ARTICLE

PBPK Model of Morphine Incorporating Developmental Changes in Hepatic OCT1 and UGT2B7 Proteins to Explain the Variability in Clearances in Neonates and Small Infants

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Morphine has large pharmacokinetic variability, which is further complicated by developmental changes in neonates and small infants. The impacts of organic cation transporter 1 (OCT1) genotype and changes in blood-flow on morphine clearance (CL) were previously demonstrated in children, whereas changes in UDP-glucuronosyltransferase 2B7 (UGT2B7) activity showed a small effect. This study, targeting neonates and small infants, was designed to assess the influence of developmental changes in OCT1 and UGT2B7 protein expression and modified blood-flow on morphine CL using physiologically based pharmacokinetic (PBPK) modeling. The implementation of these three age-dependent factors into the pediatric system platform resulted in reasonable prediction for an age-dependent increase in morphine CL in these populations. Sensitivity of morphine CL to changes in cardiac output increased with age up to 3 years, whereas sensitivity to changes in UGT2B7 activity decreased. This study suggests that morphine exhibits age-dependent extraction, likely due to the developmental increase in OCT1 and UGT2B7 protein expression/activity and hepatic blood-flow.
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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Morphine is a substrate of OCT1, which is expressed differentially during development in neonates and small infants. However, the impact of the OCT1 ontogeny profile on morphine CL in neonates still remains unclear.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This is a study using PBPK modeling to assess the impacts of the developmental changes in OCT1 and UGT2B7 protein expression/activity and blood flow on morphine CL after intravenous administration.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ The morphine PBPK model with ontogeny profiles could generate realistic age-dependent morphine CLs. Simulated studies indicated that morphine CL in 3-year-old children was more sensitive to changes in cardiac output compared with neonates; whereas CL in neonates was more sensitive to change in UGT2B7 activity.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

☑ This study demonstrates the complementary utility of PBPK modeling, which can improve mechanistic understanding of important factors contributing to the variability in morphine PKs in neonates and small infants as observed by descriptive and/or population PK analyses.

Morphine is used for pain management in neonatal and infant patients,[1,2] as well as for the treatment of neonatal abstinence syndrome.[3] Intravenous administration of morphine is commonly used for acute pain management giving a rapid and reliable response, this route circumvents the high first-pass metabolism associated with oral administration and allows for better clinical control. The pharmacokinetics of morphine is known to be highly variable in pediatric and adult patients, which is further exacerbated in neonates and small infants due to rapid developmental changes during this period. Among neonates and small infants, age-dependent variations in organ size and function (e.g., enzyme and transporter activities related to morphine disposition)[4,5] are potential factors that may explain the observed variability in plasma morphine concentrations. Physiologically based pharmacokinetic (PBPK) models are a useful tool to assess the impact of potential contributing factors to the pharmacokinetic (PK) variability of morphine, because they can account for complex changes in PK mediated by growth and development,[6,7] as well as the effects of genetic variations and/or disease.

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Morphine is known as a high extraction drug in adults.[8] This drug is considered to be mainly eliminated in the liver with a minor contribution of renal excretion. Approximately 10% of unchanged morphine was excreted into the urine after intravenous administration.[9] Most morphine was excreted into urine as 3-glucuronide and 6-glucuronide,[9] which were metabolized predominantly by UDP-glucuronosyltransferase 2B7 (UGT2B7) as well as other UGT enzymes.[10] The UGT2B7 enzyme was expressed in the liver and kidneys; however, the metabolic activity in kidney microsomes was found to be lower than that observed in liver microsomes.[11,12] Therefore, morphine is considered to be primarily metabolized by hepatic UGT2B7.

Morphine is positively charged due to its physicochemical characteristics[13] under the physiological condition (e.g., at pH 7.4) and, thus, has potentially low membrane permeability. A recent report, based on in vitro experiments, suggested that morphine is subject to organic cation transporter 1 (OCT1)-mediated cellular uptake to hepatocytes before undergoing metabolism in the liver.[14] In accordance with *in vitro* data, morphine plasma concentrations in healthy volunteers were significantly dependent on OCT1 polymorphisms after codeine administration.[14] Subsequently, our group has demonstrated that OCT1 genetic variants influence the PK of morphine after intravenous administration of morphine in children who underwent adenotonsillectomy.[15] These findings clearly showed that OCT1 plays an essential role in morphine disposition and indicated the importance of further quantitative assessments combined with the known influencing factors.

Our previous study using PBPK modeling of morphine demonstrated OCT1 and blood flow as important potential contributors to PK variability after intravenous administration in pediatric (6–16 years old) and adult subjects.[16] As for OCT1, an age-dependent increase in hepatic OCT1 protein expression was determined using pediatric liver tissues.[17,18] The hepatic blood flow also demonstrated an age-dependent increase.[6,19–21] However, the impact of age-dependent physiological changes, including development of OCT1 and UGT2B7 proteins and hepatic blood flow during the perinatal period, have not as yet been systematically characterized.

In the present study, the ontogeny profiles of OCT1 and UGT2B7 protein expression, and modified age-dependent cardiac output were incorporated into the developed PBPK model of morphine with pediatric-specific system parameters. The predictive performance of the PBPK model was tested by comparing predicted age-dependent morphine clearance (CL) values with clinical observations. Sequentially, the impact of changes in cardiac output and UGT2B7 activity on morphine CL were assessed in virtual pediatric subjects from birth up to 3 years of age using the model. Alterations in hepatic extraction ratio due to age-dependent physiological changes were also determined through the PBPK simulations.

MATERIALS AND METHODS

PBPK model development for neonatal and infant subjects

The Simcyp simulator software version 16 release 1 (Certara UK Limited, Simcyp Division Sheffield, UK) was used to develop a morphine PBPK model. A previously published morphine compound file was used in this study.[16] In the current study, the OCT1 genotype was assumed to be a wild type and the *in vitro* kinetic parameters of OCT1 **1/*1* (i.e., K_m, *in vitro* Michaelis-Menten constant and J_{max}, *in vitro* maximal rate of transporter-mediated uptake) were incorporated into the compound file to describe a sinusoidal uptake transport function. The PK simulations of morphine were conducted using the Simcyp Pediatric platform. Age-dependent anatomical and physiological parameters for virtual pediatric subjects were generated based on the equations that described the relationship between the subject's age and each parameter, as reported by Johnson *et al.*[6] In this study, the developmental changes in hepatic OCT1 and UGT2B7 expression levels and the glomerular filtration rate (GFR) were incorporated into the Simcyp Pediatric platform, as described below. The setting of age-dependent cardiac outputs was also slightly modified to fit the clinical observations reported in the literature. A schematic workflow of pediatric PBPK modeling of morphine and assessment of contributing factors to morphine CL in this study is illustrated in **Figure 1**.[6,16,17,22,23]

Conversion of scaling factor for hepatic OCT1 function

The published relative scalar of 5.1,[16] which was established using a relative scaling method, was in this study corrected for the absolute abundance of OCT1 (1.63 pmol OCT1/millions of hepatocytes from the Simcyp database[24]), hence, an overall scalar of 3.13 was used for all simulations.

Ontogeny profile of hepatic OCT1 protein expression

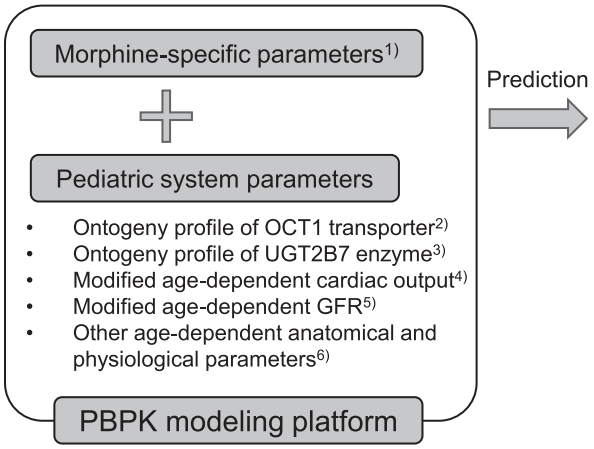
The developmental change in hepatic OCT1 protein expression was reported as the following equation[17]:

$$E_{pediatric}\,(\text{pediatric OCT1 expression}) = E_0 + \frac{E_{max} \times Age^h}{\left(Age^h + Age_{50}^h\right)} \quad (1)$$

where $E_{pediatric}$ is the protein expression of hepatic OCT1 in a pediatric subject; E_0 is the OCT1 expression at birth (mean parameter estimate, 0.58 fmol/μg membrane protein); E_{max} is the maximum protein expression from the baseline (3.98 fmol/μg membrane protein); Age_{50} is the age in years at which 50% of adult expression is observed (0.47); and h is the Hill coefficient (0.92) according to the report by Prasad *et al.*[17] (2016). In this study, the ontogeny profile was adapted to the estimated pediatric expression level of the hepatic OCT1 protein based on the adult abundance of hepatic OCT1 protein as follows:

$$\text{Abundance of hepatic OCT1 protein}_{pediatric} = \text{abundance of hepatic OCT1 protein}_{adult} \times \frac{E_{pediatric}}{E_{adult}} \quad (2)$$

where the abundance of the hepatic OCT1 protein_{adult} was 1.63 pmol/million hepatocytes. The ratio of pediatric-to-adult protein expression ($E_{pediatric}/E_{adult}$) was calculated using the protein expression of hepatic OCT1 for each age range ($E_{pediatric}$) and the maximum protein expression from the baseline (E_{adult} was assumed to be the same level as



Age-dependent morphine clearances

Scenario	OCT1 ontogeny	UGT2B7 ontogeny	Age-dependent Cardiac outputs
1	–	–	+
2	+	–	+
3	–	+	+
4	+	+	+

Status of each age-dependent physiological parameters in pediatric system model: +, active; – , inactive.

Figure 1 Schematic workflow of pediatric physiologically based pharmacokinetic (PBPK) modeling of morphine and assessment of contributing factors to morphine clearance. The impacts of the ontogeny profiles of organic cation transporter 1 (OCT1) and UDP-glucuronosyltransferase 2B7 (UGT2B7) protein expression on morphine clearance in pediatric subjects were assessed using the PBPK model with modified pediatric system parameters in the four scenarios summarized in **Table 1**. (1) Morphine-specific physiochemical and biochemical parameters were previously developed and evaluated in healthy volunteers and children aged 6–16 years old.[16] (2) Ontogeny profile of OCT1 protein expression was modified according to the report by Prasad *et al.*[17] (2016), as described in the Method section. The profile is depicted in **Supplementary Figure S1**. (3) Ontogeny profile of UGT2B7 protein expression was implemented in the Simcyp Pediatric model (version 16).[22] (4) Age-dependent cardiac output was modified to fit the clinical observations, as described in the Method section. The relationship between age and modified cardiac output (i.e., as a cardiac output scaler) is depicted in **Supplementary Figure S2**. (5) Age-dependent change in glomerular filtration rate (GFR) was modified according to the report by Rhodin *et al.*[23] (2009), as described in the Method section. (6) Other age-dependent anatomical and physiological parameters were summarized in the report by Johnson *et al.*[6] (2006).

the reported maximum effect (E_{max})[17]). The estimated abundance of the hepatic OCT1 $protein_{pediatric}$ for each age range is summarized in **Supplementary Figure S1**. When $E_{pediatric}/E_{adult}$ is set as 1.0, the ontogeny profile of OCT1 protein is deactivated. In this study, the OCT1 activity was assumed to be linear with protein abundance.

Ontogeny profile of UGT2B7 protein expression

The age-dependent UGT2B7 expression in liver microsomal protein was described with the following equation up to 20 years of age in the Simcyp simulator[22]: fraction of adult = 0.0453 × age (years) + 0.089. In addition, the age-dependent UGT2B7 expression in kidney microsomal protein was assumed to be the same as in the liver. When fraction of adult is set as 1.0 (e.g., reached at adult level), the ontogeny profile of UGT2B7 protein is deactivated.

Ontogeny profile of glomerular filtration rate

In this study, the maturation of the glomerular filtration rate (GFR) in relation to renal morphine CL was described with the following equation in the Simcyp simulator, according to the method by Rhodin *et al.*[23]:

$$\mathrm{GFR}\ (\mathrm{mL/min}) = 4.292 + \frac{(121-4.292)\times Age,\ year^{3.4}}{\left(Age,\ year^{3.4} + 0.148^{3.4}\right)} \times \left(\frac{Body\ \ weight,\ kg}{70\ \ kg}\right)^{0.75} \quad (3)$$

where age is a full-term postnatal age (in years) for a virtual subject in this study.

Age-dependent changes in cardiac output

Modification of age-dependent changes in cardiac output was conducted using the scaler function implemented in the Simcyp Simulator in order to fit simulated cardiac outputs to the clinical observations.[19–21] The detailed setting of the age-dependent scaler function for cardiac output is summarized in **Supplementary Figure S2**. In the Simcyp Simulator, cardiac output (L/h) is now estimated according to the following equation[6]:

$$Cardiac\ \ output(L/h) = BSA\ \left(m^2\right) \cdot (110 + (184.974 \cdot \left[e^{-0.0378 \cdot Age\ \ (year)} - e^{-0.2477 \cdot Age\ \ (year)}\right])) \quad (4)$$

Body surface area was estimated using body weight and height of virtual pediatric subjects generated by the Simcyp simulator according to two different methods based on the body weight in pediatric subjects (Haycock method for children with weight ≤15 kg; and the Dubois and Dubois method for children with weight >15 kg).[6]

Simulation of morphine pharmacokinetics in virtual pediatric and adult populations

Age-dependent morphine CLs were estimated using the simulation results from virtual neonatal and infant subjects. A total of 2,300 simulations were conducted ($n = 10$ virtual subjects × 10 trials for each age group) with 23 age groups covering the period from 0–3 years. In this study, virtual neonates were assumed to be full-term infants. In the simulation, morphine was intravenously administrated (0.05 mg/kg intravenous bolus, every 6 hours for 24 hours[25]) to 100 virtual subjects with an equal proportion of boys and girls (50% each) in each age group. The PK parameters, area

under the curve (AUC_t), and CL were estimated using the PBPK simulation results. The AUC_t was calculated using the simulation after the last dose according to the linear trapezoidal method. The CL estimates were calculated by dividing the morphine dose by AUC_t. Subsequently, the morphine CL was standardized to a subject *i* with a standard body weight of 70 kg using allometric scaling by applying the following equation[4,5]:

$$CL_{70kg,i} = CL_{pediatric,i} \times \left(\frac{70\ kg}{Body\ \ Weight_{pediatric,i}} \right)^{0.75} \quad (5)$$

The allometrically standardized CL estimates ($CL_{70kg,i}$) were compared with observed CL estimates from previously reported studies.[15,26–33] In addition to the simulations in virtual neonatal and infant subjects, the allometrically standardized CL estimates of morphine in children aged 6–16 years and adults were calculated using the Simcyp Simulator. In order to generate CL values in virtual subjects for older children (6–16 years) and adults, the Sim-Pediatric and Sim-Healthy Volunteer population files (databases) were used, respectively. The PK simulations for the children (6–16 years) were conducted based on yearly increments from age of 6 years, whereas hepatic OCT1 protein expression was assumed to be the same as adults. The age range for the healthy adult simulations was set from 20–50 years. Dose, administration route, and proportion of male and female subjects were the same as those used in the simulations for virtual neonatal and infant subjects as described above.

An additional PK simulation was performed in the neonatal population in order to evaluate the predictive performance of the developed PBPK model of morphine, using clinically observed PK profiles. Data plotted in the graphs reported by Dahlstrom *et al.*[34] were extracted as numerical data using GetData Graph Digitizer version 2.26 (getdata-graph-digitizer.com). The evaluation of the developed PBPK model was performed by visual predictive check and by comparing the observed and predicted values of the various PK parameters. The visual predictive check was performed by overlaying simulated and observed systemic drug concentration-time profiles. In the simulation, morphine (0.133 mg free-based morphine/kg[34]) was intravenously administrated (10 second bolus) to the virtual subjects with an equal proportion of male and female subjects (50% each). The age range for virtual subjects was set at 0.08–0.12 years old, in which the absolute abundance of hepatic OCT1 was set as 0.54 pmol/10^6 hepatocytes for this age range. The scaler setting for cardiac output was 1.85. The simulation size was 500 simulations ($n = 10$ virtual subjects $\times$ $n = 50$ trials).

Sensitivity analysis of cardiac output and UGT2B7 activity on morphine clearance in virtual pediatric subjects

Sensitivity analyses were conducted to assess the potential impact of cardiac output and UGT2B7 activity on morphine CL in virtual pediatric subjects aged from 1 day to 3 years old. The age-dependent cardiac output was defined as described above and set it as an initial 100% value for each age group. Cardiac output was modified from 50% to 150% of the defined value on the Simcyp Pediatric platform. The UGT2B7 activity was also modified from 50% to 150% by changing the maximal rate of metabolism (V_{max}) values for both morphine 3-glucuronide and 6-glucuronide formation. The details about the simulation design are summarized in **Supplementary Table S1**. For the simulation, the settings (i.e., dosing, simulation size, and proportion of female subjects) and the calculation of PK parameters were the same as described in the first paragraph of the previous section. Subsequently, using simulation results in the subjects having normal cardiac output and UGT2B7 activity, hepatic extraction was determined as a ratio of predicted CL to hepatic blood flow, which was assumed as 28% of cardiac output in this study.

RESULTS

Adjustment of age-dependent cardiac outputs from neonates to children

Age-related changes in cardiac output were simulated to fit the clinical observations[19–21] by modifying scaling of cardiac output implemented in the Simcyp Simulator (details are shown in **Supplementary Figure S2**). The comparison between the simulated and observed age-dependent cardiac outputs is depicted in **Figure 2**.[19–21] The predicted cardiac outputs in full-term newborn babies were similar to the clinical observations,[19] in which the predicted cardiac output was almost double the body surface area-based prediction, which is the default in the Simcyp Pediatric model version 16. The central tendency of simulated cardiac outputs for children fell in the middle of clinical observations, as was reported by Cattermole *et al.*[20] and de Simone *et al.*[21]

Age-dependent morphine clearances from neonates to adults

The impacts of the ontogeny profiles of OCT1 and UGT2B7 proteins on morphine CL in pediatric subjects after birth up to 3 years old were tested via the simulation analyses using the developed morphine PBPK model with modified pediatric system parameters in four different scenarios. The predicted morphine CL estimates are depicted in **Figure 3** overlaid with clinically observed CL estimates previously reported in the literature.[26–30,33] The simulated CL estimates with ontogeny profiles of both OCT1 and UGT2B7 and modified age-dependent cardiac output best captured an age-dependent increase as observed in clinical PK studies (scenario 4). The PBPK model also predicted a wide distribution of individual CL values, which reasonably captured the variability observed in clinical PK studies. In addition, the CL estimates in older children (6–16 years) and adult healthy volunteers were well-predicted in regard to the clinically observed data[15,31,32] (**Supplementary Figure S3**).

Morphine pharmacokinetics in neonates

Morphine concentration-time profiles in virtual neonates were simulated and compared with observed PK profiles from two individual patients whose full PK profiles were reported in the literature (reported as patient T.T. and

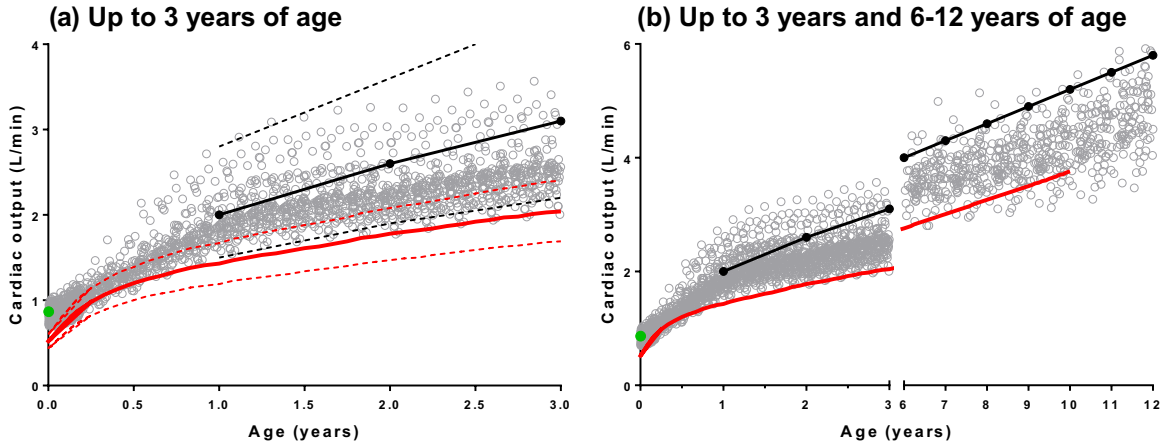


Figure 2 Simulated changes in cardiac output with age. Age-dependent cardiac outputs were generated by multiplying a cardiac output embedded in the Simcyp Simulator, using a scaling factor. The scaler settings are depicted in **Supplementary Figure S2**. Simulated cardiac output values with the Simcyp Pediatric model as a function of age. (**a**) The open gray circles represent the simulated changes in cardiac output with age (from birth up to 3 years of age); (**b**) from birth up to 3 years and from 6–12 years of age. The simulated data are overlaid with observed data: green circle, mean value reported by Walter *et al.*[19]; solid black lines, data by Cattermole *et al.*[20] (dashed lines represent the observed minimum and maximum values); solid red lines, data from de Simone *et al.*[21] (dashed lines, data calculated using the reported equation of $y = 162 - 1.33 \times$ body weight ± 25). The reported cardiac output values by de Simone *et al.*[21] were estimated using median body weights taken from the World Health Organization child growth standard.[35]

patient A.J., **Figure 4**).[34] The PK variability generated through the PBPK model captured the observed PK profiles in these individual two patients (**Figure 4a**). The population mean of simulated PK profiles was comparable to the observations in patient T.T. (**Figure 4b**), in which the mean hepatic OCT1 expression in virtual subjects was 10 nmol/liver (**Table 1**). On the other hand, the PK observation in patient A.J. was comparable to the simulated PK profile in a virtual subject having an hepatic expression of 36 nmol OCT1 protein/liver among the 500 individuals simulated (**Figure 4c**).

Accordingly, predicted AUC_{∞} and allometrically standardized CL estimate of 188 ± 80 ng/mL*hr (mean $\pm$ SD) and 29.4 ± 13.5 L/hr/70kg, respectively, were similar to the observed AUC value of 205 ng/mL*hr and the allometrically standardized CL estimate of 19.1 L/hr/70kg in patient T.T.[34] Predicted AUC_{∞} and allometrically standardized CL in the virtual subject having hepatic OCT1 expression of 36 nmol/liver were 79.0 ng/mL*hr and 57.3 L/hr/70kg, respectively, which were close to observed AUC_{∞} and CL in patient A.J. (99.7 ng/mL*hr and 40.1 L/hr/70kg, respectively[34]).

The impact of cardiac output and UGT2B7 activity on morphine clearance in virtual pediatric subjects

Sensitivity analyses were performed by changing cardiac output (**Figure 5a**) and UGT2B7 activity (**Figure 5b**) from 50% to 150%, where 100% represents the normal value in a virtual subject. The morphine CL estimate was sensitive to changes in cardiac output and UGT2B7 activity in virtual subjects from 1-day-old postnatal up to 3 years old. However, the degree of morphine CL sensitivity to these changes was different among age groups tested in this study. Impact of changes in cardiac output on morphine CL increased with age (from 1 day old to 3 year old), whereas those in UGT2B7 activity decreased with age. A 50% increase in cardiac output resulted in 12–17% escalation in predicted CL estimates in virtual infant subjects over 6 months old; however, the escalation in predicted CL estimates was <6.2% in virtual neonatal subjects. On the other hand, a 50% increase in UGT2B7 activity resulted in 16–19% escalation in predicted CL estimates in virtual neonates; however, the escalation in predicted CL estimates was <10% in virtual infant subjects over 6 months old.

In addition to the difference in sensitivity among age groups, morphine showed an age-dependent increase in predicted hepatic extraction ratio (**Figure 6**).[8] The geometric mean of the predicted hepatic extraction ratio of 3-year-old children was 3.4-fold higher than that of 1-day-old neonates.

DISCUSSION

The function of hepatic OCT1 transporter and hepatic blood flow level were previously identified as potential contributing factors to large variability in morphine clearance.[16] Regarding hepatic OCT1 function, the uptake transporter activity may vary with expression levels of the OCT1 protein. In the neonatal and infant periods, hepatic OCT1 protein expression showed an age-dependent developmental change,[17,18] as did hepatic blood flow and UGT2B7. Therefore, these developmental changes may have an impact on morphine PK in neonates and small infants. In this study, these developmental changes were implemented into a PBPK model of morphine in addition to other age-dependent anatomical and physiological changes in order to predict morphine CL in these younger populations. The inclusion of ontogeny profiles of OCT1 and UGT2B7 resulted in the most adequate prediction for an age-dependent increase in morphine CL in pediatric subjects after birth up to 3 years old. The current study also indicates that the contribution of

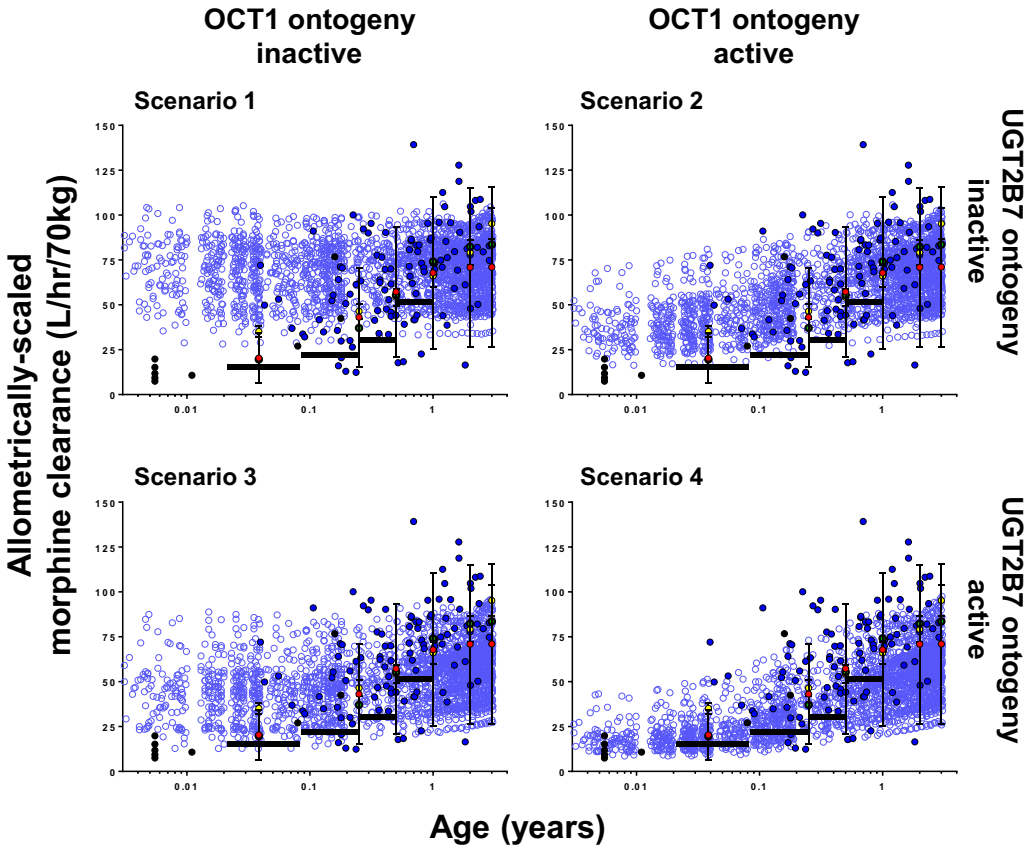


Figure 3 Comparison between predicted and observed allometrically scaled morphine clearance estimates in pediatric subjects after birth up to 3 years old. Open purple circles represent the simulation results of morphine clearance using the developed physiologically based pharmacokinetic model of morphine incorporating the ontogeny profile of hepatic organic cation transporter 1 (OCT1) or/and UDP-glucuronosyltransferase 2B7 (UGT2B7) protein expression. Four different combinations of ontogeny status were used in the simulation as indicated above and summarized in **Figure 1**. Closed circles and bars represent observed data from reported clinical studies: solid black circles, Lynn & Slattery[33]; filled blue circles, Anand *et al.*[26]; filled red circles, Bouwmeester *et al.*[27] and Krekels *et al.*[28]; filled yellow circles: Krekels *et al.*[28] and Knibbe *et al.*[29]; and black bars, McRorie *et al.*[30]

UGT2B7 to morphine CL is almost comparable to that of OCT1 during the neonatal period.

Age-dependent cardiac output implemented in the Simcyp Pediatric Simulator as a default was originally evaluated against the International Commission on Radiological Protection reference values.[6] However, the model trended to underpredict cardiac outputs in children <5 years of age. Therefore, in this study, the setting of cardiac output simulation was modified to make simulated cardiac outputs a good fit with those from several clinical studies over age.[19–21] Although the data by Cattermole *et al.*[20] (2010) were from a Chinese pediatric population, we assumed that this population has cardiac output similar to the general (white; North European) pediatric population because the reported mean body weight in the Chinese children was comparable to the median body weight at the corresponding age group in the World Health Organization child growth standard, based on multiple populations.[35] Further confirmation using clinical studies is needed to determine racial differences in developmental change in cardiac output.

Morphine is known as a substrate of UGT2B7[10,36] as well as OCT1.[14] *In vitro* immunoblot analysis using 40 individual liver samples (<1 year olds to adults) demonstrated a positive correlation between UGT2B7 protein expression and postnatal age ($r = 0.68$).[37] In that study, the expression level at aged <1 year was ~10-fold lower compared to the adult level. Interestingly, morphine glucuronidation was detectable in fetal liver, although no clear correlation was identified between the gestational age (15–27 weeks) and the glucuronidation rate.[38] These findings at least indicated that UGT2B7 protein is expressed during the fetal stage of development and gradually increases with age. A PBPK model that incorporated the ontogeny profile of UGT2B7 resulted in an ~3-fold overprediction (i.e., predicted % difference was ~-70%) of morphine CL across the body weight range in neonates and small infants, although the pattern of change was similar to clinical observations.[39] In the current study, the inclusion of OCT1-mediated hepatic uptake into the PBPK model resulted in a good prediction of morphine CL and, thus, an improvement of the previous model.[39] For neonates aged 2 weeks old, the current PBPK model predicted an allometrically weight normalized

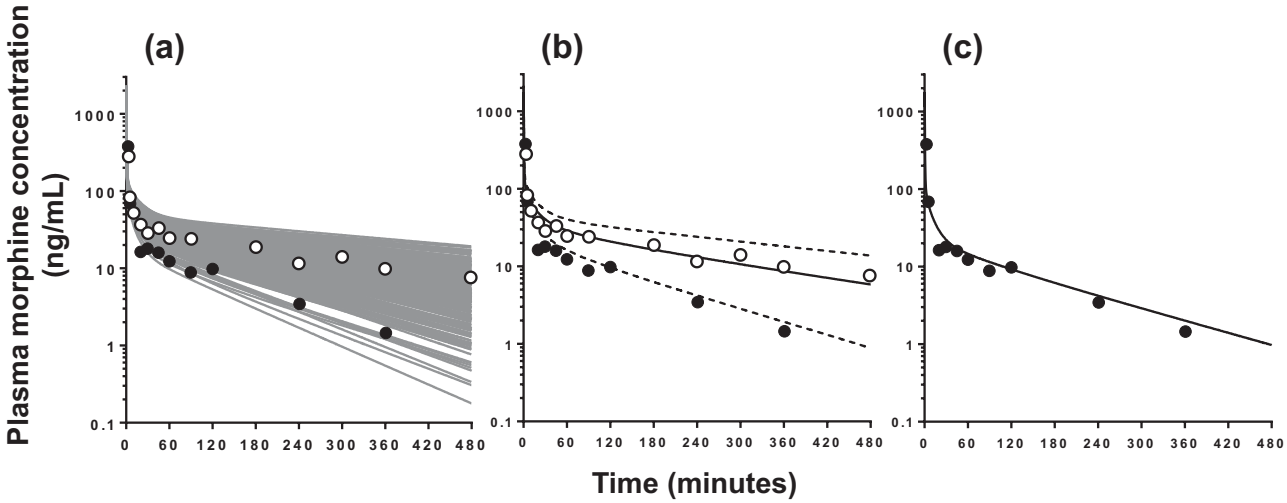


Figure 4 The physiologically based pharmacokinetic (PBPK) model-predicted vs. observed concentration-time profiles of morphine in neonates. The PBPK model predicted morphine concentration-time profiles after the intravenous administration compared with clinical observations in two individual patients in the report by Dahlstrom *et al.*[34] (patient T.T., open circles; and patient A.J., closed circles). (**a**) Solid gray lines represent individual 500 simulated pharmacokinetic profiles. (**b**) Solid and dashed lines represent mean, and 5th and 95th percentiles of 500 simulations, respectively. (**c**) Solid black line represents the simulation in a virtual subject having hepatic organic cation transporter 1 expression of 36 nmol/liver (as liver absolute abundance).

morphine CL estimate of 21.4 L/hr/70kg, which is comparable to the predicted CL (19.6–35.1 L/hr/70kg), based on population analyses using clinically observed PK data.[28] The difference between the PBPK model predictions and clinical observations provides insight into potential unknown factors that contribute to morphine disposition and emphasize the need for optimizing PBPK models with new findings, especially in the pediatric population.[40]

Large variability in morphine CL was observed in clinical studies. A part of this variability may be due to changes in the physiology of the reported patients caused by disease, surgery, and concomitant medications.[26–30,33] The PBPK model, when incorporated with the ontogeny profile of hepatic OCT1 and UGT2B7 protein expression, estimated a wide distribution of individual CL values, which reasonably captured the variability observed in clinical PK studies. This study demonstrated the impact of hepatic OCT1 expression levels – due to liver size and the abundance of hepatic OCT1 protein – on morphine PK profiles in neonates. The abundance of OCT1 protein in neonatal liver samples showed large variability. Our previous study showed that the coefficient of variation for OCT1 protein expressions was 38–67% in neonatal liver samples.[18] In order to reduce the predicted large distribution, one possible solution is to identify the variability in OCT1 activity for each genotype, as well as a genotype-dependent ontogeny profile; which will be considered in a future study. The same is also possible for the UGT2B7 enzyme.

One point to consider is the influence of cardiac surgery, as it has been reported that the morphine CL is higher after noncardiac surgery than it is after cardiac surgery in neonates and infants.[41] However, even for noncardiac surgery in neonates, the potential effects of dopamine treatment on cardiac output could be considered as another influencing factor, as dopamine was found to be used in the postoperative management,[42] in esophageal

Table 1 Predicted pharmacokinetics parameters of morphine in virtual neonates after the intravenous administration

Age years		Body weight kg	Hepatic OCT1 nmol/liver	C_{max}, $\times 10^3$ ng/mL	AUC_{∞}, ng/mL*hr	$CL_{pediatric,i}$, L/hr	$CL_{70kg,i}$, L/hr per 70kg
Predictions by PBPK modeling							
Mean	0.10	4.2	10.0	1.80	188	3.55	29.4
SD	0.01	0.6	10.4	0.16	80	1.66	13.5
Minimum	0.08	2.7	0.38	1.41	48	1.14	9.7
Maximum	0.12	6.2	96.2	2.32	474	10.7	94.3
Clinical observations							
Patient T.T.	0.1	4.0	NA	2.12	205	2.23	19.1
Patient A.J.	0.1	4.4	NA	2.54	99.7	5.04	40.1

AUC_{∞}, area under the plasma concentration-time curve extrapolated to infinity; $CL_{pediatric,i}$, pediatric clearance; C_{max}, maximum concentration for simulations (for clinical observations, concentration at time zero); NA, not available; OCT1, organic cation transporter 1; PBPK, physiologically based pharmacokinetic.
$CL_{70kg,i}$ (L/hr per 70kg), an allometrically-scaled clearance with standard body weight of 70kg (i.e., $CL_{70kg,i}$ in Eq. (5), as described in the Method section), which is calculated as follows:

$$CL_{70kg,i} = CL_{pediatric,i} \times \left(\frac{70\ kg}{Body\ \ Weight_{pediatric,i}} \right)^{0.75}$$

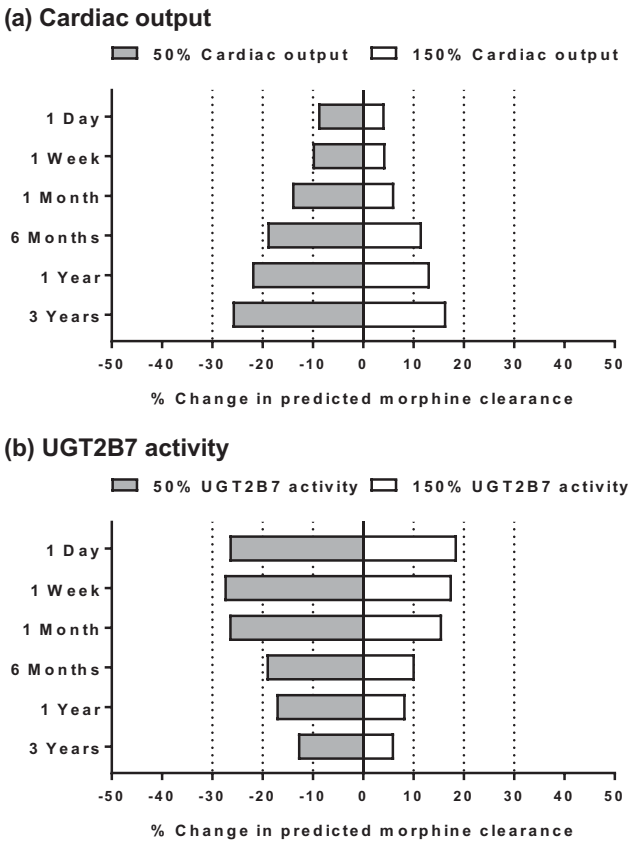


Figure 5 Impact of cardiac output and UDP-glucuronosyltransferase 2B7 (UGT2B7) activity on predicted morphine clearance in virtual pediatric subjects aged 1 day after birth to 3 years old. (**a**) Cardiac output was changed from 50% (gray column) to 150% (open column), in which 100% represents a normal value for each subject in the sensitivity analysis. Data are presented as ratio (%) of the predicted morphine clearance with change in cardiac output to that in normal condition. (**b**) The UGT2B7 activity was changed from 50% (gray column) to 150% (open column), in which 100% represents a normal value for each subject, in the sensitivity analysis. The 50% (gray column) and 150% (open column) changes represent a 50% decrease and a 50% increase in maximal rate of metabolism (V_{max}) values of morphine 3-glucuronization and 6-glucuronization in normal subjects, respectively. Data are presented as ratio (%) of the predicted morphine clearance with change in UGT2B7 activity to that in the normal condition.

atresia with or without tracheo-esophageal fistula, intestinal obstruction, and omphalocele and gastroschisis. The use of dopamine has been shown to have a variable effect on cardiac output in these populations, with some patients having a concentration-dependent increase in cardiac output, whereas others show little response to infused dopamine.[43] These differences might be due to the variable baseline of cardiac output observed in patients. In the present study, cardiac output was increased by 50% in virtual subjects to assess the sensitivity on the PBPK model-predicted morphine CL, based upon the finding that cardiac output increased 45–51% in neonatal patients that showed a cardiac response to dopamine.[43] The cardiac output was also decreased to 50% in the performed sensitivity analysis, because quantified fractional reduction in renal blood flow, which is set at 19% (male subjects) and 17% (female subjects) of cardiac output in the Simcyp Simulator, was reported at 78%, 55%, and 63% of normal blood flow in adult patients with mild, moderate, and severe chronic heart failure, respectively.[44,45] Sensitivity of morphine CL to changes in cardiac output increased with age from 1 day old to 3 years old. An increase in sensitivity to changes in cardiac output from 6-year-old children to adults was observed in the previous study. Thus, hemodynamics may contribute to the variability in morphine CL, especially in patients over 1 year old. Interestingly, in the elder population, Schaefer *et al.*[46] discussed the decline in liver size and hepatic blood flow, due to old age, resulting in a longer half-life of morphine after intravenous administration. Therefore, age-dependent changes in physiological factors affecting hepatic elimination have an impact on morphine disposition after intravenous administration for all ages, from neonates to older patients.

Morphine is reported to have a high extraction ratio of 0.7 after intravenous administration in healthy adults.[8] Therefore, hepatic blood flow is considered as the rate-limiting factor for elimination of morphine through the liver. On the other hand, this study suggested that sensitivity of predicted morphine CL to changes in UGT2B7 activity decreased with age in pediatric subjects, in a direction opposite to changes in cardiac output–which indicates an age-dependent change in the rate-determining process of morphine CL. In addition, the predicted hepatic extraction of morphine exhibited an age-dependent increase. This could be due to the developmental increase in OCT1 and UGT2B7 protein expression and hepatic blood flow in the Simcyp Simulator. Similar to this finding, it was reported

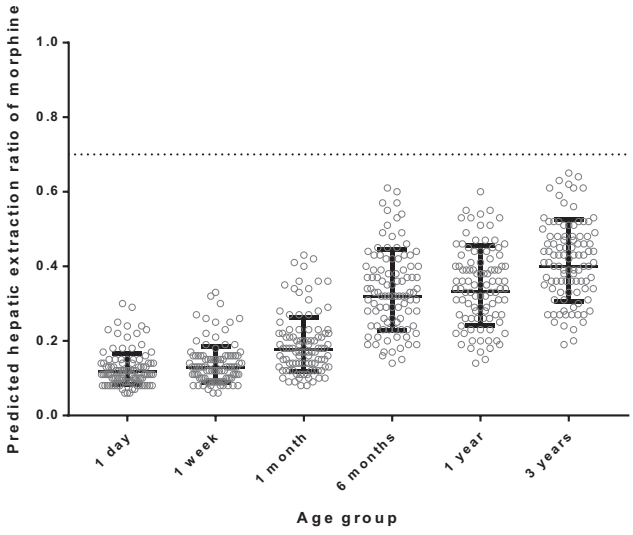


Figure 6 The age-dependent increase in physiologically based pharmacokinetic-simulated hepatic extraction of morphine. Hepatic extraction was determined as a ratio of predicted clearance to hepatic blood flow, which was assumed as 28% of cardiac output in this study. Open circles represent individual results from the simulation ($N = 100$ for each age group), as described in the Method section. Bars represent the geometric mean ± SD. The dotted horizontal line shows the hepatic extraction of morphine in healthy adults.[8]

that the hepatic extraction ratio of midazolam, a CYP3A probe substrate, varies with age due to age-dependent physiological changes, including hepatic CYP3A4 protein expression.[47] From these findings, the hepatic extraction ratio in children, especially for high extraction drugs, can be different from that in adults due to the developmental change in physiological factors over age. Therefore, this age-dependent difference in the ratio should be considered because it is associated with the bioavailability of drugs, which may directly reflect on the dose required.

One limitation of the current study is that we have not considered the pharmacogenetic effect of *OCT1* gene variants on hepatic morphine CL due to lack of data regarding the genotype-dependent developmental changes in hepatic OCT1 protein expression or activity. In our previous study, the OCT1 expression level showed a decreasing trend in connection with the OCT1-Arg61Cys (rs12208357) variant of OCT1; however, the sample size was too small to draw any conclusions (4 of 32 pediatric liver samples represented the OCT1-Arg61Cys variant).[18] Nies *et al.*[48] (2009) reported that this variant strongly correlated with decreased OCT1 protein expression in adult liver tissue ($P < 0.0001$). Therefore, our next challenge will be to include assessment of genotype-dependent effects on the developmental change in hepatic OCT1 protein expression in neonates and infants.

An advantage of PBPK modeling is that model-based predictions provide mechanistic insight into the most likely underlying sources of observed variability in drug PKs. The findings from this study indicate that developmental changes in hepatic OCT1 and UGT2B7 protein expression and hepatic blood flow contribute to changes in hepatic extraction of morphine among pediatric patients. In addition, the observed wide variability in morphine CL could be in part attributed to the differences in hemodynamics among patients in various conditions; however, further confirmation using clinical data is needed.

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