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Simcyp

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Introduction: Genetic polymorphisms in drug metabolizing enzymes and membrane transporters can result in clinically-important variations in drug pharmacokinetics (PK). One such genetic variant is the truncation of the canine *Mdr1* encoded P-gp protein in the collie dog. Typically referred to as "Ivermectin (IVM) Sensitive", collies with this phenotype mutation (ABCB1-1Δ) can exhibit toxicities not encountered in the wild-type (WT) dog. However, even in animals homozygous for the ABCB1-1Δ gene (mu/mu), there can be a range of doses tolerated before an adverse central nervous system (CNS) response is manifest.

Using Simcyp Dog (Version 17R1) a collie specific physiologically based pharmacokinetic (PBPK) model was developed for loperamide. FDA-generated data on loperamide PK and CNS toxicity in IVM sensitive dogs (**Myers *et al.,* 2015**) was used to verify the predicted plasma concentration-time (Cp-t) profiles. Although not an avermectin, loperamide is a P-glycoprotein (P-gp) substrate, gaining access to the brain in collies lacking functional P-gp which elicits CNS adverse effects. Use of breed specific PBPK models can facilitate our understanding of the multifactorial differences in drug PK and pharmacodynamics (PD) across the canine population.

Objective: The primary objective of this work is to use the PBPK Collie dog model in conjunction with the multi-compartment brain model to predict brain concentration – time (C_{Brain}-t) profiles based on passive diffusion and P-gp kinetics. The secondary objective (as an initial effort) is to verify whether the predicted C_{brain} C_{Brain}-t profiles can predict qualitatively the observed CNS toxicity in mutant collies compared to their WT counterparts.

Methods: Loperamide *in vivo* Cp-t profiles were available after oral administration of doses of 0.01, 0.05, 0.1 and 0.2 mg/kg in collies pre-screened for genotype and IVM sensitivity (7 WT, 16 heterozygotes, and 10 mu/mu collies). Ascending doses were sequentially administered until dogs exhibited onset of CNS toxicity. Four dogs (2 WT & 2 mu/mu collies) were selected for this pilot PBPK modeling (Figure 1). Since all WT dogs had similar Cp-t profiles, two collies were randomly selected (Pearl WT and Whisper WT). From the 10 mu/mu collies, Ashley Mut (highest Cp-t profiles for all doses) and Barney Mut (Cp-t profiles similar to WT) were selected (**Fig 1.**).

System Parameters: Collie anatomy and physiology data were collated from published literature (**Table 1**). Beagle data normalised (by body weight) was used when collie specific data was not available. **Drug Parameters:** Loperamide parameters were obtained from literature (**Table 2**). Using the multi-compartment brain model within the Simcyp Collie Simulator, C-t profiles in the brain interstitial fluid (ISF), brain blood, cranial CSF, and spinal CSF were predicted simultaneously. For the mu/mu dogs, it was assumed that all brain concentrations equilibrated passively with the plasma. For the WT dogs, brain C-t profiles were modeled using literature-based absolute abundance of P-gp in the Blood Brain Barrier (BBB) and Blood Cerebrospinal Fluid Barrier (BCSFB) and using Michaelis-Menten (J_{max}/Km) kinetics for Loperamide.

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14th International Congress of the European Association for Veterinary Pharmacology and Toxicology, Wroclaw, Poland; 24th – 27th June, 2018

P13.1: Section 13. Modelling and Simulation

PBPK Modelling of Collie Plasma and Brain Concentrations as Impacted by P-gp to Explore Loperamide-Induced CNS Toxicity (Pade *et al.* Slide 2 of 4)

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Figure 1: Observed & Predicted loperamide plasma Cp-t following oral administration of (A) 0.01, (B) 0.05, or (C) 0.1 mg/kg loperamide oral solution.
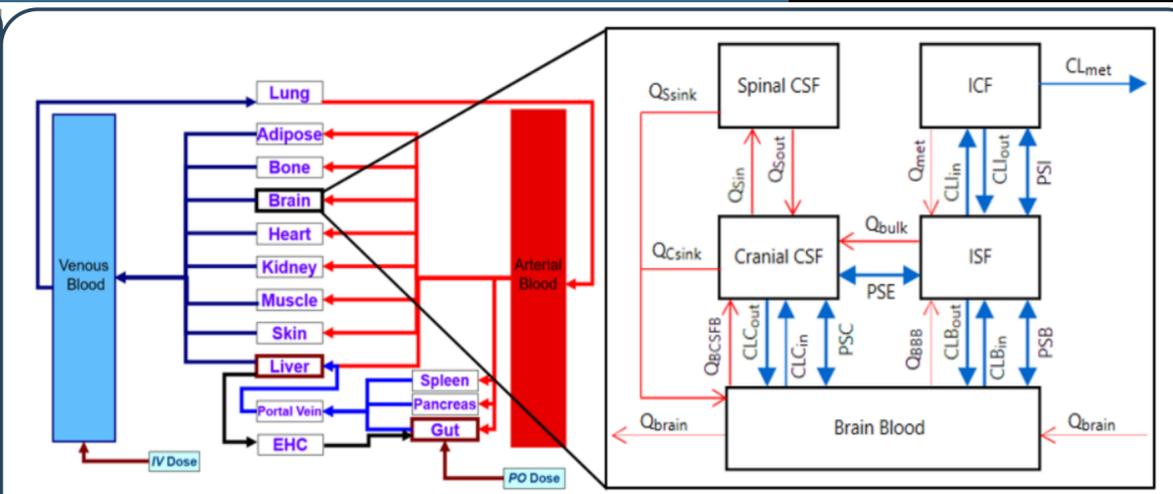
Figure 2: Simcyp Animal 5 - Compartment brain model within the full PBPK model ICF: Intra-cellular Fluid; ISF: Interstitial Fluid; CSF: Cerebrospinal Fluid

Table 1: List of Collie Breed System Parameters

Collie Parameters	Value/Source
Body Weight (kg)	23
Cardiac Output (mL/min)	2480/Collie
Whole body tissue Volumes	Collie
Tissue blood flows	Normalised using Beagle data
GI Length/Diameter	Collie
Gastric emptying	Beagle
Transit (SI & Colon)	Collie
Brain tissue volume (mL)	87.4/Collie
Brain Mass Vol (ICF + ISF) (mL)	47/Collie
CSF Volume (Spinal + Cranial) (mL)	38/Collie
CSF Production rate (mL/min)	0.08/Collie
P-gp abundance – BBB (fmol/µg protein)	6.2/Beagle
P-gp abundance – BCSFB (fmol/µg protein)	0.146/Beagle

Table 2: Five Compartment Brain model parameters

Parameter	Definition/Value
QBrain	Qbrain Cerebral blood flow (69.4 mL/min)
PSB	PS of BBB
PSC	PS of BCSFB
PSI	PS of ICF-ISF Barrier
PSE	PS of CSF-ISF-Barrier
CLBin & CLBout	Apparent clearance of BBB uptake & efflux transporters
CLCin & out	Apparent clearance of BCSFB uptake & efflux transporters
CLIin & CLIout	Apparent clearance of ICF uptake & efflux transporters
Qbulk	Bulk flow of CSF from ISF to cranial CSF (0.03 mL/min)
QCsink	CSF absorption rate from cranial CSF section
QSsink	CSF absorption rate from spinal CSF section
QSin	CSF flow rate form cranial to spinal section
QSout	CSF flow rate form spinal to cranial section
CLmet	Brain Metabolic clearance
All values in [mL/min]; PS: Passive permeability surface area product	

Table 3: Loperamide Input parameters

Loperamide (base)	Value
Mol. Wt (g/mol)	477
$LogP_{O:W}$	3.86
pKa (type)	8.91 (Base)
B:P ratio	1.08
fu plasma	0.034
SI Passive Peff (x 10^-4 cm/s)	0.13
V_{SS} (L/kg) Predicted	2.4
CL (mL/min)	Individually fitted
fu Brain (ICF & ISF)	0.01
fu CSF	1
PS-BBB (mL/min)	115
PS-BCSFB (mL/min)	50
PS-ISF-ICF (mL/min)	50
PS-ISF-CSF (mL/min)	300
P-gp J_{max} (pmol/min)	10 (fitted)
P-gp Km (µM)	0.0001 (fitted)
REF (BBB)	1.342
REF (BCSFB)	0.031

Results: For all 4 dogs, the collie PBPK model resulted in close agreement between the simulated and observed Cp-t profiles (**Figure 1**) providing justification to estimate brain C-t profiles for loperamide.

Ashley Mutant: Driven by the high blood levels (increasing in greater than dose proportionality), Ashley had very high loperamide concentrations in all brain compartments (**Figure 3**) with observed toxicity after 0.1 mg/kg (**Table 4**).

Barney Mutant: Cp-t profiles were similar to the WT, but the lack of functional P-gp led to drug being present in the ISF and ICF with CNS toxicity first occurring at the 0.1 mg/kg dose. However, Barney's brain concentrations at 0.1 mg/kg were similar to Ashely's 0.05 mg/kg (when toxicity did not occur).

Whisper WT: The Michaelis Menten J_{max} and Km values for P-gp efflux were determined by fitting, based on observed toxicity reports in dogs and humans at oral doses of ~ 3-5 mg/kg (**Hugnet 1996; Stanciu 2017**). When Whisper WT was simulated with a dose of 5 mg/kg, saturation of BBB P-gp efflux was observed at J_{max}: 10 pmol/min and Km: 0.1 nM (**Figures 4 & 5**).

PBPK Modelling of Collie Plasma and Brain Concentrations as Impacted by P-gp to Explore Loperamide-Induced CNS Toxicity
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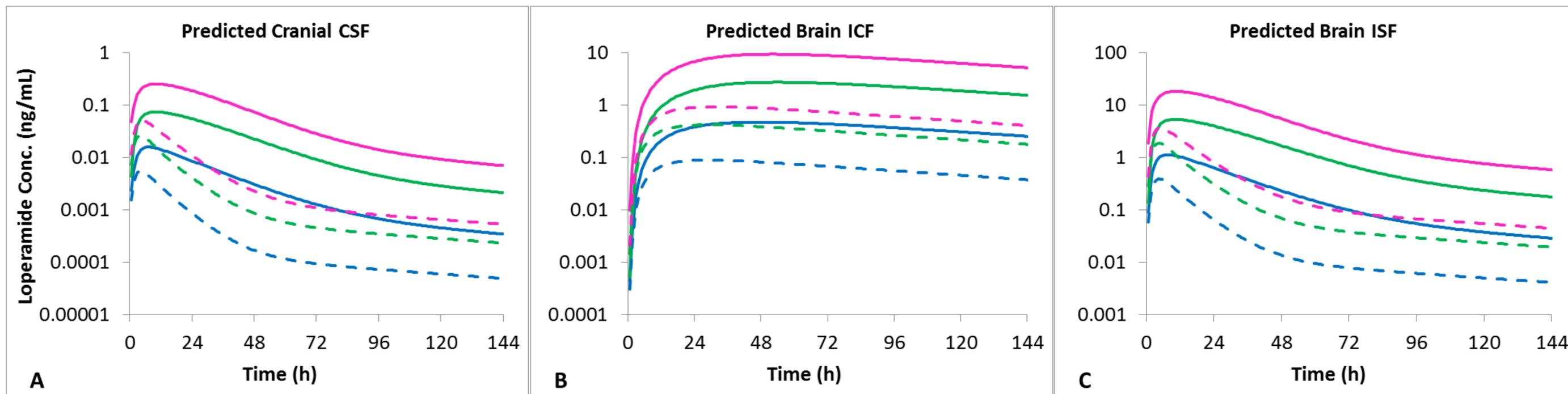
Figure 3: Predicted brain concentrations in mutant dogs **Solid line = Ashley Mut**; **Hatched line = Barney Mut.** **Blue = 0.01 mg/kg**; **Green = 0.05 mg/kg**; **Pink = 0.1 mg/kg**

Table 4: C_{max} and corresponding CNS Observations

C_{max} (ng/mL)	0.05 mg/kg	0.1 mg/kg		0.2 mg/kg		1 mg/kg	5 mg/kg
	Ashley Mut	Ashley Mut	Barney Mut	Whisper WT	Pearl WT	Whisper WT	Whisper WT
ICF	2.70	9.2	0.92	0.11	0.13	0.78	27.78
ISF	5.27	18.0	3.66	0.54	0.72	4.58	144.38
Cranial CSF	0.07	0.3	0.05	0.03	0.03	0.16	1.01
Tox. Obsd at Dose (mg/kg)	0.1	0.1	0.1	No CNS toxicity		CNS Tox. Possible	CNS Tox. Expected

Figure 4: P-gp Efflux Clearance for Whisper WT. Note P-gp saturation at 5 mg/kg (associated with CNS toxicity in humans and dogs).
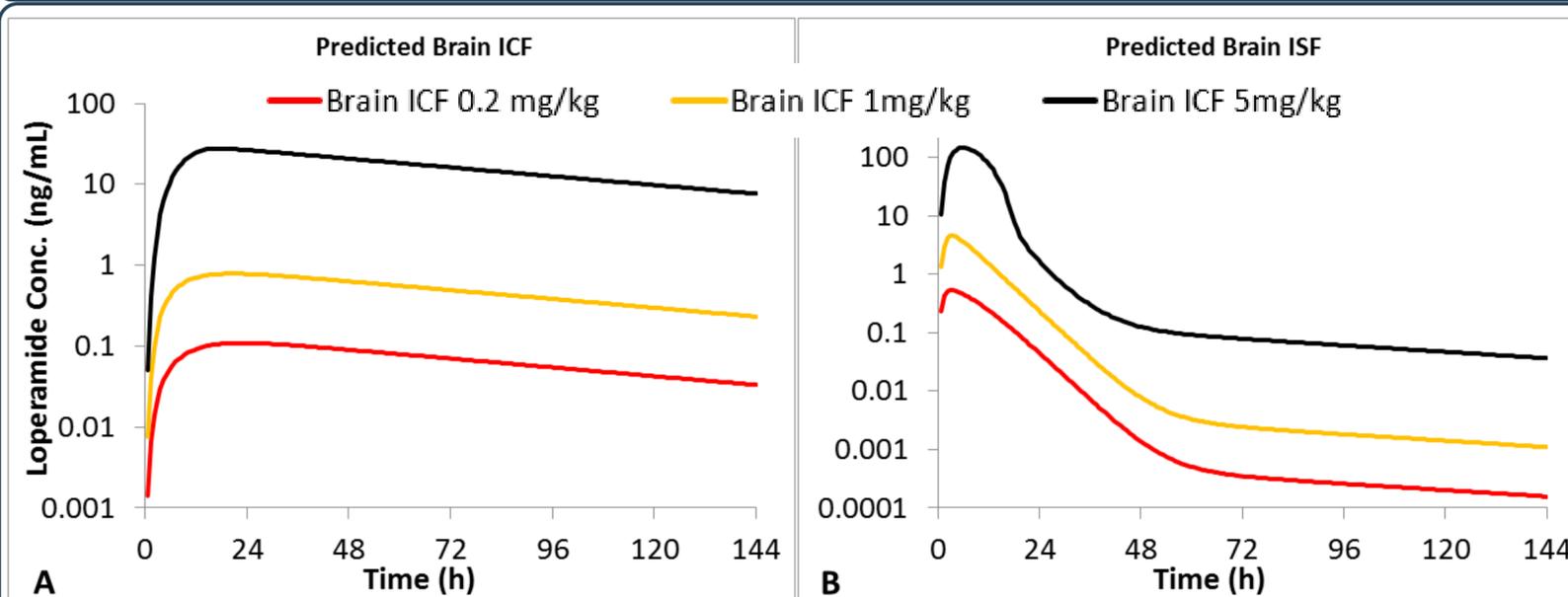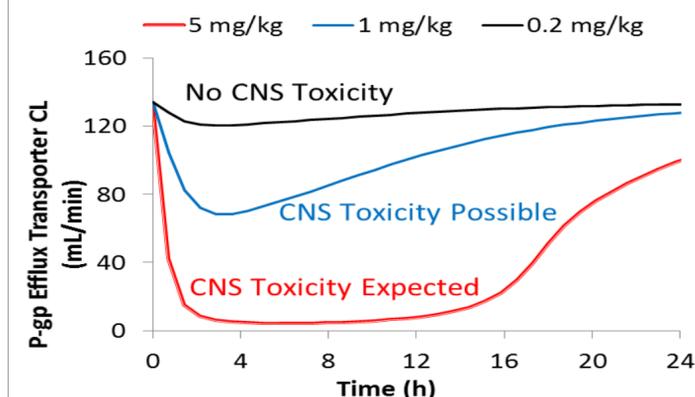
Figure 5: Predicted Brain Conc. in WT Dog (Whisper)

PBPK Modelling of Collie Plasma and Brain Concentrations as Impacted by P-gp to Explore Loperamide-Induced CNS Toxicity
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Discussion: Based on the PBPK predictions, WT dogs had CSF concentrations only slightly less than that of the mu/mu dogs for the administered doses, reflecting a compartment driven by passive diffusion. This reflects the low (or negligible) P-gp abundance in the BCSF barrier. ICF and ISF concentrations in WT dogs tended toward zero due to active efflux.

Assuming the presence of functional P-gp blocks loperamide accumulation in the ISF (**Mealey *et al.*, 2008**), we can estimate the range of ISF concentrations associated with CNS toxicity based upon data generated in the mutant dogs. This approach can then be further applied to estimate the range of P-gp activity across the heterozygotes (where some had adverse effects while others did not). The variability in CNS response for the heterozygotes ('yes' vs, 'no' toxicity) can be due to variability in proportion of functional vs. non functional P-gp. Note that the J_{max} and Km values for P-gp were estimated based on a single dose (5 mg/kg) where loperamide toxicity was reported in beagle dogs (**Hugnet *et al.*, 1996**)

Ultimately, this analysis raises four points to consider regarding the potential for adverse CNS effects for other P-gp substrates:

1. Blood levels themselves do not necessarily imply if an adverse CNS effect will occur in dogs carrying the MDR 1 mutation.
2. There appears to be marked inter-dog variability in the observed CNS toxicity for the corresponding CNS concentration.
3. Drugs other than the avermectins may be important to consider in terms of potential CNS toxicity in dogs carrying the ABCB1-1Δ gene.
4. *In silico* models can support our understanding of variability in CNS permeability of these substrates in dogs heterozygous/mutant for the *MDR-1* gene.

Next steps: PBPK modeling for all Collies:

➢ Get a more refined estimate of the P-gp saturation kinetics for a range of loperamide concentrations
➢ Predict systemic loperamide concentrations for all collie dogs (7 WT, 16 heterozygotes, 10 mu/mu) using the Collie PBPK model
➢ Use the PBPK predicted systemic concentrations to estimate brain concentrations in all Collie dogs
➢ Estimate abundance/activity of functional P-gp for the heterozygotes (responders vs non-responders) to predict brain concentrations at which toxicity was observed/not observed
➢ Evaluate the range of relative abundance/activity of P-gp in the heterozygous Collie dogs.

Conclusions:
The Simcyp Collie PBPK model in conjunction with the 5-compartment brain model can satisfactorily predict systemic & brain concentrations in WT and mutant collies. This underscores the need to consider not just PK but also PD differences due to individual differences in the sensitivity of the CNS receptors.

References:
Myers *et al.* Drug Metab. Dispos., 43:1392–1407, 2015
Stanciu *et al.* J. Psychoactive Drugs, 49 (1), 18–21, 2017
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