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Development and verification of an endogenous PBPK model to inform hydrocortisone replacement dosing in children and adults with cortisol deficiency

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A B S T R A C T

The goal of hormone replacement is to mirror physiology. Hydrocortisone granules and modified release formulations are being developed to optimise cortisol replacement in the rare disease of adrenal insufficiency. To facilitate clinical development, we built and verified a physiologically based pharmacokinetic (PBPK) model for the endogenous hormone cortisol (hydrocortisone) in healthy adults, and children and adults with adrenal insufficiency.

The model predicted immediate-release hydrocortisone pharmacokinetics in adults across the dose range 0.5 to 20 mg, with predicted/observed AUCs within 0.8 to 1.25-fold. The model also tightly predicted pharmacokinetic parameters for modified-release formulations, with AUCs within 0.8 to 1.25-fold after single and multiple dosing. Predicted modified-release formulation pharmacokinetics (PK) in 12 to 18-year olds showed PK to be similar to adults.

This hydrocortisone PBPK model is a useful tool to predict adult and paediatric pharmacokinetics of both immediate- and modified-release hydrocortisone formulations, and develop clinical dosing regimens.

1. Introduction

Cortisol is an essential adrenal glucocorticoid stress hormone, regulated by the pituitary and has a distinct circadian rhythm (Bancos et al., 2015). Diseases of cortisol deficiency, called adrenal insufficiency, are rare, resulting in an adrenal crisis and ultimately death if patients are not adequately treated. Adrenal insufficiency can be congenital or acquired and once diagnosed is a life-long condition. In childhood, the genetic condition congenital adrenal hyperplasia (CAH) is the commonest cause of adrenal deficiency, and in adults it can be either primary, due to failure of the adrenal gland (Addison's Disease), or secondary due to failure of the pituitary gland to stimulate the adrenal (hypopituitarism).

For both CAH and adrenal insufficiency, glucocorticoid replacement therapy is primarily with hydrocortisone (identical to the endogenous cortisol molecule) which is given multiple times a day due to its short half-life. In neonates early diagnosis and individualised treatment with hydrocortisone two to four times daily is vital to ensure survival and prevent poor health outcomes (Merke and Bornstein, 2005). The challenge for the paediatrician treating young children with CAH is to maintain a balance between too little hydrocortisone, with the risk of adrenal crisis, and too much, resulting in growth suppression, hypertension and obesity. This risk is increased when no paediatric appropriate formulation is available, with children dependent on compounded tablets which, one detailed study reported, in up to 25% of tested batches prescribed to paediatric patients, did not meet the European Pharmacopeia accuracy and precision criteria (Neumann et al., 2017). Recently, a taste-masked multi-particulate formulation of hydrocortisone (Infacort®), with paediatric appropriate doses, has been developed and is licensed in the European Union under the trade name Alkindi® (hydrocortisone granules in capsules for opening) (Diurnal Europe B.V., Netherlands)(Neumann et al., 2020; Neumann et al., 2018; Whitaker et al., 2015). Carrying out clinical studies in children is challenging and, in the absence of detailed paediatric clinical data, the

development of a PBPK model could better inform clinicians of how best to tailor the dose of hydrocortisone to the child as it grows.

The cortisol circadian rhythm is established early in life and is important for health (Porter et al., 2017). Current hydrocortisone therapy cannot replace the healthy cortisol circadian rhythm and this is a problem particularly in patients with CAH as they grow and develop because the failure of cortisol levels to rise in the early hours of the morning results in poor disease control and poor health outcomes (Han et al., 2014). A modified-release hydrocortisone formulation based on multi-particulate technology with a delayed release coating (Chronocort®) has been developed to meet the need for a hydrocortisone formulation that could reproduce the endogenous cortisol circadian rhythm. When given twice daily (ca. 2300 h and ca. 0700 h), this modified-release formulation provides cortisol concentrations that reflect normal physiology (Whitaker et al., 2014), and improve disease control in patients with CAH (Mallappa et al., 2015). To date, clinical testing of this modified-release formulation has only been carried out in adults and there is a need to predict PK in children, especially adolescents where disease control is poor(Finkielstain et al., 2012).

PBPK models marry the complex interplay of physiological (system) parameters with drug related properties and represent a mechanistic approach by which to quantitatively predict the PK of specific drugs in different populations (Jamei et al., 2009). Paediatric PBPK (p-PBPK) models additionally take into account the development of organs and the ontogeny of specific enzymes and transporters that determine age related pharmacokinetic (PK) profiles (Johnson and Rostami-Hodjegan, 2011; Johnson et al., 2006). The overall aim of this study was to develop adult and then paediatric PBPK models for the immediate-release, multi-particulate and modified-release formulations of hydrocortisone, with the ultimate goal of simulating the immediate-release multi-particulate formulation PK from birth to 18 years and then the modified-release PK in adolescents from 12 to 18 years of age (as

modified-release formulations are generally avoided in younger age groups).

2. Materials and Methods

2.1. PBPK approach

The PBPK models for hydrocortisone were developed using the Simcyp Simulator population-based PBPK software (Certara UK Ltd., Simcyp Division, Sheffield, UK; version 16.1). The development of the various models and populations within the Simcyp Simulator has been described previously (Jamei et al., 2009; Johnson et al., 2006). The strategy for developing the PBPK models for hydrocortisone followed the established 'best practice' using a 'Learn and Confirm' approach (Jones and Rowland-Yeo, 2013), and is shown graphically in Figure 1.

2.1.1. Model-building sequences

Adult published studies describing the PK of intravenous (IV) hydrocortisone were used to establish the initial drug parameters for distribution and elimination (Derendorf et al., 1991; Toothaker and Welling, 1982). Data from published studies describing immediate-release oral hydrocortisone PK were then used to establish the absorption model parameters for the immediate-release formulations of hydrocortisone, and to provide further verification of the model (Derendorf et al., 1991; Toothaker et al., 1982a; Toothaker et al., 1982b). Once developed, clinical studies of the immediate-release multi-particulate formulation in adults (Infacort-001 and Infacort-002; EudraCT Number: 2013-000260-28) were used to verify the final model before performing simulations in the paediatric population. Aspects of the immediate-release multi-particulate PBPK model were carried forward into development of the modified-release model, in which one clinical study in adults (DIUR-004; EudraCT number:
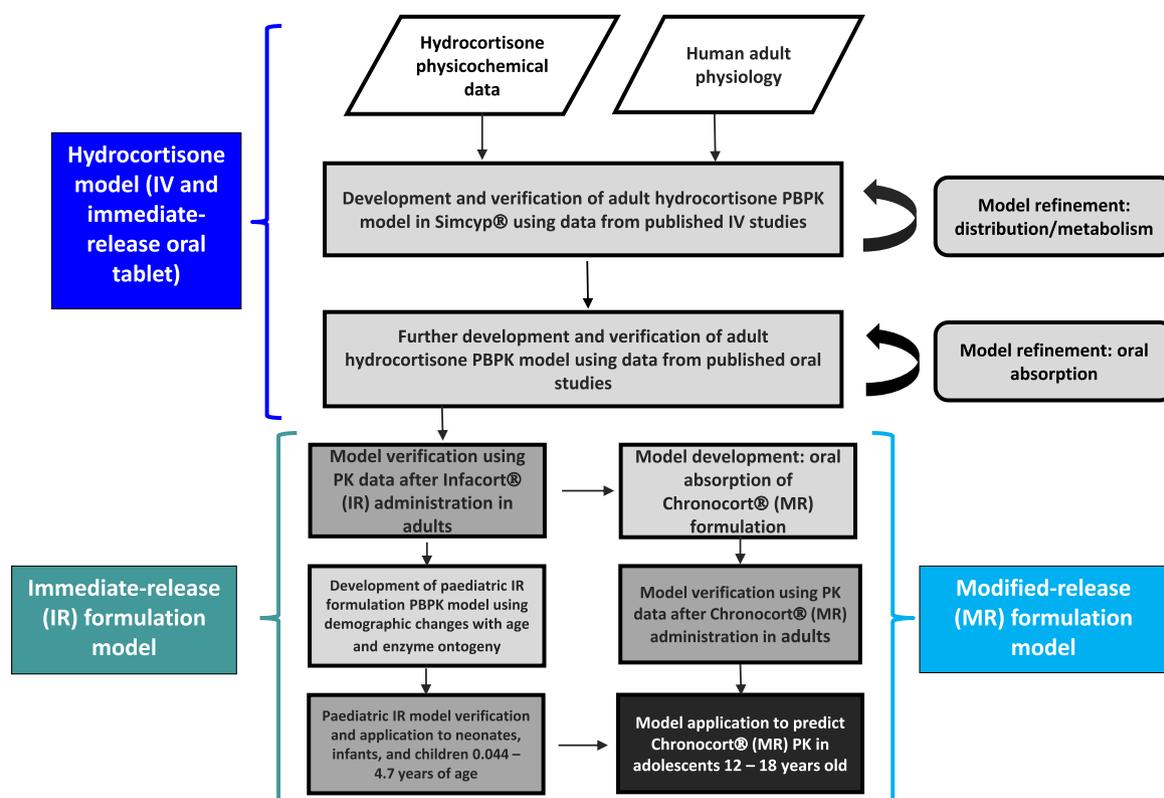
Figure 1. Workflow of hydrocortisone model development, verification, and application

Table 1

Mean observed and predicted IV and oral hydrocortisone PK parameters in adults

Published Clinical studies					
Study	Dose (mg)	Route	AUC_{0-inf} (ng/ml*h)	C_{max} (ng/ml)	T_{max} (h)
Toothaker and Welling (1982)	5	IV	410±80	312±NR	-
Predicted	**5**	**IV**	**358±108**	**225±46**	**-**
Ratio (Pred/Obs)			**0.87**	**0.72**	
Toothaker and Welling (1982)	10	IV	790±100	573±NR	-
Predicted	**10**	**IV**	**641±201**	**470±184**	**-**
Ratio (Pred/Obs)			**0.81**	**0.82**	
Toothaker and Welling (1982)	20	IV	1480±310	1095±NR	-
Derendorf et al. (1991)	20	IV	1175±285	NR	-
Predicted	**20**	**IV**	**1229±453**	**731±284**	**-**
Ratio (Pred/Obs)			**0.83/1.04**	**0.67/NR**	
Toothaker et al. (1982a,b)	5	oral (IR)	293±57	119±23	0.7±0.4
Predicted	**5**	**oral (IR)**	**318±105**	**94.1±23**	**1.2±0.19 (1.2, range 0.8 to 1.72)***
Ratio (Pred/Obs)			**1.08**	**0.79**	
Toothaker et al. (1982a,b)	10	oral (IR)	474±48	199±29	1.0±0.5
Toothaker et al. (1982a,b)	10	oral (IR)	447±75	175±119	0.8±0.5
Predicted	**10**	**oral (IR)**	**551±171**	**157±35**	**1.2±0.19 (1.2, range 0.75 to 1.73)***
Ratio (Pred/Obs)			**1.16/1.23**	**0.79/0.89**	
Derendorf *et al.*, 1991(Derendorf et al., 1991)	20	oral (IR)	1162±308	258±70	1.4±0.3
Toothaker et al. (1982a,b)	20	oral (IR)	835±148	263±55	0.9±0.5
Patel et al. (1984) (B) Patel et al. (1984)	20	oral (IR)	911±234	252±40	1.4±0.7
Patel et al. (1984) (D) Patel et al. (1984)	20	oral (IR)	858±224	285±62	1.0±0.6
Predicted	**20**	**oral (IR)**	**933±364**	**273±111**	**1.2±0.18 (1.2 range 0.7 to 1.75)***
Ratio (Pred/Obs)			**0.80/1.12/1.02/ 1.09**	**1.06/1.04/1.08/ 0.96**	
Toothaker et al. (1982a,b)	30	oral (IR)	958±113	393±58	1.0±0.5
Predicted	**30**	**oral (IR)**	**1367±509**	**433±188**	**1.2±0.2 hours (1.2, range 0.45 to 1.7)***
Ratio (Pred/Obs)			**1.42**	**1.10**	
Infacort studies					
Study	**Dose (mg)**	**Route**	**AUC_{0-inf} (nM*h)**	**C_{max} (nM)**	**t_{max} (h) (median and range)**
Infacort 001 (Melin et al., 2017; Whitaker et al., 2015)	0.5	oral (IRM)	123±28	74.8±19.9	0.5 (0.5 to 1.0)
Predicted	**0.5**	**oral (IRM)**	**101±38**	**43.3±12.9**	**1.1 (0.7 to 1.5)**
Ratio (Pred/Obs)			**0.82**	**0.58**	
Infacort 001 (Melin et al., 2017; Whitaker et al., 2015)	2	oral (IRM)	449±80	228±39	0.5 (0.5 to 1.5)
Predicted	**2**	**oral (IRM)**	**374±124**	**157±44**	**1.1 (0.7 to 1.5)**
Ratio (Pred/Obs)			**0.83**	**0.69**	
Infacort 001 (Melin et al., 2017; Whitaker et al., 2015)	5	oral (IRM)	909±152	404±61	0.5 (0.5 to 1.5)
Predicted	**5**	**oral (IRM)**	**813±249**	**333±85**	**1.1 (0.8 to 1.5)**
Ratio (Pred/Obs)			**0.89**	**0.82**	
Infacort 001 (Melin et al., 2017; Whitaker et al., 2015)	10	oral (IRM)	1603±282	600±138	0.75 (0.5 to 1.5)
Predicted	**10**	**oral (IRM)**	**1364±386**	**537±122**	**1.1 (0.8 to 1.5),**
Ratio (Pred/Obs)			**0.85**	**0.90**	
Infacort 002 (Johnson et al., 2018; Melin et al., 2017)	20	oral (IRM)	2630±714	974±284	0.75 (0.5 to 1.25)
Predicted	**20**	**oral (IRM)**	**2165±567**	**809±172**	**1.05 (0.5 to 1.5)**
Ratio (Pred/Obs)			**0.82**	**0.82**	
Chronocort studies					
Study	**Dose (mg)**	**Route**	**AUC_{0-inf} (nM*h)**	**C_{max} (nM)**	**t_{max} (h) (median and range)**
DIUR-004 (Karunasena et al., 2017) - Fasted	20	oral (MR)	2981±417	708±135	4.5 (2.5 to 6.5)
Predicted	**20**	**oral (MR)**	**2659±771**	**487±131**	**3.9 (1.1 to 8.1)**
Ratio (Pred/Obs)			**0.89**	**0.69**	
DIUR-004(Karunasena et al., 2017) - Fed	20	oral (MR)	3229±613.5	549±93.3	6.8 (3.5 to 20)
Predicted	**20**	**oral (MR)**	**2882±864.6**	**414±116**	**4.2 (1.3 to 7.9)**
Ratio (Pred/Obs)			**0.89**	**0.75**	**0.62**
DIUR-003 (Mallappa et al., 2015)	20/10	oral (MR)	4870±1218	591±112	-
Predicted	**20/10**	**oral (MR)**	**4766±1512**	**551±165**	**-**
Ratio (Pred/Obs)			**0.98**	**0.93**	**-**
DIUR-008	20	oral (MR)	2640±634	609±140	5.0 (2.0 to 6.0)
Predicted	**20**	**oral (MR)**	**2641±766**	**474±128**	**3.83 (1.2 to 8.1)**
Ratio (Pred/Obs)			**1.00**	**0.78**	

IV=intravenous; IR=immediate-release; IRM=Infacort® immediate-release multiparticulate form; MR=Chronocort® modified-release form

2014-005025-12) was used to further develop the model to be suitable for a modified-release formulation. The performance of the modified-release PBPK model was then verified using data from two additional clinical studies in adults (DIUR-003 and DIUR-008; IND number: 76485 & EudraCT number: 2016-001390-32). A summary of the studies is presented in Supplementary Table 1. Once verified, the modified-release model was used to simulate the PK of both single and multiple dosing scenarios in a virtual adolescent population aged 12 to 18 years.

PK data are presented as total (free and bound) cortisol. Although unbound cortisol is the active form in the body, total concentrations as well as pharmacodynamic and clinical endpoints are primarily used for dose modification and efficacy evaluation in the clinic.

2.1.2. Population parameters used in the PBPK models

2.1.2.1. Absorption parameters. The default gastric residence times within Simcyp Simulator were used for simulations of immediate release formulations. For modified-release simulations, the fasted mean gastric residence time was set to 2 hours to reflect the extended gastric emptying time of pellet formulations (Davis et al., 1986). In addition, the coefficient of variation (CV) for the pH values of some of the intestinal segments in the model were decreased in order to ensure that the release pH for the formulation occurred in all virtual subjects. These absorption changes are described further in the Supplementary data – Section 1.

2.1.2.2. Incorporation of cortisol binding protein. Hydrocortisone binds both to albumin and to cortisol binding globulin (CBG) in human plasma. Data on human cortisol binding globulin expression in males and females were collected from the literature (see Supplementary Data – Section 2 for references). A meta-analysis was performed and the calculated weighted mean and CV for males (n=323) and females (n=235) were 0.751μM (cv = 14%) and 0.872 μM (cv = 19%), respectively.

2.1.2.3. Ontogeny of CBG. Data on the ontogeny of CBG was taken from the literature (see Supplementary Data – Section 3 for references), with a total of 642 observed data points included in the analysis (Supplementary Figure 1). The fraction of adult expression with age was 0.22, 0.49, 0.57, 0.7, 0.82, 0.95 and 1 at 0, 0.08, 0.5, 3, 5, 7, and 18 years, respectively. The ontogeny profile was entered into the Simcyp Simulator as a scalar for the adult values.

2.1.2.4. Metabolism of hydrocortisone and ontogeny of major pathways. The elimination of hydrocortisone is complex (see Supplementary Figure 2) and was grouped into major metabolic routes for the purpose of the current PBPK models. Active hydrocortisone (cortisol) is converted to inactive cortisone by the enzyme 11β-hydroxysteroid dehydrogenase 2 (11β-HSD2)(Walker and Seckl, 2001). The other major elimination enzyme is 5α-reductase and there is a minor contribution from cytochrome P450 3A (CYP3A) in the 6β hydroxylation of cortisol. Other metabolic pathways, 20β-oxoreductase and 5β-reductase are lumped together as 'Additional metabolism'.

The ontogeny of 11β-HSD2 was derived based on urinary cortisone to cortisol ratios (Rodgers and Rowland, 2006), and that for 5α-reductase from urinary allo-Tetrahydrocortisol/Tetrahydrocortisol ratios (Wudy et al., 2007). The sources of information and equations describing 11β-HSD2 and 5α-reductase ontogeny profiles are listed in the Supplementary data – Section 3 and the final ontogeny profiles are shown in Supplementary Figure 3. The CYP3A4 ontogeny profile in the Simcyp Simulator has been described previously (Salem et al., 2014), and no ontogeny was assumed for the additional clearance pathways.

2.1.2.5. Drug parameters used in the models. Immediate- and modified-

release model input parameters and their sources of information are summarized in Supplementary Table 2. The formulation and absorption aspects incorporated into the PBPK models are described in more detail in the Supplementary data – Section 1.

2.1.2.6. Plasma protein binding. For the immediate-release model, a non-linear free fraction of hydrocortisone in plasma (fu), reflecting binding to both albumin and CBG, was calculated as described in the Supplementary Information – Sections 4 and 5. For the modified-release model the reference protein $[P]_{ref}$ value for albumin was 45 g/L (Simcyp Simulator default) and that for CBG was 0.41 μM, taken from the immediate-release multi-particulate study Infacort-002, since CBG was not in the modified-release clinical studies.

2.1.2.7. Incorporating hydrocortisone metabolic data into PBPK model. The fraction metabolised by the different metabolic pathways is shown in Supplementary Table 3. Values were calculated based on available mass balance data for total metabolites (including glucuronides) and unchanged drug. Because the 11β-HSD2 and 5α-reductase enzymes are not available in the Simulator, two of the UDP-glucuronosyltransferase enzymes were used as surrogates for these enzymes. Relative enzyme expression was used in the simulations and for the paediatric simulations, the relevant ontogeny functions were applied for 11β-HSD2 and 5α-reductase (See Supplementary Information - Section 6). The retrograde model was used to generate initial intrinsic clearance values for CYP3A4, 11β-HSD2, 5α-reductase and an additional clearance based on a weighted mean fixed fraction unbound of 0.066 and a CL_{iv} value of 20L/h.

2.1.2.8. Distribution. The minimal PBPK model with a single adjusting compartment (SAC) was used to describe the distribution of hydrocortisone. This model, akin to a 2-compartmental PK model plus liver compartment, gave similar results to using a full PBPK model for the immediate release formulation (data not shown). This model, but not full PBPK, allows the added flexibility of simulating the effects of changing fu on volume of distribution. In the immediate-release model, the volume of distribution of hydrocortisone was dynamically linked to changes in fu during the simulation. However, during the simulation of modified-release dosing in paediatrics, this setting caused an instability in the ODE (ordinary differential equation) solver output. Therefore, for modified-release modelling a fixed volume of distribution was applied. Outputs were checked for both a fixed and concentration-dependent volume of distribution and it was found that fixing the volume had no effect on the resulting simulations, so this was carried forward.

2.1.3. Simulation of clinical studies

All model development and verification simulations were run using virtual subjects matched as closely as possible with respect to age and sex to those in the corresponding actual studies (Supplementary Table 1), and according to the same study designs. However, it should be noted that the healthy volunteer virtual adult and the healthy virtual paediatric populations within Simcyp were used for all simulations, whereas the clinical studies had a mixture of healthy volunteers and CAH patients. At this time little is known of any physiological differences between healthy and CAH patients that may affect PK. For the published clinical studies, data from more than one study were combined into a single study design; more detail of the specific trial designs within the simulator are given in the Supplementary data – Section 7. The dose units for simulations were those in the clinical studies.

2.1.3.1. Paediatric model application – modified-release formulation. The developed modified-release model was used within the Simcyp Paediatric Simulator to simulate single and multiple dose pharmacokinetics in the age range of 12 to 18 years at doses based on body weight and body surface area. These paediatric simulation results were then compared

with adult simulations at the same dose. In the single dose cases, simulations were 24 hours in duration, with drug administration in the morning. In the multiple dose cases, simulations were 72 hours in duration, with drug administration at 2300 h and 0700 h each day, starting with the first dose of the modified-release formulation at 2300 h on day 1 of the simulation (actual day 2 of clinical study).

For the first paediatric simulations, doses were calculated using the body surface areas of the adult subjects (CAH patients) in the multiple-dose clinical study. The doses of 20 mg and 10 mg were divided by each individual's body surface area (calculated using the DuBois formula) to obtain BSA-based doses 11.6 and 5.8 mg/m^2, respectively.

Doses of 8 mg/m^2 in the evening and 4 mg/m^2 in the morning (12 mg/m^2 over 24 hours) were also used in the model application simulations after being rounded to available capsule size combinations. This dose is in keeping with current guidelines on the treatment of CAH (Speiser et al., 2018). These simulations were run using the Simcyp Paediatric Simulator in six month age bands ranging from 12 to 18.5 years old.

2.1.4. Data analysis

Predicted and observed concentration-time data was compared using a visual predictive check, the number of observed data points within the 5th and 95th predicted range were considered. For PK parameters the ratio of predicted to observed values was determined.

A good prediction was defined as between 0.8 and 1.25-fold of the observed (bioequivalence criteria based on $\pm$ 20% difference), reasonable prediction as between 0.67 and 1.5-fold of the observed and acceptable prediction as between 0.5 and 2-fold of the observed. Though there is no universally agreed-upon scale of PBPK model prediction quality, these ranges are often used (Abduljalil et al., 2014).

3. Results

3.1. Simulation of previously published adult hydrocortisone PK studies

The results of the simulation of published clinical hydrocortisone PK studies following intravenous (IV) and oral administration are shown in Table 1. For all three IV and 6 out of 7 oral studies the predicted AUC0-inf was within 0.8 to 1.25-fold of observed. For the 5 and 10mg oral dose simulations the C_{max} was 0.79-fold in both cases and predicted T_{max} was 1.2 vs 0.7 or 0.8 hours. The predicted PK parameters for the 20mg dose were in close agreement with the four clinical studies published. For the 30mg oral dose, the AUC was 1.42-fold of observed.

3.2. Adult immediate-release multi-particulate formulation studies

Figure 2 shows the results of the simulation of the oral Infacort-001 and Infacort-002 PK studies and a summary of PK parameter results is given in Table 1. For all simulations at the different doses, the predictions were good; the majority (90%) of individual observed concentration-time data were within the simulated 5th and 95th percentile. The predicted/observed AUC ratio was within 0.8 to 1.25-fold in for all Infacort® studies, but at the lower doses of 0.5 to 2mg there was some slight under-prediction of C_{max}. Overall, the predicted/observed C_{max} ratio was within 0.8 to 1.25-fold in 3 out of 5 cases and always within 2-fold. Simulated median T_{max} values were slightly longer than observed, however there was overlap in the observed and predicted ranges. The model successfully simulated the known non-linear cortisol binding globulin (CBG) binding of hydrocortisone, as reflected in Supplementary Figure 4.

3.3. Paediatric immediate-release multi-particulate formulation studies

Figures 3A-C show the predicted concentration-time profile
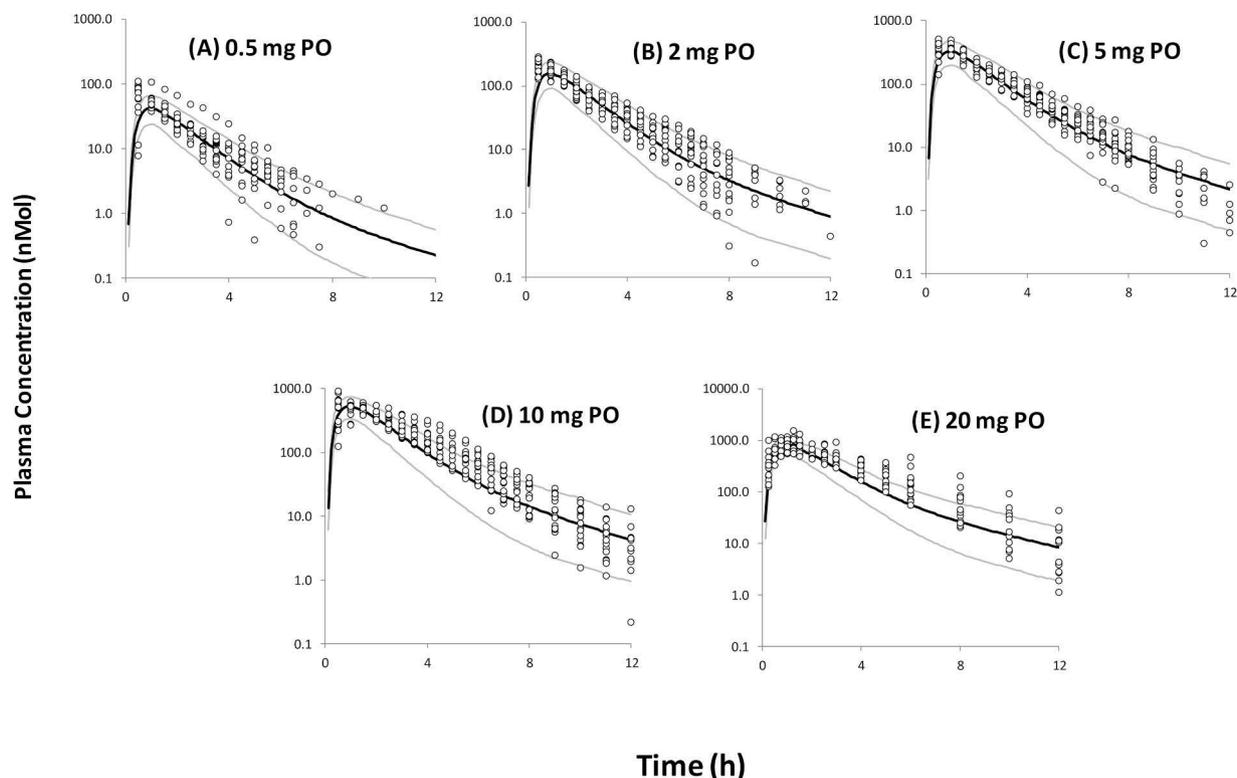
Figure 2. Predicted concentration-time profiles (mean—black lines; 5th and 95th percentiles—grey lines) and observed individual values (open circles) of immediate-release hydrocortisone in healthy dexamethasone-suppressed adults for Infacort-001 and Infacort-002 clinical studies. A) 0.5 mg; B) 2 mg; C) 5 mg; D) 10 mg; E) 20 mg

Enzyme ontogeny / No enzyme ontogeny

A) 0.16 mg/kg given to <u>children</u> 2 - 4.7 y (Cohort 1)

B) 0.22 mg/kg given to <u>infants</u> 0.3 – 1.8 y (Cohort 2)

C) 0.53 mg/kg given to <u>neonates</u> 0.044 – 0.071 y (Cohort 3)

Plasma Concentration (nMol)

Time (h)
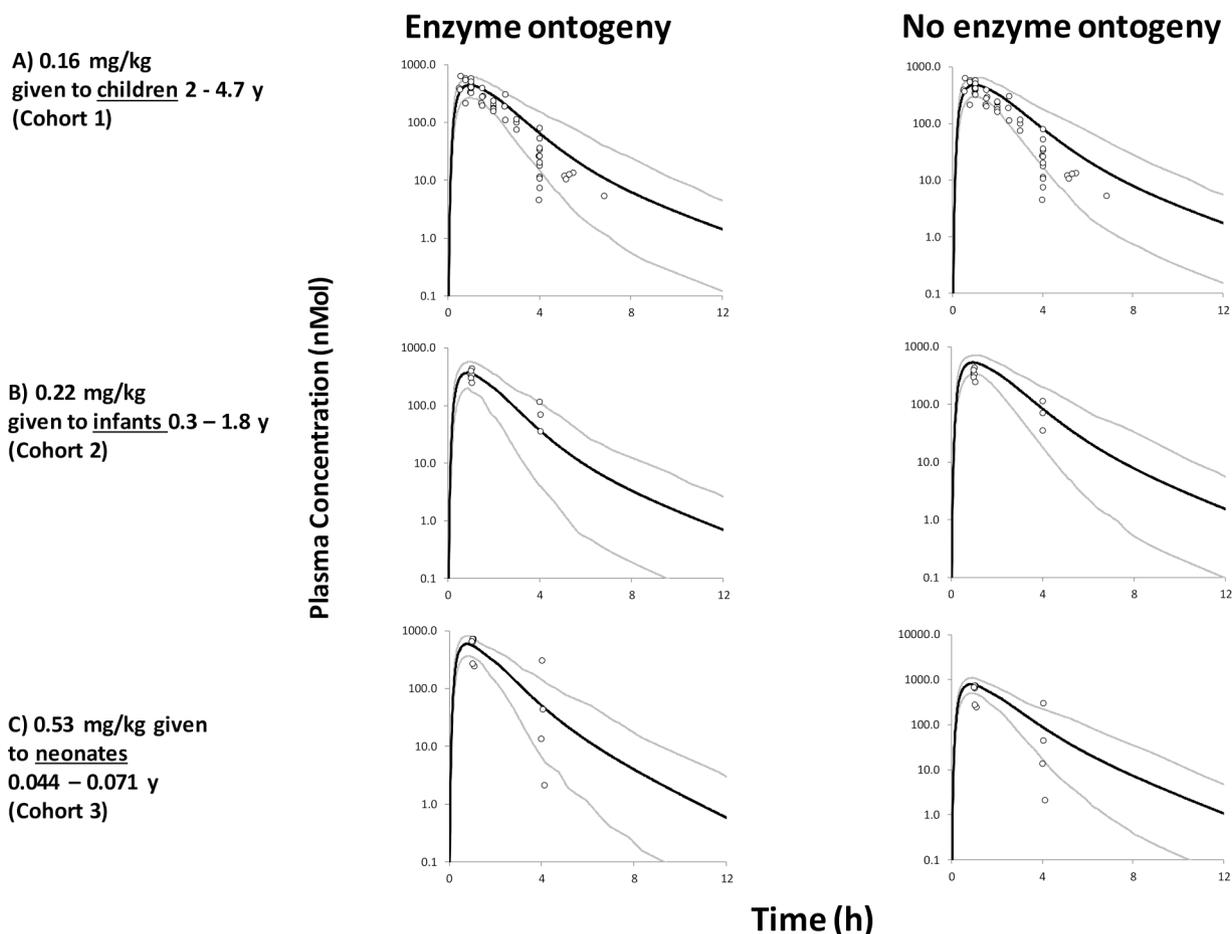
Figure 3. Predicted concentration-time profiles (mean—black lines; 5th and 95th percentiles—grey lines) and observed individual values (open circles) of immediate-release hydrocortisone in paediatric patients for Infacort-003 clinical study. The described ontogeny is incorporated in the left-hand graphs and no ontogeny assumed in the graphs on the right. A) 0.16 mg/kg given to children 2 to 4.7 y.o. (Cohort 1); B) 0.22 mg/kg given to infants 0.3 to 1.8 y.o. (Cohort 2); C) 0.53 mg/kg given to neonates 0.044 to 0.071 y.o. (Cohort 3).

compared to the clinical trial data for paediatric cohorts 1 to 3 on a log scale, LHS with modelled ontogeny for 11β-HSD2 and 5α-reductase, and RHS with no modelled ontogeny. As the observed data were sparse data, there are no calculated PK parameters. In all cases assuming ontogeny, 80 % of the observed data are within the predicted 5th and 95 percentiles compared to 68% assuming no ontogeny. For cohort 1, 2 to 4.7y (Figure 3A) the observed elimination rate appears faster than predicted. For cohorts 2 and 3, 0.3 to 1.8y (Figure 3B) and 0.044 to 0.071 y (Figure 3C) the elimination rate appears reasonably predicted.

3.4. Adult modified-release formulation studies

A comparison of simulated and observed plasma concentrations of cortisol following a single oral 20 mg dose of the modified-release formulation in the fasted state are shown in Figure 4A. Associated mean C_{max} and $AUC_{(0,24)}$ values are compared in Table 1. The simulated mean AUC values were within 1.2-fold of the observed data. Due to the variability in the observed T_{max} values in modified release, the simulated C_{max} of the mean cortisol plasma concentration profile (487 nmol/L) was lower than the mean of individual observed C_{max} values (708 nmol/L). Whilst the simulated mean plasma cortisol concentrations were in reasonable agreement with the mean observed plasma cortisol concentrations, the mean of observed individual C_{max} values was approximately 1.5-fold higher than the corresponding simulated value. The geometric mean of the fraction absorbed was 0.97 for this and all modified-release and immediate-release multi-particulate adult simulations. Eighty-six percent of observed concentrations were within the 5th and 95th percentiles of the simulated population.

A comparison of simulated and observed plasma concentrations of cortisol following a single oral 20 mg dose of the modified-release formulation in the fasted state from a different study are shown in Figure 4B in which individual concentrations for the observed and mean and 5th and 95th percentiles of the simulated population are displayed. Associated mean C_{max} and $AUC_{(0,24)}$ values are compared in Table 1. The simulated mean $AUC_{(0-24)}$ was a very close match to the observed value, whereas the mean C_{max} was somewhat under-predicted (simulated C_{max} was 78% of the observed C_{max}). The simulated median T_{max} was also earlier than the corresponding observed value (simulated T_{max} was 77% of the observed T_{max}). Eighty-eight percent of observed concentrations were within the 5th and 95th percentiles of the simulated population.

A comparison of simulated and observed plasma concentrations of cortisol on the third day following oral dosing of a 20 mg dose of the modified-release formulation in the evening and 10 mg the following morning (8 hour interval) for 3 days in CAH patients are shown in Figure 4C. Individual concentrations for the observed and mean and 5th and 95th percentile of the simulated population are displayed. Associated geometric mean C_{max} and AUC values are compared in Table 1. The simulated mean AUC and C_{max} values were within 1.1-fold of the observed values and 80% of the observed data points were within the 5th and 95th percentiles of the simulated population.

A comparison of simulated and observed plasma concentrations of cortisol following a single oral 20 mg dose of the modified-release formulation in the fed state are shown in Figure 4D. Associated mean C_{max} and $AUC_{(0,24)}$ values are compared in Table 1. The simulated mean
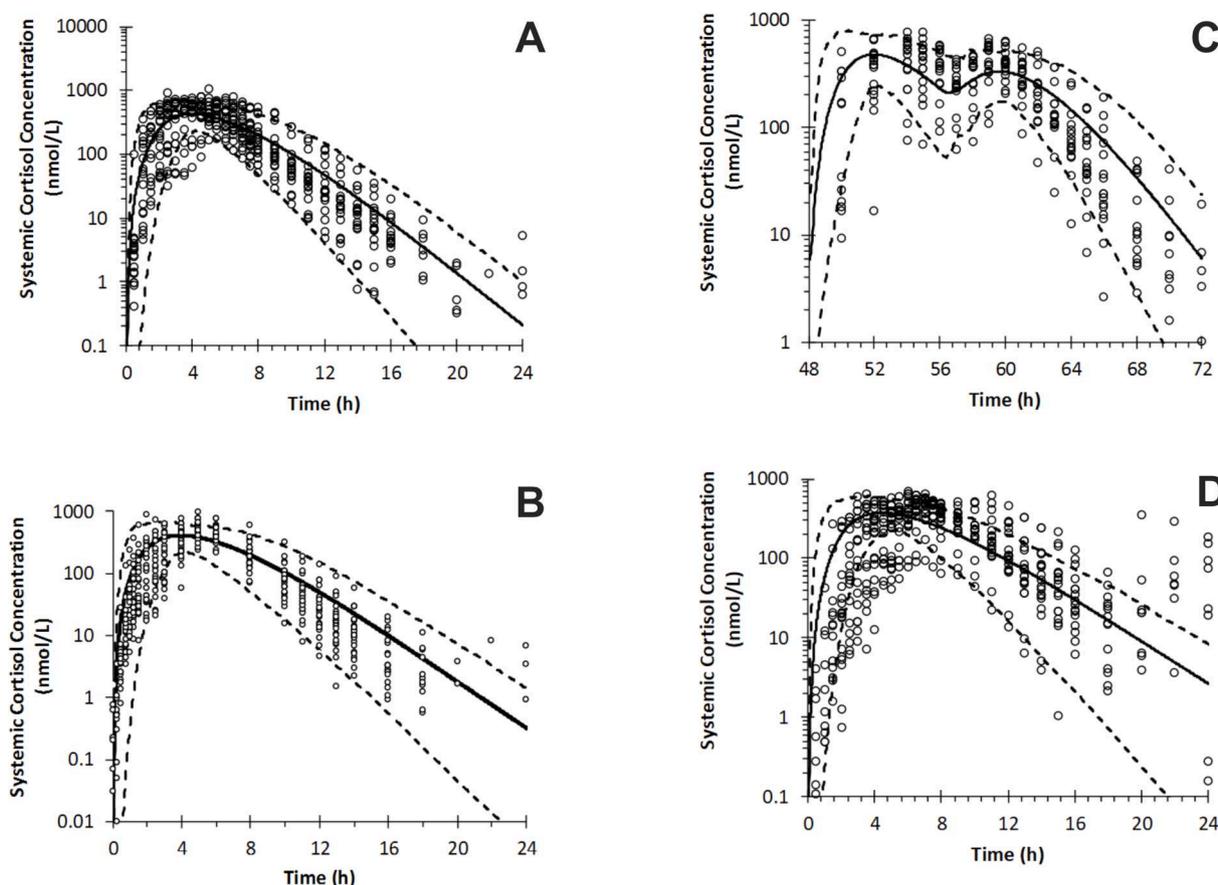
Figure 4. A) Mean simulated (line) and observed (open circles, baseline adjusted individual data points - DIUR-004 clinical study) plasma cortisol concentrations following a single oral dose of modified release hydrocortisone (20 mg) to fasted healthy individuals. The dashed lines represent 5th and 95th percentiles of the simulated population (n = 180). B) Mean simulated (line) and observed (open circles, baseline adjusted individual data points - DIUR-008 clinical study) plasma cortisol concentrations following a single oral dose of modified release hydrocortisone (20 mg) to fasted healthy individuals. The dashed lines represent 5th and 95th percentiles of the simulated population (n = 240). C) Mean simulated (line) and observed (open circles, baseline adjusted individual data points - DIUR-003 clinical study) plasma cortisol concentrations on the 3rd day after oral dosing of 20 mg modified release hydrocortisone in the evening and 10 mg modified release hydrocortisone the following morning (8 hour interval) for 3 days in CAH patients. The dashed lines represent 5th and 95th percentiles of the simulated population (n = 160). D) Mean simulated (lines) and observed (circles, baseline adjusted, individual data points - DIUR-004 clinical study) plasma cortisol concentrations following a single oral dose of modified release hydrocortisone (20 mg) to fed healthy individuals. The dashed lines represent 5th and 95th percentiles of the simulated population (n = 180).

Table 2
Results for simulations of a single 0.3 mg/kg dose as well as multiple dosing of 0.3 mg/kg in the evening and 0.15 mg/kg in the morning of the modified-release formulation in virtual adolescents (ages 12 to 18 years). C_{max} and AUC values are geometric means, T_{max} are median and range.

Modified-release dosing regimen	Population	C_{max} nmol/L	$AUC_{(0-24h)}$ nmol*h/L	T_{max} h
0.3 mg/kg orally as a single dose	Paediatric	564±166	2940±984	3.6 (1.3 to 6.8)
	Adult	543±147	2992±901	3.9 (1.2 to 8.1)
	P/A	1.04	0.98	0.92
Modified-release dosing regimen	Population	$C_{max(48-56h)}$ nmol/L	$AUC_{(48-72h)}$ nmol*h/L	T_{max} h
0.3 mg/kg q23:00h, 0.15 mg/kg q07:00h x 3 days	Paediatric	581±157	4631±1436	-
	Adult	593±166	5012±1453	-
	P/A	0.98	0.92	-
Modified-release dosing regimen	Population	C_{max} nmol/L	$AUC_{(0-24h)}$ nmol*h/L	T_{max} h
11.6 mg/m^2 orally as a single dose	Paediatric	608±170	3194±958	3.5 (1.3 to 6.8)
	Adult	529±138	2908±814	3.9 (1.1 to 8.1)
	P/A	1.15	1.10	0.90
Modified-release dosing regimen	Population	$C_{max(48-56h)}$ nmol/L	$AUC_{(48-72h)}$ nmol*h/L	T_{max} h
11.6 mg/m^2 q23:00h, 5.8 mg/m^2 q07:00 × 3days	Paediatric	617±167	5173±1500	-
	Adult	588±165	4965±1440	-
	P/A	1.05	1.04	-
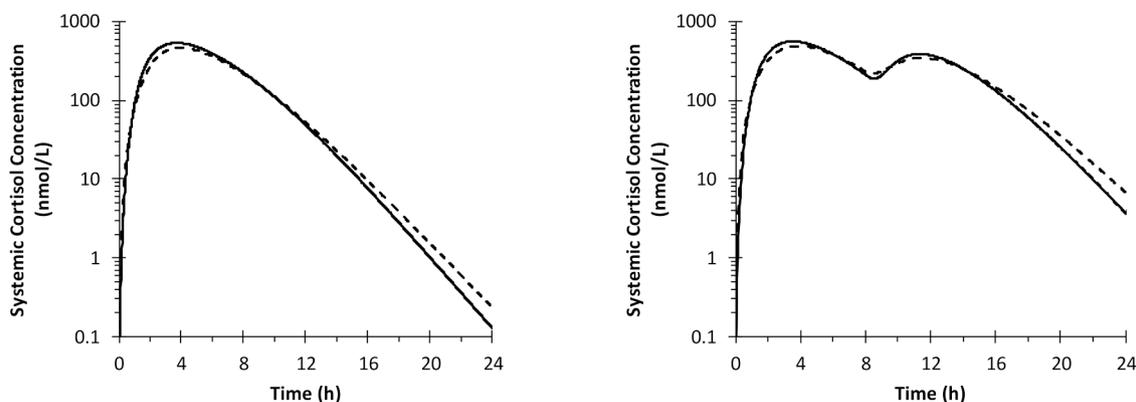
Figure 5. A) Simulated mean (n = 180) plasma cortisol concentrations after a single oral dose of 11.6 mg/m^2 modified release hydrocortisone in children aged 12 to 18 years (solid line) and in adults (dotted line). B) Simulated mean (n = 160) plasma cortisol concentrations on the 3rd day after oral dosing of 11.6 mg/m^2 modified release hydrocortisone in the evening and 5.8 mg/m^2 modified release hydrocortisone the following morning (8 hour interval) for 3 days in children aged 12 to 18 years (solid line) and in adults (dotted line).

Table 3

Predicted PK parameters in adolescents by age band after clinically proposed BSA-based dosing of 12 mg/m^2 per day Table 1. Geometric means of BSA-based dosing of 12 mg/m^2 per day simulations in virtual adolescents broken down by 6-month age bands and rounded to the nearest capsule size doses of modified-release hydrocortisone. C_{max} and AUC values are geometric means.

Age (years)	Equivalent BSA (m^2)	Calculated PM dose based on BSA (mg) (8 mg/m^2)	Calculated AM dose based on BSA (mg) (4 mg/m^2)	Nearest Capsule-based dose (mg)	$C_{max(48-56)}$ (nmol/L)	$C_{max(56-72)}$ (nmol/L)	$AUC_{(48,56)}$ (nmol*h/L)	$AUC_{(56,72)}$ (nmol*h/L)
12 – 12.5	1.3	10.4	5.2	10/5	506.4	329.2	2187	1765
12.5 – 13	1.3	10.4	5.2	10/5	483.8	313	2088	1683
13 – 13.5	1.4	11.2	5.6	10/5	463.4	298.4	1999	1609
13.5 – 14	1.4	11.2	5.6	10/5	447.6	287.1	1931	1553
14 – 14.5	1.5	12	6	10/5	434.3	277.6	1874	1507
14.5 – 15	1.5	12	6	10/5	417.7	266.1	1801	1448
15 – 15.5	1.6	12.8	6.4	15/5	523.7	276.8	2321	1565
15.5 – 16	1.6	12.8	6.4	15/5	512.1	270	2268	1529
16 – 16.5	1.6	12.8	6.4	15/5	502.2	264.2	2220	1505
16.5 - 17	1.6	12.8	6.4	15/5	493.6	259.1	2181	1478
17 – 17.5	1.6	12.8	6.4	15/5	489.5	256.7	2162	1465
17.5 – 18	1.6	12.8	6.4	15/5	485.1	254.1	2142	1452
18 – 18.5	1.6	12.8	6.4	15/5	482.9	252.9	2132	1448

AUC was within 1.2-fold of the observed data and simulated mean C_{max} within 1.25-fold of observed. However, though the mean simulated T_{max} was within 2-fold of observed (4.2 hours simulated *vs.* 6.8 hours observed), it was significantly lower, failing to capture the observed significant increase in T_{max} in the fed state as compared with the fasted. In total 65% of the observed data points were within the 5th and 95th percentiles of the simulated population.

3.5. Adolescent modified-release formulation simulations

Results for simulations of a single 0.3 mg/kg dose as well as multiple dosing of 0.3 mg/kg in the evening and 0.15 mg/kg in the morning of the modified-release formulation in virtual adolescents (ages 12 to 18 years) are shown in Table 2. Following body weight-based dosing, mean C_{max}, T_{max}, and AUC in adolescents were within 8% of adult values. Results for simulation of a single 11.6 mg/m^2 dose as well as multiple dosing of 11.6 mg/m^2 in the evening and 5.8 mg/m^2 in the morning of the modified-release formulation in virtual adolescents ages 12 to 18 years are shown in Table 2. After this body surface area-based dosing, mean C_{max}, T_{max}, and AUC in adolescents were within 15% of adult values. A comparison of the simulated mean plasma concentrations in adults and adolescents is shown in Figure 5. The recommended dose for adolescents is between 10-15 mg/m^2 (Speiser et al., 2018), we have therefore also simulated the mid-range daily dose of 12 mg/m^2 and how this would be

administered in the unit dose of modified-release hydrocortisone providing a physiological C_{max} and AUC (Table 3).

4. Discussion

We have built a PBPK model for hydrocortisone and tested it against the published literature and clinical trials using novel formulations of immediate-release hydrocortisone (Infacort®) and modified-release hydrocortisone (Chronocort®). The PBPK model predicted PK parameters in adults and children across hydrocortisone dose ranges 0.5 to 20 mg. The model captured the adult modified-release PK data after single and multiple dosing, provided predictions of PK in adolescents 12 to 18-years and allowed us to generate dosing regimens for adolescents using modified-release hydrocortisone.

In recent years there has been a renewed interest in the use of PBPK modelling in drug development, specifically in the area of drug-drug interaction prediction; however, use in paediatric and other areas of drug development is increasing (Jamei, 2016; Sato et al., 2017). Paediatric PBPK has been used to determine the age related doses for clinical trials (Leong et al., 2012; Rioux and Waters, 2016), drug-drug interactions (Olafuyi et al., 2017), formulation bridging (Johnson et al., 2014) and study design (Dumont et al., 2013). There are few published examples of PBPK simulations for humans involving replacement of endogenous substrates such as hormones (Li et al., 2014;

Nakada et al., 2018). This is the first time a PBPK model has been developed for hydrocortisone, an endogenous compound, and the model incorporates the known complexities in hydrocortisone disposition. Thus, the model includes systems information to account for non-linear binding to cortisol binding protein, metabolism by 11β-HSD2 and 5α-reductase, cortisol metabolism by 20β-oxo-reductase and 5β-reductase and how these parameters develop with age.

Overall results from the simulations of published adult immediate-release oral and IV hydrocortisone PK studies and adult immediate-release multi-particulate studies indicate that the model performs well over the dose range 5 to 20 mg for IV administration and 0.5 to 30 mg for oral administration of immediate-release hydrocortisone. There is some under-prediction of C_{max} particularly in 0.5 mg and 2 mg single dose immediate-release multi-particulate formulation simulations. It is not clear what the source of error is, but it may be a consequence of inaccuracies in defining the non-linear protein binding effects on volume of distribution or a slower absorption rate coming through the absorption model. However, for 5 mg and above the predicted/observed AUC and C_{max} ratios are within the tight range of 0.8 to 1.04-fold, these are the dose levels of more therapeutic interest in the adolescent and adult population. In general, the 5th and 95th percentiles for the simulations capture the variability in observed PK, although there is some evidence that variability in patient groups with primary or secondary adrenal insufficiency is higher compared to healthy volunteers (Werumeus Buning et al., 2017).

The paediatric immediate-release multi-particulate formulation simulations reasonably captured the concentration-time profiles down to neonates around 2 weeks of age. For the two younger age groups of neonates (less than 1 month old) and infants (1-24 month old), the simulated developmental patterns for 11β-HSD2 and 5α-reductase appear reasonable in relation to the results. However, for the older age group between 24 months and 6 years the observed elimination appears to be slightly faster than that predicted, this could be due to over-expression of other steroid metabolizing enzymes, placed together as 'additional clearance' and assumed to have no ontogeny in this age group, or misspecification of the applied ontogeny for 11β-HSD2 or 5α-reductase. The ontogeny of 11β-HSD2 and 5α-reductase are derived from urinary cortisone/cortisol and allo-Tetrahydrocortisol/Tetrahydrocortisol ratios (Wudy et al., 2007). Whilst these ratios may need correcting for age related changes in renal function and also one minus fraction reabsorbed, there is little evidence regarding the ontogeny of the latter and the assumption was made that both of these factors cancel out. The simulations assuming no ontogeny for all enzymes resulted in poorer prediction of the observed data and give further evidence for over-expression of cortisol metabolizing enzymes in the younger age groups.

Simulations of the adult modified-release PK studies showed that the final model recovered the observed plasma cortisol concentrations well in both single and multiple dosing scenarios. For the 20 mg single-dose (healthy volunteers) simulations in the fasted state, the simulated mean AUC values were within 1.1-fold of the observed data. Due to the expected variability in the observed T_{max} values from modified-release, the observed C_{max} of the mean cortisol plasma concentration profile (487 nmol/L) was lower than the mean of individual C_{max} values (708 nmol/L). Whilst the simulated mean plasma cortisol concentrations were in reasonable agreement with the mean observed plasma cortisol concentrations, the mean of observed individual C_{max} values was 1.5-fold higher than the corresponding simulated value. Single-dose simulations of 20 mg of the modified-release formulation in healthy volunteers as in study DIUR-008 yielded an exposure (as measured by AUC_{0-24h}) nearly identical to that observed. C_{max} and T_{max} were slightly under-predicted by the model but well within 2-fold of the observed geometric mean parameter values. For the 20 mg/10 mg multiple dosing study simulations in the fasted state, the simulated mean AUC and C_{max} values were within 1.1-fold of the observed data. In the single-dose simulation of 20 mg modified-release in the fed state, the simulated mean AUC and

C_{max} values were within 1.25-fold of the observed data but the T_{max} was less well predicted, though with the mean still within 2-fold of observed.

After calculation of mg/kg and mg/m^2 doses roughly equivalent to 20 mg and 10 mg of the modified-release formulation in adults, simulations were performed in a virtual paediatric population aged 12 to 18 and results compared to equivalent doses simulated in adults. The results showed minimal difference in C_{max}, T_{max}, and AUCs between adults and children. These simulation results suggest that dosing of the modified-release formulation based on either body weight (mg/kg) or body surface area (mg/m^2) will yield predictable pharmacokinetics in paediatric populations. The hydrocortisone doses recommended by guidelines for adolescents with CAH are 10–15 mg/m^2 per day (Speiser et al., 2018), and the model was used to predict a clinically appropriate dosing regimen for adolescents based on a daily dose of 12 mg/m^2.

The limitations of our model include not inputting the circadian variation in CBG in the model, however a recent PK study by Melin et al. (2019) concluded: 'the difference in cortisol exposure is <11% between times of highest and lowest CBG concentrations; therefore hydrocortisone dose adjustment based on time of dosing is not required'. There are a number of assumptions in the current PBPK models related to the metabolism of hydrocortisone. Active hydrocortisone (cortisol) is metabolised to inactive cortisone in vivo by the enzyme 11β-HSD2 and the latter converted back to cortisol as needed by 11β-HSD1. Representation of the metabolic fate of hydrocortisone required some simplification of the pathways involved. In particular, the interconversion of cortisol and cortisone was subsumed in the net forward reaction to the latter compound. Lumping of pathways accounting for 36.6% of total metabolism assumed no ontogeny has been discussed. One other assumption is that most of the metabolism is occurring in the liver with some in the kidney, in reality hydrocortisone is metabolised in a number of tissues (Walker and Seckl, 2001). This will not change the overall results but is less mechanistic than what is actually occurring in vivo.

In conclusion, the current models, verified using a large amount of available clinical data, account for the complexities in the PK of hydrocortisone. These models are potentially useful tools for understanding significant covariates within clinical studies and for dose extrapolation into other paediatric age ranges, other ethnic groups and between formulations.

Disclosure Summary

Credit Author Statement

Jennifer Bonner: Methodology, Validation, Formal analysis, Writing-Original Draft, Writing-Review & Editing, Visualization; **Howard Burt:** Methodology, Validation, Formal analysis, Writing-Review & Editing, Project administration; **Trevor N Johnson**: Methodology, Validation, Formal analysis, Writing-Original Draft, Writing-Review & Editing, Visualization, Project administration; **Martin J Whitaker**: Conceptualization, Investigation, Writing-Review & Editing, Supervision; **John Porter**: Conceptualization, Investigation, Writing-Review & Editing, Supervision; **Richard J Ross**: Conceptualization, Investigation, Writing-Review & Editing, Visualization, Supervision.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2021.105913.

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