W4313

# Use of *In Silico* Physiologically Based Pharmacokinetic (PBPK) Models to Predict Food Effects in Dogs

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## Purpose

To investigate the accuracy of *in silico* models in the prediction of *in vivo* drug absorption in dogs, using an evaluation of food effects as a first step in this assessment.

## Background

Unlike the relative rich body of information available to support the development of human *in silico* pharmacokinetic models, there exist numerous gaps confounding a corresponding use of *in silico* population predictions for dogs. Such modeling tools can be invaluable for identifying those variables that can influence *in vivo* drug product performance in the targeted canine patient population and for facilitating interspecies extrapolations (dog to human and human to dog) during the early phases of human or canine drug product development.

As a first step toward characterizing *in silico* models as a prognostic tool for canine *in vivo* drug absorption, the Simcyp software was used to predict the effect of food on two monoprotic acids, celecoxib (pKa = 11.1) and mavacoxib (pKa = 9.57). Both drugs are considered to be highly permeable (Cox et al., 2010; Paulson et al., 2001). Furthermore, both drugs exhibit substantial improvement in canine drug absorption when administered with food. However, while canine celecoxib elimination is rapid (with $t_{1/2}$ ~ approximately 2 – 4 hr) and was highly dependent upon phenotype [extensive metabolizers (EM) exhibited a total systemic clearance (CL) of 0.9876 L/hr/kg while poor metabolizers (PM) had a corresponding CL value of 0.2216 L/hr/kg], mavacoxib is primarily eliminated as unchanged parent in the feces (approximately 60 % of the dose after oral administration). Furthermore, due to its high affinity protein binding, mavacoxib is associated with a CL value of 0.0027 L/hr/kg. Lastly, to better understand *in vivo* differences that can impact the extrapolation of absorption information between humans and dogs, we compared the accuracy of our canine celecoxib food effect predictions to the accuracy achieved when human celecoxib fed/fasted predictions were generated using the human Simcyp module.

Pivotal questions addressed in this study were as follows:

- What are the model considerations needed to obtain the predictions that most closely reflect the *in vivo* food effects in dogs and humans?
  - ❖ Using intrinsic drug solubility
  - ❖ Use of segmental drug solubility as reported for the human small intestine
- What factors may be responsible for the observed celecoxib food effect in the dog versus the lack of a corresponding food effect in human?
- What are potential areas of future investigation if the prediction errors are large?

## Methods

Systemic concentration-time profiles were generated using the Simcyp Animal (V12, canine module) and Human (V12) software. Model input parameters are summarized in Table 1.

**Celecoxib**:
The data used for modeling canine and human celecoxib PK profiles were derived from the manuscript by Paulson et al., (2001). Canine and human data were both obtained following the oral administration of an identical formulation: bulk drug in capsule. The human dose was 200 mg and the canine dose was 5 mg/kg. The canine datasets were subdivided into EM and PM dogs, each modeled separately.

We note that although the Paulson study provided both canine intravenous (IV) and oral data sets, these studies were conducted in different animals. It was the dogs that were administered only the oral dose that was used for our simulated comparisons. Therefore, volume and CL estimates provided in the IV dataset needed to be slightly modified to improve the accuracy of model predictions of those animals from which the fasted and fed state data were derived.

**Table 1: Summary of the simulation input values used for the canine and human datasets (Human estimates for CL and Volume based upon Davies et al., 2000).**

| CELECOXIB | | | MAVACOXIB | |
|---|---|---|---|---|
| | Dog | Human | Dog | |
| Dose (mg) | 50 | 200 | Dose (mg) | 40 |
| Mol Wt. | 381.38 | 381.38 | Mol Wt. | 384 |
| pKa | 11.1 | 11.1 | pKa | 9.57 |
| log P | 3.050 | 3.050 | log P | 3.800 |
| B/P | 0.89 | 1 | B/P | 0.98 |
| fu | 0.015 | 0.026 | fu | 0.02 |
| Vss (L/kg) | 2.216 | 1.35* | Vss (L/kg) | 1.64 |
| CL (iv) (mL/min) (EM) | 164.6 | 667.0* | DLM Clint (µL/min/mg) | 1.718 |
| CL (iv) (mL/min) (PM) | 74.000 | | | |
| CLr (mL/min) | | 0.0407 | CLr (mL/min) | 0.040 |
| | | | | |
| Celecoxib Intrinsic Solubility (mg/mL) | | | | |
| 0.005 (Experimental) | | 0.05 (Predicted) | | 0.003** |
| Dog Segmental Solubility (mg/mL) | | Human Segmental Solubility (mg/mL) | | |
| Stomach | 0.003 | Stomach | 0.002 | |
| Duodenum | 0.003 | Duodenum | 0.046 | |
| Jejunum I | 0.006 | Jejunum I-II | 0.046 | |
| Jejunum II-V | 0.015 | | | |
| Ileum | 0.015 | Ileum I-IV | 0.046 | |
| Colon | 0.015 | Colon | 0.046 | |

*$V_{ss}$/F; CL/F
** Estimation of intrinsic solubility based upon values reported by Cox et al. (0.006 mg/mL) resulted in a marked inflation of the predicted drug concentrations. This is consistent with what we observed with celecoxib in dogs. The value of 0.003 mg/mL represents the value which resulted in the best fit predictions.

Due to the limitations in the information pertaining to the dog, a minimal PBPK model was employed. For consistency, we likewise used the minimal PBPK model for predicting human food effects (healthy Caucasian population module).

Segmental solubility assessments were based upon the celecoxib biorelevant fasted small intestinal fluid solubility values reported by Shono et al., (2009). These values were adjusted for dogs in an exploratory manner to facilitate our understanding of human/canine differences. Furthermore, since we did not have *in vivo* solubility estimates in dogs or humans, we began our evaluation with the estimate of intrinsic solubility (IS) reported in Drugbank.ca (0.005 mg/mL). We noted that although that value worked well in describing the observed canine dataset, it failed to adequately describe the corresponding human dataset. Therefore, the human predictions were to a value of 0.05 mg/mL, which coincides with the predicted IS derived with the Simcyp tool box. Potential reasons for the inconsistency in human-canine solubility estimates were explored.

**Mavacoxib**:
Canine mavacoxib data were obtained from Cox et al., 2010. Only canine data were available for this drug, which exhibits extensive, high affinity protein binding. No phenotypic differences in drug metabolism were defined in the published dataset. Due to mavacoxib's protein binding characteristics, it was necessary to estimate the intrinsic unbound hepatic clearance. This was achieved by converting the CL (from IV data) to liver microsomal intrinsic clearance based on the Simcyp Retrograde Model (Cubitt 2011). For IS, we began our predictions using the reported value of 0.006 mg/mL. This value was modified as necessary to improve our model predictions.

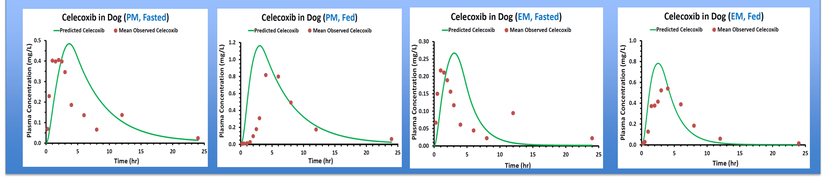
## Results

The absorption parameter values obtained for celecoxib and mavacoxib are provided in Table 2.

**Celecoxib:**

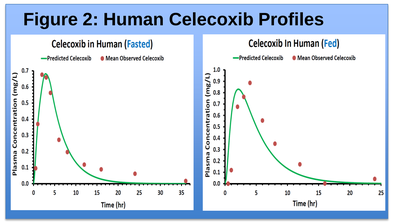
Dog:
Using the Drugbank estimate of IS for dogs, the canine Simcyp module provided a good approximation of peak ($C_{max}$) and total drug exposure ($AUC_{0\text{-}inf}$) for both the PM and EM dogs. For both canine populations, the model also provided an excellent qualitative estimate of the impact of food on drug absorption. The most noticeable error in the dataset was related to predictions of time to peak concentrations ($T_{max}$). Since the relative error was similar in EM and PM dogs, this error appears to reflect absorption model mis-specification. The resulting fitted profiles (fasted and fed) are shown in Figure 1.

**Figure 1: Canine Celecoxib Profiles**




Human:
To model the observed human data, the reported IS value needed to be increased from the 0.005 mg/mL 0.05 mg/mL. With this higher solubility estimate, the percent of drug absorbed under fasted conditions increased to 88% and therefore, there were negligible food effects. While the time to peak concentrations was well predicted in the fasted state, predictions peaked earlier than observed values in under fed conditions. Human observed versus predicted celecoxib concentration–time profiles are provided in Figure 2.

**Figure 2: Human Celecoxib Profiles**




The ratio of predicted to observed AUC and $C_{max}$ value across all datasets are provided in Figure 3. A comparison of the fed/fasted ratios for AUC and $C_{max}$ in all observed and predicted datasets is provided in Figure 4.

**Figure 3: Ratio of Pred/Obs Values**

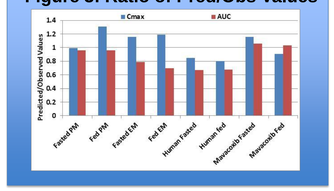



**Figure 4: Fed/Fasted Ratios**

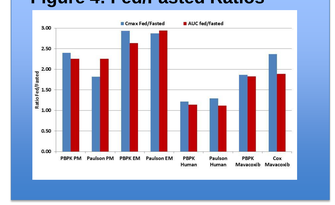



**Table 2: Summary of observed and predicted bioavailability data**

| Subj | State | Dataset | Tmax (h) | Cmax (mg/L) | AUCinf (mg/L.h) |
|---|---|---|---|---|---|
| **Celecoxib** | | | | | |
| Dog PM | Fasted | IS | 3.61 | 0.48 | 3.75 |
| | | Seg | 4.08 | 0.37 | 3.05 |
| | | Paulson | 7.50 | 0.49 | 3.90 |
| | Fed | IS | 3.00 | 1.16 | 8.45 |
| | | Seg | 3.12 | 1.24 | 8.91 |
| | | Paulson | 7.30 | 0.89 | 8.80 |
| Dog EM | Fasted | IS | 3.00 | 0.27 | 1.34 |
| | | Seg | 3.60 | 0.20 | 1.10 |
| | | Paulson | 1.50 | 0.23 | 1.70 |
| | Fed | IS | 2.53 | 0.78 | 3.52 |
| | | Seg | 2.76 | 0.74 | 3.20 |
| | | Paulson | 4.00 | 0.66 | 5.00 |
| Human | Fasted | IS | 2.89 | 0.68 | 4.40 |
| | | Seg | 3.79 | 0.33 | 2.46 |
| | | Paulson | 2.44 | 0.81 | 6.56 |
| | Fed | IS | 2.16 | 0.83 | 5.00 |
| | | Seg | 4.32 | 0.47 | 3.58 |
| | | Paulson | 3.42 | 1042.00 | 7.32 |
| **Mavacoxib** | | | | | |
| Dog | Fasted | | 9.49 | 1.21 | 724.59 |
| | | Cox | 67.40 | 1.04 | 683.00 |
| | Fed | | 9.48 | 2.24 | 1320.00 |
| | | Cox | 17.40 | 2.46 | 1290.00 |

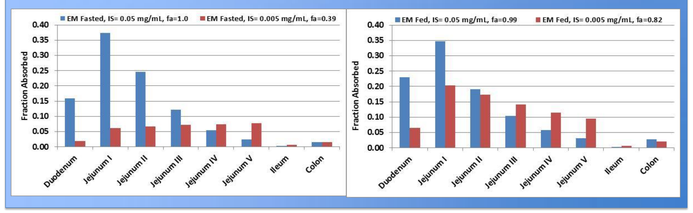
**Discrepancies in Values Human vs. Canine for Solubility**
Our first step was to show that the lowering of permeability (e.g., from 2.11 cm^-4/sec to a value of 0.2 cm^-4/sec) would not influence our prediction outcomes, thereby confirming that our examination need only focus on *in vivo* solubility and dissolution. To determine a potential reason for observed interspecies differences (both in terms of predictions and observed food effects), we used the human solubility estimate of 0.05 mg/mL and subsequently modified *in vivo* dissolution. This was accomplished by adjusting the effective diffusion layer thickness $h_{eff}$, which is inversely correlated to dissolution rate in accordance with the Noyes Whitney equation:

$$DR(t) = -N\frac{D_{eff}}{h_{eff}(t)}4\pi a(t)\left(a(t)+h_{eff}(t)\right)\left(C_{surface}-C_{bulk}(t)\right)$$

where DR =dissolution rate, t = time, N = number of particles, D*eff* = diffusion coefficient, σ=particle radius; A=particle surface area, Cs=particle surface solubility; Cb=bulk concentration

Decreasing the dissolution rate (increasing $h_{eff}$) improved our ability to estimate AUC and $C_{max}$ in the canine fasted state (despite use of the 10-fold higher human solubility estimate) but effectively eliminated the food effect (similar to the small food effect seen in the human dataset). Thus, there appears to be an inherent difference in the human and canine GI tracts that impact the *in vivo* solubility (and therefore dissolution) of some molecules. Characterization of this difference requires future examination.

**Figure 5: Regional Distribution Fraction of Dose Absorbed**




We next examined the impact of prandial state and solubility estimate on the regional intestinal absorption (Figures 5 - EM dogs only). The higher solubility values resulted in a rapid and extensive drug absorption in the proximal segments of the small intestine (thereby increasing $C_{max}$). In the fasted state, the overall increase in absorption resulted in a marked over-estimation of fasted AUC thereby negating the food effects (similar to that observed and predicted in humans). This suggests that differences in food effects in dogs and humans may not only reflect interspecies differences in intestinal solubility but also to differences in GI transit time.

Segmental vs. Intrinsic solubility:
For both human and canine datasets, the accuracy of the predicted values was further challenged by using published human *in vitro* segmental solubility. Considering the known differences in GI fluid composition of the human and the dog (Arnt et al., 2013), these results underscore the importance of obtaining canine-specific solubility data when generating *in silico* PK predictions. The greatest magnitude of error was observed when the estimates were generated using segmental solubility in the PM dogs. However, even when modeling the human data, the error associated with food effect predictions using the segmental solubility data was consistently greater than that obtained with the intrinsic solubility (Table 2)

**Mavacoxib:**
In contrast to celecoxib, mavacoxib was associated with a very long terminal depletion phase and has negligible drug metabolism. Thus, by exploring the ability to model mavacoxib in dogs, we could focus on the absorption component of the *in silico* predictions. For mavacoxib, despite the presence of a substantial food effect, we succeeded in modeling AUC values under both fed and fasted conditions. The absolute errors in the $C_{max}$ estimates were likewise similar, although $C_{max}$ was slightly over-estimated in fasted dogs and under-estimated in fed dogs. We note that similar to that observed with celecoxib, efforts to use reported values of intrinsic solubility lead to an over-inflation of the predicted concentration-time profiles. The similarity of these findings for the two drugs suggests that we are likely to introduce substantial error in our predictions when attempting to use *in vitro* physico-chemical drug characteristics as model parameters for generating blood level canine drug concentration-time profiles. Results are seen in Table 2 and Figures 3 and 4.

## Discussion and Conclusions

Although *in silico* models have the potential to serve as important prognostic tools for predicting factors influencing canine drug absorption and inter-species extrapolation of drug absorption variables, there are several factors that have been identified as requiring additional investigation:

- It would appear that the error in canine $C_{max}$ estimates largely reflect the difficulty encountered with accurately assessing not only *in vivo* drug solubility but also the precise location of drug absorption. In this regard, the dog is a far more sensitive system as compared to humans.
- We need to determine why there is a 10-fold difference in between the solubility value estimates that provided the best fit for the human versus canine datasets. Intrinsic solubility is a function of the drug properties and not a function of the species GI tract, our results show that, when predicting the in vivo solubility, the model clearly needs to consider other variables associated with the GI milieu that may be as influential on drug performance as the IS itself.
- To date, we have not studied high solubility/ low permeability compounds to ascertain the impact of our limited information on canine GI physiology and the "what if" model scenarios that can be used to better predict drug absorption in the absence of drug specific canine absorption data. Therefore, our next step in this effort is to begin exploring the prediction errors associated with drugs presenting with other physiochemical characteristics. Both the human drug classifications as defined by the BCS and the BDDS will be considered when selecting the next set of compounds for our simulation and modeling assessments.

## References

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