

Precipitation in and Supersaturation of Contents of the Upper Small Intestine After Administration of Two Weak Bases to Fasted Adults

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ABSTRACT

Purpose To evaluate precipitation in and supersaturation of intestinal contents after administration of pharmacologically relevant doses of dipyridamole and ketoconazole to 12 healthy adults.

Methods On two separate days each subject was administered in stomach 240 ml aqueous solutions of two dipyridamole doses (30/90 mg) and two ketoconazole doses (100/300 mg). Physicochemical characteristics, total drug content, and drug concentration were measured in individual intestinal contents (≤ 7 ml) aspirated at specific times post-dosing. Drug concentration after incubation (37°C/48 h) and equilibrium solubility were measured. Precipitate crystallinity was evaluated by x-ray powder diffraction.

Results Precipitated fraction was minimal (dipyridamole, $\leq 7\%$) or limited (ketoconazole, $\leq 16\%$). Ketoconazole precipitates were mostly amorphous. Depending on dose, intestinal contents with $\text{pH} > 3.6$ were supersaturated with dipyridamole up to 10 and 30 min and with ketoconazole up to 30 and 50 min post-administration. Intestinal contents with $\text{pH} > 5$ and concentration of micellar components < 5 mM were supersaturated with ketoconazole or dipyridamole, but precipitated fraction was significant only for ketoconazole. After incubation, crystalline precipitates were found in almost all samples. Slow precipitation of base and/or precipitation of other phases account for this observation.

Conclusions Intraluminal precipitation of weakly alkaline, lipophilic, high permeability drugs may not be substantial. Estimating intestinal supersaturation in regard to free base is inadequate as other phases may precipitate.

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INTRODUCTION

Due to their ionization characteristics, bases dissolve more easily at acidic pHs. Therefore, after oral administration in the fasted state, concentrations in the gastric contents may exceed solubility in the contents of the upper intestinal lumen, and a fraction may precipitate upon entering the duodenum. Solubility varies with the compound, the composition of the medium, and the method of measurement. In most cases, it refers to the equilibrium solubility, i.e. to the concentration of the drug in a saturated solution

when excess of the most stable crystalline form of the drug is present. Precipitated fraction, π ($0 \leq \pi \leq 1$), can be estimated by the following equation:

$$\pi = 1 - \frac{C}{C_t} \quad (1)$$

where C is the concentration of the drug and C_t is the total amount of drug (dissolved and precipitated) per unit volume.

However, the rate of particle growth is compound- (1) and medium- (2) dependent. *In vitro* data in simple aqueous media, simulated intestinal fluids, and human intestinal fluids suggest that it may be comparable with the upper intestinal transit rates (1,2). Therefore, after administration in the fasted state, contents of upper small intestine may be supersaturated with the base, and the flux across the intestinal mucosa may be higher than expected from equilibrium solubility considerations. The degree of supersaturation can be expressed by the relative supersaturation index, σ (3):

$$\sigma = \frac{C}{C_s} - 1 \quad (2)$$

where C and C_s are the concentration of the drug in solution and equilibrium solubility of the drug, respectively. According to equation 2, a solution can be unsaturated ($\sigma < 0$), saturated ($\sigma = 0$), or supersaturated ($\sigma > 0$).

As of the end of 2006, 37.9% of active pharmaceutical ingredients (APIs) approved in the U.S.A. after 1981 for oral administration have been weak bases (4). Nevertheless, *in vivo* data relevant to precipitation are almost non-existent. It has recently been shown that intestinal precipitation of a weak base that is under development by AstraZeneca is less of a limitation for *in vivo* drug absorption than implied from equilibrium solubility and *in vitro* test assessments (5). Although that was probably the first study dealing with *in vivo* precipitation/supersaturation of bases, observations were made indirectly (using plasma data), and, therefore, quantitative assessment of the degree of precipitation/supersaturation *in vivo* was not possible.

The goal of the present study was to evaluate on a quantitative basis the precipitation in and the supersaturation of contents of the upper small intestine after administration of two lipophilic weak bases to healthy fasted adults. Dipyridamole [2-({6-[bis(2-hydroxyethyl)amino]-4,8-bis(piperidin-1-yl)-[1,3]diazino[5,4-d]pyrimidin-2-yl}(2-hydroxyethyl)amino)ethan-1-ol, $\log P$ 2.7–4.9, pK_a 5.7–6.4] and ketoconazole [1-[4-(4-{{(2S,4R)-2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-yl)methyl)-1,3-dioxolan-4-yl}methoxy}phenyl)piperazin-1-yl]ethan-1-one, $\log P$ 4.4, pK_a 2.9 and 6.5] (6) were used as model compounds.

MATERIALS AND METHODS

Materials

Dipyridamole (DPD) was from Boehringer Ingelheim Espanã, S.A. (Malgrat De Mar, Spain, lot # 08342). Ketoconazole (KCZ) was from Janssen Cork, Janssen Pharmaceutical Ltd. (Little Ireland, Cork, Republic of Ireland, lot # 0712003723). Acetonitrile and methanol of HPLC grade were from E. Merck (Darmstadt, Germany). Egg phosphatidylcholine (Lipoid E PC[®] 99.1% pure, lot #105019-1/14) was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). Water of HPLC grade was obtained by using a Labconco[®] water pro ps. System (Kansas City, USA). All other chemicals were of analytical grade.

Human Study

The study was held in the Red Cross Hospital of Athens after receiving approval by the Greek Ethics Committee (IS-111) and by the National (Greek) Organization for Medicines (GR 210181 - EudraCT # 2008-006178-13). It was a cross-over four-phase study. Subjects were administered 240 ml of drug solution to the antrum of their stomach as follows:

- Phase 1: A low dose DPD (30 mg) solution was administered (DPD-L).
- Phase 2: A high dose DPD (90 mg) solution was administered (DPD-H).
- Phase 3: A low dose KCZ (100 mg) solution was administered (KCZ-L).
- Phase 4: A high dose KCZ (300 mg) solution was administered (KCZ-H).

For each compound, both low dose and high dose are within the range of pharmacologically relevant single doses (7).

Samples were aspirated from a location near the ligament of Treitz at specific time points and up to 70 min post dosing.

Preparation of Drug Solutions

DPD

Commercially available non-carbonated mineral water (AVRA, Greece) was acidified with HCl so that its pH became 2.7 (Solution A). The DPD-L solution was prepared by dissolving 30 mg DPD in 240 ml of solution A (0.12 mg/ml). The DPD-H solution was prepared by dissolving 90 mg DPD in 240 ml of solution A (0.38 mg/ml). The final pH of both DPD-L and DPD-H solutions was 2.7.

KCZ

Commercially available non-carbonated mineral water was acidified with HCl so that its pH became 2.6 (Solution B) or 2.4 (Solution C). The KCZ-L solution was prepared by dissolving 100 mg KCZ in 240 ml of solution B (0.42 mg/ml). The KCZ-H solution was prepared by dissolving 300 mg KCZ in 240 ml of solution C (1.25 mg/ml). The final pH of both KCZ-L and KCZ-H solutions is 2.7.

Subjects

Twelve healthy adults (10 M/2 F) with a mean age of 26 years (range 20–38 years) gave informed consent and participated in the study. One subject was 15% heavier, and the rest were up to 20% lighter than their ideal body weights. Subjects did not have history or clinical evidence of gastrointestinal (GI) disease, did not participate in another clinical study, and did not receive medicines that influence the function of GI tract up to at least 30 days prior to the study. The health status of each subject was confirmed by physical examination and screening of blood parameters for renal and hepatic functions.

One female subject had been taking birth control pills for more than 3 months prior to her participation in the study, and she took her pill after the end of each experimental day. Both female subjects were not pregnant according to their written declaration and the results of pregnancy test in the morning of each experimental day.

Study Protocol

For each subject, the study was performed on two separate experimental days. Alcohol and any over-the-counter medication were discontinued 3 days prior to and throughout each experimental day, whereas food intake was discontinued for 12 h prior to the start of each experimental day, and water was consumed *ad libitum*. At about 8 a.m. on the experimental day, the subject arrived at the clinic and was briefly screened for his/her health status by the gastroenterologist.

Experimental Day A

After spraying the back of the mouth with lidocaine, the subject was intubated nasally using a sterile two-lumen duodenal tube (Freka® Trelumina, CH/FR 16/9, 150 cm, Fresenius Kabi Deutschland GmbH, Bad Hombourg, Germany).

No attempt was made to isolate the aspiration segment from the rest of GI contents. The double bore tube was 150 cm long with an external diameter of 5.3 mm and a plastic tip at its distal end. A series of holes 55–65 cm

proximal to the tip were used to access the antrum of the stomach. A further series of hand-made holes 13.5–23.5 cm proximal to the tip were used to aspirate samples from the ligament of Treitz. Insertion of the tube was assisted by a guiding wire, and its position was monitored fluoroscopically. After reaching its final position and removing the wire, the subject lay semisupine, and 240 ml of DPD-L solution were administered using 60-ml (capacity) syringes to the antrum. Administration lasted for about 1 min, and samples (up to about 7 ml) were aspirated from the ligament of Treitz at 5, 10, 20, 30, 40, 50, 60 and 70 min after the administration of the solution. Immediately after aspiration, about 8 ml of air was pumped into the tube to clear its contents back into the lumen (total internal volume of this sampling tube was estimated to be about 4 ml). After aspiration of the last sample, the subject was administered 200 ml of non-carbonated mineral water and relaxed by lying semisupine on the bed for 2 h. At the end of the 2-h relaxing period, the subject was administered 240 ml of DPD-H solution, and samples were aspirated as described above. At the end of the experimental day and before removing the tube/discharging the subject, the final position of the tube was confirmed fluoroscopically.

Experimental Day B

Compared with experimental day A, the only difference on day B was that the drug in administered solution was KCZ. KCZ-L was administered first, and KCZ-H was administered second.

The administered DPD and KCZ solutions were measured to be stable at room temperature for at least 24 h. Previous experience with DPD and KCZ indicated that these model compounds are stable in incubated luminal contents (6,8,9).

The overall mean/median sample volume was 5.5/6.4 ml for the DPD experiments and 6.3/7.0 ml for the KCZ experiments; i.e. the total volume of intestinal fluid aspirated from the end of duodenum, during each phase was about 40–45 ml. At the end of each phase, the mean fluid volume in the stomach (4.3 ± 2.0 ml) was not higher than that prior to administration of the drug solution (10 ± 15 ml), whereas bile salts in the stomach after the end of aspirations were minimal (up to 0.05 mM). These data suggest that influx of duodenal contents due to aspirations was minimal (if any).

Median total-aspirated-volume/pH values for the contents from the stomach and from the upper small intestine prior to the first administration of DPD were 6.2 ml/1.7 and 1.2 ml/6.6, respectively, whereas prior to the second administration of DPD they were 9.0 ml/2.6 and 2.0 ml/

6.2, respectively. The corresponding numbers prior to the first KCZ administration were 3.3 ml/1.8 and 0.8 ml/7.0, respectively, whereas prior to the second administration of KCZ they were 10.0 ml/2.0 and 2.0 ml/5.5, respectively. Median amount of drug in the stomach prior to the second administration of DPD could not be quantified, whereas prior to the second administration of KCZ it was less than 33 µg. Median drug concentration in the small intestine prior to the second administration of DPD was 2.1 µg/ml and prior to the second administration of KCZ was 4.9 µg/ml. These data indicate that the 2-h period between the two administrations performed at each experimental day was adequate for the solution after the first administration to be practically removed from the upper GI lumen.

Handling of Samples

Upon collection, each sample was immediately divided in two portions: The first was used for measuring total drug content (solid and dissolved drug), C_t , in duplicate. The second was transferred in a vial that was filled with the sample and sealed (to minimize loss of bicarbonates during subsequent handling). The vial was immediately centrifuged (37°C, 12560 × g, 10 min) to remove any solid particles and the supernatant was divided in four parts: the first was used for assaying the drug concentration, C , in duplicate; the second was incubated (37°C, 48 h, 75 oscillations/min) and, after centrifugation (37°C, 12560 × g, 10 min), the drug concentration, C_{inc} , was measured; the third was incubated (37°C, 48 h, 75 oscillations/min) in presence of excess of the compound, and, after centrifugation (37°C, 12560 × g, 10 min), equilibrium solubility, C_s , was measured; equilibration times using identical experimental conditions have been reported to be much shorter than 48 h for measuring of DPD and KCZ solubility in human intestinal fluids that initially contain no drug (8); and the fourth was used for assaying individual bile salts (taurocholate (TC), glycocholate (GC), taurochenodeoxycholate (TCDC), ursodeoxycholate (USD), glycochenodeoxycholate (GCDC), glycodeoxycholate (GDC) and cholate (C)), lecithin (phosphatidylcholine and lyso-phosphatidylcholine), cholesterol, free fatty acids (linoleic, palmitic and stearic acids), monoglycerides, and total protein content.

After each centrifugation, as much as possible of the supernatant was carefully poured out of the tube so that any precipitated material remained in the tube and stored at -70°C. After all relevant assays, tubes that contained at least 0.1 mg drug particles were subjected to X-ray powder diffraction (XRPD) analysis.

Samples for measuring C_t and C were stored in glass vials at -20°C. Samples for measuring C_{inc} and C_s were immediately incubated. Samples for measuring individual

bile salts, lecithin, cholesterol, free fatty acids, monoglycerides and protein content were stored at -70°C in separate plastic vials so that each assay was performed immediately after the first thawing.

The superiority of centrifugation (37°C, 12560 × g, 10 min) over filtration through PVDF membrane filters (0.45 µm HV, Durapore® membrane filters, Millipore, Ireland) and through cellulose nitrate filters (0.45 µm, Purabind® 045, Whatman, Germany) in removing solid particles was confirmed with preliminary solubility measurements of DPD and KCZ using canine intestinal fluids collected as described previously (8). Filtrations involved the use of glass syringes and stainless steel filter holders. Both drugs required the application of excessive pressure, whereas adsorption onto the filters was substantial and variable (2.5–37% for DPD/Durapore® data, 4.5–31% for KCZ/Durapore® data and 39–97% for KCZ/Purabind® data). In addition, coefficient of variation of solubility data was 8.2% for DPD/Durapore® data and 0.9% for DPD/centrifugation data, whereas, for KCZ, centrifugation has been shown to lead to smaller CVs than filtration when measuring equilibrium solubility in colonic fluids (9).

Analysis of Samples

Measurement of C_t involved the addition of acetonitrile using a volume that ensures complete dissolution of potentially existing drug particles in the vial (1:6 v/v). After vortexing and centrifugation (10°C, 12560 × g, 10 min), the supernatant was diluted with mobile phase, and a sample was injected into the HPLC system. Measurement of C , C_{inc} and C_s involved treatment with acetonitrile, centrifugation (10°C, 12560 × g, 10 min), and appropriate dilution of supernatant with mobile phase prior to injection into the HPLC system. DPD and KCZ were assayed with HPLC-UV methods that were based on previously published methods (6). Individual bile salts, lecithin, cholesterol, free fatty acids and monoglycerides were assayed with HPLC-CAD methods (10,11). Total protein content was determined using a commercially available kit (BCA™ Protein Assay Kit, Thermo SCIENTIFIC, Rockford, USA).

Crystallinity of solid contents was assessed by XRPD in a PANalytical X'Pert Pro diffractometer Model PW3040/60 equipped with an X'Celerator detector (PANalytical B.V., Almelo, The Netherlands) using a Cu Kα radiation at 40 kV and 45 mA. Samples were scanned from 2.0° to 40.0° (2θ) using a 0.0167° 2θ step size and 31.75 s time count. The sample was prepared by mounting a few milligrams of sample on a Si wafer (zero background) plates, resulting in a thin layer of powder. In order to confirm that the obtained diffractogram referred to the drug and not to some other solid material in the lumen, samples were analyzed on a random basis with FT-IR and NMR.

Data Analysis

Raw data are presented as Box-Whisker plots showing the median value; the 10th, 25th, 75th, and 90th percentiles; and the individual outlying data points. The mean values are indicated with a solid white line. The concentration of micellar components was calculated as the sum of the molar concentration of individual bile salts, lecithin (sum of lyso-phosphatidylcholine and phosphatidylcholine), free fatty acids, monoglycerides, and cholesterol.

Precipitated fractions and relative supersaturation indices in the individual aspirates, π_{lumen} and σ_{lumens} were estimated by using equations 1 and 2, respectively. Precipitated fractions after incubation of supernatants of individual aspirates for 48 h at 37°C, π_{lumen}^{inc} , were estimated by using equation 1, concentration in lumen as C_l and C_{inc} as C . Relative supersaturation indices after incubation of supernatant for 48 h at 37°C, σ_{lumen}^{inc} , were estimated by using equation 2 and C_{inc} as C .

The significance of the precipitated fraction (i.e. assessing whether $\pi > 0$) was evaluated with one sample one-tailed t-test or one sample one-tailed Wilcoxon test, depending on the results of normality tests. The significance of the difference of supersaturation index from zero was evaluated with one

sample two-tailed t-test or one sample two-tailed Wilcoxon test, depending on the results of normality tests. Type I error was set to 0.05. The normality tests were performed using SigmaStat 3.5 (SPSS Science Inc., New York, USA). The t-tests and the Wilcoxon tests were performed using S-Plus 4.5 (Insightful Corporation, Seattle, USA).

RESULTS

Environment in the Upper Small Intestine after DPD and KCZ Administrations

Within each phase, distribution of pH values in the upper small intestine was skewed as aspirates with low pH were repeatedly collected. Median pH values (Table I) were similar to those previously measured after administration of 250 ml water (12), and, therefore, administration of acidic solutions (necessary to dissolve the dose) did not seem to affect the pH in the upper small intestine substantially. This is in line with the fact that the resulting pH after the addition of 240 ml of AVRA water in 15 ml of pH 1.8 HCl solution (the conditions that generally exist in the fasted stomach) is 3.0, not much different from pH 2.6 that results after mixing

Table I Physicochemical Characteristics of Human Intestinal Fluids During the First 70 min After Administration of the DPD and KCZ Solutions in the Present Study

	DPD-L	DPD-H	KCZ-L	KCZ-H
pH	5.8 ± 1.9 6.5(1.9–8.2)	5.7 ± 1.7 6.3(1.8–8.1)	5.7 ± 1.7 6.2(1.9–8.2)	5.6 ± 1.4 6.0(2.3–7.7)
Total Bile salts (mM) ^a	3.0 ± 3.5 1.6(0.019–17.8)	3.6 ± 3.8 2.5(0.034–19.9)	2.9 ± 4.3 1.3(0.039–26.8)	3.0 ± 3.8 2.0[LOQ(=0.002) –19.9]
Lecithin (mM) ^b	0.26 ± 0.36 0.054[LOQ(=0.004) –1.30]	0.25 ± 0.40 0.025[LOQ(=0.004) –1.70]	0.18 ± 0.33 0.046[LOQ(=0.004) –2.13]	0.28 ± 0.42 0.091[LOQ(=0.004) –1.95]
Free Fatty Acids (mM) ^c	0.54 ± 0.69 0.23[LOQ(=0.005) –3.12]	0.6 ± 1.0 0.13[LOQ(=0.005) –4.8]	0.39 ± 0.70 0.15[LOQ(=0.005) –4.73]	0.50 ± 0.63 0.22[LOQ(=0.005) –2.41]
Monoglycerides (mM)	0.021 ± 0.039 0.0063[LOQ(=0.0024) –0.184]	0.019 ± 0.041 0.0035[LOQ(=0.0024) –0.183]	0.014 ± 0.031 0.0131[LOQ(=0.0024) –0.187]	0.018 ± 0.029 0.0507[LOQ(=0.0024) –0.128]
Cholesterol (mM)	0.23 ± 0.31 0.102[LOQ(=0.001) –1.35]	0.28 ± 0.39 0.083[LOQ(=0.001) –1.53]	0.18 ± 0.26 0.0770[LOQ(=0.001) –1.57]	0.22 ± 0.27 0.078[LOQ(=0.001) –1.12]
Total Protein content (mg/ml)	1.09 ± 0.69 0.98(0.24–2.93)	1.54 ± 0.84 1.42(0.23–4.85)	1.06 ± 0.66 0.88(0.29–3.75)	1.49 ± 0.78 1.29(0.39–4.89)

Depending on the parameter, data in each cell have been collected from 48 to 89 individual aspirates. Upper numbers refer to mean ± SD, whereas lower numbers to median (range)

^a Sum of TC, GC, TCDC, UDC, GCDC, GDC and C

^b Sum of phosphatidylcholine and lyso- phosphatidylcholine

^c Sum of linoleic, palmitic and stearic acid

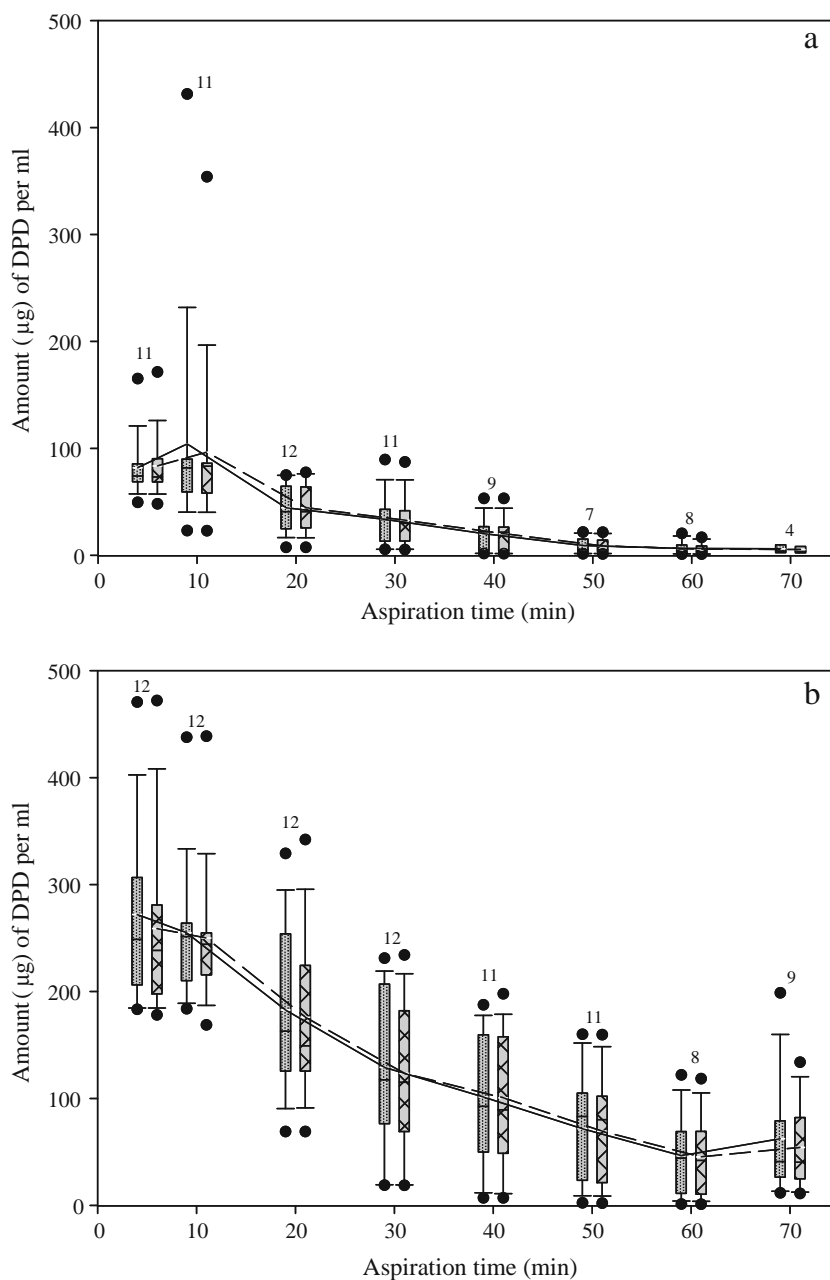
240 ml of pH 2.7 HCl solution (administered in this study) with 15 ml of pH 1.8 HCl solution.

Distribution of total bile salts levels was also skewed as high total bile salt levels were repeatedly observed (Table I). Mean total bile salts were somewhat higher than those determined by Persson *et al.* (2 mM) (13) and lower than the values reported by Bevernage *et al.* (5.4 mM) (2). The difference may be related to the smaller number of volunteers and/or the differences in aspirating/sample treatment protocols, applied in the previous studies. Median levels (Table I) were also consistent with previous reported median values (14). The overall % mean (SD) relative bile salts composition in aspirated intestinal fluids

after the administration of the four solutions was 11.9 (5.7) % TC, 36 (12) % GC, 9.7 (5.4) % TCDC, 5.0 (5.8) % UDC, 24.1 (8.8) % GCDC and 12.5 (9.4) % GDC. In two volunteers, traces of C were also detected. Therefore, primary bile salts (cholate, chenodeoxycholate) conjugated with glycine dominate, in accordance with previous findings (2,13,15).

Most lecithin in the aspirates was in form of lysophosphatidylcholine, and mean values (Table I) were similar with those reported previously by Persson *et al.* (0.2 mM) (13). Median values (Table I) were lower than values reported previously by Clarysse *et al.* (0.6 mM) (16). The latter difference may be related to the smaller number

Fig. 1 Total amount of DPD per ml, C_t (grey boxes), and concentration of DPD, C (lined grey boxes), in the upper small intestine after the administration of DPD-L (a) and DPD-H (b) solutions to fasted healthy adults. The number of adults that contributed to the construction of the specific pair of boxes is shown above each pair. Solid line connects mean C_t data, and dotted line connects mean C data.



of samples and the analytical method applied in the previous study (a commercially available kit had been used).

Mean free fatty acids values (Table I) were similar to previously reported values (0.6 mM) (17). Median values were slightly lower than previously reported values that were estimated from fewer number of samples (0.5 mM) (16).

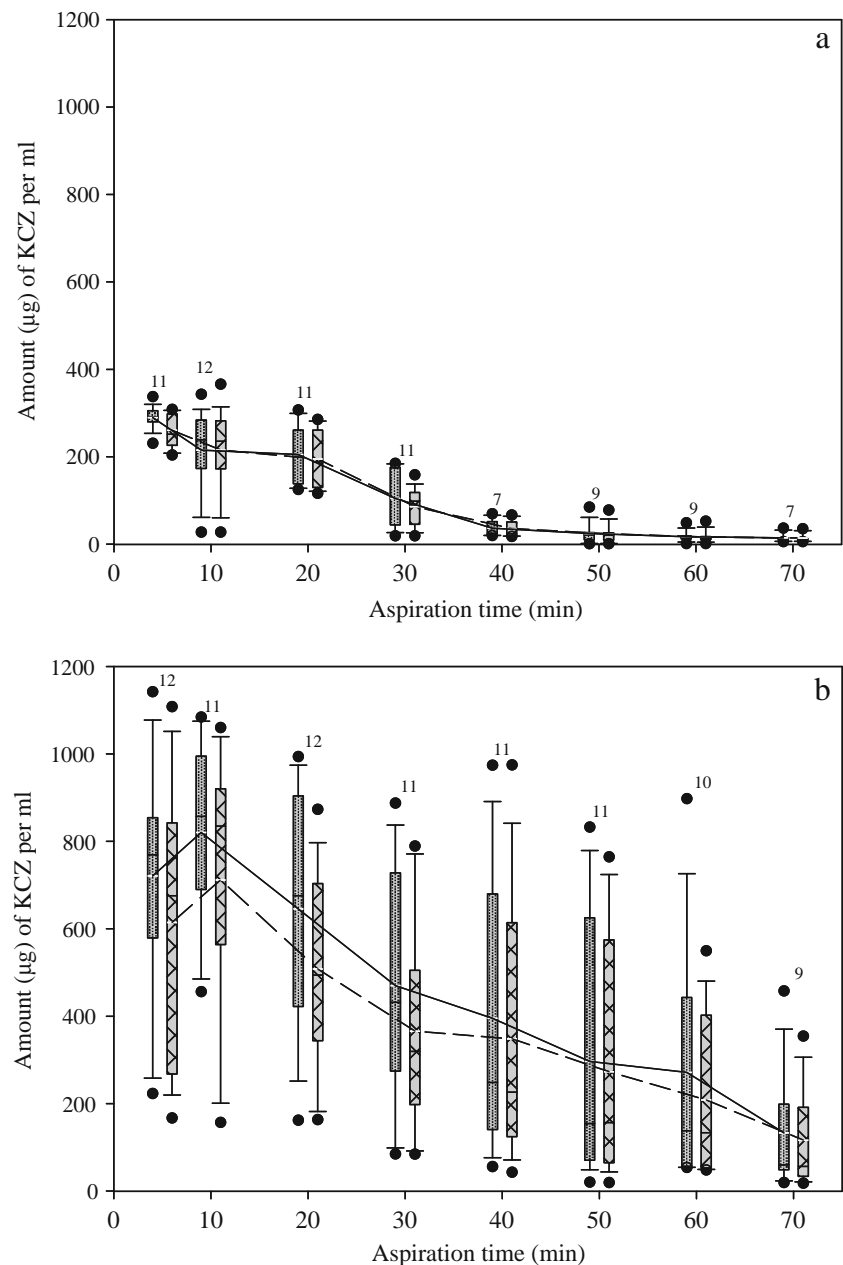
Mean values of monoglycerides and cholesterol (Table I) were higher than previously reported mean values: according to Persson *et al.* (13), the mean monoglycerides concentration was about 0.001 mM, and the mean cholesterol concentration was about 0.006 mM.

Mean total protein content (Table I) was somewhat lower than previously reported values (3.1 mg/ml (12), 7.5 mg/ml (13) and 2.1 mg/ml (18)).

Equilibrium Solubility of DPD and KCZ in Aspirates from the Fasted Upper Small Intestine

Measurement of equilibrium solubility in samples with $\text{pH} \leq 3.6$ (54 out of 336 individual aspirates in total) was associated with substantial pH change during the solubility experiments (equilibrium pH was 0.8–1.6 units higher than initial pH). Since such pH changes at this pH range affect equilibrium solubility values dramatically, the use of measured solubility values for estimating the relative supersaturation index in the lumen becomes problematic. In contrast, at $\text{pH} \geq 3.6$, changes of pH at equilibrium are much smaller, and, even though they still exist, their impact on solubility values is limited; other factors (such as bile

Fig. 2 Total amount of KCZ per ml, C_t (grey boxes), and concentration of KCZ, C (lined grey boxes), in the upper small intestine after the administration of KCZ-L (a) and KCZ-H (b) solutions to fasted healthy adults. The number of adults that contributed to the construction of the specific pair of boxes is shown above each pair. Solid line connects mean C_t data, and dotted line connects mean C data.



salts) contribute substantially to the equilibrium solubility value. Therefore, relative supersaturation index was estimated only for aspirates with $\text{pH} \geq 3.6$. However, since the bias in estimation of a mean or median solubility value would be much higher if the relevant solubility data were excluded, estimation of average solubility and comparisons with previously published values were made after including all available data ($n = 336$). Mean equilibrium solubilities of DPD in intestinal aspirates after DPD-L and DPD-H administrations were $469 \mu\text{g/ml}$ and $614 \mu\text{g/ml}$, respectively, whereas median values were $51 \mu\text{g/ml}$ and $83 \mu\text{g/ml}$, respectively. For KCZ, mean equilibrium solubility after low and high dose administration was $449 \mu\text{g/ml}$ and $404 \mu\text{g/ml}$, respectively, whereas the corresponding median values were $36 \mu\text{g/ml}$ and $73 \mu\text{g/ml}$, respectively. Mean KCZ solubilities were close to previously reported means (19), and median DPD and KCZ solubilities were close to solubilities previously reported in pooled intestinal aspirates (8). Since distributions of DPD and KCZ solubility data were skewed, it may be concluded that mean solubilities of the two compounds may not be representative of the average solubility.

For both DPD and KCZ, equilibrium solubilities in intestinal aspirates with $\text{pH} \geq \text{pK}_a$ correlated linearly with the total bile salt concentrations, ($R^2 = 0.74$ for DPD and

0.78 for KCZ). Correlations were only slightly improved when total micellar concentrations were used.

Precipitation in and Supersaturation of Luminal Contents after Administration of DPD and KCZ Solutions

Total amounts of drug per ml, C_b , and corresponding drug concentrations, C , in the upper small intestine at specific times after drug administration to fasted healthy adults are shown in Fig. 1 (DPD data) and in Fig. 2 (KCZ data). Precipitated fractions and supersaturation indices are presented in Table II (DPD data) and Table III (KCZ data). Precipitation fraction was estimated for every individual sample, whereas supersaturation index was estimated only if the sample had $\text{pH} > 3.6$.

For DPD, at both dose levels luminal precipitation was minimal, and mean precipitated fraction, $\overline{\pi}_{\text{lumen}}$, was up to 7% (Table II). Significant supersaturation was observed up to 10 min post administration of DPD-L and up to 30 min post administration of DPD-H. Interestingly, significant precipitation in the lumen was observed even when the luminal contents were not saturated with the administered DPD phase (DPD-L/50 min, Table II, first two columns). A similar observation can be made for some incubated

Table II Fraction of DPD Precipitated in Upper Small Intestine, $\overline{\pi}_{\text{lumen}} \pm \text{SD}$, and Relative Supersaturation Index, $\overline{\sigma}_{\text{lumen}} \pm \text{SD}$, at Specific Times After Administration of DPD-L (Upper Rows) and DPD-H (Lower Rows) and the Corresponding Relative Supersaturation Indices and Precipitated Fractions, After Incubation of the Supernatants for 48 h at 37°C ($\overline{\sigma}_{\text{lumen}}^{\text{inc}} \pm \text{SD}$ and $\overline{\pi}_{\text{lumen}}^{\text{inc}} \pm \text{SD}$, Respectively)

Time after DPD administration (min)	$\overline{\pi}_{\text{lumen}} \pm \text{SD}$	$\overline{\sigma}_{\text{lumen}} \pm \text{SD}$	$\overline{\sigma}_{\text{lumen}}^{\text{inc}} \pm \text{SD}$	$\overline{\pi}_{\text{lumen}}^{\text{inc}} \pm \text{SD}$
5	-0.015 ± 0.028	3.1 ± 2.1^b	0.84 ± 0.70^b	0.38 ± 0.27^a
	0.05 ± 0.11	4.4 ± 4.3^b	0.41 ± 0.72	0.41 ± 0.34^a
10	0.025 ± 0.066	1.3 ± 1.5^b	0.07 ± 0.64	0.36 ± 0.24^a
	0.020 ± 0.043	2.9 ± 3.7^b	1.2 ± 2.6	0.31 ± 0.28^a
20	-0.005 ± 0.033	-0.02 ± 0.62	-0.24 ± 0.55	0.22 ± 0.15^a
	0.031 ± 0.082	1.3 ± 1.6^b	0.65 ± 0.92	0.23 ± 0.17^a
30	0.021 ± 0.034^a	0.2 ± 1.4	-0.36 ± 0.42	0.24 ± 0.26^a
	0.037 ± 0.077	0.9 ± 1.2^b	-0.04 ± 0.46	0.33 ± 0.22^a
40	0.011 ± 0.033	-0.53 ± 0.71	-0.80 ± 0.20^b	0.42 ± 0.32^a
	0.018 ± 0.050	0.08 ± 0.93	-0.28 ± 0.60	0.19 ± 0.21^a
50	0.020 ± 0.016^a	-0.83 ± 0.17^b	-0.877 ± 0.084^b	0.16 ± 0.31
	0.051 ± 0.045^a	0.4 ± 1.3	0.06 ± 0.63	0.15 ± 0.21^a
60	0.07 ± 0.11	-0.89 ± 0.13^b	-0.944 ± 0.061^b	0.40 ± 0.22^a
	0.034 ± 0.039^a	-0.41 ± 0.48	-0.54 ± 0.32^b	0.24 ± 0.20^a
70	0.073 ± 0.093	-0.922 ± 0.056^b	-0.943 ± 0.032^b	0.21 ± 0.25
	0.07 ± 0.11^a	-0.14 ± 0.75	-0.40 ± 0.48	0.26 ± 0.17^a

Precipitated fractions were estimated using all available data; relative supersaturation indices were estimated in samples in which pH at equilibrium was up to 0.5 pH units different from pH in the beginning of solubility measurement, i.e. samples with $\text{pH} < 3.6$ were excluded

^a Statistically higher ($p < 0.05$) than zero, based on one-tailed t-test or Wilcoxon test

^b Statistically different ($p < 0.05$) from zero, based on two-tailed t-test or Wilcoxon test

Table III Fraction of KCZ Precipitated in Upper Small Intestine, $\overline{\pi}_{\text{lumen}} \pm \text{SD}$, and Relative Supersaturation Index, $\overline{\sigma}_{\text{lumen}} \pm \text{SD}$, at Specific Times After Administration of KCZ-L (Upper Rows) and KCZ-H (Lower Rows) and the Corresponding Precipitated Fractions and Relative Supersaturation Indices, After Incubation of the Supernatants for 48 h at 37°C ($\overline{\pi}_{\text{lumen}}^{\text{inc}} \pm \text{SD}$ and $\overline{\sigma}_{\text{lumen}}^{\text{inc}} \pm \text{SD}$, Respectively)

Time after KCZ administration (min)	$\overline{\pi}_{\text{lumen}} \pm \text{SD}$	$\overline{\sigma}_{\text{lumen}} \pm \text{SD}$	$\overline{\sigma}_{\text{lumen}}^{\text{inc}} \pm \text{SD}$	$\overline{\pi}_{\text{lumen}}^{\text{inc}} \pm \text{SD}$
5	0.10 ± 0.13 ^a	11.5 ± 6.0 ^b	0.50 ± 0.44 ^b	0.52 ± 0.41 ^a
	0.14 ± 0.26 ^a	8 ± 11 ^b	0.58 ± 0.80 ^b	0.40 ± 0.41 ^a
10	0.003 ± 0.034	7.3 ± 5.1 ^b	1.0 ± 1.1 ^b	0.48 ± 0.40 ^a
	0.16 ± 0.26 ^a	17 ± 13 ^b	0.27 ± 0.23 ^b	0.73 ± 0.36 ^a
20	0.050 ± 0.049 ^a	6.4 ± 3.6 ^b	1.29 ± 0.70 ^b	0.41 ± 0.33 ^a
	0.15 ± 0.24 ^a	9.4 ± 9.3 ^b	0.30 ± 0.64	0.73 ± 0.30 ^a
30	0.11 ± 0.15 ^a	2.5 ± 2.9 ^b	0.75 ± 0.74 ^b	0.24 ± 0.26 ^a
	0.16 ± 0.26 ^a	8.2 ± 7.2 ^b	0.8 ± 1.2	0.55 ± 0.37 ^a
40	0.039 ± 0.072	0.5 ± 1.6	0.0 ± 1.0	0.24 ± 0.19 ^a
	0.13 ± 0.14 ^a	10 ± 10 ^b	0.25 ± 0.76	0.48 ± 0.40 ^a
50	0.026 ± 0.041 ^a	−0.30 ± 0.97	−0.51 ± 0.71	0.19 ± 0.12 ^a
	0.090 ± 0.094 ^a	4.9 ± 5.9 ^b	0.64 ± 0.55 ^b	0.40 ± 0.32 ^a
60	0.073 ± 0.090 ^a	−0.53 ± 0.25 ^b	−0.63 ± 0.19 ^b	0.17 ± 0.11 ^a
	0.13 ± 0.13 ^a	3.0 ± 4.4	−0.20 ± 0.46	0.45 ± 0.40 ^a
70	0.045 ± 0.046 ^a	−0.23 ± 0.79 ^b	−0.52 ± 0.41	0.24 ± 0.21
	0.12 ± 0.11 ^a	1.6 ± 4.3	0.07 ± 0.92	0.22 ± 0.31 ^a

Precipitated fractions were estimated using all available data; relative supersaturation indices were estimated in samples in which pH at equilibrium was up to 0.5 pH units different from pH in the beginning of solubility measurement, i.e. samples with pH < 3.6 were excluded

^a Statistically higher ($p < 0.05$) than zero, based on one-tailed t-test or Wilcoxon test

^b Statistically different ($p < 0.05$) from zero, based on two-tailed t-test or Wilcoxon test

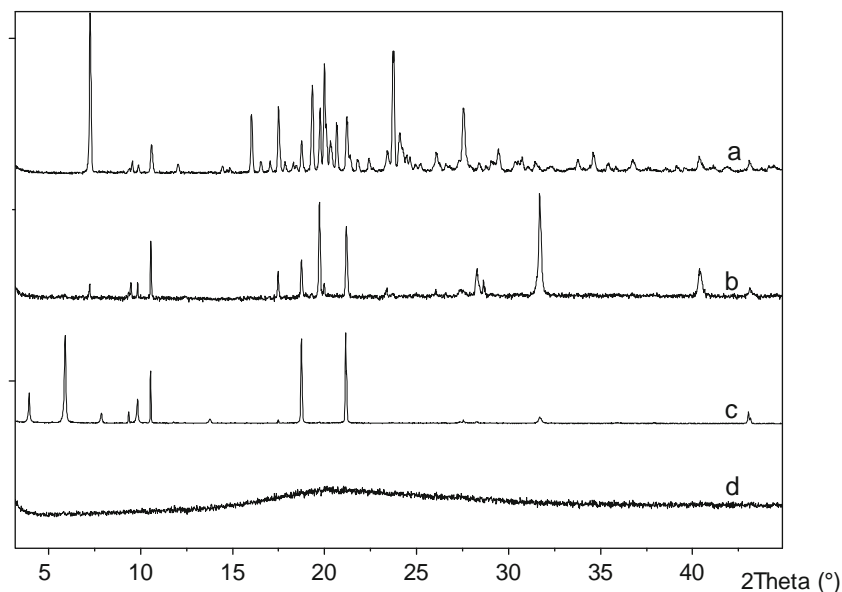


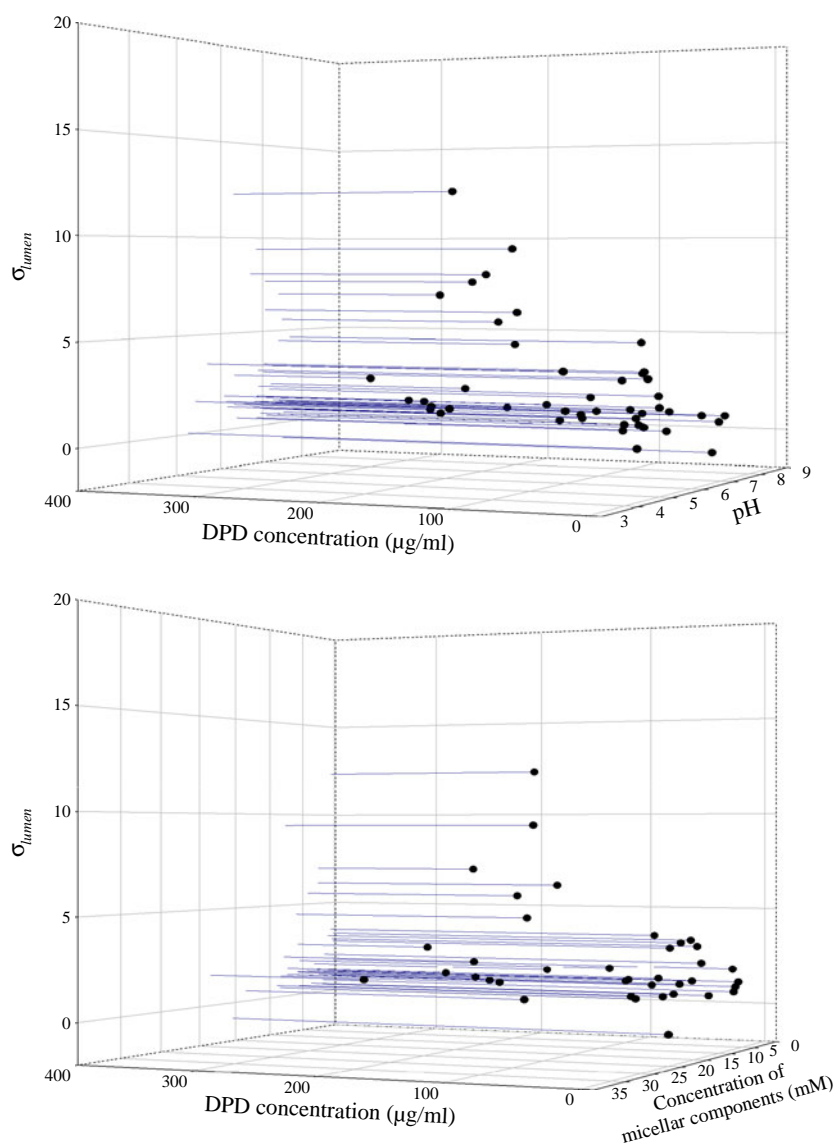
Fig. 3 Representative X-ray powder diffraction patterns of KCZ precipitates. (a) KCZ reference material (used for preparing the administered solutions and measuring equilibrium solubilities); (b) crystalline pattern that refers to the precipitate collected after the incubating (37°C, 48 h, 75 oscillations/min) the supernatant from the sample aspirated 20 min after KCZ-H administration to volunteer #4; (c) crystalline pattern that refers to precipitate collected after incubating (37°C, 48 h, 75 oscillations/min) the supernatant from the sample aspirated 5 min after KCZ-H administration to volunteer #1; (d) amorphous pattern that refers to the precipitate in the aspirated sample collected 30 min after KCZ-H administration to volunteer #10.

supernatants of luminal contents (DPD-L/40 min, DPD-L/60 min and DPD-H/60 min, Table II, last two columns). X-ray diffractograms could not be studied in detail, because the available amounts of precipitated DPD in the lumen were tiny. However, the presence of generally amorphous materials could be claimed (data not shown). Amounts in incubated samples were also tiny, because, despite the substantial precipitated fractions (Table II), they refer to very small total amount of DPD in the incubated vials.

For KCZ, significant luminal precipitation was observed at both dose levels. After KCZ-L, precipitated fraction in the upper small intestine was up to 11% (at 30 min post administration). After KCZ-H, precipitated fraction was up to 16% (at 10 and 30 min post administration). Luminal contents were supersaturated up to 30 min and up to 50 min post dosing of KCZ-L and KCZ-H, respectively. As with DPD, precipitation was observed even when luminal

contents were not saturated with the administered KCZ phase (KCZ-L/60 min and KCZ-L/70 min, Table III, first two columns). A similar observation was made for the KCZ-L/60 min sample after incubation (Table III, last two columns). X-ray diffractograms revealed that, after administration of KCZ solutions, three different solid states could exist in the small intestine: a crystalline state consistent with KCZ reference material (used for preparing the administered solutions) (Fig. 3a and b); a crystalline state that, based on the intense peak at low angle, it may be argued that is likely to be a hydrated form (Fig. 3c) (20); and an amorphous state (the most frequently observed state in the samples upon aspiration) (Fig. 3d). After incubation of supernatants for 48 h at 37°C, precipitated KCZ was always crystalline and had one of the two crystalline forms that had been observed also in the small intestine (Fig. 3b and c).

Fig. 4 The relative supersaturation index of DPD base, σ_{lumen} , as a function of luminal DPD concentration and pH [$n = 46$ aspirated samples] (*upper graph*) and as function of luminal DPD concentration and concentration of total micellar components [$n = 38$ aspirated samples] (*lower graph*). Aspirates with $\text{pH} < 3.6$ and aspirates in which precipitation was observed but the supernatants were not saturated with the base are not included.



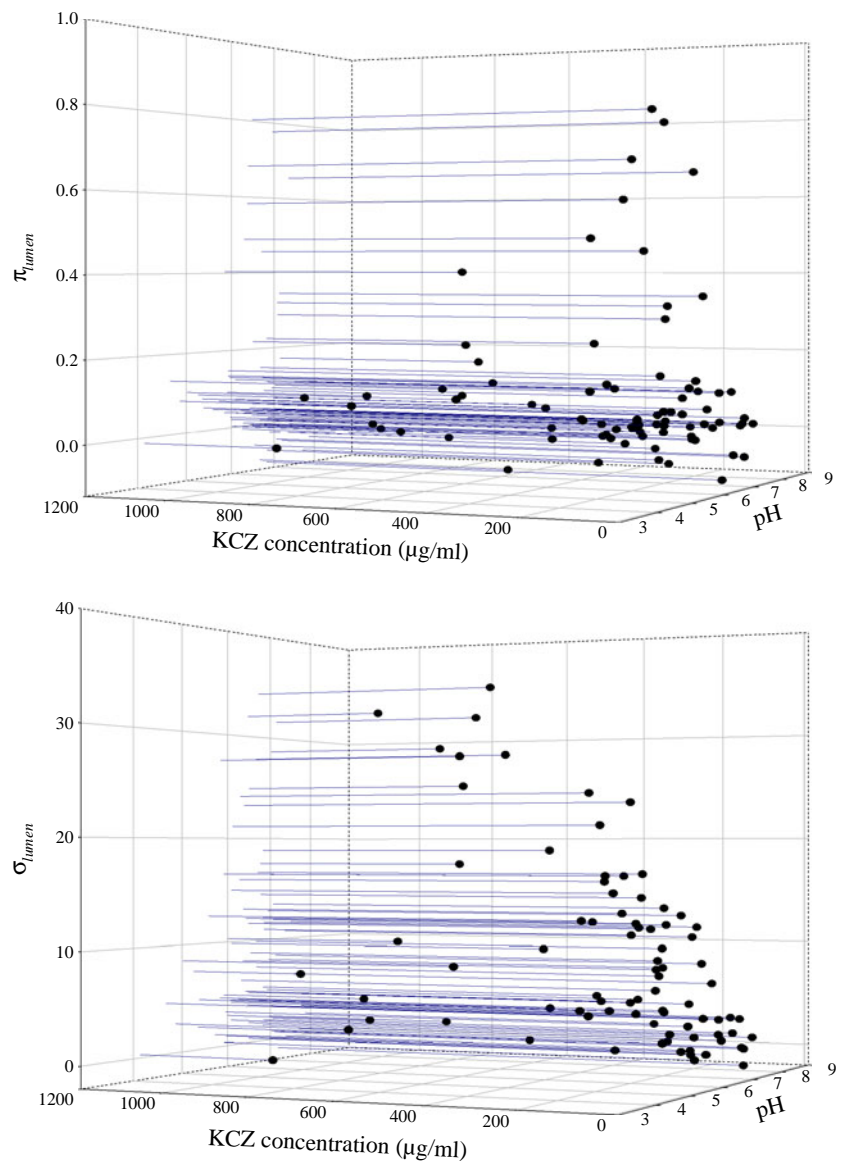
DISCUSSION

Based on this study, precipitation of two lipophilic, high permeability weak bases in the upper small intestine, after oral administration of pharmacologically relevant doses in the fasted state is, at most, limited. Using DPD solubility in pH 7.0 citrate-phosphate buffer, the dose-to-solubility ratios of DPD for the DPD-L and DPD-H doses are 9 l and 27 l, respectively. Using KCZ solubility in pH 7.0 citrate-phosphate buffer, the dose-to-solubility ratios of KCZ for the KCZ-L and KCZ-H doses are 55 l and 164 l, respectively. It may be argued, therefore, that if the dose of a highly permeable base is (almost) completely dissolved during gastric residence and the dose-to-solubility of the non-ionized form is up to about 50 l, one should expect minimal precipitation in the lumen. Significant precipitation should be expected when dose/solubility ratio

of the non-ionized form is about 160 l or higher. Such finding seems to be in agreement with recent work predicting fasted state intestinal precipitation of nelfinavir (pKa 6.0) intralumenally at a dose-to-solubility ratio of 417 l (at pH 7) (21). It is interesting, however, that precipitation can occur even at low intestinal pHs (seven aspirates with KCZ concentration 67.2–932.5 $\mu\text{g/ml}$ and $2.5 < \text{pH} < 4.4$ had $\pi_{\text{lumen}} > 0.1$). Also, in this study, in order to facilitate differentiation between precipitates and undissolved particles, gastric contents entering the small intestine were in dissolved state. After administration of a solid dosage form of a weak base, it is possible that few particles may escape dissolution during gastric residence and may act as nuclei for precipitation in the small intestine; i.e., precipitation in that case may be more pronounced.

Precipitation was observed in almost all DPD and KCZ aspirates after their incubation for 48 h at 37°C, indicating

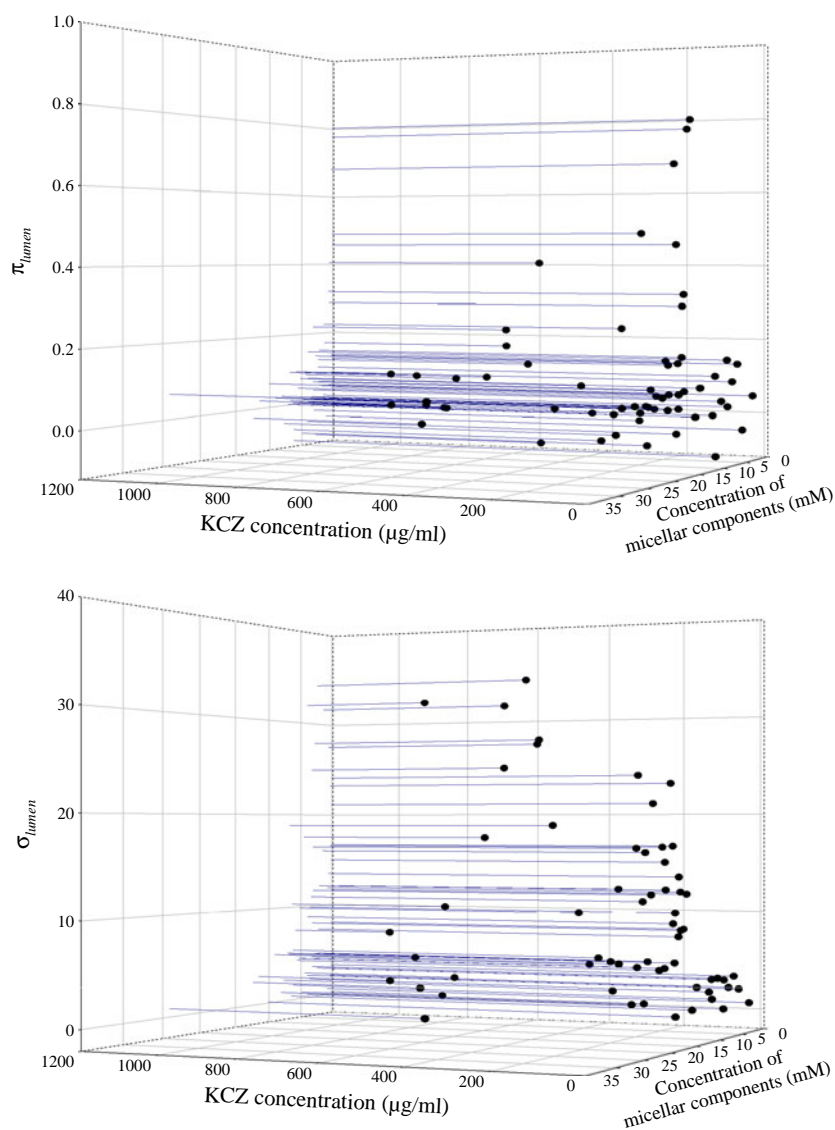
Fig. 5 The precipitated fraction of KCZ base, π_{lumen} , (upper graph) and the relative supersaturation index of KCZ base, σ_{lumen} , (lower graph) as a function of luminal KCZ concentration and pH. Both upper and lower graphs contain data from the same individual aspirates ($n = 83$). Aspirates with $\text{pH} < 3.6$ and aspirates in which precipitation was observed but the supernatants were not saturated with the base are not included.



that luminal contents are at least saturated with the administered phase and precipitation occurs at slow rates. However, precipitation was measured to be significant even in cases where luminal contents and/or incubated luminal contents were unsaturated in regard to the administered phase (Tables II and III). For example, the sample aspirated from subject #3 60 min after DPD-L administration had the following characteristics: $C_t=20.4 \mu\text{g/ml}$, $C=16.8 \mu\text{g/ml}$, $C_{inc}=11.7 \mu\text{g/ml}$ and $C_s=378.5 \mu\text{g/ml}$ (pH of the media in which these concentrations were measured was 4.5–4.8). Since equilibrium solubility of the most stable crystalline phase was measured, drug precipitation in luminal contents and/or incubated luminal contents that are unsaturated with the free base could happen if the precipitated phase is different from that used for measuring equilibrium solubility, i.e. different from the free base. This is possible if, for example, a hydrated form of the drug is

formed, as postulated for the KCZ-H/5 min precipitate after incubation of the aspirated sample of volunteer #1 (Fig. 3c). Hydrates are known to have lower solubilities than anhydrous forms (e.g. 22), and the crystalline state of most KCZ precipitates after their incubation at 37°C for 48 h was that shown in Fig. 3c and not that of the reference material (Fig. 3a) (data not shown). Precipitated phase can also be different from the free base when the pH of the aspirated sample is acidic, as precipitation of salt(s) of the base may occur. We attempted to confirm the presence of salt(s) in precipitates with highly acidic pHs using NMR, but the unavailability of reference salt(s) and the complexity of composition of the aspirated samples did not allow for clear-cut conclusions. Although there are no reports on solubility of KCZ salt(s), it has been shown that solubility of KCZ varies with the identity of the anion of buffer at pH 3. Specifically, solubility of KCZ in phosphate, citrate, D-

Fig. 6 The precipitated fraction of KCZ base, π_{lumen} , (upper graph) and the relative supersaturation index of KCZ base, σ_{lumen} , (lower graph) as a function of luminal KCZ concentration and concentration of total micellar components. Both upper and lower graphs contain data from the same individual aspirates ($n=62$). Aspirates with pH < 3.6 and aspirates in which precipitation was observed but the supernatants were not saturated with the base are not included.



tartrate, L-tartrate, and D, L-tartrate buffers with pH 3 varied from 2.07 mg/ml (in phosphate buffer) to 8.5 mg/ml (in L-tartrate buffer) at 23°C (23).

With the problems associated with the reliability of measurement of equilibrium solubility of the base at acidic pH values (due to change of pH at equilibrium and/or the formation of insoluble complexes) and the possibility of precipitation of a different phase even at pH values close to neutral, one should be cautious when considering precipitation *vs.* supersaturation. Supersaturation should refer to a specific phase, and equilibrium solubility must be measured while the characteristics of the medium remain unchanged.

Figures 4, 5 and 6 contain data from all available individual aspirates that do not have highly acidic pH (pH > 3.6), and, if precipitation has been observed, drug concentration in the aspirated sample is not lower than equilibrium solubility of the base. Due to lack of detailed information, it was decided to include data from aspirates in which the precipitate had different crystalline state than that of the solid material used for measuring equilibrium solubility. It must be acknowledged that equilibrium solubility in those samples may be slightly different than the measured equilibrium solubility of the base. If indeed the different crystalline state refers to hydrate form, it should have lower equilibrium solubility than the anhydrous form, and, therefore, values of supersaturated indices in Figs. 4, 5 and 6 may be slightly underestimated.

Due to minimal precipitation, the relationship of DPD precipitation with the pH and/or the concentration of micellar components cannot be investigated with certainty. However, at luminal DPD concentrations between 19.4 and 275.6 µg/ml, contents of upper intestinal lumen are supersaturated with the base when pH is greater than about 5 and the concentration of micellar components is less than about 10 mM (Fig. 4a and b, respectively). Based on Fig. 5, at luminal KCZ concentrations between 19.6 and 924.8 µg/ml, the base precipitates when pH in upper intestinal lumen is greater than about 5, whereas supersaturation with the base is also observed at the same pH range. Based on Fig. 6, at concentrations between 19.6 and 924.8 µg/ml, the base precipitates if the concentration of micellar components is less than about 5 mM, whereas intestinal contents are supersaturated with the base when concentration of micellar components is less than about 10 mM. Therefore, for concentration of micellar components between 5 and 10 mM, it seems that contents can be supersaturated without significant precipitation. In summary, at luminal concentrations observed after administration of pharmacologically relevant DPD or KCZ doses, the average luminal contents (pH > 5 and concentration of micellar components < 5 mM) are supersaturated with the base.

Finally, it is interesting to note that by using DPD and an *in vitro* transfer model, Kostewicz *et al.* (24) observed higher

precipitation in and supersaturation of fasted state simulated intestinal fluid than that measured in the contents of the upper small intestine of healthy adults (this study). Reasons include the higher concentrations of DPD employed in the donor (gastric) compartment of the *in vitro* setup and, perhaps, differences between the precipitated phase and the phase for which equilibrium solubility is measured. Using concentrations closer to those measured in this study and a different *in vitro* transfer model, Gu *et al.* (25) also overestimated the luminal precipitation of DPD observed in the present study, although to a lesser extent.

In conclusion, extensive intraluminal precipitation of lipophilic, high permeability, weak bases may be relatively rare in the human intestine, restricted to compounds with a significantly worse risk profile than DPD and KCZ. Intraluminal precipitation for explaining changes on plasma levels of BCS Class II weak bases in pharmacokinetic studies should be used with caution. Evaluation of precipitation in *vs.* supersaturation of luminal contents after administration of weak bases in the fasted state becomes complicated by the possibility of precipitation of various drug phases and also by the difficulty in measuring equilibrium solubility at intraluminal acidic pHs. These issues in addition to using biorelevant drug concentrations are important for the predictability of *in vitro* setups.

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