Chapter 7

Top-Down and Bottom-Up Modeling; The Physiological and Physicochemical Basis for the Maturation of UGT2B7-Mediated Drug Glucuronidation

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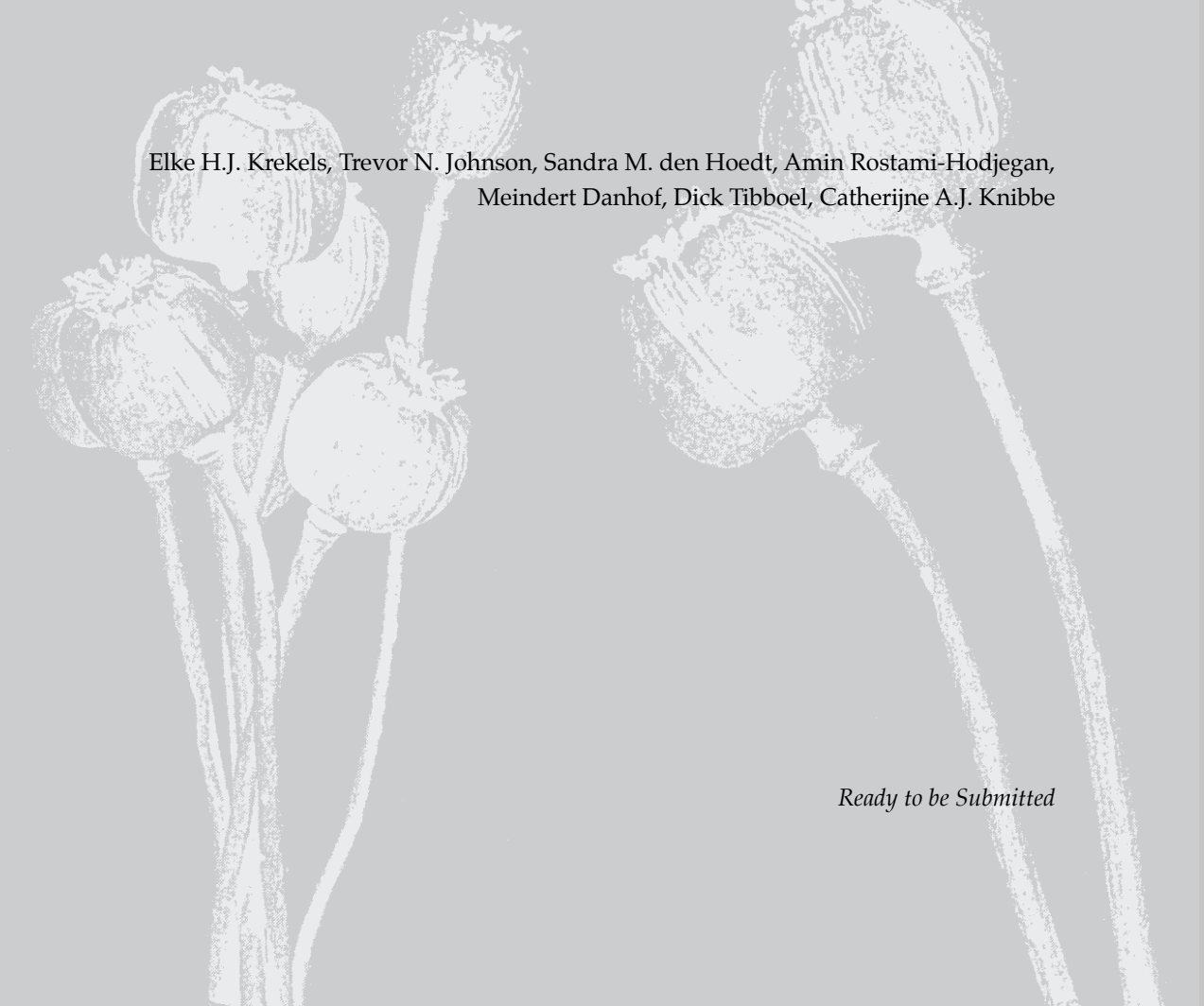

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Abstract

There is little insight in the extrapolation potential of paediatric covariate models between drugs that share a common elimination pathway. In this study, the physiological and physicochemical basis of a semi-physiological covariate model quantifying the developmental changes in *in vivo* UGT2B7-mediated drug glucuronidation in children younger than three years in population models (top-down models), was untangled using a physiologically-based model (bottom-up model).

Simcyp version 11 was used to simulate *in vivo* clearance values for morphine and zidovudine, both selective UGT2B7 substrates, in children younger than three years. The contribution of changes in system-specific parameters (hepatic blood flow, liver volume, microsomal protein per gram of liver, UGT2B7 ontogeny, unbound drug fraction) to changes in *in vivo* drug clearances were quantified. Additionally, the influence of physicochemical drug properties (molecular mass, logP, pKa) on the *in vivo* clearance of hypothetical UGT2B7-substrates was determined.

Using currently available *in vitro* data, morphine and zidovudine clearances were under-predicted by the physiologically-based model. However, the predicted developmental profile in glucuronidation clearance was similar to the clinically observed profile across the first three years of life, with the exception of the first two weeks of life. Changes in system-specific parameters explained 79% and 41% of the increases in *in vivo* morphine and zidovudine clearance, respectively, with the influence of liver size and UGT2B7 ontogeny being most pronounced. Physicochemical drug parameters did not affect the developmental glucuronidation profile, although logP and pKa did both influence the absolute value of clearance.

In conclusion, liver size and UGT2B7 ontogeny were identified as the main physiological drivers of the increases in UGT2B7-mediated clearance in the first three years of life. As physicochemical drug parameters only alter the absolute value of paediatric *in vivo* glucuronidation, the semi-physiological paediatric covariate model for drug glucuronidation can be used to predict the developmental clearance profile of other UGT2B7 substrates. Situations involving non-linear clearance as well as blood flow dependence due to high drug extraction ratios need further investigation.

7.1 Introduction

Research to develop evidence-based rather than empiric or consensus-based dosing algorithms for the paediatric population is complicated by practical, ethical, and legal constraints. These issues can be overcome by the application of population modeling approaches as is also recommended by the FDA and EMA [1,2]. Population modeling relies on outcome measures (i.e. observed concentrations) that can be obtained during routine clinical practice and allows for data analysis on the basis of sparse samples [3]. However, despite the multi-factorial nature of the ontogeny of drug clearance, paediatric population models only describe net observed changes in ontogeny with a limited number of covariate relationships. Therefore these models are only applicable to specific drugs in a specified population, requiring collection of the same type of data and full analysis of these data for every drug in every population of interest. Recently physiologically-based modeling has gained in popularity. These models use *in vitro* data on drug kinetics in combination with anatomical measurements, physiological parameters and mathematical equations to quantify physiological processes and the interaction of a molecule with certain physicochemical properties with this system. The usefulness of this type of modeling in paediatric pharmacokinetics has also been recognized by regulatory agencies [4]. Not all parameters in the physiologically-based models can be easily obtained, especially in the paediatric population. However, most parameters are 'system specific' and therefore not restricted to specific drugs or populations. As a result physiologically-based models are more generalizable.

Only rarely have the advantages of physiologically-based modeling, known as the bottom-up approach, been combined with the top-down approach of population modeling to augment each other. We have recently developed and validated a population pharmacokinetic model for morphine glucuronidation in children younger than three years (Chapters 3 and 4). It was found that the paediatric covariate model that quantifies the developmental changes in morphine clearance can be directly incorporated in the paediatric population model for the clearance of zidovudine (Chapter 6). Since morphine and zidovudine are both primarily eliminated through UGT2B7-mediated glucuronidation, this confirmed our hypothesis that covariate relationships in paediatric population models quantify developmental changes in the underlying physiological system, and thereby constitute system-specific information that can be extrapolated between drugs that share a common elimination pathway. Pharmacokinetic modeling based on this concept was called semi-physiological modeling, as it combines analyzing outcome measures with population modeling and the mechanistic insight of physiologically-based modeling.

The current study focuses on the semi-physiological developmental glucuronidation model for UGT2B7-mediated glucuronidation clearance in preterm and term neonates to children younger than three years, which was developed using morphine and zidovudine as model drugs (Chapters 3, 4, and 6). We aimed to dissect the various sources of developmental changes in the physiological system to identify which changes are the main drivers of the net observed changes in UGT2B7-mediated glucuronidation in neonates and infants, thereby also identifying patient characteristics that potentially limit the applicability of the semi-physiological developmental glucuronidation model. Additionally, we investigated whether certain physicochemical drug properties could affect the utility of the semi-physiological developmental model for compounds that share the UGT2B7-mediated elimination pathway.

7.2. Materials and Methods

7.2.1 Physiologically-Based Simulations

The physiologically-based pharmacokinetic modeling software Simcyp version 11 (Simcyp Ltd, Sheffield, UK) was used to investigate the influence of system-specific and drug-specific parameters on the ontogeny of *in vivo* UGT2B7-mediated drug glucuronidation in the first three years of life.

The paediatric database in Simcyp was selected for the physiologically-based pharmacokinetic simulations and parameters were set to include patients with a maximum age of three years. One thousand individuals were simulated and the same random number generation seed was used for repeated simulations. This yielded exactly the same set of individuals for each simulation and allowed for a direct comparison of clearance predictions between individuals in simulations with varying model parameter values. A uniform age distribution was used and the male/female ratio was set to 1. For each of the 1000 simulated individuals, age and sex appropriate bodyweight and height were determined based on UK reference growth charts taking inter-individual variability into account [5]. Body surface area was calculated according to Haycock *et al.* [6] for children with a bodyweight less than 15 kg and according to DuBois and DuBois [7] for heavier children. The parallel-tube model was used to derive hepatic drug clearances from *in vitro* intrinsic clearances.

In the current study, morphine and zidovudine were used as model compounds. Both drugs are specific substrates for the UGT2B7 isoenzyme [8–10] and used in the proof-of-concept studies for the development of the semi-physiological paediatric model (Chapters 3 and 6). In the simulations, intravenous bolus doses of 0.1 mg/kg morphine or 3 mg/kg zidovudine were administered, representing clinically relevant doses.

7.2.1.1 System-Specific Parameters

Hepatic blood flow, liver volume, milligram microsomal protein per gram of liver (MPPGL), UGT2B7 ontogeny and unbound drug fraction were the five system-specific parameters of the physiologically-based pharmacokinetic model that were investigated in the current study. The term UGT2B7 ontogeny is used to describe the fractional expression and function of the UGT2B7 isoenzyme in children compared to adults. The percentage change of each of the five parameters was calculated for patients in i) the first three months of life (0 – 3 months, group I), ii) the second three months (3 – 6 months, group II), iii) the second half year (6 – 12 months, group III), iv) the second year (1 – 2 years, group IV), and v) the third year (2 – 3 years, group V). This was done by calculating the mean parameter value of all individuals with an age in the first two weeks and the last two weeks of each interval and determining the percentage increase in these values per group.

Subsequently, one at a time, the values of each of the five system-specific parameters were increased within a physiologically relevant range, based on the mean increase in the parameter values in each of the five age-groups. For hepatic blood flow and liver volume this was 23%, for MPPGL this was 2%, for the UGT2B7 ontogeny factor this was 21%, and for the unbound drug fraction this was 0.51% for morphine and 0.33% for zidovudine. To quantify the influence of these changes on the *in vivo* drug clearance a 'sensitivity ratio' was calculated for each individual by dividing the difference in physiologically-based morphine or zidovudine clearance prediction by the percentage difference in the system-specific parameter value. This sensitivity ratio quantifies how sensitive *in vivo* drug clearance is to changes in the underlying system-specific parameters. For example, a sensitivity ratio of 0.80 indicates that a 10% increase in a system-specific parameter, would increase *in vivo* drug clearance by 8%. Mean sensitivity ratios were calculated for each system-specific parameter in each of the age-groups described above. By multiplying the percentage change in a system-specific parameter in each age-group by the mean sensitivity ratio for that age-group, the percentage change in *in vivo* glucuronidation clearance as a result of the changes in the underlying parameter value in that age-range was determined.

Since the user cannot alter UGT2B7 ontogeny in the Simcyp software package, an alternative scenario was simulated that represents a situation with a 21% increase in the UGT2B7 ontogeny factor. This ontogeny factor is a scalar for the intrinsic glucuronidation clearance that takes place in both the liver and the kidneys. Similarly, liver density is a scalar for intrinsic UGT2B7-mediated glucuronidation in the liver, while milligram microsomal protein per gram of kidney (MPPGK) is a scalar of intrinsic UGT2B7-mediated glucuronidation clearance in the kidney. Therefore simulation of a scenario in which both liver density and MPPGK are increased by 21%, were used to represent a situation with a 21% increased UGT2B7 ontogeny factor.

7.2.1.2 Drug-Specific Parameters

Morphine and zidovudine were added to the compound database in Simcyp by obtaining their drug-specific parameters from literature. In the simulations the assumption was made that there is no morphine or zidovudine elimination through other pathways than UGT2B7-mediated glucuronidation, that there is no biliary clearance of these drugs, and that there is no active drug transport into or out of hepatocytes.

The obtained drug-specific parameters for each drug are presented in Table I. With respect to the Michaelis-Menten parameters, values for the formation of the two morphine glucuronides were obtained from a study using human adult liver microsomes from five separate individuals [20] and values for the glucuronidation of zidovudine were obtained from a study using liver microsomes from four adults [14]. To verify the obtained drug-specific parameters, morphine and zidovudine clearances were predicted by the physiologically-based model for 1000 healthy adult volunteers and compared to reported clearance values in adults of 93 L/h for morphine [21–23] and 91.2 L/h for zidovudine [15,24,25]. This yielded a 75% mean under-prediction for morphine and a 71% mean under-prediction for zidovudine. Based on literature reports that showed that for UGTs the presence or absence of albumin and fatty acids in *in vitro* assays influence K_m values but not V_{max} values [26,27], the physiologically-based clearance predictions for morphine and zidovudine in adults were optimized by adjusting the K_m values of these drugs. For morphine the optimized K_m value was 115.8 μM for the formation of both M3G and M6G, and for zidovudine this was a value of 4 μM. These were the K_m values that were used in the subsequent paediatric simulations.

To identify how physicochemical drug properties influence paediatric UGT2B7-mediated glucuronidation, *in vivo* clearance values of hypothetical small molecular UGT2B7-specific substrates with various physicochemical properties, were simulated with Simcyp. In these simulations, the implicit assumption was made that the changes in physicochemical properties influenced neither the active transport of the hypothetical drug into or out of the hepatocytes nor the interaction of the hypothetical drug with the UGT2B7 isoenzyme. For the hypothetical drugs, molecular weights of 100, 200, 500, 800, and 1000 g/mol were used in combination with octanol/water partition coefficients (logP) of 0.01, 1, and 5.5. Neutral compounds were simulated as well as monoprotic acids and bases with acid dissociation constants (pKa) of 2 or 5 and 8.5 or 12 respectively, and diprotic acids and bases with pKa values of 2 and 5 and 8.5 and 10 respectively. An ampholyte was used with a pKa of 5 and 9. These values are summarized in table I. The Simcyp toolbox was used to calculate the blood/plasma ratio and unbound drug fraction for each hypothetical drug based on logP and pKa values. The Michaelis-Menten parameters obtained for morphine were used and in the simulations an intravenous drug dose of 0.1 mg/kg was administered.

Table I. *Drug-specific parameters for morphine, zidovudine, and hypothetical drugs used in the physiologically-based simulations.*

Parameter [unit]	**Parameter values**			**references**
Physicochemical parameters	*Morphine*	*Zidovudine*	*Hypothetical drug*	
molecular mass [g/mol]	285.34	267.24	100 - 1000	
logP	0.77	0.05	0.01 - 5.5	[11–13]
pKa1	7.93	9.68	2 - 12	[14,15]
pKa2	9.63	-		
Blood binding parameters				
blood / plasma ratio	1.08	0.86	derived	[13,16,17]
fraction unbound in adults	0.62	0.77	derived	[13,18,19]
Enzyme kinetic parameters				
K_m [μM]	115.8	4	115.8	
V_{max} [pmol/min/mg protein]	9250, M3G 1917, M6G	1166	9250 1917	[14,20]

logP = octanol/water partition coefficient, pKa = acid dissociation constant, K_m = Michaelis-Menten constant, V_{max} = maximum formation rate, NA = not applicable

The influence of the changes in physicochemical drug parameters on the predicted *in vivo* clearance were assessed by changing one parameter value while keeping all other parameter values constant and calculating the percentage difference between the clearance predictions between two simulations. When the highest individual prediction difference was less than 5%, the parameter was classified as not significantly influencing drug glucuronidation. When the highest individual prediction difference was more than 5% and the difference between mean prediction difference of individuals in the first month of life and individuals in the 35th month of life was less than 5% a constant was classified as influencing the absolute value of drug glucuronidation. When the difference between mean prediction difference of individuals in the first month of life and individuals in the 35th month of life was more than 5%, the parameter was classified as influencing the ontogeny profile in addition to influencing the absolute value of drug glucuronidation clearance.

7.2.2 Semi-Physiological Developmental Glucuronidation Model

The semi-physiological developmental glucuronidation model is represented by the covariate model quantifying the net observed developmental changes in drug glucuronidation clearance in children under the age of three years including preterm

and term neonates. This is the model obtained and validated in a previous population analysis of paediatric morphine data (Chapters 3 and 4) which was also directly extrapolated to zidovudine (Chapter 6). In this model the overall developmental changes in drug glucuronidation in children younger than three years are quantified according to equation 1:

$$CL = a \cdot f_{neonate<10} \cdot BW^{1.44} \qquad \text{(equation 1)}$$

in which *CL* represents the drug glucuronidation clearance, *a* is a constant that represents the absolute value of clearance, $f_{neonate<10}$ represents a reduced glucuronidation fraction in neonates younger than ten days, and *BW* represents the bodyweight of an individual paediatric patient in kilograms. The absolute value of clearance for each drug (i.e. value of *a* in equation 1) is estimated from concentration-time data in a population analysis. The reduction in glucuronidation clearance in neonates with a postnatal age younger than ten days ($f_{neonate<10}$) is 50% and is independent from gestational age. The final element of equation 1 quantifies the overall ontogeny of *in vivo* drug glucuronidation in this young population using bodyweight as a surrogate descriptor in an exponential equation with an exponent of 1.44.

Using the currently available *in vitro* data as input parameters, the morphine and zidovudine clearance predictions by the physiologically-based pharmacokinetic model in Simcyp were compared to the clearance values according to the semi-physiological developmental glucuronidation model. This was done by plotting clearance values from both models *versus* bodyweight, which is the primary covariate in the semi-physiological developmental glucuronidation model, and by plotting the prediction difference between the physiologically-based clearance values and the semi-physiological clearance values for each of the 1000 simulated individuals *versus* bodyweight. Additionally, for each of the 1000 individuals in the simulation dataset of the current study, the morphine and zidovudine clearances according to the semi-physiological model were determined as well. The percentage increase in morphine and zidovudine clearance predictions in each of the five age-groups according to the semi-physiological developmental glucuronidation model were calculated as described above.

7.3. Results

7.3.1 System-Specific Parameters

Table II ranks the five system-specific parameters by their relative contribution to the developmental changes in *in vivo* drug glucuronidation, as depicted in the last two

columns. The percentage change in clearance as a result of developmental changes in the underlying system-specific parameters is calculated from the percentage change in the system-specific parameter according to the physiologically-based model in Simcyp and the sensitivity ratio quantifying the sensitivity of drug clearance to these changes. It can be seen that the contribution of each system-specific parameter to the developmental changes in clearance is different for morphine and zidovudine. With respect to the different age-groups, the contribution of the parameters to developmental changes in *in vivo* clearance is highly non-linear and may even be bi-directional. Despite these differences, liver volume can overall be regarded as the main driver of developmental changes in UGT2B7-mediated glucuronidation, by causing an increase in *in vivo* clearance in the different age-groups between 13% and 31% for morphine and 7.3% and 22% for zidovudine, with an especially large contribution in the first three months of life. The increase in *in vivo* morphine and zidovudine clearance as a result of UGT2B7 ontogeny in the different age-groups ranges between 10% and 29%, and 7.4% and 18% respectively. The influence of hepatic blood flow on developmental changes in morphine clearance is below 5% in all age-groups and can therefore be regarded negligible, while for zidovudine clearance the contribution of changes in hepatic blood flow to increases in *in vivo* clearance ranges between 3.7% and 7.9%. For both drugs, the contribution of changes in MPPGL and unbound drug fraction is negligible in all age-groups.

7.3.2 Drug-Specific Parameters

Simulations with hypothetical small molecular UGT2B7 substrates with physicochemical properties in the ranges depicted in table I revealed that physicochemical drug properties do not influence the ontogeny profile of *in vivo* UGT2B7-medidated glucuronidation clearance. It was found that molecular mass, in the range between 100 g/mol and 1000 g/mol, did not influence UGT2B-mediated glucuronidation clearance of the simulated hypothetical drugs at all, assuming that the increase in mass did not alter the uptake or efflux of the drug by hepatocytes or the interaction of the drug molecule with the UGT2B7 isoenzyme. Increasing the octanol/water partition coefficient (logP) between 0.01 and 5.5 while keeping all other parameters constant, yielded a slight decreasing trend in the predicted absolute value of drug glucuronidation. On the other hand, increasing the acid dissociation constant (pKa) between 2 and 12 while keeping other parameters constant, yielded a trend towards an increasing predicted absolute value of drug glucuronidation. No strong relationship was observed between the physicochemical properties of the hypothetical drugs and the absolute value of glucuronidation clearance, nor was there a relationship between the derived blood to plasma ratio of the hypothetical drugs and the absolute value of drug glucuronidation. There was however a strong linear correlation ($r = 0.978$) between the mean glucuronidation clearance predicted by the physiologically-

based model for the hypothetical drugs in each of the 1000 simulated individuals and the unbound drug fraction of the hypothetical drug in plasma, which was derived from the logP and pKa value using the Simcyp toolbox. According to this relationship, while keeping the Michaelis-Menten parameters constant at the values obtained for morphine, every 0.1 increase in unbound drug fraction of the hypothetical drug resulted in an increase in *in vivo* drug clearance of 1.5 L/h.

Table II. *System-specific parameters investigated in the current study. The percentage increase in parameter value in each of the five age-groups is given, as well as the mean sensitivity ratios of the clearance of morphine and zidovudine in each group. The calculated percentage change in in vivo morphine and zidovudine clearance as a result of the change in the underlying system-specific parameters are also presented.*

Parameter	Percentage change in parameter value	Mean sensitivity ratio		Percentage change in clearance as a result of changes in parameter	
		morphine	zidovudine	morphine	zidovudine
Liver volume	I: 38% II: 18% III: 19% IV: 17% V: 21%	I: 0.82 II: 0.81 III: 0.79 IV: 0.76 V: 0.72	I: 0.58 II: 0.56 III: 0.50 IV: 0.43 V: 0.35	I: 31% II: 15% III: 15% IV: 13% V: 15%	I: 22% II: 10% III: 9.5% IV: 7.3% V: 7.4%
UGT2B7 ontogeny	I: 12.7% II: 11.4% III: 20.2% IV: 33.8% V: 25.2%	I: 0.90 II: 0.90 III: 0.88 IV: 0.85 V: 0.81	I: 0.66 II: 0.65 III: 0.60 IV: 0.52 V: 0.45	I: 11% II: 10% III: 18% IV: 29% V: 20%	I: 8.4% II: 7.4% III: 12% IV: 18% V: 11%
Hepatic blood flow	I: 33% II: 17% III: 19% IV: 22% V: 24%	I: 0.059 II: 0.061 III: 0.081 IV: 0.103 V: 0.127	I: 0.24 II: 0.22 III: 0.29 IV: 0.22 V: 0.29	I: 1.9% II: 1.0% III: 1.5% IV: 2.3% V: 3.0%	I: 7.9% II: 3.7% III: 5.5% IV: 4.8% V: 7.0%
Milligram microsomal protein per gram of liver	I: 0.71 % II: 0.70% III: 1.63% IV: 3.3 % V: 3.1%	I: 0.83 II: 0.83 III: 0.81 IV: 0.78 V: 0.75	I: 0.62 II: 0.60 III: 0.55 IV: 0.47 V: 0.39	I: 0.6% II: 0.6% III: 1.3% IV: 2.6% V: 2.3%	I: 0.4% II: 0.4% III: 0.9% IV: 1.6% V: 1.2%
Unbound drug fraction	Morphine / zidovudine I: -1.6% / I: -0.93% II: -0.72% / II: -0.45% III: -1.5% / III: - 0.89% IV: -0.05% / IV: -0.008% V: 1.2% / V: 0.74%	I: 0.92 II: 0.92 III: 0.90 IV: 0.87 V: 0.85	I: 0.71 II: 0.70 III:0.65 IV:0.58 V: 0.51	I: -1.5% II: -0.67% III:-1.4% IV: -0.04% V: 1.02%	I: -0.66% II: -0.32% III:-0.58% IV: -0.005% V: 0.38%

Age-groups: I: 0 – 3 months, II: 3 – 6 months, III: 6 – 12 months, IV: 1 – 2 years, V: 2 – 3 years.

7.3.3 Semi-Physiological Developmental Glucuronidation Model

Figure 1 shows the total morphine glucuronidation clearance and zidovudine glucuronidation clearance values in children younger than three years according to the semi-physiological developmental glucuronidation model and the physiologically-based pharmacokinetic model in Simcyp. The graphs in figure 1 indicate an under-prediction of the morphine and zidovudine glucuronidation in children older than ten days (solid circles) by the physiologically-based model, which is reflected in a mean percentage difference for this subpopulation of -68.3% for morphine and -19.1% for zidovudine in the bottom graph. In children younger than ten days (asterisks), the reduction in glucuronidation capacity as quantified by the semi-physiological developmental glucuronidation model, is not observed in the predictions by the physiologically-based pharmacokinetic model. This yields a mean percentage difference of -19.4% for morphine and 105% for zidovudine. These results illustrate large differences in the prediction of developmental changes in *in vivo* glucuronidation clearance between the semi-physiological model and the physiologically-based model in the first two weeks of life. In older infants and children, the prediction difference remains constant throughout the bodyweight-range, suggesting that in this older subpopulation the ontogeny profile predicted by the physiologically-based model mainly differs from the semi-physiological developmental glucuronidation model in absolute value while it is rather similar in shape.

Developmental changes in *in vivo* morphine and zidovudine clearance relative to birth are depicted in figure 2 for the semi-physiological model (grey lines) and the physiologically-based model (black lines), including the individual contribution of each system-specific parameter in the physiologically-based model (non-solid black lines). It can be seen that for both morphine and zidovudine, the largest contribution to the increase in *in vivo* glucuronidation is coming from the increase in liver volume (dotted black line) and the ontogeny of the UGT2B7 isoenzyme (long dashed black line). When not taking into account the rapid increase in drug glucuronidation predicted by the semi-physiological model at the age of ten days (dashed grey line), the combined influence of the changes in the five system-specific parameters investigated in the current study explains 79% of the clinically observed increases in morphine and 41% of the clinically observed increases in zidovudine clearance in the first three years of life.

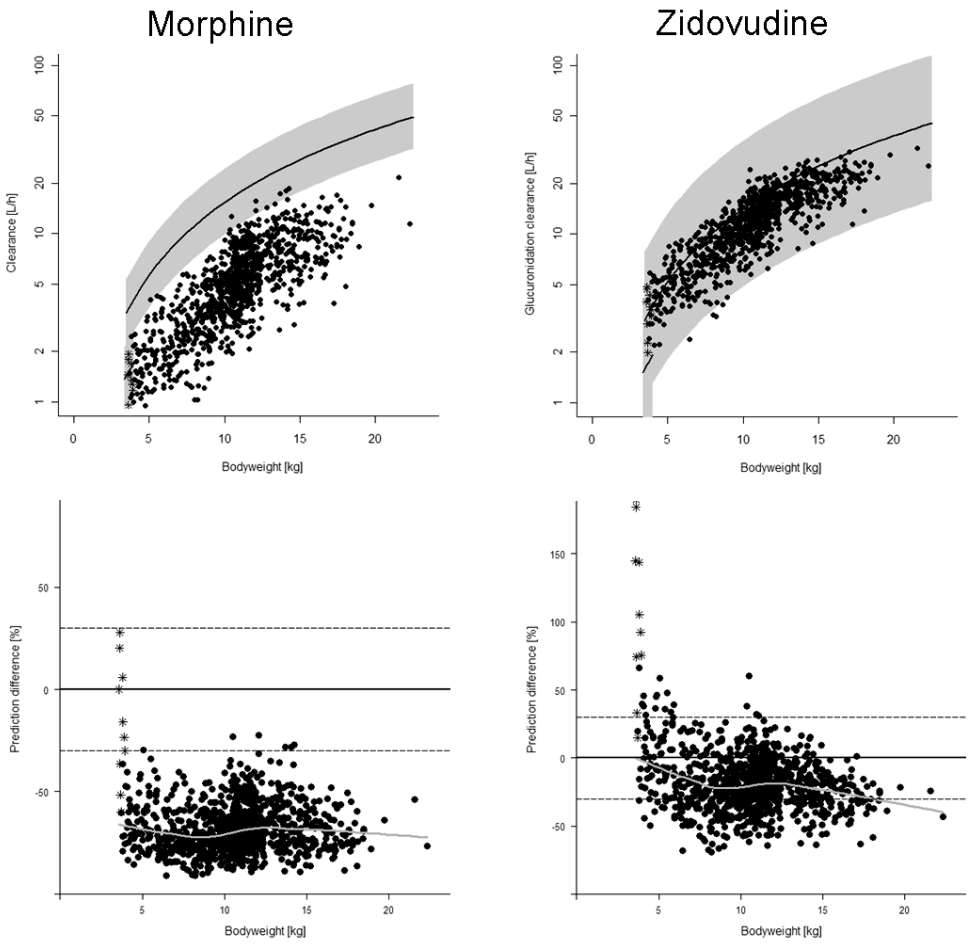

Figure 1. *Predicted in vivo morphine clearance (top left) and zidovudine clearance (top right) versus bodyweight in children younger than three years by the physiologically-based pharmacokinetic model (asterisk for neonates younger than ten days, and solid dots for children older than ten days) and the semi-physiological developmental glucuronidation model (lines are population predictions and shaded area indicates the 95% prediction interval). The prediction difference between the two models is depicted versus bodyweight for both drugs (bottom). The horizontal lines in these graphs show 0% prediction difference (solid line) and ± 30% prediction difference (dotted lines) and the grey line represents the loess curve of the data.*

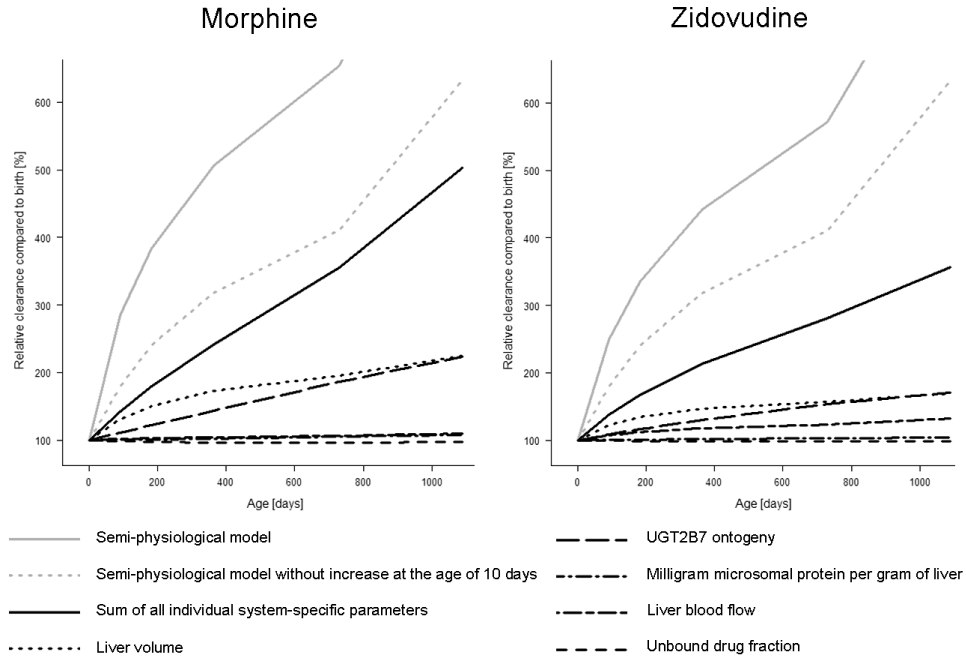

Figure 2. *Developmental changes in in vivo morphine and zidovudine clearance in the first three years of life relative to birth. The solid grey line represents the total increase predicted by the semi-physiological model and the dotted grey line represents the clearance increase predicted by the semi-physiological model without taking the rapid increase at the age of ten days into account. The solid black line represents the sum of the changes by all five system-specific parameters with the non-solid black lines representing the individual contribution of each system-specific parameter.*

7.4. Discussion

The clinically observed *in vivo* maturation pattern for morphine glucuronidation in children has been extensively studied and quantified in a paediatric covariate model before (Chapters 3 and 4) and it was shown that this covariate model could be directly extrapolated to the glucuronidation of zidovudine in a semi-physiological modelling concept (Chapter 6). It is of interest to investigate to what extent this paediatric developmental glucuronidation model can be extrapolated to other patient populations or other UGT2B7 substrates. Therefore the current study investigated the physiological and physicochemical basis of the semi-physiological developmental covariate model for UGT2B7-mediated glucuronidation clearance, using the physiologically-based

modelling software Simcyp. Detailed investigation of the influence of individual system-specific and drug-specific parameters on the ontogeny pattern of *in vivo* glucuronidation revealed that increases in liver volume and in the ontogeny of UGT2B7 isoenzymes are the main physiological drivers of the developmental changes in drug glucuronidation (table II and figure 2). The logP and pKa of a drug, but not the molecular mass, influence the absolute value of the drug glucuronidation clearance without however influencing the pattern of developmental changes.

In figure 1 it can be observed that the clearance predictions by the physiologically-based model (symbols), using the currently available *in vitro* information on morphine and zidovudine clearance, are generally lower than the clearance values obtained from the semi-physiological developmental glucuronidation model. Additionally, as can be observed in figure 2, the combined influence of the age-related increases in the five system-specific parameters investigated in the current study (solid black line) do not fully explain the clinically observed increase in morphine and zidovudine clearance (solid grey line), not even when the rapid increase in glucuronidation clearance predicted by the semi-physiological model at the age of ten days are not taken into account (dashed grey line). There are a number of possible explanations for the discrepancies in clearance values obtained by the physiologically-based model and the semi-physiological model.

The UGT enzyme kinetic parameters for morphine and zidovudine were obtained from two studies using liver microsomes described in literature. Confidence that these values accurately represent *in vivo* enzyme kinetic values is limited by the fact that numerous incubation conditions influence measured UGT enzyme kinetics, causing difficulties in obtaining good predictions on *in vivo* UGT enzyme kinetics from microsome studies [26–30]. In fact, in the current study the reported K_m values had to be adjusted to values that yielded accurate clearance predictions by the physiologically-based model in adults. In a sensitivity analysis, when changing V_{max} and K_m values from values ten-fold lower to ten-fold higher than the values used in the current analysis, predicted paediatric glucuronidation clearances changed with a factor two for M6G formation and a factor twelve for both zidovudine glucuronidation and M3G formation. This illustrates, that imprecise *in vitro* values for enzyme kinetics may influence clearance predications by the physiologically-based model significantly.

Further discrepancies may be the result of the assumption made in the simulations with the physiologically-based model. For instance, morphine and zidovudine were both assumed to be solely eliminated through glucuronidation. While glucuronidation is believed to be the major elimination pathway for these drugs, a small contribution of other elimination pathways cannot be excluded. Some paediatric morphine studies have for instance suggested that morphine is to a small degree eliminated through sulphation

or unchanged elimination in the very young [31,32]. Additionally, in the physiologically-based model active drug uptake or efflux by hepatocytes and biliary clearance of morphine and zidovudine were assumed to be zero. Although it is unclear how accurately this reflects the clinical situation, animal studies with morphine have shown energy-dependent carrier-mediated uptake of morphine in hepatocytes [33,34], while others found active hepatic uptake to not limit hepatic morphine metabolism [35]. Additionally, biliary clearance of morphine was reported in rat livers [35].

Of the system-specific parameters in the physiologically-based model, the ontogeny profile for liver volume is based on a large number of observations [36], while the paediatric information on other system-specific parameters is more limited, decreasing the level of confidence for the ontogeny profiles of these parameters in the physiologically-based model. Especially for the ontogeny of UGT2B7, expression and function of this isoenzyme in the physiologically-based model increases linearly with age from 8.9% from adult values at birth to adult values at the age of 20 years. Review of literature data however, suggests the expression and function of UGT isoenzymes to increase rapidly in the first few weeks of life (Chapter 2). Given that morphine and zidovudine clearances were found to be rather sensitive to changes in the UGT2B7 ontogeny factor, which is expressed in average sensitivity ratios of 0.87 and 0.58 respectively, a suboptimal representation of this ontogeny profile may explain the discrepancy in the morphine and zidovudine clearance values according to the semi-physiological model and physiologically-based model in figure 1 and the discrepancy between the predicted increases in morphine and zidovudine clearances by the physiologically-based model (solid grey line) and the semi-physiological model (solid black line) in figure 2. Therefore, improving the UGT2B7 ontogeny profile in the physiologically-based model may improve the predictions for paediatric UGT2B7-mediated glucuronidation.

The relatively large impact of changes in liver volume on *in vivo* drug glucuronidation observed in this study, may limit the applicability of the semi-physiological developmental glucuronidation model in patients with a reduced liver size, as a result of for instance liver resection, or a reduced liver function, for instance in patients with hepatic dysfunction as a result of virus associated hepatic disease or liver cirrhosis. Literature reports have indeed shown morphine and zidovudine clearance to be significantly reduced in adult patients with cirrhosis [37–40]. Reduced liver size or liver function may have clinical implications for dosing UGT2B7 substrates in these patients. Interestingly, hepatic blood flow was found have a limited impact on morphine glucuronidation in the current study, while cardiac surgery was found to have a clinically significant influence on paediatric morphine clearance, which was attributed to changes in hepatic blood flow resulting from changes in cardiac output [41,42].

Physicochemical parameters that influence the ontogeny profile of drug glucuronidation clearance in children would limit the application of the semi-physiological developmental glucuronidation model to UGT2B7 substrates with these properties in a population modeling approach. However, assuming that changes in the physicochemical properties of drugs do not influence drug uptake or efflux by hepatocytes or the interaction of the drug molecule with the UGT2B7 isoenzyme, the molecular weight of hypothetical drugs did not influence drug glucuronidation, while the logP and pKa of the hypothetical drug molecule only influenced the absolute value of the drug glucuronidation clearance. The absolute value of the drug glucuronidation clearance is reflected in the value of *a* in equation 1, these results thereby further support the hypothesis that this is a drug-specific constant. Since in a semi-physiological modeling approach for new UGT2B7 substrates the value of this constant has to be estimated based on the population analysis of outcome measures, these results suggest that the semi-physiological developmental glucuronidation model can predict developmental changes in glucuronidation clearance of all small molecular substrates for UGT2B7.

However, although results showed that the unbound drug concentration of hypothetical drugs in plasma is the main driver of the absolute value of drug glucuronidation, the Michaelis-Menten parameters were kept constant in all simulations with hypothetical drugs, in this case at the values used in the simulations for morphine. Michaelis-Menten parameters are major drivers of the absolute value of drug metabolism, but due to the non-linear correlation between substrate concentration and intrinsic drug clearance, interpretation of the results from simulations with varying Michaelis-Menten constants is complex. These simulations were not performed in the current study, however such simulations are necessary to further investigate the applicability of the semi-physiological developmental glucuronidation model for drugs with saturable glucuronidation kinetics.

Additionally, the application of the semi-physiologically-based developmental glucuronidation model in scenarios of drugs with varying extraction ratios needs to be further investigated. The ontogeny of UGT isoenzymes may have a one to one effect on low extraction UGT substrates since in this case *in vivo* clearance closely reflects intrinsic clearance. High extraction UGT substrates on the other hand, may be affected less by an increased level in UGT activity as their clearance will be limited by hepatic blood flow. Morphine and zidovudine have similar extraction ratios of 0.5 and 0.65 respectively [43–45]. The influence of changes in the underlying physiological system is therefore expected to be rather similar for both drugs, which resulted in the same ranking of system-specific parameters for both drugs in table II and the possibility to extrapolate the semi-physiological developmental glucuronidation model between these drugs (Chapter 6). The small difference in extraction ratio that does exist between these two drugs may

explain why in table II the percentages change in morphine and zidovudine *in vivo* clearance as a result of the changes in the underlying system-specific parameters are not the same, and why the influence of hepatic blood flow is for example more pronounced for zidovudine than for morphine. It is expected that direct extrapolation of paediatric covariate models in a semi-physiological modelling approach between UGT2B7 substrates is possible when both substrates that have similar extraction ratio's, however extrapolation between drugs with different hepatic extraction ratio's may require further deconvolution of the maturational changes in the underlying physiological processes.

5. Conclusion

The current analysis illustrates that the key physiological driver of the maturation of UGT2B7-mediated hepatic morphine and zidovudine glucuronidation in children younger than three years, as quantified by the semi-physiological developmental glucuronidation model, are liver blood flow and ontogeny of UGT2B7 expression and function. The logP and pKa are two important physicochemical drug properties that influence the absolute value of the glucuronidation clearance, but not the maturation profile, with a strong correlation between the unbound drug fraction and the absolute clearance value for drugs with similar Michaelis-Menten parameters. The results of this study suggest that in patients with normal liver function, the ontogeny pattern in clearance of new UGT2B7 substrates in children under the age of three years can be predicted by the semi-physiological developmental glucuronidation model and that only the absolute clearance value of the new substrate needs to be estimated. The generalizability of the semi-physiological modelling concept to patients with reduced liver size or liver function and to scenarios with non-linear clearance or large differences in hepatic drug extraction rations requires further investigation.

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