

Veterinary Application of In Vitro Dissolution Data and the Biopharmaceutics Classification System

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ABSTRACT The Biopharmaceutics Classification System (BCS) has been developed for human pharmaceutical compounds to predict oral drug absorption. A similar approach for evaluating in vitro data regarding dissolution, solubility, and permeability of veterinary oral dosage formulations has not been applied to predict oral absorption in animals. However, if reliable data can be generated, it may be possible to apply these principles to veterinary drugs. Before this can happen, obstacles must be overcome. Because of differences in anatomy and physiology between animals and people, extrapolations to veterinary drug formulations may not be applicable. Veterinary drug formulations also may differ in their size, excipient content, and use compared to human drug formulations. There is a clear need to examine the application of in vitro data regarding dissolution and permeability for product evaluation and regulatory decisions. This article and future scientific presentations will explore the potential for this application.

Upon resolution of these questions and following appropriate adjustments to testing methods for permeability, solubility, and dissolution assessments, we will be able to apply, with confidence, BCS principles to oral formulations intended for use in dogs. We believe that the extrapolation of BCS principles to oral formulations for use in canines will prove to be an extremely valuable contribution to veterinary medicine.

This *Stimuli* article on the potential applications of BCS to veterinary drugs was endorsed by the USP Veterinary Drugs Expert Committee. Reader comments about the suggested applications of in vitro solubility, permeability, dissolution data, and the BCS are invited. These comments should be directed to Ian F. DeVeau, Ph.D., at the Department of Standards Development, U.S. Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852-1790, telephone: 301.816.8178; fax: 301.816.8373; e-mail: ifd@usp.org.

INTRODUCTION

The USP Veterinary Drugs Expert Committee (EC) formed an ad hoc committee to examine and make recommendations about in vitro dissolution and oral absorption of veterinary pharmaceutical compounds. The basis for this initiative has been the development of the Biopharmaceutics Classification System (BCS) for human pharmaceutical compounds. The BCS is one of the most significant tools recently developed to facilitate product development

and regulatory decisions (1). By understanding a compound's solubility and permeability characteristics, pharmaceutical scientists can develop a mechanistic approach to predict the influence of variables such as food, formulation, dosing regimen, and disease on drug absorption from the gastrointestinal (GI) tract. By understanding the relationship between a drug's in vivo absorption profile and the in vitro solubility and dissolution characteristics, one can identify conditions under which the in vitro data could serve as a surrogate for in vivo bioequivalence testing. The correlation between in vitro data and in vivo absorption is abbreviated as IVIVC.

Pharmaceutical compounds can be grouped into one of the following categories:

CLASS I: High Solubility, High Permeability: generally very well absorbed compounds.

CLASS II: Low Solubility, High Permeability: exhibit dissolution rate–limited absorption.

CLASS III: High Solubility, Low Permeability: exhibit permeability-limited absorption.

CLASS IV: Low Solubility, Low Permeability: very poor oral bioavailability.

The rate of product dissolution may influence the resulting plasma concentration/time profile if the drug is Class II. However, for Class I or III drugs, other factors may influence drug absorption. For example, for Class I compounds (highly soluble, highly permeable) the rate of gastric emptying rather than product performance is the rate-limiting step in determining the bioavailability characteristics. For these formulations, marked difference in in vitro dissolution profiles may occur without any resulting differences detected in product bioavailability (2, 3, 4). Similarly, highly soluble, poorly permeable compounds (Class III) dissolve rapidly. However, in these cases, it is not the rate of drug dissolution that is usually rate-limiting but rather diffusion across biological membranes. Therefore, we can again assume that for Class III compounds, if dissolution is faster than the rate of gastric emptying then differences in product dissolution will not affect product bioavailability. For Class III compounds, as long as absorption occurs via linear processes, the absolute amount of drug absorbed can be increased with a higher dose (5).

On the other hand, for high-permeability, low-solubility compounds (Class II), the rate and extent of product dissolution will have a significant role in defining the resulting blood concentration/time profile (6). This may be attributable to problems associated with either particle size (termed dissolution-limited absorption) or drug solubility (termed solubility-limited

absorption). When absorption is limited by solubility, particle size exerts minimal effect on the fraction of drug absorbed. In this situation, fraction of drug absorbed can be improved only by enhancing drug solubility. For example, solubility can be improved by including in the product formulation a surfactant or other excipient that may improve solubility. Conversely, for some compounds particle size also exerts a significant effect on absorption. In these cases, solubility is not a limiting factor, and improvement in the fraction of drug absorbed can be achieved by decreasing particle size, which increases surface area.

FDA CURRENT USE OF BCS CONCEPTS

FDA's Center for Drug Evaluation and Research (CDER) has incorporated BCS concepts into several guidance documents, including several pertaining to scale-up and post-approval changes, as well as a guidance for the waiver of in vivo bioequivalence study requirements for high-solubility/high-permeability drug products (Class I) based on in vitro dissolution data (7). To be granted a waiver of in vivo bioequivalence study requirements, CDER recommends that in vitro dissolution tests be conducted under the following conditions:

- The test apparatus should be USP Apparatus 1 at 100 rpm or Apparatus 2 at 50 rpm (8). Testing should be conducted in 900 mL of each of the following dissolution media: (1) 0.1 N HCl or Simulated Gastric Fluid USP without enzymes; (2) a pH 4.5 buffer; and (3) a pH 6.8 buffer or Simulated Intestinal Fluid USP without enzymes.
- For each formulation, a minimum of 12 dosage units should be evaluated and a dissolution profile generated. Two dissolution profiles are considered similar when the f_2 value is ≥ 50 .
- To allow the use of mean data, the coefficient of variation should not exceed 20% at the earlier time points (e.g., 10 minutes) or 10% at all other time points. If, under all dissolution conditions, both the test and reference products dissolve 85% or more within 15 minutes, profile comparisons are not necessary.

Controlled-Release Dosage Forms

The absorption of controlled-release dosage forms is, by definition, dissolution rate–limited. Because drug permeability characteristics must be sufficiently high to ensure the presence of sink conditions within the GI tract, the appropriate candidates for these products are Class I or II compounds (9). Moreover, to develop an IVIVC for these products, test procedures must account for the changing environments to which the oral dosage form will be exposed as it traverses the GI tract. These include:

- The impact of changes in fluid volume, surfactants, and motility patterns on product

dissolution and drug solubility

- Regional anatomic differences in intestinal permeability and surface area
- Regional physiologic differences in intestinal metabolism and secretion.

WHY CONSIDER VETERINARY APPLICATION OF BCS PRINCIPLES?

The scientific basis for drug classification in humans has been thoroughly tested, and the criteria for drug classification and biowaivers were shown to accurately predict product in vivo performance. Recently, Kassim et al. (10) developed BCS classifications for 325 medicines contained in the World Health Organization (WHO) Essential Drug List. Permeability was classified based on correlations of human intestinal permeability of 29 reference drugs with the estimated logarithm of the partition coefficient (Log P) or CLogP lipophilicity values. Metoprolol was chosen as the reference compound for permeability and Log P or ClogP. Log P and CLogP were linearly correlated ($r^2 = 0.78$) for 104 drugs. A total of 53 (43.1%) and 62 (50.4%) drugs on the WHO list exhibited Log P and CLogP estimates, respectively, that were greater than or equal to the corresponding metoprolol value and were classified as high-permeability drugs.

Considering the economic constraints under which veterinary product development, regulation, and practice must function, the incorporation of BCS principles would be extremely beneficial to veterinary medicine. The cost of development of veterinary pharmaceutical formulations is very expensive compared to the financial return in a market that is comparatively smaller than the human market. The benefits of applying BCS principles to veterinary drug formulations would be felt by the drug sponsor with lower costs of development, and veterinarians and animal owners would benefit from greater availability of medications. Unfortunately, the extrapolation of these criteria to veterinary species is not straightforward. There remain numerous interspecies differences in GI physiology that may influence clear-cut interpretation of permeability classifications. In addition, unlike the situation for human medicine, body size (among breeds of dogs, for example) determines the dosage strength used in veterinary species. Tablet sizes in veterinary medicine tend to span a large range so that the drugs can be safely and conveniently administered. Thus, the use of a set volume of fluid and dosage strength for defining drug solubility may not be appropriate in veterinary medicine. Lastly, the current criteria used for defining a rapidly dissolving product may not be appropriate in animal species for which the GI transit rate can be markedly greater than that observed in humans.

The potential importance of these variables needs to be considered prior to using BCS concepts as a predictive tool in veterinary species. Nevertheless, there can be enormous

value in having such a system available for classifying veterinary compounds. Upon considering the many veterinary species for which medications may be prescribed, the USP ad hoc committee agreed that the greatest benefit would derive from developing this tool for use in dogs, rather than considering all other species at this time.

Limit the Analysis to Formulations for Dogs

From a regulatory perspective, addressing this question first in dogs will have the largest impact on the development and regulation of veterinary pharmaceuticals. Most oral veterinary solid drugs are developed for dogs. In addition, dogs are often included as one of the preclinical species in which drugs are tested prior to use in humans. Consequently, there is a wealth of information already generated in dogs due to their ease of handling and because they are one of the species traditionally used for preclinical drug development/toxicological evaluation. There are fewer oral drugs for cats. Many oral drugs for horses tend to be in a paste or granule formulation. Cattle medications are most often given by injection or are orally mixed in water or feed. Therefore, the practical approach is to initially limit our study to oral formulations for dogs.

It is our hope that by examining those aspects of solubility, permeability, and in vitro dissolution test criteria that may be different in dogs versus humans we can better understand how interspecies differences may influence drug classification. The aim of the ad hoc USP committee is to develop guidelines that may be used for dogs, and these guidelines may eventually be developed into a new USP General Chapter on evaluation of veterinary oral pharmaceutical formulations. As a follow-up to this process, we will ultimately strive to extend our findings to support drug classification in other animal species.

BENEFITS DERIVED FROM ESTABLISHING THE BCS FOR DOGS

Numerous potential benefits could be derived by establishing BCS principles for facilitating the development and regulation of drugs and oral drug products for use in dogs. These include:

- Use in initial drug candidate selection
- Use in the development of product formulations
- Improvement of our understanding of drug products for which bioavailability may be different from that observed in humans (extralabel use) and those drugs that may have significant bioavailability problems when obtained from compounding pharmacies
- Regulatory applications:

- Waiver of Class I (and possibly Class III) compounds
- Help establish in vitro dissolution methods and specifications that have in vivo relevance and can be used to determine conditions under which additional in vivo bioavailability study data may not be necessary (the development of in vivo/in vitro correlations—IVIVC).

OBJECTIVES OF THIS USP INITIATIVE

The Veterinary Drug EC established this ad hoc USP committee with the mission to review the pertinent literature, examine original research, collect data on oral absorption of compounds in dogs, obtain public input, and possibly direct new research initiatives with the following goals in mind:

- To delineate ways in which in vivo dissolution and drug permeability can differ between humans and dogs.
- To develop a list of points to consider when developing in vitro dissolution methods intended to reflect potential formulation effects on product bioavailability in dogs.
- To explore the possibility of developing canine IVIVC and consider whether or not the criteria used to support waivers in human subjects is appropriate for supporting waivers of in vivo bioequivalence study requirements in dogs (particularly with regard to Class I and III compounds).
- Because of the recent interest in nonsteroidal anti-inflammatory drugs (NSAIDs) in canine veterinary practice, the criteria for these drugs should be examined even though they are considered Class II according to BCS review.
- To develop a list of excipients that can affect drug absorption in dogs. There may be excipients that can affect product bioavailability in a manner that may not be identified under in vitro test conditions. This list would be used to compare with a list of corresponding excipient effects recognized to occur in humans.

One of the first initiatives of the USP ad hoc committee will be to prepare a list of drugs for which data are available in dogs and classify them based on the model developed for compounds included on the WHO Essential Drug List (10). In this classification scheme, the following criteria will be considered:

- Is a related drug used under clinical practice conditions in dogs?
- Are there adequate data available to evaluate oral absorption in dogs?
- Is the drug approved within the U.S. for oral administration to dogs?
- Which of these human drug products may be subject to extralabel use in dogs?

- Are there differences in the dosage forms of these compounds when administered to humans versus dogs?
- Are there drugs that are frequently compounded from the human formulation for animal use by compounding pharmacies?

PROBLEMS WITH INTERSPECIES EXTRAPOLATIONS

One of the obstacles to overcome in applying the BCS to formulations of compounds used in dogs is interspecies extrapolation. Drugs administered to people may cause different absorption profiles in dogs because of a variety of factors. Some comparisons of oral absorption of drugs in humans versus dogs have been reviewed (11, 12, 13). The differences in oral absorption between dogs and humans can be partially explained by differences in anatomy and physiology (11, 14, 15). Interspecies differences in drug bioavailability are most often the consequence of variables such as GI transit time, in vivo dissolution, presystemic metabolism, physicochemical interactions with gut contents, bacterial digestion, and site-specific differences in absorptive surface area. The diversity in GI anatomy and function reflects differences in dietary habits of the animals (16). For example, carnivores, such as dogs, possess a relatively simple colon but well-developed small intestine (long villi), which is consistent with a diet that is low in fiber but high in fat and protein. As a generalization, gastric emptying time influences the systemic appearance of rapidly dissolved, well-absorbed drugs.

Differences in Gastric and Intestinal pH between Dogs and People

Dogs have lower basal acid secretion than do people (14, 11). The effect of food on gastric pH also differs across species. Generally, the gastric pH of fasted dogs is highly variable, ranging between 3 and 8 (17). In addition, a higher pH in the intestine of dogs by comparison with humans may result in better absorption of drugs that are weak bases, but this can be influenced by feeding. Following a meal, gastric acid secretion rates in dogs exceed those of humans and are slower to return to baseline. The postprandial gut pH in humans tends to exceed that observed in dogs due to the strong buffering action of the diet, returning to baseline values within approximately one hour (14).

A radiotelemetric technique (Heidelberg capsule) for monitoring canine GI function provided continuous pH profiles for baseline (fasted) and postprandial states (18). During a 6-hour test period in 4 Beagles, the baseline (fasting) gastric pH ranged between 0.9 and 2.5 (first 30 minutes of testing). The postprandial gastric pH values varied between 0.5 and 3.5 (first 30 minutes of testing). Baseline duodenal pH values during the initial 5 minutes after gastric emptying varied from 4.5 to 7.5. They varied between 4.5 to greater than 8.0 (during the

initial 60 minutes following gastric emptying). The intestinal pH tended to increase linearly over the initial 60-minute postprandial period. The mean gastric residence time of the capsule was 74 minutes in the fasted state (range 0 to 240 minutes), and postprandially the capsule remained in the stomach for the duration of the 6-hour observation period. This study highlights the variability in pH and gastric motility that can occur even within a single breed of dog.

In some cases, interspecies differences in product bioavailability are a result of dissimilarity in GI pH. Because dogs have higher basal intestinal pH and lower basal acid secretion in the stomach than do people, these differences appeared to be responsible for dissimilarities in the bioavailability of indomethacin (19), metronidazole (20), and cinnarizine (21). In other situations, pH dependence appears to be related to product formulation rather than the active compound, as was the case when the relative bioavailability of two formulations of L-735,524, an HIV protease inhibitor, was different when tested in rats versus dogs (22). Two extended-release formulations of theophylline developed for humans did not show an extended-release profile for dogs (23). The elimination half-life of theophylline in dogs was only slightly longer for extended-release oral formulations compared with the IV formulation, which would not be considered sufficient for extended release. Theophylline from these capsules and tablets was rapidly and completely absorbed and had to be administered every 12 hours in dogs to maintain therapeutic plasma concentrations. These studies were performed in unfed dogs, in which intestinal pH is higher than humans. It is possible that different findings would occur if there had been comparisons with fed dogs.

Differences between Dogs and People in Gastric Emptying

Particle dispersion is important for optimizing in vivo dissolution characteristics of low-solubility or slowly dissolving dosage forms. In humans, particle density has been demonstrated to significantly impact gastric emptying time, with heavier particles (e.g., 2.8 g/cm³) being retained longer than less-dense particles (e.g., 1.5 g/cm³) (24).

As is the case for humans, particle size affects gastric emptying time in veterinary species. In dogs, very small particles (e.g., 1 g/cm³, 1.6 mm diameter) empty more rapidly than do particles whose diameter exceeded 2.4 mm (25). Particles greater than 7 mm were not emptied from the canine stomach until 6–8 hours after food intake during a late phase of digestion (26). When one compares the gastric emptying of fasted humans, dogs, and minipigs, the order in rate of gastric emptying is dogs > humans > minipigs (27). These differences are observed both with tablets (enteric coated aspirin, diameter 5.8 mm, 1.24 g/cm³ and barium sulfate tablets, 6.0 mm diameter, 1.52 g/cm³) and granules (diameters = 0.1 mm, density = 1.17 and 1.34 g/cm³ respectively). Tablets empty more rapidly than do

granules in dogs, but they are cleared at a similar rate in humans. For example, in dogs, the absorption of drug from granules continued to occur even after 10 hrs postdose. In contrast, peak concentrations in humans occurred within about 5 hours after administration.

Despite the faster gastric emptying observed for dogs versus humans in the fasted state, food appeared to result in a substantially greater delay in the emptying of large particles (tablets) and pellets in dogs as compared to humans (28). These effects of particle size were demonstrated with aspirin tablets. When human-labeled enteric coated aspirin tablets were administered to dogs (29) the tablets were poorly digested and retained in the stomach. These tablets (diameter 13 mm) were coated with phthalate, which prevented digestion of the tablets. If animals were fed, the enteric coated tablets were more likely to be retained in the stomach, which was demonstrated by comparing once-daily feeding to thrice-daily feeding. However, neither particle size nor prandial state affects the rate of intestinal transit (30, 24).

Density influences gastric emptying, and particles with densities closest to that of the gastric contents are emptied the fastest. Very light or very heavy particles emptied with greater difficulty. In part, the slower emptying of large particles may be due to retropulsion induced by gastric contraction. Similarly, viscosity affects the rate of gastric emptying, and time to emptying and the volume of gastric contents increase in accordance with the viscosity of the ingesta (31).

Although much attention has been given to the impact of particle size, luminal fluid volume is also a critical variable in determining the time for gastric emptying. Studying gastric emptying in dogs, Gupta and Robinson (32) demonstrated that fluid volume markedly affects the gastric emptying of particles, regardless of particle density. Greater fluid volumes tended to increase the initial rate of gastric transit, although the time for 100% emptying of particles was completed more rapidly at smaller fluid volumes. Thus, viscosity, fluid volume, and particle size are all critical variables for ensuring that particle dispersion will occur within the GI tract (32).

Differences between Dogs and People Regarding Intestinal Drug Absorption

The majority of orally administered drugs are absorbed via passive transcellular transport (33). Transcellular transport generally occurs when the compound is un-ionized, although there are examples of ionized molecules that are absorbed via transcellular processes (34, 35). Molecular movement across biological membranes is complicated by differences in membrane polarity, hydrophobicity, and density along the gastrointestinal tract (36). Most drugs are absorbed in the small intestine, and little absorption occurs in the colon. In addition to cellular membrane barriers to drug diffusion, significant impedance is also affected by the components of the gastric and intestinal mucous layer such as phosphatidyl choline,

cholesterol, and linoleic acid, which retard the diffusion of small lipophilic molecules such as propranolol and hydrocortisone (37). Small hydrophilic molecules such as mannitol appear to freely diffuse through this lipid barrier. Mucous gel-forming components, such as mucin and DNA, exert far less negative effects on the diffusion of lipophilic molecules. However, they may serve to block the diffusion of peptides and proteins.

For passive diffusion mechanisms, whether a drug is absorbed via paracellular or transcellular mechanisms is determined by both physicochemical and physiological factors. Although the primary determinant is usually related to the drug's properties, the physiological characteristics of the animal, such as membrane diffusion surface, diffusion distance, and membrane permeability also can play a key role. Lennernas (38) noted that changing certain physiological characteristics such as the effective permeability of the membrane (P_{eff}) or the time available for drug absorption (intestinal residence time) can alter the fraction of drug absorbed.

In addition to passive mechanisms, active transport is important to the absorption of several compounds. Beta-lactam antibiotics, such as amoxicillin, as well as other amino β -lactams, are absorbed in the proximal small intestine via a carrier-mediated system that can be saturated (39). The carrier-mediated absorption system for aminopenicillins is most likely the dipeptide carrier system (40). Both active and passive transport mechanisms may occur simultaneously for the same molecule. A determination of which mechanism(s) has the dominant role tends to be compound specific and may not be well predicted by in vitro systems (33). Nevertheless, it must be remembered that even active transport mechanisms require that the drug penetrate the intestinal cells via the transcellular route.

Different intestinal length and transit can influence comparative interspecies drug absorption. Intestinal transit time influences the absorption of drugs with limited mucosal permeability, carrier-mediated uptake, drugs subject to intestinal degradation, or products whose dissolution is the rate-limiting step for systemic absorption (41, 42). Dogs have a shorter absolute intestine length and shorter intestinal length to body length ratio compared to people. Corresponding to a shorter intestinal length in dogs is a shorter gastrointestinal transit time compared to that of people (2 hours vs. 4 hours, respectively). The impact of interspecies differences in GI transit time was underscored by the failure of Beagle dogs to adequately model the human bioavailability of acetaminophen sustained-release tablets (42), griseofulvin tablets (43), valproic acid (44), and ampicillin (45).

Intestinal transit time can be a critical determinant of product bioavailability for dissolution rate-limited formulations. In this regard, marked interspecies differences are again observed. For example, when fluid or particulate markers are administered via intragastric

administration, the percent of dose excreted in the feces from hrs 0 to 24 in dogs and mature swine are 55% and 7% for the fluid markers, respectively, and 40% and 2% for particulate markers, respectively (46). Some of this difference can be accounted for by differences in intestinal length between pigs and dogs. The ratio of body length to intestine length is 1:14 and 1:6 in pigs and dogs, respectively (46). (Ratios for humans are similar to those of pigs.) Sustained-release preparations (eroding matrix) of the lipophilic compound propylthiouracil demonstrate very poor bioavailability in dogs because of the very short GI transit time. Generally, the product will reach the canine colon (2–3 hours) before having had an opportunity to dissolve (47). Accordingly, we can expect that a low-solubility compound or a product formulated to provide slow dissolution characteristics will tend to exhibit a poorer bioavailability in the canine compared to the swine.

For Class III drugs, intestinal permeability rather than in vitro dissolution is the rate-limiting step in drug absorption. For compounds exhibiting permeability-limited absorption, dissolution rate is generally less important than the rate of intestinal transit. Therefore, some formulations of Class III and IV compounds can have markedly different dissolution rates without affecting the blood concentration/time profile (48, 49).

Dogs can compensate for the shorter intestinal length with longer intestinal villi, which provide more surface area for absorption. Because they possess greater surface area for diffusion compared to people, dogs actually absorb many drugs orally much better than do humans (12). The bioavailability of small hydrophilic compounds tends to be greater in species such as dogs where both pore diameter and surface area tend to exceed that in humans (11).

The crypts, villi, and microvillae are critical for increasing the GI surface area. Highly permeable drugs generally are absorbed upon contact with the intestinal membrane, and the majority of absorption occurs at the villus tip (50). Because the radial surface area does not markedly differ across species, we would expect there to be minimal (if any) differences in the absorption of highly permeable compounds across animal species. Therefore, there is an assumption that the basic composition of intestinal epithelial cells is relatively similar across species, and intestinal membrane permeability will be comparable across target animal species (51). However, significant interspecies differences in intestinal absorptive surfaces can result from differences in the size, number of pores, and shape of the intestinal villi (14, 50). In addition, dogs have higher bile salt secretion, which increases the solubility of some poorly water-soluble drugs.

Paracellular absorption (between cells) also can be important, and interspecies differences in this pathway should not be discounted. Paracellular diffusion involves both diffusion and a

convective volume flow through water-filled intercellular channels whose diameter is approximately 3–10 Å in humans (38). Accordingly, the size and number of paracellular spaces influence the intestinal absorption of most hydrophilic compounds. This, in turn, is affected by the mucosal surface area and by cellular density (52). In humans, the small intestinal surface area for paracellular absorption is approximately 0.01% of the total membrane surface area. For this reason, unless the molecule is extremely small (e.g., <200 Da), paracellular transport will have a minor role in drug absorption (33).

Greater paracellular transport observed in dogs may be attributable to either interspecies differences in pore diameter or a difference in the number of pores on the intestinal villi, the latter reflecting differences in the effective surface area across regions of the small intestine (53). This difference in surface area for paracellular absorption may impact the relative bioavailability of small hydrophilic (low-permeability, high-solubility) compounds such as furosemide (54). There was also greater bioavailability of large molecular weight polyethylene glycol (PEG) in dogs compared to rats, which has been ascribed to the presence of larger and more frequent pores in the canine intestine (55).

Permeability is not necessarily constant throughout the GI tract. Although for some compounds drug absorption appears to be site independent (56), for others it is site dependent (57). When drug absorption is site dependent, the availability of dissolved drug at the absorption site can be a limiting factor in product bioavailability. Across species, the location in the intestinal tract where the free drug is presented can markedly influence product absorption. In people, amoxicillin (and possibly other β -lactam antibiotics) is preferentially absorbed in the duodenum and jejunum but poorly absorbed from the more distal regions of the small intestine (ileum), and no absorption occurs distal to the ileum from the various segments of the large intestine (57). Differences in site-specific drug metabolism are known to occur across animal species (58). This may be a reason why dicloxacillin, although not an aminopenicillin, demonstrates only poor oral availability in dogs (23%) (59) but much higher oral availability in people (50–85%).

FACTORS THAT AFFECT THE BCS CLASSIFICATION

Although differences in canine versus human GI characteristics, as described in this article, will clearly influence interspecies differences in drug and drug product bioavailability, the question remains whether these differences will also influence the BCS classification of a compound. Current guidelines for in vitro dissolution of oral dosage forms used in humans specify a specific volume into which the drug is dissolved. This volume is meant to simulate the volume of the stomach (for example, 900 mL). This volume needs to be rescaled for

dogs. However, dog breeds obviously cover a wide range of sizes from small toy breeds to the giant breeds. Because the administered dosage strength for dogs is related to body weight, this leads to questions of whether or not gastric volume scales in proportion to body size. To date, there are no data available upon which to resolve this question.

Defining the Test Conditions

Because there may be a need to use a smaller volume for in vitro dissolution testing of oral products for dogs, the solubility of the product may not be extrapolated directly from human data. This uncertainty leads to the potential need to reconsider the BCS definition of solubility when applied to oral products for dogs. Based on the USP definition, solubility is defined as the extent to which molecules from a solid are removed from its surface by a solvent (60):

Very soluble	Less than 1 part solvent needed to dissolve 1 part solute
Freely soluble	From 1 to 10 parts solvent needed to dissolve 1 part solute
Soluble	From 10 to 30 parts solvent needed to dissolve 1 part solute
Sparingly soluble	From 30 to 100 parts solvent needed to dissolve 1 part solute
Slightly soluble	From 100 to 1,000 parts solvent needed to dissolve 1 part solute
Very slightly soluble	From 1,000 to 10,000 parts solvent needed to dissolve 1 part solute
Practically insoluble	More than 10,000 parts solvent needed to dissolve 1 part solute

FDA's Center for Drug Evaluation and Research (CDER) uses somewhat different definitions of solubility (7). One of the questions to resolve for developing BCS concepts for formulations in dogs is whether a USP-like definition of solubility is more appropriate than the definition currently used by CDER. For example, which definition (above) corresponds to “highly soluble?”

Similarly, the question of the pH of the medium must be addressed. Currently, a highly soluble drug is expected to dissolve under all pH conditions. However, we recognize that there are species-specific differences in gastric and intestinal pH that may not only affect drug solubility but also the conditions under which in vitro testing should occur. As discussed previously in this article, pH conditions in the stomach and intestine vary tremendously in dogs and cannot be extrapolated from humans.

OBJECTIVES OF THE USP COMMITTEE

The USP Veterinary Drugs Expert Committee formed the ad hoc committee to organize and facilitate gathering data and information that will address many of the questions presented above. This committee intends to hold a scientific meeting that will bring together experts from veterinary academia, the pharmaceutical industry, and FDA to share information. The goals of the meeting are multifold. First, we would like to obtain public comment on the value

of this initiative to veterinary medicine. If there is agreement that this initiative will have a positive impact on the practice and development of pharmaceuticals intended for use in dogs, we will explore answers to the questions that need to be addressed, define potential research initiatives, and determine strategies for moving this initiative forward.

SPECIFIC QUESTIONS TO ADDRESS

During this time for public comment, we will explore the following questions that must be resolved before BCS principles can be extrapolated to facilitating pharmaceutical decisions in dogs:

Solubility

Currently CDER defines a highly soluble drug as one in which the highest dosage strength completely dissolves in 250 mL or less of aqueous buffer, pH 1–7.5. This volume reflects the gastric fluid volume of a fasted human after consuming an oral dose with 8 oz of liquid.

- In veterinary species, is 250 mL an appropriate volume for evaluating solubility? This question is critical because it is impractical to expect animal owners to encourage their pets to drink a cup of water after swallowing an oral medication.
- What is the volume of the canine stomach (mL/kg), and does canine gastric volume vary linearly with body weight?
- Is the dissolution pH range specified for humans appropriate for the canine situation?

Permeability

Can we simply extrapolate permeability classification across animal species, or are there sufficient data available to consider interspecies differences in both transcellular and paracellular pathways?

- Are there canine-specific in vitro systems available?
- In the CDER guidance, in some cases highly permeable drugs can be defined by a systemic availability (F) of >90%. In dogs, however, there is a greater tendency for high first-pass drug loss by comparison with that observed in humans. Highly permeable drugs may still have poor systemic availability due to first-pass metabolism. Therefore, how can we readily assess highly permeable drugs under these circumstances?
- When tests are properly validated (7), animal in situ intestinal perfusion data can be used to support human permeability classifications for passively absorbed compounds. (Caution is expressed with regard to potential misclassification when

efflux transporters are present.) Is it feasible to have similar systems validated for developing permeability classifications for dogs?

Dissolution

Are the currently available in vitro dissolution specifications appropriate for pharmaceutical formulations administered to dogs?

- Consider the differences in gastric emptying time: Dogs tend to have more rapid gastric emptying than humans. The magnitude of this difference is a function of particle size. In contrast, in some cases food appears to have a more profound effect on inhibiting gastric emptying compared to humans. Therefore, what is an appropriate time frame for defining a rapidly dissolving compound in dogs? Currently, CDER guidance on biowaivers (7) states that dissolution must be $\geq 85\%$ for both products to be considered rapidly dissolving. For slower dissolution, the f_2 criterion must be applied. Will this criterion adequately cover the dog?
- What is the potential impact of differences in ionic composition of GI fluids?
- What is the appropriate pH range? Canine fasted gastric pH is reported to range from 3 to 8. In most human subjects, fasted gastric pH values range from 1 to 3.
- What is the appropriate agitation speed of the apparatus?

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