Use of developmental Midazolam and 1-hydroxymidazolam data with pediatric physiologically based modelling to assess CYP3A4 and UGT2B4 ontogeny in vivo.

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Running title: Assessing ontogeny of CYP3A4 and UGT2B4 using pediatric PBPK

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Abbreviations
AUC, Area Under Concentration-Time curve; CL, Absolute clearance; CLint, Intrinsic clearance; Cmax, maximum concentration; CYP, Cytochrome P450; DDI, Drug-Drug Interaction; GA, Gestational Age; ME, Mean Error; MSE, Mean Squared Error; MDZ, Midazolam; 1OHMDZ, 1-hydroxymidazolam; PE, Prediction Error; PK, Pharmacokinetic; PBPK, Physiologically Based Pharmacokinetic model; P-PBPK, Pediatric Physiologically Based Pharmacokinetic model; PMA, Post Menstrual age; PNA, Post Natal Age; UGT, Uridine diphosphate glucuronosyl transferase.
Abstract

Pediatric physiologically based pharmacokinetics modeling in drug development has grown in the past decade but uncertainty remains regarding ontogeny of some drug metabolizing enzymes. In this study a midazolam and 1-hydroxymidazolam PBPK model was developed and used to define the ontogeny for hepatic CYP3A4 and UGT2B4. Data for model development and pharmacokinetic studies on iv midazolam in adults and pediatrics, were collated from the literature. The PBPK model was verified in the adult population and then used to compare the performance of two ontogeny profiles for CYP3A4 in terms of parent drug elimination in pediatrics. Four studies also published data on the 1-hydroxymidazolam and this was used to evaluate the known ontogeny for UGT2B4.

For midazolam elimination the Upreti CYP3A4 ontogeny performed better than Salem; mean error (bias) and mean squared error (precision) were 0.14 and 0.064 compared to 0.69 and 1.21, respectively. For 1-hydroxymidazolam elimination, the Simcyp default ontogeny of UGT2B4 appeared to perform best for studies covering the age range 0.5 to 15.7 years whilst for a study in younger ages 0 to 1 years it was the Badee UGT2B4 ontogeny. In preterm neonates overall expression of UGT appeared to be around 10% of that in adults.

Identifying the optimal model of CYP3A4 ontogeny is important for the regulatory use of PBPK. The results for midazolam are conclusive but research about other CYP3A4 metabolized compounds will underpin generalizability of the CYP3A4 ontogeny. UGT2B4 ontogeny is less certain, but this study indicates the most likely scenarios.
Significance statement

A PBPK model for midazolam and 1-hydroxymidazolam was developed to test various ontogeny scenarios for CYP3A4 and UGT2B4. The CYP3A4 ontogeny of Upreti resulted in more accurate prediction of midazolam CL across 9 clinical studies, age range birth to 18 years. 1-hydroxy midazolam was used as a marker of UGT. The Simcyp default ‘no ontogeny’ profiles for UGT2B4 performed the best; however for < 1 year of age there was some evidence of over-activity of this enzyme compared to adults.
Introduction

In recent years there has been a rapid increase in the application of Physiologically based Pharmacokinetic (PBPK) models in clinical pharmacology and particularly in drug development (El-Khateeb et al., 2021). Extending these models to special populations such as pediatrics (P-PBPK), where they integrate additional information on developmental physiology and ontogeny of enzymes and transporters, has been a natural progression in their development (Verscheijden et al., 2020). There has also been a rapid rise in the use of P-PBPK for clinical, model and drug development applications (Johnson et al., 2022), in terms of PBPK regulatory submissions to the US Food and Drug Administration; in 2018/19, 9% of these were for pediatrics (Zhang et al., 2020).

One of the more common applications of P-PBPK is in dose projection as part of a pediatric study (PSP) or investigation (PIP) plan. However, there are few examples where pediatric studies have been replaced (Johnson et al., 2014). Although regulatory authorities acknowledge the value of these models and encourage their use in pediatric drug development (Leong et al., 2012) they highlight a need for appropriate levels of model qualification related to context of use and more research in terms of uncertain parameters (EMA, 2016; Kuemmel et al., 2020).

Inclusion of ontogeny data into P-PBPK models is important in terms of predicting drug CL in certain age groups (Wagner et al., 2015) and there have been many studies in this area (Emoto and Johnson, 2022). Despite this, there remains uncertainty around the optimal ontogeny of certain enzymes either due to conflicting data e.g. hepatic CYP3A4 or general lack of data e.g. UGT2B4. For CYP3A4 there are two in vivo based studies that show different CYP3A4 ontogeny profiles; that of Salem et al (2014) shows a hyperbolic pattern mirroring in vitro data (de Wildt et al., 1999; Alcorn and McNamara, 2002) while that of Upreti and Whalstrom (2016) suggests over-expression in infant and early childhood groups.
compared to adults. For UGT2B4 there are in vitro data from one study showing over-expression in neonates and infants (Badee et al., 2019) but the same study also shows an over-expression of UGT2B7 which is in contrast with other in vitro data (Zaya et al., 2006). Research in this area is held back by lack of clinical data in early age groups, particularly in those less than 2 years of age and by lack of reliable probe drug markers for some of the enzymes that can feasibly be administered to children.

Intravenous midazolam (MDZ) is routinely used in both adult and pediatric medicine and in both cases has previously been used as a marker of hepatic CYP3A4/5 activity (Watkins, 1994; van Groen et al., 2019). The rapid t_{1/2} distribution of the drug (<0.3 hours) (Kanto, 1985) means that thus, relatively early time points are influenced more by drug elimination. Although CYP3A5 is involved in metabolism, there is evidence to suggest no ontogeny for this enzyme post birth (Stevens et al., 2003; Hines, 2007; Upreti and Wahlstrom, 2016), making MDZ useful for assessing CYP3A4 ontogeny. The main metabolite is 1-hydroxymidazolam (1-OHMDZ) which has around half the sedative activity of the parent drug (Mandema et al., 1992; Johnson et al., 2002). The PK of 1-OHMDZ has been studied in humans (Mandema et al., 1992) and its metabolism assessed using in vitro systems (Zhu et al., 2008; Seo et al., 2010; Nguyen et al., 2016). The metabolism occurs predominantly via UGT2B4 (fraction metabolized (fm) = 47%), and UGT2B7 (31%) with additional contribution from UGT1A4 (22%). Hence 1-OHMDZ has potential for assessing the development of UGT enzymes with age (de Wildt et al., 2010) and because the ontogeny of UGT2B7 (Pacifici et al., 1982; Choonara et al., 1989; Pacifici et al., 1989; Strassburg et al., 2002; Zaya et al., 2006; Bhatt et al., 2019) and UGT1A4 (Miyagi and Collier, 2007; Bhatt et al., 2019) is relatively well described in the literature, the ontogeny of UGT2B4 can be further evaluated.
As improving the predictive accuracy of P-PBPK models is important, the aims of the current study are:

- Use midazolam iv clinical data in the pediatric age range, with emphasis on early ages, to determine the relative accuracy of midazolam CL predictions using the hepatic CYP3A4 ontogeny of Salem et al (2014) and Upreti and Wahlstrom (2016).
- To develop and verify a 1-OHMDZ PBPK model linked to parent drug.
- For studies reporting 1-OHMDZ PK data in the pediatric population assess the currently utilized hepatic UGT2B4 ontogeny profile in Simcyp against that recently published by Badee et al (2019).

**Methods**

**Model development**

The default Sim-Midazolam file was used in all simulations. This has been extensively verified in the adult population and summary data is available on request. The 1-OHMDZ model was built as a primary metabolite file with formation from midazolam, via CYP3A4 and CYP3A5. The input parameters and sources of information for 1-OHMDZ are shown in Table 1.

CYP3A7 is highly expressed in preterm neonates (Stevens et al., 2003) and may have a more important role in MDZ kinetics in these populations. Thus, the existing Sim-Midazolam model was modified to include CYP3A7 for the preterm population. The relative contribution of CYP3A7 in the metabolism of MDZ to 1-OHMDZ has been described previously based on recombinant enzyme kinetics (Williams et al., 2002); intrinsic clearances were reported as 3.34, 3.31 and 0.02 ml/min/nmol for CYPs 3A4, 3A5 and 3A7, respectively. Based on the current Sim-Midazolam file, an additional contribution from CYP3A7 was calculated based on the above data with minor correction based on differences in CLint.
CYP3A4/5 in Simcyp compared to Williams et al (2002); this was CLint = 0.0224 and 0.041 µL/min/pmol for conversion to 1-OHMDZ and 4-OHMDZ, respectively.

PBPK modelling approach

The accepted learn and confirm approach was used in each case with development and initial verification of each drug model (MDZ and 1-OHMDZ) in the adult population before moving on to pediatric application (Leong et al., 2012). A literature search was performed using Pubmed to identify clinical studies presenting iv midazolam PK data, with or without 1-OHMDZ in the adult and pediatric populations and these are shown in Table 2.

Simulations trial design

All simulations were undertaken using Simcyp v21.1 (Certara UK Limited), in adults using the Sim-Healthy volunteer population, in pediatrics using the Sim-Paediatric or Sim-Japanese Paediatric population and preterm using the Sim-Preterm population. Simulations were carried out using a trial design matched as closely as possible to the clinical study for age range and body weight (where indicated), number of individuals, proportion of males and females, dose and dosing intervals and duration of study and disease (Table 2). Simulations were carried out using 10 to 20 trials and the number of individuals to result in not less than 100 virtual subjects. The observed mean and standard deviation for plasma concentration-time profiles were digitalised from the references using GetData and overlaid in the simulation results. The exception to this was for van Groen where authors made the data available. For the study by van Groen (2019), as the individual data for age and weight was available, the Custom Trial Design feature was used to incorporate this information into the simulations. For the preterm study of de Wildt et al. (2001) a multiple population set up was utilized; four populations of 60 subjects each with GA set at 26, 29, 32 and 34 weeks and PNA set between 0.0084 and 0.03 years (3 to 11 days) were used in the simulations.
**Investigating the optimal hepatic ontogeny for CYP3A4**

The nine pediatric iv midazolam studies in Table 2 (Tolia to van Groen) were used in this investigation. Two CYP3A4 ontogeny profiles, Salem et al (2014) and Upreti and Wahlstrom (2016) (Figure 1A) were incorporated sequentially into the different workspaces and a comparison made between the two. The same fixed seed random distribution was retained between simulations to mimic a crossover study design with the same virtual subjects between simulations.

**Investigating UGT ontogeny**

Only the Upreti ontogeny for the CYP3A4-mediated metabolism of parent MDZ to 1OHMDZ was used in these simulations. The studies of van Groen et al (2019), Neupane et al (2022) and Reed et al (2001) were used in this analysis. Because the UGT1A4 default ontogeny in Simcyp is similar to that reported by Badee et al (2019), and also due to the relatively low fm of 22%, this was left fixed in the analysis. Likewise, as the UGT2B7 ontogeny in Simcyp was derived from six different studies (Stassburg et al., 2002; Pacifici et al., 1982; Pacifici et al., 1989; Zara et al., 2006; Choonara et al., 1989; Bhatt et al., 2019), it was also fixed as it was considered to be relatively robust. The ontogeny for UGT2B4 was varied between simulations assuming either default ‘no ontogeny’ or the ontogeny of Badee et al (2019). The different UGT ontogeny profiles for UGT1A4, 2B4 and 2B7 are shown in Figure 1B.

**Simulations in preterm neonates**

The default CYP3A4 ontogeny profile for the Sim-Preterm population in Simcyp was used (Abduljalil et al., 2020). The CYP3A7 ontogeny, based on data from multiple sources including Hines et al (2007 and Stevens et al (2003) is defined in equation 1.

\[
Fraction = C_0 + C_1 \cdot PMA \\
\text{Equation 1}
\]

Where \( C_0 = 50.79 \) and \( C_1 = -39.77 \)
All UGTs were treated the same regarding ontogeny. A sensitivity analysis was performed to identify the optimal UGT profile among the different scenarios; no expression of UGT, adult expression of UGT, 20% and finally 10% of adult UGT expression.

**Data analysis**

Simulated mean, 5th and 95th percentiles of plasma concentration-time profiles were compared with observed data and their variability. Predicted and observed AUC$_{0-\infty}$ or AUC$_{0-t}$ (1-OHMDZ data from Neupane et al only) were compared in terms of 2-fold, 1.5-fold or 0.8 to 1.25-fold error.

For the sensitivity analysis regarding optimal ontogeny, the mean squared error and mean error were used as measures of precision and bias according to equations 2 and 3.

\[
MSE = \frac{1}{N} \times \sum_{n=1}^{N} PE^2 \quad \text{Equation 2}
\]

\[
ME = \frac{1}{N} \times \sum_{n=1}^{N} PE \quad \text{Equation 3}
\]

Where \( PE = \frac{\text{Predicted PK parameter}}{\text{Observed PK parameter}} - 1 \), \( n \) is the individual value and \( N \) is the overall number of values.

**Results**

**Model verification**

There was good prediction of both MDZ and 1-OHMDZ concentration-time profiles in adult subjects for all three studies (Figure 2); most mean observations were close to the mean prediction line and within the 5th and 95th percentiles. Based on the profiles shown in Figure 2 (A,B; C,D: E,F), predicted to observed (P:O) AUC$_{0-\infty}$ values for MDZ were 1.27, 1.16 and 1.01 and corresponding AUC$_{0-\infty}$ values for 1-OHMDZ were 1.26, 1.17 and 1.11, respectively.

**CYP3A4 ontogeny comparison**

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Visual comparison of the MDZ results generally shows improved prediction using the Upreti compared to Salem ontogeny (Figure 3, supplementary Figure 1); the only exception to this is for the studies of Payne et al (1989) and Tolia et al (1991) where there is little difference. The observed and predicted AUC$_{0-\infty}$ or CL values are shown in Table 3. Across the nine pediatric studies, P:O AUC$_{0-\infty}$ ratios ranged from 0.84 to 3.95 with the Salem ontogeny and from 0.85 to 1.4 with the Upreti ontogeny. The improved prediction is particularly marked for the youngest age group study of van Groen et al (0.0109 to 0.942y) and to a lesser extent Rey et al (1.75 to 4y). For these two studies the predicted error for AUC$_{0-\infty}$ was 2.94 and 0.47, respectively with the Salem ontogeny and fell to 0.14 and 0.024 with the ontogeny of Upreti. There was a fall in prediction error for AUC$_{0-\infty}$ across all studies but for Payne et al (1989), Tolia et al (1991) and Reed et al (2002) this was marginal. Overall, changing ontogeny from Salem to Upreti, the mean squared error (precision) based on AUC$_{0-\infty}$ prediction fell from 1.21 to 0.064 and mean error (bias) fell from 0.69 to 0.14 thus indicating higher precision and less bias for the latter. Prediction of Cmax was reasonable across most studies (Figure 3 and Supplementary Figure 1) with some under-prediction for Neupane et al., 2022.

**Exploring UGT ontogeny using 1-OHMDZ as a probe.**

A visual comparison of the UGT2B4 ontogeny combinations for the studies is shown in Figure 4, the full results are shown in Table 4. The default UGT2B4 ontogeny performed best overall in terms of lowest predictive error (0.164 compared to -0.23 for Badee et al., 2019) and for the studies of Reed et al (2002) and Neupane et al (2022) was visually better. However, for the data in the youngest subjects from van Groen et al (2019) the UGT2B4 ontogeny from Badee et al (2019) performed best with a predictive error of -0.105 compared to 0.62 for the default.
Extending the UGT analysis to preterm neonates, the default CYP3A4 and CYP3A7 ontogeny were used in the simulations and resulted in reasonable prediction of the concentration-time data for MDZ especially when the default non-expression of UGT1A4 is applied (Figure 5A). When UGT1A4 ontogeny is set to ‘No ontogeny’ the model uses the adult expression and there is some over-prediction of MDZ elimination (Figure 5C) as this enzyme also has minor involvement in parent MDZ elimination. The sensitivity analysis in the preterm population to predict 1-OHMDZ PK, going from no UGT expression (Figure 5B) to full adult UGT expression (Figure 5D) suggests that these subjects have around 10% of the overall adult UGT activity (Figure 5H) as this assumption resulted in the closest prediction of the 1-OHMDZ concentration-time profile in these subjects.

**Discussion**

Several *in vitro* ontogeny studies for CYP3A4 have been published and applied to PBPK models (Edginton et al., 2006; Johnson et al., 2006). However, the performance of some of these have been questioned (Leong et al., 2012) and subsequent models have been derived; Salem et al (2014) based on iv midazolam and Upreti and Wahlström (2016) based on iv sufentanil. Both research groups report the improved performance of their *in vivo* derived compared to *in vitro* based ontogeny although that of Salem is actually similar to the *in vitro* profile (Johnson et al., 2006). In recent publications, simulations have been performed using both CYP3A4 ontogeny profiles to support client and regulatory queries regarding dose (Emoto et al., 2019; Johnson et al., 2019) and DDI liability (Cleary et al., 2021). As pediatric PBPK models are used to support PSP and PIPs this is clearly an unsatisfactory situation with an urgent need to establish the most optimal ontogeny pattern. Comparison of the two ontogeny profiles in this study, using an expanded iv midazolam dataset in pediatrics, indicated that the Upreti ontogeny is performing better than that of Salem despite the latter
being based on midazolam data. Based on the younger age studies (Rey et al., 1991; van Groen et al., 2019), the clearance may be slightly faster and there is scope to refine the Upreti model.

Whilst the current study supports the use of the Upreti ontogeny going forward, it needs to be recognized that this was developed based on one CYP3A4 substrate sufentanil. This is a high extraction drug \( (E_H = 0.7) \) and will be sensitive to hepatic blood flows used in the deconvolution procedure (Scholz et al., 1996). Furthermore, three of the MDZ studies used to verify ontogeny were of short duration \( (< 3 \text{ hours}) \) (Rey et al., 1991; Tolia et al., 1991 and Neupane et al., 2022) and should have ideally included sampling over a longer period. However, given the rapid distribution of the drug (Kanto, 1985) the data are likely to be mainly influenced by elimination. This is supported by the PBPK simulations which account for both phases simultaneously. For MDZ the influence of ontogeny can clearly be seen for the study of Rey et al. (1991) where the predicted concentration-time data reflects the observed data in the 0-2h time period when the Upreti ontogeny is applied. For 1-OHMDZ, the influence of ontogeny can also be seen on predicted concentrations when compared against data from the study of Neupane et al (2022) over the same time span.

For the preterm population the ontogeny of CYP3A4 is around 7-8% of the adult value and changes with post menstrual age (Abduljalil et al., 2020). Because of the high expression of CYP3A7 in these subjects, around 300pmol/mg microsomal protein (Stevens et al., 2003) the metabolism via this enzyme was also included based on in vitro data (Williams et al., 2002). The PK profile for iv midazolam was reasonably well captured; examination of the fractional contribution of each pathway, \( fm \) for CYP3A7 was around 21% and CYP3A4 around 68%, reflecting the lower capacity of CYP3A7 to metabolize many CYP3A4 substrates (Williams et al., 2002; Takahiro et al., 2015). More research is needed on the contribution of CYP3A7 to drug metabolism in the pre-term population, not just in relation to drug elimination, and
the switch between CYP3A7 and CYP3A4 with both gestational and post-natal age, but also its contribution to drug-drug interactions (Godamudunage et al., 2018; Salerno et al., 2021). Expanding PBPK models to include drug metabolites is especially important if the metabolite in question elicits a therapeutic effect such as the case with 1-OHMDZ (Johnson et al., 2002). 1-OHMDZ PK has been studied in humans (Mandema et al., 1992) following administration of the actual metabolite which facilitated model development in this case. 1-OHMDZ has previously been proposed as a general marker of UGT ontogeny in preterm infants (de Wildt et al., 2010), clearly its formation rate is dependent on CYP3A4/5 activity with age and thus the reason for optimizing the ontogeny of these enzymes above. The metabolite is an attractive marker for UGT ontogeny due to availability of data in pediatrics and ease of measurement (van Groen et al., 2019).

There are several recent studies on the ontogeny of UGT2B4 and UGT2B7, the major enzymes responsible for 1-OHMDZ elimination. Badee et al (2019) reported 2-fold over-activity of both enzymes in the early years which then decline to adult values. For completeness this combination was tested in the current analysis but shown to result in under-prediction of concentration-time data (Supplementary Figure 2). Bhatt et al (2019) reported a sigmoidal developmental pattern for UGT2B7 reaching adult values by around 7 years of age, based on both protein expression and activity. This data was included in the current Simcyp UGT2B7 default ontogeny, verification of which is based on morphine (Emoto et al., 2017; Emoto et al., 2018) and zidovudine (Salem et al., 2022) PK data. The former may be less than ideal as this is a high extraction drug and depending on age more influenced by liver blood flow and OCT1 transport into the hepatocytes (Emoto et al., 2018). The verification data for default UGT2B7 ontogeny shows good performance across the pediatric age range. Compared to CYP3A4 the UGT2B4 results are less clear. Based on the current analysis, the default ontogeny appears to be the best model in terms of lowest predictive error but there
were differences in results between the two older compared to the younger age studies. For the younger subjects of less than 1 year of age in the study of van Groen et al (2019) the Badee UGT2B4 ontogeny was better.

There is little ontogeny data for UGT in preterm infants and in these subjects, this should consider not only the gestational age (GA) at birth but also post-natal age, a recent publication has highlighted this in relation to development of glomerular filtration rate (Salem et al., 2022). Using a sensitivity analysis approach the UGT enzymes ontogeny had to be set at 10% of adult values to replicate the 1-OHMDZ PK data in a preterm population GA 26 to 34 weeks, Bhatt et al (2019) reported values in neonates of 1.8 and 13% for UGT1A4 and 2B7, respectively.

Limitations of the current study are stated hereafter. 1-OHMDZ is not a pure marker for UGT2B4 (fm = 0.47) and we are assuming the current ontogeny for UGT2B7 (fm = 0.31) and UGT1A4 (fm =0.22) are reasonably robust. The optimal study duration and PK sampling times for this metabolite as a UGT marker have also not been established. Despite these limitations, the PBPK model has allowed an exploratory analysis to be conducted; key findings are that the ontogeny of UGT2B4 appears to increase rapidly around the time of birth or just after birth and may be over-expressed in younger age groups as also indicated by Badee et al (2019).

Disease effects have been largely ignored, for the evaluation of CYP3A4 ontogeny; seven of the studies were performed in relatively healthy children. However, one study (van Groen et al., 2019) was in 0-2 y aged children on a pediatric intensive care unit and another (Mathews et al., 1988) was in subjects undergoing cardiac surgery. For the former study, whilst exclusion criteria included hepatic or renal impairment, and various forms of dialysis, infection was not an exclusion criterion and various cytokines are known to suppress CYP3A4 activity (Morgan, 2009; Schmitt et al., 2011; Vet et al., 2016). For the latter study,
a correction was incorporated to account for reduced cardiac output and thus liver blood flow post-surgery.

PBPK models are a useful research tool and allow the flexibility to perform ‘what if’ scenarios and evaluate these against clinical data as in this and previous studies (Johnson et al., 2016). Whilst this study uses the Simcyp Simulator, several other PBPK platforms could be used for this analysis. One of the things holding back this approach is the lack of clinical studies performed in discrete age bands where ontogeny is important, such as the one by van Groen et al (2019). Whilst the difficulties of performing studies in preterm and term neonates and infants are well known (Allegaert et al., 2022) advances in PK methodology such as sparse sampling, dried blood spots (Mizuno et al., 2021) and appropriate use of microdose methods (van Groen et al., 2019) are helping to overcome these.

In this study only iv MDZ studies were considered to evaluate hepatic CYP3A4 ontogeny and not combined hepatic and intestinal CYP3A4 ontogeny as for oral MDZ. A future research application of P-PBPK models could be in the evaluation of intestinal CYP3A ontogeny in relation to what is already known (Johnson et al., 2001; Kiss et al., 2021). In the meantime, there is clearly a need expand the analysis described here to other iv CYP3A substrates used across the pediatric age range e.g. alfentanil, sildenafil and itraconazole. To further optimize the CYP3A4 ontogeny a simultaneous fitting approach is being investigated with multiple CYP3A4 substrates and available PK data across pediatric age ranges. Fitting hepatic CYP3A4 abundance at specific ages will allow an overall in vivo ontogeny profile to be generated.

In conclusion, a 1-OHMDZ model was established and successfully verified in the adult population. The combined MDZ/1-OHMDZ model was then used to evaluate both CYP3A4 and UGT2B4 ontogeny. Evaluation of the UGT2B4 ontogeny indicates it is likely to be either fully expressed at birth or develop very quickly to above adult levels after birth. In a
comparison of the Upreti and Salem ontogenies for CYP3A4, the former demonstrated superior performance in recovering observed profiles and CL data for an expanded midazolam dataset in infants and neonates. Thus, this should be factored into decision making when providing dose recommendations in these individuals, whilst ongoing efforts are made to underpin the generalizability of the optimal CYP3A4 ontogeny for other CYP3A4 metabolized compounds.
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Data Availability

The authors declare that all the data supporting the findings of this study are available within the paper and its Supplemental Data.

Author contributions

Participated in research design  TNJ

Conducted simulations  EMH, TNJ

Contributed external data SNdW, MAT

Performed data analysis  EMH, TNJ

Wrote or contributed to writing of manuscript TNJ, EMH, SNdW, MAT, KRY
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Neupane B, Pandya H, Pandya T, Austin R, Spooner N, Rudge J, and Mulla H (2022) Inflammation and cardiovascular status impact midazolam pharmacokinetics in


Figure Legends

Figure 1. Different ontogeny profiles for CYP3A4 (A) and UGT1A4/2B4/2B7 (B). The references, including for the default Simcyp UGT2B7 ontogeny are in main text.

Figure 2. Simulated means (black lines) and 5th and 95th percentiles (grey lines) for midazolam and 1OHMDZ with overlaid mean observed data (circles) in adult subjects. A, B are following a 0.1mg/kg iv dose (Mandema et al 1992), C and D are following a 0.3mg/kg iv dose Clausen et al., 1988) and E and F are following 5mg iv dose (Kupferschmidt et al 1995).

Figure 3. Predicted and observed concentration time profiles for midazolam in different pediatric studies comparing the CYP3A4 ontogeny profiles of Salem et al 2012 (LHS) and Upreti and Wahlstrom 2016 (RHS). Black lines are the mean predicted profiles, grey lines are the 5th and 95th percentiles and black circles are the corresponding observed data (A,B) van Groen et al 2019, 36.6 ng/kg in age 0.0109 to 0.942y; (C,D) Rey et al 1991, 0.2 mg/kg in age 1.75 to 4 years; (E,F) Reed et al 2001, 0.15mg/kg in age 0.5 to 12 years; (G,H) Salonen et al 1987, 0.15mg/kg in age 1.1 to 11.1 years; (I,J) Neupane et al 2022, 34.6 mcg/kg in age 0.62 to 15.7. All studies show mean data except van Groen (A,B) and Neupane (I,H) which are individuals. Other studies are shown in Supplementary Figure 1.

Figure 4. Predicted and observed concentration time profiles for 1-OHMDZ in van Groen et al (2019), Reed et al (2002) and Neupane et al 2022 comparing the default UGT2B4 ontogeny profile in Simcyp v21 with of Badee et al (2019). Black lines are the mean predicted profiles, grey lines are the 5th and 95th percentiles and black circles are the corresponding observed data in individuals.

Figure 5. Simulated means (black lines) and 5th and 95th percentiles (grey lines) for midazolam and 1OHMDZ with overlaid mean observed mean data (circles) in preterm neonates. following a 0.1mg/kg iv dose (de Wildt et al 2002), A&B are assuming no
expression of UGT1A4, 2B4 and 2B7, C & D are assuming adult expression, E&F are assuming expression is 0.2 of adult and G&H are assuming expression is 0.1 of adult
Table 1: Input parameter values used for 1-OH midazolam

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<td>3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Blood binding parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fu</td>
<td>0.15</td>
<td></td>
<td>(Nguyen et al., 2016)</td>
</tr>
<tr>
<td>B:P ratio</td>
<td>1</td>
<td>Assumed</td>
<td></td>
</tr>
<tr>
<td><strong>Distribution parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V_{ss} (L/kg)</td>
<td>1.71</td>
<td>Method 3, full PBPK model in Simcyp</td>
<td></td>
</tr>
<tr>
<td><strong>Elimination parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UGT1A4 CL_{int} (pmol / min / mg protein)</td>
<td>67.33</td>
<td>Scaled HLM data, UGT isoforms assigned using rUGT data</td>
<td>(Zhu et al., 2008; Seo et al., 2010; Nguyen et al., 2016)</td>
</tr>
<tr>
<td>UGT2B4 CL_{int} (pmol / min / mg protein)</td>
<td>141.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UGT2B7 CL_{int} (pmol / min / mg protein)</td>
<td>93.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP3A4 CL_{int} (pmol / min / mg protein)</td>
<td>14.89</td>
<td>Scaled HLM data</td>
<td>(Nguyen et al., 2016)</td>
</tr>
</tbody>
</table>
Table 2. Summary of midazolam iv clinical studies Adult, Pediatric and Preterm

<table>
<thead>
<tr>
<th>Age range</th>
<th>n</th>
<th>Trial design in Simcyp (Trials x Subjects)</th>
<th>Prop Female</th>
<th>Dose (iv)</th>
<th>Bolus / infusion time</th>
<th>Disease</th>
<th>1-OHMDZ data</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 to 25y</td>
<td>8</td>
<td>20 x 8</td>
<td>0.5</td>
<td>0.1 mg/kg</td>
<td>15 mins</td>
<td>No</td>
<td>Yes</td>
<td>(Mandema et al., 1992)</td>
</tr>
<tr>
<td>23 to 32y</td>
<td>8</td>
<td>20 x 8</td>
<td>0</td>
<td>0.3 mg/kg</td>
<td>5 seconds</td>
<td>No</td>
<td>Yes</td>
<td>(Clausen et al., 1988)</td>
</tr>
<tr>
<td>20 to 30y</td>
<td>8</td>
<td>20 x 8</td>
<td>0</td>
<td>5mg</td>
<td>Unknown assumed 3 mins</td>
<td>No</td>
<td>Yes</td>
<td>(Kupferschmidt et al., 1995)</td>
</tr>
<tr>
<td>8 to 17y</td>
<td>20</td>
<td>10 x 20</td>
<td>0.35</td>
<td>0.08 mg/kg</td>
<td>5 mins</td>
<td>No</td>
<td>No</td>
<td>(Tolia et al., 1991)</td>
</tr>
<tr>
<td>0.5 to 13.7y*</td>
<td>34</td>
<td>5 x 34</td>
<td>0.44</td>
<td>0.15 mg/kg</td>
<td>Unknown assumed 3 mins</td>
<td>Status Epilepticus</td>
<td>No</td>
<td>(Hamano et al., 2019)</td>
</tr>
<tr>
<td>0.62 to 15.7y</td>
<td>35</td>
<td>5 x 35</td>
<td>0.286</td>
<td>34.6 mcg/kg</td>
<td>Unknown</td>
<td>No (surgical arm only)</td>
<td>Yes</td>
<td>(Neupane et al., 2023)</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Age Range</th>
<th>Study Duration</th>
<th>Study Sample</th>
<th>Assumed Weight</th>
<th>Assumed Sedation</th>
<th>Outcome 1</th>
<th>Outcome 2</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 to 11.12y</td>
<td>6</td>
<td>20 x 6</td>
<td>0.5 assumed</td>
<td>0.45 mg/kg</td>
<td>No</td>
<td>No</td>
<td>(Salonen et al., 1987)</td>
</tr>
<tr>
<td>0.5 to 10.26y</td>
<td>6</td>
<td>20 x 6</td>
<td>0.4</td>
<td>0.3 mg/kg</td>
<td>Yes (30% reduction in cardiac output included)</td>
<td>No</td>
<td>(Mathews et al., 1988)</td>
</tr>
<tr>
<td>0.5 to 12y</td>
<td>12</td>
<td>10 x 12</td>
<td>0.5</td>
<td>0.15 mg/kg</td>
<td>No</td>
<td>Yes</td>
<td>(Reed et al., 2001)</td>
</tr>
<tr>
<td>3 to 10y</td>
<td>8</td>
<td>20 x 8</td>
<td>0.5 assumed</td>
<td>0.15 mg/kg</td>
<td>No</td>
<td>No</td>
<td>(Payne et al., 1989)</td>
</tr>
<tr>
<td>1.75 to 4y</td>
<td>6</td>
<td>20 x 6</td>
<td>0.5 assumed</td>
<td>0.2 mg/kg</td>
<td>No</td>
<td>No</td>
<td>(Rey et al., 1991)</td>
</tr>
<tr>
<td>0.0109 to 0.942 y</td>
<td>15</td>
<td>10 x 15</td>
<td>0.5 assumed</td>
<td>37.6 ng/kg</td>
<td>Yes (pediatric intensive care)</td>
<td>Yes</td>
<td>(van Groen et al., 2019)</td>
</tr>
<tr>
<td>GA 26 to 34 wks</td>
<td>24</td>
<td>10 x 24</td>
<td>0.67</td>
<td>0.1 mg/kg</td>
<td>Yes (neonatal intensive care)</td>
<td>Yes</td>
<td>(de Wildt et al., 2001)</td>
</tr>
</tbody>
</table>
days

GA = gestational age; PNA = post-natal age

*Japanese Pediatric population used for this simulation
Table 3. Summary of mean ±SD observed and predicted AUC$_{0-\infty}$ (or clearance) parameters in pediatric studies for MDZ comparing the different CYP3A4 ontogeny profiles.

<table>
<thead>
<tr>
<th>Study</th>
<th>Observed AUC$_{0-\infty}$ or CL*</th>
<th>Predicted AUC$_{0-\infty}$ or CL*</th>
<th>Predicted:Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salem et al</td>
<td>Upreti</td>
<td>Salem et al</td>
</tr>
<tr>
<td>Tolia et al 1991</td>
<td>10 ±5* ml/min/kg</td>
<td>8.41 ±3.33*</td>
<td>8.48 ± 3.2*</td>
</tr>
<tr>
<td>Hamano et al 2019</td>
<td>202 ng.h/ml</td>
<td>352±263</td>
<td>282±155</td>
</tr>
<tr>
<td>Neupane et al 2022</td>
<td>51* L/h/70kg</td>
<td>35.2*</td>
<td>40.8*</td>
</tr>
<tr>
<td></td>
<td>44.7 ng.h/ml</td>
<td>74.9±33.6</td>
<td>60.4±28.8</td>
</tr>
<tr>
<td>Salonen et al 1987</td>
<td>576 ±89 µg.h/L</td>
<td>936±445</td>
<td>754±323</td>
</tr>
<tr>
<td>Matthews et al 1988</td>
<td>417 ±232 ng.h/ml</td>
<td>720 ± 314</td>
<td>575 ± 211</td>
</tr>
<tr>
<td>Study</td>
<td>AUC0-∞ (ng.h/ml)</td>
<td>Cmax (µg.h/L)</td>
<td>Ratio</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------</td>
<td>--------------</td>
<td>--------</td>
</tr>
<tr>
<td>Reed et al 2001</td>
<td>281 ± 127</td>
<td>313 ± 149</td>
<td>254 ± 109</td>
</tr>
<tr>
<td>Payne et al 1989</td>
<td>276 ± 63.4</td>
<td>306 ± 143</td>
<td>251 ± 106</td>
</tr>
<tr>
<td>Rey et al 1991</td>
<td>283 ± 106</td>
<td>417 ± 209</td>
<td>290 ± 126</td>
</tr>
<tr>
<td>van Groen et al 2019</td>
<td>48.9 (34.2-219)</td>
<td>193 (28 to 1032)</td>
<td>55.8 (20.3-357)</td>
</tr>
</tbody>
</table>

*clearance all other values are AUC0-∞, \( ^{3} \) AUC0-∞ values calculate using NCA in Phoenix 32 Build 8.0.0.3176 using age binned mean concentration-time data, \(^{#}\) median and range

**Table 4.** Summary of the median (range) or mean±SD observed and predicted AUC & Cmax parameters in pediatric studies reporting 1-OHMDZ comparing the different UGT2B4 and UGT2B7 ontogeny combinations. In all cases the Upreti ontogeny was used for MDZ.
<table>
<thead>
<tr>
<th>Study</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng/L<em>h)</em></th>
<th>Cmax (ng/L)*</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng/ml*h)</th>
<th>Cmax (ng/ml)</th>
<th>AUC&lt;sub&gt;0-2h&lt;/sub&gt; (ng/ml*h)&lt;sup&gt;§&lt;/sup&gt;</th>
<th>Cmax (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Observed</strong></td>
<td>12.39</td>
<td>NR</td>
<td>62.7</td>
<td>32.4</td>
<td>10.37</td>
<td>8.91</td>
</tr>
<tr>
<td><strong>Predicted</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Default UGT2B4 ontogeny</td>
<td>20.0</td>
<td>7.4</td>
<td>66.9</td>
<td>25.0</td>
<td>8.41</td>
<td>6.16</td>
</tr>
<tr>
<td>P:O</td>
<td>1.61</td>
<td>-</td>
<td>1.06</td>
<td>0.77</td>
<td>0.81</td>
<td>0.69</td>
</tr>
<tr>
<td>Prediction error</td>
<td>0.616</td>
<td>0.067</td>
<td>-0.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Predicted</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Badee UGT2B4 ontogeny</td>
<td>11.05</td>
<td>4.82</td>
<td>49.0</td>
<td>20.0</td>
<td>6.43</td>
<td>4.87</td>
</tr>
<tr>
<td>P:O</td>
<td>0.89</td>
<td>-</td>
<td>0.78</td>
<td>0.62</td>
<td>0.62</td>
<td>0.55</td>
</tr>
<tr>
<td>Prediction error</td>
<td>-0.104</td>
<td>-0.22</td>
<td>-0.38</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Median values

<sup>§</sup> Calculated in Microsoft Excel using the trapezoidal rule
**Conflict of Interest:** TNJ, EMH and KRY are employees of Certara UK Limited (Simcyp Division). TNJ, EMH, SdW, MAT and KRY have declared no competing interests for this work.

**Funding:** No funding was received for this work.
Fig 1

(A) CYP3A4 Salem et al 2014

(B) UGT2B7 Simcyp default
UGT2B4 Simcyp default
UGT2B4 Badee et al 2019
UGT1A4 Simcyp default