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Research Paper

Canine Intestinal Contents *vs.* Simulated Media for the Assessment of Solubility of Two Weak Bases in the Human Small Intestinal Contents

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Purpose. This study was conducted to assess the relative usefulness of canine intestinal contents and simulated media in the prediction of solubility of two weak bases (dipyridamole and ketoconazole) in fasted and fed human intestinal aspirates that were collected under conditions simulating those in bioavailability/bioequivalence studies.

Methods. After administration of 250 mL of water or 500 mL of Ensure plus® [both containing 10 mg/mL polyethylene glycol (PEG) 4000 as nonabsorbable marker], intestinal aspirates were collected from the fourth part of the duodenum of 12 healthy adults and from the mid-jejunum of four Labradors. Pooled samples were analyzed for PEG, pH, buffer capacity, osmolality, surface tension, pepsin, total carbohydrates, total protein content, bile salts, phospholipids, and neutral lipids. The shake-flask method was used to measure the solubility of dipyridamole and ketoconazole in pooled human and canine intestinal contents and in fasted-state-simulating intestinal fluid (FaSSIF) and fed-state-simulating intestinal fluid (FeSSIF) containing various bile salts and pH-buffering agents.

Results. For both compounds, solubility in canine contents may be predictive of human intralumenal solubility in the fasting state but not in the fed state. The poor agreement of results in canine and human aspirates can be attributed to the higher bile salt content in canine bile. Solubility in FaSSIF containing a mixture of bile salts from crude bile predicted satisfactorily the intralumenal solubility of both drugs in the fasted state in humans. Solubility in FeSSIF, regardless of the identity of bile salts or of the buffering species, deviated from intralumenal values in the fed human aspirates by up to 40%. This was attributed to the lack of lipolytic products in FeSSIF, the higher bile salt content of FeSSIF, and the lower pH of FeSSIF.

Conclusions. FaSSIF containing a mixture of bile salts from crude bile, and FeSSIF containing lipolytic products and, perhaps, having lower bile salt content but slightly higher pH, should be more useful than canine intestinal aspirates for predicting intralumenal solubilities in humans.

KEY WORDS: canine intestinal fluid; dipyridamole; FaSSIF; FeSSIF; human intestinal fluid; ketoconazole; solubility.

INTRODUCTION

Drug concentration at the gut wall is a function of the dose, the intralumenal solubility, and the intralumenal dissolution/release kinetics (1). Theoretical estimations of intraintestinal solubility (i.e., maximum possible concentration at the gut wall) are not yet possible, whereas direct assessment of intraintestinal solubility in human aspirates is problematic due to limited availability and high costs. Therefore, the use of alternative media for estimating luminal solubility, i.e., intestinal contents from animals or simulated media, would be highly desirable.

To the best of our knowledge, there are only two publications in which solubility data in canine intestinal contents have been compared with solubility data in human intestinal aspirates. The first is an abstract in which the solubility of three nonionizable compounds (danazol, felodipine, and griseofulvin) in canine intestinal fluid collected in the fasted state is reported to be higher than in fasting human intestinal aspirates due to differences in bile salt content (2). The second is a more recent article that deals with contents collected in the fed state and in which the solubility of four nonionizable compounds (danazol, felodipine, griseofulvin, and cyclosporine) in canine jejunal contents was found to correspond well to solubility in fed human jejunal aspirates

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(differences less than 30%) (3). Although there are no methodological details provided in the first study, one issue in the second study is that the experimental procedures for administering and collecting samples differed between humans and dogs (3).

Media simulating the fasted intestinal contents and containing a simple bile salt adequately predicted solubility of a moderately lipophilic nonionizable compound (hydrocortisone) in fasted intestinal aspirates (4). Adequate estimation of intralumenal solubility of another nonionizable compound with higher lipophilicity (i.e., of danazol) required the inclusion of phospholipids in the medium (5). Furthermore, results with a medium simulating the fed intestinal contents [fed-state-simulating intestinal fluid (FeSSIF)] (6) enabled satisfactory prediction of the absorption rates of two highly dosed lipophilic compounds [one nonionizable (atovaquone) and one weak acid (troglitazone)], with predicted input profiles differing from mean actual profiles by no more than 30% (7). On the other hand, in the recent study of Persson *et al.* (3), the solubility of danazol, griseofulvin, felodipine, and cyclosporine in fed human intestinal aspirates was shown to be underestimated by the solubility data in FeSSIF (8).

In this study we assessed the relative usefulness of canine intestinal contents (collected at various times after meal administration) and of simulated media (containing various bile salts and buffer species) in the prediction of solubility of two weak bases (dipyridamole and ketoconazole, Table I) in fasted and fed human intestinal aspirates. Both canine and human aspirates were collected under conditions simulating the bioavailability/bioequivalence studies (16). Compared to nonionizable or acidic compounds, estimation of intraluminal solubility of weak bases using data collected in media from animals or in simulated media may be more problematic because the identity of the anions of the buffering species may affect the solubility product of the salt of the ionized base (17).

MATERIALS AND METHODS

Materials

Dipyridamole was donated by Boehringer Ingelheim GmbH (lot 02116, Ingelheim, Germany) and ketoconazole by Recordati Espana S.L. (lot 03000051, Beniel, Spain). Purified sodium taurocholate (>97% pure) was purchased from Sigma-Aldrich Chemie GmbH (cat. no. T4009, St. Louis, MO, USA). Crude sodium taurocholate (~32% taurocholic acid) from ox bile was purchased from Fluka Chemie GmbH (cat. no. 86340, lot 386645/1 41201, Buchs, Switzerland). By using Enzabile® (Nycomed Pharma AS, Norway) the 3-α-hydroxy bile salts content was measured to be 87.9 ± 2.2% (17). Soybean partially hydrolyzed phosphatidylcholine (Lipoid S 100, >94% phosphatidylcholine and 3% lysophosphatidylcholine) was donated by Lipoid GmbH (lot 790181-1, Ludwigshafen, Germany). All other chemicals were of analytical grade.

Table I. Lipophilicity, Ionization Constants, and Aqueous Solubility of Dipyridamole and Ketoconazole (9,10,11–15)

	Dipyridamole	Ketoconazole
log *P*	2.74	4.45
pK_a	5.7–6.4 (alkaline)	2.94 and 6.51 (both alkaline)
Solubility	5 μg/mL, pH 7, phosphate buffer	6.9 μg/mL, pH 6.5, PBS buffer

Human Intestinal Fluids

Human intestinal aspirates (HIFs) were collected from the distal duodenum (D4) of 12 healthy adults (8 men and 4 women) that arrived in the clinic fasted, and, before aspirations, they were administered 250 mL of water [containing 10 mg/mL polyethylene glycol (PEG) 4000] or 500 mL of Ensure plus® (containing 10 mg/mL PEG 4000) (18). Administrations were performed on a crossover basis. The study was held at the Red Cross Hospital of Athens after receiving approval by the Scientific and the Executive Committee of the Hospital. Details on the clinical part of the study as well as meal administration and aspiration procedures have been recently described (16). Samples were aspirated 30 min after administration of water and 30, 60, 120 and 180 min after administration of Ensure plus®.

Upon aspiration, each sample was stored for 1–18 months at −70°C. On the day of the solubility experiment, all samples were brought to room temperature. Samples aspirated after administration of water were pooled by taking 3 mL from each sample to create the *HIF fasted* sample. The same pooling procedure was followed for samples after administration of Ensure plus® to create *HIF fed 30*, *HIF fed 60*, *HIF fed 120*, and *HIF fed 180* samples.

The physicochemical characteristics of all pooled HIFs and solubilities of the compounds in the pooled HIFs were measured concurrently immediately after the pooling procedure.

Canine Intestinal Fluids

Canine intestinal fluids (CIFs) were collected from four male Labradors (29–35 kg) having a chronic nipple valve fistula at mid-jejunum (approximately 76 cm below the pylorus). Dogs were fasted from the afternoon before the experimental day. On the experimental day, each dog was given 250 mL of water (containing 10 mg/mL PEG 4000) or 500 mL Ensure Plus® (containing 10 mg/mL PEG) (18). Administrations were performed on a crossover basis. Intestinal contents were recovered via the jejunal fistula on a volume basis (~22 mL per vial) and the collection period lasted until no fluid could be recovered from the fistula or for 180 min. The collection procedure was approved by the Animal Ethics Committee (Gothenburg, Sweden) and followed the tenets of the Principles of Laboratory Animal Care (NIH publication no. 85-23, revised 1985).

Upon collection, contents were stored for 2–4 months at −70°C. Each vial was brought to room temperature, a sample was obtained for assaying its PEG content, and the vial was returned to −70°C. On the day of the solubility experiment, all vials were brought to room temperature. For each dog, intestinal contents collected after administration of water were pooled into three samples. The first pooled sample

consisted of all contents collected from time zero until the time by which 20% of the administered meal (based on the quantity of PEG recovered from the fistula) was recovered. The second pooled sample was created from all contents recovered subsequently until the second 20% of the meal was recovered from the fistula (corresponding to the 20–40% cumulative amount of PEG). The third pooled sample was created from all contents recovered subsequently until the third 20% of the meal was recovered from the fistula (corresponding to the 40–60% cumulative amount of PEG). Having three pooled samples from each dog, those containing the first 20% of the meal were pooled further ($n = 4$ dogs) to create the *CIF 20 fasted* sample. The *CIF 40 fasted* and the *CIF 60 fasted* samples were formed similarly. The same pooling procedure was followed for the contents collected after administration of Ensure Plus® to create the *CIF 20 fed*, *CIF 40 fed*, and *CIF 60 fed* samples.

The physicochemical characteristics of all pooled CIFs and solubilities of the compounds in the pooled CIFs were measured concurrently immediately after the pooling procedure.

Simulated Media

Because it is not practical to use natural buffer species (bicarbonates) in media simulating the fasting small intestinal contents, phosphates and maleates have been previously proposed to achieve the required buffer capacity and pH value (17). Therefore, four media simulating the fasted small intestinal fluid were tested:

- Standard FaSSIF (6),
- FaSSIF prepared with crude sodium taurocholate from ox bile ($FaSSIF_{cr}$) (17),
- FaSSIF containing maleate anhydride as buffering species ($FaSSIF_{m}$) (17), and
- FaSSIF prepared with crude sodium taurocholate and containing maleate anhydride as buffering species ($FaSSIF_{cr,m}$) (17).

For similar reasons both citrates and acetates have been previously proposed for simulating the fed intestinal conditions [in this case even bicarbonates would probably be not physiologically relevant (17)]. Therefore, four media simulating the fed small intestinal fluid were tested:

- Standard FeSSIF (6),
- FeSSIF prepared with crude sodium taurocholate from ox bile ($FeSSIF_{cr}$) (17),
- FeSSIF containing citrates as buffering species ($FeSSIF_{c}$) (17), and
- FeSSIF prepared with crude sodium taurocholate and containing citrates as buffering species ($FeSSIF_{cr,c}$) (17).

Simulated media were prepared freshly on the day of each solubility experiment.

Solubility Studies

Solubilities were measured in triplicate using the shake-flask method. Medium (4.5 mL) and pure drug powder in excess (45 mg for dipyridamole and 135 mg for ketoconazole) were transferred into Erlenmeyer flasks (ca. 25 mL). Flasks were then covered with parafilm and put in a shaking water bath (37°C).

Equilibration time was measured in FaSSIF and in FeSSIF and found to be less than 3 and 5 h, respectively, for both dipyridamole and ketoconazole. Based on these data, 3 h were considered adequate for the fasted canine and human samples collected after water administration and 5 h were considered adequate for the fed canine and human samples collected after Ensure plus® administration.

At equilibrium, the pH in each flask was measured.

Samples containing human or canine intestinal fluids were centrifuged for 10 min at 4000 rpm and at 15°C (Universal 32R, Hettich Labborapparate, Tuttlingen, Germany). One milliliter of the supernatant was transferred to another tube and 2 mL of acetonitrile was added. This mixture was centrifuged again for 10 min at 4000 rpm and at 15°C and the supernatant was subjected to high-performance liquid chromatography (HPLC) assay. Samples containing simulated media were immediately filtered with regenerated cellulose filters (RC 17 mm, 0.45 μm Titan, Wilmington, DE, USA) and, after the first 0.2 mL was discarded, the filtrate was subjected to HPLC assay.

Because HIFs and CIFs contained varying amounts of PEG, the effect of PEG on solubility data was also studied. For this reason, the solubility of both dipyridamole and ketoconazole in all media simulating the fasting intestinal contents was measured in the presence of 3 mg/mL PEG. Similarly, the solubility of both dipyridamole and ketoconazole in all media simulating the fed intestinal contents was measured in the presence of 10 mg/mL PEG.

Analytical Methods

PEG 4000 was determined by the method proposed by Malawer and Pwell (19) and modified by Buxton *et al.* (20). For the PEG assay, the limit of quantification (LOQ) (21) was 3.3 mg/mL. pH values were measured by a pH electrode (ER350B, Metrohm, Herisau, Switzerland). Buffer capacities were measured in just one pH direction (16) by dropwise addition of HCl. Osmolality was measured by the freezing point depression technique (semimicro osmometer Typ Dig L, Knauer, Berlin, Germany). Surface tension was measured by the DeNouy ring method (Sigma70, KSV Instruments, Monroe, CT, USA). Total 3-α-hydroxy bile acid levels were determined with a commercially available kit (Enzabile, Nycomed, Sweden) and the LOQ (21) was 500 μM (17). Solid-phase extraction followed by HPLC with evaporative light scattering (ELS) detection were used to determine the lipid content in the intestinal fluids (3). LOQs for phosphatidylcholine and lysophosphatidylcholine were 0.01 and 0.05 mM, respectively. LOQs for free fatty acids, cholesterol, cholesterol ester, monoglycerides, diglycerides, and triglycerides were 0.4, 0.03, 0.005, 0.01, 0.11, and 0.03 mM, respectively.

Dipyridamole was assayed with a modification of the HPLC-UV method proposed by Gu *et al.* (9). The analytical column was a Hypersil® ODS C18 (250 × 4.6 mm, 5 μm). The mobile phase consisted of water/acetonitrile/diethylamine (50:50:0.2) and the flow rate was 1 mL/min. The injection volume was 20 μl and the retention time was about 6.2 min. The detection wavelength was 280 nm.

Table II. Physicochemical Characteristics of the Pooled Human Intestinal Aspirates (HIFs) that were Used as Solubility Media[a]

Pooled sample	PEG content (mg/mL)	pH	Buffer capacity (mmol/L/ΔpH)	Osmolality (mOsmol/kg)	Surface tension (mN/m)	Bile salts (mM)	Phospholipids[b] (mM)
HIF fasted	3.4	6.7	5.6	197	33.6	2.82	NM
HIF fed 30	8.6	6.6	28	408	28.1	10.7	5.77
HIF fed 60	7.6	6.5	30	416	27.8	11.8	4.31
HIF fed 120	8.5	5.8	22	453	28.6	6.62	2.99
HIF fed 180	6.5	4.9	23	368	NM	3.72	<LOQ

HIF fasted = HIF collected 30 min after administration of water; HIF fed 30 = HIF collected 30 min after administration of Ensure Plus®; HIF fed 60 = HIF collected 60 min after administration of Ensure Plus®; HIF fed 120 = HIF collected 120 min after administration of Ensure Plus®; HIF fed 180 = HIF collected 180 min after administration of Ensure Plus®; NM, not measured; LOQ = limit of quantification.
[a] Measurements were performed in the pooled samples.
[b] Phosphatidylcholine and lysophosphatidylcholine.

Ketoconazole was also assayed with a modification of the HPLC-UV method proposed by Poelma *et al.* (10). The analytical column was a Hypersil® ODS C18 (250 × 4.6 mm, 5 μm). The mobile phase consisted of methanol/acetonitrile/diethylamine (75:25:0.1) and the flow rate was 1 mL/min. The injection volume was 50 μl and the retention time was about 8 min. The detection wavelength was 254 nm.

For both drugs, quantification of solubility data was made according to standard curves prepared in the corresponding solubility medium. Depending on the medium, the quantification limits (21) for dipyridamole ranged from 0.48 to 7.19 μg/mL, with the highest value observed in fed canine intestinal contents. Depending on the medium, the quantification limits for ketoconazole ranged from 0.98 to 5.56 μg/mL, with the highest value observed, again, in fed human intestinal aspirates.

Data Analysis

Statistical evaluation of the data was performed with SigmaStat® (version 2.03, SPSS Inc., Chicago, IL, USA). Solubility data in fed canine intestinal contents and human intestinal aspirates were compared with one-way analysis of variance followed, where appropriate, by the Tukey's *post hoc* test. Solubility data in simulated media and in canine intestinal fluids were compared with data in human intestinal fluids with unpaired *t* test. In all cases, differences were considered significant at the 0.05 level.

RESULTS

Physicochemical Characteristics of HIFs, CIFs, and Simulated Media Used in Solubility Studies

The physicochemical characteristics of HIFs, CIFs, and simulated media that were used as solubility media are presented in Tables II–V.

PEG content was compared for human and canine aspirates in samples collected after water administration. In humans, the duodenal contents at 30 min after water administration contained significant amounts of secretions (16). In dogs, the initial 40% of the meal arrived at midgut only slightly diluted by secretions, whereas the following 20% of the meal was slightly concentrated. The pH of HIF fasted was ~0.5 units lower than the pH of CIF fasted (Tables II and IV) and similar to the pH of media simulating the fasted intestinal contents (Tables II and V). In a previous study, it was found that the pH of duodenal aspirates increased by up to 6% within 20 min storage at room temperature (16), presumably due to a slow transformation of bicarbonates to carbon dioxide under zero-convection conditions (22). Such an increase was also observed in the present study; the median pH value measured immediately after aspiration was 6.2 (16), whereas the initial pH of HIF fasted was 6.7 (Table II). However, this increase was not enough to affect buffer capacity; buffer capacity of HIF fasted was similar to the median value measured in a previous study immediately after aspirations (16). The buffer capacity of HIF fasted was higher than the buffer capacity of CIF fasted (Tables II and IV) but lower than the buffer capacity of the fasting state simulating media (Tables II and V). HIF fasted and CIF fasted were hypoosmotic, whereas media simulating the fasting contents were isoosmotic. Surface tension of HIF fasted was similar to the values in CIF fasted but lower than the value of simulated media, presumably due to the absence of various surface active agents in the simulated media such as enzymes. Apart from the CIF 60 fasted sample, bile salt levels in CIF fasted and in media simulating the fasting contents were similar to the values in HIF fasted. Canine

Table III. Mean (SD) Concentrations (mM) of Neutral Lipids in the Pooled Human Intestinal Fluids (HIFs) that were Used as Solubility Media

	30 min	60 min	120 min	180 min
Fatty acids	39.1 (1.2)	42.1 (2.3)	41.7 (1.9)	34.7 (2.5)
Cholesterol	1.30 (0.7)	0.90 (0.7)	0.40 (0.2)	0.30 (0.2)
Cholesterol ester	0.12 (0.1)	0.23 (0.2)	0.12 (0.1)	0.17 (0.1)
Monoglycerides	5.90 (1.9)	7.30 (4.2)	6.40 (3.8)	4.20 (4.7)
Diglycerides	1.70 (1.1)	7.80 (6.6)	4.20 (2.4)	5.00 (4.2)
Triglycerides	<LOQ	<LOQ	1.90 (1.2)	0.60 (0.3)

Measurements were performed in the individual samples collected after administration of Ensure plus® (i.e., in the fed state). Ensure plus® contains 47.5 mg/mL fats of which 4.2 mg/mL are saturated, 15.8 mg/mL are polyunsaturated, 26.3 mg/mL are monounsaturated, and <0.021 mg/mL is cholesterol (http://rpdcon40.ross.com/mn/Ross+MN+Nutritional+Products.nsf/web_Ross.com_XML/4654062A8AEB363485256464006CD72D?OpenDocument). LOQ = limit of quantification.

Table IV. Physicochemical Characteristics of the Pooled Canine Intestinal Contents (CIFs) that were Used as Solubility Media[a]

Pooled sample	PEG content (mg/mL)	pH	Buffer capacity (mmol/L/ΔpH)	Osmolality (mOsmol/kg)	Surface tension (mN/m)	Bile salts (mM)	Phospholipids[b] (mM)
CIF 20 fasted	7.8	7.1	1.4	69	31.1	2.41	<LOQ
CIF 40 fasted	8.9	7.1	1.4	62	31.8	2.94	<LOQ
CIF 60 fasted	12.9	7.1	4.2	207	36.5	9.39	8.12
CIF 20 fed[c]	6.5	6.0	30	841	28.3	17.0	7.60
CIF 40 fed[c]	11.6	6.0	24	667	28.5	12.8	4.36
CIF 60 fed[c]	10.2	6.1	29	679	28.7	18.0	19.4

CIF 20 fasted = CIF containing the initial 20% of PEG that arrived at the jejunum after administration of water; CIF 40 fasted = CIF containing the second 20% of PEG that arrived at the jejunum after administration of water; CIF 60 fasted = CIF containing the third 20% of PEG that arrived at the jejunum after administration of water; CIF 20 fed = CIF containing the initial 20% of PEG that arrived at the jejunum after administration of Ensure plus®; CIF 40 fed = CIF containing the second 20% of PEG that arrived at the jejunum after administration of Ensure plus®; CIF 60 fed = CIF containing the third 20% of PEG that arrived at the jejunum after administration of Ensure plus®; LOQ = limit of quantification.

[a] Measurements were performed in the pooled samples.

[b] Phosphatidylcholine and lysophosphatidylcholine.

[c] These fluids contain neutral lipids that were not quantified.

gallbladder in the fasting state shows brief alternating excursions of filling and emptying with the number of emptying events exceeding the filling events during phase II (23). Therefore, if one takes into account that bile salt levels in canine gallbladder are about 3-fold higher than in human gallbladder (24), partial emptying of canine gallbladder during fasting may explain the higher bile salt levels CIF 60 fasted. The relatively decreased water content in this sample (based on relevant PEG values) may also partly account for the observed differences in the bile salt level.

After Ensure Plus®, HIFs contained small amounts of secretions, whereas CIFs were slightly concentrated at late meal emptying times. Up to 120 min postmeal administration, the pH of HIFs fed was similar to the pH of CIFs fed but higher than the pH of media intended to simulate the fed intestinal contents. The pH of HIF fed 180 was similar to the pH of simulated media. In a previous study, it was found that the pH of duodenal aspirates decreased slightly (by approx. 3%) within 10 min of storage at room temperature, presumably due to the progress of lipid digestion (16). Compared to data collected immediately after aspirations (16), the initial pH of HIFs was slightly lower only for the HIF fed 120 and the HIF fed 180 (by 5.7 and 13%, respectively). The buffer capacity of HIFs fed was similar to the median buffer capacity estimated immediately after aspirations (16) and also similar to the buffer capacity of CIFs fed (Tables II and IV). However, the buffer capacity of HIFs fed was less than half of the value of media simulating the fed state (Tables II and V). HIFs fed, CIFs fed, and media simulating the fed state were hyperosmotic, but the ranking was HIFs fed < simulated media < CIFs fed. Surface tension of HIFs fed and CIFs fed were similar and lower than the surface tension of media simulating the fed human intestinal contents. Bile salt levels in HIFs fed were lower than bile salt levels in CIFs fed or in simulated media. The higher bile salt levels in CIFs can again be attributed to the increased bile salt content of the canine gallbladder (24). Phospholipid content of the HIFs fed bracketed the value used in the simulated medium, whereas the CIFs fed phospholipid content was much higher than HIFs fed. The ratio of phospholipids/bile salts was shown to be 1:2 to 1:3 in human samples, 1:1 to 1:3 in canine samples, and 1:4 in simulated media. The presence of neutral lipids was substantial in all HIFs fed (Table III). It is noteworthy, however, that triglycerides were not quantified 30 min after meal administration. Two factors that could contribute to the "sudden" appearance of triglycerides are the LOQ of the analytical method and the slow gastric emptying process (16). Also, in accordance with previous data (3) and for the entire sampling period, the most abundant species were free fatty acids and monoglycerides with the ratio between them being 5.8–8.2 during the entire sampling period. The rather constant fatty acid levels in the duodenum have been observed by others, too (25).

Table V. Physicochemical Characteristics of the Simulated Media that were Used as Solubility Media[a]

Medium	PEG content (mg/mL)	pH	Buffer capacity (mmol/L/ΔpH)	Osmolality (mOsmol/kg)	Surface tension (mN/m)	Bile salts (mM)	Phospholipids[b] (mM)
Fasted state simulating intestinal media, regardless of bile salt and/or buffer species identity	–	6.5	12	270	49.8	3.00	0.75
Fed state simulating intestinal media, regardless of bile salt and/or buffer species identity	–	5.0	76	635	36.9	15.0	3.75

[a] These media do not contain neutral lipids.

[b] Phosphatidylcholine and lysophosphatidylcholine.

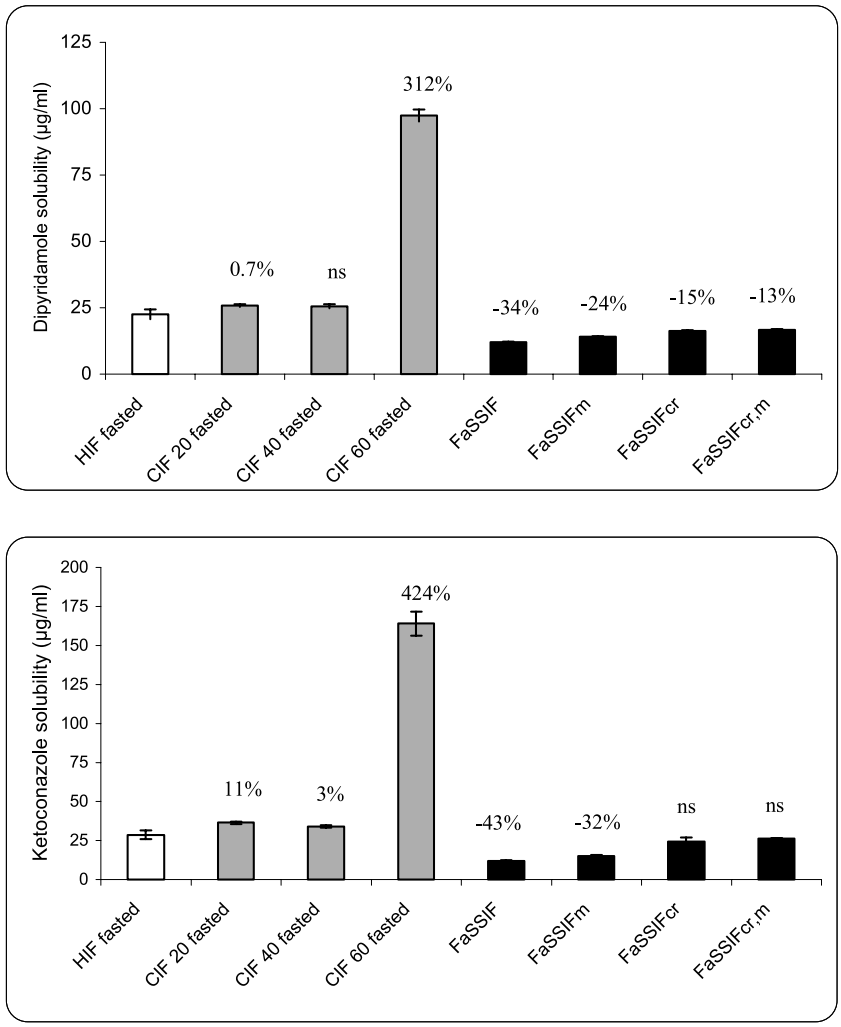

Fig. 1. Mean ± SD solubility data of dipyridamole (top) and ketoconazole (bottom) in human intestinal aspirates (HIFs) after administration of 250 mL water containing 10 mg/mL PEG (white bars), in canine intestinal fluids (CIFs) after administration of 250 mL water containing 10 mg/mL PEG (gray bars), and in various media simulating the fasting small intestinal conditions (black bars). "ns" denotes nonsignificant difference from the value in "HIF fasted." A number on top of a bar denotes the magnitude of the statistically significant difference between the mean solubility in the relevant medium and the mean solubility in HIF fasted. Abbreviations are explained in "Materials and methods."

pH Changes at Equilibrium Solubility in the Various Media

At equilibrium drug solubility, pH values in human and canine contents collected after administration of water were increased due to the low buffer capacity of these aspirates (Tables II and IV) and the excess of weak base present. In HIF fasted, pH values increased from 6.7 to 7.4 for dipyridamole and to 7.5 for ketoconazole experiments. In CIF fasted, pH values increased from 7.1 to 7.5–7.6, for dipyridamole and to 7.5–7.7 for ketoconazole.

Based on the ionization properties of dipyridamole [one alkaline pK_a for which reported values range from 5.7 to 6.4 (9,11,12)] and ketoconazole [two alkaline pK_as with reported values of 2.94 and 6.51(13)], the above-mentioned pH increases in HIF fasted and CIF fasted may slightly shift solubility to lower values.

PEG Effects on Solubility Data

Based on Table VI, regardless of the composition of the medium and/or the concentration of added PEG (3 or 10 mg/mL), PEG increased dipyridamole's solubility up to 6.8% and ketoconazole's solubility up to 11% in simulated media.

Solubility Data

Solubility data are graphically presented in Figs. 1 and 2. It is interesting to note that in HIF fasted the mean solubilities of dipyridamole (22.5 μg/mL) and ketoconazole (28.8 μg/mL) were four times higher than the solubilities in simple aqueous solutions having similar pH values [5 μg/mL and 6.9 μg/mL for dipyridamole (14) and ketoconazole (10),

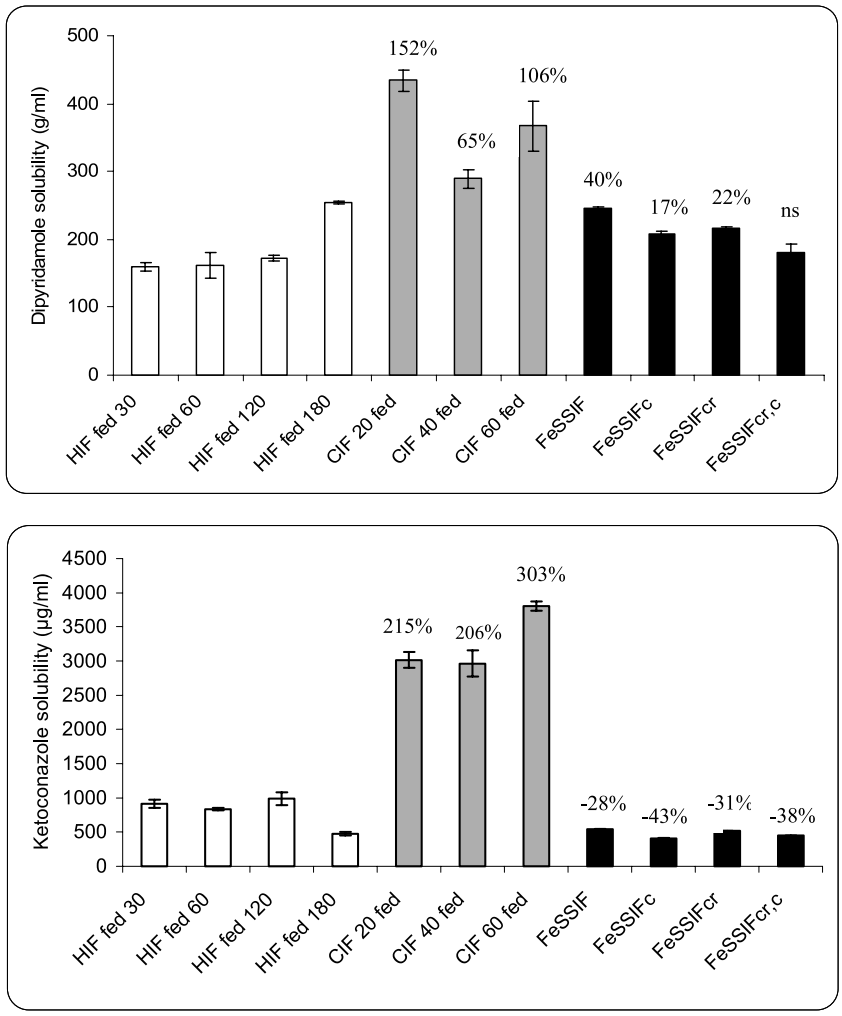

Fig. 2. Mean ± SD solubility data of dipyridamole (top) and ketoconazole (bottom) in human intestinal aspirates (HIFs) after administration of 500 mL Ensure Plus® containing 10 mg/mL PEG (white bars), in canine intestinal fluids (CIFs) after administration of 500 mL Ensure Plus® containing 10 mg/mL PEG (gray bars), and in various media simulating the fed small intestinal luminal conditions (black bars). "ns" denotes nonsignificant difference from the grand mean solubility in HIF fed 30, HIF fed 60, and HIF fed 120. A number on top of a bar denotes the magnitude of the statistically significant difference between the mean solubility in the relevant medium and the grand mean solubility in HIF fed 30, HIF fed 60, and HIF fed 120. Abbreviations are explained in "Materials and methods."

respectively], whereas differences between solubilities in HIFs fed and simple aqueous solutions were much higher.

For both compounds, solubility in CIF 20 fasted or in CIF 40 fasted was higher than solubility in HIF fasted, but the difference ranged from nonsignificant up to only 11%. In contrast, for both compounds, solubility in CIF 60 fasted was more than four times higher than in HIF fasted (Fig. 1); CIF 60 fasted had three times higher concentration of bile salts than HIF fasted, CIF 20 fasted, or CIF 40 fasted (Tables II and IV).

For both compounds, solubility in FaSSIF and $FaSSIF_m$ was 24–43% lower than in HIF fasted. In contrast, when crude sodium taurocholate was used, the difference was only 13–15% for dipyridamole and nonsignificant for ketoconazole (Fig. 1). Taking into account the possible overestimation of solubility in HIF fasted due to the presence of PEG (Table VI), solubility data in $FaSSIF_{cr}$ or $FaSSIF_{cr,c}$ predicted intralumenal solubility of compounds very well.

Solubility of both compounds in human aspirates collected after administration of 500 mL Ensure plus® was not significantly affected by aspiration time for times up to 120 min after administration of Ensure plus®. Values ranged from 159.9 to 172.7 µg/mL for dipyridamole and from 834.5 to 988.6 µg/mL for ketoconazole (Fig. 2). For dipyridamole, solubility increased significantly (to 253.5 µg/mL) in aspirates collected 180 min after meal administration, presumably due to the pH drop in the relevant sample (Table II). In contrast, ketoconazole's solubility was significantly decreased (to 475.7 µg/mL) 180 min after Ensure plus®, presumably due to the decreased bile salt, phospholipid, and neutral lipid contents in this sample (Tables III and IV); compared with dipyridamole, bile salts, phospholipids and neutral lipids seem to

Table VI. Effect of PEG on the Solubility Data of Dipyridamole and Ketoconazole in Media Simulating the Contents of the Small Intestine

	Dipyridamole	Ketoconazole
% Increase of the mean solubility value when 3 mg/mL PEG 4000 was added to the medium		
FaSSIF	6.1*	9.5*
$FaSSIF_m$	1.0	NM
$FaSSIF_{cr}$	6.8*	0
$FaSSIF_{cr,m}$	0	NM
% Increase of the mean solubility value when 10 mg/mL PEG 4000 was added to the medium		
FeSSIF	4.8*	11*
$FeSSIF_c$	2.1	5.9
$FeSSIF_{cr}$	5.2	0
$FeSSIF_{cr,c}$	3.5	0

Data were calculated using the mean value of each data set ($n = 3$). The coefficient of variation of each data set was 0.27–9.8%. Asterisks denote statistically significant values (t test, $\alpha = 0.05$). NM = not measured.

be more important for ketoconazole, most likely because it is more lipophilic than dipyridamole (Table I).

For both compounds, solubility in CIFs fed overestimated the solubility in HIF fed 30, HIF fed 60, or HIF fed 120 by approximately double for dipyridamole and up to three times for the more lipophilic ketoconazole (Fig. 2). The higher bile salt and phospholipid content of CIFs fed are at least partly responsible for these differences (Table III *vs.* Table IV).

Solubility of dipyridamole in media simulating the fed intestinal contents predicted well or overestimated up to 40% the solubility in HIF fed 30, HIF fed 60, or HIF fed 120. In contrast, solubility of ketoconazole in media simulating the fed intestinal conditions underestimated the solubility in HIF fed 30, HIF fed 60, and HIF fed 120 by 28–43%. HIFs fed had lower concentration of bile salts and higher pH than simulated media, and, unlike simulated media, they contained PEG and dietary lipids (Tables II and III *vs.* Table IV). As this and previous (26) studies suggest, the impact of lipolytic products seems to increase with the lipophilicity of the compound.

DISCUSSION

A major issue when addressing solubility issues of ionizable compounds relates to the buffering capacity of the medium and whether or not at equilibrium the initial conditions have been disturbed due to the dissolution of the pH-modifying compound. Although in this study the pH difference was not important to the results and the conclusions, the issue of the pH change during equilibrium solubility estimations could be eliminated if solubility were to be estimated with alternative methods, such as the miniaturized rotating disk dissolution apparatus operated under sink conditions (3).

Species differences in gastrointestinal anatomy and physiology are well documented in the literature (27). This study showed that, in the fasted state, canine intestinal contents might be useful for assessing intralumenal solubilities in humans if the gallbladder contractions of the dog could be minimized. In the fed state, differences in solubility data collected in human and canine intestinal fluids were more pronounced due to the increased bile salt content of the canine gallbladder. Recently (3), differences between solubilities in canine and human fed intestinal fluids were found not to be substantial. However, in that study, human intestinal contents were aspirated during perfusion of a jejunal segment, and solubility data in those aspirates were compared with solubility data in canine intestinal contents collected via a jejunal fistula after single oral administration of a meal. In addition, in that study (3), the total meal volume and energy content was much smaller than the meal administered in this study and/or the meals usually administered in bioavailability/bioequivalence studies. It has been shown that the intensity of gallbladder contractions in dogs is dependent on the ingested calories and meal lipid content (28).

FaSSIF (6) reflects reasonably well the intraintestinal conditions after administration of a glass of water. However, one issue is the higher buffering capacity of FaSSIF. In addition, this study showed that the use of a crude mixture of bile salts and maleates, in addition to substantially reducing costs, might actually improve the prediction of intralumenal solubility.

Deviation of physicochemical characteristics of FeSSIF (6) from HIFs fed was greater than the deviation of FaSSIF from HIF fasted. FeSSIF has a pH value about one unit lower than the intralumenal contents, has a higher buffer capacity, osmolality, and surface tension, has higher bile salt content, and lacks lipolytic products. It would be interesting to see whether a correlation between a specific physicochemical parameter of HIFs fed and solubility data exists. However, this would require solubility measurements in individual aspirates and, perhaps, application of the shake-flask method at miniscale level.

Despite the differences between certain physicochemical parameters of FeSSIF and HIFs fed, solubility data in FeSSIF were closer than data in CIFs fed to data in HIFs fed. Although the lack of dietary lipid simulation did not lead to great underestimation of intralumenal solubility, it should be kept in mind that the one unit lower pH of FeSSIF might have partially hidden the importance of lipolytic products on the solubility of the lipophilic weak bases tested. Based on Fig. 2, however, solubility in simulated media in which there was no simulation of presence of lipoplytic products is not much different from solubility in aspirates. Moreover, the same simulated media have been successfully used to predict the plasma profile of compounds with lipophilicities of about 4 (7), and, when lipolytic products were included in the simulated media, the resulting profiles did not lead to better prediction of the plasma profile (M. Vertzoni, Ph.D. thesis, National & Kapodistrian University of Athens, 2004). Therefore, for compounds with log P values less than about 4, the effect of lipolytic products on their solubility may not be as dramatic as previous studies have suggested either directly (3) or indirectly (26) for compounds with higher lipophilicity. For more lipophilic compounds, the inclusion of lipolytic products is necessary. Based on Table III, and given that at high concentrations (30–40 mM) fatty acids cannot be easily incorporated into an aqueous solution *in vitro*, inclusion of a monoglyceride (at about 5–10 mM, as higher concentrations

would again not be easily accommodated in the aqueous phase) into FeSSIF seems to be a logical way to simulate the presence of lipolytic products in the small intestine.

CONCLUSIONS

Assessment of intralumenal solubility remains an ongoing objective because direct measurements in humans are not practical and estimations based on data collected in alternative media have not been established for a broad range of compounds to date. Confirming previous observations, solubility of dipyridamole and ketoconazole in human aspirates was found to be at least four times higher than solubility in simple aqueous media. Based on this study, intralumenal solubility in the fasted state could be adequately estimated by using FaSSIF containing crude taurocholic acid. In the fed state, the simulated medium should probably be adjusted to a slightly lower bile salt content and a slightly higher pH than has been previously recommended (6 instead of 5), whereas if the molecule is highly lipophilic, addition of lipolytic products should be considered. Solubility in the fed canine luminal contents overestimates intralumenal solubility due to the increased presence of bile salts and phospholipids, making intralumenal contents aspirated from dogs less suitable for predicting solubility in humans.

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