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Application of a Physiologically-Based Pharmacokinetic Model for the Prediction of Bumetanide Plasma and Brain Concentrations in the Neonate

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Running title: Bumetanide and babies: PBPK predictions

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Conflict of Interest Disclosure

Maria Donovan, Khaled Abduljalil, Geraldine Boylan, John Cryan and Brendan Griffin have no conflicts of interest to declare.

Application of a Physiologically-Based Pharmacokinetic Model for the Prediction of Bumetanide Plasma and Brain Concentrations in the Neonate

Abstract

Bumetanide is a loop diuretic that is proposed to possess a beneficial effect on disorders of the central nervous system, including neonatal seizures. Therefore, prediction of unbound bumetanide concentrations in brain is relevant from a pharmacological prospective. A physiologically-based pharmacokinetic (PBPK) model was developed for the prediction of bumetanide disposition in plasma and brain in adult and pediatric populations.

A compound file was built for bumetanide integrating physicochemical data and *in vitro* data. Bumetanide concentration profiles were simulated in both plasma and brain using Simcyp PBPK model. Simulations of plasma bumetanide concentrations were compared against plasma levels published in literature. The model performance was verified with data from adult studies before predictions in the pediatric population were undertaken.

The adult and pediatric intravenous models predicted pharmacokinetic factors, namely area under the concentration-time curve, maximum concentration in plasma and time to maximum plasma concentration, within two-fold of observed values. However, predictions of plasma concentrations within the neonatal intravenous model did not produce a good fit with observed values.

The PBPK approach used in this study produced reasonable predictions of plasma concentrations of bumetanide, except in the critically ill neonatal population. This PBPK model requires more information regarding metabolic intrinsic clearance and transport parameters prior to further validation of drug disposition predictions in the neonatal population. Given the lack of information surrounding certain parameters in this special population, the model is not appropriately robust to support the recommendation of a suitable dose of bumetanide for use as an adjunct antiepileptic in neonates.

ClinicalTrials.gov trial registry, NCT01434225

Keywords: Bumetanide, Brain, Physiologically-based pharmacokinetic modeling, Pediatrics, Disposition

Abbreviations

AUC: area under the concentration-time curve; BBB: blood-brain barrier; Cl: clearance; CL_{int}: hepatic intrinsic clearance; Cmax: peak plasma concentration; CNS: central nervous system; f_u: fraction unbound; IV: intravenous; $Kp_{,brain}$: brain-to-plasma partition coefficient; MRP4: multidrug resistance protein 4; NKCC1: Na-K-Cl cotransporter; OAT3: organic anion transporter 3; Oatp1a4: organic anion-transporting polypeptide 1a4; Obs: observed; PBPK: physiologically-based pharmacokinetic modeling; Pred: predicted; tmax: time to reach peak plasma concentration.

Introduction

Bumetanide is an inhibitor of Na-K-2Cl cation chloride cotransporter (NKCC1) that was initially developed as a loop diuretic for the treatment of edema in adults and children (Cook et al., 1988; Holazo, Colburn, Gustafson, Young, & Parsonnet, 1984; Lau, Hyneck, Berardi, Swartz, & Smith, 1986; Marcantonio et al., 1982; Marshall, Wells, Letzig, & Kearns, 1998; Oberbauer, Krivanek, & Turnheim, 1995). It had been suggested that bumetanide may be used as an adjunct antiepileptic with phenobarbital in the treatment of neonatal seizures and a dose-finding clinical trial of bumetanide has been undertaken in a critically ill neonatal cohort (Dzhala, Brumback, & Staley, 2008; Pressler et al., 2015). The mechanism underlying bumetanide's adjunct antiepileptic activity has been elucidated to be due to the inhibition of intracellular chloride accumulation through NKCC1, thereby facilitating the excitatory to inhibitory switch in gamma-aminobutyric acid signaling (Ben-Ari, 2002). However, permeability of bumetanide across the blood-brain barrier (BBB) has been predicted and shown experimentally to be a limiting factor, as it is a highly plasma protein bound, diprotic acid with pKa values of 3.6 and 7.7 (Fiori et al., 2003), therefore is >99% ionized at physiological pH and bumetanide has been shown to be a substrate of human organic anion transporter 3 (OAT3), murine organic anion-transporting polypeptide 1a4 (Oatp1a4) and human multidrug resistance protein 4 (MRP4), which all operate as efflux transporters at the BBB at physiological pH (Donovan, O'Brien, Boylan, Cryan, & Griffin, 2015; M. D. Donovan, H. Schellekens, G. B. Boylan, J. F. Cryan, & B. T. Griffin, 2016; Puskarjov, Kahle, Ruusuvuori, & Kaila, 2014; Römermann et al., 2017). Despite knowing that the half-maximal inhibitory concentration of bumetanide for NKCC1 is between 200 and 300nM, it is unknown if, and at what systemic dose, this unbound concentration of bumetanide in the brain can be achieved (Puskarjov et al., 2014).

Bumetanide is not licensed for use in the pediatric population as ‘no clinical trials have been carried out in children’, but its use has been reported in critically ill edematous children (Health Products Regulatory Authority, 2016; Marshall et al., 1998; Sullivan, Witte, Yamashita, Myers, & Blumer, 1996). Bumetanide pharmacokinetics in healthy adult volunteers have been described; absorption following oral administration occurs rapidly, with a reported bioavailability of 95% and the maximum plasma concentration measured by two hours post dosing (Ward & Heel, 1984). Bumetanide is highly plasma-protein bound (up to 95%), but the range of volumes of distribution is nonetheless large (12-35 litres). The elimination half-life of bumetanide is approximately 1.25 hours. Half of the administered dose is eliminated unchanged in urine, with hepatic metabolism and biliary excretion accounting for the remainder of the excretion (Halladay, Carter, Glenn Sipes, Brodie, & Bressler, 1975; Ward & Heel, 1984). The main Phase I metabolic route for bumetanide is oxidation of the N-butyl side chain forming inactive alcohol metabolites. Glucuronidation by UDP-glucuronosyl transferase occurs prior to excretion in the urine and bile (Zisaki, Miskovic, & Hatzimanikatis, 2015). It has been estimated that up to 20% of the dose is excreted via the feces (Halladay et al., 1975). Age is a major source of variability in all aspects of pharmacokinetics, including absorption, distribution, metabolism and elimination, and any of the above pharmacokinetic processes could potentially lead to inter-population (adults vs neonates) variation due to developmental changes (Marshall, Wells, Letzig, & Kearns, 1998). Despite these potentially large pharmacokinetic differences, legislation requiring pediatric clinical drug trials to be conducted is relatively new (Leong et al., 2012).

The extensive practice of off-label prescribing in the pediatric population not only leads to a higher risk of adverse drug reactions, but also to low levels of efficacy due to suboptimal dosing of the therapeutic agent (Leong et al., 2012). One of the reasons for this is that maturation of pharmacokinetic processes, such as metabolism and elimination, are not linear with age or bodyweight, thus allometric scaling of adult drug doses to pediatric dosing regimens results in unpredictable and often inaccurate pediatric doses (Espie, Tytgat, Sargentini-Maier, Poggesi, & Watelet, 2009; T. N. Johnson, Rostami-Hodjegan, & Tucker, 2006). Allometric scaling methods, including bodyweight, the three-quarters exponent of bodyweight and body surface area, have been shown to both over- and under-predict suitable doses across the pediatric dose range, emphasizing that children are not just small adults (Bouzom & Walther, 2008; T. N. Johnson, 2008). Conversely, the physiologically-based pharmacokinetic (PBPK) approach, which integrates both physicochemical properties of the drug and physiological properties in a physiologically relevant compartmental structure, has demonstrated usefulness in the prediction of drug pharmacokinetics and inter-individual variability (Nestorov, 2003; Rostami-Hodjegan, 2012; Sager, Yu, Ragueneau-Majlessi, & Isoherranen, 2015). When applied to a pediatric population, the system properties are parameterized with age-dependent physiological parameters, such as tissue volumes and enzyme ontogeny (T. N. Johnson & Rostami-Hodjegan, 2011).

PBPK models are reported to produce superior predictions of concentration-time profiles compared to classic compartmental models, but are burdened by many disadvantages also, such as the need for detailed physiological and drug data which may not be readily available (Bouzom & Walther, 2008; Sager et al., 2015). The PBPK model can be used to predict drug exposure in different tissues as it encompasses each organ as a separate compartment, including inaccessible compartments such as the brain (Sager et al., 2015). In this study, a PBPK model is employed to predict the concentration-time profiles produced by bumetanide in plasma in the adult population and extend it to pediatric population. Validations of these predictions are based on human data which has been published and is available in the literature. One of the main aims of this research was to predict the concentration of bumetanide achieved in the brain compartment of a simulated neonatal population. Ideally, observed brain or cerebrospinal fluid (CSF) concentrations would be compared with predicted values in adults initially, if available, prior to predicting brain/CSF concentrations in neonates. This would improve confidence in the predictions made (Maharaj, Barrett, & Edginton, 2013). CSF sampling in adults of bumetanide concentration is unlikely to be feasible or ethical, as the treatment of seizures with bumetanide is particular to pediatric populations. Nonetheless, the maximum concentrations achieved in brain in the neonatal population can be estimated; however the reliability of this data is weak, as brain/CSF predictions were not validated in an adult population first and CSF samples were not taken from the neonatal study of bumetanide, NEMO (Treatment of NEonatal seizures with Medication Off-patent: evaluation of efficacy and safety of bumetanide, Clinicaltrials.gov identifier - NCT 01434225). This bumetanide PBPK model will enrich our understanding of the mechanisms that underpin the pharmacokinetic differences seen between adults and neonates. This study shows the usefulness of *in silico* predictions in bridging between preclinical *in vitro* and *in vivo* experiments to first-in-neonate trials in the context of this particular clinical situation (bumetanide in critically-ill neonates with seizures).

Materials and Methods

Model structure

Simcyp Simulator V14 (Simcyp Ltd, Sheffield, UK) was used to provide the general structure of the developed PBPK model. The 4-compartmental permeability-limited brain 4brain model which is incorporated into the human whole body PBPK model of the Simcyp Simulator and has been described previously (Gaohua, Neuhoff, Johnson, Rostami-Hodjegan, & Jamei, 2016) was used to predict drug concentrations in the central nervous system (CNS) for this study. Simcyp® is a population-based simulator that performs 'bottom-up' mechanistic modeling and simulation of absorption, distribution, metabolism and excretion processes to predict pharmacokinetic profiles and parameters of drugs and their variation between virtual subjects (Jamei et al., 2009).

Model development

The model of bumetanide was developed as described in Figure 1. The human PBPK model was used to predict bumetanide concentrations in a virtual adult population of healthy volunteers divided into ten trials with ten patients in each trial. Pharmacokinetic parameters from the simulated trials were compared with actual published plasma data from healthy volunteer adults from a total of five different studies (Cook et al., 1988; Holazo et al., 1984; Lau et al., 1986; Marcantonio et al., 1982; Oberbauer et al., 1995). The developed model was ultimately used to predict the pharmacokinetics of bumetanide using Simcyp's Paediatric PBPK model, which was checked for accuracy using plasma samples collected from two trials, one involving a heterogeneous population of nine children with edema (ranging from 3 months to 11.5 years, with one participant aged 25 years) and the NEMO clinical study with fourteen critically-ill neonates with seizures (Marshall et al., 1998; Pressler et al., 2015; US National Institutes of Health, 2015). Simulated trial designs, including the age ranges, male/female ratios and dosing schedules for each respective virtual trial were based on published clinical trials.

Relevant physicochemical properties of bumetanide such as molecular weight, lipophilicity, ionization constants, and fraction unbound in plasma were collated from publications and entered as model inputs (Table 1). Total intravenous clearance of bumetanide was calculated as the mean of the intravenous clearance reported in three studies, weighted based on sample size (Marcantonio et al., 1982; Oberbauer et al., 1995; Pentikainen, Pasternack, Lampainen, Neuvonen, & Penttila, 1985). Tissue partition coefficients of bumetanide were calculated using the Rodgers and Rowland algorithm (Simcyp Kp prediction method 2) (Rodgers & Rowland, 2007). The brain: plasma partition coefficient (Kp_{brain}) was manually changed from that predicted by the Rodgers and Rowland method from physicochemical properties of bumetanide (0.058) to values reported in rodents (0.01 in adult rats, 0.015 in adult mice, 0.06-0.26 in both adult and juvenile rats with seizures induced using different seizure models (Brandt, Nozadze, Heuchert, Rattka, & Loscher, 2010; Donovan et al., 2015; Tollner, Brandt, Romermann, & Loscher, 2015). There are a number of limitations with the predictions of bumetanide concentrations in brain: large interindividual variability in observed plasma concentrations makes it very difficult to compare the concentrations predicted by the model with observed concentrations; there are differences between the predicted Kp_{brain} values based on physicochemical properties and observed Kp_{brain} values in rodents; the translatability of rodent data such as Kp_{brain} to humans, especially critically ill neonates, may not be accurate (Zamek-Gliszczynski et al., 2013) and Kp_{brain} in critically ill neonates is unknown. Thus, the prediction of bumetanide concentrations in brain is a theoretical exercise to explore if predicted concentrations are in the effective range. All available Kp_{brain} values were used to predict brain concentration. The default physiological parameters for healthy adult volunteers and pediatric populations were used. The pediatric population within Simcyp

Simulator has extensive information on pediatric demography, developmental physiology and biochemistry built into it (T. N. Johnson, Zhou, & Bui, 2014). Due to the lack of quantitative *in vitro* data on the hepatic metabolism of bumetanide, hepatic intrinsic clearance was back calculated from the weighted mean of adult intravenous clearance using the retrograde model available in the Simcyp Simulator. Once the hepatic clearance is established in adult, then the adult hepatic intrinsic clearance is scaled to neonates based on the blood flow, liver size, protein binding and ontogeny in children. Thus, a perfusion-limited model incorporating adult clearance values was utilizsed for adult and pediatric simulations.

Model verification

Simulated trials yielded predictions of both pharmacokinetic parameters and concentration-time profiles. Pharmacokinetic parameter predictions were compared against observed values expressed in the literature. Predictions of pharmacokinetic parameters within two-fold of the observed value were considered to be reasonable predictions, as this is a frequently used criterion for accuracy (Musther et al., 2015). Predicted concentration-time profiles were reported as mean, 5th and 95th percentile confidence intervals. Visual predictive checks against the observed mean concentrations ± standard deviations were used to confirm the model accuracy.

Results

Model Simulations of Plasma Bumetanide in Adult Populations

In humans, the PBPK model of bumetanide was verified in healthy adult volunteers using intravenous data (Table 2 and Figure 2). The simulated output demonstrated a two-compartment profile, consistent with published pharmacokinetic models (Jullien et al., 2015; Marcantonio et al., 1982; Popovic et al., 2013). Simulations of intravenous bolus injections generated reasonable pharmacokinetic predictions of area under the concentration-time curve (AUC) (0.71-1.15), peak plasma concentration (Cmax) (0.62-1) and time to reach peak plasma concentration (tmax) (0.5-1) as shown by the predicted/observed ratios (Table 2) and concentration-time profiles overlaid the observed data (Figure 2). As intravenous clearance differed between studies, a weighted mean of clearance was calculated from these studies based on sample size (Marcantonio et al., 1982; Oberbauer et al., 1995; Pentikainen et al., 1985). Renal clearance values were similar across all studies, so a mean value was chosen (Marcantonio et al., 1982; Oberbauer et al., 1995). The predictions made using the Simcyp model were within two-fold of observed values in adults, which meets the criteria for reasonable pharmacokinetic predictions (Table 2).

Model Simulations of Plasma Bumetanide in Pediatric Populations

A pediatric PBPK model for bumetanide was developed using a similar approach to the adult model, this time using the in-built pediatric population in Simcyp®. The pharmacokinetic parameters estimated by the pediatric intravenous model are compared to observed values (Table 3 and Figure 3). The predicted pharmacokinetic parameters from a single intravenous dose of bumetanide in a virtual pediatric population comprising ten trials with ten patients each reflected observed data from a heterogeneous edematous pediatric population well with predicted/observed ratios for AUC, Cmax and tmax calculated as 1, 1.39 and 1 respectively.

Bumetanide plasma levels in neonates were shared with us by the consortium of NEMO trial members, and were used to compare to predicted bumetanide profiles in a virtual neonatal population (Table 4 and Figure 4) (Jullien et al., 2015; Pressler et al., 2015). Since the true ontogeny is unknown, both the fast ontogeny and slow ontogeny scenario were explored. The slow ontogeny function within Simcyp® was used to scale clearance of bumetanide to the pediatric population. However, AUC, Cmax and tmax predicted by the neonatal model fell outside of acceptable limits in certain instances, which could be due to a number of reasons. The observed AUC was taken as the range (1.9-17.7 mg/L.h) reported for AUC of the first bumetanide dose which represents a 9.3-fold difference between maximum and minimum observed AUC, reflecting the large inter-individual variability in this critically ill neonatal population (Jullien et al., 2015). Moreover, there is wide variability between the predicted: observed ratios for Cmax (0.87-2.24) and tmax (0.76-2.4) depending on the dose. This may be partially due to sampling times as observed tmax and Cmax data were taken from the sampled data instead of extrapolation of the sampled point and therefore may not reflect the actual peak values. The estimation of tmax is complicated by the administration of bumetanide as a slow intravenous infusion.

Model Simulations of Brain Bumetanide in Pediatric Populations

The Kp brain was first estimated using Rodgers and Rowland mechanistic method in Simcyp, which predicts drug distribution from physicochemical properties and in vitro data: this gave a predicted Kp brain in the pediatric population of 0.058. While Kp_{brain} could have been estimated from previous *in vitro* experiments in which apparent permeability of bumetanide was measured (Maria D. Donovan, Harriët Schellekens, Geraldine B. Boylan, John F. Cryan, & Brendan T. Griffin, 2016), *in vitro* models have been noted to be poor at predicting of *in vivo* distribution of drugs and a battery of *in vitro* models would be required for accuracy (Garberg et al., 2005). If brain bumetanide concentrations in adults were available to verify predicted concentrations, a similar approach to that taken by Johnson *et al.*, in which *in vitro* data was generated to determine apparent permeability and the efflux ratio of antipsychotics at the BBB, could be utilised to determine bumetanide permeability at the human BBB (M. Johnson et al., 2016). It has been proposed that animal data on Kp can be incorporated into a PBPK model to ensure accurate distribution if such information is available (Musther et al., 2015). Kp_{brain} values are available in the literature for bumetanide in rat (Kp 0.01 (Donovan et al., 2015)), mouse (Kp 0.015 (Tollner et al., 2015)) and rats with seizures (Kp 0.06-0.26, Donovan, O'Driscoll et al., unpublished (Brandt et al., 2010)). An estimation of total bumetanide concentration in brain mass was achieved by using each of these reported plasma: brain values to predict total brain bumetanide concentrations (Figure 5 and Table 5). Across the four doses administered to neonates, there was a 14-fold difference between the Cmax achieved when Kp_{brain} was 0.01 compared to 0.26. The predicted Cmax in brain ranged from 0.0043 mg/L to 0.361 mg/L. These Cmax concentrations represent predictions of total brain concentrations of bumetanide; however, by taking into account that bumetanide has been shown to be highly brain-tissue bound (77% bound), the only predicted concentration which achieves pharmacologically relevant unbound concentrations (0.073 mg/L and 0.109 mg/L) is 0.361mg/L.

Discussion

The main objective of this study was to investigate if an *in silico* PBPK model could be used to predict bumetanide concentrations accurately in plasma and brain tissue in neonates with seizures. PBPK modeling is a method for the prediction of temporal drug concentrations in various organs, including the CNS (Wyska, Swierczek, Pociecha, & Przejczowska-Pomierny, 2015). Using PBPK, it is possible to gain a prospective mechanistic understanding of the pharmacokinetics of a drug in the pediatric population, which facilitates optimal pediatric clinical trial design (Bjorkman, 2005). PBPK predictions in neonates are acknowledged to be difficult due to the rapidly evolving physiology in this age-group, including the changes in volume of distribution, hepatic and kidney function, and remodeling of the vasculature, leading to large inter-individual variability, as demonstrated in the predictions reported here (Bjorkman, 2005). Nonetheless, it may be possible to define a systemic dose of bumetanide that would be predicted to lead to pharmacologically relevant unbound concentrations in the brain using *in silico* PBPK models.

There are reports of the successful use of PBPK in the prediction of pediatric pharmacokinetics and plasma concentration time profiles of oseltamivir (Parrott et al., 2011), clobazam and stiripentol (Ogungbenro & Aarons, 2015), valproic acid (Ogungbenro & Aarons, 2014), voriconazole (Zane & Thakker, 2014), quetiapine (T. N. Johnson et al., 2014), theophylline and midazolam (Bjorkman, 2005), cyclosporine (Gerard et al., 2010), moxifloxacin (Edginton, 2011), sotalol (Khalil & Läer, 2014), acetaminophen ((Jiang, Zhao, Barrett, Lesko, & Schmidt, 2013), lorazepam (Maharaj, Barrett, & Edginton, 2013), theophylline, sildenafil and phenytoin (Abduljalil, Jamei, Rostami-Hodjegan, & Johnson, 2014), paracetamol, alfentanil, morphine, theophylline and levofloxacin (Edginton, Schmitt, & Willmann, 2006). Even though many of the aforementioned drugs are used for disorders of the CNS, total and/or free drug concentrations in the brain are not reported or verified against animal or human data in these studies. This highlights the paucity of information available on the pharmacologically-relevant concentrations at the site of action of many neuroactive drugs in humans. Following verification of the bumetanide PBPK model built for this study using observed human adult data, bumetanide plasma/brain concentration profiles were predicted in a pediatric and a neonatal population. Predicted concentration-time plasma profiles in pediatrics and neonates were compared to available published data and concentration-time profiles shared with the group by the NEMO consortium, respectively. NEMO, a dose-finding clinical study in neonates, investigated the effect of administering up to four doses of bumetanide 0.05mg/kg-0.3mg/kg at 12-hourly intervals on seizure control and reported an adverse benefit: risk ratio, which is likely to be partially due to low concentrations of bumetanide reaching the brain (Pressler et al., 2015).

It is imperative that concentrations of neuroactive drugs at the site of action can be predicted, as they may differ greatly from exposure in other compartments due to a variety of factors including tissue volume, biochemical composition, active transport processes and metabolic clearance (de Lange, Ravenstijn, Groenendaal, & van Steeg, 2005; Kielbasa & Stratford, 2012). The accurate prediction of drug concentrations in the human CNS from preclinical *in vitro* and *in vivo* data is known to be a challenging task (Westerhout, Ploeger, Smeets, Danhof, & de Lange, 2012). It has previously been shown that a bottom-up whole body PBPK modeling program simulated total brain concentrations accurately in rats (Ball, Bouzom, Scherrmann, Walther, & Decleves, 2014). In this study, both predicted and reported Kp_{brain} values from rodent preclinical models were used to estimate total brain concentration of bumetanide in humans, since these values are not available for human populations (Brandt et al., 2010; Donovan et al., 2015; Tollner et al., 2015). Given that the half-maximal inhibitory concentration of bumetanide for inhibition of NKCC1 is between 200nM and 300nM, the unbound concentration of bumetanide achieved in brain has to be within this range for efficacy as a neuro-active therapeutic agent, thus free bumetanide in the brain should be present at concentrations between 0.073mg/L and 0.109mg/L (Puskarjov et al., 2014). As bumetanide displays high non-specific binding to brain tissue, we have calculated that only one of the brain mass concentrations in Table 5 is likely to result in pharmacologically relevant levels of unbound bumetanide in brain i.e. a dose of 0.3mg/kg given to a neonate with a Kp_{brain} of 0.26 (M. D. Donovan et al., 2016; Puskarjov et al., 2014). There are many limitations with this approach, including that Kp_{brain} is only measured at a singular time-point and the output displays total brain tissue concentration as opposed to the unbound active concentration. Furthermore, it should be noted that only one neonate received the highest dose of 0.3mg/kg, and was administered three of the four possible doses at 12-hourly intervals (Pressler et al., 2015). While no rescue antiepileptic medicines were required in this participant indicating drug efficacy, adverse effects of dehydration and hearing loss, which are at least partially bumetanide-related, were reported (Pressler et al., 2015).

Following assessment of this *in silico* model's predictions, we can cautiously postulate that the concentration of bumetanide reaching the CNS in the neonates enrolled in NEMO was sub-therapeutic. Strategies which can increase unbound concentrations of bumetanide in the brain in a safe and effective manner, such as OAT3 efflux transporter inhibition or prodrug administration need further exploration (Donovan et al., 2015; M. D. Donovan et al., 2016; Erker et al., 2016; Tollner et al., 2015; Tollner et al., 2014) However, close monitoring would be required in either of these scenarios to ensure any risk of toxicity is minimized as all of these simulations were carried out retrospectively and fitted to clinical data (Edginton et al., 2006). *In silico* models are a useful preclinical tool for estimation of concentrations in pediatric and neonatal populations; however, the paucity of physiological and transporter data in critically ill neonates with seizures, along with a lack of adult data, means that currently, the PBPK model developed here is not sufficiently robust to be used to completely bridge the knowledge gap between *in vitro* and *in vivo* studies to first-in-neonate dosing of bumetanide. This research has clearly shown that *in silico* prediction depends on the model input information and reflects the unmet need for the in-depth study of neonatal physiology, pharmacokinetics and pharmacodynamics to understand the kinetics and pharmacological action of drugs when they are indicated solely in the pediatric patient.

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Table 1 ***Summary of bumetanide physicochemical properties and pharmacokinetic parameters used for model development in Simcyp.***

Parameter	**Input to Human Model**
Molecular weight (g/mol)	364.4
Compound type	Diprotic Acid
LogP	2.6
pKa_1	3.6 (Fiori et al., 2003)
pKa_2	7.7 (Fiori et al., 2003)
Blood to plasma ratio	0.55 (estimated, weak acid→ 1-haematocrit)
Distribution model	Full PBPK model
Prediction model	Rodgers and Rowland (method 2)
Fraction unbound in plasma (f_u)	0.03 (Shim, Lee, & Lee, 1991)
Total intravenous (IV) Clearance (Cl)	10.17 L/h (Weighted mean from intravenous clearance reported in (Marcantonio et al., 1982; Oberbauer et al., 1995; Pentikainen et al., 1985))
Renal Clearance	4.84 L/h (Marcantonio et al., 1982; Oberbauer et al., 1995)
Hepatic Intrinsic Clearance (CL_{int}) (μL/min/10^6 cells)	17.14 (calculated using retrograde model from intravenous clearance)

Table 2 Summary of predicted versus observed bumetanide pharmacokinetic parameters in the adult population after single intravenous doses

Observed data	**AUC (ng/ml.h)**			**Tmax (h)**			**Cmax (ng/ml)**		
	Pred	**Obs**	**Pred/Obs Ratio**	**Pred**	**Obs**	**Pred/Obs Ratio**	**Pred**	**Obs**	**Pred/Obs Ratio**
Lau et al.: (Lau et al., 1986) 5mg IV bolus	492	635	**0.77**	0.04	0.05	**0.8**	847	1375	**0.62**
Cook et al.: (Cook et al., 1988) 3mg IV bolus	302	292.3	**1.03**	0.04	0.08	**0.5**	510	600	**0.85**
Marcantonio et al.: (Marcantonio et al., 1982) 1mg IV bolus	96.5	136.74	**0.71**	0.04	0.08	**0.5**	154	180	**0.86**
Oberbauer et al.: (Oberbauer et al., 1995) 0.5mg IV bolus	48.7	56.4	**0.86**	0.04	0.04	**1**	83.7	100	**0.84**
Holazo et al.: (Holazo et al., 1984)	102	89	**1.15**	0.04	0.04	**1**	200	200	**1**

Table 3 Summary of predicted versus observed bumetanide pharmacokinetic parameters in the pediatric population after single intravenous doses

Observed data	**AUC (mcg/ml.min)**			**Tmax (h)**			**Cmax (mcg/ml)**		
	Pred	**Obs**	**Pred/Obs Ratio**	**Pred**	**Obs**	**Pred/Obs Ratio**	**Pred**	**Obs**	**Pred/Obs Ratio**
Marshall et al.: (Marshall et al., 1998) 0.1mg/kg IV bolus Retrograde CL_{int} from weighted mean of adult IV clearance, slow ontogeny	36.24	36.1	**1**	0.04	0.04	**1**	1.32	0.95	**1.39**

Table 4 Summary of predicted versus observed bumetanide pharmacokinetic parameters in the neonatal population after multiple slow intravenous infusions

Observed data	**AUC$_{t\ first\ dose}$ (mg/L.h)**			**Tmax* (h)**			**Cmax (mcg/ml)**		
	Pred	**Obs**	**Pred/Obs Ratio**	**Pred**	**Obs**	**Pred/Obs Ratio**	**Pred**	**Obs**	**Pred/Obs Ratio**
Jullien et al., 2015(Jullien et al., 2015) Retrograde CL_{int} from weighted mean of adult IV clearance, slow ontogeny 0.05mg/kg slow IV infusion	0.97	1.9-17.7	**0.05-0.47**	0.72	0.45	**1.6**	0.4	0.46	**0.87**
0.1mg/kg slow IV infusion	1.94	1.9-17.7	**0.11-1.02**	0.72	0.3	**2.4**	0.79	0.71	**1.11**
0.2mg/kg slow IV infusion	3.88	1.9-17.7	**0.219-2.04**	0.72	0.5	**1.44**	1.58	1.06	**1.49**
0.3mg/kg slow IV infusion	5.81	1.9-17.7	**0.33-3.06**	0.72	0.95	**0.76**	2.38	1.06	**2.24**

* Observed tmax is the time to maximum sampled concentration

Table 5 Summary of maximum brain tissue concentration of bumetanide predicted with four Kp_{brain} values

Dose bumetanide administered	**C_{max} brain (mg/L)**			
	Kp 0.01	*Kp 0.015*	*Kp 0.058*	*Kp 0.26*
0.05mg/kg	0.0043	0.0066	0.024	0.0602
0.1mg/kg	0.0087	0.013	0.048	0.12
0.2mg/kg	0.0173	0.0263	0.0959	0.241
0.3mg/kg	0.026	0.0394	0.144	**0.361**

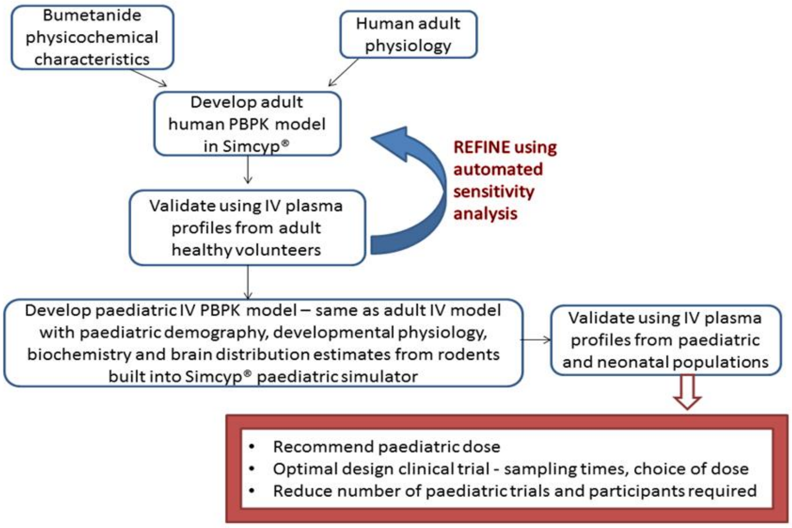

Figure 1 Workflow of bumetanide model development and verification in Simcyp®. IV = intravenous. Plasma concentrations from all populations used for validation were found in the literature and individual studies are referenced throughout.

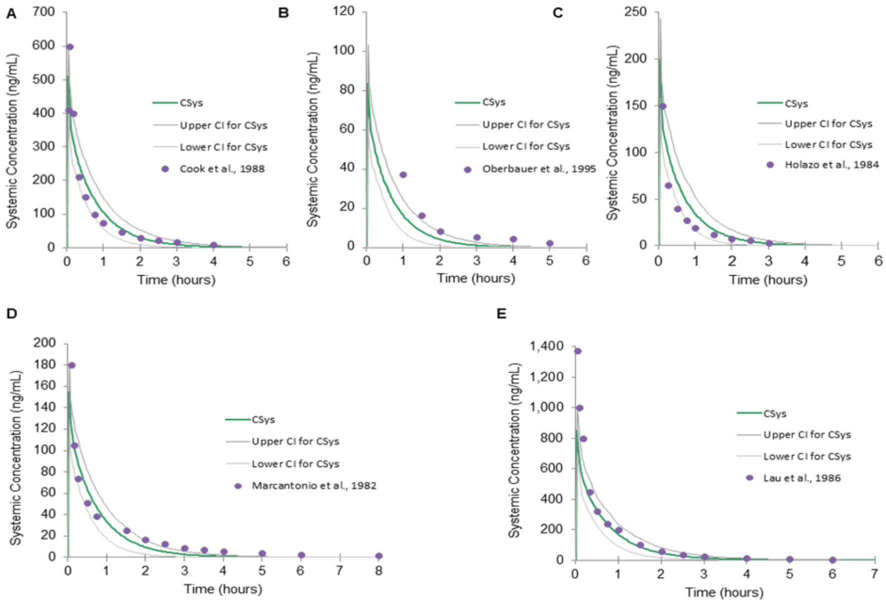

Figure 2 Predicted versus observed bumetanide pharmacokinetic profiles in the adult population after single intravenous doses A) Cook et al. trial, B) Oberbauer et al. trial, C) Holazo et al. trial, D) Marcantonio et al. trial and E) Lau et al. trial. Retrograde CLint calculated from weighted mean of intravenous clearance reported in three studies.

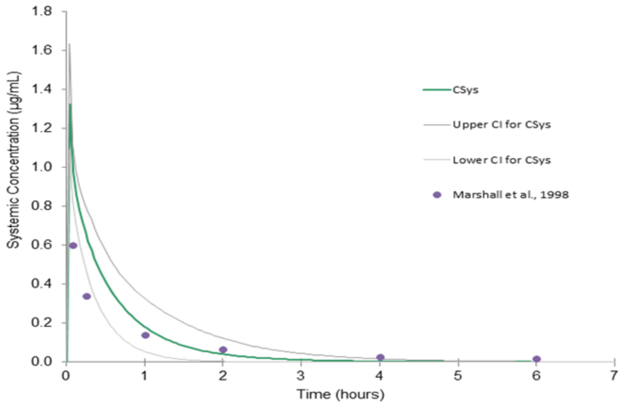

Figure 3 Predicted versus observed bumetanide pharmacokinetic profiles in a pediatric population (Marshall et al. trial) after single intravenous doses. Retrograde CLint calculated from weighted mean of intravenous clearance reported in three studies and slow ontogeny applied to elimination processes.

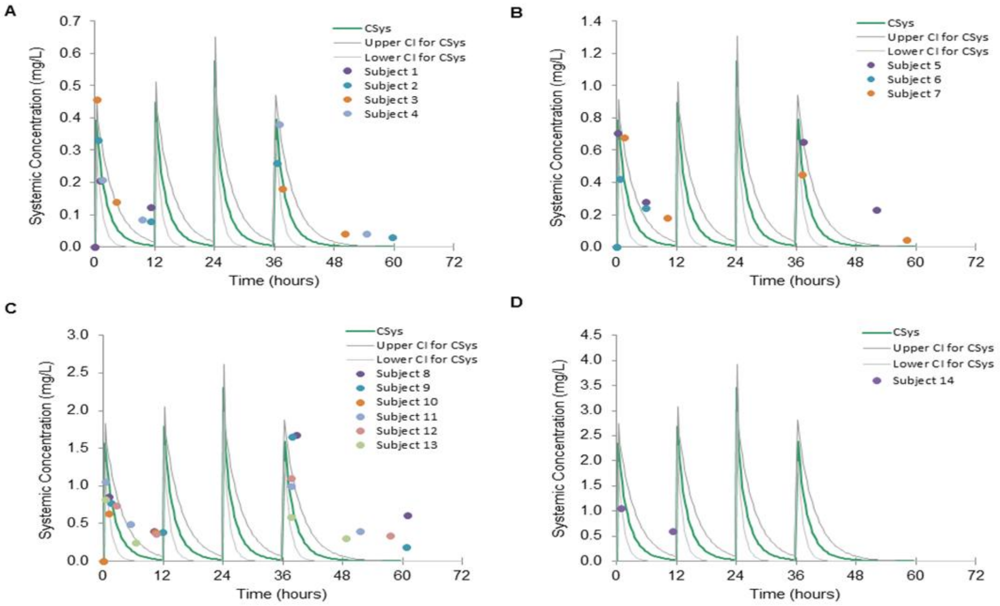

Figure 4 Predicted versus observed bumetanide pharmacokinetic profiles in a neonatal population after multiple (up to four) slow intravenous infusions at 12-hourly intervals (NEMO dose-finding trial) of A) 0.05mg/kg bumetanide, B) 0.1mg/kg bumetanide, C) 0.2mg/kg bumetanide and D) 0.3mg/kg bumetanide. Plasma samples were drawn from each neonate in the observed trial at up to four different time-points. Retrograde CL_{int} calculated from weighted mean of intravenous clearance reported in three studies and slow ontogeny applied to elimination processes.

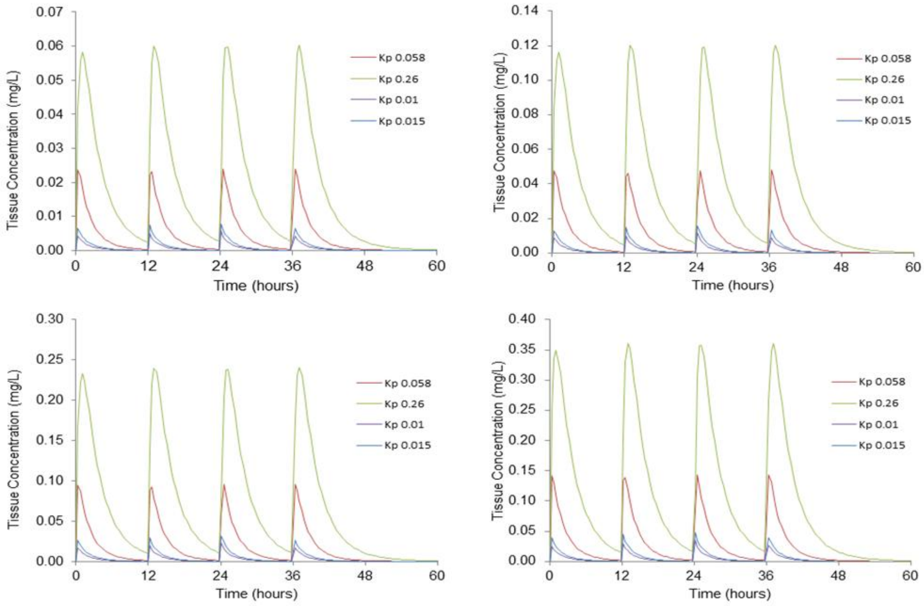

Figure 5 Predicted brain tissue concentrations of bumetanide following intravenous administration every 12 hours of A) 0.05mg/kg bumetanide, B) 0.1mg/kg bumetanide, C) 0.2mg/kg bumetanide and D) 0.3mg/kg bumetanide in neonates. Kp $_{brain}$ values estimated from Simcyp and preclinical studies.