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Use of the pentagastrin dog model to explore the food effects on formulations in early drug development

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ABSTRACT

The ability to extrapolate dosage performance from *in vitro* to *in vivo* situations has an important role in early drug development. In parallel, the Beagle dog has come to represent a useful animal model for extrapolation to humans especially when drugs formulated for humans are to be tested. In this article, the pentagastrin-induced Beagle dog model was validated internally to show that in the colony the dogs were generally responsive to known doses of pentagastrin that produces effects similar to gastrin in the stomach, i.e., increasing gastric acid production and lowering gastric pH. The effect was observed with a short time course, maximum pH lowering was observed between 0.5 and 1 h with return to baseline at 2–4 h. The dog stomach pH is a better representative of the human fasted stomach with this pretreatment. The ultimate goal was to use these animals in a food effect studies to predict the behavior of formulations in humans. The results for 4 compounds were provided in the dog and a clear relationship between the effect of food in the dog and the effect of food in humans was observed. While the directionality (positive or negative) of the effect could be adequately predicted, the extent of the effect could not be predicted for all the tested formulations of the 4 compounds. The data will be used to generate a database of known compounds from which a correlation can be drawn to make future predictions using the pentagastrin dog model.

1. Introduction

The ability to extrapolate dosage performance from *in vitro* to *in vivo* situations has been under investigation for more than 2 decades (Kararli, 1995). When formulated drugs are to be tested in humans, effects beyond drug dissolution from the formulation in biorelevant conditions cannot today be well predicted from *in vitro* and/or in silico tools. These are namely effects of excipients on permeability, solubility, transit or the behavior of colloidal formulations or amorphous systems. The OrBiTo project will address these current caveats of the *in vitro*/in silico tools, and in parallel, will provide information on which *in vivo* animal model would be most predictive of the formulation performance in humans. The drug product performance in an animal model would allow for *in vitro–in vivo* extrapolations or correlations to be made, which might give the formulator insight into how best to design the next dosage form in early drug development to maximize exposure or control drug release or to mitigate potential effects of food on absorption. The Beagle dog has come to represent a useful animal model for extrapolation to humans. Beagles are a typical species for toxicology studies, relatively easy to maintain, and generally available commercially. The anatomic and physiologic processes in a dog are similar to humans without the extra expense and labor required for the maintenance of a nonhuman primate colony. However, the literature is not conclusive on the exact pH under fasting conditions of the Beagle stomach where dissolution and drug release occurs (Chiou, 2000; Zwart, 1999; Neervannan, 2006; Akimot M et al., 2000; Merck Index Online, 2013; Lentz et al. 2007). Thus, extrapolations may be skewed due to these apparent differences. The range of pH has been quoted from 1.5 to 6.7 depending on the source (Neervannan, 2006; Dressman, 1986). Some of the gastrointestinal physiologic similarities and differences are listed in Table 1 (Zwart, 1999; Dressman, 1986). In addition, there may be differences in intestinal permeability of the dog versus human and certainly the type and roles of known transporters in the intestine have not yet been clearly delineated for either species (Chiou, 2000). In particular, the effective intestinal permeability coefficient (P_{eff}) in dog for some drugs (e.g. danazol, griseofulvin) were estimated to be 3-fold smaller than those in humans, due to the differences in the microscopic structure of the intestinal wall (Sugano and et al., 2009). In contrast, in cases of paracellular permeation, P_{eff} in dogs could be larger than in humans because of the species difference in the pore size of the paracellular structure (He and et al., 1998). Some authors

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Table 1
Comparisons of the gastrointestinal physiology of human and dog.

Parameter	Human		Dog	
	Fasted	Fed	Fasted	Fed
pH (stomach)	Variable, 1–5	5–7	1.5–6.7	~2
Gastric motility	Cyclic (~4 phase over 4 h)	Regular tonic contractions	Cyclic (qualitatively similar to human)	Regular tonic contractions
Gastric emptying (solid meal)	8–15 min	1–3 h	Similar to human	3 h

References Zwart et al. (1999) and Dressman (1986).

argue that the P_{eff} difference for transcellular absorption is cancelled out by the difference of the intestinal radius resulting in similar absorbed dose fraction making dog a relevant model to mimic human absorption (Lentz, 2008; Sugano and et al., 2009). However, many of the other attributes of dog intestinal anatomy and physiology corresponds well with what is known for humans, such as gastric emptying, gastrointestinal transit time and diet.

In this manuscript, the stomach pH of 21 dogs was evaluated to ascertain the nominal fasted pH in this dog colony. In addition, a canine diet was formulated to represent the high fat diet administered to humans in a food effect study (Mod Testdiet, xxxx). In order to determine an optimal study design in the dog, the timing of food relative to pentagastrin administration, a pH lowering agent, was examined. In all cases the Sanofi-Aventis Research compound was administered orally to the Beagle after food administration to best mimic the FDA guidance on bioavailability and bioequivalence studies (Food-Effect Bioavailability and Fed Bioequivalence Studies, 2002).

Four Sanofi compounds were used to examine the ability of the dog model to "predict" the effect of food on the exposure of development formulations in the human. The timing of the companion human study was retrospective; meaning typically for these compounds there was some preliminary data from humans on the effect of food. The types of formulations used or developed were dependent on the program and stage of development of each candidate. Thus the study tested a small, non-uniform chemical space with each candidate having a different solution or requirement to reduce the effect of food. The results were examined in terms of ratio of area-under-the plasma concentration time curve (AUC) under fed conditions relative to AUC under fasting conditions. While an understanding of variability for these compounds in humans was available, the same degree of understanding was not always available for the dog. Therefore, Reduce, Refine and Replace, a 3Rs approach, was taken to attempt to maximize the predictability of the output and control variability while trying to minimize the number of animals that were needed to measure relative exposure after administration of food. The output was compared to known food effects of formulations in humans.

2. Methods

2.1. Drugs, formulations and reagents

Drug A: MW ~ 320 g/mol; Log P = 2.1; Solubility: 1.5 mg/mL in 0.1 N HCl, <0.1 mg/mL in water buffered at pH 4.5, >20 mg/mL in 95% ethanol, 1 mg/mL in FaSSIF and 3 mg/mL in FeSSIF; Dissociation constant (pK_a): 5.4 (weak base). The apparent permeability coefficient (P_{app}) in CaCo2-TC7 at pH 6.5 and 7.4: 184×10^{-7} cm/s suggested high permeability along the intestine. Drug A was not an inhibitor of P-glycoprotein (P-gp). Based on the dose range employed in clinical studies and the high permeability and low solubility, this compound was classified as Biopharmaceutical classification system (BCS) II.

Drug A formulation tested: a Capsule and a Tablet, both at 50 mg.

Drug B: MW ~ 400; practically insoluble in water at 25 °C; slightly soluble in 96% ethanol. At pH 6.5 and 7.4, P_{app} was equal to 322×10^{-7} cm/s and 313×10^{-7} cm/s, respectively suggesting that this was a highly permeable compound. It was not an inhibitor of P-gp.

Drug B formulation tested: a Capsule and a Tablet, both at 200 mg. Based on the dose range employed in clinical studies and the high permeability and low solubility, this compound was classified as BCS II.

Drug C: MW ~ 500; practically insoluble in aqueous medium over a wide range of pH values. At pH 7.4, P_{app} was equal to 2.2×10^{-7} cm/s and was not an inhibitor of P-gp in Caco-2-TC7cells. Based on the dose range employed in clinical studies and the high permeability and low solubility this compound was classified as BCS II.

Drug C formulation tested: a tosylate salt capsule and a lipcap (free base) capsule, both at 50 mg.

Drug D: MW ~ 600; practically insoluble in water at 25 °C. Based on the dose range employed in clinical studies and the high permeability (Pgp substrate with a saturation of the efflux at clinical dose leading to a high permeability in these conditions) and low solubility, this compound was classified as BCS II.

Pentagastrin was purchased from Sigma (St. Louis, MO). All other analytical reagents were purchased from commercial sources and are of at least standard laboratory reagent grade.

2.2. Preparation of pentagastrin

Pentagastrin is almost insoluble in water, and ethanol (Merck Index online, 2013). In order to assure that the pentagastrin was in solution, at a constant concentration during the validation, a stock solution of a pentagastrin was prepared using dimethyl formamide (DMF) in which it was soluble. The final concentration, 24 μg/mL in 1% DMF, was obtained by dissolving 2.4 mg pentagastrin in 1.0 mL DMF. The solution was gently mixed until completely dissolved, and then vortexed until clear. This "stock solution" was aliquoted into sterile vials, 10 μL each, and stored at −20 °C. On the day of dosing one aliquot was removed from the freezer for 15 min, and a sufficient volume of 0.9% sterile saline was added to prepare 10 mL which was then vortexed until clear. The entire solution was filtered through a 0.22 μ filter prior to dosing.

2.3. Animals

Male Beagle dogs were maintained as an in-house colony, with body weights at dosing approximately 10 kg. The in-life portions of the study were performed in designated rooms in our in-house vivaria. A certified laboratory canine diet was offered once daily. Water from the in-house automatic water system was available *ad libitum* through the study. The animal rooms were lighted entirely with artificial fluorescent lighting, with a controlled 12-h light/dark cycle. Temperature and humidity in the animal room were monitored by an automated system, 24 h a day, throughout the study. Normal temperature and relative humidity ranges in the animal room were 22 ± 4 °C and 50 ± 20%, respectively. The