A Comparison of the Solubility of Danazol in Human and Simulated Gastrointestinal Fluids

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INTRODUCTION

In the fasted state, there is a varying amount of bile components in the upper intestinal fluids. Levels of 0.1–13.3 mM bile salts have been reported (1). The solubility of hydrocortisone in human intestinal fluids (HIFs) from fasting subjects has been reported to be slightly higher than the solubility in buffer and in simulated intestinal fluids (SIFs) representing fasted state (2). There was no correlation between the bile salt contents of the fluids and the solubility of hydrocortisone, which might be due to its relatively low lipophilicity. The purpose of the present study was to investigate the solubility in human gastric and intestinal fluids of danazol, which is more lipophilic than hydrocortisone. The log P of hydrocortisone and danazol are 1.6 and 4.5, respectively (3,4). Danazol is assumed to be a class II drug, however, no permeability data were found in the literature. In order to elucidate inter-subject variations the solubility of danazol was compared with characteristics of the human fluids: bile salt content, lecithin content, pH, surface tension, and osmolality. In addition, the solubilities of danazol in simulated gastric and intestinal fluids were determined in order to make comparisons with the solubilities in the human aspirates.

MATERIALS AND METHODS

Materials

Danazol was donated by Sanofi Winthrop Ltd (Newcastle Upon Tyne, UK). Sodium glycocholate 97% pure (GC) and sodium taurocholate 97% pure (TC) were purchased from Sigma (St. Louis, USA). L-α-egg lecithin (L) as Lipoid EPC 98% pure with an average MW of 780.2 g/mol was donated by Lipoid (Ludwigshafen, Germany). Sodium lauryl sulfate (SLS) 96.6% pure was purchased from Unikem (Copenhagen, Denmark) and chloramphenicol Ph.Eur. grade was purchased from Nordisk Droge/Kemikalie (Copenhagen, Denmark). All other chemicals were of analytical grade and all materials were used as received.

Dissolution Media

Ten HIFs and five human gastric fluids (HGFs) were used. The fluids originated from 13 healthy male and female subjects who had fasted for 10 hours. For two subjects both the intestinal and gastric fluid were included in the study. The method for aspiration has been described in detail by Lindahl *et al.* (1) and the study was approved by the Ethics Committee of the Medical Faculty, University of Uppsala, Sweden. The intestinal fluids were collected in the proximal jejunum approximately 60 cm distally of the pylorus. The gastric fluids were collected from the antrum region of the ventricle. After collection the GI fluids were stored at −80°C for 3–4 years before the solubility test and the characterization of the fluids. No significant changes were found in the pH after 3 years of storage at −80°C. To prevent microbial growth during the dissolution studies, 6 mM NaN_3 and 0.01 mM chloramphenicol were added.

The simulated fluids represent the gastric and the upper intestinal fluids in fasted state and are presented in Table 1.

Solubility Studies

The solubility studies were performed at 37°C by tumbling 3 mg danazol and 5 ml dissolution medium in 15 ml glass flasks with teflon screw stoppers. Samples were taken after at least 17 hours ($n = 3$). The samples from the simulated fluids and the reference media were centrifuged for 10 min. at 5,000 rpm (Labofuge A, Heraeus Sepatech). The supernatant was filtered (Minisart RC4 0.45 μm, Sartorius) and the sample was added equal volume of acetonitrile and transferred to autosampler glass vials.

The samples from the human fluids were centrifuged for 20 min. at 5,000 rpm (Labofuge A, Heraeus Sepatech) and equal volume of acetonitrile was added to the supernatant. This mixture was centrifugated for 5 min. at 5,000 rpm (Labofuge A, Heraeus Sepatech) and the supernatant was transferred to autosampler glass vials.

Surface Tension

The surface tensions of the human gastric and intestinal fluids were measured at 37°C by the Wilhelmy plate method (Krüss K10T; $n = 3$). The CMC in the simulated media was determined by surface tension measurements. To investigate if saturation with danazol would change the CMC in the simulated fluids, surface tension was measured in solutions containing GC and GC + lecithin both in the absence of and saturated with danazol ($n \geq 3$). Furthermore, surface tension was measured in solutions containing varying amounts of SLS in the absence of danazol ($n = 4$).

Analytical Methods

Danazol was quantified by a modified HPLC method (5). As analytical column, a 4.6 × 250 mm Spherisorb®S5 ODS2

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ABBREVIATIONS: CMC, critical micellar concentration; GC, sodium glycocholate; GC/L, sodium glycocholate and lecithin; HGF, human gastric fluids; HIF, human intestinal fluid; SGF, simulated gastric fluids; SIF, simulated intestinal fluid; SLS, sodium lauryl sulfate; TC, sodium taurocholate; TC/L, sodium taurocholate and lecithin.

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Table 1. The Concentrations of the Surfactants in the Simulated Fluids: Intestinal and Gastric

Surfactants	Concentration (mM)	Abbrev.
Simulated intestinal fluids (SIFs)[a]		
Sodium glycocholate	1.00	GC1
	4.00	GC4
Sodium glycocholate + Lecithin (4:1)[b]	1.00/0.25	GC/L1
	4.00/1.00	GC/L4
Sodium taurocholate	1.00	TC1
	4.00	TC4
Sodium taurocholate + Lecithin (4:1)[b]	1.00/0.25	TC/L1
	4.00/1.00	TC/L4
Reference medium	n.r.[c]	Buffer
Simulated gastric fluids (SGFs)[d]		
Sodium lauryl sulfate	3.47	SGF(l)
	8.67	SGF(m)
	13.87	SGF(h)
Reference medium	n.r.[c]	SGF

[a] SIFs: pH = 6.5 ± 0.1 (50 mM phosphate buffer), 3 mM NaN_3, NaCl added to a total Na^+ concentration of 150 mM.
[b] The ratio of bile salt to lecithin in human bile is between 2:1 and 5:1 (6).
[c] n.r.: not relevant.
[d] SGFs: pH = 1.2 ± 0.1, 34.2 mM NaCl. The SLS concentration of 8.7 mM is according to FIP guidelines from 1997.

(Waters, USA) was used with a pre-column Syncropak RSC C18, 5 μm, 40 × 4 mm (Microlab, Denmark). The mobile phase consisted of demineralized water, methanol and acetonitrile (25:35:40% v/v). The flow rate of 1 ml/min resulted in a retention time of 10 min. The peak area were recorded at 286 nm (HP1050 UV Detector) and converted to concentrations by comparison with external standards.

The concentration of bile salts in the human fluids was determined by colorimetric determination of total 3α-hydroxy bile acids (Enzabile®, Nycomed). Due to the high bile salt level in the human fluids, the samples were diluted with serum to a concentration within the calibration curve for the kit. The bile salt concentration in the human GI fluids was the difference between the diluted sample and the serum, taking the dilution factor into account. The concentration of phosphatidylcholine (lecithin) was determined by an enzymatic colorimetric determination of choline using the kit MPR 2 (Boehringer Mannheim) and was performed by Medi-Lab (Copenhagen). Both lecithin and lysolecithin are included in this analysis. The pH (MeterLab PHM 220, Radiometer) was determined as well as the osmotic pressure (Osmomat 030-D, Gonotec).

Data Analysis

The solubilities in the human and simulated fluids were tested against the solubility in the reference solution by performing a t-test assuming equal variance and $p < 0.05$ was considered significant. A linear regression was performed on the solubilities in the SGFs versus the SLS content (Corel Quatro Pro® 7). To investigate if there was a correlation between the solubility in the HIFs and the bile salt content, pH, surface tension and osmolality a Spearman rank correlation was performed (Statgraphics 7.0).

RESULTS

The solubility of danazol was only correlated with the bile salt content in the HIFs giving a Spearman rank correlation coefficient of 0.83 ($p = 0.013$) (Fig. 1). The mean solubilities in the human fluids, the bile salt content, pH, surface tension and osmolality are shown in Table 2. The content of lecithin was below the limit of quantification of 0.2 mM for all human fluids, except for the intestinal fluid from subject 9, which had a lecithin content of 0.3 mM.

Interestingly, the pH and osmolality of the HIFs gave a Spearman correlation coefficient of 0.90 ($p = 0.007$).

The solubilities of danazol in the HIFs and SIFs fluids are compared in Fig. 2a. All solubilities in the SIFs and HIFs were statistically different from the solubility in buffer except for the solubilities in the GC1 and TC1 fluids.

The solubilities of danazol in the HGFs and SGFs are compared in Fig. 2b. There was a linear relationship between the danazol solubility and the concentration of SLS in the SGFs ($r^2 = 0.998$).

The CMC was 0.86 mM GC in the GC solutions without danazol, and when the GC solutions were saturated with danazol, a lower CMC of 0.52 mM GC was obtained. For the GC/L solutions, CMC was 0.016 mM GC, and when the GC/L solutions were saturated with danazol, a slightly lower CMC of 0.011 mM was obtained. The surface tension measurements in solutions containing SLS gave a CMC of 0.41 mM SLS.

DISCUSSION

Human and Simulated Intestinal Fluids (HIFs and SIFs)

At increasing bile salt concentration in the HIFs an increasing danazol solubility was observed. The presence of bile salts in the intestinal fluids suggests that other bile components such as lecithin and cholesterol are present as well. The concentration

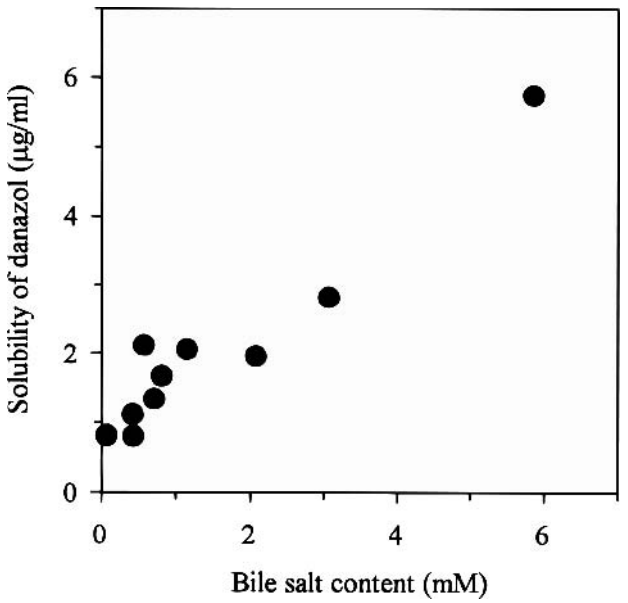

Fig. 1. Comparison of the solubility of danazol in ten human intestinal fluids (HIFs) with the bile salt content. The Spearman rank correlation coefficient was 0.83 ($p = 0.013$).

Table 2. The Solubility of Danazol, the Bile Salt Content, pH, Surface Tension, and Osmolality in the Human Intestinal and Gastric Fluids from Fasted Subjects

Fluids	Solubility of danazol (μg/ml)	Bile salt (mM)	pH	Surface tension (mN/m)	Osmolality (mOsm/kg)
Intestinal fluids	2.04 (±1.45)	1.52 (±1.77)	6.7 (±0.9)	33.7 (±2.8)	278 (±16)
Gastric fluids	1.61 (±0.05)	0.82 (±0.57)	2.8 (±1.1)	33.6 (±5.9)	221 (±15)

Note: Mean ± s.d.

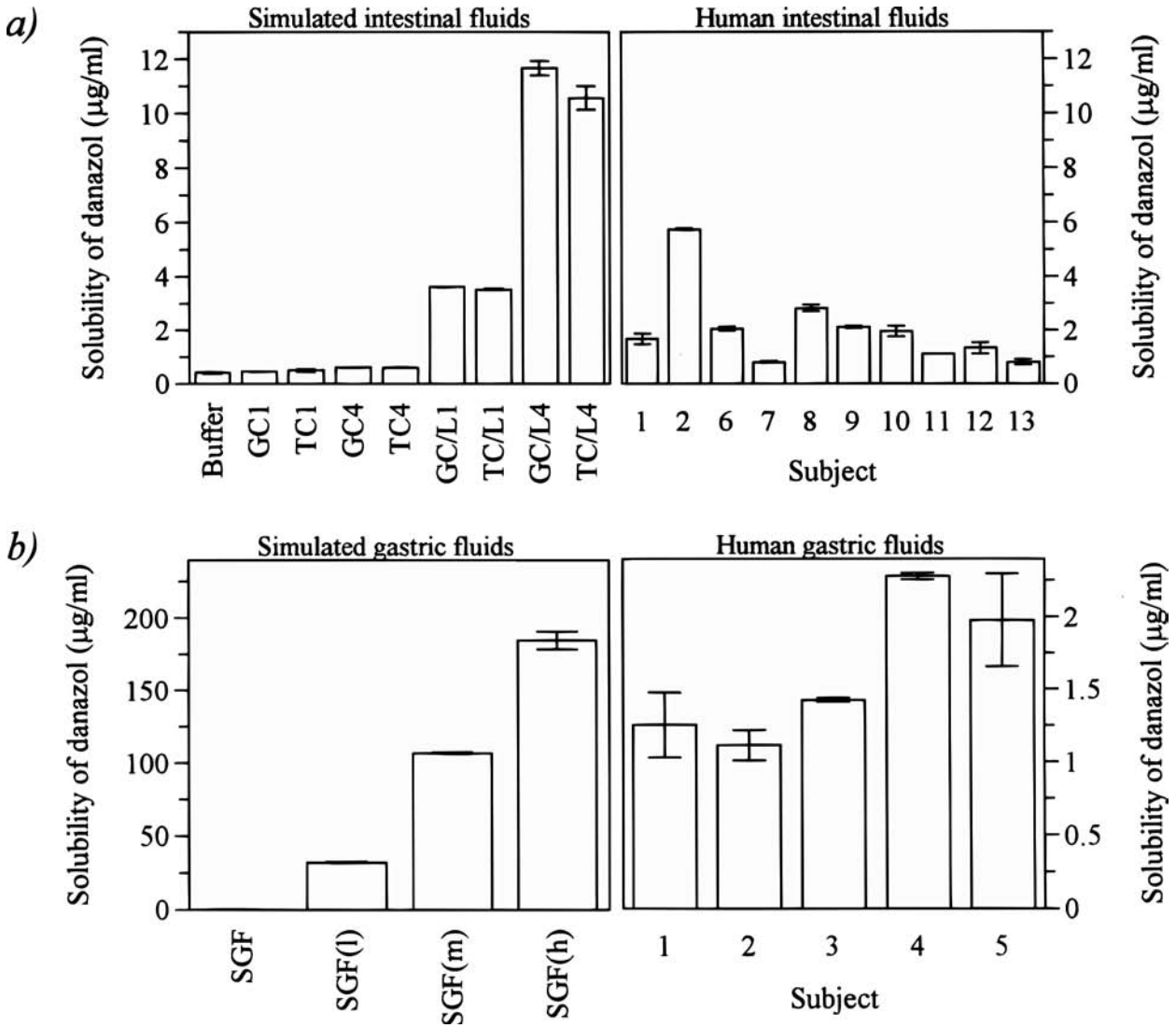

Fig. 2. Comparison of the solubility of danazol in human and simulated GI fluids (n = 3, mean ± s.d.). *a*) Comparison of the solubility of danazol in simulated and human intestinal fluids. Simulated intestinal fluids (SIFs): GC1 and GC4 are 1 mM and 4 mM sodium glycocholate, respectively; TC1 and TC4 are 1 mM and 4 mM sodium taurocholate, respectively; GC/L1 is 1 mM sodium glycocholate and 0.25 mM lecithin; GC/L4 is 4 mM sodium glycocholate and 1 mM lecithin; TC/L1 is 1 mM sodium taurocholate and 0.25 mM lecithin; TC/L4 is 4 mM sodium taurocholate and 1 mM lecithin. *b*) Comparison of the solubility of danazol in simulated and human gastric fluids. Simulated gastric fluids (SGFs): SGF is USP's simulated gastric fluid without pepsin; SGF(1), SGF(m), and SGF(h) are SGF with 3.47, 8.67, and 13.87 mM SLS, respectively.

of lecithin was, however, lower than expected in most samples. The ratio of bile salt to lecithin molecules has been reported to be about 2:1 to 5:1 (6) and it was 1.9:1 in the fluid from subject 9 but above 30:1 in the fluid from subject 2 having the highest bile salt concentration. The characterization of the HIFs regarding bile salt content, pH and osmolality is in accordance with previously published data on similar fluids (1,2).

The mean solubility of danazol in the HIFs was comparable with the solubilities in the GC/L1 and TC/L1 fluids and these SIFs are, therefore, relevant dissolution media for evaluating the intestinal solubility of danazol at fasted state conditions. The solubility of danazol was dramatically increased when lecithin was added to the SIFs together with the bile salt (see Fig. 2a). This might be due to the increasing number of micelles in these fluids as a result of the lower CMC. It might also be due to a greater solubilization capacity of the micelles, since the GC/L mixed micelles have an approximately 2.5 times greater hydrodynamic radius than the GC micelles (2). The solubility of danazol measured by Naylor *et al*. (5) in a similar fluids (TC/L4) was higher (29.30 $\pm$ 6.59 μg/ml) than the solubility measured in this study (10.55 $\pm$ 0.43 μg/ml). One explanation might be that different qualities of lecithin were employed in the two studies.

Human and Simulated Gastric Fluids (HGFs and SGFs)

The solubility in the HGFs was 3.3 times higher than the solubility in the SGF without SLS suggesting that the human fluids contain agent(s) that are able to solubilize danazol. This increase might be due to micelles or mixed micelles of as yet unknown origin, for example lysolecithin (CMC 0.02 mM (7)) and/or free fatty acids refluxed from the small intestine. At gastric pH the phospholipids are known to be hydrolyzed to lysophospholipids and free fatty acids (8).

Due to reflux of intestinal content into the stomach there was a bile salt content of 0.82 $\pm$ 0.57 mM in the HGFs. The bile salt content and osmolality in the HGFs were in accordance with the levels found by others (1,9). The pH of 1.9–4.7 in the HGFs was similar to previously reported data in gastric juice from healthy subjects (1,10) but it was slightly higher than values of 1–2 in gastric juice from eight patients (9). Even though there is a variation of the pH in gastric juice at fasted state, it is generally accepted to be about 2 (10). The surface tension in three gastric fluids (28, 29, and 32 mN/m) were below the values published by others in the range of 35–51 mN/m in gastric juice from healthy subjects and patients (9,11). This lower surface tension might be caused by a greater contamination of duodenal juice. Finholt *et al.* have shown that the surface tension in gastric juice was dramatically lowered by admixture of up to 20% duodenal juice (11).

The mean solubility of danazol in the HGFs was much lower than the solubilities measured in the SGFs containing SLS. The recommended amount of 8.7 mM SLS (12,13), gives an overestimation of the solubility. It is 66 times higher than the solubility in the HGFs. The aim of adding SLS to dissolution media is to lower the surface tension in an attempt to mimic in-vivo conditions (12) and this gives a better wetting of pharmaceutical powders than the wetting obtained with dissolution media without surfactants. The surface tensions in the SLS solutions above CMC were between 31.4–34.2 mN/m which is in the interval of the surface tensions measured in the HGFs. The linear regression of the solubility of danazol versus concentration of SLS showed that a concentration of 1.4 mM SLS would give a relevant solubilization effect and maximum wetting effects since it is above the CMC. The CMC of SLS is, however, dependent on the purity of the SLS lot employed as well as the ionic strength of the solution (14).

In closing, further studies need to be made with model drugs of differing lipophilicity to clarify what characteristics of the gastrointestinal fluids determine the solubility profile. In the attempt to mimic the gastrointestinal fluids for dissolution studies, it is important to incorporate the relevant factors in choice of dissolution medium. The present study has shown that bile components, here represented as bile salts and lecithin, are of great importance in simulating intestinal fluids. The present study was done with a non-ionizable compound, but when dealing with an ionazible compound it is crucial to further include a possible pH effect in the design of the dissolution medium.

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