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Research Article

Reconciling Human-Canine Differences in Oral Bioavailability: Looking beyond the Biopharmaceutics Classification System

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Abstract. The extrapolation of oral bioavailability (F) information between dogs and humans has had an important role in the drug development process, whether it be to support an assessment of potential human pharmaceutical formulations or to identify the bioavailability challenges that may be encountered in dogs. Accordingly, these interspecies extrapolations could benefit from a tool that helps identify those drug characteristics consistent with species similarities in F. Our initial effort to find such a tool led to an exploration of species differences as it pertained to the biopharmaceutics classification system (BCS). However, using a range of compounds, we concluded that solubility and permeability alone could not explain interspecies inconsistencies in estimates of F. Therefore, we have now extended our evaluation to include canine versus human comparisons of F based upon the biopharmaceutics drug disposition classification system (BDDCS) and the extended clearance classification system (ECCS). Using the same data as that in our initial BCS assessments, we conclude that although neither the BDDCS nor the ECCS can reliably improve our ability to determine when F will be similar in humans and dogs, the ECCS provides a mechanism to help define possible causes for observed human-canine inconsistencies.

KEY WORDS: biopharmaceutics drug disposition classification system; dog oral bioavailability; extended clearance classification system; human oral bioavailability; interspecies extrapolation.

INTRODUCTION

The dog is often used as a preclinical species during human drug formulation development, resulting in the need to identify and adjust for interspecies differences that can influence the applicability of canine-derived oral bioavailability (F) data to the human patient. However, we also know that estimates of F can be markedly different between humans and dogs. Similarly, human drug products may be administered to dogs under the care of a licensed veterinarian. In these situations, much of the information about these drug products are extrapolated from data generated in humans.

Causes for the bias associated with interspecies extrapolations typically reflect the differences in the dog versus human gastrointestinal (GI) tracts. However, our understanding of these differences is negatively impacted by the gaps in our understanding of canine GI physiology (e.g., the segment-specific distribution and activity of influx and efflux transporters and of metabolizing enzymes). Until such information are generated, there is a need for a tool to help identify those drugs for which interspecies extrapolations may be considered "acceptable." This need provided the impetus for exploring the potential utility of published drug classification systems.

Our initial attempt to reduce the magnitude of these disparities (1) was based on a segregation of compounds in accordance with the biopharmaceutics classification system (BCS) (2). Using published information, we compared human versus canine ratios of the area under the curve (AUC) values following oral versus intravenous (IV) drug administration (where the AUC's were normalized for the administered oral and IV dose). However, after studying 46 compounds (a total of 50 data sets, which included 4 instances of duplicated compound), we concluded that the BCS does not improve our ability to identify those drugs for which

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interspecies extrapolations can be reliably applied. Thus, we needed to consider alternative ways to improve our understanding of the underlying causes for some of the observed human-canine bioavailability differences.

One of the challenges impacting such interspecies comparisons is the numerous processes that can influence the estimation of F such as in vivo drug dissolution, drug solubility and permeability across the enterocyte membrane (f_a), potential drug loss within the intestine (lumen or enterocyte), and the fraction of the absorbed dose entering the portal circulation and traversing the liver without undergoing presystemic hepatic metabolism (3). Each of these factors needs to be considered when attempting interspecies extrapolations.

Because of differences in interspecies GI fluid volume and composition, the existing disparity in the solubility classification for some compounds was not surprising. However, reasons for permeability differences were not straightforward. There is a general belief that passive transcellular permeability is similar in humans and dogs (4). Thus, for a given set of permeability characteristics, differences in the fraction of dissolved drug entering the enterocyte is likely reflective of differences in absorptive surface area, intestinal transit time, or intestinal transporter characteristics. For compounds where the pH of GI fluids approach that of its pKa, human-canine differences in GI pH can affect the magnitude of drug existing in an ionized form and therefore, drug permeability and movement through the unstirred boundary layer.

Upon considering the known species-specific physiological attribute of dogs versus humans, the following critical factors were identified as potential causes for observed interspecies inconsistencies:

1. pH and bile salt composition of GI fluid (5–7): The manner in which interspecies differences in drug pH affects its solubility and permeability will depend upon drug pKa and whether it is an acid, base, or zwitterion. Furthermore, the influence of bile salt composition will depend upon the pKa and ionization characteristics of the drug (7).
2. Length of the GI tract (8): If the drug is absorbed primarily in the ileum and colon, the shorter intestinal segmental lengths and GI transit time encountered in the dog could lead to marked differences in f_a.
3. Paracellular absorption: The higher frequency, size, and/or selectivity of the tight junctions of intestinal cells in dogs could lead to higher bioavailability for some drug molecules in dogs than in humans (9).
4. Presystemic drug metabolism: This could occur in the intestinal lumen, within the enterocyte, or in the liver. We know that there can be substantial disparity between the species with respect to the nature and extent of metabolism associated with certain compounds/pathways. For example, with some compounds (e.g., the prodrug, candesartan cilexetil), dog-human differences in proximal small intestinal pH and pancreatic enzymes resulted in a 4-fold higher Vmax in dog versus human intestinal fluids (10). Thus, we see the possibility of species differences in drug luminal stability due to their respective differences in pancreatic secretions. We also know that the segmental location of the various intestinal enzymes can differ in the dog and human (11). Therefore, if a drug is associated with a pre-systemic metabolism, there could be a substantial risk for human-canine divergence in the bioavailable fraction. Moreover, the segmental location of the intestinal metabolism could increase the impact of any existing differences in drug dissolution.
5. Transporters: Given the known species-specific characteristics of transporter kinetics, drug affinity, segmental location, and abundance, classifications that consider the role of transporters in the absorption and hepatic uptake of drugs may help identify drugs that are more likely to exhibit species-specific differences in oral bioavailability (12). Species differences in the segmental preponderance of various influx and efflux transporters are known to exist. In this regard, as indicated by Wu and Benet (13), a classification of highly permeable does not necessarily imply high transcellular permeability. In addition, as is the case with intestinal metabolism, interspecies inequality in transporter activity (particularly when located in the upper small intestine) may lead to differences in the rate and extent of dissolution for some low solubility drugs by affecting the luminal dissolved concentrations against which additional dissolution must occur.

Thus, for this second phase of our effort, two classification systems were considered. The first was the biopharmaceutics drug disposition classification system (BDDCS), which divides drugs into classes based upon their solubility and metabolism.

Class 1: High solubility, extensive metabolism, rapid dissolution
Class 2: Low solubility, extensive metabolism
Class 3: High solubility, minimal metabolism
Class 4: Low solubility, minimal metabolism

It is also recognized that those drugs that fall within BDDCS class 2 are likely to be substrates for hepatic uptake transporters (14,15). Thus, because of its consideration of drug metabolism and transporter affinity, we considered the BDDCS to be a potential tool for identifying those compounds with the highest likelihood for similar dog and human values of F.

The other tool we evaluated was the extended clearance classification system (ECCS). Unlike the BDDCS, this system considers molecular charge (acid, base, zwitterion, neutral), molecular weight, and the primary mechanism of elimination as rate and extent of elimination (renal versus hepatic metabolism). The ECCS consists of 6 different molecular classifications (15):

Class 1A: Molecular weight (MW) $\leq$ 400, acids or zwitterions that are well absorbed and for which systemic clearance is mediated by cytochrome 2C6 (CYP2C6), esterases or by one or more of the uridine 5′-diphosphoglucuronosyltransferase enzymes (UGTs). These compounds tend to escape intestinal first pass metabolism and have most of the absorbed drug escaping hepatic first pass metabolism.

Class 1B: MW ≤ 400, acids or zwitterions whose oral absorption is typically high, and whose predominant clearance mechanism involves hepatic uptake mediated by organic anion transporting polypeptide (OATP) transporters, followed by metabolism involving CYP2C, UGTs, esterases, or the CYP3A enzymes. These compounds usually escape intestinal first pass metabolism if they are not substrates for CYP3A enzymes.

Class 2: High permeability neutral /basic drugs with high oral absorption and metabolism mediated generally by CYP3A, CYP2D6 and UGTs. These compounds are well absorbed but tend to have a high risk of intestinal first pass drug metabolism.

Class 3A: MW ≤ 400. Acids or zwitterions that show moderate absorption with the dominant elimination pathway being renal clearance involving organic anion transporters (OAT)1/3 transporters. These drugs tend to escape intestinal first pass metabolism.

Class 3B: MW > 400, acids or zwitterions with low to moderate absorption with hepatic or renal uptake mediated by the OATP transporters. Their affinity for the renal versus hepatic transporters dictates the contribution of active hepatic uptake or renal clearance. These compounds may also be eliminated unchanged in the bile.

Class 4: Base or neutral compounds that show moderate absorption with the dominant route of clearance involving OAT or organic cation transporters (OCT)2 transporters.

Thus, although not directly factored into the ECCS, it is evident that inherent within this classification is a natural segregation of high versus low permeability compounds as well as the factors influencing drug aqueous solubility (pKa, ionization characteristics, and molecular weight). The further classification of drugs most likely to be associated with high first pass drug loss or transporter activity supports an identification of compounds likely to be poor candidates for interspecies extrapolation of oral bioavailability.

This manuscript summarizes our effort to identify a potential framework that can be used to support an extrapolation of oral bioavailability data between humans and dogs.

METHODS

To compare the various classification systems, the drugs, BCS classification, and F values included in the initial exploration by Papich and Martinez (1) were used in this analysis. Our evaluation included 46 sets of values. All studies used in this evaluation considered only total (free+ protein bound) drug concentrations. Therefore, to complete this assessment, we explored the possibility of human-canine differences in protein binding/binding affinity. Although the hepatic extraction of certain highly protein bound drugs may be more efficient than would be expected based upon their unbound fractions (16–19), we assumed that this would be a rare event and therefore this possibility was not considered in our evaluation of interspecies relationships. The BCCS and ECCS classifications of most compounds on this list (human information) were obtained from (20–22). Human drug doses used in this comparison were based upon the maximum approved dose within the USA. Canine doses were derived from information contained in Papich and Martinez (1). To account for differences between human and canine body weights, doses in humans were approximated as administered dose (mg) divided by 70 kg body weight. For dogs, since the majority of studies were conducted in beagles, we estimated the mg/kg dose based upon an assumption that the average body weights were approximately 10 kg.

For many of the dog studies, the drugs were administered either as the human formulation or as a compounded product. We assumed that if the issue of formulation effects was the primary cause of the identified disparities, differences in F would be more pronounced in low than in high solubility compounds. However, this was not the case, even with BCS 1 compounds (something that we found to be surprising and is further explored in the discussion).

For each drug included in our analysis, the human metabolism and transporter characteristics were summarized from published information. In some cases, the information was derived from drug databases such as Drugbank or Pubchem (see Supplemental Material 1). Since absorption, distribution, metabolism, and elimination (ADME) data are often unavailable for the dog, drug ADME characteristics solely reflected human PK attributes.

Because of the relatively small number of compounds included in each class, assessment of the utility of the BDDCS and ECCS for identifying likely drugs for which human-canine values of F will be comparable was largely assessed from a qualitative perspective. Nevertheless, for the purpose of visualization, a linear regression of dog F versus human F as a function of BDDCS or ECCS class is provided. The regression equation, confidence intervals (alpha = 0.10), prediction intervals and coefficients of determination were calculated using the Proc Reg procedure in SAS (version 9.4). Because of the number of observations available for ECCS 1B, 3B, and BDDCS 4, these classes were excluded from the analysis.

When assessing interspecies differences in plasma protein binding, several drugs were excluded due to the absence of canine protein binding data. Given the wide range of compounds, data limitations (particularly for dogs), and range of experimental conditions employed, the method for estimating free fraction was not included as a critical variable during this interspecies comparison. Rather, trends were identified as a function of ECCS class. When more than one published report contained protein binding values for a given drug and species, our assessments were based upon the averaged values.

RESULTS

A spreadsheet containing the physicochemical information, ADME characteristics, BCS, BDDCS, and ECCS classifications for all 46 compounds is available in Supplemental Material 1. The relationship between the BCS, BDDCS and ECCS classifications of the compounds included in this evaluation is provided in Table I. Table I also describes the aqueous solubility and LogP of each compound.

In our analysis, we arbitrarily applied the limits of 0.75 to 1.25 to reflect similarity in dog and human oral bioavailability. For 28 of the 46 compounds, dog F/human F ratios were less than 1; 18 of the 46 drugs had ratios ≥ 1. Half of the compounds (23/46) were associated with dog F /human ratios of F ranging between 0.75 and 1.25.

Table I. Comparison of compound characteristics and classification

Drug name	Solubility (mg/mL)	Log P	BCS*	BDDCS*	ECCS*	F_{human}	F_{dog}	Max dose (mg/kg) human	Dog dose (mg/kg)	Ratio of F dog/human
Acetaminophen	0.1	0.89	3	1	2	*0.87*	*0.63*	7.14	14.00	*0.72*
Amlodipine	1	0.29	3	1	2	0.74	0.88	0.14	2.50	1.19
Amoxicillin	4	− 0.58	3	3	3A	0.93	0.77	7.14	27.20	0.83
Atenolol	26.5	0.16	3	3	4	*0.54*	*0.83*	1.43	40.00	*1.54*
Azithromycin	0.01	4.02	2	3	4	*0.34*	*0.97*	8.57	24.00	*2.85*
Bisoprolol	1000	1.94	1	3	2	0.80	0.91	0.14	1.10	1.14
Carvedilol	0.01	3.14	2	2	2	*0.25*	*0.14*	0.36	1.10	*0.57*
Cephalexin	1	− 0.67	3	3	3A	*0.95*	*0.57*	7.14	24.00	*0.60*
Chlorpheniramine	100	3.77	1	1	2	0.41	0.36	0.06	10.00	0.88
Cimetidine	1	0.79	3	3	4	0.62	0.75	11.43	10.00	1.21
Ciprofloxacin	10	1.32	2/4	4	3A	0.60	0.66	10.71	25.00	1.10
Clindamycin	100	2.16	1	1	2	0.87	0.73	4.29	15.00	0.83
Clomipramine	100	5.65	1	1	2	0.87	0.73	0.36	5.62	0.83
Codeine	100	1.45	3	1	2	*0.48*	*0.16*	0.86	4.50	*0.33*
Cyclosporine	0.01	2.95	2	2	2	*0.50*	*0.04*	1.43	5.00	*0.08*
Diazepam	1	2.98	1	1	2	*0.28*	*0.23*	0.14	4.00	*0.82*
Diltiazem	100	2.64	1	1	2	*0.38*	*0.26*	1.71	3.00	*0.68*
Digoxin	0.01	1.95	1	3	4	0.70	0.58	0.00	0.04	0.83
Dolasetron	100	0.062	1	1	2	0.07	0.07	1.43	6.50	1.03
Doxycycline	0.1	− 3.66	1	3	3B	*0.93*	*0.37*	1.43	10.00	*0.39*
Fluoxetine	10	4.27	1	1	2	0.95	0.72	0.71	2.00	0.76
Furosemide	0.01	0.74	4	4	3A	*0.61*	*0.77*	1.14	40.00	*1.26*
Gabapentin	100	− 1.12	3	3	3A	*0.60*	*0.80*	11.43	85.00	*1.33*
Hydralazine	40	0.73	3	1	2	*0.35*	*0.77*	0.71	10.40	*2.20*
Hydroxyzine	0.01	3.32	2		2	0.60	0.72	1.43	4.25	1.20
Ibuprofen	0.01	3.14	2	2	1A	0.80	0.77	11.43	8.75	0.96
Ketoprofen	0.051	3.31	2	2	1A	0.90	0.90	1.07	10.00	1.00
Levetiracetam	1040	2.79	1	3	4	1.00	1.00	10.71	50.00	1.00
Linezolid	1	0.58	1	1	2	1.00	0.97	8.57	25.00	0.97
Lorazepam	0.08	2.39	1	1	2	*0.93*	*0.60*	0.03	0.94	*0.64*
Meloxicam	0.01	0.97	4	2	1A	0.97	1.00	0.21	0.26	1.03
Metoclopramide	0.01	1.48	1/3	3	2	*0.82*	*0.48*	0.14	2.30	*0.58*
Metronidazole	10	− 0.46	3	1	2	0.99	0.80	7.14	43.56	0.81
Minocycline	33	0.05	3	1	1B	*0.95*	*0.50*	1.43	10.00	*0.53*
Morphine	62.5	1.19	3	1	2	*0.24*	*0.05*	0.14	1.50	*0.22*
Naproxen	33	2.86	2	2	1A	0.99	0.84	7.14	10.00	0.85
Phenobarbital	0.1	1.52	1	1	1A	1.00	0.97	1.43	36.45	0.97
Phenytoin	0.01	2.14	2	2	1A	*0.90*	*0.36*	1.43	15.50	*0.40*
Piroxicam	0.023	3.06	2	2	1A	0.90	1.00	0.29	0.42	1.11
Procainamide	5	0.88	3	3	4	0.83	0.85	7.14	25.00	1.02
Propranolol	33	2.65	1	1	2	*1.00*	*0.27*	1.29	1.00	*0.27*
Ranitidine	100	0.63	3	3	4	*0.27*	*0.81*	4.29	3.85	*3.00*
Rofecoxib	0.01	1.83	2	2	1A	*0.93*	*0.26*	0.71	5.00	*0.28*
Sildenafil	1	2.11	1	1	2	*0.38*	*0.54*	1.43	1.50	*1.42*
Theophylline	8.33	− 1.03	3	1	2	0.99	0.91	4.29	16.00	0.92
Tramadol	33	2.52	1	1	2	0.73	0.65	0.71	8.78	0.89

*BCS, BDDC, and ECCS classifications are based upon human drug characteristics. Italicized entries is intended to highlight those drugs with dog F/human F ratios outside the arbitrary bounds of 0.75–1.25. Due to interstudy and intrastudy variability in subject weights, human doses are estimated as administered dose divided by 70 kg. For dogs, this is administered dose divided by 10 kg

Comparative Protein Binding

When dealing with total drug concentrations, as we are doing in this manuscript, species differences in plasma protein binding can influence the apparent total drug clearance of a low extraction ratio drug during its movement through the liver. Therefore, to explore the possibility of bias attributable to reported total rather than free drug concentrations, the unbound fraction (fu) of these 46 drugs in humans and dogs were compared. We observed scatter in the relationship between the fu in dogs and humans (Fig. 1a). To determine if there was any influence of drug ECCS class and human-canine estimates of fu, trends in this relationship was evaluated as a function of ECCS class (Fig. 1b–d).

The smallest magnitude of interspecies disparity was observed in ECCS 1A and 2. However, we cannot exclude the

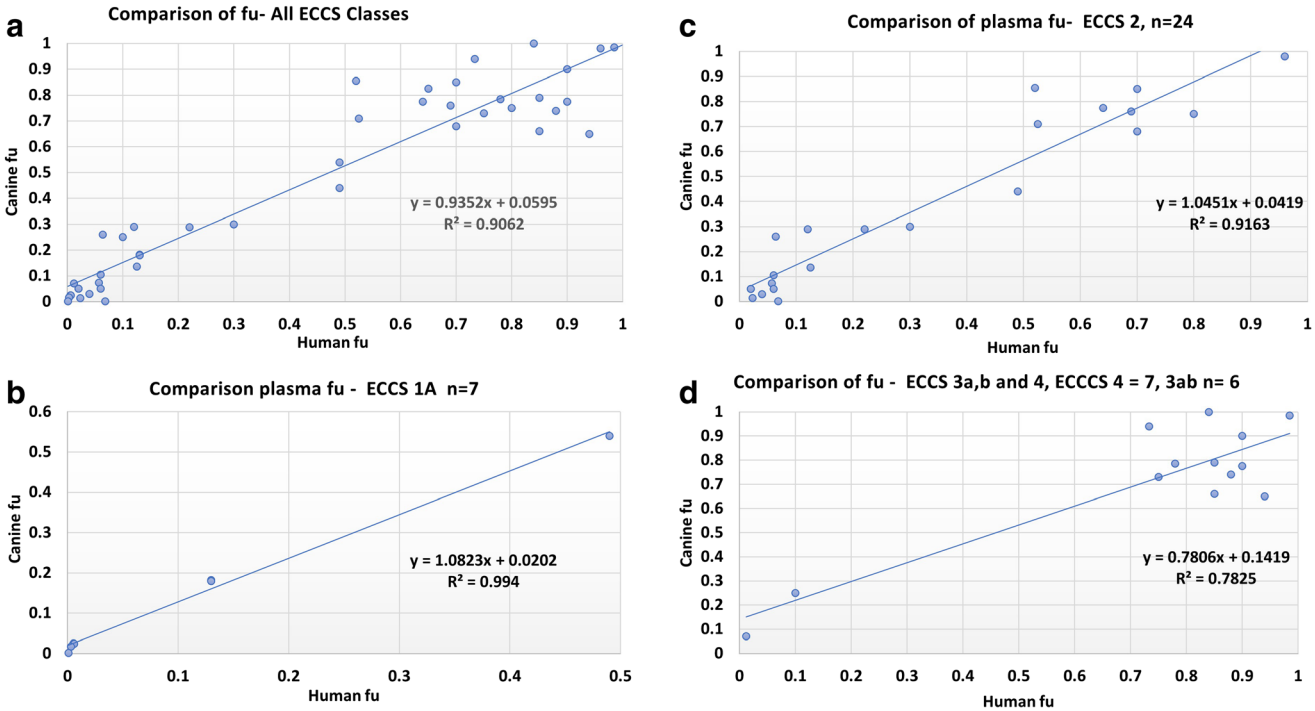

Fig. 1. **a**–**d** Relationship of unbound fraction (fu) in humans versus dogs as a function of ECCS class. **a** Comparison of fu in dogs and humans across all ECCS classes. **b** Interspecies comparison of fu for ECCS 1A. **c** Interspecies comparison of fu for ECCS 2. **d** Interspecies comparison of Fu for ECCS classes 3A, 3B, and 4

possibility that the minimal disparity was influenced by the small number of compounds included in this comparison. In contrast, the largest disparity was observed with ECCS 3A, 3B, and 4. Interestingly, the class 4 (bases and neutral) compounds were associated with high levels of fu (human fu = 0.66–1.0). The data used in this analysis and the corresponding sources of information are provided in Supplemental Material 2.

With most compounds having $\leq$ 85% protein binding, we considered any interspecies differences to have negligible impact on drug first pass drug loss. However, this raised the question of how similar was binding for those compounds where fu < 10%, where small deviation in fu can have a substantial impact on the relative fraction of free drug in the blood. Therefore, we plotted those compounds separately.

For the drugs with protein binding in humans > 90%, there was substantial variability in the canine/human relationship in fu. Interestingly, in nearly all cases where fu < 0.1, the canine fu exceeded that of humans (Fig. 2). Most of these compounds were either ECCS 2 or 1A.

When assessing the dog F/human F values for these 13 highly protein bound compounds, we see that 9 of the 13 ratios were between the values of 0.75–1.25. The other four compounds ranged from 0.08 to 1.62. Therefore, based upon the available information, it is concluded that we cannot attribute difference in dog versus human first pass drug loss to species-specific plasma protein binding characteristics.

BDDCS

A linear regression describing the relationship between dog F and human F estimates for three of the four BDDCS classes is provided in Fig. 3a–c. One class (class 4) did not contain enough observations to support this kind of evaluation. The low coefficient of determination across all three BDDCS classes is consistent with the challenges associated with efforts to use solubility and metabolism alone for identifying drug characteristics more likely to exhibit interspecies similarities in F.

Classifying drugs in accordance with the BDDCS did not improve the ability to identify those drugs for which human-canine extrapolations are appropriate. Considering those compounds for which dog F/human F ratios were within the limits of ± 25%, 10 /23 (43%) were from BDDCS class 1, 5/9 (55%) were from BDDCS class 2, and 6/12 (50%) were from BDDCS class 3. Only two of the 46 drugs were BDDCS class 4. Given the similarity in results across the three BDDCS

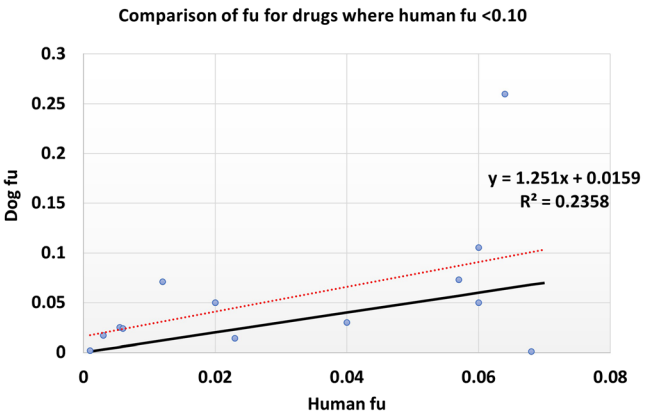

Fig. 2. Relationship between unbound fraction (fu) in dogs versus humans for those drugs associated with > 99% protein binding in at least one species. The dark black line describes unity while the red hatched line reflects the linear regression of human versus canine fu

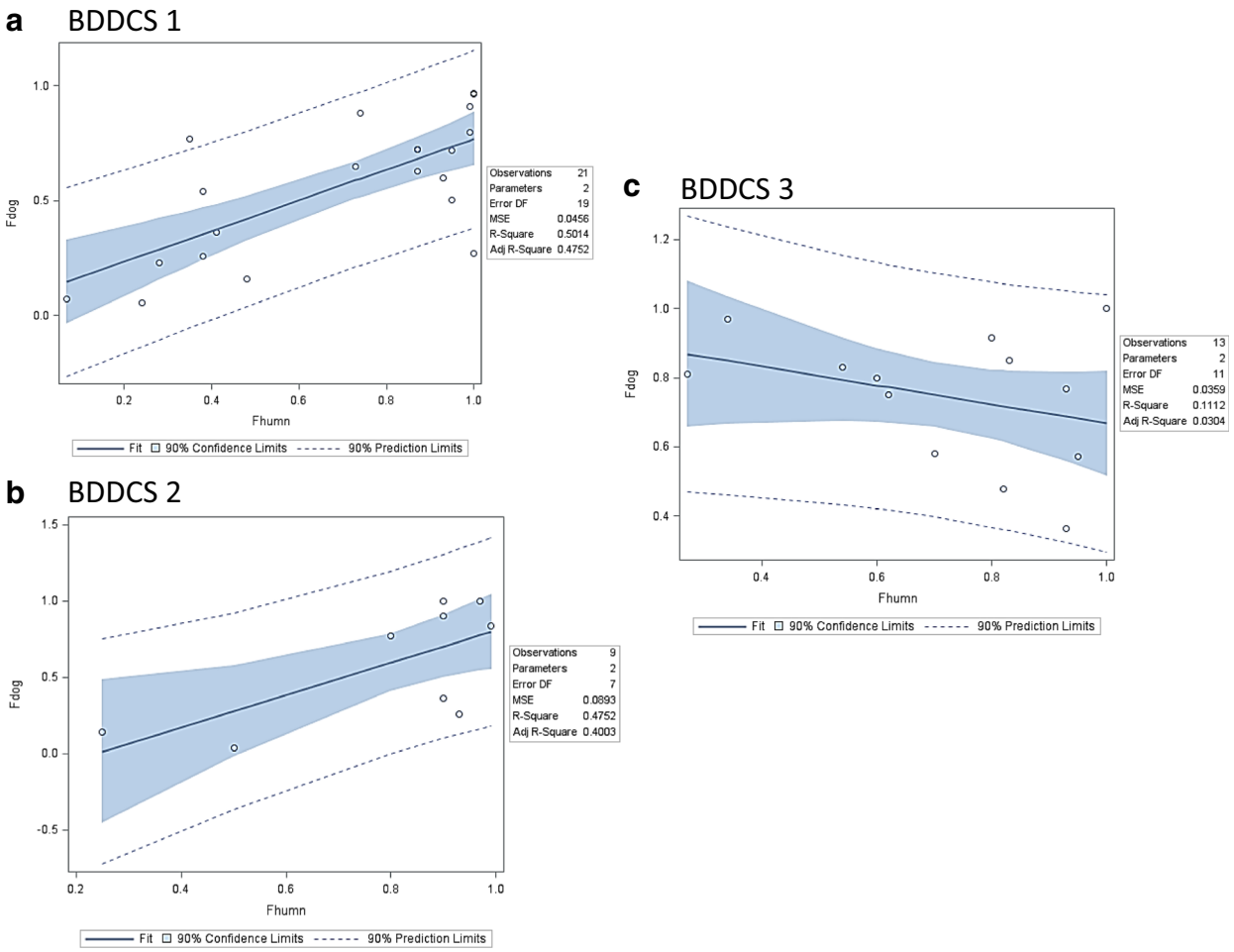

Fig. 3. **a**–**c** Linear regression of dog F versus human F as a function of BDDCS classification. **a** BDDCS 1. **b** BDDCS 2. **c** BDDCS 3. A description of the statistical evaluation is provided in "Methods"

classes, this method of drug classification is concluded to provide no additional insights for predicting compounds likely to exhibit a high level of correlation in the respective human-canine estimates of F (Table II).

ECCS

A linear regression describing the relationship between dog F and human F estimates for four of the six ECCS classes is provided in Fig. 4a–d. Two classes (classes 1B and 3B) did not contain a sufficient number of observations to support this kind of evaluation. Like that seen with the BDDCS, the low coefficient of determination across all four ECCS classes is consistent with the challenges associated with efforts to use drug classification systems as a high-level tool for identifying those drugs that are more likely to exhibit interspecies similarities in F. The limited number of observations included in classes 1A, 3A, and 4 was considered when concluding that the assessment of the ECCS as a tool is best evaluated on a qualitative basis.

A summary of the dog F/human F ratios as a function of ECCS class is provided in Table III.

Compared to the other ECCS classes, those drugs contained in class 1A (acidic, highly metabolized drugs) exhibited the largest proportion of dog F/human F ratios within the limits of 0.75–1.25 (75% = 6/8). Compounds in this ECCS class are typically well absorbed, are metabolized primarily by hepatic CYP2Cs, esterases and UGTs, and not subject to extensive intestinal first pass metabolism. However, more compounds need to be examined to determine if this trend in species similarity can be maintained.

A successful correlation for class 2 compounds was observed in 52% (13/25) of the compounds. Despite their high permeability, these drugs also present with the highest likelihood of intestinal first pass drug metabolism. Metabolism may have contributed to the wide range of dog F/human F ratios seen in this ECCS class (0.22–2.20).

For class 3A, only 40% (2/5) of the drugs met our ±25% criterion, which may reflect the involvement of transporters. However, the range of these ratios was relatively narrow as compared to that in other classes (0.60–1.33). These compounds also exhibit minimal presystemic loss and its primary route of elimination is renal (thus avoiding species-specific characteristics for presystemic drug losses). Again, due to the small number of compounds evaluated, it is difficult to draw reliable conclusions from the available information.

For class 4 compounds, 57% (4/7) compounds were associated with higher estimates of F in dogs than in humans

Table II. Dog F/human F ratios segregated by BDDCS classification. Italicized entries indicate values that outside of the bounds of 0.75 to 1.25

Class 1	Class 2	Class 3	Class 4
0.72	*0.57*	0.83	1.10
1.19	*0.08*	*1.54*	*1.26*
0.88	0.96	*2.85*	
0.83	1.00	1.14	
0.83	1.03	*0.60*	
0.33	0.85	1.21	
0.82	*0.40*	0.83	
1.03	1.11	*0.39*	
0.76	*0.28*	*1.33*	
2.20		1.02	
0.97		*3.00*	
0.64		1.00	
0.81		*0.58*	
0.53			
0.22			
0.97			
0.27			
1.42			
0.92			
0.89			
0.68			

(Table III). Although the sample size is small, this trend of dog F > human F is different from that seen in the other drug classes, suggesting a possible unique characteristic in drugs in ECCS 4 that should be considered.

DISCUSSION

Critique of the Results

Several potential sources of interspecies differences need to be considered when assessing potential reasons for observed disparities in F.

1. First pass drug loss in the liver or in the small intestine (as suggested by our findings in ECCS class 3A).
2. The involvement of intestinal influx or efflux transporters.
3. Paracellular absorption processes.
4. Biliary recycling.
5. Drug ionization in the GI tract.

For example,

1. Paracellular transport: This passive process is influenced both by charge and molecular size/geometry, and for a given size, the process appears to be selective for cationic rather than anionic and neutral drugs (23). As we observed in our previous paper (1), dogs have larger pore diameter and a greater abundance of intestinal pores as compared to other species (9). This difference in pore number and diameter may have contributed to the observed species differences. In the paper by Van De Waterbeemd *et al.* (24), examples were provided of drugs with higher F in dogs than humans due to greater paracellular transport in dogs. In the current evaluation, this process appeared to be a potential cause of interspecies differences both for acids (ECCS class 3A) and for cationic (ECCS class 4) compounds:
   a. Furosemide (ECCS class 3A, dog F/human F = 1.26): with a pKa of 3.9, we expect that this weak acid may dissolve better in the canine than human stomach (gastric pH tends to be higher in dogs). While this may have contributed to the higher bioavailability in dog (as would be argued by Matsumura *et al.* (25), it may also reflect its greater paracellular absorption in the dog (26).
   b. Atenolol (ECCS class 4, F dog/F human = 1.54): with a pKa of 9.6, it is by unlikely that solubilization is a cause for the difference in the oral bioavailability of this weak base. Rather, considering the physicochemical characteristics and the absence of substantial first pass metabolism, the most probable cause for this human-canine difference is the role of paracellular absorptive processes (27).
   c. Ranitidine (ECCS class 4, F dog/F human = 3): with a pKa of 8.08, it is unlikely that solubilization is a cause for the difference in the oral bioavailability of this weak base. Rather, the much higher oral bioavailability in dogs is likely attributable to the role of paracellular transport (28).
2. Possible dose-associated bias: Rofecoxib ((ECCS class 1A, F dog/F human = 0.28): with a pKa exceeding 14, it is unlikely that solubilization is a cause for the difference in the oral bioavailability of this weak acid. In humans, the estimated 93% oral bioavailability remained consistent when administered doses ranging from 12.5 to 50 mg. However, in dogs, exposure (as represented by $AUC_{0-\infty}$) increased in a dose proportional manner following IV administration (1–4 mg/kg) but was substantially less than dose proportional after oral administration (2 to 10 mg/kg) (29). In that same study, based on mean canine $AUC_{0-\infty}$ following oral versus IV administration, F was estimated as 58% (2 mg/kg dose), 32% (5 mg/kg dose) and 17% (10 mg/kg dose). {Note that in that paper, there is a slight difference between these values and the estimate of 28% described in the abstract and discussion versus the ratio of mean values that was estimated from the data provided in the published table. The explanation for this slight discrepancy is undetermined, but it does not impact our overall conclusions}. From this information and from the relative amount of drug in canine feces after oral versus IV administration, low F for the dog is attributed to poor absorption across the mucosa rather than to first pass drug loss. The reported F for humans is based upon the administration of a 50 mg dose (approximately 0.71 mg/kg). Because of the large difference in the mg/kg dose administered to humans compared to dogs, we cannot determine if the observed inequivalence is a function of differences in administered mg/kg dose or to a true difference in oral bioavailability at comparable doses. Optimum evaluation of interspecies differences should use comparable doses whenever possible.

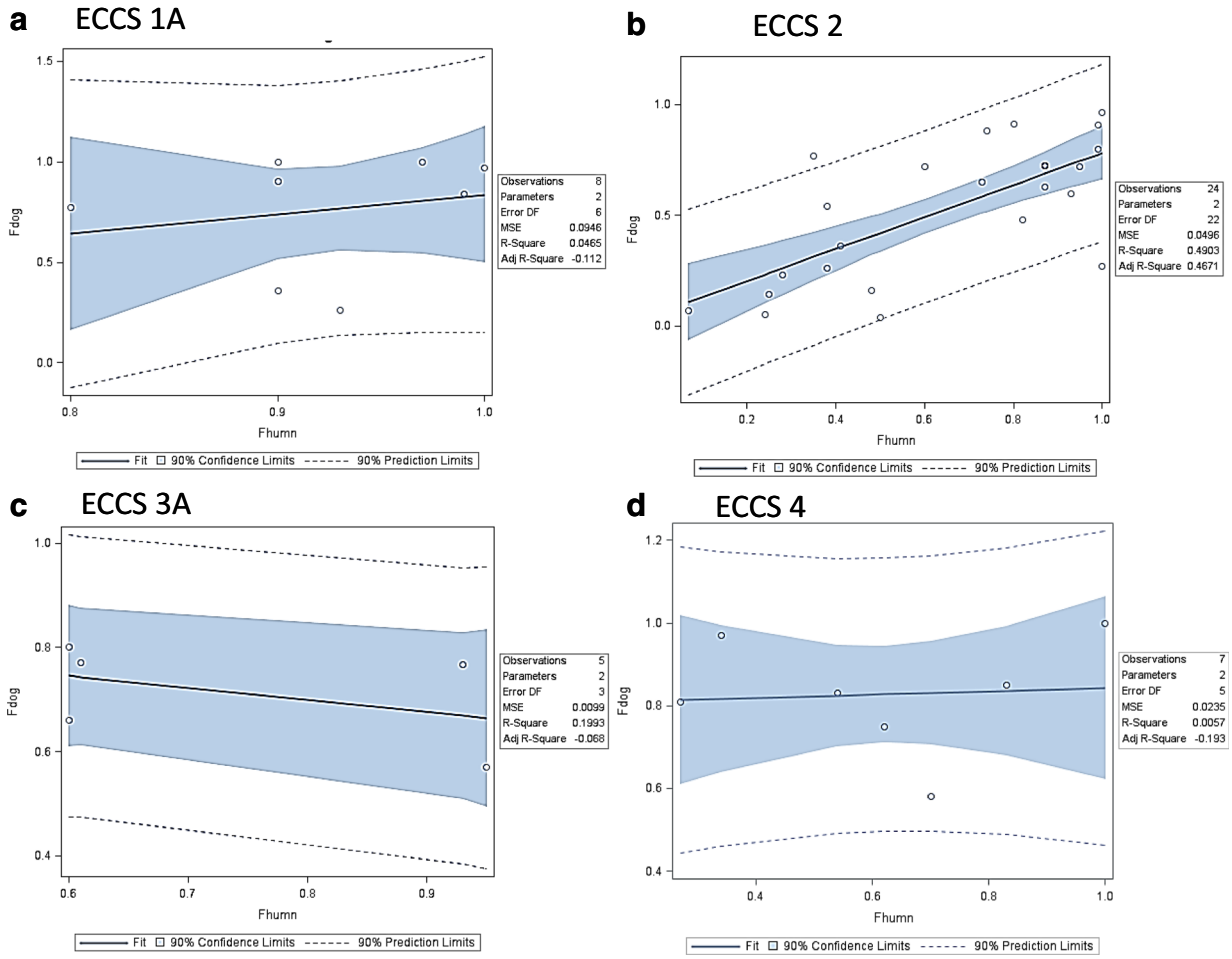

Fig. 4. **a**–**d** Linear regression of dog F versus human F as a function of BDDCS classification. **a** ECCS 2. **b** ECCS 1A. **c** ECCS 3A. **d** ECCS 4. A description of the statistical evaluation is provided in "Methods"

3. Possible species differences in luminal events: Minocycline (ECCS 1B dog F/human F = 0.53): with an acidic pKa of 2.9 and a basic of about 7.9, it is unlikely that aqueous solubility is a cause for the observed interspecies difference. There also does not appear to be a high first pass drug loss in humans, and elimination in dogs has been attributed to renal clearance (30). This nullifies the explanation that human-canine oral bioavailability differences are attributable to first pass drug loss. Thus, the cause of this disparity remains undefined. However, because chelation of tetracyclines by divalent cations can have a profound effect on oral absorption, it is possible that this can affect dogs more than humans. Future studies should consider whether there are differences in the cation concentration in the contents of the human versus canine gut and if such differences could contribute to interspecies differences in the oral bioavailability of some compounds.
4. Possible impact of species differences in GI transit times: Phenytoin (ECCS class 1A, dog F/human F = 0.40). Despite being a weak acid with a pKa of 8.33, its low solubility may translate to a need for longer residence time in the GI tract. Given the shorter intestinal length in dogs than in humans, this may explain the poorer absorption in the dog as compared to the human [e.g., see (31)].
5. Possible impact of species differences in regional transporter expression: Azithromycin (ECCS Class 4, dog F/human F = 2.85, base, pKa = 8.74). Interestingly, based upon a study in ileostomy patients, azithromycin was found not to exhibit substantial first pass drug loss in humans and the poor oral bioavailability in humans may largely reflect incomplete absorption (32). P-glycoprotein (P-gp), biliary excretion, and OATP (uptake transporter) appear to influence the oral bioavailability of azithromycin. P-gp has the greatest abundance in the dog colon with little expression in the upper small intestine of the normal healthy dog (33). In contrast, the human expression of P-gp can be seen throughout the small intestine with the greatest amounts being found in the lower small intestine (34). This difference in the region of expression for this efflux transporter may contribute to the lower oral bioavailability in humans.

One of the issues confronted when conducting these cross-study comparisons was the higher mg/kg doses typically used when studying oral bioavailability in dogs versus humans. Consequently, it is important to consider the possible

Table III. Ratio of dog/human estimate of F for each ECCS class. Italicized entries indicate values that are outside of the bounds of 0.75 to 1.25

Class 1A	Class 1B	Class 2	Class 3A	Class 3B	Class 4
0.96	*0.53*	*0.72*	0.83	*0.39*	*1.54*
1.00		1.19	*0.60*		*2.85*
1.03		1.14	1.10		1.21
0.85		*0.57*	*1.26*		0.83
0.97		0.88	*1.33*		1.00
0.40		0.83			1.02
1.11		0.83			*3.00*
0.28		*0.33*			
		0.08			
		0.82			
		0.68			
		1.03			
		0.76			
		2.20			
		1.20			
		0.97			
		0.64			
		0.81			
		0.22			
		0.27			
		1.42			
		0.92			
		0.89			
		0.58			

influence of dose on the solubilized fraction of administered dose in dogs versus humans, its potential impact on intestinal permeability, first pass metabolism, and on in vivo dissolution. For this, it is worthwhile to review the information from the original BCS article by Papich and Martinez (1).

a. For compounds where a classification of highly soluble in humans is linked to the administration of doses that result in a low dose number, there is the possibility that a BCS 1 or 3 drug in humans may behave as a BCS 2 or 4 drug in dogs. Discussion of this point was addressed in our first manuscript (1).
b. In terms of the higher dose leading to a decrease in first pass extraction (saturable elimination processes), this would have resulted in dogs having a higher rather than the frequently observed lower estimate of F as compared to that of humans. Furthermore, in the Supplemental Material 1, we have identified those compounds that are associated with a high first pass drug loss in humans. Therefore, it is unlikely that saturable first pass drug loss is responsible for most observed instances of the poor correlation.
c. In terms of transporters, higher doses in dogs could potentially saturate influx transporters, resulting in lower estimates of estimates of F. Again, although influx transporter information is largely lacking in dogs, the Supplemental Material 1 provides drug-specific information for humans. Based upon the few drugs where this would be an issue, it is unlikely that saturation of influx transporters was responsible for the species differences in F.
d. Lastly, higher doses could be problematic in cases of dissolution-rate limited drugs, especially considering the more rapid GI transit in dogs. An example of this was shown by Martinez *et al.* (12) where dissolution-rate limited formulations performed far more poorly in dogs as compared to humans. Assuming that problems associated with in vivo dissolution will be a minor issue for immediate release formulations of drugs that are highly soluble and highly permeable, we would anticipate that drugs in this BCS class would show substantially closer interspecies estimates of F than what is seen across other BCS classifications. However, this was not the case (1).

The other point of potential bias in this study was the use of different formulations in dogs vs humans in several of the cited studies. One possible argument worthy of consideration is that this would have led to formulation-induced differences in dog versus human in vivo dissolution and therefore differences in oral drug absorption. However, we have reason to believe that if this was a problem, it would have minimal influence on the conclusions derived from our survey. For example, we would anticipate that formulation effects would have been more pronounced for low solubility than for high solubility compounds. Furthermore, if the dog formulation had resulted in less drug moving past an absorption window for low (versus high) permeability compounds, we would have expected to see a far better interspecies correlation (less of a risk of formulation-induced differences in oral drug absorption) associated with BCS I compounds. There also may have been fewer formulation effects associated with BCS III than with either BCS II or IV drugs. In terms of the use of the ECCS, we have moved away from using solubility and dose number as a criterion for classification (although it is indirectly incorporated into the criteria by considering lipophilicity, pKa, and mechanism of elimination, where those drugs primarily eliminated by the kidneys would be expected to be hydrophilic). Nevertheless, this too failed to define those compounds likely to exhibit similar values of F in humans and dogs. Thus, we conclude that although the problem associated with the use of published literature was present, it did not bias the conclusions generated with our evaluation.

Ultimately, although none of the classification systems succeeded in categorizing compounds in a manner that provided an assurance of similar F values in dogs and humans, the ECCS did help to refine our focus on the potential reasons for observed interspecies differences. The segregation of drugs in accordance with their molecular and ADME characteristics helped to discern if the basis for these differences is ionization (which could influence in vivo dissolution, the proportion of dose in a state amenable for transcellular movement or movement through the unstirred water layer (7,35)), first pass drug loss, segmental differences attributable to species differences in intestinal transporter distribution, or the potential for paracellular absorption in the dog.

Comparison of Drug Classifications Using the BDDCS Versus the ECCS

As shown in Fig. 5, molecular classification based upon the ECCS has several important distinctions from that of the

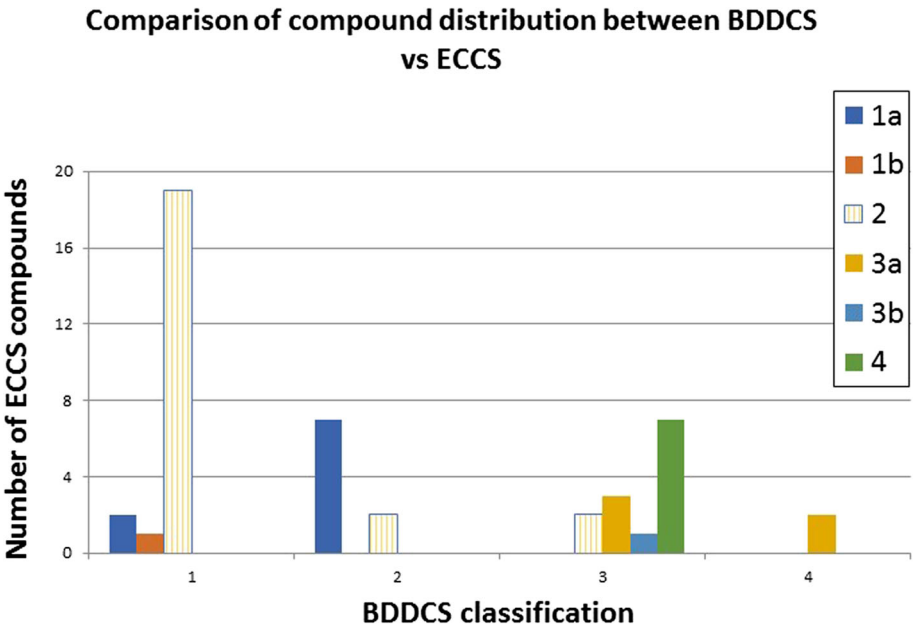

Fig. 5. The number of compounds within each of the ECCS classifications that fall within a specific BDDCS class (*X*-axis). Within each BDDCS class, each bar represents one of the six ECCS drug classes

BDDCS. For example, ECCS classes 1A, 2 and 3a contained compounds that were included in two or more BDDCS classifications. Conversely, of the drugs examined, all ECCS class 4 drugs were classified as BDDCS 3. Too few drugs were contained within ECCS classes 1B and 3B to draw any conclusions. Because the total number of drugs included in this evaluation is limited, more examples are needed to determine if this outcome represents a reproducible trend.

View from an Alternative Perspective

Matsumura *et al.* (25) examined the fraction of an oral dose that was absorbed across the enterocyte membrane (*fa*) in humans versus dogs. Their method of evaluation was markedly different from ours in that we evaluated F, but they estimated *fa* based upon drug solubility and species intestinal characteristics. Their work focused on BCS class 2 compounds (note that in our previous publication (1), we encountered problems when trying to extrapolate BCS class from humans to dogs). Nevertheless, interesting information was derived from their study. Based upon their assessment of 5 compounds with diverse characteristics, Matsumura *et al.* (25) concluded that the f_a values of neutral compounds will be similar in dogs and humans but that for acidic compounds, dogs tend to have the higher estimate of f_a as compared to humans. This was not consistent with our observations of F where it was primarily ECCS Class 4 drugs that were associated with higher values of F in dogs than humans. However, an important point that these authors did provide was that when dealing with acidic compounds, solubility within the canine intestinal tract will likely be greater than that in humans due to inherent differences in GI fluid composition and pH. A similar point was raised by Walsh *et al.* (7) who observed that substantially higher solubility in canine versus human fasted state simulated intestinal fluids was largely attributable to the higher pH and bile salt composition in the dog. We did not specifically consider this issue, but it is evident that when in vivo solubilization is the rate-limiting step, there may be a greater risk of error when trying to extrapolate the F (or *fa*) of acidic compounds across species.

CONCLUSIONS

The ECCS positioned us to identify the potential causes and the molecular characteristics associated with an increased likelihood for similar values of F in dogs and humans:

1. There typically is a better correlation observed with those compounds that are weak acids or zwitterions as compared to bases. However, as noted by Matsumura *et al.* (25), pKa needs to be considered relative to that of the canine versus human GI fluid pH.
2. The presence of high first pass drug loss (intestine or liver) increases the likelihood of poor canine-human correlations in oral bioavailability.
3. There is a range of molecular weights that increase the likelihood of differences in membrane transport processes (transcellular versus paracellular).
4. It is important to ensure that similar doses (on mg/kg basis) are compared in dogs and humans because there is the risk of saturation (transporter, metabolism) that may artificially result in observed differences in the estimates of F in dogs and humans. There also is the risk of greater solubility issues due to the limited amount of fluid within the canine GI tract.
5. Location of transporters are critical (influx and efflux). Even if the same transporter is involved, the unique distribution of transporters in the dog and humans can alter product bioavailability, particularly for those compounds that more slowly dissolve within the GI

tract. For the influx transporters located in the proximal small intestine, the shorter intestinal length and faster transit time for dogs as compared to humans may limit the "window" available for canine drug absorption. In this case, canine oral bioavailability be more sensitive to factors affecting in vivo drug dissolution. This is where tools such as physiologically based pharmacokinetic models can be invaluable, providing insights into intestinal characteristics that can bias the interspecies extrapolation.

As a result of the parameters considered within the framework of the ECCS, this classification system increases our ability to identify the relationship between a drug's physicochemical characteristics and host physiology. This understanding will be valuable for future interspecies extrapolations in oral bioavailability and for understanding potential species differences in rate-limiting factors that can impact the formulation of future medicines.

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