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RESEARCH PAPER

Biorelevant Media to Simulate Fluids in the Ascending Colon of Humans and Their Usefulness in Predicting Intracolonic Drug Solubility

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ABSTRACT

Purpose To develop media simulating human colonic fluids (HCFs), to evaluate their use in predicting intracolonic solubility of ketoconazole, danazol and felodipine and to compare solubilities in HCFs with previously determined solubilities in gastric (HGFs) and small intestinal (HIFs) fluids.

Methods Fasted state simulated colonic fluid (FaSSCoF) and fed state simulated colonic fluid (FeSSCoF) were designed to reflect fluids previously collected from the ascending colon in healthy adults. Solubilities of the three model compounds were determined in HCFs, simulated HCFs, and plain buffers.

Results For ketoconazole, solubilities in FaSSCoF and FeSSCoF were closer than those in the corresponding plain buffers to the solubility in HCFs. For danazol and felodipine, solubilities in FaSSCoF and FeSSCoF predicted solubilities in HCFs. In the fasted state, solubilities of danazol and felodipine in HCFs were higher than or similar to in HGFs or HIFs, while the ketoconazole solubility was lower. In the fed state, solubilities of all three model compounds in HCFs were lower than in HGFs or HIFs.

Conclusions FaSSCoF and FeSSCoF more closely predict solubility of poorly soluble compounds in HCFs than plain buffers. In most cases, solubility in HCFs differs from those in HGFs and HIFs.

KEY WORDS ascending colon · drug solubility · FaSSCoF · FeSSCoF · simulated colonic fluids

INTRODUCTION

Our knowledge about the environment of the healthy adult upper gastrointestinal (GI) lumen has been substantially improved during the last decade. As a result, more reliable simulation of the environment in the upper GI lumen has been made possible, and since oral absorption is usually complete in the upper-middle small intestine, our ability to predict oral drug absorption has also been improved (1).

However, in cases where the dose dissolves slowly in the small intestine, where the drug has low permeability in the small intestine but can also be absorbed to some extent through the colonic mucosa, where an extended release dosage form is administered, or where the dosage form targets the drug to the colonic mucosa for local action, conditions in the lower GI lumen will influence drug/dosage form. Information about the environment of the lower GI lumen of healthy adults relevant to drug/dosage form performance has appeared in the literature only recently (2,3), and, therefore, simulation of the environment in the lower GI lumen to date has been poor (e.g. 4).

In the present investigation, media simulating the physicochemical characteristics of the ascending colon in the fasted and fed states (3) were developed and evaluated for their usefulness in estimating intracolonic

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solubility of three lipophilic compounds: ketoconazole (weak base), danazol and felodipine (non-ionized compounds). Since solubility data of all three model compounds in samples aspirated from the upper GI lumen have been published, a third objective was to compare solubilities of these compounds in the fluids of the upper and lower GI lumen.

MATERIALS AND METHODS

Materials

Ketoconazole was from Recordati Espana S.L., Spain (lot # 03000051), danazol from Sanofi-Aventis A.E.B.E, Greece (batch No F510357), and felodipine from AstraZeneca, Sweden (batch No 134-01).

Egg phosphatidylcholine (Lipoid E PC®, 97.9% pure) was from Lipoid GmbH, Germany. Ox bile salt extract was from Fluka Chemie GmbH, Germany (product No 86340, lot # 386645/1 12601). According to our HPLC-CAD analysis (5) the total bile salts content is 71.5% w/w, and the following bile acids are present: taurocholic 22.2%w/w, glycocholic 24.7%w/w, taurochenodeoxycholic 1.7%w/w, ursodeoxycholic 7.1%w/w, glycochenodeoxycholic 1.3% w/w, cholic 7.5%w/w, glycodeoxycholic 7.1%w/w. Bovine serum albumin was from Fluka Chemie GmbH, Germany (lyophilized, Cat No 05480, Lot S31291 087). All other chemicals were of analytical grade, except for solvents which were of HPLC grade.

Simulated Colonic Fluids

The physicochemical characteristics and composition of fasted-state simulated colonic fluid (FaSSCoF) and fed-state simulated colonic fluid (FeSSCoF) are shown in Table 1 in comparison with the average physicochemical characteristics and composition of fluids of ascending colon of healthy adults (3).

Since the required pH and buffer capacity levels could not be achieved by using biorelevant buffer species (e.g. short chain fatty acids), tris/maleate buffer systems were used in FaSSCoF and FeSSCoF. Total protein content in the ascending colon corresponds to the sum of peptides (3

Table 1 Physicochemical Characteristics and Composition of Fasted State Simulated Colonic Fluid (FaSSCoF) and Fed State Simulated Colonic Fluid (FeSSCoF) in Comparison with the Average Physicochemical Characteristics and Composition of Fluids of Ascending Colon of Healthy Adults in the Fasted and in the Fed States (3)

	Fasted state		Fed state	
	Average[a] intracolonic data	FaSSCoF	Average[a] intracolonic data	FeSSCoF
pH	7.8[b] 7.5 ($n=2$)	7.8	6.0[b] 6.2 ($n=4$)	6.0
Buffer Species	Various, including SCFAs	Tris/maleates[d]	Various, including SCFAs	Tris/maleates[d]
Buffer Capacity[c] (mmol/L/ΔpH)	21.4[b]/10.3[b] 10/5 ($n=2$)	16/26	37.7[b]/16.4[b] 12/13 ($n=2$)	15/14
Osmolality (mOsm/kg)	81	196	227	207[e]
Surface tension (mN/m)	42.7	51.4	39.2	50.4
Proteins/peptides (mg/ml)	9.7	3[f]	6.9	3[f]
Total carbohydrates (mg/ml)	8.1	0	14	14[g]
Total bile acids (μM)	115	150[h]	587	600[h]
Long chain fatty acids (μM)	120	100[i]	225	200[i]
Phosphatidylcholine (μM)	362	300	539	500
Cholesterol (μM)	594	0	1,502	0

[a] Means, apart from pH and cholesterol (which are medians); $n=12$, unless specified differently; SCFAs are short chain fatty acids. Variability of the average data is provided in ref (3).

[b] These data refer to total ascending colonic contents. All other intracolonic data refer to the fluid part of ascending colon aspirates

[c] By titrating with HCl/by titrating with NaOH

[d] Please see "Simulated Colonic Fluids" section for details

[e] Adjusted with NaCl (34 mM)

[f] Bovine serum albumin

[g] Glucose

[h] Bile salt extract

[i] Palmitic acid

or more amino acids) and proteins that are present in HCF (3). Based on this analysis, a single protein at a concentration lower than the measured total protein content was incorporated in FaSSCoF and FeSSCoF. Elastase, an enzyme that originates from pancreas, binds sterols (6), and is stable enough to reach the feces (7), and ferritin, a protein also known to reach the colon after oral administration (8,9), were two potential candidates. However, their use is restricted by high costs and perhaps limited availability in the required amounts (e.g. www.sigma-aldrich.com). As a result, the relatively inexpensive and easily accessible bovine serum albumin (BSA) at a concentration of 3 mg/ml was used (Table 1). Glucose was used for simulating intracolonic carbohydrate content (Table 1), except in cases where it would have adversely affected osmolality. Bile salt extract was selected because it consists primarily of cholates (3), and it is affordable for routine solubility (and drug release) studies. Palmitic acid, one of the fatty acids that form cell membrane bilayers (10,11), was selected because it has been quantified in the colonic fluids (3) and because it is easier to incorporate into the FaSSCoF and FeSSCoF than other fatty acids. Cholesterol was not included in either FaSSCoF or FeSSCoF, based on earlier observations that bile salts and phospholipids promote cholesterol crystallization (12), and data from this study that are presented in "Solubility Data in Artificial Media *vs.* HCFs" section.

Preparation of FaSSCoF

Initially, the tris/maleate buffer solution is prepared by dissolving 5.5 g Tris(hydroxymethyl)-aminomethane (Tris) and 8.8 g of maleic acid in water (HPLC grade), adjusting the pH to 7.8 with ~240 ml NaOH 0.5 M, and diluting to 1 l with water. ~450 ml of the buffer solution is transferred into a 1-l round-bottom flask, to which 0.113 g of the bile salt extract is added and dissolved. Then, 0.222 g phosphatidylcholine and 0.026 g palmitic acid are each dissolved in 3 ml dichloromethane, and the two resulting solutions (total of 6 ml) are transferred into the round-bottom flask. Dichlomethane is driven off the flask by using a rotary evaporator under vacuum at 40°C until a clear solution having no perceptible odor of dichlomethane is attained. The volume of the solution is then adjusted to 1,000 ml with buffer. Finally, 3 g of bovine serum albumin is dissolved by gently agitating the medium with a magnetic stirrer to obtain a lightly turbid solution (Fig. 1).

Preparation of FeSSCoF

The tris/maleate buffer solution is prepared by dissolving 3.7 g Tris and 3.5 g of maleic acid in water (HPLC grade), adjusting the pH to 6.0 with ~33 ml NaOH 0.5 M, and diluting to 1 l with water. ~450 ml of the buffer solution is transferred into a 1-l round-bottom flask, to which 0.451 g of the bile salt extract is added and dissolved. Then, 0.370 g phosphatidylcholine and 0.051 g palmitic acid are each dissolved in 3 ml dichloromethane, and the resulting solutions (total of 6 ml) are transferred into the round-bottom flask. The dichlomethane is evaporated as described for FaSSCoF, and the volume of the solution is adjusted to 1,000 ml with buffer. Finally, 2 g NaCl, 14 g glucose, and 3 g bovine serum albumin are dissolved by gently agitating the medium with a magnetic stirrer to obtain a lightly turbid solution (Fig. 1).

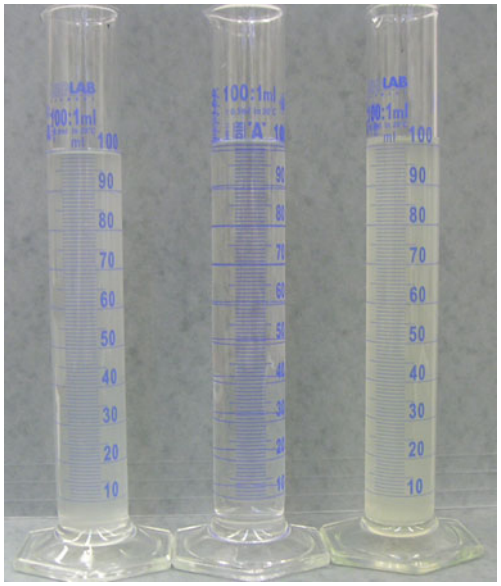

Fig. 1 Macroscopic appearance of fasted state simulated colonic fluid (FaSSCoF, *left*) and fed state simulated colonic fluid (FeSSCoF, *right*) *versus* the appearance of distilled water (*middle*).

In this study, both FaSSCoF and FeSSCoF were used fresh. To assess the possibility of earlier preparation and to confirm that their composition does not change during incubation at 37°C, physical stability of both media was evaluated by measuring cholates and palmitic acid concentration (3,5) in presence and in absence of bovine serum albumin

- immediately after medium preparation,
- after standing of the medium for 24 h at room temperature, and
- after standing of the medium for 24 h at room temperature and for 8 h at 37°C in the shaking water bath.

In all cases, deviation of cholate concentration from that measured immediately upon medium preparation ranged from −6.1% to +8.3%. For palmitic acid, corresponding deviation ranged from −17.0% to +13.0%. No trend for increased deviation after the 8 h/37°C incubation was observed.

Human Colonic Fluids (HCFs)

Colonoscopies were held in the Red Cross Hospital of Athens after receiving approvals by the Scientific and the Executive Committee of the Hospital (AP 23783 and AP 27573). Inclusion and exclusion criteria were identical to those reported recently (3). Contents of the ascending colon were collected under anaerobic conditions from 8 healthy adults in both the fasted and fed states on a crossover basis. In Phase I, subjects were fasted for 16 h before and during colonoscopy, and in Phase II, they consumed a standard breakfast (960 kcal) 6 h prior to colonoscopy and a light lunch 2 h prior to colonoscopy. Composition of meals is described in Diakidou *et al.* (3). Times at which colonic contents were collected correspond to the times at which orally administered drug products are expected to arrive in the ascending colon during bioavailability/bioequivalence (BA/BE) studies. After each collection, the pH was measured, the colonic contents were filled into test tubes with an 11 ml capacity, a cocktail for inhibiting proteolysis and lipolysis was added (20 μL/mL (13)), the tubes were sealed, ultracentrifuged (30,000 g, 20 min, 25°C), and the supernatant was collected (Human Colonic Fluids, Phase I: HCF_{fasted}; Phase II: HCF_{fed}).

Solubility Measurements in Simulated Colonic Fluids and in Plain Buffers

Equilibrium solubility measurements were made in FaSSCoF, FeSSCoF, FaSSCoF containing no bovine serum albumin ($FaSSCoF_{sBSA}$), FeSSCoF containing no bovine serum albumin ($FeSSCoF_{sBSA}$), FaSSCoF containing 4 mg/ml bovine serum albumin ($FaSSCoF_{4,BSA}$), FeSSCoF containing 4 mg/ml bovine serum albumin ($FeSSCoF_{4,BSA}$), and blank FaSSCoF and FeSSCoF, i.e. in plain (tris/maleate) buffer pH 7.8 and pH 6.0, respectively. In each medium, measurements were performed in triplicate.

For each measurement, 5 ml of the medium and pure drug powder in excess (75 mg for ketoconazole, and 5 mg for danazol and felodipine) were transferred into 10-ml polypropylene vials. Vials were then sealed with high density polypropylene caps and put in an oscillating water bath (37°C). Based on equilibration times observed previously using similar media volumes (1–5 ml) and the same *in vitro* setup for measuring solubilities of the model compounds in human aspirates from the upper GI lumen and in biorelevant media (14–16), vials were oscillated for 8 h (ketoconazole), 4 h (danazol) and 16 h (felodipine). To evaluate the adequacy of centrifugation *vs.* filtration, two samples were taken from each vial after equilibrium had been reached. The first was filtered through 0.45 μm filters (regenerated cellulose, 17mm, Titan®, Wilmington, USA). After discarding approximately the first 0.2 ml, the pH of the filtrate was measured, and an aliquot of the filtrate was diluted with acetonitrile, centrifuged (10 min, 11,400 g (10,000 rpm), 10°C), and assayed by HPLC. The second sample was centrifuged for 10 min at 11,400 g at 37°C, the pH was measured, and, after appropriate dilution of supernatant with acetonitrile and centrifugation (10 min, 11,400 g, 10°C), assayed by HPLC.

Solubility Measurements in HCFs

Measurements were made in individual HCF samples. Due to limited availability of volumes, solubility in individual supernatants could be measured only once. For each measurement, 1 ml of HCF and pure drug powder in excess (15 mg for ketoconazole, and 1 mg for danazol or felodipine) were transferred into 2-ml polypropylene vials. Vials were then sealed with polypropylene caps and put in an oscillating water bath (37°C) for periods identical to those used in the experiments in simulated colonic fluids (please see above). Since HCFs could not be filtered through 0.45 μm filters, samples were centrifuged for 10 min at 11,400 g (10,000 rpm) at 37°C after reaching equilibrium. Adequacy of centrifugation was confirmed by measuring solubility in selected (according to volume availability) individual HCF_{fasted} and HCF_{fed} samples in triplicate; low variability (high reproducibility) of the estimate would indicate adequacy of centrifugation as a separation method. After measuring the pH, the supernatant was appropriately diluted with acetonitrile, centrifuged again (10 min, 11,400 g, 10°C) and assayed for drug concentration by HPLC.

Analytical Methods

All model compounds were assayed with HPLC-UV methods.

For ketoconazole, a previously reported method was applied (17) by using a Hypersil® BDS C18 column (250× 4.6 mm, 5 μm). The mobile phase consisted of water: acetonitrile:diethylamine (73:27:0.1 v/v/v), and the flow rate was 1 ml/min. The detection wavelength was 240 nm. The injection volume was 20 μl, and the retention time about 9 min.

For danazol, a modification of a previously proposed method (18) was applied. The analytical column was a Hypersil® BDS C18 (250×4.6 mm, 5 μm). The mobile phase consisted of water:acetonitrile (45:55 v/v), and the flow rate was 1.2 ml/min. The detection wavelength was 286 nm. The injection volume was 50 μl, and the retention time about 12 min.

For felodipine, a previously published method was applied (15). The column was Hypersil® BDS C18 (150× 4.6 mm, 5 μm). The mobile phase consisted of water:

acetonitrile (50:50 v/v), and the flow rate was 1.5 ml/min. The detection wavelength was 240 nm. The injection volume was 20 μl, and the retention time about 10 min.

For each drug/medium combination, quantification was based on linear standard curves constructed using the same medium and identical sample handling procedure.

Data Treatment

Comparisons of solubility data in HCF_{fasted} samples or in plain buffer pH 7.8 with data in FaSSCoF and of solubility data in HCF_{fed} samples or in plain buffer pH 6.0 with data in FeSSCoF were carried out with two-sided unpaired *t*-tests. The effect of bovine serum albumin on drug solubility was evaluated with one way-ANOVA and Tukey's post hoc test. Comparison of solubility data in HCF_{fasted} samples with data in HCF_{fed} samples was carried out using a two-sided, paired *t*-test.

In all cases, differences were considered significant at the 0.05 level.

RESULTS

Separation of Solids from Liquid at Equilibrium

Solubility data in FaSSCoF, FeSSCoF, and plain buffers obtained by centrifuging and by filtering the samples at equilibrium are presented in Table 2.

In FaSSCoF and FeSSCoF, separation of solid from dissolved material by centrifugation and filtration resulted in similar solubility values for ketoconazole and danazol. For felodipine, higher concentrations after filtration than after centrifugation indicated that submicron particles of felodipine may have passed through the 0.45 μm filters. Similar data were obtained in $FaSSCoF_{sBSA}$, $FeSSCoF_{sBSA}$, $FaSSCoF_{4,BSA}$, and $FeSSCoF_{4,BSA}$ (data not shown).

For all model drugs, solubility data in plain buffers collected by filtering the samples at equilibrium (Table 2) are similar with those published previously reported in buffers with similar pHs [ketoconazole (19)] and in water (danazol and felodipine (20,21)). In plain buffers, centrifugation did not sediment ketoconazole and danazol particles adequately; particles could still be observed visually after the procedure. In contrast, centrifugation and filtration were equally successful in separating felodipine particles (Table 2). Differences on particle density, particle size and powder wettability may all account for the variable ability of the applied centrifugation conditions to separate solids from liquid.

For ketoconazole, the relative standard deviation (RSD, $n=3$) of solubility measured in an HCF_{fasted} sample and in an HCF_{fed} sample after centrifuging the samples at equilibrium was 1.5% and 6.7%, respectively. For danazol, the corresponding RSD values were 2.7% and 0.7%, and for felodipine, 9.5% and 4.3%, respectively. Therefore, solubility data in HCFs measured after centrifuging the samples at equilibrium can be considered reliable for these compounds. It is worth pointing out that centrifugation conditions applied in the present study may not be adequate for separation of solid particles of other drugs from HCFs (data not shown).

Solubility Data in Artificial Media *vs.* HCFs

Mean+SD solubility data of ketoconazole, danazol, and felodipine in simple buffers and in simulated colonic fluids *vs.* individual and mean+SD solubility data in HCFs are shown in Figs. 2 and 3. In all cases, pH at equilibrium was at most ±0.1 pH unit different from initial pH and close to the pH of contents of ascending colon measured immediately upon collection. In ketoconazole studies, specifically, median pH of HCF_{fasted} and HCF_{fed} samples at equilibrium were 7.6 and 6.3, respectively.

Solubility in plain buffers fails to estimate intracolonic solubility of lipophilic compounds. For ketoconazole, mean solubilities in HCF_{fasted} and HCF_{fed} are far higher than solubility in plain buffers with correspondingly similar pH values, i.e. 51 vs 2.550 μg/ml and 82 *vs.* 12.00 μg/ml, respectively. For danazol, the corresponding comparisons

Table 2 Centrifugation *vs.* Filtration of Samples on Equilibrium Solubility Data (Mean(SD), μg/ml, $n=3$) of Ketoconazole, Danazol and Felodipine in FaSSCoF, FeSSCoF, Plain Buffer pH 7.8, and Plain Buffer pH 6.0

	Ketoconazole		Danazol		Felodipine	
	Centrifugation	Filtration	Centrifugation	Filtration	Centrifugation	Filtration
FaSSCoF	17.2(1.0)	18.30(0.83)	8.4(2.1)	8.59(0.51)	13.27(0.75)	29.63(0.38)
FeSSCoF	24.5(1.1)	27.77(0.18)	7.03(0.61)	6.59(0.35)	15.51(0.70)	23.90(0.22)
Plain buffer pH 7.8	N.A.[a]	2.550(0.079)	N.A.[a]	0.332(0.034)	1.00(0.11)	0.802(0.027)
Plain buffer pH 6.0	N.A.[a]	12.00(0.42)	N.A.[a]	0.346(0.037)	1.267(0.087)	0.94(0.12)

[a] Not applicable, because particles remained suspended after centrifugation

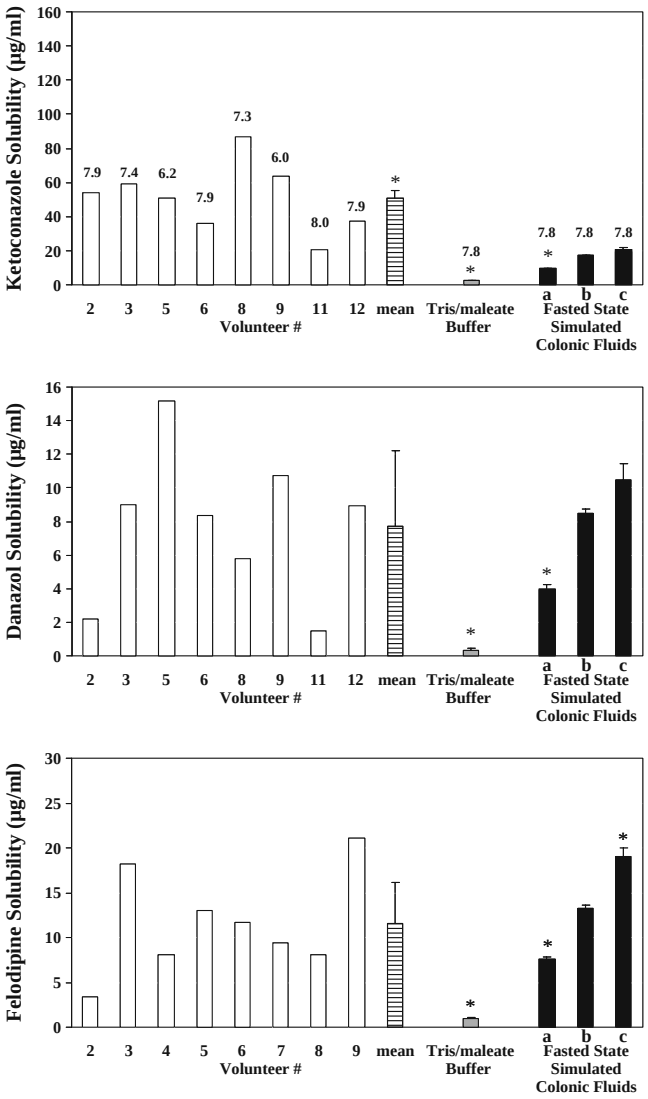

Fig. 2 Solubility of ketoconazole (*upper*), danazol (*middle*) and felodipine (*lower*) in HCF_{fasted} samples (*white bars*: individual data corresponding to a specific subject #; *lined bars*: Mean + SD ($n = 8$)), in plain buffer pH 7.8 (*grey bar*: Mean + SD ($n = 3$)), and in media simulating the colonic fluids in the fasted state (*black bars*: mean + SD ($n = 3$); (a) $FaSSCoF_{sBSA}$, (b) FaSSCoF, (c) $FaSSCoF_{4,BSA}$). A *number above* ketoconazole solubility data indicates the medium pH at equilibrium (up to 0.1pH unit different from initial medium pH). An asterisk above a mean + SD bar exists only when the difference is statistically significant from FaSSCoF.

are 7.70 *vs.* 0.332 μg/ml and 6.1 *vs.* 0.346 μg/ml, while for felodipine the values are 11.6 *vs.* 0.802 μg/ml and 12.2 *vs.* 0.940 μg/ml, respectively.

Although the mean solubility of ketoconazole in HCF_{fasted} and HCF_{fed} is significantly higher than its solubility in FaSSCoF and FeSSCoF (51 *vs.* 17.2 μg/ml ($p = 0.022$) and 82 *vs.* 24.5 μg/ml ($p = 0.012$), respectively), the estimates are much closer than those from the plain buffers. Since ketoconazole is partially ionized at pH 6 ($pka_1 = 2.9$, $pka_2 = 6.5$, both basic (14)), the superiority of FaSSCoF over plain buffer pH 7.8 (17.2 *vs.* 2.550 μg/ml) is more dramatic than superiority of FeSSCoF over plain buffer pH 6.0 (24.5 *vs.* 12.00 μg/ml). With ketoconazole, unlike with other compounds, no specific trend has been observed between solubility and concentration of biorelevant components simulating the environment in the upper small intestine (22). Therefore, we believe that further data using ionizable compounds are needed to obtain a clearer picture of the usefulness of FaSSCoF and FeSSCoF to predict intracolonic solubility of acids and bases in general.

For danazol and felodipine, which are both non-ionized within the physiologically relevant pH range, mean solubilities in FaSSCoF are similar to solubilities in HCF_{fasted}, and mean solubilities in FeSSCoF are similar to solubilities in HCF_{fed}. For neither drug were the differences statistically significant ($p > 0.444$ in all cases). These data show that FaSSCoF and FeSSCoF can far better predict the intracolonic solubility of danazol and felodipine than plain buffers.

For every model compound, solubility in FaSSCoF was not statistically different than solubility in FeSSCoF, and solubility in HCF_{fasted} was not different than solubility in HCF_{fed} ($p > 0.123$ in all cases). The lack of differences may, at least partly, relate to the low power of the performed tests (less than 0.23 in all cases). Therefore, more data are needed before concluding that solubilities are generally similar in these media. Although for non-ionizable compounds a single simulated colonic fluid may be adequate for predicting solubility in the ascending colon, for ionizable compounds, the pH change in the contents of the ascending colon with the dosing conditions should be considered.

Since intracolonic solubility of ketoconazole was not predicted one-to-one by FaSSCoF and FeSSCoF, two potential alterations in FaSSCoF and FeSSCoF composition were considered.

First, the effect of albumin concentration on drug solubility in simulated colonic fluids was evaluated. It has been shown that albumin's effect on solubility of non-ionized lipophilic compounds varies with the level of phospholipids in the medium (23). Therefore, prior information on the interaction of model compounds with albumin may not be directly applicable in the present study. Data from this study suggest that solubility in simulated colonic fluids increases with the concentration of albumin when the drug is not ionized (ketoconazole in FaSSCoF/danazol and felodipine in FaSSFoF and FeSSCoF) but remains unaffected when the drug is (partly) ionized (ketoconazole in FeSSCoF) (Figs. 2 and 3). Depending on the isoelectric point of the protein, the interaction with (partly) ionized molecules might be protein specific, and, therefore, protein effects on ketoconazole solubility in FeSSCoF may vary with the protein used for simulating the protein content of the ascending colon. Elimination of

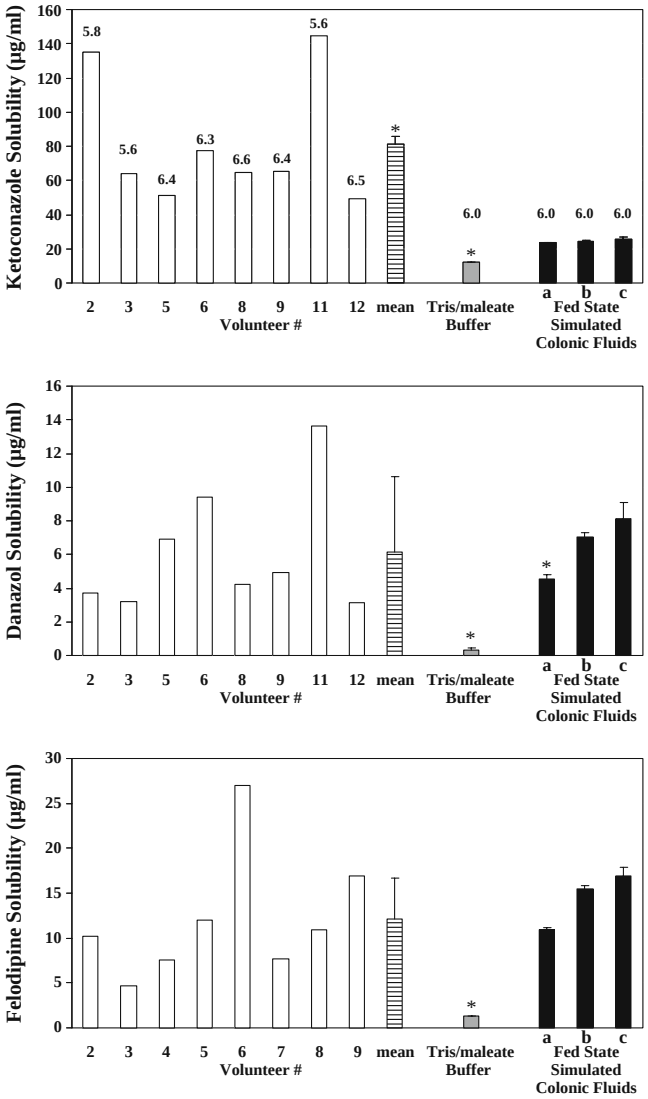

Fig. 3 Solubility of ketoconazole (*upper*), danazol (*middle*) and felodipine (*lower*) in HCF_{fed} samples (*white bars*: individual data corresponding to a specific subject #; *lined bars*: Mean + SD ($n = 8$)), in plain buffer pH 6.0 (*grey bar*: Mean + SD ($n = 3$)), and in media simulating the colonic fluids in the fasted state (*black bars*: mean + SD ($n = 3$); (a) $FeSSCoF_{sBSA}$, (a) FeSSCoF, (c) $FeSSCoF_{4,BSA}$). A *number above* ketoconazole solubility data indicates the medium pH at equilibrium (up to 0.1 pH unit different from initial medium pH). An *asterisk* above a mean + SD bar exists only when the difference is statistically significant from FeSSCoF.

bovine serum albumin from FaSSCoF or FeSSCoF leads to greater deviation of the solubility of non-ionized compounds from their solubility in HCFs, in most cases (Figs. 2 and 3), whereas increasing concentration of bovine serum albumin from 3 mg/ml to 4 mg/ml does not affect the solubility data significantly in most cases (Figs. 2 and 3). Therefore, the concentration of bovine serum albumin in FaSSCoF and FeSSCoF (3 mg/ml, Table 1) seems to be appropriate.

Second, the possibility of including cholesterol in FaSSCoF and FeSSCoF and the subsequent effect(s) on drug solubility was evaluated. *In vitro* studies have shown that cholesterol crystallization occurs depending on relative amounts of lipids, and excess cholesterol may exceed solubilizing capacity of mixed bile salt—phospholipid micelles (12). In line with those observations, biorelevant concentrations of cholesterol (Table 1) restricted the maximum phosphatidylcholine concentration in FaSSCoF and FeSSCoF to 37.5 µM and 75 µM, much lower than the phosphatidylcholine concentration in human colonic contents (Table 1). Solubility of ketoconazole and danazol in fasted-state simulated colonic fluid containing physiological concentrations of cholesterol but lower than physiological concentrations of phosphatidylcholine were 13.8(0.32) µg/ml and 5.7(1.0) µg/ml, respectively, i.e. lower than solubilities in FaSSCoF (Table 2) and less predictive of solubilities HCF_{fasted} (50.93(20.34) µg/ml *vs.* 7.70(4.49) µg/ml, respectively). Similarly, for the comparison of ketoconazole and danazol solubility data in fed-state simulated colonic fluid containing cholesterol but less phosphatidylcholine, solubility was 15.2(0.4) µg/ml and 3.3(0.5) µg/ml, respectively, i.e. also lower than the solubility in FeSSCoF (Table 2) and less predictive of the corresponding aspirates, HCF_{fed} (81.51(37.18) µg/ml *vs.* 6.14(3.71) µg/ml, respectively). Therefore, inclusion of cholesterol in FaSSCoF or FeSSCoF does not improve predictions of intracolonic solubility.

DISCUSSION

Solubilities of all three model compounds were much higher in the colonic aspirates than would be predicted from plain buffers at the equivalent pH values. This observation suggests that bile acids, phosphatidylcholine, and palmitic acid at total concentration of about 1 mM (0.6 mM in the fasted state and 1.3 mM in the fed state, respectively) and, perhaps, peptides/proteins that are present in the ascending colon exert significant solubilization effects.

Table 3 compares equilibrium solubility values of ketoconazole, danazol, and felodipine in aspirates from the upper GI lumen (literature data), and in HCFs (this study).

For ketoconazole, solubility in the ascending colon is generally lower than in the upper GI lumen (Table 3) under both dosing conditions, due to the higher pH value and/or reduced presence of solubilizing agents in the colon. As presented in the previous section, solubility in HCF_{fasted} is not statistically different from solubility in HCF_{fed} samples (Table 3). Based on individual samples, there is a trend for increased solubility of ketoconazole in HCF_{fed} (Fig. 2 *vs.* Fig. 3). As pH is only one of the factors affecting solubility of (partially) ionized lipophilic compounds in HCF samples,

Table 3 Mean ± SD Equilibrium Solubility Data (μg/mL, 37°C) for Ketoconazole, Danazol, and Felodipine in Human Gastric Fluids (HGF), in Fluids Collected from the Upper/Middle Small Intestine of Humans (HIF), and in Fluids of Ascending Colon (HCF) Collected in the Fasted and in the Fed State

	Ketoconazole	Ref #	Danazol	Ref #	Felodipine	Ref #
HGF_{fasted}	9,025 ± 133 (20–40 min, pooled sample from 12 adults)	26	1.61 ± 0.05 (5 adults)	27	0.410 ± 0.077 (20–40 min, pooled sample from 12 adults)	26
HIF_{fasted}	28.8 ± 2.7 (30 min, pooled sample from 12 adults)	14	2.04 ± 1.45 (10 adults)	27	14.3 ± 0.03 (pooled sample from 12 adults)	28
	240 ± 238 (30 min, 4 adults)	24	4.9 ± 1.1 (pooled sample from12 adults)	28		
	346 ± 260 (60 min, 3 adults)		16 ± 21 (30 min, 4 adults)	24		
	181 ± 133 (90 min, 3 adults)		18 ± 18 (60 min, 4 adults)			
	625 ± 742 (120 min, 3 adults)		13 ± 7 (90 min, 3 adults)			
			15 ± 18 (120 min, 3 adults)			
HCF_{fasted}	51 ± 20 (5 h, 8 adults)	This study	7.7 ± 4.5 (5 h, 8 adults)	This study	11.6 ± 5.8 (5 h, 8 adults)	This study
HGF_{fed}	626 ± 63 (30 min, pooled sample from 12 adults)	16	Not available		77 ± 35 (90 min, pooled sample from 12 adults)	29
	3,150 ± 135 (60 min, pooled sample from 12 adults)				43 ± 21 (150 min, pooled sample from 12 adults)	
	4,749 ± 82 (120 min, pooled sample from 12 adults)				57 ± 4 (180 min, pooled sample from 12 adults)	
HIF_{fed}	913 ± 60 (30 min, pooled sample from 12 adults)	14	101 ± 38 (pooled sample from 6 adults)[a]	28	413 ± 52 (pooled sample from 6 adults)[a]	28
	834 ± 18 (60 min, pooled sample from 12 adults)					
	989 ± 95 (120 min, pooled sample from 12 adults)					
	476 ± 24 (180 min, pooled sample from 12 adults)		37 ± 11 (30 min, 5 adults)	24		
	1,050 ± 895 (30 min, 5 adults)	24	27 ± 18 (60 min, 5 adults)			
	933 ± 857 (60 min, 5 adults)		25 ± 21 (90 min, 5 adults)			
	536 ± 178 (90 min, 5 adults)		14 ± 12 (120 min, 5 adults)			
	534 ± 171 (120 min, 5 adults)		18 ± 26 (150 min, 5 adults)			
	780 ± 361 (150 min, 5 adults)		12 ± 10 (180 min, 5 adults)			
	744 ± 741 (180 min, 5 adults)		9 ± 5 (210 min, 5 adults)			
	314 ± 174 (210 min, 5 adults)		14 ± 5 (240 min, 5 adults)			
	437 ± 176 (240 min, 5 adults)		12 ± 8 (270 min, 5 adults)			
	160 ± 122 (270 min, 5 adults)		6 ± 5 (300 min, 5 adults)			
	83 ± 69 (300 min, 5 adults)					
HCF_{fed}	82 ± 37 (6 h, 8 adults)	This study	6.1 ± 3.7 (6 h, 8 adults)	This study	12.2 ± 7.0 (6 h, 8 adults)	This study

If a pooled sample of aspirates was used, SD (standard deviation) reflects the experimental variability in solubility measurement from three replicates. In all other cases, individual aspirates were used and SD reflects inter-individual variability in intraluminal solubility. Times are after administration of water (fasted state) or liquid meal (fed state) in stomach. Fasted state data without time specification have been collected in samples aspirated without any prior administration of water.

[a] Instead of administration in the stomach, liquid meal had been perfused through the intestinal segment of adults during a period of 90 min, and HIF_{fed} samples (in which solubility was measured) had been collected 20–60 min after the start of the perfusion.

more data and, perhaps, multivariate analysis would have been needed to confirm the possible dominant effect of pH on ketoconazole solubility in HCF samples. Another reason that makes identifying a statistically significant pH effect on solubility of ionized compounds difficult may relate to the limited number of individual samples and the non-linear pH-solubility profile; if few of the individual samples have substantially different pH, solubility in those samples may be orders of magnitude different than the rest and, thus, dominate the mean solubility value, perhaps to the opposite direction. The physiological variation in sample pH may explain the big variation of average solubility data of

ketoconazole in HIF_{fasted} (Table 3); Data of Kalantzi *et al.* (14) suggest an average solubility of 28.8 μg/ml (measured in a pooled sample from 12 aspirates collected 30 min after water administration in the fasted state), whereas data of Clarysse *et al.* (24) suggest average solubility between 144 and 620 μg/ml (means of 5 measurements in individual aspirates collected at various times (30–120 min) after water administration in the fasted state).

In the fasted state, solubilities of the non-ionized compounds in the ascending colon are similar or higher than those in the upper GI lumen (Table 3). A similar observation has been recently made by others with prednisolone (25). Apparently, in the fasted state, proteins and/or other unidentified solubilizing agents in the ascending colon compensate for the solubilization effects of bile salt micelles in the upper small intestine, and they lead to higher drug solubility in the colon than in the stomach (Table 3). In the fed state, the higher solubility values in the upper GI lumen reflect the increased presence of solubilizing agents in this region.

In summary, while solubilities of ketoconazole in FaSSCoF and FeSSCoF were closer to solubilities in HCF_{fasted} and HCF_{fed} samples, respectively, than those in plain buffers with similar pHs, ways of more accurately predicting intracolonic solubility of ketoconazole need to be investigated further, perhaps with the study of other weak bases. For danazol and felodipine, solubilities in FaSSCoF and FeSSCoF predicted solubilities in HCF_{fasted} and HCF_{fed} samples. The ability of these media to predict intracolonic solubility needs to be confirmed with a wider range of neutral compounds.

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