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Title: Impact of hepatic CYP3A4 ontogeny functions on DDI risk in paediatric PBPK/PD modelling: critical literature review and ivabradine case study

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Running head: Paediatric PBPK-PD modelling: evaluation of DDI risk

Abstract

Clinical assessment of drug-drug interaction (DDI) in children is not a common practice in drug development. Therefore, physiologically-based-pharmacokinetic (PBPK) modelling can be beneficial for informing drug labelling. Using ivabradine and its metabolite (both CYP3A4 substrates), the objectives were (i) to scale ivabradine-metabolite adult PBPK-PD to paediatrics, (ii) to predict the DDI with strong CYP3A4 inhibitor and (iii) to compare the sensitivity of children to DDI using two CYP3A4 hepatic ontogeny functions: Salem and Upreti. Scaled parent-metabolite PBPK-PD model from adults to children satisfactorily predicted PK and PD in 74 children (0.5-18 years) regardless of CYP3A4 hepatic ontogeny function applied. However, using Salem ontogeny, mean predicted parent and metabolite AUC_{12h} and heart rate change from baseline (ΔHR) were 2-, 1.5- and 1.4-fold higher in young children (0.5-3 years) compared to Upreti ontogeny, respectively. Despite these differences, choice of appropriate hepatic CYP3A4 ontogeny was challenging due to sparse PK and PD data. Different sensitivity to ivabradine-ketoconazole DDI was simulated in young children relative to adults depending on the choice of hepatic CYP3A4 ontogeny. Predicted ivabradine and metabolite $AUC_{DDI}/AUC_{control}$ were 2-fold lower in the youngest children (0.5-1 years) compared to adults (Salem function). In contrast, Upreti function predicted comparable ivabradine DDI across all age groups, although predicted metabolite $AUC_{DDI}/AUC_{control}$ was 1.3-fold higher between youngest children and adults. In case of PD, differences in predicted DDI were minor across age groups and between both functions. Current work highlights importance of careful consideration of hepatic CYP3A4 ontogeny function and implications on labelling recommendations in paediatric population.

1 Introduction

2 Physiologically-based-pharmacokinetic (PBPK) modelling is a quantitative translational tool that
3 enables prediction of drug exposure in the systemic circulation and tissues by accounting for
4 physiological and drug-related parameters.¹ In early stages of drug development, PBPK modelling
5 can be used for optimisation of clinical study design e.g., first-in-human doses or sampling scheme².
6 In later stages, PBPK model predictive performance can be improved by refining input parameters
7 with clinical data (middle-out approach/reverse translation³⁻⁵). This results in greater confidence in
8 model extrapolation to untested scenarios (i.e. drug-drug interactions (DDI)) and/or special
9 populations (i.e. paediatrics, organ impairment⁶).

10 Pharmaceutical companies and regulatory authorities have shown an increasing confidence in
11 application of PBPK modelling in decision making.⁷⁻¹⁰ Between 2008-2017, paediatric PBPK
12 modelling was the second main application in regulatory submission (after evaluation of the DDI risk),
13 representing 15% of total number of new drug applications to the U.S. Food and Drug Administration
14 (FDA). Dosing recommendations and optimisation of paediatric study design were the most common
15 purposes of paediatric PBPK modelling⁷. Although PBPK-based prediction of DDI in adults is widely
16 accepted¹¹, this application remains sparse in children.^{12,13} As paediatric DDI studies are particularly
17 challenging for ethical and practical reasons, mechanistic modelling could be an ideal alternative.¹⁴
18 However, the lack of understanding and/or knowledge in maturation of certain processes can limit the
19 extrapolative power and confidence in this approach.¹⁵

20 Maturation of metabolic enzymes, renal function or transporter-mediated disposition has been the
21 subject of many studies reported in the literature.¹⁶⁻²⁰ Most efforts have focused on delineating the
22 ontogeny of cytochrome P450 enzymes with variable conclusions^{21,22}. This inconsistency is evident
23 even in the case of CYP3A4 despite being the most investigated enzyme. The most commonly used
24 CYP3A4 ontogeny functions assume different age variations in enzyme activity. The ontogeny
25 function proposed by Salem et al.²¹ suggests that hepatic CYP3A4 increases from an early age and
26 reaches adult level by the age of 2.5 years. In contrast, Upreti and Wahlstrom²² suggested that
27 CYP3A4 maturation increases from an early age and exceeds adult level between 0.1 to 11 years. All
28 above highlights the necessity to understand the impact of different assumptions regarding CYP3A4
29 age-related changes on the PBPK model predictive performance. To that end, reported applications

of paediatric PBPK modelling for CYP3A4 substrates were reviewed critically in the current study, aiming to highlight current status and challenges in the prospective PBPK modelling in this population.

Ivabradine, a selective and specific I_f current inhibitor, is indicated in chronic heart failure (CHF) and stable angina pectoris in adults. The marketing authorization in children with CHF between 6 months-18 years old was recently approved by FDA.²³ Ivabradine and its main metabolite are eliminated via CYP3A4 and both entities demonstrated pharmacological effect on the heart rate (HR).²⁴ The link between pharmacokinetics (PK) and pharmacodynamics (PD) in adults and children has been explored using a population-PK approach.²⁴⁻²⁶ A joint parent-metabolite PBPK-PD model for this drug has recently been developed in adults and verified against multiple clinical studies.⁵ The current study is an extension of that PBPK-PD model to children between 6 months and 18 years. Furthermore, ivabradine-metabolite PBPK-PD model scaled to children was utilised for the assessment of DDI risk with a strong CYP3A4 inhibitor with the aim to compare sensitivity of children across different age groups to CYP3A4-mediated DDI relative to adults. Finally, evaluation of the impact of different hepatic CYP3A4 ontogeny functions on DDI-related changes in ivabradine PK and PD has been performed.

Methods

Overview of paediatric PBPK modelling of CYP3A4 substrates

Critical evaluation of the literature reported examples of paediatric PBPK modelling was performed; inclusion criteria are detailed in Supplementary Section 1.

Clinical data for ivabradine

Individual data in children were collected from a clinical study conducted by Servier as summarised previously²⁵. The clinical study followed ethical principles of the Declaration of Helsinki and study protocol was reviewed and approved by Ethical Review Boards. Study participants provided and signed an informed consent prior to inclusion in the studies. Data from 74 children in total with dilated cardiomyopathy and CHF were available. Four age groups were defined: 6-12 months (Class 1), 1-3 years old (Class 2), 3-18 years old with a body weight below 40 kg (Class 3) and above 40 kg (Class 4). Patients received up to five increasing doses of ivabradine, administered as an oral liquid paediatric formulation or tablet for children older than 6 years old. The starting dose for Class 1 was 0.02 mg/kg b.i.d. and was increased to 0.05, 0.10, 0.15 and 0.20 mg/kg b.i.d. For the classes 2 and 3,

the starting dose was 0.05 mg/kg and was increased to 0.10, 0.15, 0.20 and 0.30 mg/kg b.i.d. For the oldest age group (Class 4), subjects received a starting dose of 2.5 mg of ivabradine b.i.d. and was increased to 5, 7.5, 10 and up to 15 mg b.i.d. Blood concentrations for ivabradine and metabolite were measured at five sampling times: on day 13, 1 and 2 h after the second drug intake, on day 14, prior to and 4 h after the first drug administration and on day 1 of the maintenance period (highest administered dose), 7 h after the first drug administration. HR values at baseline were recorded for each patient and before each blood PK sample by 12-lead electrocardiogram (ECG), finger pulse recorder or ECG scope. HR baseline values were used in the PBPK/PD model.

PBPK-PD model structure

An ivabradine joint parent-metabolite disposition model was developed in adults⁵ using Matlab® v2016 and optimised using Bayesian method implemented in NONMEM® v7.4. This model was extended to account for oral absorption and DDI with CYP3A4 inhibitors by implementing a mechanistic intestinal model. Distribution of ivabradine and metabolite to the site of action was described by a five-compartment heart model. Lastly, the pharmacological effects of both ivabradine and the metabolite on HR were linked through a PD model. Schematic of the model structure is illustrated in Figure 1. Model equations and parameters are reported in Lang et al.⁵ and detailed in the Supplementary Sections 2-4.

In the ivabradine PBPK model, non-eliminating organ tissues were modelled following flow-limited assumptions (Equation 1). In order to reduce the model complexity, a peripheral (adipose tissue, skin, bones, rest of body) and a splanchnic (spleen, pancreas, stomach wall and the intestinal serosa) compartments were included based on the proper lumping method²⁷. These lumped compartments receive blood supply from an arterial blood flow and the blood flow returns to the venous blood or the portal vein via the peripheral rate K_{per} or splanchnic rate K_{spl} , respectively (Equations 2-4).

$$V_T \frac{dC_T}{dt} = Q_T \cdot \left(C_{art} - \frac{C_T}{K_{b,T}} \right) \quad \text{Equation 1}$$

$$\frac{dA_{per}}{dt} = Q_{per} \cdot C_{art} - A_{per} \cdot K_{per} \quad \text{Equation 2}$$

$$\frac{dA_{spl}}{dt} = Q_{spl} \cdot C_{art} - A_{spl} \cdot K_{spl} \quad \text{Equation 3}$$

$$K_{spl} = \frac{Q_{in}}{4 \cdot K_{b,in} \cdot V_{in}} + \frac{Q_{pa}}{4 \cdot K_{b,pa} \cdot V_{pa}} + \frac{Q_{sp}}{4 \cdot K_{b,sp} \cdot V_{sp}} + \frac{Q_{st}}{4 \cdot K_{b,st} \cdot V_{st}} \quad \text{Equation 4}$$

where A, C, V, Q, K_b are amount, concentration, volume, blood flow, tissue-to-blood partition coefficient; subscripts art, T, per, spl, in, pa, sp and st represent the arterial blood, tissue, peripheral, splanchnic, intestinal serosa, pancreas, spleen and stomach serosa.

Ivabradine mechanistic gut model followed the structure defined previously⁵; each intestinal segment was separated to the intestinal lumen and the intestinal wall, containing the enterocytes, site of CYP3A4-mediated metabolism. Regional differences in physiological parameters and CYP3A4 expression were considered.^{28,29}

The mechanistic heart model included five compartments where each compartment corresponded to a different tissue layer: the pericardial fluid, the extracellular matrix, the epicardium, the midmyocardium (site of drug action) and the endocardium. Diffusion of ivabradine and metabolite was described by passive permeability, as in vitro studies did not show evidence of transporter-mediated uptake.⁵ The PD model was an Emax model adapted from Peigne et al.²⁵ and was linked to the joint PBPK-PD model via ivabradine/metabolite unbound concentrations in the midmyocardium (Equation 5).

$$HR = Base \times \left[1 - E_{max} \times \left(\frac{\frac{Cheart_P}{EC_{50P}} + \frac{Cheart_M}{EC_{50M}}}{1 + \frac{Cheart_P}{EC_{50P}} + \frac{Cheart_M}{EC_{50M}}} \right) \right] \quad \text{Equation 5}$$

where Base, E_{max} , $Cheart_P$, $Cheart_M$, EC_{50P} and EC_{50M} are the HR value at baseline, maximal drug effect, unbound heart tissue concentrations for parent and metabolite and concentrations which yield 50% of parent drug and metabolite effect, respectively.

Scaling of ivabradine and metabolite PBPK/PD model from adults to children

The adult model implemented in Matlab® v2016 was scaled by integrating age-dependent changes in physiological parameters for children between 0.5 to 18 years collated from the literature³⁰. Tissue blood flows were defined as a fraction of the cardiac output, which was defined as a function of age and body surface area, whereas tissue volumes were integrated as age-dependent fraction of total body weight¹⁶. Ivabradine and metabolite unbound fractions in plasma were calculated by accounting for changes in α -acid glycoprotein,¹⁶ whereas renal clearance integrated maturation of the renal

function.¹⁷ Edginton et al. (2006)³¹ reported no effect of age-related differences in tissue composition on predicted K_b , muscle in children for selected drugs, in contrast to adipose where the effect was more apparent. Potential effect of differences in tissue composition between children and adults was implemented here by allometric scaling of K_{per} (a composite parameter of K_b s and tissue volumes), considering that adipose is part of peripheral compartment in ivabradine PBPK/ PD model. Peripheral and splanchnic rates were scaled using allometric scaling based on weight and a 0.75 exponent³². Age-dependent changes in hepatic intrinsic clearance (CL_{int}) were implemented via liver weight in children and CYP3A4 maturation function described by Salem et al.²¹ and Upreti and Wahlstrom²². The impact of the choice of CYP3A4 hepatic ontogeny function on the predicted magnitude of DDI was evaluated in different paediatric populations, both on PK and PD levels. The intestinal CL_{int} implemented an intestinal CYP3A4 maturation function described by Johnson et al.¹⁶ Additional changes in gut physiology in children relative to adults are detailed in Supplementary Section 2. Food has been reported to increase ivabradine AUC_{inf} by 42%²³, but this information was not available in the paediatric study and was therefore not considered in the model development. Relevant equations for calculation of some drug-related parameters are summarised in Figure 2 and scaled parameters are in Table 1. Values for age-independent model parameters are the same as reported in adults⁵ (see Supplementary Table S2).

Prediction of PK and PD DDI with ketoconazole in children

Inhibition of CYP3A4 by a strong inhibitor (ketoconazole) was simulated for each age group using the paediatric module in Simcyp Simulator® v18. Model development is detailed in Supplementary Section 5 and input parameters are summarised in Supplementary Table S3. Ketoconazole simulations were performed for a 3.3 mg/kg dose (oral solution) once daily for 5 days (equivalent to a 200 mg dose in adults) and a single oral dose of ivabradine on day 5 (0.10 mg/kg for classes 1 to 3 or 5 mg for class 4). Evaluation of perpetrator model in the population of relevance is an important consideration; therefore, simulated ketoconazole systemic exposure was compared to the reported clinical data with the same formulation. To account for CYP3A4 inhibition in both liver and intestine, the unbound ketoconazole concentrations in the liver and the portal vein were simulated, assuming that portal vein concentrations reflect ketoconazole concentrations in the enterocytes.³³ The impact of CYP3A4 hepatic ontogeny functions on DDI magnitude was evaluated by calculating the ratio between ketoconazole co-administered and control groups for PK parameters (area-under-the curve AUC_{inf}

and maximal concentrations C_{max} for ivabradine and metabolite). Analogous evaluation was done by comparing the PD effect (maximal HR change from baseline, Δ HR) between ketoconazole and control phase. The impact of both ontogeny functions on predicted DDI with ketoconazole-equivalent inhibitor was evaluated for a range of adult $f_{mCYP3A4}$ values keeping all other parameters as for ivabradine (i.e. minimal intestinal interaction, Supplementary Figure S2).

Results

Review of examples of paediatric PBPK modelling

The literature search identified 28 studies that focused on evaluation of PK in children using PBPK modelling (Table 2, Supplementary Table S4). In most studies, investigated paediatric population included children >3 year old and few focused on neonates and younger children (<1 year; 12 out of 28). The most commonly used strategy for paediatric model development was (i) development of an adult PBPK model (using a bottom-up or middle-out approach) and evaluation against clinical data (drug alone, 79% of cases) or with CYP3A4 modulators (21% of studies), followed by (ii) scaling from adults to children with model refinement (18%) and verification against paediatric clinical data (89%). The main reported objectives were optimisation of dosing or sampling times and evaluation of model predictive performance for this population, with only six studies exploring the prediction of DDI in children. No studies investigated possible link between PK and PD and the consequences of potential age-dependent variations on these.

User-friendly software platforms were mostly used (64% Simcyp®, 18% PK-sim®) and the default intestinal and hepatic CYP3A4 ontogeny functions were generally applied. *In vitro*- or *in vivo*-derived CYP3A4 ontogeny functions using a particular study drug were also explored in four studies. All CYP3A4 reported ontogeny relationships are plotted in Supplementary Figure S1. *In vitro*-derived hepatic ontogeny functions using diverse reference drugs (e.g. midazolam, sildenafil) showed an increase in CYP3A4 abundance and/or activity over age^{16,34–38}. Although several studies using *in vivo* data were in agreement with these *in vitro*-derived functions, recently reported maturation functions showed a different profile.²² Upreti and Wahlstrom²² indicated an increase in hepatic CYP3A4 fraction of the adult value up to 2 years old. This fraction exceeded value of 1 between 0.1 and 12 years, suggesting higher CYP3A4 abundance/activity compared to adults which may significantly affect model predictions in children, especially under 3 years old. Contrary to the liver function, intestinal

CYP3A4 maturation has been less explored and the ontogeny function published by Johnson et al.¹⁶ is currently the only available option.

Ivabradine paediatric PBPK-PD modelling

Observed and predicted ivabradine/metabolite concentration-time and HR over time profiles are shown in Figure 3 (linear scale shown in Supplementary Figure S2 and predicted unbound heart tissue concentration-time profiles is shown in Supplementary Figure S3); impact of using either Salem²¹ or Upreti²² ontogeny functions is illustrated. Additional plots exploring impact of age on various PK parameters are illustrated in Supplementary Figures S4-S8. Predicted PK parameters and Δ HR for each age group and for different ontogeny functions are summarised in Table 3. Both models adequately described sparse PK and PD data in all paediatric age groups. However, model predictions for parent drug and metabolite PK were slightly different between the two ontogeny functions in the youngest children (Classes 1 and 2). Predicted parent C_{max} and AUC_{12h} for children in Class 1 using Upreti ontogeny function were lower by 55.1% and 46.9%, respectively compared to the parameters predicted by the Salem function. As age increased, the percentage difference in these parameters between the two ontogeny functions decreased and parameters were comparable for the oldest children (Class 4).

Although differences in model predictions for the metabolite were evident depending on the choice of hepatic ontogeny function, the extent of difference between two approaches was not as pronounced as in the case of ivabradine (predicted AUC_{12h} and C_{max} in Class 1 were 41.8% and 19.4% lower, respectively using Upreti function). The percentage difference in metabolite parameters between ontogeny functions followed the same order with age as seen for ivabradine. Metabolite concentration-time profiles using either ontogeny function were overlapped for Classes 3 and 4 (Figure 3B).

The joint parent-metabolite PBPK-PD model was also applied to predict Δ HR in different paediatric populations; predictions were in agreement with sparse clinical observations (Figure 3C). Choice of hepatic CYP3A4 ontogeny function resulted in up to 35% difference in predicted Δ HR at the highest administered dose for paediatric Classes 1 and 2. No significant differences (<2%) were predicted for older children (Classes 3 and 4).

DDI risk assessment with ketoconazole

In the initial step, PK parameters derived from simulated ketoconazole concentrations in children were compared to reported PK parameters in the literature.^{39,40} Good agreement was seen despite the large inter-individual variability and a wide age range in the studies (simulated ketoconazole median $AUC_{0-6h}=27.0$ ng.h/mL and median $C_{max}=6.44$ µg/mL compared to observed AUC_{0-6h} range = [2.6-36.4] ng.h/mL and C_{max} range=[0.3-8.8] µg/mL for an oral dose of 5mg/kg³⁹).

Using Salem ontogeny function²¹, predicted effect of ketoconazole on parent and metabolite PK exposures ($AUC_{DDI}/AUC_{control}$ ratios) increased with age and was more pronounced in adolescents and adults (Figure 4A and 4C), in agreement with age-related changes in fm_{CYP3A4} (Table 1). Children between 0.5 and 1 year old had the lowest predicted ivabradine and metabolite $AUC_{DDI}/AUC_{control}$ ratios (mean±SD=3.53±1.13 and 0.69±0.54 for ivabradine and metabolite, respectively) compared to adults (7.03±3.07 and 1.46±1.08). However, DDI magnitude at the level of PD effect (assessed by $\Delta HR_{DDI}/\Delta HR_{control}$ ratios) was not significantly different between groups (Class 1 vs adults: mean±SD=1.70±0.59 vs. 1.79±0.87). However, inter-individual variabilities increased across age groups as shown by the larger boxplot lengths in adolescents and adults (Figure 4E).

With Upreti ontogeny function²², simulated ketoconazole effect on parent drug PK was not significantly different between children and adults (Figure 4B). Ivabradine $AUC_{DDI}/AUC_{control}$ ratios were comparable between Class 1 and adults (6.38±2.68 and 7.09±3.42, respectively); similar trends were seen across other age groups. In the case of metabolite, the highest mean increase in metabolite exposure was simulated for youngest children (from 0.5 to 3 years) (metabolite $AUC_{DDI}/AUC_{control}$ ratios=1.63±1.24 and 1.68±1.17 for Classes 1 and 2, respectively). Children beyond age of 3 years were predicted to be less sensitive to ketoconazole effect on the metabolite exposure (1.27±1.15 and 1.14±0.79 for Classes 3 and 4, respectively); large standard deviations were evident in all cases. With respect to PD effect, magnitude of DDI was overall comparable across five age classes (Figure 4F). $\Delta HR_{DDI}/\Delta HR_{control}$ ratios were slightly higher in children compared to adults (Class 1 vs adults: 2.02±0.80 vs. 1.82±1.05).

Discussion

This study illustrates the extrapolation of a joint ivabradine parent-metabolite PBPK-PD model from adults to children and its application for the investigation of DDI with a strong CYP3A4 inhibitor. In

addition, this study explored the impact of different hepatic CYP3A4 ontogeny functions on the predicted DDI magnitude with ketoconazole in children from different age groups, both from the PK and PD perspective. The extension to paediatrics was done by integrating age-related changes in relevant parameters in our previously published joint ivabradine-metabolite PBPK-PD model.⁵ To our knowledge, this analysis is the first example of the evaluation of different CYP3A4 hepatic ontogeny functions and the impact of the choice of maturation function on the predicted PK and PD DDI risk in children.

Critical evaluation of the literature identified 28 reported examples of paediatric PBPK modelling of CYP3A4 substrates with predominant applications in dose optimisation and/or study design refinement (Table 1). Prediction of the DDI in children using PBPK modelling was rarely explored although many challenges related to conduct of paediatric DDI studies still remain¹⁴. Previous studies⁴¹ have reported differences in the observed DDI magnitude between adults and children for a range of drugs and highlighted different sensitivity to DDI between these two populations, but also in paediatric subgroups (<1 year old). Lack of consistency in the selection of hepatic CYP3A4 ontogeny was evident, with majority of reported paediatric PBPK modelling examples applying the Salem function²¹. The age variations in CYP3A4 based on Salem et al. were in agreement with *in vitro* data from paediatric human liver microsomes (HLM) (Supplementary Table S4). However, some studies did not corroborate this relationship; e.g., voriconazole CL_{int} measured in paediatric HLM (2-10 years) was 2.5-fold higher than in adult HLM.³⁵ In the case of tramadol, the fraction of adult activity in HLM from 1-month children was reported to be higher than in microsomes from 3-month children.³⁷ Although these studies suggest higher hepatic CL_{int} in children compared to adults, these comparisons did not consider CYP3A4 expression differences between two populations. Additionally, paediatric HLM were often pooled samples from children with a large or narrow age range, which did not allow a more extensive comparison of CYP3A4 activity/abundance across age.

Existing examples of applications of CYP3A4 hepatic ontogeny functions resulted in differing model predictive performance (Table 1). In the case of mefloquine, midazolam and alfentanil, Upreti ontogeny improved model PK predictions in young children (0.5-2 years).⁴² In contrast, tacrolimus paediatric PBPK model demonstrated that Salem ontogeny adjusted for disease effect in children (1-16 years) in intensive care unit provided more consistent results.⁴³ In the case of imatinib PBPK

model (children >2 years), no significant differences were observed.⁴⁴ However, no studies reported systematic comparisons of these hepatic CYP3A4 ontogeny functions for the evaluation of DDI risk in children.

Exploration of paediatric PK modelling has been reported previously for ivabradine and its metabolite.^{25,45} However, these studies did not consider prediction of DDI magnitude in paediatrics and the population PK/PD model assumed the same relationship and drug potency between two populations (details in Supplementary Section 6). Here, scaling of ivabradine-metabolite PBPK-PD model from adults to children was carried out following a stepwise approach for paediatric PBPK modelling.^{16,46} The scaled PBPK-PD model using either of CYP3A4 hepatic ontogeny function adequately predicted ivabradine and metabolite PK and PD effect. The main differences in the predicted ivabradine and metabolite PK were evident for the youngest age groups (Classes 1 and 2), consistent with the higher CYP3A4 activity in very young children proposed by Upreti function relative to Salem function. Predicted ivabradine hepatic CL_{int} in the youngest children (Classes 1 and 2) were up to 3-fold higher with Upreti ontogeny function, hence lower ivabradine and metabolite exposure were predicted in these children (0.5-3 years). With respect to PD evaluation, the PBPK-PD model provided comparable overall results when using either Salem or Upreti ontogeny (Figure 3). Differences in predicted HR change in young children (0.5-3 years) between the two ontogeny functions were minor (Table 3), consistent with the low sensitivity of heart rate change to variations in ivabradine plasma PK observed in adults.⁵ Due to sparse PK and PD sampling, choice of the most appropriate hepatic CYP3A4 ontogeny function could not be conclusive for ivabradine.

The simulated DDI magnitude with ketoconazole accounted for CYP3A4 inhibition in the intestine and liver. Due to ivabradine high intestinal availability ($F_G > 0.8$), the predicted DDI magnitude with ketoconazole was mainly driven by the inhibition of hepatic CYP3A4. It also provided a good framework for investigation of the impact of different hepatic ontogeny functions on the predicted DDI magnitude. Depending on the implemented hepatic ontogeny function, young children (0.5-3 years) were predicted to have different sensitivity to CYP3A4 DDI. Using Salem ontogeny, this paediatric population was predicted to be less sensitive to concomitant administration of ketoconazole compared to adults (predicted mean parent $AUC_{DDI}/AUC_{control}$ ratio of 3.5 vs. 7.1 in Class 1 and adults, respectively). Similar results were obtained when predicting the metabolite PK and in agreement with

some reported examples of regulatory submissions with this ontogeny function (deflazacort-clatithromycin, Table 1).

In contrast to Salem function, use of Upreti hepatic CYP3A4 ontogeny resulted in similar predicted DDI magnitude for ivabradine PK between children and adults (predicted mean parent $AUC_{DDI}/AUC_{control}$ ratio of 6.4 vs. 7.1 in Class 1 and adults, respectively). Despite differing hepatic CYP3A4 expression in children between 0.1 and 11 years old, the sensitivity to CYP3A4 inhibition was very comparable. As the hepatic availability is a composite parameter of hepatic blood flow and hepatic clearance, age-dependency in hepatic blood flow could explain this outcome⁴⁷. In the case of metabolite, children in Classes 1 and 2 had slightly higher mean predicted metabolite $AUC_{DDI}/AUC_{control}$ compared to adults with Upreti function but large variability in those AUC ratios was evident. Despite differences in predicted PK between the two ontogeny functions, CYP3A4 age variations did not have a significant impact on the fold-change in ivabradine HR ($\Delta HR_{DDI}/\Delta HR_{control}$) between Class 1 children and adults (Figure 4E and 4F).

Depending on the age group, predicted ivabradine DDI with ketoconazole-equivalent inhibitor would be classified as moderate ($2 < AUC_{DDI}/AUC_{control} < 5$) to strong ($AUC_{DDI}/AUC_{control} > 5$) with Salem function, reflecting age-dependent predicted fm_{CYP3A4} ⁴⁸ (0.59 and 0.86 for Class 1 and adults, respectively). In contrast, predicted fm_{CYP3A4} based on Upreti ontogeny function was consistent across age groups and comparable to ivabradine adult fm_{CYP3A4} , resulting in predicted strong DDI in both children and adults. Hence, paediatric labelling would indicate a contraindication with strong CYP3A4 inhibitors regardless. Additional DDI simulations were performed for a range of adult fm_{CYP3A4} (0.2-1), assuming minimal intestinal interaction, as in the case of ivabradine (Supplementary Figure S9). For a victim drug with adult fm_{CYP3A4} of 20%, predicted mean parent $AUC_{DDI}/AUC_{control}$ for young children (Class 1) was 1.65 and 2.44 using Salem and Upreti ontogeny functions, respectively; in agreement with the lower predicted fm_{CYP3A4} in youngest children with Salem function (0.06) vs. Upreti function (0.14) (Supplementary Figure S9). Differences in predicted classification of DDI (weak vs. moderate) would result in different paediatric labelling recommendations⁴⁹, highlighting the importance of careful consideration of CYP3A4 maturation function in the DDI risk evaluation.

Conclusion

Joint ivabradine-metabolite PBPK-PD model was successfully scaled from adults to children (0.5 to 18 years) by integrating age differences in system- and drug-related parameters. This analysis highlighted the impact of hepatic CYP3A4 ontogeny on the evaluation of DDI risk in particular in youngest children (<1 year). Use of Upreti hepatic ontogeny function provided a worst-case scenario as the predicted ivabradine DDI with ketoconazole was comparable between youngest children and adults. In contrast, Salem function predicted a lower DDI magnitude in youngest children. In ivabradine case, the predicted DDI-related changes in the heart rate were not significantly different between children and adults. In the absence of clinical data in children, the Summary of Product Characteristics indicated a contraindication with strong CYP3A4 inhibitors based on adult DDI data. Given the current lack of agreement on hepatic CYP3A4 activity in children and potential impact on labelling recommendations, evaluation of both Salem and Upreti ontogenies in paediatric PBPK modelling of DDIs should be considered.

Study highlights:

What is the current knowledge on the topic?

Hepatic CYP3A4 increases from an early age to adult age, but there is a lack of consensus regarding the rate of CYP3A4 maturation.

What question did this study address?

What is the impact of selection of hepatic CYP3A4 ontogeny functions on PBPK-PD model predictions and evaluation of DDI risk in children?

What does this study add to our knowledge?

Based solely on comparisons of model predictions and ivabradine PK and PD clinical data, choice of the appropriate ontogeny function was challenging. Ivabradine PBPK-PD modelling predicted different sensitivities to CYP3A4 inhibition in very young children (<1 year) depending on the applied hepatic CYP3A4 ontogeny.

How might this change clinical pharmacology or translational science?

In the absence of a consensus in paediatric PBPK modelling, careful consideration of both hepatic CYP3A4 ontogeny function is recommended, together with implications on drug product labelling in children.

Author contributions

J.L., K.O., L.V., M.C. and A.G. wrote the manuscript; J.L., K.O. and A.G. designed the research; J.L. performed the research; JL analyzed the data.

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Table captions

Table 1. Drug-related parameters of the PBPK-PD model of ivabradine and metabolite for all age groups

Table 2. Evaluation of literature collated applications of paediatric PBPK modelling for CYP3A4 substrates

Table 3. Ivabradine-metabolite PBPK-PD model predictions using Salem ontogeny or Upreti ontogeny in four age classes

Figure captions

Figure 1. Schematic of ivabradine PBPK-PD model for adults and children (abbreviation definitions can be found in Methods section). Model equations and parameters are reported in Lang et al.⁵ and detailed in the Supplementary Sections 2-4.

Figure 2. Drug-related parameter scaling from adults to children between 6 months and 18 years. AAG: Alpha-acid glycoprotein (g/L); $f_{u,p_{ped}}$ and $f_{u,p_{ad}}$: paediatric and adult unbound fraction in plasma; f_{ub} : unbound fraction in blood; AS: active secretion; BW: body weight (kg); CL_r: renal clearance (L/h); Q_{renal} : renal blood flow (L/h); CL_{int,r}: renal intrinsic clearance (L/h); $MF_{hepCYP3A4}$: hepatic CYP3A4 maturation function; PMA: post-menstrual age (weeks); CL_{int,h_{ped}} and CL_{int,h_{ad}}: paediatric and adult hepatic intrinsic clearance (L/h); $MPPGL_{ped}$ and $MPPGL_{ad}$: paediatric and adult microsomal protein per gram of liver (33.3 mg/g); liver wt_{ped} and liver wt_{ad} : liver weight (g) in children and adult; $MF_{intCYP3A4}$: intestinal CYP3A4 maturation function; CL_{int,g_{ped}} and CL_{int,g_{ad}}: paediatric and adult intestinal intrinsic clearance (L/h).

Figure 3. Predicted ivabradine (A) and metabolite (B) concentration over time profiles and predicted heart rate (C) over time profiles. Thick solid line represents predicted median using Salem ontogeny and thin solid lines represent the 95% prediction interval using Salem ontogeny. Thick dotted line represents predicted median using Upreti ontogeny and thin dotted lines represent the 95% prediction interval using Upreti ontogeny. Triangle symbol represents individual ivabradine blood concentrations; square symbol represents individual metabolite blood concentration; circle symbol represents the individual heart rate measurements.

Figure 4. Predicted fm_{CYP3A4} using either Salem (A) or Upreti (B) hepatic CYP3A4 ontogeny function and simulations of drug-drug interaction risk with ketoconazole and ivabradine in children using the PBPK-PD model and Salem (C, E and G) or Upreti (D, F and H) hepatic CYP3A4 ontogeny. Class 1: 0.5-1 years, Class 2: 1-3 years, Class 3: 3-18 years (body weight < 40 kg), Class 4: 3-18 years (body weight > 40 kg); AUC: area-under-the curve (from time 0 to infinity); DDI: drug-drug interactions; ΔHR : maximal heart rate change from baseline. Each boxplot displays the minimum, first quartile, median, third quartile and maximum. Reported observed $AUC_{DDI}/AUC_{control}$ in adults was 6.7 and 1.6 for ivabradine and metabolite, respectively (Lang et al., 2020)⁵

Parameter	Definition	Unit	0.5-1 year	1-3 years	3-18 years (BW < 40kg)	3-18 years (BW > 40kg)	Adults > 18 years	Adult value reference	
IVABRADINE	fu,p	Unbound fraction in plasma	%	[40-42]	[39-40]	[37-39]	37	37	24
	Qgut	Hybrid parameter of blood flow and permeability	L/h	1.69	2.26	4.22	5.9	6.35	Calculated ¹
	Kper	Peripheral rate	L/h	0.84	1.12	2.07	3.39	4.16	Optimised ²
	Kspl	Splanchnic rate	L/h	15.2	17.9	12.4	20.3	25.2	31
	CLint,r	Renal intrinsic clearance	L/h	15.2	17.9	24	22.6	21	24
	CLint,h	Hepatic intrinsic clearance - Salem	L/h	22.3	47.5	98	152	171	Optimised ²
	fm _{CYP3A4}	Fraction metabolised - Salem		0.59	0.73	0.80	0.87	0.86	Calculated ³
	CLint,h	Hepatic intrinsic clearance - Upreti	L/h	60.7	84.4	114	151	171	Optimised ²
	fm _{CYP3A4}	Fraction metabolised - Upreti		0.80	0.83	0.83	0.87	0.86	Calculated ³
METABOLITE	fu,p	Unbound fraction in plasma	%	[28-29]	[26-28]	[25-26]	25	25	24
	Qgut	Hybrid parameter of blood flow and permeability	L/h	0.36	0.444	0.696	0.923	0.996	Calculated ¹
	Kper	Peripheral rate	L/h	0.81	1.08	2	3.27	4.01	Optimised ²
	Kspl	Splanchnic rate	L/h	3.07	4.1	7.45	12.3	15.2	31
	CLint,r	Renal intrinsic clearance	L/h	18.6	22	20.2	17.6	26.2	24
	CLint,h	Hepatic intrinsic clearance - Salem	L/h	18.7	39.8	80.7	125	145	Optimised ²
	fm _{CYP3A4}	Fraction metabolised - Salem		0.50	0.64	0.80	0.88	0.85	Calculated ³
	CLint,h	Hepatic intrinsic clearance - Upreti	L/h	50	70.8	93.5	127	145	Optimised ²
	fm _{CYP3A4}	Fraction metabolised - Salem		0.73	0.76	0.82	0.88	0.85	Calculated ³

¹calculated using apparent permeability and enterocytic blood flow (Supplementary Section 2)

²adult value was optimised within a Bayesian framework⁵

³calculated using $fm_{CYP3A4} = CL_{int,h} / (CL_{int,h} + CL_{int,r})$

Hepatic CYP3A4 ontogeny function		CYP3A4 substrates ¹	Included age range (yr) ²	Software ²	Main applications ²
<i>in vitro</i>-derived		Midazolam, voriconazole, sildenafil, clindamycin, vincristine	< 0.1 (1) 0.1-1 (2) 1- 3 (4) 3-18 (5)	Matlab (1) PK-sim (2) Simcyp (2)	- leverage knowledge gap in CYP3A4 maturation (4) - integrate <i>in vitro</i> CYP3A4 metabolism using HLM data specific to study drug (2) - compare predictive performances of population-PK and PBPK approaches (1)
<i>in vitro/in vivo</i>-derived	Johnson et al., 2006 ¹⁶	Quetiapine, docetaxel	1- 3 (1) 3-18 (2)	Simcyp (2)	- prediction of PK for a new formulation (1) - dose optimisation and optimal sampling design (1) - compare predictive performances of population-PK and PBPK approaches (1)
<i>in vivo</i>-derived	Edginton et al., 2006 ¹⁹	Etoposide, rivaroxaban	< 0.1 (1) 0.1-1 (3) 1- 3 (3) 3-18 (3)	PK-sim (3)	- evaluate PBPK model predictive performance (1) - compare predictive performances of population-PK and PBPK approaches (1) - investigate DDI risk in children (1) - guide dosing prior to paediatric phase I study (1)
	Salem et al., 2014 ²¹	Sirolimus, tramadol, clobazam, artemether, lumefantrine, ivabradine, deflazacort, mefloquine, itraconazole, ondansetron, sufentanil, tacrolimus, sunitinib, imatinib, entrectinib, cyclosporin A, chloroquine	< 0.1 (4) 0.1-1 (6) 1- 3 (11) 3-18 (15)	Matlab (1) Simcyp (14)	- leverage knowledge gap in CYP3A4 maturation (3) - evaluate PBPK model predictive performance (2) - compare predictive performances of population-PK and PBPK approaches (2) - compare Salem and Upreti ontogeny functions (3) - dosing and/or sampling optimisation (6) - inform drug submission file to regulatory authorities (2) - investigate DDI risk in children (4)
	Upreti and Wahlstrom, 2016 ²²	Mefloquine, itraconazole, ondansetron, tacrolimus, nilotinib	< 0.1 (1) 0.1-1 (2) 1- 3 (4) 3-18 (4)	Simcyp (4)	- evaluate PBPK model predictive performance (1) - leverage knowledge gap in CYP3A4 maturation (1) - compare Salem and Upreti ontogeny functions (3)
None		Midazolam, guanfacin	1-3 (1) 3-18 (2)	Nonmem (1) Simcyp (1)	- leverage knowledge gap in CYP3A4 maturation using a population-PBPK approach (1) - dose optimisation (1) - inform drug submission file to regulatory authorities (1) - investigate DDI risk in children (1)

¹Details of individual studies with corresponding references are provided in Supplementary Table S4

²Number in brackets indicates number of studies

		Class 1 - 0.5-1 year 0.05mg/kg Salem Upreti %diff ³			Class 2 - 1-3 years 0.0.5mg/kg Salem Upreti %diff ³			Class 3 - 3-18 years [BW<40kg] 0.05mg/kg Salem Upreti %diff ³			Class 4 - 3-18 years [BW>40kg] 2.5mg Salem Upreti %diff ³		
Ivabradine	AUC _{12h} ¹ (ng.h/mL)	27.2 [11.6-62.2]	12.3 [5.12-28.8]	54.8%	22.4 [9.27-52.4]	14.1 [5.69-34.5]	37.1%	26.5 [9.99-70.4]	23.4 [8.40-63.8]	11.7%	34.9 [13.8-86.1]	35.0 [13.7-89.3]	-0.3%
	C _{max,ss} ¹ (ng/mL)	11.7 [5.40-26.0]	6.18 [2.70-13.9]	47.2%	9.47 [4.20-21.7]	6.65 [2.81-15.2]	29.8%	8.06 [3.48-19.0]	7.30 [3.09-17.8]	9.4%	9.87 [4.28-22.9]	9.85 [4.26-23.0]	0.2%
Metabolite	AUC _{12h} ¹ (ng.h/mL)	7.65 [3.10-17.4]	4.85 [1.81-11.9]	36.6%	8.07 [3.27-19.0]	5.62 [2.18-13.9]	30.4%	10.1 [3.73-26.3]	9.25 [3.23-26.6]	8.4%	14.2 [5.35-34.9]	14.4 [5.23-36.4]	-1.4%
	C _{max,ss} ¹ (ng/mL)	2.10 [0.92-4.47]	1.81 [0.752-4.29]	13.8%	2.31 [1.02-4.88]	1.92 [0.833-4.23]	16.9%	1.99 [0.856-4.60]	1.90 [0.798-4.57]	4.5%	2.74 [1.13-6.29]	2.69 [1.15-6.40]	1.8%
Maximal ΔHR change ²		5.48 [4.72-6.37]	3.26 [2.99-3.77]	40.5%	5.26 [4.77-6.18]	3.58 [3.07-3.90]	31.9%	7.22 [5.94-8.52]	6.13 [5.01-7.19]	15.1%	4.87 [4.29-6.02]	4.98 [4.49-5.95]	2.3%

¹given as mean [5% prediction interval-95% prediction interval]; ²absolute relative heart rate change from baseline ³percentage difference between predictions using Salem and Upreti hepatic CYP3A4 ontogeny function

PBPK model

PD model

$$HR = Base \times \left[1 - E_{max} \times \left(\frac{\frac{Cheart_P}{EC_{50_P}} + \frac{Cheart_M}{EC_{50_M}}}{1 + \frac{Cheart_P}{EC_{50_P}} + \frac{Cheart_M}{EC_{50_M}}} \right) \right]$$

Fraction unbound in plasma

$$AAG = \frac{0.887 \cdot Age^{0.38}}{8.89^{0.38} + Age^{0.38}}$$

$$fu, p_{ped} = \frac{AAG_{ad} \cdot fu, pad}{1 + (1 - fu, pad) \cdot AAG}$$

Renal clearance (active tubular secretion)

$$AS = BW^{0.040} \cdot (1.14 - 0.465 \cdot e^{-0.185 \times Age})$$

$$CL_{int, r} = \frac{AS \cdot CL_r}{fu, b \cdot (1 - CL_r / Q_{renal})}$$

Hepatic clearance

$$\text{Salem ontogeny: } MF_{hep \text{ CYP3A4}} = \frac{PMA^{3.9}}{71^{3.9} + PMA^{3.9}}$$

$$\text{Upreti ontogeny: } Age < 2.5 \text{ yr: } MF_{hep \text{ CYP3A4}} = 0.05 + \frac{(1.7 - 0.05) \cdot Age^{1.3}}{0.1^{1.3} + Age^{1.3}}$$

$$Age > 2.5 \text{ yr: } MF_{hep \text{ CYP3A4}} = 0.7 + e^{-0.1 \cdot Age - 0.5}$$

$$CL_{int, h_{ped}} = \frac{CL_{int, h_{ad}} \cdot MPPGL_{ped} \cdot liver \ wt_{ped} \cdot MF_{hep \text{ CYP3A4}}}{Liver \ wt_{ad} \cdot MPPGL_{ad}}$$

Intestinal clearance

$$MF_{int, \text{CYP3A4}} = int \ ab_{\text{CYP3A4}} \cdot \left(0.42 + \frac{0.639 \cdot Age}{2.36 + Age} \right)$$

$$CL_{int, g_{ped}} = CL_{int, g_{ad}} \cdot MF_{int, \text{CYP3A4}}$$

Class 1
6 to 12 months

Class 2
1 to 3 years

Class 3
3 to 18 years (BW < 40kg)

Class 4
3 to 18 years (BW > 40kg)