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Research paper

Dissolution media simulating the proximal canine gastrointestinal tract in the fasted state

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ABSTRACT

Human biorelevant media have been shown to be a useful tool in pharmaceutical development and to provide input for *in silico* prediction of pharmacokinetic profiles after oral dosing. Dogs, in particular Beagles, are often used as animal models for preclinical studies. Key differences in the composition of human and canine gastric and intestinal fluids are described in the literature and underscore the need to develop a discrete set of biorelevant media, adapted to the conditions of the proximal canine gastrointestinal (GI) tract, to improve forecast and interpretation of preclinical results using *in vitro* dissolution studies. Canine biorelevant media can also be used in the development of oral dosage forms for companion animals, which is a rapidly growing market. The compositions of Fasted State Simulated Gastric Fluid canine (FaSSGFc) and Fasted State Simulated Intestinal Fluid canine (FaSSIFc) are adapted to the physiological composition of the corresponding gastrointestinal fluids in terms of pH, buffer capacity, osmolality, surface tension, as well as the bile salt, phospholipid, and free fatty acid content (in terms of concentration and reported subtypes). It was demonstrated that canine Fasted State Simulated Intestinal Fluid (FaSSIFc) is superior in predicting the solubility of model compounds in Canine Intestinal Fluid (CIF) compared to the human biorelevant media (FaSSIF and FaSSIF-V2). Two different versions of FaSSGFc, composed at pH 1.5 and pH 6.5, offer the possibility to design *in vitro* studies which correspond to the *in vivo* study design, depending on whether pentagastrin is used to decrease the gastric pH in the dogs or not. Canine biorelevant media can therefore be recommended to achieve more accurate forecasting and interpretation of pharmacokinetic studies of oral drug products in dogs.

1. Introduction

After p.o. administration, the limiting factors to absorption are typically poor solubility and low dissolution rate, inadequate permeability, extensive first pass metabolism and in some cases insufficient stability under gastrointestinal conditions [1]. Many drugs show low solubility in water as well as in buffers and in the compendial media proposed by the pharmacopoeias for running dissolution tests. Galia et al. [2] introduced biorelevant media (FaSSIF and FeSSIF), which are adapted to human small intestinal fluids in terms of pH, buffer capacity, osmolality as well as bile salt and phospholipid concentration. The solubility determined in these biorelevant media better reflects the physiological solubility of poorly soluble compounds [3] and can therefore be used to assess correctly the absorption-relevant solubility. Vertzoni et al. [4] developed a biorelevant dissolution medium simulating the fasted state human gastric conditions (FaSSGF). Jantratid et al. [5] completed the set of biorelevant media to simulate the human proximal gastrointestinal (GI) tract by developing a Fed State Simulated Gastric Fluid (FeSSGF) and revising Galia's compositions. Today, these biorelevant media are often used in pharmaceutical development.

Especially in developing new drugs, animal studies are often conducted at early stages to compare performance of formulation prototypes. Dogs, in particular Beagles, are generally used as laboratory animal models for preclinical studies and are particularly popular for comparing oral dosage forms because the dimensions of the GI tract are similar enough to permit the administration of dosage forms intended for subsequent testing in humans. Moreover, dogs are easier to handle than other species of similar size such as minipigs and monkeys [6]. In addition, fasted state gastric motility patterns are qualitatively similar in dogs and humans [7,8]. Although the physiological similarities clearly support the use of dogs as a model for oral drug absorption in humans, the use of human biorelevant dissolution media to predict solubility or dissolution rates in canine GI fluids is questionable.

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Literature sources mention differences in the composition of gastric and intestinal fluids of humans and dogs. For example, differences in gastric and intestinal pH in humans and dogs [6,9–11] can influence the solubility of compounds with pH-dependent solubility (i.e., weak acids and bases). Differences in the composition and concentration of bile salts [11–14] have also been described and may affect drug solubility, especially for compounds with high log*P*. Further, the surface tensions of the human FaSSGF, FaSSIF, and FaSSIF-V2 media do not match the surface tensions observed in the corresponding canine gastrointestinal aspirates. Surface tension is known to strongly influence wetting effects [15] and therefore having an impact on dissolution rates [16] and prediction of pharmacokinetics from dissolution results.

These differences underscore the need to develop a discrete set of biorelevant media, adapted to the conditions of the proximal canine gastrointestinal (GI) tract, to improve interpretation of preclinical results in the animal model via *in vitro* studies. Creating a link between the *in vitro* and *in vivo* performance of dosage forms in dogs paves the way for establishing similar links in human studies and also helps to interpret the *in vivo* canine data better in terms of what can be expected in humans.

Animal health products are a rapidly growing market. The total sales volume in 1998 was 11 Billion US-Dollar [17] and increased to 18.6 Billion US-Dollar in 2010 [18]. While the sale of livestock animal veterinary drugs is relatively stable, the market for companion animal medicines grew 100% between 1993 and 1999 [17]. The IFAH-Europe 2011 annual report estimates that 60 million dogs are kept as companion animals in Europe [19]. Requirements for approval of veterinary drug products are now comparable to those for humans and have caused expenses for research and development of these products to rise substantially. The availability of biorelevant media to simulate the proximal canine GI tract would add a sophisticated tool in the formulation development.

In this publication, we seek to combine findings from analysis of canine gastric fluid (CGF) and canine intestinal fluid (CIF) samples with a survey of recently published literature to compose biorelevant media designed especially for dogs: canine Fasted State Simulated Gastric Fluid (FaSSGFc) and canine Fasted State Simulated Intestinal Fluid (FaSSIFc). The compositions of the canine biorelevant media are then checked by comparing the solubility of model compounds in CGF and CIF to their solubility in the two new media.

2. Materials and methods

2.1. Materials

Maleic acid (99% pure, lot 029K5409) was purchased from Sigma–Aldrich Chemie GmbH, Steinheim, Germany. Egg phosphatidylcholine (97.5% pure, lot 108072-04/065) was donated from Lipoid GmbH, Ludwigshafen, Germany. Lysophosphatidylcholine (85.7% pure, lot PHA S 1104027) was donated from biorelevant.com, Muttenz, Switzerland. Sodium taurocholate (lot 2007100274) and sodium taurodeoxycholate (lot 2011030093) were used as received from Prodotti Chimici e Alimentari SpA, Basaluzzo, Italy. Sodium hydroxide solution (0.1 N NaOH), hydrochloric acid (37% HCl), and hydrochloric acid solution (0.1 N HCl) were purchased from VWR International GmbH Darmstadt, Germany. Dichloromethane, sodium chloride (NaCl), sodium dihydrogen phosphate monohydrate, and sodium hydroxide (NaOH) pellets were all of analytical grade and purchased from Merck KGaA, Darmstadt, Germany. Sodium oleate (82.7% pure, lot 51110) was obtained from Riedel-de Haën, Seelze, Germany.

Cinnarizine (lot 127K1457), danazol (lot 1436047V), dipyridamole (lot BCBC1928), and griseofulvin (lot 010M0537V) were purchased from Sigma Aldrich, Steinheim, Germany. Celecoxib (lot SRP01888c), cyclosporine a (lot SRP04670c), felodipine (lot SRP00425f), and nitrendipine (lot SRP00418) were obtained from Sequoia Research Products, Pangbourne, UK. Ketoconazole (lot 10057525) was purchased from Caelo, Hilden, Germany.

2.2. Methods

2.2.1. Physicochemical parameters of canine gastric fluid (CGF), canine intestinal fluid (CIF), and corresponding biorelevant media

Aspirates (CGF and CIF) for these studies had been collected in previous studies [13,20]. Pooled samples containing either CGF or CIF aspirates, which had been stored at −20 °C in the interim, were made available for the current studies.

2.2.1.1. pH. A freshly calibrated pH meter (model 720A, Orion Research Inc., Beverly, USA) was used for pH measurements.

2.2.1.2. Buffer capacity. The buffer capacity was determined using a Titroline Alpha Plus apparatus (Schott Instruments, Mainz, Germany) by addition of 0.1 N sodium hydroxide or 0.1 N hydrochloric acid solution, measuring the volume required to change the pH by one unit.

2.2.1.3. Osmolality. The osmolality was measured using a Osmomat O30 (Gonotec, Berlin, Germany) by determining the freezing-point depression.

2.2.1.4. Surface tension. Surface tension measurements were conducted using a Delta-8 multi-channel microtensiometer (Kibron Inc., Helsinki, Finland). The instrument can be used for medium throughput screening on a 96 well plate according to a technology based on the Du Nuoy ring method. Instead of a ring, fine metal rods are employed to record the maximum pull force exerted by the surface tension [21]. The microtensiometer was calibrated with Milli Q-water to 72.8 mN m^{-1} at a measuring temperature of 20 °C. Published surface tension values of sodium lauryl sulfate solutions (0.02% and 0.1%) [22] were confirmed and used as a control for lower surface tensions.

2.2.2. Composition of canine fasted state simulated gastric and intestinal fluids

The pH values of the canine fasted state simulated fluids were chosen based on the results of the GI pH study in fasted state Beagles and common literature sources.

Buffers were selected according to their ability to maintain the desired pH and buffer capacity. The Van Slyke Eq. (1) was used to calculate the buffer concentrations needed to obtain the target buffer capacity [23].

$$\beta = 2.3C\frac{\mathrm{Ka}[\mathrm{H_3O^+}]}{(\mathrm{Ka} + [\mathrm{H_3O^+}])^2} \quad (1)$$

In Eq. (1), β is the buffer capacity, C is the total buffer concentration, that is, the sum of the molar concentrations of acid and salt, and $[H_3O^+]$ is the molar concentration of the hydronium ion.

The amount of sodium chloride, which is needed to adjust the biorelevant medium to the physiological osmolality was calculated on the basis of the freezing-point depression according to the Raoult's law relation (2) and refined experimentally [24].

$$\Delta T_f = i * K_f * m \quad (2)$$

In Eq. (2), ΔT_f is the freezing-point depression, i is the van't Hoff factor, accounting for the number of individual ions formed by a compound in solution, K_f is the cryoscopic constant, which is -1.858 K kg mol^{-1}, and m is the concentration in moles of solute per kilogram of solvent (mol kg^{-1}) or molality of the solution.