



## Review Article

Theme: Celebrating Women in the Pharmaceutical Sciences

Guest Editors: Diane Burgess, Marilyn Morris and Meena Subramanyam

# Comparison of Canine and Human Physiological Factors: Understanding Interspecies Differences that Impact Drug Pharmacokinetics

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**Abstract.** This review is a summary of factors affecting the drug pharmacokinetics (PK) of dogs *versus* humans. Identifying these interspecies differences can facilitate canine-human PK extrapolations while providing mechanistic insights into species-specific drug *in vivo* behavior. Such a cross-cutting perspective can be particularly useful when developing therapeutics targeting diseases shared between the two species such as cancer, diabetes, cognitive dysfunction, and inflammatory bowel disease. Furthermore, recognizing these differences also supports a reverse PK extrapolations from humans to dogs. To appreciate the canine-human differences that can affect drug absorption, distribution, metabolism, and elimination, this review provides a comparison of the physiology, drug transporter/enzyme location, abundance, activity, and specificity between dogs and humans. Supplemental material provides an in-depth discussion of certain topics, offering additional critical points to consider. Based upon an assessment of available state-of-the-art information, data gaps were identified. The hope is that this manuscript will encourage the research needed to support an understanding of similarities and differences in human *versus* canine drug PK.

**KEY WORDS:** ADME; canine-human interspecies extrapolation; metabolism; scaling factors; transporters.

## BACKGROUND

The dog is not only a preclinical species for human medicine but also a potential patient, presenting with numerous analogues of human diseases. This includes cancer, inflammatory bowel disease

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(IBD), diabetes, and cognitive dysfunction (1). In fact, because of the numerous homologous cancers occurring in dogs and humans, the National Cancer Institute established the Comparative Oncology Program (<https://ccr.cancer.gov/comparative-oncology-program>) (2). Similarly, canine cognitive dysfunction mimics Alzheimer's disease (3, 4), containing spontaneously deposited human-type amyloid- $\beta$  that shows 98% homology with that of the human protein (3).

To support these important interspecies extrapolations, there is a need to understand the respective physiological differences that can exist in drug absorption, distribution, metabolism, and elimination. Such information can also help predict interspecies differences in drug toxicity (5–9). Therefore, this review provides a comparison of canine *vs.* human physiological characteristics, transporter abundance, activity and location, and enzyme abundance, activity, and location. In some cases, this information is derived from studies conducted in the individual species. In other situations, data are from studies where both species were simultaneously assessed and compared.

Since most canine studies employ healthy adult Beagles or purpose bred mongrels, this review focuses on information generated in individuals assumed to represent a wild-type population (i.e., without genetic mutations identified in the study reports). Population variability known to exist in humans (10–12) and dogs (13–15) is not covered.

The main body of the manuscript provides a high-level comparison of the canine *versus* human differences impacting drug PK. A more in-depth discussion of some of the topics covered in the manuscript can be found in the [Supplemental material](#). To further support an examination of the points covered in this review, an extensive list of references is provided.

## COMPARISON OF CANINE VS. HUMAN PHYSIOLOGY, INCLUDING TRANSPORTER ABUNDANCE, ACTIVITY AND LOCATION, AND ENZYME ABUNDANCE, ACTIVITY, AND LOCATION

### Gastrointestinal (GI) Physiology

#### Gastric Crushing Force

Dogs and humans exhibit similar area-normalized torque (approximately 3700 dynes/cm<sup>2</sup>), reflecting comparable 2-dimensional rotational force, but very different 3-dimensional crushing force (8). Using identical pressure sensitive pills in dogs and humans, the area-normalized gastric emptying force (dynes/cm<sup>2</sup>) was 606 and 962 in fasted and fed humans and was 3858 and 3639 dynes/cm<sup>2</sup> for fasted and fed dogs (16). Similar relative differences were observed using the destructive force dependent release system (DDRS) (17, 18). This difference in crushing force can render the dog a poor model for testing human oral modified release dosage forms.

#### Pylorus Restrictions

The dog pylorus tends to be more restrictive than that of humans, with beads of 17 mm diameter passing through the human pylorus (fasted state, 6 ounces of water), but the dog stomach retaining particles of 5–10 mm for up to about 1.5 h (19, 20). In the fed state, particles greater than 5 mm in diameter in the dog stomach were retained for over 7.5 h (19). The corresponding timeframe in humans was more difficult to define because of its dependence on meal composition (21).

#### The Interdigestive Migrating Motor Complex (IMMC)

The IMMC both of humans and dogs is characterized by 4 phases. However, while phase 3 of the IMMC (rapid peristaltic contractions) is similar in both species under fasted conditions (approximately every 120 min in humans and 100 min in dogs), the time to phase 3 activity following a meal is 2.6 to 5 h in humans and 5–13 h in dogs (22, 23).

#### Gastric Emptying Time

The estimation of gastric emptying time can be highly dependent upon the experimental method employed (20). Using the SmartPill<sup>®</sup> GI Monitoring system, (24) observed a mean gastric emptying time of 34 min in fasted dogs and a median of 30 min (min/max = 7 to 202 min) in fasted humans (25). Species differences emerged in the fed state. While in (Beagle) dogs, the duration ranged from 1.11 to 4.53 h, it ranged from 3.08 to 19.20 h in humans (Fig. 1). Similarly, when considering those cases where the SmartPill<sup>®</sup> exited the

stomach at the time of the first meal, GET estimated obtained across several canine breeds was comparable to estimates found in humans (24, 26, 27). Contrasting this to what was discussed for the IMMC, these results may reflect temporary entrapment of the SmartPill<sup>®</sup> within the human (25) and canine (28) stomach after a solid meal. Since the SmartPill<sup>®</sup> is in fact a capsule, the dimensions of this device may influence estimates of gastric emptying time and small intestinal residence time in humans (26) and dogs (27). Despite this problem, these devices are routinely used for monitoring local pH and formulation release mechanisms (26).

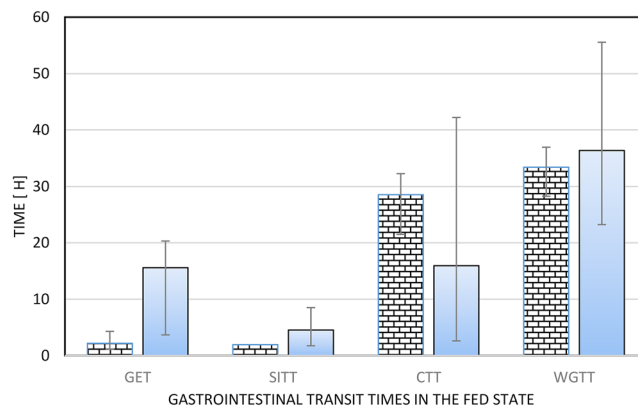
#### Small Intestinal Transit Time

Most studies indicate a faster and more variable small intestinal transit time in dogs (1–2 h and not affected by the food intake) *versus* humans (24, 29) (Fig. 1).

#### Gastric pH Under Fasted and Fed Conditions

**Fasted State.** Given the large variability that can occur within the canine fasted stomach, the United States Pharmacopoeia (USP) recommends that drug gastric solubility in the dog be evaluated over a pH range of 1.2–6.5 (24, 29). In contrast, a single pH value (1.6) is recommended for testing in human fasted simulated gastric fluid (USP General Chapter <1236>).

**Fed State.** The USP General Chapter <1236> includes a description of gastric fluids for the fed state for humans but describes only the fasted state for dogs. Under fed conditions, humans tend to exhibit an initial rise in pH due to the buffering effect of food, but pH slowly returns to pre-meal conditions in a period of 60–90 min. In contrast, the dog does not exhibit this initial rise due to the buffering effect, which may be attributable to a higher basal acid secretion (29), the



**Fig. 1.** Mean, minimum, and maximum GI transit time measured via a SmartPill swallowed in 19 fed humans and 6 fed Beagle dogs. Meals were of comparable caloric value but differed in terms of consistency (pureed for dogs but not for humans). Graph is based upon digitized values (GetDataGraph Digitizer, version 2.6, Softnews Net SRL, SOFTPEDIAR, Sectorul 3, Romania) derived from information presented by Koziolok *et al.* (2015 and 2019) (24, 25). Key (based on terms used in the referenced citation): GET = gastric emptying time, SITT = small intestinal transit time, CTT = colon transit time, WGTT = whole gut transit time. Patterned columns = dog; solid columns = humans

amount (grams) of food consumed (30), and to housing conditions [using Bravo capsules, there tended to be a slightly more acidic postprandial gastric pH when dogs were fed under home cage *versus* study cage conditions (31)].

### Intestinal Fluid Composition

Weak acids are typically more soluble in the canine *vs* human fasted intestinal fluids. For neutral molecules and for weak bases with a pKa of less than 3, the difference was largely a function of the species-specific extent of bile micellization. Above pH 4, the solubility differences for weak acids were associated both with ionized and unionized drug solubilization (32).

Although the USP Chapter <1236> describes human fed and fasted intestinal and colonic fluids, only fasted small intestinal fluids are described for dogs (33–35). Recommended fasted state small intestinal pH is 6.5 and 7.5 for humans and dogs, respectively (where the intestinal segment is not identified for either species). These values are consistent with reports for dogs (Heidelberg capsule), where intestinal pH under fasted conditions ranged from around 6.0 immediately after gastric emptying and progressively increased to pH 7.5 at 120 min after gastric emptying (29), and for humans (IntelliCap® system) where the proximal small intestinal pH was around 6 and increased to about 7–8 in the more distal regions (36). Additional information is available in Supplemental Section 1, Table 1S.

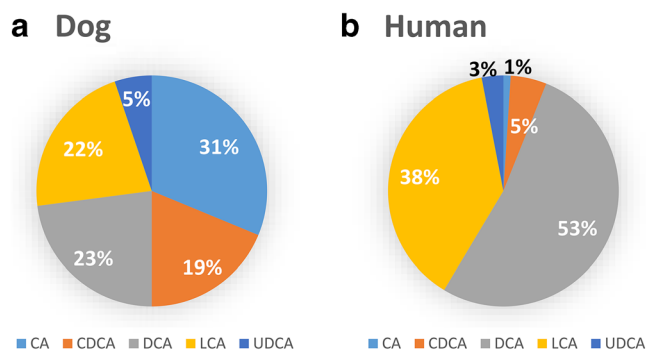
### Canine-Human Differences in Bile Composition

The impact of bile salts on drug absorption may be drug and animal species specific (32, 37). The recommended composition of canine fasted state simulated intestinal fluids (FaSSIFc) includes 5 mM sodium taurocholate and sodium taurodeoxycholate (34), while for humans, the recommendation is only 3 mM sodium taurocholate (38). The numerous canine-human differences in primary and secondary bile abundance and type (from fecal samples) (39, 40) are provided in Fig. 2. While the ratio of human bile acid conjugation to glycine (primary) *versus* taurine (glycine/taurine) is 3, there is negligible conjugation to glycine in dogs, with nearly all conjugation being to taurine.

The largest canine-human differences in pH and bile salt content occur during the postprandial state. For example, in a study comparing the bile salt and phospholipid content in the mid-jejunum of four Labradors and in the duodenal segment of healthy human adults following a 500-mL meal of Ensure Plus<sup>R</sup> (41), the bile salt content was 18 mM and 11.8 mM in dogs and humans, respectively, at 60 min following the meal. The corresponding phospholipid content (mM) was 19.4 mM and 4.31 mM in dogs and humans, respectively.

The bile salt composition in dogs varies as a function of diet, a result believed to be linked to diet-induced modification of the gut microflora (42, 43), and potentially reflecting similar diet-associated variation in bile salt composition in humans.

There also appears to be a difference in the canine *vs* human bile associated ratios ( $\mu\text{M}/\mu\text{M}$ ) of phospholipid



**Fig. 2.** Relative fecal composition of bile salts in dogs (a) *versus* humans (b). Note that the dog relative amounts are based upon average of individual dog values estimated across all diets studied. Values are based upon information provided by Bazzoli *et al.* (1982) (39) and Herstad *et al.* (2018) (40). Key: CA = cholic acid, CDCA = chenodeoxycholic acid, DCA = deoxycholic acid, LCA = lithocholic acid, UDCA = ursodeoxycholic acid

(cytoprotective) to bile acid (0.19 dog *vs* 0.3 humans) and of cholesterol (prevents phospholipid cytoprotection) to phospholipid (0.044 *vs* 0.2 for dog and humans, respectively) (44). Thus, although the relative amount of phospholipid may be lower in dogs as compared to humans, the markedly higher content of cholesterol in humans relative to dogs may have implications regarding differences in hepatotoxicity in the presence of elevated bile acid concentrations (45).

### Unstirred Boundary Layer (UBL)

The UBL (the complex colloidal system that coats the intestinal mucosa) can create a barrier to the absorption of a number of compounds (46), particularly those which have low solubility and high permeability (47). Although its thickness has been estimated to be about 40  $\mu\text{m}$  in dogs and humans (based upon intestinal perfusion studies involving glucose and warfarin) (47), its composition and thickness is recognized to vary as a function of intestinal segments (48). Unfortunately, there is not sufficient information describing the differences in the mucous layers in humans *versus* dogs or its relationship to diet, genetics, or disease (49).

### GUT MICROBIOME

The gut microbiota has been implicated as a potential source of population variability in human drug PK (50–52) and for drugs undergoing enterohepatic circulation in dogs (52, 53). Alterations in gut microbiome have also been implicated in a number of pathologies, including irritable and IBD and psychiatric disorders in humans (54–56) and dogs (57).

The dog gut anatomy, physiology, and diet have adapted to that of humans during domestication (58, 59). Consequently, a 60% taxonomic and functional overlap was identified between the gut microbiota of dogs and humans, in contrast to only 33% and 10–20% similarity observed between humans *versus* pigs and mice, respectively [based upon short sequencing reads from each host associated gut microbiome (57)]. Furthermore, the gut microbial changes observed in the presence of colorectal cancer (40), IBD (60, 61), and diabetes mellitus (62) are comparable between

humans and dogs. This makes health and drug-specific PK consequences that may accompany changes in the canine microflora relevant not only from a veterinary perspective but also in terms of its potential extrapolation to humans.

The predominant fecal bacteria both in humans and dogs are *Firmicutes*, *Bacteroidetes*, and *Clostridium* but unlike humans, *Fusobacteria* also appears to be abundant in dog feces. However, the microbiome captured in the feces (luminal microflora) may not fully correlate with the microbiome adhering onto the gut wall (63–65).

## COMPARISON OF DRUG PERMEABILITY AND ABSORPTION

The interrelationship of factors influencing effective permeability ( $P_{\text{eff}}$ ) is provided in Supplement Section 1.

### Membrane Passive Intrinsic Transcellular Permeability ( $P_{\text{trans},0}$ )

Although  $P_{\text{trans},0}$  tends to be similar in humans and dogs, the overall  $P_{\text{eff}}$  needs to be considered from the perspective of absorptive surface area. In terms of intestinal length, unlike the human small intestine (cadaver) which is largely comprised of the ileum (66), the predominant segment of the canine cadaver GI tract is the jejunum (67). The shorter canine small intestine segments and rapid intestinal transit time can lead to challenges in optimizing drug product dissolution and subsequent absorption (68). In contrast, although the canine small intestinal length is about half that of humans, the long and slender villi of the canine small intestine enhances absorptive surface area, providing at least partial compensation for the shorter intestinal length (69, 70). However, the surface area in humans is further increased by the presence of the plicae circularis, which is not present in the dog (71). This additional component needs to be factored into the differences in absorptive surface area between the two species.

### Paracellular Absorption

Based upon an *in vitro* permeability study using Caco-2 cells (which are reflective of immature small intestinal enterocytes), human intestinal pore diameter was estimated to include a restriction component of 4.3–4.5 Å (71). As indicated by bioavailability studies of both drugs and polyethylene glycol oligomers of varying molecular mass (Daltons, Da), the intestinal pore diameter and abundance in dogs exceeds that of humans (72, 73), leading to a tendency for greater canine paracellular drug absorption (Supplemental Section 1, Table 2S). Generating a semi-log plot of these values, we estimated that 50% polyethylene glycol bioavailability via the paracellular pathway occurs at a pore radius of 6 Å in humans ( $R^2$  for regression = 0.97; ~PEG350) and 7.14 Å in dogs ( $R^2$  for regression = 0.88; ~PEG700).

This difference in pore radius may also affect the magnitude of bioavailability enhancement associated with absorption-modifying excipients (74, 75).

*Active Transport (See Intestinal Transporter Section Below)*

### Lymphatic Absorption

Data pertaining to lymphatic uptake in humans are not available because such investigations necessitate invasive and non-reversible surgery of the intestinal and/or thoracic lymphatic duct. (76, 77). Nevertheless, such data are available in lymph-cannulated dogs, showing that food increases the cumulative amount of triglycerides in lymph from 0.3 to 18.4 g during the first 4 h and cumulative lymph flow (g) from 174 (fasted) to 483 (fed). The impact of prandial state is even greater 8–12 h after meal (78).

While qualitatively similar effects of food on the absorption and metabolism of drugs such as halofantrine (Hf) have been reported in humans and dogs (79, 80), when administered as an amorphous solid dispersion, the fed state increased canine Hf lymphatic transport from 1.3% in the fasted state to 54% in the fed state (79). Postprandial administration increased Hf oral bioavailability by 3-fold in humans and 12-fold in Beagles and reduced the hepatic Hf metabolism by 2.4-fold in humans and by 6.8-fold in dogs (79). Similar findings of meal-enhanced lymphatic absorption and oral bioavailability were observed when the antiparasitic agent, moxidectin, was administered via intragastric lavage to lymph-cannulated dogs (81).

The canine-human comparison of lymphatic uptake and its relationship to drug physicochemical characteristics remains a topic for additional investigation.

## EXTRAPOLATING DRUG CLASSIFICATION BETWEEN DOGS AND HUMANS

To better understand the impact of the differences described above, the appropriateness of extrapolating drug classifications developed for humans to dogs was explored using either the Biopharmaceutics Classification System (BCS), the Biopharmaceutics Drug Disposition Classification System (BDDS), or the Extended Clearance Classification System (ECCS) (82–84). While none of these systems could reliably predict human vs dog differences in drug oral bioavailability, the ECCS suggested greater canine-human correlations for weak acids, zwitterions, and compounds not undergoing pre-systemic metabolism (82).

## DRUG DISTRIBUTION

### Interspecies Differences in Organ-Specific Distribution of Cardiac Output (CO)

The CO (in L/h/kg body weight) is much higher in the Beagle dog as compared to that of the human [e.g., one estimate is 12.3 and 4.54 for the dog and human, respectively (Supplemental Tables 3S and 4S)]. The dog CO values exceed that predicted on the basis of allometric scaling (see Supplemental material for further discussion). The brain receives about 15–20% of the CO in young adult humans but this decreases with age (cerebral blood flow/CO ratio decreased by 1.3% per decade due to changes in cerebral blood flow) (85). In contrast, canine cerebral blood flow comprises only about 2–3% of the CO (86). Other than the

brain (and possibly muscle), the Beagle-human CO distribution to all other organ systems is similar (85 and Supplemental Section 2, Table 3S).

### Interspecies Differences in Steady State Volume of Distribution ( $V_{d,ss}$ )

Using data published on 152 marketed compounds covering a range of physicochemical characteristics, only 57% of the  $V_{d,ss}$  values (L/kg body weight) measured in dogs were within 2-fold that of humans (87). Plasma protein binding could not explain this disparity (88). It was concluded that species variation in  $V_{d,ss}$  reflected the tissue composition (protein *vs* lipid) and pH (for ionizable compounds).

Alternatively, differences in resting potential (mV) across the tissue membranes could be an indicator of possible canine-human differences influencing the diffusion of ionized drug molecules across cell membranes which, in turn, could contribute to differences in the  $V_{d,ss}$  for certain drugs. However, reported canine-human tissue-specific resting membrane potentials tended to be similar [e.g., red blood cells (−9, −11), muscle (−90, −80), and gut tissue (−58, −44) for dog and humans, respectively] (89–93). With regard to pH differences, an attempt to compare the intracellular pH of canine *versus* human healthy tissues revealed the sparseness of data contained within the published literature. Available data (brain, lung, muscle, red blood cells, plasma) indicate similar intracellular pH values (i.e., within a range of 7.0–7.4). Assessments of small differences in tissue intracellular pH and its impact on drug  $V_{d,ss}$  would need to consider the drug pKa, Log P, blood-plasma ratio, plasma protein binding, intracellular tissue binding, and finally the relative proportion of that particular tissue within the given species.

Given the likelihood that pH differences have negligible influence on the  $V_{d,ss}$  of most drugs, the primary cause of observed species inconsistencies appears to be differences in species tissue composition. To that end, additional insights into factors influencing canine *versus* human drug  $V_{d,ss}$  values were obtained from the work of Ruark *et al.* (94). These investigators conducted a meta-analysis of tissue composition and organ characteristics. Using a bottom-up approach, they evaluated the passive tissue/plasma water partition coefficient ( $K_{t,p}^{passive}$ ) as a function of compound characteristic and species. The predicted  $K_{t,p}^{passive}$  for neutral substances with low Log  $P_{O,W}$  was greater in dogs than humans, similar at Log  $P_{O,W} \sim 2$ , but much greater in humans than dogs for Log  $P_{O,W} \geq 3$ . As compound lipophilicity increased, the  $K_{t,p}^{passive}$  of all organs examined tended to be substantially higher in humans than dogs. These differences are consistent with that reported by others (87, 95). Nevertheless, similar canine-human binding to brain tissue homogenates is observed, which has been attributed to the markedly higher lipid *vs* protein content of the brain and therefore higher non-specific binding to the lipid in the dog brain *versus* the potential for specific binding to proteins (96).

### Interspecies Differences in Free Drug Concentrations

In humans, albumin comprises 50–60% of blood plasma proteins, translating to about 3.5–5.5 g/dL ([\[emedicine.medscape.com/article/2054430-overview\]\(https://www.emedicine.medscape.com/article/2054430-overview\)\). Normal amounts of albumin in dogs range from 2.5 to 4.3 g/dL \(<http://webcanine.com/category/health/lab-normal-values/>\). The alpha1-acid glycoprotein \(AAG\) in healthy humans and Beagle dogs range from 0.45–1.12 mg/mL and 0.25 to 0.5 mg/mL, respectively \(97\). While human AAG has a wide drug binding region for basic, acidic, and neutral compounds, dogs have basic and steroid binding sites that overlap and affect each other. The dog does not appear to contain an acidic ligand binding region \(98\).](https://</a></p>
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Colclough *et al.* (2014) evaluated the plasma protein binding of 574 compounds (acids, bases, neutral compounds, and zwitterions) (99). The ratio of % drug unbound in dog to human ranged from 0.065 to 122, with the majority of ratios between 0.5 and 5 (Fig. 3a, b).

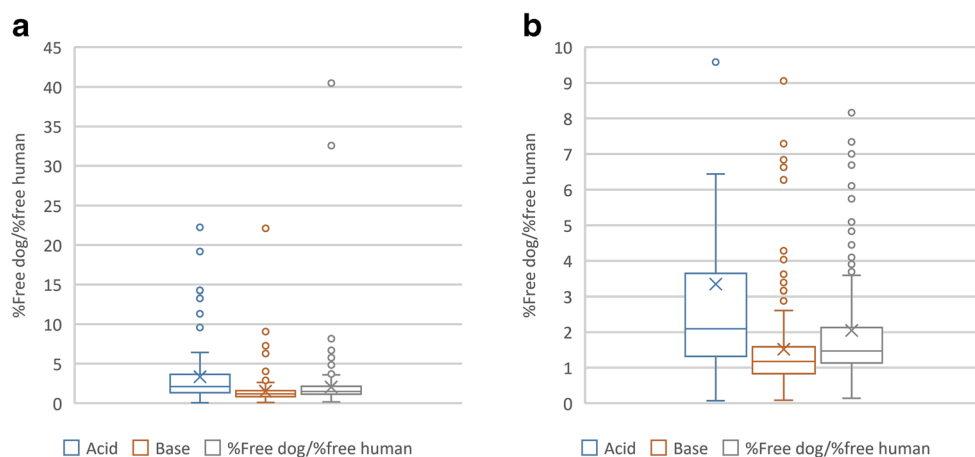
The authors concluded that for the given dataset, although on average, plasma protein binding in dogs was 1.5 times lower than to that of humans, there were also some drugs where the binding in dogs exceeded human plasma protein binding. The authors acknowledged that they did not distinguish between the different plasma proteins and assumed serum albumin to be the major binding protein.

With regard to binding to serum albumin, acidic drugs exhibit the largest differences in dog *versus* human protein binding while that of bases and possibly zwitterions are the most consistent. Potential reasons for this observation include interspecies differences in the amino acid composition of serum albumin and drug binding sites. For example, despite high levels of binding to human Sudlow site I (by hydrophobic or by static interactions) for warfarin and phenylbutazone, similar Sudlow site I binding for both compounds appeared to be absent in dogs. This was attributed to canine-human difference in the microenvironment of the binding site and its effects on the size and/or hydrophobicity of the binding pocket (100). In contrast, for drugs like diazepam and ibuprofen, Sudlow site II binding kinetics, appeared to be similar in dogs and humans (100, 101).

Drug binding sites (Sudlow sites I and II) are located within subdomains IIA and IIIA (102). Although human and canine albumin share 79.8% amino acid sequence homology, there are critical differences in the helical domains (102). As compared to human albumin, canine serum albumin exhibits an expansion of Sudlow site I, leading to both greater space and water accessibility within that binding site, thereby interfering with albumin binding to warfarin and phenylbutazone (102). It is the Sudlow site I that appears to exhibit the greatest magnitude of dissimilarity in drug binding in dogs as compared to that seen in humans.

Species differences in globulins, free fatty acids, bilirubin, or metabolites can either competitively displace drugs from albumin binding sites and/or induce conformational changes (98). Moreover, albumin can also undergo posttranslational modifications affecting its ability to interact with some drugs (103).

Species differences in drug distribution can occur due to differences in plasma constituent fractions, such as proportion of plasma lipoprotein, cholesterol, triglycerides, and apolar lipids (104). This can influence drug distribution characteristics within the blood. For example, as compared to humans, dogs have a significantly higher proportion of intravenously administered free and liposomal nystatin carried within the high-density lipoproteins, potentially influencing drug PK, toxicity, and activity (104).



**Fig. 3.** Boxplot of ratio of % unbound drug in dogs *versus* humans as a function of drug acid/base properties. **a** Full range of values. **b** Y-axis truncated to define mean, median, and quartiles. Where X = mean, upper and lower limits of box are the 25 and 75 percentiles of values. Line in the middle = median. Whiskers are Q1 + 1.5 interquartile range, and points = outliers. Based upon data by Colclough *et al.* (2014) (99)

## METABOLISM

Humans possess *N*-acetyltransferase 1 (NAT1) in all tissues, and *N*-acetyltransferase 2 (NAT2) in the liver and intestine. In contrast, dogs do not express the NAT enzymes, thereby affecting their ability to activate or deactivate carcinogenic metabolites via this pathway (105, 106). Similarly, while humans are capable of synthesizing a single functional aldehyde oxidase (AOX1), the dog does not produce hepatic aldehyde oxidase (107).

Dog liver microsomes are about 55-fold more efficient than human in the conversion of cyclophosphamide (CP, a prodrug) to 4OHCP, the cytotoxic metabolite (108). In humans, this conversion is primarily a function of CYP2B6, with a far smaller involvement by the CYP2C subfamily (where the role of CYP2C19 exceeds that of CYP2C9) (109, 110). In dogs, Cyp2c21 and Cyp2c41 are the isoforms primarily responsible for this enzymatic conversion (108).

Rifampin is a strong inducer of CYP3A4 in humans. While induction of canine Cyp3a12 does occur (111), it is far less potent than in humans (based upon changes in mRNA levels observed in human hepatocytes *versus* canine liver slices) (112). Furthermore, for 22 xenobiotics (including nicardipine, rifampin, clotrimazole, and nifedipine), there was a lack of correlation between the resulting fold change in mRNA expression levels in the human (> 30-fold induction) *versus* canine (approximately 8-fold induction) gene. To understand the reason for this difference, the amino acid sequence of the dog and human pregnane X receptor (PXR), the predominant nuclear receptor responsible for CYP3A expression, was compared. Despite 86% homology, existing amino acid differences may cause a slight reduction in the hydrophobic region of PXR, which in turn, may impact its interaction with an inducer (112).

### Metabolism Enzyme Abundance

A comparison of the fractional total composition of the various CYPs in dog *versus* human liver (113, 114) and intestine (113, 115) is shown in Fig. 4a and b, respectively. Human liver and intestinal cytochrome P450 distributions are provided by Paine *et al.* (2006) (113) and Ho *et al.* (2017) (117).

## Liver Enzymes

*Human.* The percent contribution, based on total immunoquantified P450 content, is 40% CYP3A, 25% CYP2C, 18% CYP1A2, 9% CYP2E1, 6% CYP2A6, 2% CYP2D6, and < 1% CYP2B6 (114). Similar values were reported by Achour *et al.* (2014) (118), who examined the pmol CYP enzymes/mg hepatic microsomal protein.

*Dog.* Considering the average protein abundance of 11 cytochrome P-450 isoforms and 2 accessory proteins across 59 dogs of varied breeds (14, 119):

- the most highly expressed enzymes (> 120 pmol/mg microsomal protein) are Cyb5a > Cyp2d15 > Cyp3a12.
- intermediately expressed (40–89 pmol/mg microsomal protein) are Cyp1a2 = Cyp2b11 = Cyp2e1 > Cyp2c21 > P450 oxidoreductase (POR)
- poorly expressed (< 12 pmol/mg microsomal protein): Cyp2a13 = Cyp2a25 = Cyp2c41 (the latter absent in the livers of many dogs) > Cyp3a26 = Cyp1a1.

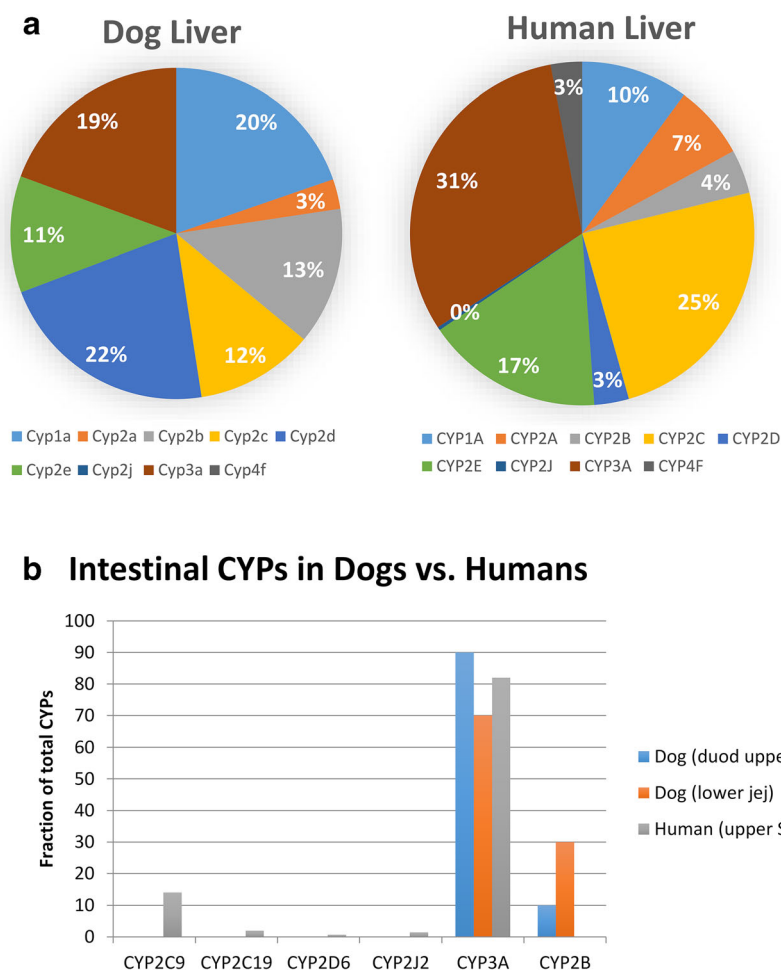
In terms of interindividual variability in protein abundance:

- high = Cyp1a1 > Cyp1a2 (%CV = 105–276%).
- intermediate = Cyp2c41 = Cyp2a13 > Cyp2b11 = Cyp3a26 = Cyp2a25 > Cyp3a212 = Cyp2c21; (%CV = 36 to 67%)
- low = POR = Cyp2e1 = Cyp2d15 > Cyb5a (%CV = 16–28%).

## Intestinal Enzymes

The abundance and diversity of the cytochrome P450's is far greater in the liver than in the intestine of dogs and humans (Fig. 4a, b).

While Cyp3a and 2b11 provide the largest contribution in dogs, in humans, CYP2C9, 2C19, 2D6 (detected in most



**Fig. 4.** Comparison of cytochrome P450 composition (expressed as fraction of total CYPs) in the liver and intestine of dogs and humans. **a** Comparison in canine (116) versus human liver (Paine *et al.* (2006) (113); Shimada *et al.* (1994) (114)). **b** Comparison of dog (digitized values estimated from Heikkinen *et al.* (2015) (115) versus those of humans (113) as a function of intestinal segment and cytochrome P450

but not all individuals), and 2J2 have also been identified (113). The canine equivalent of CYP2J2 has not as yet been identified, although we have reason to believe that low levels may also be present in the canine upper small intestine (120).

The most abundant canine (Beagle) intestinal enzymes are Cyp3a12 (primary) and Cyp2b11 (secondary), with abundance and activity declining from small intestine to colon (based on enterocytes lining the intestinal villi) (121). Cyp3a12 and Cyp2b11 represent 90 and 10%, respectively, of the intestinal cytochrome P450's in the upper small intestine (duodenum and upper jejunum), changing to 70 vs 30%, respectively, in the lower portions of the jejunum and below (116, 122). Despite the importance of intestinal drug metabolism on limiting the fraction of drug making it through the gut into the portal circulation, the same enzymes represent only a fraction of the pmol amount of expressed enzyme in the liver (116). For example, assuming 0.88  $\mu\text{mol}$  and 1.45  $\mu\text{mol}$  for Cyp2b11 and Cyp3a12, respectively, in Beagle whole liver, the relative expression levels across the small intestinal segments ranged from  $1.59 \times 10^{-4}$  (ileum) to  $5.76 \times 10^{-3}$  (duodenum/upper jejunum) for Cyp2b11 and from  $2.92 \times 10^{-4}$  (ileum) to  $6.59 \times 10^{-3}$  (duodenum/upper jejunum) for

Cyp3a12. Similarly, activities of testosterone 6 $\beta$ - and 16 $\alpha$ -hydroxylation (6 $\beta$ - and 16 $\alpha$ -OHT), a marker of Cyp3a12 activity was markedly higher in the liver as compared to the small intestine, and this activity decreased along the length of the small intestine (123).

In contrast to the availability of human abundances and activities of uridine 5'-diphospho-glucuronosyltransferases (UGTs) in the literature, these data are sparse in dogs. In humans, the most abundant intestinal UGT is UGT2B17, followed by UGT1A1 and UGT1A7. In terms of canine Ugt, Ugt1a11 is the most abundant in the intestine, but also present is 1a1, 1a2, 1a3, 1a4, and 1a9 (115, 124). We are not aware of a human orthologue of Ugt1a11.

## TRANSPORTERS

A summary of the assorted influx and efflux transporters found throughout the human body has been published in the 2019 international transporter consortium report (125). A synopsis of the various human and canine transporters involved in intestinal absorption (126–128), hepatic

metabolism (10, 129), biliary secretion (130), renal elimination (131), and the blood-brain barrier (132–137) is provided in Fig. 5.

For the sake of brevity, our discussion will focus primarily on transporters of the liver and kidney. Data on the brain can be found in the Supplement Section 6.

## Liver

Published reports need to be interpreted with caution, because the interspecies comparisons are frequently based upon levels of mRNA, even though mRNA levels are often poorly correlated with the abundance of expressed protein or of transporter activity (121, 138).

In contrast to the human liver, where Solute Carrier Organic Anion Transporter Polypeptide (OATP) OATP1B1 and OATP1B3 are the most abundant transporters (about 29% of the hepatobiliary transporters), the most abundant canine transporter is Oatp1b4 (about 50% of the canine hepatobiliary transporters) (138). Using plated hepatocytes, Matsunaga *et al.* (139) observed that while the intracellular protein binding in humans and dogs was similar, transporter activity was not. Estimates of cell to medium concentration ratio for unbound drug ( $K_{p_{uu}}$ ), which reflects the interplay of active uptake, passive diffusion, and metabolism/biliary secretion, were markedly different in dogs and humans (corrected for species hepatocellularity, refer to Table 10S). Moreover, the relationship between human *vs.* canine uptake efficiency was compound specific. Although Wilby *et al.* (2011) observed somewhat greater similarity in the activity of Oatp1b4 and OATP1B3, they too found this to be substrate specific (140).

Interference in bile salt transport can result in drug-induced liver injury (DILI). Inhibition of bile salt export protein (BSEP) activity (*in vitro* hepatocyte assay of 24 compounds) also showed species differences, with 7 compounds having much lower  $IC_{50}$  values in dogs than humans, 10 of similar sensitivity (dog/human  $IC_{50}$  ratios ranging between 0.60 and 4.4), 3 being somewhat less sensitive in dogs ( $IC_{50}$  dog/human ratios of 7.39–24), and 3 compounds for which the dog failed to reflect compound's ability to inhibit human BSEP (141).

In order of abundance, after the OATPs, the next most abundant family of transporters in the human liver are the organic cation transporters (specifically hepatic OCT1) and the multidrug resistance protein 2 (Mrp2) in dogs. In terms of the sinusoidal efflux transporters, the MRP/Mrp 3-6 were typically present in relatively small amounts in the healthy liver both of dogs and humans (138). Although this cited study did not account for transporter losses due to tissue digestion and membrane extraction [e.g., see (142)], it did provide one of the few reports containing a within-study canine-human transporter comparison.

The relative expression estimated between species provides the basis of comparisons of human *versus* dog as shown in Fig. 6b. Although the dog data were obtained from Wang *et al.* (2015) (138), the human data were taken from the meta-analysis results included in the Simcyp Simulator, Version 19. The human data provided in Fig. 6 represents data for healthy humans (adult, 20–50 years, Caucasian).

Further discussions of the relative abundance of the hepatic transporters in dogs and humans are provided in Supplemental Section 3, Table 5S. In addition, Supplemental Section 4 describes how two hepatic transporters (BSEP/Bsep and MDR3/Mdr3) can interact in the formation of bile micelles.

## Kidney

The toxicity for certain renally eliminated compounds in dogs, such as the phenoxyacetic acids and related organic acids (143), has been linked with the lower capacity of dogs to secrete organic acids from the kidney. Conversely, for the acyclic nucleoside phosphonates, lower OAT-associated accumulation of these compounds in the kidney of dogs *versus* humans has been implicated in the corresponding lower renal toxicity of these compounds in dogs (144). This dissimilarity likely reflects the deficiency of transporters in the SLC22 family in dogs [e.g., including Oat 2, 3, 4, Oct1 (present, but at very low quantities), Oct2, Oct3, and Octn1]. Also absent in the dog is Oatp4c1 (145). Conversely, the higher affinity (lower  $K_m$  value) of human *vs* canine OAT1 for acyclic nucleoside phosphates was responsible for renal accumulation and nephrotoxicity of many antiviral compounds in humans but not in dogs (144).

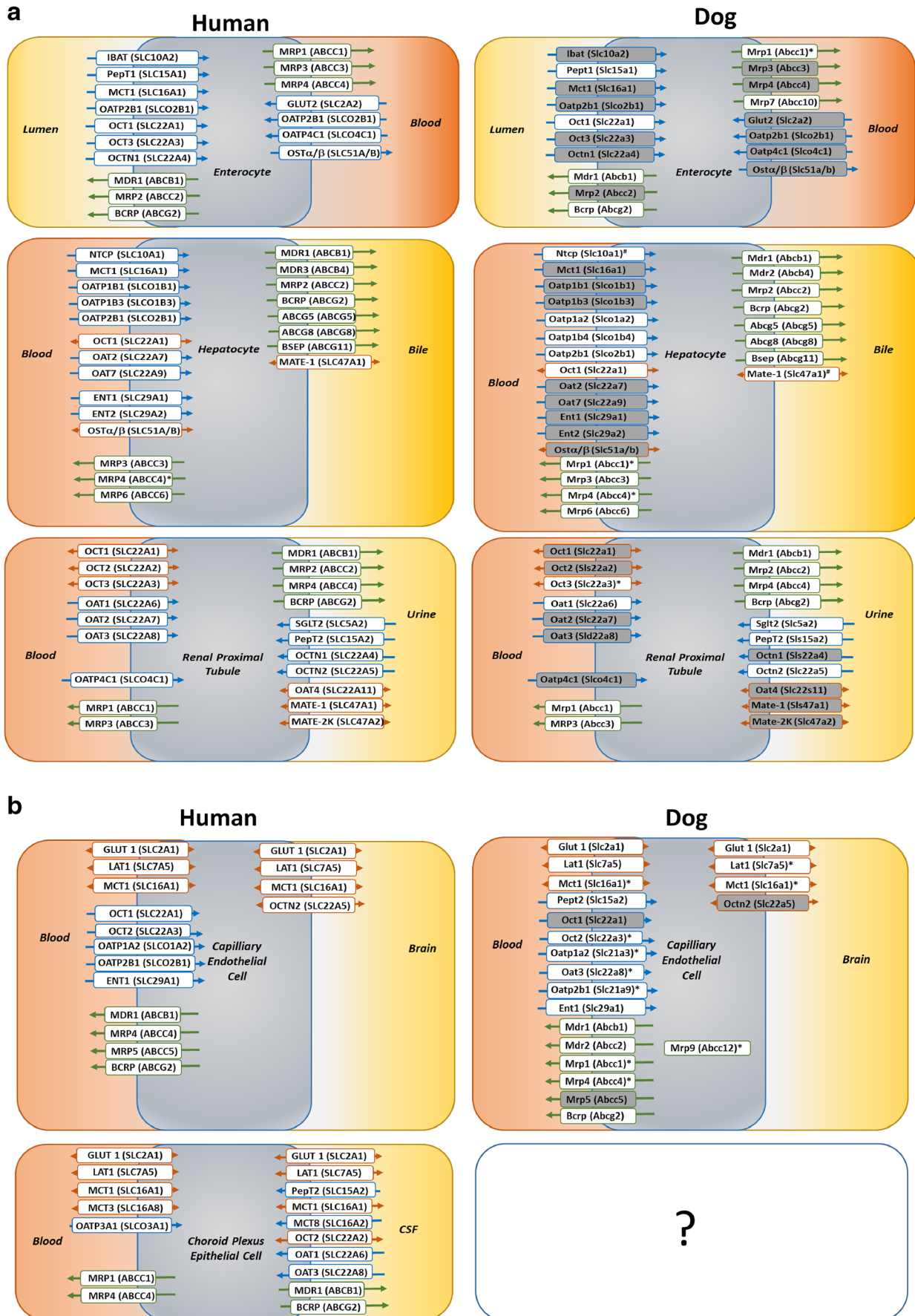
In terms of the ABC transporters, although Basit *et al.* (2019) concluded that breast cancer resistance protein (Bcrp) is not conserved in the canine proximal tubule (131), Wittenburg *et al.* (2019) were able to quantify this transporter using LC/MS/MS in the canine renal cortex (137).

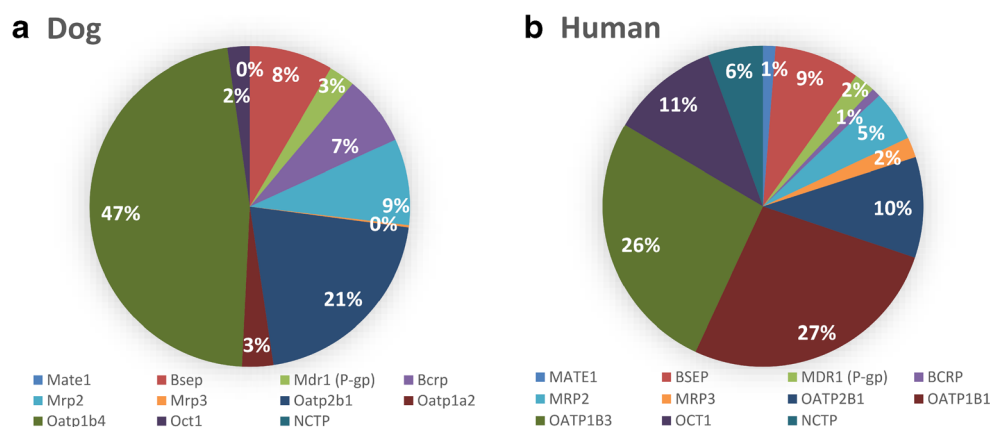
A comparison of the human and canine renal transporter abundance data based upon the findings of Basit *et al.* (2019) is provided in Table I (131).

Scaling of the renal transporter abundance to amount per gram of dog or human kidney is provided in Supplemental Section 5 and Tables 6S and 7S.

Fig. 5a Influx and efflux transporters in the human and canine liver, kidney, and intestine (based upon figures provided by Lai 2009 (130), Pan 2019 (10), Basit *et al.* (2019) (131), Estudiante *et al.* (2013) (126), the US FDA/University of Southern California collaborative transporter portal <http://transportal.compbio.ucsf.edu/organs/small-intestine/>, Burt *et al.* (2016) (129), Harwood *et al.* (2019) (128), Giacomini *et al.* (2010) (127), and Morris *et al.* (2017) (134). **b** Influx and efflux transporters in the humans and dogs at the blood-brain barrier (135–140). The authors also wish to express our appreciation to Dr. Matthew Harwood for conducting the meta-analysis of dog brain transporters (see Supplemental Section 6, Table 7S). Abbreviations: apical sodium-dependent bile acid transporter (ABST), breast cancer resistance protein (BCRP), bile salt efflux transporter (BSEP), equilibrative nucleoside transporter (ENT), L-amino acid transporters (LAT), multi-antimicrobial extrusion protein (MATE), monocarboxylate transporter (MCT), multidrug resistance proteins (MRP), organic anion transporter (OAT), solute carrier organic anion transporter protein (OATP), organic cationic transporter (OCT), sodium/bile acid cotransporter (NCTP), organic solute transporter (OST), peptide transporter (PEPT), P-glycoprotein (P-gp, also known as multidrug resistance protein 1, MDR1), plasma membrane monoamine transporter (PMAT). Greyed out canine transporters have not been investigated to date. # not detected \* <LLOQ (below the lower limit of quantification of the analytical method)







**Fig. 6.** Relative contribution of hepatic transporter in the dog (a) and humans (b). The data for dogs are from Wang *et al.* (2015) (138). The data for humans were extracted from the figure captions

### Other Tissues

*Dog.* Tissues from healthy dogs were obtained during necropsies conducted at Colorado State University (breed not indicated) (145). In the canine brain (Supplemental Table 8S), the most abundant cortical ABC transporter is Bcrp, followed by P-gp. Bcrp was present in the liver, renal cortex, duodenum, and jejunum (none in the ileum). Mdr2 was found in low amounts in dog brain and liver. Mrp 6 and 7 were seen only in the liver and duodenal samples, respectively. P-gp was in the brain, liver, renal cortex, duodenum, and jejunum (duodenum higher than jejunum) and Mrp2 was only found in the renal cortex. Among the ATP-binding cassette transporters evaluated in canine tissues, the MRP family had the lowest expression

in most dogs, being either present or not identified in some animals. However, tissue transporter content was based on an assumption of a linear relationship between the amount of signature peptide whose reference sequence was derived from a single boxer dog, leading to potential bias if signature peptide sequences contain variants from that of the boxer.

*Human.* The relevance of tissue transporter function was underscored by statin movement into cultured human skeletal muscle fibers transfected with adenovirus to over-express several transporters (146). Incubation of these *in vitro* muscle fibers in statin-containing media led to an association between OATP2B1 expression and muscle toxicity while MRP1 expression tended to be

**Table 1.** Comparison of Proximal Tubule Protein Abundance in Dog Versus Humans for Those Transporters Expressed in the Dog Kidney (Based upon Information in Basit *et al.* (2019) (131)). Values Were Based on pmol/gm Kidney Using LC/MS/MS Methodology

	Human [pmol/g kidney]	Dog [pmol/g kidney]	Dog/human
SLC22A6/OAT1	107.7 ± 56.83	75.4 ± 43.07	0.70 (blood to tubule)
1.1. SLC22A7/OAT2	19.8 ± 8.36	NC	
1.2. SLC22A8/OAT3	78.5 ± 37.38	NC	
1.3. SLC22A11/OAT4	10.6 ± 5.64	NC	
1.4. SLCO4C1/OATP4C1	0.3 ± 0.03	NC	
1.5. SLC22A1/OCT1	BLQ	NC	
1.6. SLC22A2/OCT2	164.2 ± 53.27	NC	
1.7. SLC22A3/OCT3	0.03 ± 0.01	BLQ	
1.8. SLC22A4/OCTN1	27.2 ± 13.44	NC	
1.9. SLC22A5/OCTN2	13.1 ± 5.8	16 ± 5.84	1.22 (bidirectional tubule cell and urine)
1.10. ABCB1/MDR1	42.3 ± 16.16	32.1 ± 9.34 (32.6 ± 8.06)	0.76 (tubule cell to urine)
1.11. SLC47A1/MATE1	105.6 ± 47.52	NC	
1.12. ABCG2/BCRP	NC	NC (16.13 ± 11.01)	
1.13. ABCC1/MRP1	20.1 ± 4.54	42.2 ± 10.95	2.10 (tubule cell to urine)
1.14. ABCC2/MRP2	30.1 ± 16.52	NC (5.632 ± 1.408)	
1.15. ABCC3/MRP3	20.1 ± 1.78	NC (4.096 ± 1.536)	
1.16. ABCC4/MRP4	19.5 ± 20.58	NC (9.47 ± 7.68)	
1.17. SLC5A2/SGLT2/	76.4 ± 41.25	356.7 ± 84.85	4.67 (urine to tubule cell)
1.18. ATP1A1/Na+K+ATPase	20.1 ± 1.17	70.7 ± 15.19	3.52 (tubule cell to blood)

Numbers in brackets are estimates from Wittenburg *et al.* (2019), who expressed data as pmol/gram membrane protein using an LC/MS/MS quantification procedure (137). For unit conversion, see Supplemental Section 5, Table 5S  
NC, not conserved peptide; BLQ, below the limit of quantification

cytoprotective. Benet *et al.* (2019) further expand upon the importance of understanding transporter function in interpreting potential population variability both in terms of clearance and  $V_{d_{ss}}$  (147).

Using data generated on the basis of either protein or mRNA expression, a wide array of transporters were identified on the blood-brain barrier (BBB) and in the blood cerebrospinal fluid barrier (BCSFB) (134). The suggested arrangement in the human brain is included in Fig. 5b and Supplement Section 6, Table 8S.

### USE OF IN SILICO PHYSIOLOGICALLY BASED PK MODELS TO SUPPORT INTERSPECIES EXTRAPOLATION

The interspecies PK extrapolation from preclinical species to humans using physiologically based pharmacokinetic (PBPK) models has gained much traction in recent years. Despite successes, as shown in Supplemental Section 7, Table S9, challenges remain regarding the extrapolation of drug metabolism or transport between the dog. A comparison of hepatic and intestinal scaling factors of dogs versus humans is provided in Supplemental Section 7, Table S10 (120, 148–152).

### FUTURE TECHNOLOGIES TO SUPPORT INTERSPECIES EXTRAPOLATIONS

Consistent with efforts to streamline methods for characterizing candidate drugs, there is increasing interest in the development and use of miniature organs (so-called 3D organoids) derived from stem cells collected in animal or human patients (153). The goal is that through the use of these *in vitro* systems, it may be possible to increase our understanding of the species-specific differences in drug transport and metabolism that can influence systemic and local exposure to both parent drug and its metabolites. This information, when used to inform our PBPK models, could greatly improve our interspecies predictions.

Recent research has demonstrated that 3D intestinal organoids can be successfully maintained from healthy dogs and from dogs with naturally occurring IBD (154–157). Organoids have also been developed from dogs with urothelial carcinoma (158). These 3D systems have the potential to positively impact healthcare (e.g., in patients with bladder cancer) and streamline the development of new therapeutic strategies in humans and dogs.

### CONCLUDING COMMENTS

This review reflects an effort to provide a broad overview of the factors that can lead to differences in the drug PK characteristics in dogs and humans. Despite recognized population variability that can occur in both species, an appreciation of where the greatest degree of similarities exist can support a determination of when dog-human interspecies extrapolations may be most appropriate.

One of the challenges is the proportionally small amount of information on canine enzymes and

transporters as compared to that in humans. Furthermore, a major drawback is the frequent reliance on mRNA for identification of location and changes in transporters and drug-metabolizing enzymes (159). It is difficult to compare human and canine enzyme and transporter activity on the basis of anything other than expressed activities.

Given the utility of parallel drug product development, particularly as it comes to shared diseases, our goal is to encourage research efforts that fill remaining data gaps using technologies such as (but not limited to) human or canine organoids that can support use of PBPK models to accelerate veterinary and human drug development. With increased harmonization between human and animal drug development, there will be expanded opportunities to address the therapeutic needs both of humans and dogs.

### GAP ANALYSIS—QUESTIONS FOR FUTURE RESEARCH

Based upon our analysis, these are the current gaps where additional research is needed:

- Bile salts in dog intestinal fluids under fed and fasted conditions.
- Factors that can affect canine GI fluid volumes and composition under fasted and fed conditions and how these fluids may be altered by experimental conditions.
- Mucous layer thickness and composition in dog and humans.
- Speciation of canine regional gut microflora, the impact of diet on gut microflora, and how changes in this microbial population can influence drug PK and health.
- UGT expression and activity in dogs.
- Species differences in drug-associated nuclear receptor (e.g., PXR) activation.
- Information on canine transporter across the various tissues and their abundance and activity relative to that in humans.
- Canine-specific organ scalars (e.g., MPPGL, HPGL, PTCPGK)
- Quantitative information on the lymphatic pathway in humans and dogs.

Lastly, there is the need to develop reproducible and easy to use organoid assays that are linked via *in vivo-in vitro* extrapolation to PBPK models. If we can address these gaps, then organoids and PBPK modeling will be better suited to address the 3R's and will indeed reduce, refine, and replace studies in animal and humans equally.

### SUPPLEMENTARY INFORMATION

The online version contains supplementary material available at <https://doi.org/10.1208/s12248-021-00590-0>.

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## DECLARATIONS

**Ethics in Animal and Clinical Investigations** As only available study data were used in the development of this manuscript, no additional human or animal testing occurred for the purpose of generating this review.

**Conflict of Interest** JPM is a founder and COO of 3D Health Solutions, Inc. SN and DP are employees and shareholders of Certara UK, Simcyp Division.

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