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***Physiologically-Based Pharmacokinetic model for Ciprofloxacin in children with complicated Urinary Tract Infection***

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**ABSTRACT**

In a recent multicenter population pharmacokinetic study of ciprofloxacin administered to children suffering from complicated urinary tract infection (cUTI), the apparent volume of distribution ($V_d$) and total plasma clearance (CL) were decreased by 83.6% and 41.5% respectively, compared to healthy children. To understand these differences, a physiologically-based pharmacokinetic model (PBPK) for ciprofloxacin was developed for cUTI children. First, a PBPK model in adults was developed, modified incorporating age-dependent functions and evaluated with paediatric data generated from a published model in healthy children. Then, the model was then adapted to a cUTI paediatric population according to the degree of renal impairment (KF) affecting renal clearance ($CL_{Renal,}$) and CYP1A2 clearance ($CL_{CYP1A2}$). Serum and urine samples obtained from 22 cUTI children were used for model evaluation. Lastly, a parameter sensitivity analysis identified the most influential parameters on V and CL. The PBPK model predicted the ciprofloxacin exposure in adults and children, capturing age-related pharmacokinetic changes. Plasma concentrations and fraction excreted unchanged in urine ($f_e$) predictions improved in paediatric cUTI patients once $CL_{renal}$ and $CL_{CYP1A2}$ were corrected by KF. The presented PBPK model for ciprofloxacin demonstrates its adequacy to simulate different dosing scenarios to obtain PK predictions in a healthy population from 3 months old onwards. Model adaptation of $CL_{Renal}$ and $CL_{CYP1A2}$ according to KF explained partially the differences seen in the plasma drug concentrations and $f_e$ vs time profiles between healthy and cUTI children. Nevertheless, it is necessary to further investigate the disease-related changes in cUTI to improve model predictions.

**KEYWORDS:** PBPK; Ciprofloxacin; Children; Paediatric; complicated Urinary Tract Infection; cUTI; Renal Impairment

**List of abbreviations**

ADME: Absorption, Distribution, Metabolism and Excretion

BBD: bladder bowel dysfunction

BSA: body surface area

CAKUT: congenital anomalies of the kidneys and urinary tract

CF: Cystic Fibrosis

$CL_{Bil}$: biliary clearance

$CL_{CYP1A2}$: CYP1A2 mediated clearance

$CL_{Renal}$: renal clearance

cUTI: complicated urinary tract infection

CYP1A2: cytochrome P450 1A2

FDA: Food and Drug Administration

$f_e$: fraction excreted unchanged in the urine

$f_u$: fraction unbound in plasma in humans

GFR: glomerular filtration rate

IV: intravenous

KF: kidney function

Kp: tissue-to-plasma partition coefficient

logP: logarithm of the octanol-water partition coefficient of the neutral form (lipophilicity)

MW: Molecular Weight

PBPK: Physiologically-Based Pharmacokinetics

PK: pharmacokinetics

pKa acid: negative logarithm of the acid dissociation constant

pKb basic: negative logarithm of the basic dissociation constant

PO: oral administration

TS_$CL_{int}$: tubular secretion clearance

$V_d$: volume of distribution

## 1. INTRODUCTION

Complicated urinary tract infection (cUTI) is a disease occurring in a patient with a structural or functional abnormality of the genitourinary tract[1], and remains one of the most common causes of febrile illness in the paediatric population. At ages up to 8 years old, 2% of males and 8% of females suffer from at least one episode of UTI[2]. Children with congenital anomalies of the kidneys and urinary tract (CAKUT) are at major risk for complications of UTI such as sepsis, pseudohypoaldosteronism, renal scarring or reflux nephropathy, which could result in chronic kidney disease[3,4].

Ciprofloxacin is a second generation fluoroquinolone with a broad antibacterial spectrum, approved for treatment of cUTI in children[5]. Development of resistance of different uropathogens to fluoroquinolones, mainly due to their inappropriate use, represents a current and alarming unmet clinical need[6]. Given that ciprofloxacin's antibacterial effects are determined by the AUC over the MIC[7], it is fundamental to characterize its pharmacokinetics (PK) in the target populations to optimize drug exposure avoiding toxicity and minimizing resistance. Such characterization is not trivial to achieve in the case of paediatric patients suffering from certain diseases like cUTI, as shown below.

Ciprofloxacin undergoes glomerular filtration and tubular secretion ($TS\_CL_{int}$), which together, account for about 60% of the total clearance (CL) in adults. Non-renal CL involves CYP1A2 mediated metabolism ($CL_{CYP1A2}$) and biliary excretion ($CL_{Bil}$). Ciprofloxacin´s oral bioavailability is approximately 70% and its binding to albumin ranges between 20% to 40%[5]. Ciprofloxacin´s PK have been characterized for a cystic fibrosis (CF) adult population using a two compartment model with a total apparent volume of distribution ($V_d$) of 1.1 L/kg and a total clearance (CL) of 0.34 L/h/kg[8]. Age, body weight, serum creatinine concentration and CF status have been identified as covariates in different previous population analyses in the paediatric population[9,10].

Recently, Meesters et al.[11] have developed a population pharmacokinetic model of ciprofloxacin in children with cUTI finding that $V_d$ and total plasma CL were 83.6% and 41.5% reduced, respectively, with respect to healthy children and paediatric patients with CF[9,10]. These findings are in accordance with previous observations, indicating that renal impairment affects drug disposition beyond the impact on renal clearance[12].

Moreover, those results highlight the difficulty to establish an optimal dose and predict individual patients' dosing as the label specifies that paediatric patients with moderate to severe renal insufficiency were excluded from the clinical trial of cUTI and pyelonephritis[5]. In this context, physiologically-based pharmacokinetic (PBPK) modelling appears best suited to identify and potentially predict the impact of physiopathological alterations occurring in cUTI on the PK of ciprofloxacin in children, particularly in young infants, as PBPK models incorporate growth changes and maturation in processes that are known to alter drug disposition[13], together with disease related factors. The current evaluation appears timely as the interest in PBPK modelling is increasing in general, and also in the particular case of ciprofloxacin, where a PBPK model covering the full human life span has been recently published for healthy subjects [14].

PBPK models are regularly being applied to describe a compound's behaviour in otherwise healthy children, but their use in paediatric disease states affecting one or more of the eliminating organs is less well documented. Based on the above considerations, the goal of the present study was to develop a PBPK model in the paediatric population with cUTI, and identify physiological aspects that may be responsible for altered PK in children with cUTI as compared to a healthy population.

## 2. METHODS

### 2.1. Description of the PBPK model development workflow, clinical data and software used

In this work, an FDA guidance-based workflow for PBPK model development in children was used[15]. In a first step, a PBPK model for ciprofloxacin in the adult population was developed and challenged to predict systemic concentrations after intravenous (IV) and oral (PO) administration. Then, the established PBPK model in adults was extrapolated, incorporating age-dependent physiological and anatomical changes to generate plasma ciprofloxacin concentrations for IV and PO administration in children. Finally, the PBPK model developed in healthy children was adapted to the paediatric population suffering from cUTI according to the patient´s individually determined kidney function (KF). Lastly, a parameter sensitivity analysis was performed to identify the most influential parameters on V and CL.

Fig. 1 shows the workflow used in this work specifying each step of the model development, the type of data and the corresponding source used for model building. Briefly, mean ciprofloxacin plasma concentration versus time profiles in adults after IV or PO administration were obtained from literature[16–21]. The population PK model developed by Rajagopalan and Gastonguay[9] for ciprofloxacin in children with normal kidney function or mild renal impairment, was used to simulate typical ciprofloxacin PK profiles in plasma of healthy children. Raw data obtained from a clinical study performed in paediatric patients with cUTI were used to adapt the PBPK model established for that particular paediatric population. Supplementary Table S1 and Table 1 summarize the design characteristics of those studies performed in adults and children.

**Fig1.** Model building steps, type of data and the corresponding source. *IV* intravenous, *PO* oral.

The software WebPlotDigitizer® v.3.8 was used to extract the concentration versus time data from the adult literature studies. The software used for the PBPK model building was PK-Sim® v.7.2.1 (http://open-systems-pharmacology.org). NONMEM® v.7.4 (Icon Development Solutions, Ellicott City, MD, USA) was used to perform the simulations of the PK profiles in plasma using the model and parameters publicly available[9]. R® v.3.3.2 and RStudio® v.1.0.136 were used for the graphical representation and preparation of the data sets.

### 2.2. Adult PBPK model development and evaluation

The standard whole human body for PBPK modelling in PK-Sim® consists of 18 compartments, representing relevant organs and tissues of the body connected with arterial and venous blood flows to allow inter-compartmental mass transport. Each compartment is additionally subdivided into four sub-compartmental structures (plasma, red blood cells,

interstitial space and intracellular space). Supplementary Fig. S1 shows a schematic representation of the default PBPK model implemented in PK-Sim®[22]. Drug-related information must be provided to characterize the absorption, distribution and elimination processes. Physicochemical properties were gathered from the literature (Table 2), and clearance inputs were estimated as described below. Tissue-to-plasma partition coefficients (Kp) were predicted using the equations proposed by Rodgers et al. [23]. The physiological model parameters implemented in PK-Sim® v.7.2.1 are described in the software manual and they can be found by exporting a simulation from PK-Sim® to MOBI® and afterwards, exporting to MATLAB®[22].

Ciprofloxacin undergoes both renal and non-renal elimination. Renal clearance ($CL_{Renal}$) exceeds the value of the product between $f_u$ and the glomerular filtration rate (GFR; 125 mL/min/1.73 $m^2$) indicating a contribution of active tubular secretion[5]. Because the specific transporter(s) involved in the tubular secretion of ciprofloxacin are not well defined[27,28], and to ensure full characterization of the renal elimination, an efflux transporter was considered at the apical side of the kidney with a contribution to $CL_{Renal}$, in addition to glomerular filtration, represented by $TS\_CL_{int}$ with a value that was estimated as explained below. CYP1A2 mediated metabolism[5] and biliary excretion were represented by $CL_{CYP1A2}$ and $CL_{Bil}$, respectively. Taking into account that (i) approximately 15% of an IV dose is recovered unchanged in faeces within 5 days after dosing[5], (ii) biliary secretion is not age dependent in the range from 3 months to 18 years[28] and (iii) ciprofloxacin possesses a total plasma CL of 8.34 mL/min/kg for a healthy 70 kg adult[16,17,21], the value of $CL_{Bil}$ used in our analysis was assumed to be 1.25 ml/min/kg.

Mean plasma concentration and fraction of the administered dose excreted unchanged in urine ($f_e$) profiles were used as raw data following IV administration[16,17,21] to estimate the parameters $TS\_CL_{int}$, and $CL_{CYP1A2}$ assuming linear PK[5] and average demographic characteristics (supplementary material Table S1) using a Monte Carlo estimation method.

Once disposition was characterised, mean data obtained after PO administration[16–20] were described, using information regarding the type of pharmaceutical formulation, and assuming that the full dose administered is released from the formulation. The tablet particle dissolution was quantified by a Weibull function with no lag time, a shape factor of 0.80 and 4 hours for the 50 % dissolution time[25]. Subsequently, transcellular specific intestinal permeability was optimized.

### 2.3. Paediatric PBPK model development in healthy children

Ciprofloxacin physicochemical properties, absorption, distribution, metabolism and excretion (ADME) parameters were retained in the paediatric model as determined in the adult model. Age-dependent algorithms implemented in PK-Sim® were used to generate anatomical and physiological parameters, along with height, body water, body weight, organ weights, blood flows, cardiac output and lipid and protein concentrations. Children´s ciprofloxacin $f_u$ was estimated using the value of $f_u$ in adults, and the default albumin ontogeny function based on the McNamara and Alcorn algorithm[30].

With respect to elimination, GFR and $CL_{Bil}$ were adjusted using age-dependent functions proposed by Rhodin et al. [31] and Johnson et al. [29] respectively, as implemented in PK-Sim®. The ontogeny model published by Hayton[31] developed to describe the excretion of p-aminohippuric acid was applied for TS_$CL_{int}$ in the current exercise, whereas for the age-dependent CYP1A2 activity function, the one proposed by Edginton et al. [33] was considered.

Typical pharmacokinetic profiles for 3, 6, and 18 months, 4, 8, and 12 years old male children were then generated assuming a single administration of 9 mg/kg as a 60 min IV infusion, or a 15 mg/kg tablet[9], and challenged against the typical predictions obtained from the population PK model reported by Rajagopalan and Gastonguay[9] for subjects without cystic fibrosis. Typical pharmacokinetic profiles were obtained selecting the individual´s weight according to the corresponding age value obtained from the ICRP European population[34].

### 2.4. PBPK model for children with cUTI

The clinical characteristics of the cUTI clinical trial population [11] are summarized in Table 1.

Renal impairment is a potential major consequence of cUTI[4]. The ontogeny model implemented in PK-Sim® uses the following physiological variables to predict GFR as suggested by Rhodin et al[31]: postmenstrual age, body weight, height, adult GFR and gender. However, the model does not incorporate a component accounting for renal insufficiency.

In order to overcome that limitation, we first calculated individual patient GFR using the approach suggested by Chehade[35] and represented in the following expression, since serum creatinine (mg/dL), and cystatin C (mg/L) values were available from the patient population, as well as gender, height (cm) and post menstrual age (years):

$$GFR = 0.42 \times \frac{Height}{Serum\ Creatinine} - 4 \times 10^{-4} \times \left(\frac{Height}{Serum\ Creatinine}\right)^{2} \times Cystatin\ C + 0.69 \times Age + \begin{cases} 21.88, Males \\ 18.52, Females \end{cases}$$

Subsequently, the degree of renal impairment (KF) was calculated for each patient as the ratio of the above calculation and the ontogeny GFR value (after normalizing it by BSA) using the Rhodin et al.[31] approach, and lastly, renal clearance (including filtration and tubular secretion) was normalized by KF.

It has been widely demonstrated that not only renal elimination is altered in patients with impaired KF, also hepatic drug metabolism can be affected, either by inducing or suppressing expression of hepatic enzymes[12]. Rowland et al. reported a CYP1A2 activity of 63 and 46% compared to healthy in the case of chronic kidney disease of stage 3 or 4-5, respectively[12]. Accordingly, $CL_{CYP1A2}$ was reduced by those fractions in patients with KF values between 0.33-0.65 or lower than 0.33 of the normal (KF= 1), respectively.

With respect to the change in plasma protein concentrations occurring as a consequence of chronic kidney disease, given the low degree of binding of ciprofloxacin ($f_u$ ranges between 20 and 40%), its impact on plasma protein binding was considered to be negligible (as shown in supplementary material Fig. S3).

### 2.5. Model evaluation

For all models developed and described in sections 2.2-2.4, the PBPK based generated profiles either in plasma or in urine were displayed together with either mean published profiles, typical population PK model simulations, or individual observed profiles. In addition, PBPK based simulated data were plotted against challenged data for predictive performance analysis, considering them acceptable when they were within a two-fold prediction error. The mean absolute performance error was computed for each individual from section 2.4 as:

$$PE\% = \frac{100}{n}\sum^{n}\left|\frac{Pred - Obs}{Obs}\right|$$

Where n is the number of points while Pred and Obs refer to the model predicted and observed ciprofloxacin plasma concentration or $f_e$. As can be seen, observations are very well captured by the model for most of the best and the good fitted individuals.

### 2.6. Parameter sensitivity analysis

To identify physiological aspects that may be responsible for altered PK in children with cUTI as compared to a healthy population, a local sensitivity analysis was performed to assess the influence of every model parameter on the CL and V, except for the parameters related to the drug properties. For this analysis in PK-Sim®, every input parameter ($P_i$) was varied around the value in the simulation by 10% and a new simulation was performed keeping all other input values constant. The change in the PK parameter estimate ($\Delta PK_j$) was calculated as the difference between the values in the new simulation versus the original simulation. The sensitivity of the PK parameter to the input parameter was calculated as the ratio of the relative change of that PK parameter ($\Delta PK_j/PK_j$) and the relative variation of the input parameter ($\Delta P_i/P_i$) with the equation: $(\Delta PK_j/\Delta P_i)*(P_i/PK_j)$[22].

## 3. Results

### 3.1. Adult PBPK Model

Data from several studies following IV or PO administration, conferring a total of 11 administration protocols, were extracted from the literature [16–21] (supplementary material Table S1). The final parameter estimates using the aforementioned data and the drug properties from Table 2, are listed in Table 3. As shown in Fig. 2, simulations according to the PBPK model well represented the typical observed profiles reported in the studies for different types of drug exposure (total plasma concentration, fraction excreted unchanged in the urine, and saliva concentration) and for a variety of scenarios including (i) intravenous and oral administration, (ii) single and multiple dosing, (iii) different dosage regimens, and (iv) dose levels ranging from 200 mg to 750 mg. A slight over- or underprediction at lower concentrations can be observed, which may be due to the between-study variability. Supplementary Fig. S2A shows that for all scenarios, the observed vs. model simulated concentration pairs fall within the two-fold limits of acceptance, in 100% of the cases for IV and 93.1% for PO.

**Fig2.** Mean observed (dots) and simulated (lines) ciprofloxacin concentrations and $f_e$ after simulating

different administration protocols from the literature[16–21]. Supplementary Table S1 summarizes the design characteristics of those studies. Blue, orange and yellow represent ciprofloxacin plasma concentration, saliva concentration and $f_e$, respectively. *IV* intravenous, *PO* oral, $f_e$ fraction excreted unchanged in the urine.

### 3.2. Paediatric PBPK Model

Generally, the scaling approach gave adequate results. The profiles represented in Fig. 3 indicate that the total plasma ciprofloxacin concentrations predicted by the PBPK model are in close agreement with the simulations obtained from the published population PK model[9] for each of the five age groups explored. A moderate underprediction of IV simulations and a modest overprediction of PO can be seen for younger children at lower concentrations. In supplementary Fig. S2B it can be seen that 96.9% of the population PK simulated data vs. PK-Sim® simulated concentrations fall within the two-fold limits of acceptance for the IV administration and 95.1% for PO.

**Fig3:** Results of the comparison of PK-SIM® predicted (lines) vs. individual NONMEM simulated profiles in children (symbols) after IV and PO ciprofloxacin administration for the six representative paediatric ages. Simulations represent a single administration of 9 mg/kg as a 60 min IV infusion or 15 mg/kg tablet PO.

The implemented ontogeny functions developed in healthy children for GFR[31] and $CL_{Bil}$[29], and the functions incorporated for active secretion[32] and CYP1A2 activity[33] shown in Fig. 4 are appropriate to predict the observations for term children from 3 months to 18 years old (postnatal age).

**Fig4.** Ontogeny functions implemented in the PK-Sim® software (Rhodin et al.[31] for GFR and McNamara and Alcorn algorithm[30] for albumin) or incorporated based on the current analysis (Hayton[32] for active renal tubular secretion and Edginton et al.[33] for CYP1A2 activity). Light blue shadow covers the post menstrual age from the cUTI trial children.

### 3.3. PBPK Model in children with complicated UTI

In Fig. 5, the PBPK predicted concentration-time profiles are shown for six patients receiving ciprofloxacin either IV or PO corresponding to the best, good and worst predictions, based on the mean absolute performance error. Only data related to the dosing interval when sampling took place are represented. The full profile can be seen in Fig. S4 of the supplementary material.

**Fig5.** Individual model based simulated (lines) and observed plasma concentrations or $f_e$ (solid circles) vs time profiles for the best, good, and worse predicted patients. Blue coloured data points and lines represent plasma concentrations while the yellow colour represents urine data. The mean absolute performance error obtained for each selected patient for plasma concentration and $f_e$, respectively, was: ID17: 65%,19%; ID14: 76%,8%; ID19: 146%, 9%; ID4: 21%,12%; ID11: 16%, 46%; ID3: 214.6%, 3%. $f_e$ fraction excreted unchanged in the urine, *KF* kidney function.

Supplementary Fig. S2C shows the PBPK model based predicted vs observed ciprofloxacin plasma concentration and $f_e$ after IV or PO administration in cUTI paediatric individuals. In the case of PO administration, the percentage of predictions located within the two-fold limits of acceptance increased from 63% to 75% (plasma), and from 52% up to 72% (urine) when

changes in kidney function were considered. On the contrary, differences in those percentages were found to be negligible after IV administration. Remarkably, in the current studied population, the renal function showed a mean reduction of 16 % with respect to normal, ranging from 0.07 to 1. For IV administration, only 30% of subjects presented model predicted reduction in KF, whereas in the case of patients receiving ciprofloxacin orally, that percentage was increased up to 92%.

### 3.4. Sensitivity analysis

The results from the sensitivity analysis (Supplementary Table S2) revealed that $V_d$, for both healthy (KF=1) and renal impaired (KF=0.5), was greatly affected by the intracellular and blood cells pH. Additionally, $f_u$ as well as plasma and interstitial pH, together with kidney and muscle volume showed a ≥ 2.5% increase or decrease in $V_d$ when the parameters were increased by 10%. For CL, the contribution of the $f_u$, kidney and liver volume, tubular and biliary secretion were the most influential parameters both healthy (KF=1) and really impaired (KF=0.5). All other tested parameters did not result in a ≥ 2.5% increase or decrease in $V_d$ or CL when the parameter was increased 10% from the corresponding default value.

Renal function related patterns were observed for the impact of pH (intracellular and blood) and kidney volume in $V_d$, and for liver and kidney volumes impact in CL.

## 4. Discussion

Dose selection and optimization represents a challenge, especially in the paediatric population and even more so in diseased children. Under the premise that drug exposure is one of the main factors responsible for treatment success, understanding drug disposition is mandatory. Whereas top-down approaches (i.e., compartment-based models) have been shown useful where minor extrapolation is required, mechanistic, bottom-up or middle-out approaches are warranted to predict drug levels in the context of major changes in body physiology, with paediatric PBPK being the recommended paradigm.

In the current analysis, the PBPK approach has been used to mechanistically describe the systemic and urinary ciprofloxacin exposure in children suffering from cUTI, a disease causing serious alterations in processes involved in its PK. In fact, a recent pharmacokinetic study of ciprofloxacin administered to cUTI children showed that the apparent $V_d$ and the total plasma CL were decreased compared to healthy children (up to a 83.6% and 41.5%, respectively), presenting a degree of inter-individual variability not explained by any of the patient's factors ranging from 60 to 160% [11]. The lack of literature examples extrapolating from healthy subjects to the patient population, thus beyond ontogeny, and towards the population of interest, exemplify the novel contribution of the current work within the extensive literature on both PBPK and ciprofloxacin[9,14,16–21,36].

Data availability in the current project was limited to serum ciprofloxacin concentrations and amounts excreted in urine, as well as the patient characteristics and drug properties listed in Tables 1 and 2, respectively, i.e. not enough to develop a stand-alone model and promoting the use of validated PBPK platforms such as PK-Sim®. The model building approach focused first on model assumptions accounting for permeability, tissue partition coefficients[23] and

contributions of different elimination pathways. The agreement between simulated profiles and published data in healthy adults indicate model robustness, given the different dosing scenarios considered. Second, ontogenic relationships corresponding to renal filtration and active secretion, as well as CYP1A2 activity[31–33] were used to generate profiles in children, which were compared with those obtained based on a previously published population pharmacokinetic model[9], showing good agreement. Remarkably, the ontogeny function developed for $CL_{CYP1A2}$, based on probe substrates methoxyresorufin, caffeine and ropivacaine[33], performed better than the standard ontogeny model[22]. With respect to renal clearance mediated by secretion, its degree of maturation was adopted from the model developed for aminohippuric acid[32], which was shown to be appropriate for ciprofloxacin. Our model is simpler in its structure, especially regarding the absorption process, than the recent model covering the full life span[14], with adequate model performance as can be seen in Figs. 2 and 3. Indeed, more than 95% of the observed vs. simulated concentration pairs fall within the two-fold limits of acceptance for both healthy adults and children. Therefore, the model can be considered appropriate to be used in such population.

The third step was to adapt the PBPK model developed to the physio-pathological condition represented by cUTI. Markers of renal function available for each child comprised serum creatinine and cystatin C levels. Renal insufficiency in children was assumed to be reflected by the fractional change in the glomerular filtration rate between patients and healthy subjects combining the two markers and demographics as suggested by Chehade et al.[35]. It is known that chronic renal disease can alter drug disposition beyond renal excretion, for example decreasing enzyme expression/activity as it has been reported in the past[37]. In the current model, metabolic clearance in children with cUTI was reduced accordingly. Impact of chronic renal disease on plasma protein levels has also been reported[38]. Given the low degree of binding for the case of ciprofloxacin (between 20-40%), it is however unlikely to have a relevant effect on its unbound fraction in plasma. The aforementioned approach agrees with previous PBPK works describing PK profiles in patients with altered renal function in which scaling factors for different parameters and conditions allowed an accurate PK description of different compounds in a renally impaired population[39].

The physiologically-based model described the time-profiles of both plasma and urine after IV and PO administration reasonably well, even though a slight model misspecification, especially after IV administration, can be observed. It should be taken into account that the only adjustment performed was based on creatinine or cystatin C. In fact, this approach allowed a better prediction, yet there are a number of drawbacks. For example, the KF estimation method is generally associated with GFR assuming that tubular secretion is proportionally related to GFR, which might not represent the real scenario. This approach does not take other alterations in kidney physiology into account, i.e. 52% of the children from the cUTI trial had an estimated KF higher than 90% of the healthy function, suggesting other non-GFR-related kidney problems. Indeed, most of the individuals were suffering from CAKUT involving vesicoureteral reflux and/or bladder bowel dysfunction together with a UTI bacterial infection accompanied by acute pyelonephritis (Table 1).

The sensitivity analysis was conducted to identify model uncertainties and to identify how kidney impairment affects systemic exposure. It was observed that the impact of pH

(intracellular plasma and blood) and $f_u$ greatly affected $V_d$. Interestingly, Hinderling and Hartmann[40] studied the pH dependence of drug binding to plasma proteins in human and they observed that $f_u$ varied along with the pH value depending on the drug. Metabolic acidosis is a common complication of chronic kidney disease, which might play a relevant role in the PK of the cUTI population. Therefore, it would be very interesting to further study the internal pH state in cUTI children and the extent of pH-induced changes in the $f_u$ of ciprofloxacin under acidemia or alkalemia conditions.

It should not be ignored that ciprofloxacin appears to be a substrate of OAT3 and P-glycoprotein (P-gp) transporters. Both transporters are expressed in the proximal tubule of the kidney. OAT3 allows its substrates to be up taken from blood, and P-gp to be cleared from the proximal tubules to urine [27,28]. Furthermore, P-gp is also expressed in brain and in bile canaliculi. Alterations in the expression of those transporters or their functionality due to their binding to uremic toxins and inflammatory proteins in different tissues under renal disease conditions have been reported [12]. Those alterations may explain a decrease in volume of distribution and/or a decreased clearance, opening a research question in the case of ciprofloxacin.

## 5. Conclusion

In conclusion, the presented PBPK model for ciprofloxacin has demonstrated to be adequate to simulate different dosing scenarios and to obtain accurate PK predictions in a healthy population from 3 months old onwards. Model adaptation of GFR, $TS\_CL_{int}$ and $CL_{CYP1A2}$ according to KF partially explained the differences seen in the plasma drug concentrations and $f_e$ vs. time profiles between healthy and cUTI children. Nevertheless, it is necessary to further investigate the disease-related changes in cUTI due to the known large heterogeneity in patients suffering from CAKUT[11]. This study provides not only an evaluated PBPK model of ciprofloxacin for healthy children, but also essential key parameters to suggest hypotheses for further fine-tuning of the PBPK model in a cUTI population.

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**Competing interests:** An Vermeulen is also an employee of Janssen R&D (80%) and holds stock/stock options of J&J.

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## Tables

**Table 1.** Clinical characteristics summary of the children from the cUTI clinical trial[11] separated by administration type. Table adapted from [11].

| | **IV** | **PO** |
|---|---|---|
| Number of participants | 10 | 13 |
| Proportion females | 8 (80%) | 6 (46.2%) |
| Dose | 30-60 minutes infusion of 10-15 mg/kg every 12 hours | 15-20 mg/kg suspension or tablet (maximum 750 mg) |
| Diagnosis | Acute pyelonephritis: 9<br>Cystitis with oral intolerance: 1 | Acute pyelonephritis: 9<br>Cystitis: 2<br>Recurrent lower UTI: 2 |
| Comorbidities | CAKUT: 1<br>CAKUT, heart failure and vesicoureteral reflux: 1<br>CAKUT and stone disease: 1<br>BBD: 2<br>Cerebral palsy: 2<br>None: 3 | CAKUT: 7<br>CAKUT and chronic renal failure: 1<br>CAKUT and BBD: 1<br>CAKUT and stone disease: 1<br>Neurogenic bladder: 1<br>BBD: 1<br>Stone disease: 1<br>None: 1 |
| Age in years (median, range) | 9.86 (0.51 – 15.5) | 6.43 (0.31 – 15.4) |
| Serum cystatin C (mg/L) (median, range) | 0.71 (0.63 - 0.84) | 0.86 (0.61 – 2.88) |
| Serum creatinine (mg/dL) (median, range) | 0.49 (0.28 - 0.81) | 0.64 (0.38 – 1.54) |

**CAKUT**: Congenital anomalies of the kidneys and urinary tract, **BBD:** Bladder bowel dysfunction. 22 participants in total (1 patient received both formulations).

**Table 2.** Ciprofloxacin physicochemical properties.

| Parameter | Value | Source |
|---|---|---|
| MW (g/mol) | 331 | [24] |
| MW* (g/mol) | 314 | [25] |
| logP | 1.63 | [26] |
| pKa acidic | 6.10 | [24] |
| pKa basic | 8.60 | [24] |
| Solubility at pH7 (mg/ml) | 6.18 | [25] |
| $f_u$ | 0.67 | [25] |

**MW*:** Effective Molecular Weight (ciprofloxacin contains a fluorine atom, which leads to a reduction of the effective Molecular Weight), **logP:** logarithm of the octanol-water partition coefficient of the neutral form (lipophilicity), **pKa acid:** negative logarithm of the acid dissociation constant, **pKa basic:** negative logarithm of the basic dissociation constant, **$f_u$:** fraction unbound.

**Table 3:** Ciprofloxacin specific intestinal permeability and elimination parameters

| Parameter | Value | Source |
|---|---|---|
| Specific Intestinal permeability (transcellular) (cm/min) | $3x10^{-6}$ | Estimated from the plasma drug concentration |
| $CL_{CYP1A2}$ (ml/min) | 20.61 | Estimated from the plasma drug concentration (IV and PO) [16–21] |
| GFR (ml/min/g of organ) | 0.266 | [5,25] |
| $TS\_CL_{int}$ (L/min/kg tissue) | 1.32 | Estimated from the plasma drug concentration (IV and PO) [16–21] |
| $CL_{Bil}$ (ml/min/kg) | 1.25 | [5] |

**$CL_{CYP1A2}$:** cytochrome P450 1A2 mediated clearance, **GFR:** glomerular filtration rate, **$TS\_CL_{int}$:** tubular secretion clearance, **$CL_{Bil}$:** biliary clearance.

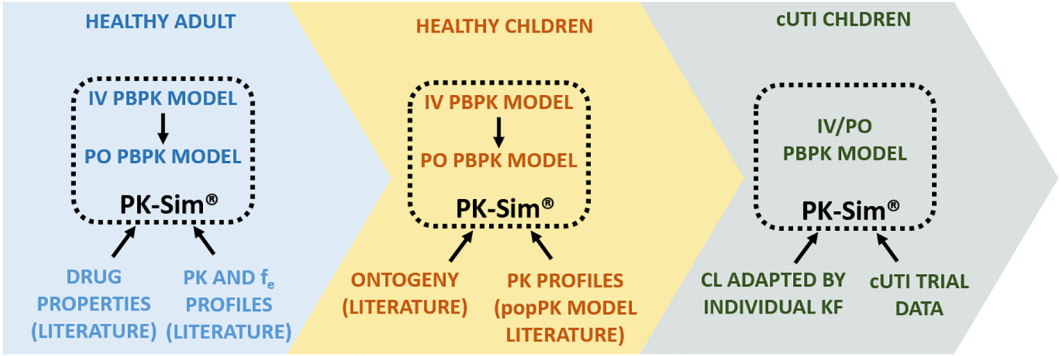
HEALTHY ADULT
IV PBPK MODEL
PO PBPK MODEL
PK-Sim®
DRUG PROPERTIES (LITERATURE)
PK AND $f_e$ PROFILES (LITERATURE)
HEALTHY CHLDREN
IV PBPK MODEL
PO PBPK MODEL
PK-Sim®
ONTOGENY (LITERATURE)
PK PROFILES (popPK MODEL LITERATURE)
cUTI CHLDREN
IV/PO PBPK MODEL
PK-Sim®
CL ADAPTED BY INDIVIDUAL KF
cUTI TRIAL DATA

Figure 1

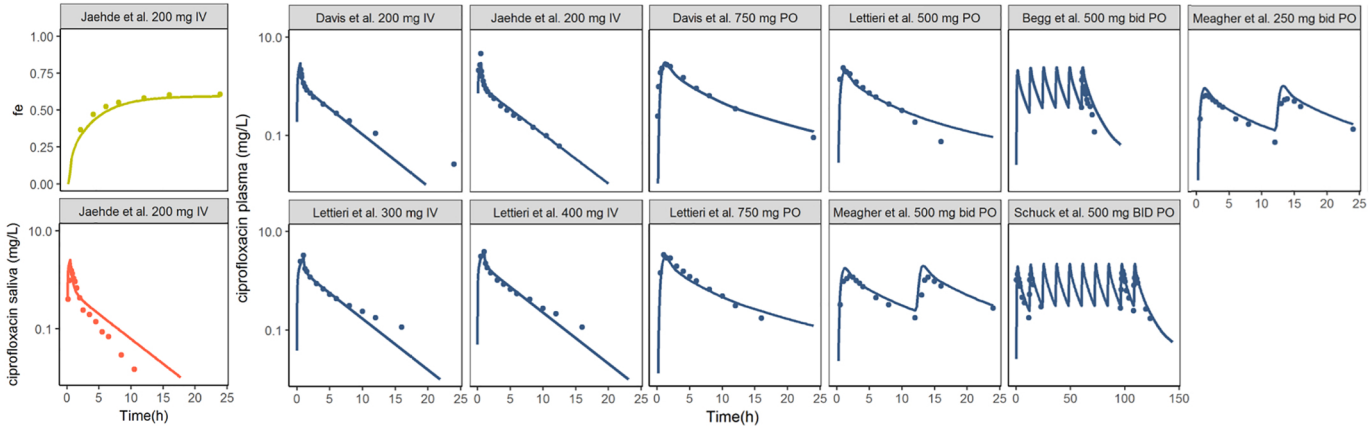
Jaehde et al. 200 mg IV
fe
1.00
0.75
0.50
0.25
0.00
Jaehde et al. 200 mg IV
ciprofloxacin saliva (mg/L)
10.0
0.1
0
5
10
15
20
25
Time(h)
Davis et al. 200 mg IV
Jaehde et al. 200 mg IV
Davis et al. 750 mg PO
Lettieri et al. 500 mg PO
Begg et al. 500 mg bid PO
Meagher et al. 250 mg bid PO
Lettieri et al. 300 mg IV
Lettieri et al. 400 mg IV
Lettieri et al. 750 mg PO
Meagher et al. 500 mg bid PO
Schuck et al. 500 mg BID PO
ciprofloxacin plasma (mg/L)
10.0
0.1
0
5
10
15
20
25
50
100
150
Time(h)

Figure 2

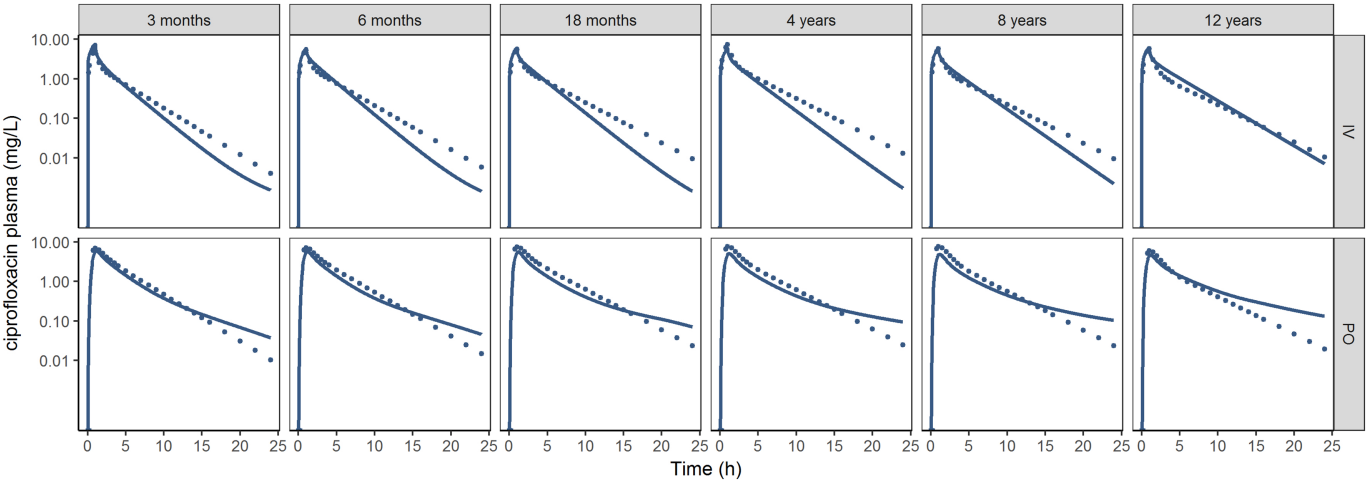
3 months
6 months
18 months
4 years
8 years
12 years
IV
PO
ciprofloxacin plasma (mg/L)
10.00
1.00
0.10
0.01
0
5
10
15
20
25
Time (h)

Figure 3

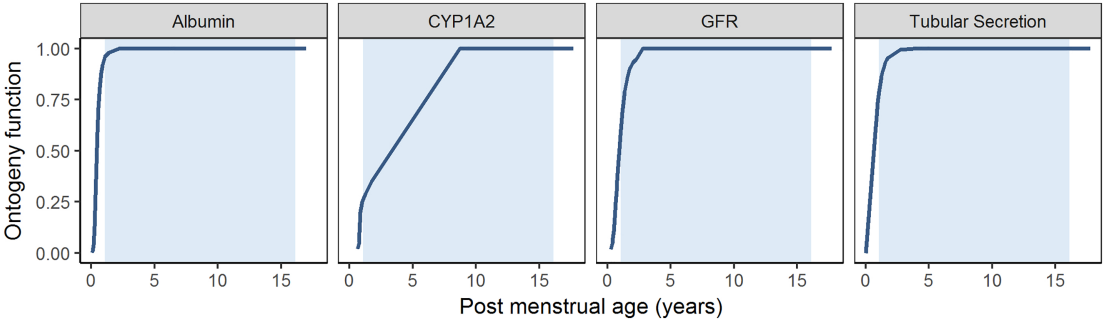
Albumin
CYP1A2
GFR
Tubular Secretion
Ontogeny function
1.00
0.75
0.50
0.25
0.00
0
5
10
15
Post menstrual age (years)

Figure 4

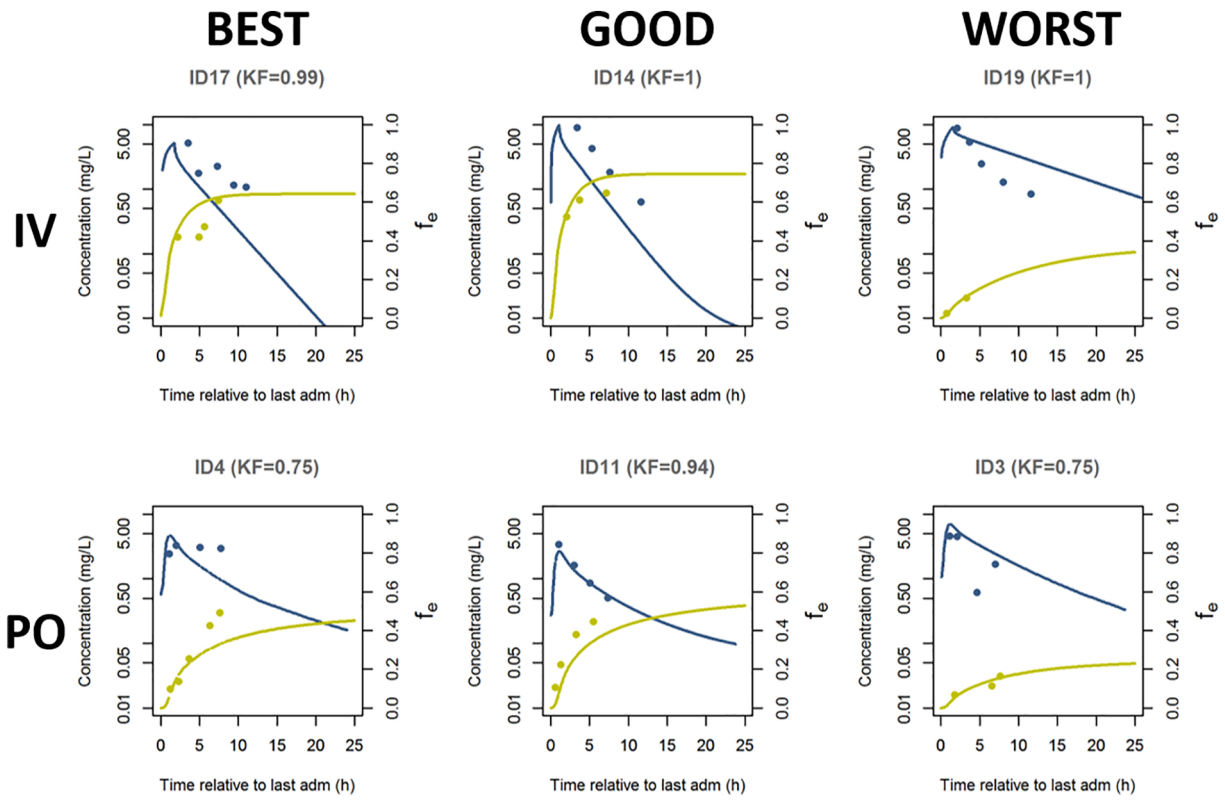
BEST
GOOD
WORST
IV
PO
ID17 (KF=0.99)
ID14 (KF=1)
ID19 (KF=1)
ID4 (KF=0.75)
ID11 (KF=0.94)
ID3 (KF=0.75)
Concentration (mg/L)
$f_e$
Time relative to last adm (h)

Figure 5