the structural model, both one and two compartment models were tested. Concerning the statistical model, the inter-individual variability was assumed to be log-normal distributed in an individual i (*post hoc* value) and is given by the following equation:

$$\theta_i = \theta_{TV} \bullet e^{\eta i} \qquad \text{(Equation 1)}$$

in which θ_{TV} is the typical value of the parameter and η_i is assumed to be a random variable with mean value zero and variance ω^2. For the intra-individual variability and residual error (statistical submodel), proportional (equation 2), additive (equation 3) and combination (equation 4) error models were tested:

$$Y_{ij} = C_{pred,ij} \bullet (1+\varepsilon_{ij}) \qquad \text{(Equation 2)}$$

$$Y_{ij} = C_{pred,ij} + \varepsilon_{ij} \qquad \text{(Equation 3)}$$

$$Y_{ij} = C_{pred,ij} \bullet (1+\varepsilon_{1,ij}) + \varepsilon_{2,ij} \qquad \text{(Equation 4)}$$

where Y_{ij} is the *j*th observation in the *i*th individual, $C_{pred,ij}$ is the predicted concentration and ε_{ij} is a random variable from a normal distribution with a mean of zero and estimated variance of σ^2.

Discrimination between structural and statistical models was based on different diagnostic tools [25]. A difference in objective function (OFV) of 3.9 points or more was considered as statistically significant ($p<0.05$ based on X^2 distribution). Furthermore, the goodness-of-fit plots (observed *versus* individual predicted

concentrations, observed *versus* population predicted concentrations, conditional weighted residuals *versus* time, conditional weighted residuals *versus* population predicted concentrations) of all data, stratified by drug and age categories were used for diagnostic purposes. Finally the total number of parameters, visual improvement of individual plots, correlation matrix, confidence intervals of parameter estimates, ill-conditioning [26] and shrinkage [27] were assessed. Ill-conditioning was tested by calculating the condition number by dividing the largest eigenvalue by the smallest eigenvalue.

Covariate model

The pharmacokinetic model was developed by simultaneously analyzing the data of gentamicin, tobramycin and vancomycin. On the basis of a systems-based pharmacology approach, within the model a distinction was made between system-specific and drug-specific information [5, 6]. Using this approach, it was assumed that the covariate model contains system-specific information derived from the developmental changes in clearance across the pediatric age range from neonates to adults of the underlying physiological systems, in this case GFR. As a result, the covariate relationships on clearance for all three drugs were not tested separately for each drug but the same covariate relationship was tested on clearance of all three drugs [7]. The population value for clearance and volume of distribution and the covariate models on volume of distribution were considered to contain drug-specific information and were therefore estimated by NONMEM for each drug separately.

The following covariates were tested: bodyweight, age, serum creatinine concentrations (< three times the age-related upper limit of the reference value in order to exclude severe renal dysfunction) and co-administration of ibuprofen, indomethacin, diuretics, amoxicillin and aminoglycosides. Since during the first five days of life serum creatinine values are considered to reflect maternal renal function [10, 28], these creatinine values were excluded from the analysis. According to the origin of the data [15-18], serum creatinine was measured using the enzymatic or uncompensated Jaffé method. In order to evaluate the influence of creatinine as a covariate on clearance different approaches were used:

(1) Evaluation of creatinine value normalized to age. According to the measuring technique, enzymatic or Jaffé respectively, different age-related reference values were used [20-24].

(2) Evaluation of creatinine clearance. Different formulas were used to estimate creatinine clearance (mL/min) in the *i*th individual: Cockroft-Gault formula, Schwartz

formula and Modification of Diet in Renal Disease (MDRD) formula.

Cockroft-Gault:

$$CL_{Cr}i = \frac{(140 - age) \bullet weight}{72 \bullet S_{Cr}} \qquad (x\ 0.85\ if\ female) \qquad \text{(Equation 5)}$$

where age is expressed in years, weight in kg and Scr is the serum creatinine (mg/dL).

Schwartz formula:

$$CL_{Cr}i = \frac{k \bullet length}{S_{Cr}} \qquad \text{(Equation 6)}$$

where k=0.33 for preterm babies in the first year of life, k=0.445 for full term infants and k=0.55 for infants and children between 1 and 12 years of age, Scr is the serum creatinine (mg/dL) and length was expressed in cm and was determined using the growth charts of the World Health Organization.

MDRD formula:

$$CL_{Cr} = 186 \bullet S_{Cr}^{-1.154} \bullet age^{-0.203} \qquad (x\ 0.742\ if\ female) \qquad \text{(Equation 7)}$$

where age is expressed in years and S_{Cr} in mg/dL.

Creatinine clearance was tested as covariate on clearance using the above mentioned formulas as well as the combination of the Schwartz formula < 12 years of age and the Cockroft-Gault or MDRD formula > 12 years of age.

Continuous covariates were separately entered into the model using a linear or power function, as shown in equation 8

$$P_i = P_p \bullet \left(\frac{Cov}{Cov_{Median}}\right)^k \qquad \text{(Equation 8)}$$

where P_i indicates the individual or *post hoc* value of the parameter for the *i*th subject, P_p is the population value of the parameter and COV is the appropriate covariate. In case of a power function, k represents the exponent value, while for a linear relationship k is fixed to 1. For creatinine, linear or power functions were tested in the denominator since a negative relationship was seen between creatinine concentrations and clearance.

In addition, as it often has been reported that the exponent k (Equation 8) on clearance is higher in neonates and young children (scaling exponent >1) [29, 30] compared to older children and adults (scaling exponent <1), a recently developed bodyweight-dependent exponent function (BDE) was tested in which the scaling exponent varied with bodyweight [31-33]. In an analysis undertaken by Wang *et al.*[31], this BDE model (Equation 9) was first used, in which the exponent for propofol clearance was found to vary between 1.35 for neonates and 0.57 for adults. The bodyweight-dependent exponent function (BDE) used in this analysis is given in Equation 9:

$$CL_{GFR} = CL_{Drug} \bullet (\frac{BW}{4kg})^{BDE} \qquad and \qquad BDE = L1 \bullet BW^{M}$$

(Equation 9)

in which CL_{GFR} is clearance in the *i*th individual with bodyweight *BW*; CL_{drug} is the clearance of the drug (gentamicin, tobramycin, vancomycin) in a full term neonate with a bodyweight of 4 kg; *BW* is bodyweight of an individual *i*; L1 is the intercept in the scaling exponent and M is the exponent which allows the scaling exponent to change with bodyweight.

The significance of a covariate was statistically evaluated by the use of the objective function. In the forward inclusion a p value <0.005 was considered as statistically significant while a more stringent p value <0.001 was used in the backward deletion. In addition, the reduction in interindividual variability in the parameter studied was evaluated upon inclusion of the covariate in the model. When two or more covariates were found to significantly improve the model, the covariate that reduces the objective function the most was retained into the model and served as a basis for subsequent inclusion of additional covariates. The choice of covariate model was further evaluated as discussed previously under structural and statistical model whereby the results of the model validation were also considered.

9.2.3. Model validation

Validation of the model was performed using the normalized prediction distribution error method [34, 35]. The dataset was simulated 500 times in NONMEM and the observed and simulated concentrations were compared using the NPDE package in R. A histogram of the NPDE distribution and the scatterplots showing the NPDE *versus* time and *versus* predicted concentrations were subsequently used to evaluate the final model.

9.3. Results

9.3.1. Patients and Data

The analysis was based on a total number of 4146 observations from three different drugs (gentamicin, tobramycin and gentamicin) collected in 1760 patients varying in age between 1 day and 18 years of age and with a bodyweight that varied between 0.415 and 85 kg. A summary of the clinical characteristics is given in Table 1.

9.3.2. Pharmacokinetic modeling: system-based approach

In the pharmacokinetic analysis based on the simultaneous analysis of gentamicin, tobramycin and vancomycin data, a two compartment model parameterized in terms of clearance (CL), intercompartmental clearance (Q), volume of distribution of the central compartment (V1) and volume of distribution of the peripheral compartment (V2) was superior over a one compartment model. Since no covariance step could be obtained, the model was simplified by equalizing Q and V2 to CL and V1, which was supported by the results of the two compartment model. The interindividual variability was only included on clearance values of gentamicin, tobramycin and vancomycin as it could not be estimated on volume of distribution of the three drugs, probably because of overparameterization. The residual variability was best described using a combined error model.

As mentioned in the methods section, the model consisted of drug-specific and system-specific parameters. The covariate model on clearance for these three drugs was considered system-specific information while the population values for clearance and volume of distribution and the covariate model on volume of distribution was considered as drug-specific information. Concerning the system-specific part of the model, a power function on the basis of bodyweight as covariate in which

the exponent varied with bodyweight (Equation 9) was found to best describe the developmental changes in clearance of the three different drugs across the entire pediatric life-span. As shown in equation 9, clearance was standardized to a full term neonate with a bodyweight of 4 kg, while it is emphasized that given the nature of this function also the median weight of the population or 70 kg could have been chosen. Implementation of this bodyweight-dependent exponent model on clearance of the three different drugs caused a drop in objective function of 3607 points ($p < 0.005$). The scaling exponent BDE was found to change in neonates from 1.42 for a neonate of 1000g to 1.34 for a neonate of 2500g to 1.3 for a neonate for 4000g to 1.0 in adults of 18 years old with a bodyweight of 70kg. A higher objective function (104 points) was found when bodyweight was implemented using a power function (Equation 8) on clearance of the three drugs. Bodyweight was also identified as most important covariate on volume of distribution of the central compartment for all three drugs. Bodyweight was implemented using a power function for gentamicin and tobramycin, while a linear function was identified for vancomycin causing in total a drop in objective function of 2438 points ($p < 0.005$). By implementing these covariates, a large part of the interindividual variability on clearance of gentamicin (62%), tobramycin (87%) and vancomycin (77%) was explained. Although the influence of creatinine on the clearance of the three different drugs was thoroughly evaluated using different methods as described in section Methods, creatinine nor creatinine clearance was not identified as a covariate in the final pharmacokinetic model. This may be explained by two different reasons: 1) only children with creatinine concentrations below 3 times the age-related reference values were included, 2) two different methods (Jaffé and enzymatic method) were used to measure creatinine in the different studies. Consequently when serum creatinine values are considered to be normal with one technique, this holds not true for the other technique.

The parameter estimates of the final pharmacokinetic model with the system-specific function for GFR mediated clearance are given in table II. The individual *post hoc* and population predicted clearance values *versus* bodyweight are illustrated in figure 1a, 1b, 1c. These figures show that for each drug the individual *post hoc* values are equally distributed around the population clearance values even though one function is used to capture maturational changes in clearance of each of the three drugs. The observed *versus* population predicted concentrations per drug and per age category are depicted in figure 2 while in figure 3 the individual and population predicted clearance values of the final system-specific pharmacology model are illustrated *versus* bodyweight. Based on the correlation matrix, a high correlation was seen between LI and M (>95%). The condition number (428) was far below the critical value of 1000 which indicates that the model was not overparameterized.

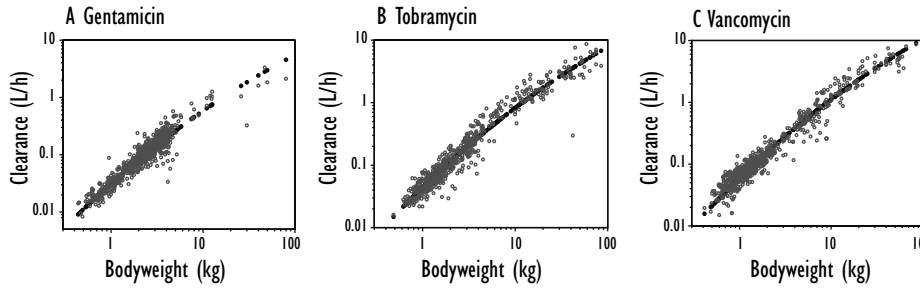

Figure 1: Individual *post hoc* (grey) and population predicted (black) clearance values (L/h) *versus* the most predictive covariate bodyweight for the three different drugs using the final system-specific pharmacology model (a,b,c).

To evaluate the performance of this system-specific pharmacology model, it was compared with independent reference models which were developed separately for each drug using a systematic covariate analysis. In accordance to the system-specific pharmacology model, a bodyweight dependent exponent model was found to best describe the developmental changes in clearance for each drug. Furthermore bodyweight was also found as covariate on volume of distribution. Figure 4 illustrates the population predicted clearance values *versus* bodyweight for the final system-specific pharmacology model and the independent reference models for the three different drugs.

9.3.3. Model validation

The system-specific model was internally validated using the normalized prediction distribution error method. The results of the NPDE analysis of the final system-specific model (figure 5) show that the model can predict the median concentrations accurately, even though a slightly over prediction of the variability was also seen. Finally, no trend was observed between the NPDE *versus* time and *versus* predicted concentrations.

9.4. Discussion

In order to support data analysis, to develop predictive models and to develop rational drug dosing schemes in children, new approaches are needed. One of the approaches, which is applied in the current investigation, is to characterize the developmental changes of important metabolic and excretion pathways from neonates until adults by the use of model drugs. Since maturation of renal function is

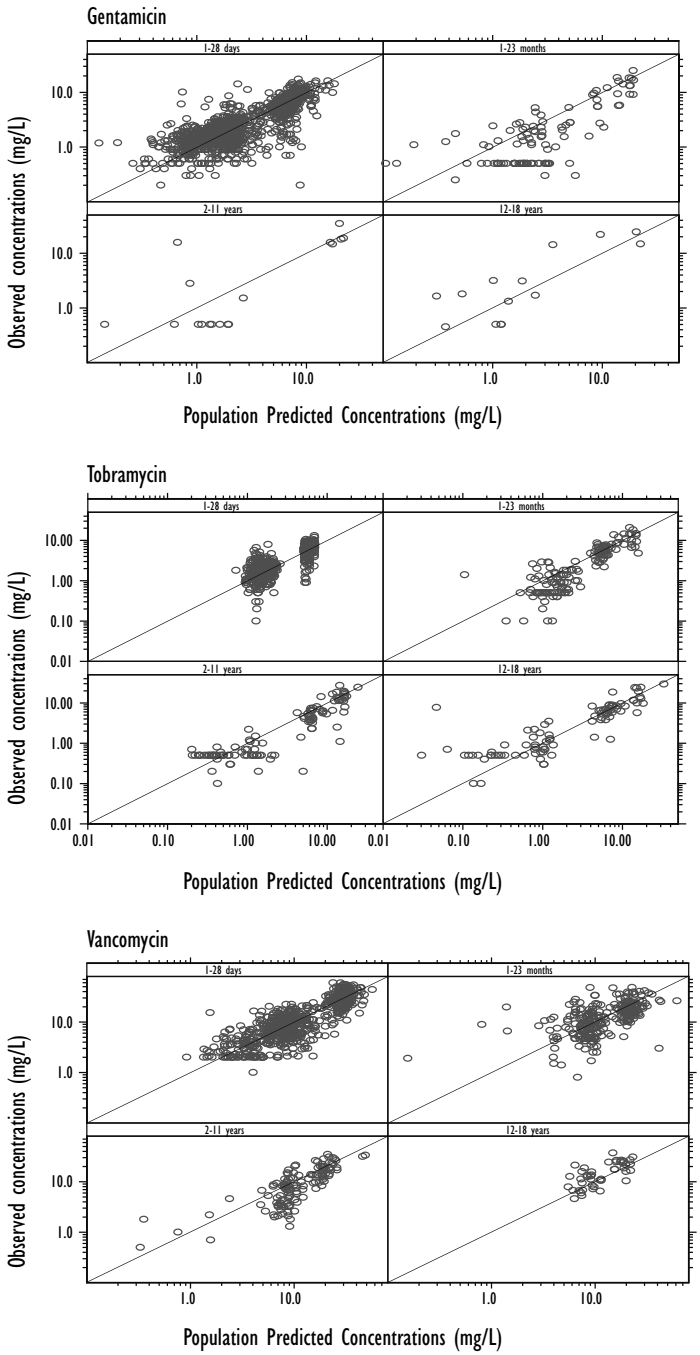

Figure 2: Observed *versus* population predicted concentrations of the final system-specific pharmacology model for gentamicin, tobramycin and vancomycin, split by four age categories.

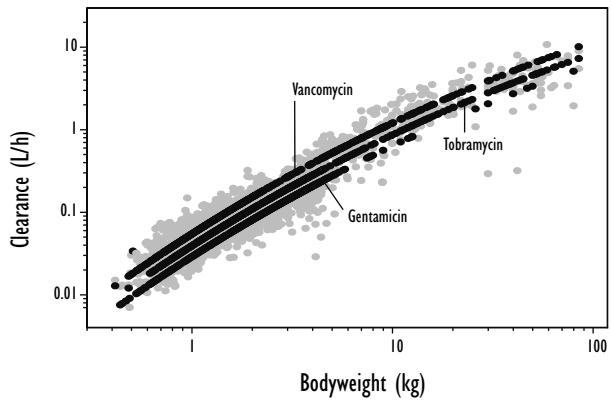

Figure 3: Individual (grey) and population predicted (black) clearance values for gentamicin, tobramycin and vancomycin *versus* bodyweight (kg) for the final system-specific pharmacology model.

age dependent, resulting in differences in glomerular filtration rate at different stages of development, the aim of this study was to characterize the maturation of GFR throughout the pediatric age range on the basis of three different renally excreted model drugs. To perform this analysis a system-specific pharmacology model [5] was developed in which a distinction was made between drug-specific and system-specific information. In this model, the developmental changes in clearance of all three drugs from preterm neonates to adults were considered system specific information and were characterized on the basis of one bodyweight-dependent exponent model [31-33] in which the exponent was found to vary with bodyweight from 1.4 in neonates to 1.0 in adults for all drugs. While this approach resulted in adequate description of the data for the entire pediatric life-span (figure 3), it is emphasized that the description of the developmental changes in renal clearance, performed in this analysis can also be viewed as empirical because an (advanced) allometric function is used. We prefer however the use of the term semi-physiological because this approach meets in the middle of a standard population pharmacokinetic analysis and a full physiologically based pharmacokinetic analysis because both drug specific and system specific information are estimated in one model.

The performance of this system-specific pharmacology model was compared with independent reference models which were developed separately for each drug using a systematic covariate analysis. In figure 4 clearance values are plotted *versus* bodyweight for the system-specific pharmacology model and for the independent reference models for the three different drugs. While for tobramycin and vancomycin, similar clearance values are observed over the entire pediatric age

Table II: Population parameter estimates of the final system-specific pharmacology model with the system-specific function for GFR mediated clearance and drug-specific information on gentamicin, tobramycin and vancomycin.

Parameter	Final pharmacokinetic covariate model (CV%)
Fixed effects	
System specific parameters: (Eq. 9)	
LI	2.23 (6.23)
M	-0.065 (-12.1)
Drug Specific parameters:	
CLgenta $_{4kg}$ (L/h)	0.21 (2.01)
CLtobra $_{4kg}$ (L/h)	0.28 (2.47)
CL vanco $_{4kg}$ (L/h)	0.39 (2.72)
VIgenta $_{4kg}$ (L)	1.45 (2.94)
VItobra $_{4kg}$ (L)	1.90 (1.99)
VIvanco $_{4kg}$ (L)	2.22 (2.63)
VIgenta $=V_{4kg} \times (BW/4kg)^{k2}$ (Eq. 8)	
k2	0.759 (4.35)
VItobra $= V_{4kg} \times (BW/4kg)^{k3}$ (Eq. 8)	
k3	0.735 (2.56)
VIvanco $= V_{4kg} \times (BW/4kg)^{k4}$ (Eq. 8)	
k4	1 FIX
Qgenta = CLgenta	-
Qtobra = CLtobra	-
Qvanco = CLvanco	-
V2genta = VIgenta	-
V2tobra = VItobra	-
V2vanco = VIvanco	-
Interindividual variability	
ω^2 on CLgenta	0.143 (12.5)
ω^2 on CLtobra	0.158 (16.5)
ω^2 on CLvanco	0.171 (10)
Residual variability	
σ^2 (proportional)	0.0886 (5.21)
σ^2 (additive) (mg/L)	0.0494 (22.7)

CL = clearance, CL_{4kg} = clearance for a full term neonate of 4kg, Q = intercompartmental clearance, VI= volume of distribution of the central compartment, V2 = volume of distribution of the peripheral compartment, BW = bodyweight (g), LI = coefficient of the bodyweight dependent exponent function, M = bodyweight dependent exponent, k2 = the exponent of bodyweight on VI of gentamicin, k3 = the exponent of bodyweight on VI of tobramycin, k4 = the exponent of bodyweight on VI of vancomycin

range, a difference between the two approaches is observed for gentamicin at the higher clearance values. For example the estimates for clearance for a neonate of 4 kg were for gentamicin 0.21 and 0.20 L/h, for tobramycin 0.28 and 0.29 L/h and for vancomycin 0.39 and 0.38 L/h for the system-specific and independent reference model, respectively. For a child of 20 kg, the estimates for clearance were 1.38 and 1.07 L/h for gentamicin, 1.84 and 2.08 L/h for tobramycin and 2.56 and 2.29 L/h for vancomycin, while for an individual of 60kg the estimates for clearance were 4.00 and 2.54 L/h for gentamicin, 5.34 and 6.07 L/h for tobramycin and 7.43 and 6.34 L/h for vancomycin for the system-specific and independent reference model, respectively. This difference for gentamicin in the higher clearance values between the two different approaches can probably be explained by the fact that for gentamicin data of only 9 individuals were available in the age range between 2 and 18 years (figure 2). Compared to the independent reference model of gentamicin, in the system-specific pharmacology model this information is supported by information on tobramycin and vancomycin for which much more information was available between in the age range between 2 and 18 years. It is therefore anticipated that for gentamicin the system-specific pharmacology model may be more reliable than the independent reference model for the higher bodyweight ranges.

In this analysis, the developmental changes in GFR were described from neonates until adults using only bodyweight as covariate on clearance. In an article of Rhodin *et al.*[36], maturation of renal function was described from premature neonates to adults using a pooled dataset of 8 different studies in which GFR was evaluated based on clearance of Cr-EDTA, mannitol, inulin, iohexol and sinistrin. Both bodyweight and postmenstrual age were identified as covariates to describe the maturational changes in GFR. Bodyweight was included on clearance using an allometric function with an exponent of 0.75 while postmenstrual age was included using a sigmoidal hyperbolic function. In our analysis which was based on a systematic covariate analysis on the

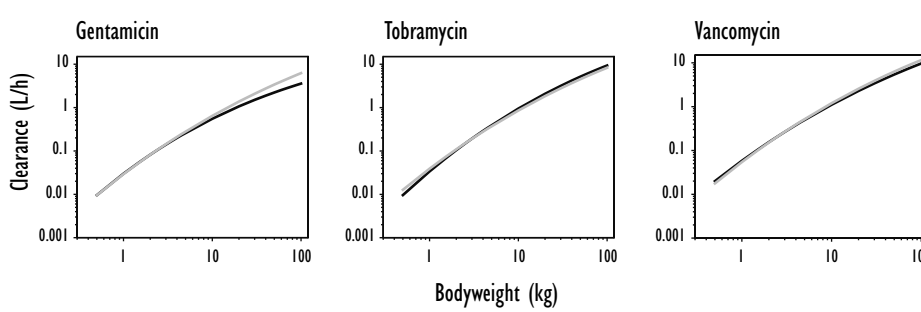

Figure 4: Population predicted clearance values *versus* bodyweight for the final system-specific pharmacology model (grey) and independent reference models (black) for the three different drugs.

basis of statistical principles, bodyweight was identified as most important covariate on clearance. More specifically, it was found that bodyweight was best implemented on clearance using a bodyweight-dependent exponent model in which the exponent based on bodyweight was found to range from 1.4 in neonates to 1 in adults (figure 6). These findings confirm the results of previous studies in which it was also shown that the scaling exponent on clearance is higher in neonates and young children compared to older children and adults [29, 30, 32]. Moreover the difference in scaling exponent signifies that the largest increase in clearance of these different drugs, which in their turn reflect GFR, is seen in the first weeks of life until 1 year after birth [8] (figure 1). As suggested before, this can be due to hemodynamic changes leading to an increase in renal blood flow and decrease in vascular resistance [9, 37].

Previously, a pharmacokinetic model was developed describing the developmental changes in clearance of amikacin in preterm and term neonates on the basis of birth bodyweight and postnatal age, representing antenatal and postnatal maturation of the kidney, respectively [29]. In that model, that proved of predictive value for other renally excreted antibiotics in neonates [38, 39] a decrease in clearance was seen when ibuprofen was co-administered. Since the combination of birth weight and postnatal age is not applicable for older children, bodyweight and age were studied as covariates. In the current study in which clearance of three different renally excreted drugs was described from neonates until adults, bodyweight was included on clearance using the bodyweight-dependent exponent model, because it proved superior over age. Although the final system-specific pharmacology model based on bodyweight was able to describe the observed concentrations without bias in all age categories, including neonates, for all drugs (figure 2), it needs to be evaluated whether the model based on birth weight and postnatal age [29] for the neonatal population would be superior in precision over the current model. Finally ibuprofen was not identified in this current study as a covariate on clearance. Probably this is due to the limited available information on co-administration of ibuprofen or indomethacin. Although it

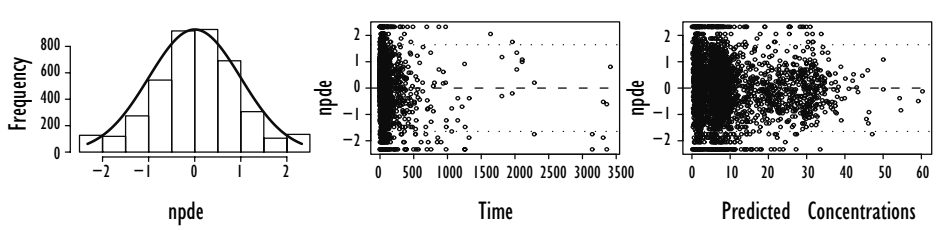

Figure 5: NPDE results of the final system-specific pharmacology model for the three different drugs. Left panel: Histogram of the NPDE distribution with the solid line representing a normal distribution, middle panel: NPDE *versus* time, right panel: NPDE *versus* predicted concentrations.

can not be excluded that separate models are needed to describe more accurately the developmental changes in neonates, in the current study we were able to successfully describe the developmental changes over the entire pediatric age range.

In this analysis, an influence of serum creatinine or creatinine clearance could not be identified, even though different approaches were tested (methods). This seems an unexpected finding because patients with creatinine values up to three times the age-related reference values [20-24] were included in the analysis. Potentially, this result may in part be explained by the fact that two different methods (Jaffé and enzymatic method) were used to measure creatinine concentrations in the different studies. Due to interferences with proteins, ketoacids, cephalosporins and bilirubin, the Jaffé method overestimates creatinine concentrations compared to the enzymatic method [40-42]. In adults it is seen that serum creatinine concentrations are overestimated by the Jaffé method by about 30% compared to the enzymatic method [43, 44]. In neonates and children this overestimation could not be exactly quantified or changes continuously [22, 45]. Moreover, this difference in creatinine measurement also affects the formulas used to calculate creatinine clearance to estimate GFR [42]. Consequently these formulas need to be adapted based on the used measuring technique. Finally, the numbers of patients with a three times increased serum creatinine concentration across the entire age range was low (5%), which should be considered when interpreting this result. Therefore, it seems that care should be taken to apply the model to children with a creatinine concentration between the two- and three times the age-related reference value. We should however notice that the final system-specific pharmacology model is able to describe the observed concentrations of all different age ranges of the three drugs adequately and without bias, even though creatinine was not included in the final model. Moreover without inclusion of creatinine on clearance, a large part of the interindividual variability was explained for the three drugs (gentamicin: 62%, tobramycin: 87%, vancomycin: 77%).

This analysis based on the use of three different renally excreted drugs to characterize GFR from neonates until adults has in addition to a number of advantages (e.g. information can be obtained directly from clinical practice causing no additional burden for patients) also some restrictions. First of all, it should be emphasized that the model developed in this study describes the developmental changes in GFR in patients without severe renal impairments. To evaluate maturation of GFR in patients with an impaired renal function, new studies need to be performed. Furthermore, it should be taken into account that data are obtained from patients staying at the intensive care units for which factors of critical illness or augmented renal clearance may have an influence on renal function.

In conclusion, in this study, we were able to develop a system-specific pharmacology model describing maturation in GFR from neonates to adults based on three different renally excreted drugs using a bodyweight-dependent exponent function. In a next step, it will be evaluated whether this model can be used to predict other renally excreted drugs, which has been shown before for a neonatal GFR model [29, 39]. In addition, it would be useful to analyze the sensitivity of this relationship to other model parameterizations and to characterize the exact influence of differences in pharmacokinetic and physicochemical properties. Furthermore, besides the extension of this system-specific pharmacology model to other renally excreted drugs the possibility to describe the developmental changes in tubular processes across the entire pediatric age using this system-specific GFR model can be explored when analyzing clearance of a drug undergoing both GFR and tubular excretion. By applying a more system-based approach the development of pharmacokinetic models will be advanced and the development of evidence-based and individualized dosing regimen in children be facilitated.

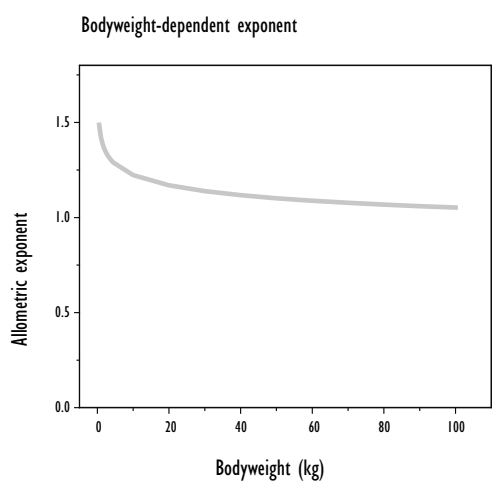

Figure 6: The relationship between the allometric exponent in the final system-specific pharmacology model and bodyweight (kg) in the bodyweight-dependent exponent model (Equation 9).

9.5. Conclusions

In this study the developmental changes in GFR mediated clearance in neonates, infants, toddlers, children and adolescents were described by describing the pharmacokinetics of three renally excreted drugs, gentamicin, tobramycin and vancomycin. Based on a distinction between drug-specific and system-specific parameters, a semi-physiological function for GFR mediated clearance was derived that can potentially be used to facilitate sparse data analysis and evidence based dosing regimens of renally excreted drugs in children.

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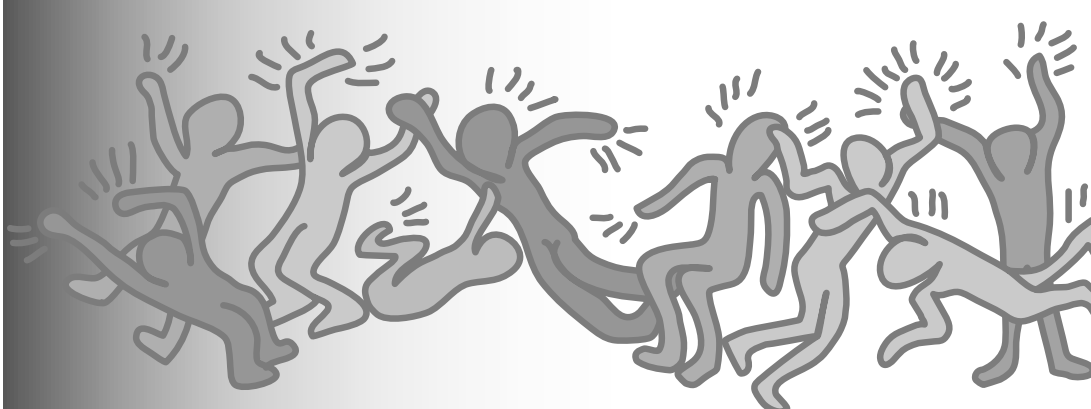
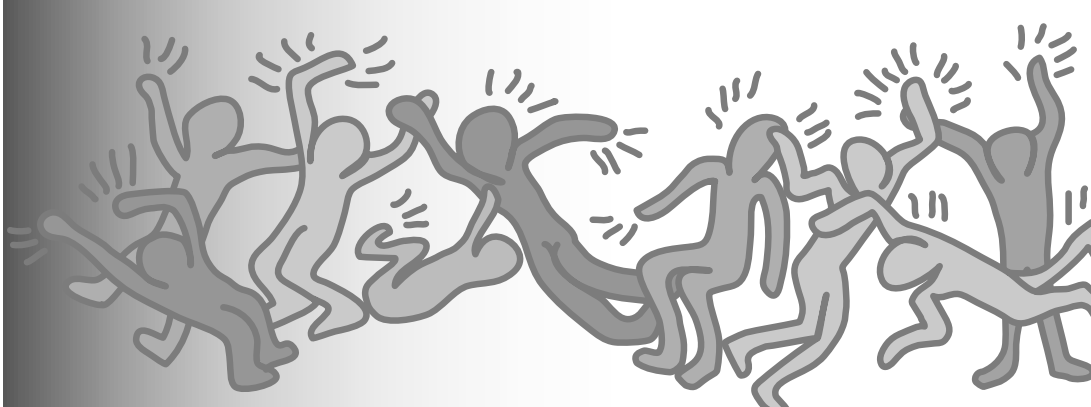
Section VI

Summary, Conclusions and Perspectives



Chapter 10

Conclusions and Perspectives



10.1 Towards a system-based pharmacology approach to predict developmental changes in renal drug clearance

To date, dosing guidelines in children are often empirically derived from dosing guidelines in adults based on linear extrapolations based on bodyweight. However, children can not be considered small adults. During development, changes in body composition, cardiac output and blood flow are seen as well as developmental changes in drug metabolizing enzymes, liver and kidneys. All of these factors may influence the pharmacokinetics of drugs. Furthermore differences in pharmacological response may be seen between children and adults due to differences in expression of receptors or differences in disease status, which influence the pharmacodynamics. The magnitude of these changes may not be solely reflected by differences in bodyweight. Therefore it is of utmost importance to characterize these developmental changes in pharmacokinetics (PK) and pharmacodynamics (PD) to develop evidence-based and individualized dosing regimen [1]. The absence of this information poses otherwise significant risks to over- or underdosing leading to adverse or even toxic effects or therapeutic failure, respectively [2,3].

Renal clearance is responsible for the elimination of a large number of water-soluble drugs and metabolites and is therefore of large importance when characterizing the pharmacokinetics of drugs. Renal clearance includes glomerular filtration, tubular secretion and reabsorption and each of these processes is subject to different developmental changes [4]. To estimate the renal clearance of drugs in children, a thorough understanding of these developmental changes in the different subprocesses contributing to renal function is needed. Therefore the aim of the research described in this thesis was to characterize the developmental changes in renal function over the entire pediatric age range. To this end, a system-based pharmacology approach was applied implicating that within the models for the different subprocesses contributing to renal function a distinction was made between system-specific and drug-specific properties [5].

In **chapter 2** of this thesis we have highlighted the potential value of population pharmacokinetic and pharmacodynamic modeling in pediatrics. Performance of clinical studies in children is associated with several ethical, practical and economical issues. Since it is unethical to perform clinical studies in healthy children, studies are performed in children suffering from a disease. As a consequence only a limited number of patients are available. Moreover the small blood volume should be taken into account limiting the number and volume of blood samples. To overcome these

issues with regard to the analysis of pediatric data, the population approach should be applied. This population approach using non-linear mixed effect modeling allows for the analysis of dense, sparse, balanced or unbalanced data, making the application highly suitable in pediatric clinical practice. Additionally, it permits the exploration of the influence of different covariates such as bodyweight, age and other covariates, to explain the variability in drug response. Finally, using this approach, PK-PD studies can be designed in the most efficient manner in order to obtain the maximum information on the PK-PD parameters with the highest precision. Once a population PK and/or PD model is developed, internal and external validations should be performed [6]. If the model performs well in these validation procedures, model simulations can be used to define a dosing regimen which in turn needs to be tested and challenged in a prospective clinical trial [6]. This methodology will improve the efficacy/safety balance of dosing guidelines, which will be of benefit to the individual child. The population approach using non-linear mixed effect modeling was applied in this thesis to describe the developmental changes in renal clearance for different drugs across the pediatric age range.

10.2. Developmental changes in GFR in preterm and term neonates by describing the pharmacokinetics of renally excreted antibiotics

Previously it has been described that during the first month of life, a rapid rise in glomerular filtration is seen. Therefore in **chapter 3** of this thesis the developmental changes in glomerular filtration were described using data of amikacin in 874 preterm and term neonates (birth bodyweight 385-4650g, postnatal age 1-30 days, gestational age 24-43 weeks). Amikacin was used as a paradigm compound to reflect GFR because it is almost entirely eliminated through GFR, Postmenstrual age proved to be the most significant covariate on clearance based on the systematic covariate analysis. However, birth bodyweight and postnatal age, representing antenatal and postnatal maturation of the kidney, respectively, proved to be superior over postmenstrual age alone. Birth bodyweight was implemented on clearance using a power function with an exponent of 1.34 and postnatal age was implemented using a linear function with a slope of 0.2. Furthermore a decrease (16%) in clearance was seen when ibuprofen was co-administered. Based on the final pharmacokinetic model, which was validated both internally and externally, simulations were performed to illustrate exposure to amikacin in preterm and term neonates following currently used dosing regimens. Based on the simulations it could be concluded that the currently used dosing regimens should be revised as they may possibly increase the risk of toxicities since

target through values between 1.5-3 mg/L were often not reached. Consequently a new model-based dosing regimen was developed for preterm and term neonates aged between 1 and 30 days, and based on current bodyweight, postnatal age and co-administration of ibuprofen.

In **chapter 4**, this new model-based dosing regimen was prospectively evaluated in 579 preterm and term neonates (median birth bodyweight 2285g (range 420-4850g), postnatal age 2 days (range 1-30 days), gestational age 34 (range 24-41 weeks)). The analysis showed that across the entire neonatal age range the observed amikacin concentrations were accurately predicted by the final pharmacokinetic model without bias. Moreover the accuracy of the model was confirmed by the NPDE. Based on the Monte Carlo simulations, it was shown that peak concentrations above 24mg/L were reached in almost all patients with different bodyweight, postnatal age and use of ibuprofen. Trough concentrations below 3 mg/L were found for 78-100% of the individuals when ibuprofen was co-administered and for 45-96% of the individuals when ibuprofen was not co-administered.

Based on the prospective study, it can be concluded that the novel model-based dosing algorithm for amikacin leads to optimized peak and trough concentrations in preterm and term neonates with varying birth bodyweight, current bodyweight, postnatal age and ibuprofen co-administration. The model-based approach for dosing drugs in the highly variable population of neonates, as applied here for amikacin, substantially contributes to the individualization of dosing drugs in neonates.

To develop rational, evidence-based and individualized dosing regimen for specific drugs, pharmacokinetic and/or pharmacodynamic models need to be developed and validated [6,7] as seen in chapter 3. However to facilitate model development for groups of drugs, a more system-based pharmacology approach is needed [5]. This approach was applied in **chapter 5,** in which it was evaluated whether the covariate model for amikacin, describing the developmental changes in GFR in preterm and term neonates (chapter 3), could be extrapolated to other renally excreted drugs. To perform this analysis five different neonatal datasets on netilmicin, tobramycin, vancomycin and gentamicin were used. Using this approach a distinction was made between system-specific and drug-specific information [5]. The covariate model that included birth bodyweight, postnatal age and co-administration of ibuprofen was considered to be system-specific while the population value was considered drug-specific (equation 1).

$$CL_i = CL_p \bullet \underbrace{(\frac{bBW}{bBW_{Median}})^{1.34} \bullet (1+(0.213 \bullet \frac{PNA}{PNA_{Median}})) \bullet 0.838_{ibuprofen}}_{\text{Amikacin covariate model}}$$

Drug specific property

(Equation 1)

Subsequently the descriptive and predictive performance of the models using the amikacin covariate model was compared to the independent reference models, which were developed based on a systematic covariate analysis. Based on the analysis, it was concluded that the descriptive and predictive properties of the models developed using the amikacin covariate model were good and fairly similar to the independent reference models, as expressed by the goodness-of-fit plots and the normalized prediction distribution error method. Finally, the same covariates as in the covariate model of amikacin, i.e. birth bodyweight and postnatal age, were identified as the most important descriptors of clearance in the independent reference models. Consequently it was concluded that pediatric covariate models contain system-specific information describing the developmental changes in the underlying physiological processes. This approach in which information of one drug is extrapolated to another drug eliminated through the same route will lead to optimization of study design, sparse data analysis and will facilitate the development of individualized and evidence-based dosing regimen.

10.3. Developmental changes in renal function (GFR and tubular processes) in preterm and term neonates by describing the pharmacokinetics of cefazolin

Chapter 6 of this thesis focused on describing the pharmacokinetics of cefazolin in preterm and term neonates. In adults it is know that cefazolin is eliminated by both GFR and active tubular secretion [8,9] and that protein binding varies between 70-90% [10,11,12,13]. However in children and certainly in neonates only limited information is available on the pharmacokinetics of cefazolin. Therefore in chapter 6 of this thesis the pharmacokinetic properties of cefazolin were described in 36 preterm and term neonates (birth bodyweight 540-4200g, postnatal age 1-30 days, gestational age 24-40 weeks). Based on total and unbound cefazolin concentrations, a one compartment model was developed in which total and unbound concentrations were linked by estimation of the protein binding (B_{max}) and the dissociation constant (K_D) which

were estimated to be 136 mg/L and 46.5 mg/L, respectively. Birth bodyweight and postnatal age were found as most important covariates on clearance of cefazolin using a power and linear function, respectively. Furthermore, it was found that albumin was linearly correlated with B_{max}. Based on this final model, Monte Carlo simulations were performed to illustrate the exposure to cefazolin following the currently used dosing regimens. According to the results, it was suggested to adjust the dosing regimen proposed by the Dutch Children's Formulary [14] to attain unbound concentrations during 60% of the dosing interval above a concentration of 8 mg/L, which corresponds to the minimal inhibitory concentration according to The Clinical and Laboratory Standards Institute (CLSI) for susceptibility of Staphylococcal species [15] and to guarantee a similar exposure in all patients.

10.4. Renal and hepatic elimination of propylene glycol in preterm and term neonates

Drug formulations often contain excipients to increase solubility and/or stability of drugs. One of the frequently used excipients is propylene glycol (PG). Propylene glycol is normally considered to be safe. However toxic effects have been reported in the adult, pediatric and neonatal population and may include bradycardia, depression of the central nervous system, increase in anion gap, lactic acidosis, hepatic dysfunction and kidney injury [16,17,18,19]. As a consequence both the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) have established guidelines considering the maximum daily dose of propylene glycol. However, a large discrepancy is seen between both guidelines. While the FDA established an acceptable daily intake of 25mg/kg bodyweight, the EMA proposed a maximum daily dose of 400mg/kg in adults and 200mg/kg in children [20]. This discordance in both guidelines indicates the lack of information on the safe use of propylene glycol in adults but also in the pediatric age range. To date, no pharmacokinetic studies in the pediatric age range were available on propylene glycol. Only a limited number of reports was found in literature informing on the toxic effects of propylene glycol [18,21,22,23,24,25]. Therefore in **chapter 7**, the pharmacokinetics of propylene glycol co-administered intravenously with paracetamol (800mg PG/1000mg paracetamol) or phenobarbital (700mg PG/200mg phenobarbital) were described in 62 preterm and term neonates (birth bodyweight 630-3680g, postnatal age 1-30 days, gestational age 24-41 weeks). A one compartment model was developed in which birth bodyweight and postnatal age were found as most important covariates on clearance. Current bodyweight was found as most important covariate on volume of distribution and proved 1.77 times higher when co-administered with phenobarbital compared to

paracetamol. Based on this final pharmacokinetic model, simulations were performed to illustrate propylene glycol exposure when co-administered with paracetamol and phenobarbital. Based on the simulations, it was shown that the population mean propylene glycol peak and trough concentrations ranged between 33-144 and 28-218 mg/L (peak) and 19-109 and 6-112 mg/L (trough) for paracetamol and phenobarbital, respectively, depending on birth bodyweight and age of the neonates.

In **chapter 8** of this thesis, renal and hepatic elimination of propylene glycol was quantified in these preterm and term neonates. In adults, it is known that 45% of propylene glycol is eliminated through the renal route and 55% is metabolized in the liver by alcohol dehydrogenase (ADH) to lactate and pyruvate [26,27]. Due to immaturity of the renal function, renal clearance of propylene glycol may be expected to be lower in neonates compared to adults. Even though the pharmacokinetics of propylene glycol have been characterized in preterm and term neonates in chapter 7, no distinction could be made between renal and hepatic elimination of propylene glycol in that analysis. It is however important to characterize the magnitude of both pathways in neonates because when it appears that one pathway or the other is more dominant in neonates this may play a role in the significance of age-specific drug-drug interactions. Therefore in chapter 8, renal and hepatic elimination of propylene glycol was characterized. The pharmacokinetic analysis was performed based on concentrations of propylene glycol in both plasma and/or urine collected in 69 (pre) term neonates (birth bodyweight 630-3980g, postnatal age 1-30 days gestational age 24-41 weeks). Birth bodyweight and postnatal age were identified as most important covariates on hepatic clearance. Since a time-dependent trend was seen in the renal excretion of propylene glycol, different models were tested based on time after first dose, urine volume and amount of creatinine in urine. Renal clearance was 15% of the total clearance after the first dose but increased over time to 25% at 24hours after the first dose. This increase was best described by a hyperbolic function based on time after the first dose. Although renal clearance increased with time after first dose up to 25%, renal clearance in neonates was substantially lower compared to adults for which renal clearance of propylene glycol was reported to be about 45% of the total clearance. As a consequence, since in neonates hepatic clearance of propylene glycol is determined as the most important elimination route, this may implicate that drug-drug interactions at the alcohol dehydrogenase enzyme are more important in neonates compared to adults. Furthermore, this may potentially also indicate that renal failure is of less importance in neonates compared to adults considering the total elimination of propylene glycol. It is concluded that, to evaluate whether the increase in renal clearance of propylene glycol indicates an auto-induced increase in renal secretion or failure of tubular reabsorption of propylene glycol, further studies are needed.

10.5. Developmental changes in GFR from neonates until adults described using different renally excreted antibiotics

Glomerular filtration is responsible for the elimination of a large number of water-soluble drugs and their metabolites. GFR is well defined in adults and is estimated to be around 120 mL/min [4]. More uncertainty rises in the pediatric age range as GFR is supposed to reach adult levels at about 6 months – 1 year of age [4]. However an exact quantification of GFR throughout the pediatric age range is missing. Therefore, the aim in **chapter 9** was to describe the developmental changes in GFR from (pre) term neonates until adults (N=1760 patients, bodyweight 415g-85kg, age 1 day-18 years) by describing the pharmacokinetics of gentamicin, tobramycin and vancomycin, which are drugs that are almost entirely eliminated through GFR. Since the analysis was based on data of three different drugs combined into one analysis, a system-based pharmacology approach was used. This means that, as explained in chapter 5, a distinction was made between system-specific and drug-specific properties. The covariate model tested on clearance was considered to contain system-specific information reflecting the developmental changes in GFR applicable to all drugs while the population value was considered to be a drug-specific parameter. Across the entire pediatric age range, from premature neonates until adults, bodyweight was found as most significant covariate on clearance and volume of distribution. The effect of bodyweight was best described on clearance using an allometric function in which the exponent changed with bodyweight from 1.4 in neonates to 1 in adults. This indicates that the largest increase in clearance of these different drugs, reflecting GFR, is seen during the first weeks of life. This maturation function, developed in **chapter 9**, may possibly be used to describe evidence-based and individualized dosing regimen for renally excreted drugs over the entire pediatric age range.

10.6. Perspectives

The aim of the research described in this thesis was to describe the developmental changes in renal function. Renal function consists of glomerular filtration, tubular secretion and reabsorption. In this thesis we primarily focused on describing the developmental changes in glomerular filtration on the basis of analyses on drugs that are primarily excreted through GFR. Once the developmental changes in GFR are described, this information can be used to describe the developmental changes in tubular processes by studying drugs that are excreted on the basis of these different subprocesses.

Glomerular filtration is rapidly rising during the first weeks of life. Moreover, also large differences in GFR are seen between preterm and term neonates. Therefore in the first part of this thesis we focused on describing the developmental changes in GFR in neonates. Subsequently the maturation of the renal function was described across the entire pediatric age range. As explained in the introduction of this thesis (chapter 1), several methods can be used to measure GFR. The most practical manner to assess GFR in healthy individuals is by measuring creatinine clearance. Since creatinine is an endogenous compound, the burden to evaluate GFR can be kept to a minimum for each individual. However, a few remarks should be considered when creatinine is used to evaluate GFR. First of all, creatinine is not only filtered by GFR but also in part secreted by tubular secretion [4,28]. In addition, the measurement of creatinine clearance based on plasma samples can be complicated since the formation of creatinine depends on muscle mass, age and gender [4]. Furthermore in the first days of life creatinine values reflect maternal renal function [29,30,31]. Finally, the Schwartz formula which is often used to estimate GFR in children based on serum creatinine and body length, often leads to overprediction of GFR [32]. Consequently, due to the reasons mentioned above, creatinine is not the best marker to assess GFR. Other markers like inulin or radioisotopes [33,34] have amongst other things the disadvantage that these compounds are exogenous implicating that the burden for each individual will be increased when evaluating GFR compared to creatinine. Therefore to perform these analyses the pharmacokinetics were described of amikacin, netilmicin, tobramycin, vancomycin and gentamicin, which are drugs that are almost entirely eliminated through GFR. Since GFR was evaluated by describing data of renally excreted drugs, data could be directly obtained from clinical practice without administering diagnostics or other compounds specific for this analysis. The latter is of course of major importance for children so that the burden for each patient can be kept to a minimum. However, this also implicates that the developmental changes in glomerular filtration were characterized in non-healthy or sick patients.

In chapter 3 the GFR model for neonates was developed based on data of amikacin obtained in 874 preterm and term neonates, covering an extensive range in gestational age, birth bodyweight and postnatal age. This analysis in which a tremendous amount of data of amikacin was used to characterize developmental changes in GFR in preterm and term neonates was never performed before as previous analyses were based on a smaller number of patients and a more narrow age range compared to our analysis. Moreover, the model developed in chapter 3 was both internally and externally validated. Consequently this model, describing the developmental changes in GFR based on amikacin clearance, could then be used to describe clearance of other renally excreted drugs in neonates (chapter 5). This extrapolation to the other renally excreted drugs was performed to populations

with the same clinical characteristics and disease status compared to the amikacin dataset since all patients were admitted to the neonatal intensive care unit and were treated with aminoglycosides or glycopeptides when sepsis was suspected. However, since critical illness (i.e. sepsis) may have an influence on clearance, these models should not be applied in other patient populations until the accuracy of the model has been evaluated in those populations. In a previous study by Ince *et al.* [35] it was reported that critical illness severely reduced the CYP3A4-mediated clearance of midazolam. Therefore, the effects of critical illness on the developmental changes in glomerular filtration should be further analyzed, even though renal excretion can not be compared to CYP3A4 metabolism. Moreover the use of this kind of drugs (aminoglycosides, glycopeptides) may cause renal toxicity after repetitive dosing [36]. Furthermore, the model performance should also be evaluated in patients on extracorporeal membrane oxygenation (ECMO) treatment. Previously it has been shown that the very invasive ECMO treatment influences the pharmacokinetic parameters of various drugs [37,38,39,40,41,42]. In a study of Dodge *et al.* [43], it was concluded that neonates on ECMO receiving gentamicin had a higher volume of distribution and a lower clearance of gentamicin compared to neonates off ECMO. In conclusion, this means that the model developed in chapter 3 should not be applied to other patient populations until the accuracy and predictability of this model is evaluated in this population. The model can however be seen as a primary basis in which a large amount of data was used. Moreover, besides the fact that the model was both internally and externally validated, it was also used to predict other renally excreted drugs.

As explained in the introduction of this thesis, the objective of this thesis was to describe the developmental changes in renal function by the use of a more system-based pharmacology approach. The key feature of this approach is that a distinction was made between system-specific and drug-specific properties. This approach was first applied in chapter 5 of this thesis. The amikacin covariate model for neonates, which was considered to be system-specific, was extrapolated to netilmicin, tobramycin, vancomycin and gentamicin, which are drugs that are almost entirely eliminated through GFR (chapter 5). The applicability of this GFR model, based on amikacin was also illustrated in an analysis performed by Zhao *et al.* [44], in which the model of amikacin was used to predict clearance of vancomycin. Based on that study, it was concluded that the model describing the developmental changes in GFR based on amikacin can be used to predict dosage regimens of other renally excreted drugs by GFR in preterm and term neonates. However, it should be emphasized that the final pharmacokinetic models for amikacin, netilmicin, tobramycin, gentamicin and vancomycin as developed in section II of this thesis are only of significance in a specific age range namely preterm and term neonates. The exponential increase in clearance

with birth bodyweight (exponent of 1.34) seen in preterm and term neonates will not be applicable to older children and adults as the renal function will be gradually flattening. Therefore, in the last section of this thesis (chapter 9) the developmental changes in GFR were quantified over the entire pediatric age range based on three renally excreted drugs using a bodyweight-dependent exponent model, in which the exponent changes depending on bodyweight. This bodyweight-dependent exponent function permits that the exponent gradually changes according to bodyweight and is able to characterize more rapid changes in neonates compared to adults. Previously similar bodyweight-dependent exponential covariate models were developed to scale clearance of propofol [45,46], busulfan [47], midazolam [48] and morphine [49] from neonates until adults. In all these models, a higher exponent was found in neonates and young children (exponent >1) compared to older children and adults as found in the model describing the developmental changes in GFR from neonates (exponent of 1.4) until adults (exponent of 1). In our opinion, the innovative and progressive aspect of the model quantifying the developmental changes in GFR over the pediatric age range, described in this thesis, is that it was based on the combination of three renally excreted drugs, gentamcin, tobramycin and vancomycin allowing for the distinction between drug and system-specific properties [5]. It should however, be highlighted that further research is needed to evaluate the generalizability of the models describing the developmental changes in glomerular filtration in neonates (section II) and from neonates until adults (chapter 9). Since in the current analyses, all drugs have fairly similar physicochemical and pharmacokinetic drug properties, the influence of different physicochemical and pharmacokinetic drug properties to the extrapolation to other drugs should be characterized on the basis of physiologically-based modeling. This was also done in an analysis by Krekels *et al.* [50], in which the influence of differences in physicochemical properties was evaluated on the extrapolation possibilities of the glucuronidation function developed using morphine data. Finally, in a next step, as performed for amikacin in neonates (chapter 3), the final pharmacokinetic models should be used to evaluate the currently used dosing regimens for amikacin, gentamicin, tobramycin, netilmicin and vancomycin in neonates and over the entire pediatric age range. If it appears that the currently used dosing regimen should be revised, new model-based dosing regimens should be developed and tested in a prospective analysis in a similar manner as amikacin (chapter 4).

Although renal function consists of glomerular filtration, active tubular secretion and reabsorption, we primarily focused on describing the maturation in glomerular filtration. Once the developmental changes in GFR were characterized, we hypothesized that this information could be used to describe the developmental changes in tubular processes by studying drugs that are excreted by both GFR and tubular processes. Development of tubular processes starts from 36 weeks of

gestation and continues during childhood [4]. In comparison with glomerular filtration, development of tubular processes is delayed [4,51,52]. For the glomerular filtration rate, it is known that adult levels are reached at approximately 6-12 months of age while for the tubular processes adult levels are not reached until 1-5 years of age [52]. Since large differences are seen in renal function between preterm and term neonates during the first month of life, we decided to initially start with the quantification of the developmental changes in tubular secretion in preterm and term neonates, as performed for GFR using amikacin as a model drug. Since cefazolin is a drug which is both eliminated by GFR and active tubular secretion [8,9], this drug was used as a paradigm compound to quantify the maturational changes in tubular secretion in neonates. To perform this analysis it was supposed that when clearance of cefazolin was higher than the clearance of GFR, it was due to active tubular secretion. Consequently based on these assumptions, the semi-physiological GFR model based on amikacin clearance from chapter 3 was directly incorporated on cefazolin clearance. This implicated that birth weight was implemented on clearance using an allometric function with an exponent of 1.34 as well as postnatal age using a linear function with a slope of 0.213 (chapter 3). Although the population clearance value is considered a drug-specific parameter, it was fixed to the value obtained in the final pharmacokinetic model of amikacin (equation 2). The reason for this approach was that we found in chapter 5, in which the amikacin covariate model reflecting GFR in (pre)term neonates was extrapolated to four other renally excreted drugs, that all initial population values were very similar for all these different drugs. Therefore, in this analysis the initial population clearance value was not estimated but fixed to the value (0.0493 L/h for a neonate with a birth bodyweight of 1750g and a PNA of 2 days) obtained in final pharmacokinetic model of amikacin.

$$CL_i = \underbrace{\{\, CL_{p\ amikacin} \bullet \left(\frac{BWb}{BWb_{Median}}\right)^{1.34} \bullet (1 + (0.213 \bullet \frac{PNA}{PNA_{Median}}))\,\}}_{\text{Developmental changes in GFR based on amikacin clearance}} + \underbrace{\{\, CL_{p\ cefalozin} \bullet \text{Covariates}\,\}}_{\text{Developmental changes in tubular processes}}$$

(Equation 2)

The remaining part was then considered to describe clearance through active tubular secretion. Consequently, to quantify these developmental changes in active tubular secretion, a systematic covariate analysis was performed based on free cefazolin concentrations collected in the 36 preterm and term neonates used for the analysis in chapter 6. Similar to GFR, birth bodyweight and postnatal age were identified as most relevant covariates for active tubular secretion. By fixing the developmental changes of GFR to the results obtained with amikacin, we were able to isolate and quantify the developmental changes in tubular secretion. Table I gives an

Table I: Population pharmacokinetic parameter estimates of the simple model, the final pharmacokinetic model and the bootstrap analysis.

Parameter	Simple model	Final pharmacokinetic model	Bootstrap final pharmacokinetic model
	Value (CV%)	Value (CV%)	Value (CV%)
Fixed effects			
Glomerular filtration			
CL_{GFR} p in $CL_{GFR} = CL_{GFR}$ p x (bBW/median)^{m} x (1+(PNA/median)xn)	0.0493 FIX	0.0493 FIX	0.0493 FIX
m	1.34 FIX	1.34 FIX	1.34 FIX
n	0.213 FIX	0.213 FIX	0.213 FIX
Tubular processes			
CL_{Tub} (L/h) = CL_{Tub} p	0.0848 (31.7)	-	-
CL_{Tub} p in $CL_{Tub} = CL_{Tub}$ p x (bBW/median)^{o} x ((PNA/median)^{p})	-	0.147 (15.0)	0.146 (16.0)
o	-	1.99 (28.8)	2.03 (31.0)
p	-	0.271 (43.5)	0.266 (47.7)
V (L) = Vp	1.86 (8.1)	-	
Vp in V = Vp x (cBW/median)^{q}	-	1.98 (5.5)	1.97 (6.1)
q	-	1.19 (13.1)	1.21 (14.8)
Interindividual variability (ω^2)			
ω^2 CL	0.243 (31.9)	0.12 (37.2)	0.108 (40.1)
ω^2 V	0.253 (31.1)	0.0649 (33.3)	0.06 (36.9)
Residual variability (σ^2)			
σ^2 (proportional)	0.0469 (27.5)	0.0473 (27.3)	0.047 (28.5)

CL_{GFR} = clearance through glomerular filtration, CL_{GFR} p= population value for clearance through GFR, CL_{Tub} = Clearance through tubular processes, CL_{Tub}p= population value for clearance through tubular processes, V = Volume of distribution, Vp = population value for volume of distribution, bBW = bodyweight at birth, cBW = current bodyweight, PNA = postnatal age, median values for the covariate model on GFR are based on the GFR model based on amikacin, median values for the covariate model on tubular processes and volume of distribution are based on the currently used cefazolin dataset

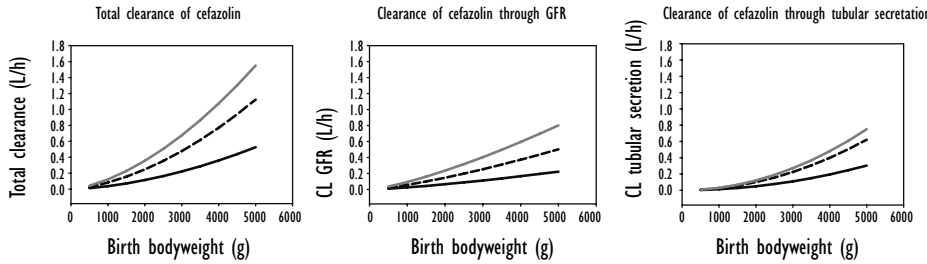


Figure 1: Model-based predicted total clearance (= sum of clearance through GFR and tubular processes) (left), clearance through GFR (based on the developmental changes in GFR seen with amikacin (equation 2) (middle) and clearance through tubular secretion (based on free cefazolin concentrations) (left) of cefazolin *versus* birth bodyweight for postnatal age of 1 (black line), 14 (dotted line) and 28 days (grey line).

overview of the parameter estimates of the simple and final pharmacokinetic model together with the values obtained from the bootstrap analysis. The model-based total clearance (sum of clearance through GFR and tubular processes), clearance through GFR and clearance through tubular processes of cefazolin *versus* birthweight for PNAs of 1, 14 and 28 days is illustrated in figure 1. Figure 1, in which clearance through tubular processes is illustrated *versus* birth bodyweight for PNAs 1, 14 and 28, indicates that the largest increase in tubular secretion is seen during the first 14 days. This may be explained by the upregulation of transporters (organic anion or cation transporters) in the kidney during the perinatal period to compensate the increased concentrations of various compounds after birth. Furthermore, this figure illustrates a lower clearance of cefazolin by tubular processes compared to glomerular filtration in neonates below 3.5 kg during the first 14 days. However, in neonates with a birth bodyweight above 3.5 kg the tubular clearance of cefazolin seem to transcend the glomerular filtration. Nevertheless on day 28, glomerular filtration is the most important elimination route of cefazolin in all neonates, which corresponds well with previously reported results that renal tubular development is delayed compared to GFR. The applicability of this model in which the developmental changes in tubular secretion were characterized in preterm and term neonates should subsequently be tested using other renally excreted drugs, which are undergoing both glomerular filtration as tubular processes. Finally in next step the maturational changes in tubular secretion should be described from neonates until adults. To perform that analysis and to be able to characterize the developmental changes in tubular secretion over the pediatric age range, the model described in chapter 9 can be used as a basis as this model is describing the developmental changes in GFR across the pediatric age range.

In summary we can claim that the developmental changes in glomerular filtration were described from preterm and term neonates to adults using a system-based pharmacology approach [5]. This implicated that a distinction was made between system-specific and drug-specific properties making it possible to extrapolate information of one drug to another drug. Further studies need to be performed to evaluate to what extent this approach is applicable to other drugs with different physicochemical drug properties. The preferred approach to perform this analysis is by the use of physiologically-based pharmacokinetic modeling approaches because this allows for simulations for a variety of drugs and physiological situations. Furthermore, the developmental changes in the other subprocesses that contribute to renal clearance (tubular secretion and reabsorption) should be characterized across the entire pediatric age range. To perform the latter analyses, the models describing the developmental changes in GFR can be used as a basis. The transition to a more system-based pharmacology approach and the combination of different strategies (extrapolation to other drugs, adult data or non-clinical data) will result in an approach focusing on the underlying system instead of focusing on the drugs and may facilitate development of pharmacokinetic models and evidence-based dosing regimens in the pediatric population.

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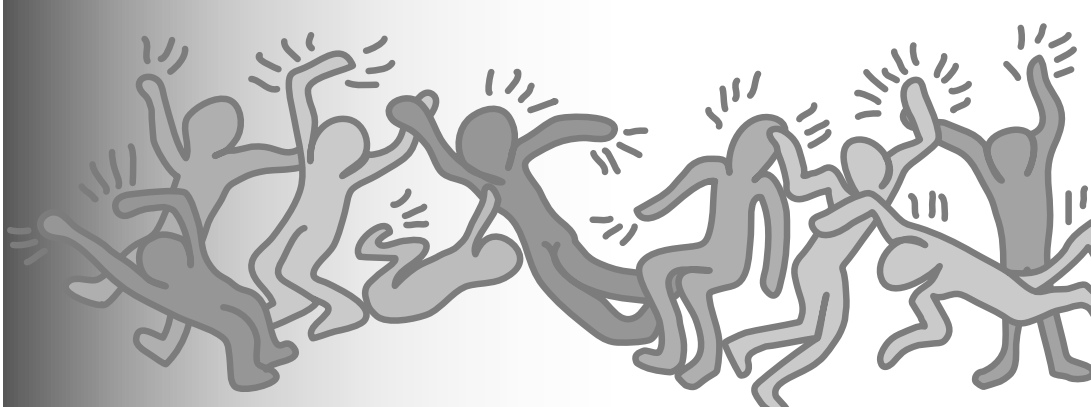
Chapter 11

Nederlandse Samenvatting



11.1. Voorspelling van de verandering in de ontwikkeling van renale klaring in kinderen aan de hand van een systeem farmacologische benadering

Doseervoorschriften bij kinderen worden vaak afgeleid uit doseervoorschriften bij volwassenen op basis van verschillen in lichaamsgewicht of lichaamsoppervlak. Aangezien kinderen niet beschouwd kunnen worden als kleine volwassenen, leidt dit echter vaak tot over- of onderdosering. Gedurende de ontwikkeling van het kind vinden er vele veranderingen plaats als gevolg van veranderingen in de grootte van het lichaam, veranderingen in de lichaamssamenstelling, en veranderingen in lever en nierfunctie. Dit heeft invloed op twee processen die bepalend zijn voor de werking van geneesmiddelen, de farmacokinetiek en de farmacodynamiek. De farmacokinetiek heeft betrekking op de absorptie, de verdeling en de eliminatie van geneesmiddelen in het lichaam, en bepaalt het verloop van de concentratie van het geneesmiddel als functie van de tijd. De farmacodynamiek heeft betrekking op de processen die ten grondslag liggen aan de werking van het geneesmiddel (de binding aan het doeleiwit, de activatie en de transductie mechanismen) en bepalend zijn voor de intensiteit van het effect als functie van de concentratie. Doseerregimes bij kinderen, die zijn afgeleid zijn uit doseerregimes bij volwassenen gebruik makend van lineaire extrapolaties op basis van lichaamsgewicht, houden geen rekening met deze veranderingen. Het is daarom noodzakelijk om de factoren die bepalend zijn voor de veranderingen in farmacokinetiek en farmacodynamiek van geneesmiddelen, die gebruikt worden bij kinderen, te karakteriseren. Dit zal uiteindelijk leiden tot wetenschappelijk onderbouwde en geïndividualiseerde doseerregimes. Om onderzoek naar de veranderingen in farmacokinetiek en farmacodynamiek bij kinderen te stimuleren, is er zowel in Europa als in de Verenigde Staten een nieuwe regelgeving met betrekking tot geneesmiddelonderzoek bij kinderen tot stand gekomen. Deze regelgeving houdt in dat onderzoek in kinderen verplicht is voor nieuwe, op de markt te brengen geneesmiddelen. Ondanks de positieve gevolgen van deze regelgeving voor de dosering van nieuwe geneesmiddelen bij kinderen, is er vooralsnog geen oplossing voor geneesmiddelen die al op de markt zijn, waardoor in de praktijk vele geneesmiddelen nog steeds off-label gebruikt worden bij kinderen.

Vele wateroplosbare geneesmiddelen en geneesmiddel-metabolieten worden geëlimineerd via de nieren. Renale klaring bestaat uit een samenspel van drie verschillende processen, glomerulaire filtratie, tubulaire secretie en tubulaire reabsorptie. Tijdens de ontwikkeling van het kind treden er belangrijke veranderingen op in elk van deze deelprocessen. Om veranderingen in de renale klaring van

geneesmiddelen in kinderen te karakteriseren, is een grondige kennis vereist van de veranderingen in elk van deze deelprocessen.

Het doel van het onderzoek in dit proefschrift was om de ontwikkelings-veranderingen in de nierfunctie te karakteriseren in verschillende leeftijdsgroepen. Daarbij is toegewerkt naar een systeem farmacologische benadering, waarbij veranderingen in de nierfunctie werden bestudeerd aan de hand van veranderingen in de excretie van modelstoffen. Bij het opstellen van de wiskundige modellen werd een onderscheid gemaakt tussen enerzijds systeem-specifieke en anderzijds geneesmiddel-specifieke eigenschappen.

In **hoofdstuk 2** van dit proefschrift, wordt een overzicht gegeven van de voordelen van de zogenaamde "populatie" analyse. Het uitvoeren van klinische studies in kinderen wordt beperkt door ethische, praktische en economische factoren. Bovendien wordt het als onethisch beschouwd om onderzoek uit te voeren bij gezonde kinderen waardoor vrijwel uitsluitend studies kunnen worden uitgevoerd in kinderen die lijden aan een ziekte. Dit heeft tot gevolg dat slechts een beperkt aantal kinderen beschikbaar is voor onderzoek. Bovendien is het bloedvolume bij kinderen beperkt waardoor het aantal en het volume van de bloedmonsters die kunnen worden afgenomen beperkt is. De beste methode om met deze beperkingen om te gaan, is door gebruik te maken van de populatie analyse. Deze methode maakt het mogelijk farmacokinetische en farmacodynamische processen te beschrijven op basis van beperkte en onevenwichtig opgebouwde datasets. Deze methode is daarom bijzonder geschikt voor het uitvoeren van onderzoek in kinderen. Bovendien is het mogelijk om de invloed van verschillende covariabelen zoals lichaamsgewicht, leeftijd en comedicatie te bestuderen om de variabiliteit tussen verschillende individuen te verklaren. Dat is belangrijk omdat op basis van de gevonden covariabelen de dosering kan worden geïndividualiseerd. Alvorens over te gaan tot toepassing in de praktijk, is het noodzakelijk dat het gevonden populatiemodel wordt geëvalueerd in een prospectieve klinische studie. Het onderzoek in dit proefschrift maakte gebruik van de populatie analyse, waarbij op basis van veranderingen in de renale uitscheiding van verschillende model-geneesmiddelen, de ontwikkelingsveranderingen in renale klaring bij kinderen in kaart werden gebracht.

11.2. Ontwikkelingsveranderingen in glomerulaire filtratie in preterme en a terme neonaten op basis van een farmacokinetische analyse van de renale excretie van antibiotica

Hoewel de nierfunctie goed gekarakteriseerd is in volwassen, zijn er slechts beperkte gegevens beschikbaar over ontwikkeling van de nierfunctie bij kinderen. Gedurende de eerste levensmaand wordt een snelle stijging gezien in de glomerulaire filtratie waarbij, rekening houdend met lichaamsoppervlak, volwassen waarden worden bereikt op een leeftijd van 6 maand tot 1 jaar. Tubulaire processen, daarentegen, lijken zich veel langzamer te ontwikkelen, waarbij volwassenen waarden worden bereikt bij een leeftijd tussen de 1-5 jaar. Omdat glomerulaire filtratie een belangrijke component is van de renale klaring van de meeste geneesmiddelen, was het primaire doel van ons onderzoek het karakteriseren van de ontwikkelingsveranderingen in glomerulaire filtratie. Om de glomerulaire filtratie te schatten, kunnen verschillende methoden worden toegepast waarbij de klaring van endogene (creatinine) of exogene (inuline, radio-isotopen) componenten wordt bepaald. Aan elk van deze methoden zijn verschillende nadelen verbonden, wat de routinematige toepassing bij kinderen en zeker bij neonaten moeilijk maakt.

Bijgevolg in het onderzoek dat is beschreven in dit proefschrift werd een methode gehanteerd, waarbij de glomerulaire filtratie geschat werd door bepaling van de klaring van een geneesmiddel dat volledig via glomerulaire filtratie wordt geëlimineerd. Het beschrijven van glomerulaire filtratie aan de hand van de klaring van een geneesmiddel heeft bovendien als voordeel dat de nodige informatie kan worden verkregen in de klinische praktijk zonder dat de patiënten hiervoor extra belast moeten worden. Het onderzoek dat wordt beschreven in **hoofdstuk 3**, heeft betrekking op de farmacokinetiek van amikacine in 874 preterme en a terme neonaten (geboortegewicht 385-4650g, postnatale leeftijd 1-30 dagen, zwangerschapsduur 23-43 weken). Amikacine werd gebruikt als model-geneesmiddel om de maturatie in glomerulaire filtratie weer te geven aangezien het bijna volledig geëlimineerd wordt door glomerulaire filtratie. Op basis van een systematische covariaatanalyse kon worden vastgesteld dat postmenstruele leeftijd de meest significante covariaat was voor de variatie in klaring. De combinatie van geboortegewicht en postnatale leeftijd, die respectievelijk de prenatale en postnatale maturatie reflecteren, bleek echter superieur te zijn over postmenstruele leeftijd. Op basis van deze bevindingen werd geconcludeerd dat in de onderzochte leeftijdsgroep, de veranderingen in

klaring van amikacine best beschreven werden met een exponentiële functie op basis van geboortegewicht met een exponent van 1.34 en een lineaire functie met een richtingscoëfficiënt van 0.2 op basis van postnatale leeftijd. Tenslotte werd vastgesteld dat de klaring van amikacine 16% lager was wanneer ibuprofen ook werd toegediend aan deze neonaten. Het huidige lichaamsgewicht werd gevonden als belangrijkste covariabele op verdelingsvolume. Om vast te stellen of het model kan worden gebruikt om concentraties te voorpellen, werd het model uitvoerig gevalideerd. Daarbij werd zowel een interne als een externe validatie verricht. Voor de externe validatie werden twee externe datasets gebruikt. Externe datasets worden gedefinieerd als datasets die afkomstig zijn van patiënten, die geen onderdeel vormden van de groep patiënten waarvan de data werden gebruikt om het model te ontwikkelen. Met behulp van verschillende analyses werd vastgesteld dat op basis van het model dat werd ontwikkeld in hoofdstuk 3, de concentraties van de patiënten in de externe datasets kunnen worden voorspeld op basis geboortegewicht, postnatale leeftijd, toediening van ibuprofen en huidig lichaamsgewicht. Nadat was gebleken dat op basis van het model goede voorspellingen kunnen worden gemaakt, werden simulaties uitgevoerd om de blootstelling aan amikacine in preterme en a terme neonaten te voorspellen op basis van de huidige in de praktijk toegepaste doseerregimes. Op basis van deze simulaties kon worden vastgesteld dat de huidige doseerrichtlijnen moeten worden herzien omdat deze naast gebrek aan effect ook aanleiding kunnen geven tot een verhoogd risico op toxiciteit omdat de gewenste dalconcentraties tussen 1.5-3 mg/L vaak niet werden bereikt. Op grond hiervan werd een nieuw doseerregime ontwikkeld op basis van de gevonden covariabelen in het finale model van amikacine (huidig lichaamsgewicht, postnatale leefijd en gelijktijdige toediening van ibuprofen) voor preterme en a terme neonaten met een leeftijd tussen 1 en 30 dagen. Tot slot werd geopperd dat dit model eventueel ook kan gebruikt worden om de farmacokinetiek van andere renaal geklaarde geneesmiddelen te beschrijven, omdat het ontwikkelde model in hoofdstuk 3 de glomerulaire filtratie reflecteert.

In **hoofdstuk 4** werd het nieuwe doseerregime voor amikacine, zoals dat werd ontwikkeld in hoofdstuk 3, geëvalueerd in de klinische praktijk. In deze prospectieve studie werden 579 preterme en terme neonaten geïncludeerd (geboortegewicht 420-4850g, postnatale leeftijd 1-30 dagen, zwangerschapsduur 24-41 weken). In een eerste analyse werden de waargenomen amikacine piek- en dalconcentraties vergeleken met de concentraties die werden voorspeld op basis van het finale model uit hoofdstuk 3. Deze analyse toonde aan dat over het gehele neonatale leeftijdsbereik de waargenomen amikacine concentraties nauwkeurig werden voorspeld zonder bias. Bovendien werd de juistheid bevestigd door het uitvoeren van de NPDE. Om de blootstelling aan amikacine te evalueren in preterme en terme neonaten werden tot slot ook Monte Carlo simulaties uitgevoerd. Op basis hiervan, werd

aangetoond dat piekconcentraties boven de gewenste waarde van 24 mg/L bereikt werden in bijna alle patiënten met verschillend lichaamsgewicht, postnatale leeftijd en gebruik van ibuprofen. Dalconcentraties onder 3 mg/L werden bereikt bij 78-100% van de neonaten wanneer ibuprofen werd toegediend en 45-96% van de neonaten wanneer geen ibuprofen werd toegediend. Op basis van deze prospectieve studie, kan er besloten worden dat het nieuwe doseerregime voor amikacin, dat gebaseerd werd op het farmacokinetisch model, ontwikkeld in hoofdstuk 3, vaker leidt tot de gewenste piek- en dalspiegels voor amikacine bij preterme en a terme neonaten met een verschillend lichaamsgewicht, geboortegewicht, postnatale leeftijd en gebruik van ibuprofen. Deze benadering, zoals toegepast in hoofdstuk 3 en 4 voor amikacine in preterme en a terme neonaten, vormt de basis voor een verdere individualisatie van doseerregimes bij neonaten.

Zoals beschreven in hoofdstuk 3 en 4, kunnen rationele, wetenschappelijk onderbouwde en geïndividualiseerde doseerschema's worden ontwikkeld op basis van gevalideerde farmacokinetische en/of farmacodynamische modellen. Dit is echter een heel tijdsrovend en intensief proces, wat het onmogelijk maakt dit uit te voeren voor elk geneesmiddel en in elke leeftijdscategorie. Om die reden werd in **hoofdstuk 5** een systeem farmacologische benadering gebruikt, waarbij systeem-specifieke informatie die is verkregen op basis van de analyse van een geneesmiddel, wordt geëxtrapoleerd naar andere geneesmiddelen die via dezelfde weg geëlimineerd worden. Met andere woorden, werd in hoofdstuk 5 nagegaan of het covariaten model van amikacine, wat de ontwikkelingsveranderingen beschrijft in glomerulaire filtratie, kan worden geëxtrapoleerd naar andere geneesmiddelen die voornamelijk via glomerulaire filtratie worden uitgescheiden. Om deze analyse uit te voeren, werden vijf verschillende datasets gebruikt die betrekking hebben op de renale excretie van netilmicine, tobramycine, vancomycine en gentamycine in kinderen. Het uitgangspunt bij deze analyse was als volgt: het covariaten model, op basis van geboortegewicht, postnatale leeftijd en toediening van ibuprofen, werd beschouwd als een systeem-specifieke beschrijving van de leeftijdsafhankelijke veranderingen in glomerulaire filtratie. De populatiewaarden klaring en verdelingsvolume worden daarentegen beschouwd als geneesmiddel-specifieke parameters. Dat betekent dat de waarden van deze parameters moeten worden geschat in de populatie analyse.

$$CL_i = CL_p \bullet \left(\frac{bBW}{bBW_{Median}}\right)^{1.34} \bullet \left(1+\left(0.213 \bullet \frac{PNA}{PNA_{Median}}\right)\right) \bullet 0.838_{ibuprofen}$$

Geneesmiddel specifieke eigenschap

Amikacin covariaten model = Systeem specifieke eigenschap

(Vergelijking 1)

Om de beschrijvende en voorspellende waarde van het systeem farmacologisch model, dat is ontwikkeld op basis van amikacine te evalueren, werden de modellen vergeleken met zogenaamde referentie modellen die ontwikkeld werden op basis van een systematische covariaatanalyse voor elke stof afzonderlijk. Op basis van deze vergelijking, kon worden vastgesteld dat de modellen ontwikkeld met het covariaten model van amikacine de data even goed beschreef als de onafhankelijke referentie modellen. Bovendien werden bij de referentie modellen dezelfde covariabelen, geboortegewicht en postnatale leeftijd, geïdentificeerd als meest belangrijke covariabelen op klaring. Op basis hiervan kan worden geconcludeerd dat pediatrische covariaten modellen systeem-specifieke informatie bevatten die onderliggende fysiologische processen beschrijven. Deze benadering, waarbij informatie van het ene geneesmiddel geëxtrapoleerd wordt naar het andere dat geëlimineerd wordt via dezelfde route, zal leiden tot optimalisatie van het studie opzet en zal de ontwikkeling van geïndividualiseerde en wetenschappelijk onderbouwde doseerschema's bevorderen.

11.3. Ontwikkelingsveranderingen in de glomerulaire filtratie en tubulaire secretie in preterme en a terme neonaten op basis van een farmacokinetische analyse van de renale excretie van cefazoline

Zoals hierboven beschreven, bestaat renale klaring niet alleen uit glomerulaire filtratie maar ook uit tubulaire processen: tubulaire secretie en reabsorptie. Cefazoline is een geneesmiddel dat zowel via glomerulaire filtratie als via actieve tubulaire secretie wordt geëlimineerd. **Hoofdstuk 6** van dit proefschrift heeft betrekking op de farmacokinetiek van cefazoline in preterme en a terme neonaten. Op basis van zowel totale als vrije cefazoline concentraties in 36 neonaten (geboortegewicht 540-4200g, postnatale 1-30 dagen, zwangerschapsduur 24-40 weken) werd een één compartimenten farmacokinetisch model ontwikkeld waarbij samenhang tussen totale en vrije concentraties werd beschreven door het schatten van parameters die de mate van de eiwitbinding (B_{max} = 136 mg/L) en de dissociatieconstante (K_D = 46.5 mg/L) beschrijven. In het ontwikkelde model waren geboortegewicht en postnatale leeftijd de belangrijkste covariabelen voor klaring van cefazoline. De verandering in cefazoline klaring werd best beschreven met een exponentiële functie op basis van geboortegewicht en een lineaire functie voor postnatale leeftijd. Bovendien werd een lineaire relatie gevonden tussen de albumine concentratie en de B_{max} voor de plasma eiwitbinding. Op basis van dit finale model, werden Monte Carlo simulaties

uitgevoerd om de blootstelling aan cefazoline te voorspellen wanneer de huidige doseerregimes worden opgevolgd. Op basis van de resultaten, wordt voorgesteld om het doseerregime zoals beschreven in het Nederlands Kinderformularium, enigszins aan te passen opdat gedurende 60% van het doseerinterval de waarden van de vrije concentraties boven 8 mg/L zijn. Deze 8 mg/L komt overeen met de minimaal inhiberende concentratie voor de vatbaarheid van Staphylococcen volgens het "Clinical and Laboratory Standards Institute". Met deze kleine aanpassing van het Nederlandse doseerregime, nl. toevoeging van één extra doseergroep, kon een vergelijkbare blootstelling in alle patiënten worden verzekerd.

11.4. Renale en hepatische eliminatie van propyleen glycol in preterme en a terme neonaten

Geneesmiddelen bevatten vaak hulpstoffen om de stabiliteit of de oplosbaarheid van de actieve componenten te vergroten. Propyleen glycol is één van de veel gebruikte hulpstoffen. Hoewel hulpstoffen als inactief en veilig zouden moeten kunnen worden beschouwd, werden toch bijwerkingen en toxische effecten gerapporteerd zoals bradycardie, depressie van het centraal zenuwstelsel, lactaatacidose, lever- en nierlijden na toediening van hoge en/or langdurige doses propyleen glycol bij volwassenen, kinderen en neonaten. Hoewel er richtlijnen bestaan, opgesteld door zowel de "Food and Drug Administration" (FDA) als de "European Medicine Agency" (EMA) die betrekking hebben op de maximale dagdosis van propyleen glycol, zijn er grote verschillen in deze richtlijnen van beide instanties. De maximale dagdosis voor propyleen glycol volgens de FDA is 25 mg/kg lichaamsgewicht terwijl de EMA een maximale dagdosis van 400 mg/kg in volwassen en 200 mg/kg in kinderen voorstelt. Deze verschillen tussen beide richtlijnen is een gevolg van het gebrek aan klinische informatie voor het veilig gebruik van propyleen glycol in volwassenen en kinderen. Bij volwassenen zou ongeveer 45% van propyleen glycol geëlimineerd worden via de nieren en 55% gemetaboliseerd worden door de lever door het enzym alcoholdehydrogenase waarbij het wordt omgezet naar lactaat en pyruvaat. Tot op heden zijn er echter geen gegevens uit farmacokinetische studies bij kinderen beschikbaar omtrent propyleen glycol. Er is enkel een beperkt aantal meldingen beschikbaar in de literatuur waarbij de toxische effecten na toediening van propyleen glycol worden gerapporteerd. Doordat de nieren nog niet volledig ontwikkeld zijn en de renale rijping niet noodzakelijk simultaan verloopt met de hepatische rijping, is de verwachting dat de renale klaring bij kinderen en zeker bij neonaten laag is in vergelijking met volwassenen. Om die reden was het doel in Sectie III van dit proefschrift om de renale en hepatische eliminatie van propyleen glycol te beschrijven in preterme en a terme neonaten.

Hoofdstuk 7 heeft betrekking op de farmacokinetiek van propyleen glycol, wanneer toegediend in combinatie met paracetamol of fenobarbital, in 62 preterme en a terme neonaten (geboortegewicht 630-3680g, postnatale leeftijd 1-30 dagen, zwangerschapsduur 24-41 weken). Op basis van de data werd een één compartimenten model ontwikkeld met zowel geboortegewicht en postnatale leeftijd als de belangrijkste covariabelen op klaring. De meest belangrijke covariabele op het verdelingsvolume bleek huidig lichaamsgewicht te zijn. Bovendien werd vastgesteld dat het verdelingsvolume 1.77 keer hoger was voor propyleen glycol wanneer dit werd toegediend met fenobarbital in vergelijking met paracetamol. Om de blootstelling aan propyleen glycol in neonaten te evalueren wanneer toegediend in combinatie met paracetamol of fenobarbital werden simulaties uitgevoerd. Op basis van de simulaties werd vastgesteld dat afhankelijk van geboortegewicht en postnatale leeftijd van de neonaten, de propyleen glycol concentraties varieerden tussen 33-144 en 28-218 mg/L (piekconcentraties) en 19-109 en 6-112 mg/L (dalconcentraties) wanneer toegediend met respectievelijk paracetamol en fenobarbital.

In **hoofdstuk 8** van deze thesis kon zowel de hepatische als renale klaring van propyleen glycol gekwantificeerd worden in 69 neonaten (geboortegewicht 630-3980g, postnatale leeftijd 1-30 dagen, zwangerschapsduur 24-41 weken) gebruik makend van propyleen glycol concentraties in zowel plasma als urine. Geboortegewicht en postnatale leeftijd werden gevonden als belangrijkste covariabelen op hepatische klaring. Omdat een tijdsafhankelijkheid werd gezien werd in de renale excretie van propyleen glycol, werden verschillende modellen getest om deze verandering te kunnen beschrijven, waaronder: modellen gebaseerd op tijd na eerste dosis, op urine volume of op de hoeveelheid creatinine in de urine. Uiteindelijk bleek dat de renale klaring 15% was van de totale klaring na de eerste dosis maar dat deze steeg tot 25% 24 uur na de eerste dosis. Deze stijging werd best beschreven door middel van een hyperbolische functie op basis van tijd na de eerste dosis. Hoewel dit onderzoek laat zien de renale klaring van propyleen glycol in neonaten toeneemt met tijd na de eerste dosis, is de renale klaring substantieel lager in neonaten in vergelijking met volwassenen, waar de renale klaring van propyleen glycol 45% van de totale klaring bedraagt. Omdat bij neonaten de hepatische eliminatie van propyleen glycol het belangrijkst is, heeft dit tot gevolg dat geneesmiddelinteracties ter hoogte van het alcoholdehydrogenase enzym belangrijker zijn in neonaten in vergelijking met volwassen. Om vast te stellen of deze stijging in renale klaring een auto-geïnduceerde stijging is in renale secretie of falen of tubulaire reabsorptie is, moeten meer studies worden uitgevoerd.

11.5. Ontwikkelingsveranderingen in glomerulaire filtratie van neonaten tot volwassen op basis van een farmacokinetische analyse van de renale excretie van antibiotica

Hoewel glomerulaire filtratie goed gedefinieerd is in volwassen en geschat wordt op ongeveer 120 ml/min, is er slechts een beperkte kennis van de maturatie van glomerulaire filtratie over de verschillende leeftijdscategorieën. Glomerulaire filtratie wordt verondersteld volwassenen waarden (uitgedrukt per lichaamsoppervlak) te bereiken op een leeftijd tussen 6 maand tot 1 jaar, maar een exacte kwantificatie ontbreekt. Dit lijkt te worden bevestigd in hoofdstuk 3 waar de veranderingen in glomerulaire filtratie in preterme en a terme neonaten werden beschreven. Echter in deze studie wordt geen rechtstreekse vergelijking met volwassenen gemaakt. Het doel van het onderzoek in hoofdstuk 9 is de ontwikkeling van een systeem-specifiek model voor de maturatie in de glomerulaire filtratie in de leeftijdsgroep van neonaten tot volwassen (N = 1760). Dit model werd ontwikkeld op basis van een analyse van de farmacokinetiek van drie renaal geëlimineerde geneesmiddelen, m.n. tobramycine, gentamycine en vancomycine. Door opnieuw een onderscheid te maken tussen systeem-specifieke en geneesmiddel-specifieke eigenschappen, konden de data van deze drie geneesmiddelen gezamenlijk worden geanalyseerd in één analyse. Daartoe werd een uniek covariaten model toegepast voor de beschrijving van de variatie in renale klaring. De populatie waarden werden op hun beurt weer beschouwd als geneesmiddel-specifieke eigenschappen en geschat voor elk geneesmiddel apart. Op basis van deze analyse in 1760 individuen (lichaamsgewicht 415 g - 85 kg, leeftijd 1 dag - 18 jaar) werd lichaamsgewicht gevonden als meest significante covariabele op zowel de klaring als het verdelingsvolume. Het effect van lichaamsgewicht op klaring werd best beschreven door gebruik te maken van een allometrische functie waarbij de exponent varieerde met lichaamsgewicht van 1.4 in neonaten tot 1.0 bij volwassenen. Dit is in overeenstemming met andere studies waarbij de waarde van de exponent bij neonaten en jonge kinderen hoger was dan bij oudere kinderen en volwassen. Dit wijst erop dat de grootste stijging in de glomerulaire filtratie, optreedt gedurende de eerste levensweken. Deze maturatie functie, ontwikkeld in hoofdstuk 9, kan mogelijk gebruikt worden om wetenschappelijk onderbouwde en geïndividualiseerde doseerschema's te ontwikkelen voor renaal geëlimineerde geneesmiddelen over het volledige leeftijdsbereik van neonaten tot volwassenen.

11.6. Conclusie

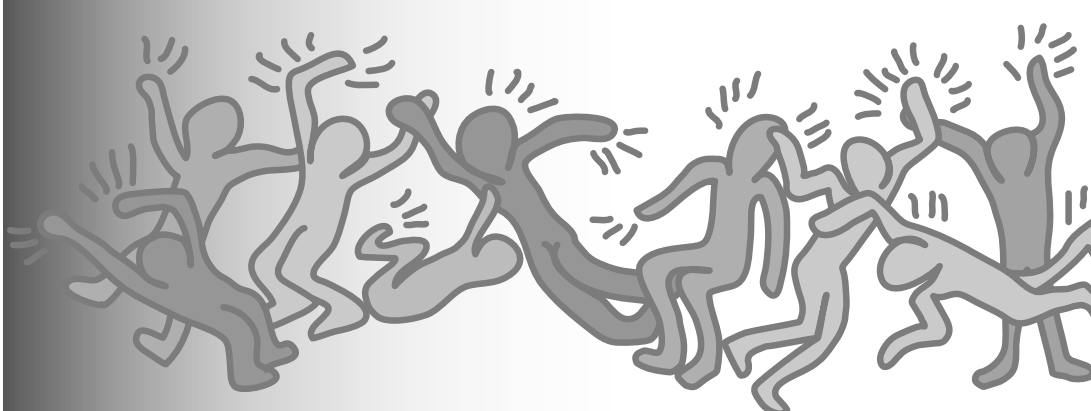
Gedurende de groei en ontwikkeling vinden veel veranderingen plaats in het lichaam. Aangezien de geneesmiddelblootstelling door deze veranderingen in groei en ontwikkeling wordt beïnvloed, moeten deze worden gekarakteriseerd. In dit proefschrift hebben we onderzoek gedaan naar de ontwikkelingsveranderingen in glomerulaire filtratie van neonaten tot volwassenen, op basis van een analyse van de veranderingen in farmacokinetiek van (model)-geneesmiddelen die bijna uitsluitend via glomerulaire filtratie worden geëlimineerd. In eerste instantie hebben we ons hierbij gericht op karakteriseren van glomerulaire filtratie in preterme en a terme neonaten, aangezien in deze groep grote veranderingen worden gezien. Op basis van het ontwikkelde farmacokinetisch model, werd vervolgens na een uitgebreide interne en externe validatie, een nieuw doseerregime opgesteld voor amikacine in preterme en a terme neonaten. Vervolgens werd in een klinische studie het nieuwe doseerregime, afgeleid uit de farmacokinetische analyse, geëvalueerd.

Om te vermijden dat voor elk geneesmiddel een tijdsrovende systematische farmacokinetische en/of farmacodynamische analyse moet worden uitgevoerd, werd vervolgens een nieuwe benadering toegepast, de zogenaamde systeem farmacologische benadering. Dit betekende dat informatie van het ene geneesmiddel geëxtrapoleerd werd naar het andere geneesmiddel, door een onderscheid te maken tussen systeem-specifieke eigenschappen en geneesmiddel-specifieke eigenschappen. Tot slot werden de ontwikkelingsveranderingen in glomerulaire filtratie gekarakteriseerd van neonaten tot volwassen, waarbij tevens deze systeem farmacologische benadering werd gebruikt. Dit had tot gevolg dat 3 verschillende geneesmiddelen, tobramycine, gentamycine en vancomycine konden gecombineerd worden in één analyse. In bovenstaande analyses is het echter wel belangrijk om op te merken dat deze systeem farmacologische benadering werd toegepast op de renale excretie van geneesmiddelen met vergelijkbare fysicochemische eigenschappen. Om de exacte invloed van verschillen in fysicochemische en farmacokinetische eigenschappen te evalueren, is het belangrijk meer studies uit te voeren om na te gaan in hoeverre deze nieuwe benadering kan worden toegepast. Deze analyse wordt best uitgevoerd door gebruik te maken van "fysiologische gebaseerde farmacokinetische modeling" omdat met deze benadering het mogelijk is simulaties uit te voeren met geneesmiddelen met verschillende fysiologische en farmacokinetische eigenschappen. Tot slot is het ook belangrijk de ontwikkelingsveranderingen te beschrijven over de verschillende leeftijdscategorieën in de andere subprocessen die bijdragen tot renale klaring namelijk tubulaire secretie en reabsorptie. Hierbij kunnen de modellen, beschreven

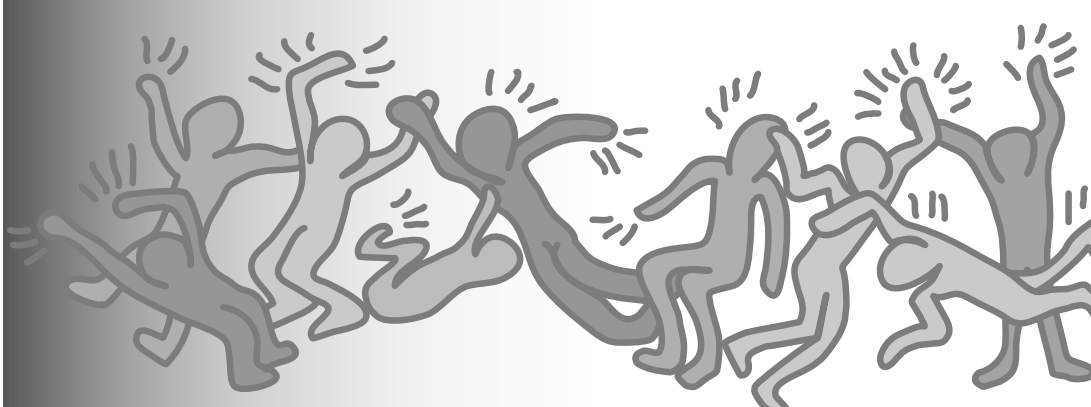
in dit proefschrift, die de ontwikkelingsveranderingen beschrijven in glomerulaire filtratie gebruikt worden als basis.

De transitie naar een systeem farmacologische benadering en de combinatie van verschillende strategieën (extrapolatie naar andere geneesmiddelen, gebruik van data uit volwassenen of niet klinische data) zal uiteindelijk leiden tot benaderingen die zich meer focussen op het functioneren van onderliggende systemen dan op geneesmiddelen. Dit zal uiteindelijk de ontwikkeling van farmacokinetische modellen en wetenschappelijk onderbouwde doseerregimes in de pediatrische populatie vereenvoudigen.

Appendix



Nawoord
Curriculum Vitae
List of Publications



Nawoord

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Ik heb geschreven,

Roosmarijn

Curriculum Vitae

Roosmarijn De Cock was born on April 16, 1985 in Dendermonde, Belgium. After her graduation in 2003 at the Sint-Lodewijkscollege in Lokeren, Belgium, she started her training to become a pharmacist. In 2008 she graduated with honours at the University of Ghent, Belgium.

In March 2009 she started as a PhD student at the division of Pharmacology of the Leiden Academic Center for Drug Research under the supervision of Prof. Catherijne Knibbe, Prof. Meindert Danhof and Prof. Karel Allegaert. During her PhD research she performed an internship to become a clinical pharmacologist at the St. Antonius Hospital, Nieuwegein, the Netherlands.

Since May 2013, Roosmarijn works at the Medical department of Bayer Healthcare, Belgium.

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