Pharmaceutical Research, Vol. 22, No. 12, December 2005 (© 2005)
DOI: 10.1007/s11095-005-8192-x

Research Paper

The Effects of Food on the Dissolution of Poorly Soluble Drugs in Human and in Model Small Intestinal Fluids

Eva M. Persson,[1] Ann-Sofie Gustafsson,[2] Anders S. Carlsson,[2] Ralf G. Nilsson[3], Lars Knutson,[4] Patrik Forsell[4], Gunilla Hanisch,[2] Hans Lennernäs,[1] and Bertil Abrahamsson[2,5]

Received June 9, 2005; accepted August 25, 2005

Purpose. This study was conducted to determine the effect of food on drug solubility and dissolution rate in simulated and real human intestinal fluids (HIF).
Methods. Dissolution rate obtained via the rotating disk method and saturation solubility studies were carried out in fed and fasted state HIF, fed dog (DIF), and simulated (FeSSIF) intestinal fluid for six aprotic low solubility drugs. The intestinal fluids were characterized with respect to physical–chemical characteristics and contents.
Results. Fed HIF provided a 3.5- to 30-times higher solubility compared to fasted HIF and FeSSIF, whereas fed DIF corresponded well (difference of less than 30%) to fed HIF. The increased solubility of food could mainly be attributed to dietary lipids and bile acids. The dissolution rate was also 2 to 7 times higher in fed HIF than fasted HIF. This was well predicted by both DIF and FeSSIF (difference of less than 30%).
Conclusions. Intestinal solubility is higher in fed state compared to fasted state. However, the dissolution rate does not increase to the same extent. Dog seems to be a good model for man with respect to dissolution in the small intestine after intake of a meal, whereas FeSSIF is a poorer means of determining intestinal saturation solubility in the fed state.

KEY WORDS: bile acids; dissolution rate; food effects; poorly soluble drugs; solubility.

INTRODUCTION

Several clinically useful drugs are highly hydrophobic and poorly soluble in water. Dissolution is a prerequisite for oral absorption and having a low solubility could, therefore, significantly limit the systemic bioavailability of a drug. It is thus of great importance in drug development to understand the *in vivo* dissolution process. For example, such knowledge could serve as a basis for the design of physiologically relevant *in vitro* dissolution tests or computer simulations of oral drug absorption to be used in selection of new chemical entities and in the development of pharmaceutical formulations.

Substantial increases in bioavailability have been observed after the coadministration of drugs such as halofantrine (1), griseofulvin (2,3), and danazol (4–6) with lipid-based formulations or food, which has been attributed to improved drug dissolution. Lipids in the food stimulate the release of bile acids and phospholipids, resulting in the formation of different colloidal phases that could solubilize lipophilic drugs. The character of these phases is controlled by the relative concentrations of bile salts, biliary lipids, and lipid digestion products and is, therefore, continually changing (7,8) (Persson *et al.*, unpublished data). The solubility of poorly soluble drugs has been examined in simulated intestinal fluids and found to increase with increasing bile salt concentration, in amounts corresponding to the levels found during fed conditions (9). Addition of phosphatidylcholine, which is excreted in the bile, to bile acid solutions creates mixed micelles and further improves the solubility

[1] Department of Pharmacy, Uppsala University, Box 580, S-751 23 Uppsala, Sweden.
[2] Department of Preformulation & Biopharmaceutics, AstraZeneca R&D, S-431 83 Mölndal, Sweden.
[3] Department of DMPK & Bioanalysis, AstraZeneca R&D, S-431 83 Mölndal, Sweden.
[4] Department of Surgery, University Hospital, S-751 85 Uppsala, Sweden.
[5] To whom correspondence should be addressed. (e-mail: bertil.abrahamsson@astrazeneca.com)

ABBREVIATIONS: BCS, biopharmaceutical classification system; *C*s, saturation solubility; CV, coefficient of variation; DIF, dog intestinal fluid; D_n, dissolution number; D_o, dose number; ELS, evaporative light scattering; FeSSIF, fed simulated small intestinal fluid; H_{acc}, number of hydrogen acceptors; H_{don}, number of hydrogen donors; HIF, human intestinal fluid; ΔH_m, change in melting entrophy; HPLC, high-performance liquid chromatography; λ, Wavelength; log*P*, octanol/water patition coefficient; LOQ, limit of quantification; M_0, the dose drug administered; M_w, molecular weight; m/z, mass to charge; PLS, partial least squares; S_{aq}, aqueuos solubility; t_{diss}, the time required for one particle of the drug to dissolve; T_m, melting point; t_{res}, the mean residence time; UV, ultraviolet; V_0, the initial gastric volume.

(10,11). Monoglycerides and free fatty acids from the diet can also be incorporated into the bile salt micelles and form mixed micelles with a larger radius. *In vitro* lipolysis models have been developed in recent years to study the solubility of poorly soluble drugs during the degradation of dietary lipids (12–15). These models have demonstrated the importance of dietary lipids in the solubilization of lipophilic drugs. In a study by Kossena *et al.* (16), it was shown that the solubilities of griseofulvin and danazol were greatly increased when fatty acids and monoglycerides were added to a bile acid/phosphatidylcholine solution. Zangenberg *et al.* (12) performed a similar study with probucol and danazol, where they added triglycerides to a bile salt solution. The solubility of both substances increased when the triglycerides were degraded, producing monoglycerides and free fatty acids. Further studies conducted using *in vitro* lipolysis models were performed by Kaukonen *et al.* (13) and Christensen *et al.* (17). These studies showed that the length of the fatty acid chain on the triglycerides has no effect on solubilization capacity. The secretion induced in the fed state and the components of a meal could not only affect the saturation solubility in the intestine, but also influence the dissolution rate. For example, bile acids could increase the dissolution rate of different drugs by increasing the solubility and/or through the wetting effect (18). However, dissolution rate is not necessarily increased to the same extent as solubility by increasing the concentration of intestinal solubilizers (19). Indeed, examples exist where the drug dissolution rate was decreased in the presence of mixed micelles in spite of an improved solubility (20).

The present knowledge on intestinal drug solubility and dissolution under the fed condition has been obtained by using artificial fluids in an attempt to mimic the *in vivo* conditions. The most frequently used medium is the fed simulated small intestinal fluid (FeSSIF) developed by Galia *et al.* (21). Dog intestinal fluid was also studied as an alternative to human intestinal fluid (HIF) (22). However, it was shown that the *in vitro* dissolution rate of poorly soluble drugs in simulated media in the fasted state do not always correlate with the dissolution rate in aspirated intestinal fluids (22,23). The correspondence of drug solubility/dissolution between human and simulated or dog intestinal fluids in the fed state has not been investigated as yet, and well-controlled dissolution rate studies are limited to very simple systems not containing any lipids of nutritional origin. Thus there is a need to perform such studies in real intestinal fluids to validate previous findings based on artificial or animal fluids and, thereby, to further improve the understanding of *in vivo* drug dissolution and absorption under fed conditions.

Human intestinal fluid can be collected via the previously developed Loc-I-Gut method (24,25), where a tube through which the intestinal fluid is collected is positioned in the jejunum. This technique has been utilized for sampling of intestinal fluid for characterization (10,23,26), and in dissolution (10,22,23) studies in the fasted state. However, this marks the first time that this technique was used to sample intestinal fluid under fed state conditions for use in solubility and dissolution studies.

The aim of the present study was to study food-induced effects on drug solubility and dissolution in human intestinal fluid. Another aim was to investigate the relevance of FeSSIF, and of dog intestinal fluid as a model for the situation in man.

MATERIALS AND METHODS

Intestinal Fluids

The sampling of the intestinal fluid was performed at the Clinical Research Department of the University Hospital in Uppsala, Sweden, and was approved by the Ethics Committee of the Medical Faculty at Uppsala University. Fed and fasted human intestinal fluid (HIF) was collected from 6 and 12 healthy volunteers, respectively, aged 24–40 years and weighing 66–86 kg (males) and 50–70 kg (females). All had given informed consent to their participation in this study. The subjects had fasted overnight before a perfusion tube (Loc-I-Gut) was positioned in the proximal part of the jejunum by oral intubation (24,25). Location of the administration and sampling holes was established fluoroscopically. The perfusion tube was a 175-cm-long (external diameter: 5.3 mm) multichannel polyvinyl tube with two inflatable balloons, and a tungsten weight at the tip. Only the lower balloon was inflated. This prevented the fluid from continuing down the gastrointestinal tract, which guaranteed complete sampling of the intestinal contents. Another tube was positioned in the stomach to drain gastric juice during the experiment to prevent nausea. The subjects were given a well-defined nutritional drink used for parenteral administration to patients with mild to moderate catabolism (NuTRIflex®; Braun, Berlin, Germany) (nitrogen 0.8 g, amino acids 5.8 g, glucose 11.5 g, lipids 7.2 g, energy 576 kJ) to simulate fed conditions; this drink was administered directly to the small intestine through the Loc-I-Gut tube. The nutritional drink contained partly metabolized triglycerides and proteins similar to the degradation in the stomach prior to emptying into the small intestine. The amount of fat administered in the nutritional drink was low, corresponding to about 1/4 of the amount given in a standard FDA breakfast meal. The nutritional liquid was continuously perfused through the intestinal segment for 90 min at a flow rate of 2 mL min^{-1} to simulate the gastric emptying rate. The fed intestinal fluid obtained during bile excretion (indicated by increased bile acid concentrations; Persson *et al.*, unpublished data), 20–60 min after the start of the perfusion (Persson *et al.*, unpublished data), was collected on ice, pooled, and stored at −70°C prior to the dissolution and solubility experiments. Fasted state fluid was collected using the procedure described previously (26). In brief, after an overnight fasting, the Loc-I-Gut tube was introduced through the subject's mouth and positioned in the jejunum. The intestinal fluid was collected by vacuum drainage without administration of fluid. Dog intestinal fluid (DIF) was collected from three male Labradors, aged 3.5–6.5 years, fistulated at mid jejunum. The dogs were administered 200 mL of the same nutritional liquid as given to the human subjects (NuTRIflex®) through an orogastric tube. The DIF was collected through the fistulae and centrifuged for 10 min at 4°C and 3000 rpm, to remove large particles. The supernatants were pooled and stored at −70°C prior to performing the experiments. This study was approved by the Animal Ethics Committee in Gothenburg.

The enzyme inhibitor orlistat (Apin Chemicals, UK) was added (1 mg mL^{-1}) prior to the *ex vivo* studies to inhibit the enzymatic degradation of lipids in fed state HIF and DIF (27).

Fed simulated small intestinal fluid (FeSSIF) was prepared according to the method described by Galia *et al.* (21).

Characterization of DIF and HIF

DIF and HIF were characterized in terms of pH, buffer capacity, surface tension, protein concentration, and lipid content. The buffer capacity, surface tension, and pH measurements were performed at 37°C and all other measurements at room temperature. The surface tension was determined with the Wilhelmy plate method (Sigma 70; KSV Instruments Ltd., Turku, Finland). A bicinchoninic acid protein assay reagent kit (Pierce, Rockford, IL, USA) was used to determine the total protein concentration. Buffer capacity was determined by titration. Solid phase extraction and high-performance liquid chromatography (HPLC) with evaporative light scattering (ELS) detection were used to determine the lipid content in the intestinal fluids (Persson *et al.*, unpublished data). In brief, the intestinal fluid was purified and divided into three lipid classes: bile acids, phospholipids, and neutral lipids (triglycerides, diglycerides, monoglycerides, cholesterol, and cholesterolester) including free fatty acids using prepacked 500 mg C18 columns and 300 mg Si columns (Isolute International Sorbent Technology, Hengoed, UK). The bile acids were separated on a Zorbax C18 Extend column (150 × 4.6 mm, 3.5 μm; Agilent Technologies, Wilmington, DE, USA). A binary gradient was used to elute the bile acids. The solvents were as follows: (A) methanol/buffer (ammonium acetate 15 mM, 0.2% triethylamine, 0.5% formic acid, pH 3.15) 60:40 (v/v); (B) methanol/buffer 95:5 (v/v). Phospholipids were separated on a YMC-Pack Diol column (250 × 2.1 mm, 5 μm) (YMC Inc., Wilmington, DE, USA). A binary gradient was used to elute the phospholipids. The two solvents were as follows: (A) hexane/2-propanol/acetic acid/triethylamine 82:18:0.5:0.014 (v/v/v/v) and (B) 2-propanol/H_2O/acetic acid/triethylamine 85:15:0.5:0.014 (v/v/v/v). Neutral lipids and free fatty acids were separated by using an Apex II Diol column (150 × 4.6 mm, 5 μm) (Jones chromatography, Denver, CO, USA). A binary gradient was used to elute the neutral lipids. The two solvents were as follows: (A) hexane/acetic acid 99:1 (v/v) and (B) isohexane/2-propanol/acetic acid 84:15:1 (v/v/v).

Model Substances

Six model drugs were chosen with low solubility and moderate to high lipophilicity (Table I). The substances used were felodipine (AstraZeneca R&D, Mölndal, Sweden), griseofulvin (97% pure; Acros Organics, Morris Plains, NJ, USA), danazol and cyclosporine (98 and 95% pure, respectively; Sigma-Aldrich, Schnelldorf, Germany), probucol (ICN Biomedicals, Eschwege, Germany), and ubiquinone (99.5% pure; ABCR GmbH, Karlsruhe, Germany) (Fig. 1). These are all low solubility drugs according to the biopharmaceutical classification system (BCS) (32). The chosen drugs are all aprotic in order to exclude pH effects on intestinal drug dissolution.

The substances were characterized in terms of their water solubility, melting point (T_m) and melting enthalpy (ΔH_m), and hydrogen acceptors (H_{acc}) and donors (H_{don}) (results are displayed in Table I). A Monte Carlo simulation (Biochemical and Organic Simulation System (33)) was used to obtain the number of hydrogen acceptors and donors in the various molecules. The melting point and enthalpy were determined with differential scanning calorimetry (DSC Q1000; TA Instruments, New Castle, DE, USA). The log *P* value and water solubility were obtained from the literature when available. The log *P* value for ubiquinone was determined in this study by calculation (ACD Labs Version 8.0) and water solubility for both probucol and ubiquinone was experimentally determined according to the procedure described in this study.

Solubility

The solubility of griseofulvin, felodipine, danazol, and cyclosporine was studied in FeSSIF, in fasted and fed HIF and in fed DIF in triplicates at 37°C. The solubility of probucol and ubiquinone was only determined in fasted and fed HIF. An excess (1 mg mL^{-1}) of drug substance was added to vials containing 1.2 mL of the different media and 250-μL samples were withdrawn after 1, 5, and 24 h. The samples were centrifuged (Rotina 46R; Hettich labinstrument AB, Sollentuna, Sweden) at 10,000×*g* for 15 min at 37°C and 100 μL of the supernatant was withdrawn for subsequent analysis of the drug content. The recovery of the substances after adding known amounts below saturation solubility to the

Table I. Physicochemical Parameters of the Model Substances including Molecular Weight (M_w), Water Solubility (S_{aq}), Lipophilicity (log *P*), Melting Point (T_m), Change in Melting Enthalpy (ΔH_m), and the Number of Hydrogen Acceptors (H_{acc}) and Donors (H_{don})

Substance	Danazol	Felodipine	Cyclosporine	Griseofulvin	Probucol	Ubiquinone
M_w (g mol^{-1})	337	384	1202	352	516	863
S_{aq} at 37°C (μg mL^{-1})	1[a]	1[b]	7[a]	14[c]	0.006	0.0007
log *P*	5[a]	5[b]	3[a]	2[a]	10[d]	21
T_m (°C)	225	144	150	220	126	48
ΔH_m (kJ mol^{-1})	42	31	34	52	36	119
H_{acc}	5	4	[e]	6	2	[e]
H_{don}	1	1	[e]	0	0	[e]

[a] Mithani *et al.* (28).
[b] Scholz *et al.* (29).
[c] Gramatte, 1994 (30).
[d] Calculated value (31).
[e] Missing.

intestinal fluids was between 95 and 100%. Thus, the solubility results were not affected by binding to laboratory equipment or by degradation in the fluids.

Experiments were performed with griseofulvin in FeSSIF, with and without orlistat, to examine whether the addition of orlistat affected the solubility of the model drugs. The solubility of griseofulvin was found to be unaffected by the addition of orlistat.

Dissolution Rate

The dissolution rate of griseofulvin, felodipine, danazol, and cyclosporine was studied, whereas probucol and ubiquinone were excluded because they were present in concentrations that were too low for accurate quantification. The dissolution rates were studied in triplicate via a rotating disc method (USP 28) which was scaled down in size (disc diameter 3 mm) in comparison to the pharmacopoeial method. The disks were compacted in a tablet press (Kilian D-50735 SP300; Kilian & Co GmbH, Cologne, Germany). The disks were examined by X-ray powder diffraction and Raman spectroscopy to determine if the crystal structure of the substances was alternated during compression. The disks of griseofulvin and cyclosporine showed signs of minor amorphous elements being present on the surface, whereas the structure of felodipine and danazol remained unaltered (Fig. 1). The surface of the disks was blown off with nitrogen gas to remove loose particles immediately prior to the experiments. The surface of the disks was studied via a scanning electron microscope to ascertain that the disks were not damaged (Quanta 200; FEI Company, Czech Republic). The speed of rotation was set to 1000 rpm and the temperature of the dissolution media was maintained at 37° C. Each experiment ran for 20 min, and during this time sink conditions were maintained. The dissolution rate was determined by linear regression from the initial linear phase in the amount dissolved *vs*. time profile.

Drug Assay

Griseofulvin, felodipine, and danazol samples from the solubility and dissolution experiments were analyzed by HPLC (Waters 717 plus autosampler, 515 HPLC pump; Waters, Milford, MA, USA) with a UV detector (Waters 2487 Dual λ Absorbance Detector; Waters). Table II shows the analytical conditions (mobile phase, wavelength, and columns) used for the different drugs. The limit of quantification (LOQ) for felodipine, danazol, and griseofulvin was 0.01 μg mL^{-1}, and the standard curves were linear in the range 0.01–100 μg mL^{-1}. Reproducibility, expressed as the coefficient of variation (CV), was below 20% for low, medium, and high concentrations (n = 10 for each concentration). Cyclosporine was quantified by a modified HPLC method with electrospray ionization mass spectrometry (34). Probucol and ubiquinone were analyzed with HPLC (Agilent HP1100 Binary Pump; Agilent Technologies) using atmospheric pressure photo ionization tandem mass spectrometry [Waters (Micromass) Ultima Pt equipped with a Syagen APPI interface; Waters]. Probucol and ubiquinone were ionized in the positive ion mode using collision energies set at 20 and 30 eV, respectively, and a cone voltage of 35 V. The analytical conditions for cy-closporine, probucol, and ubiquinone are shown in Table III. LOQ was 0.001 μg mL^{-1} for cyclosporin and 0.5 ng mL^{-1} for probucol and ubiquinone. The standard curve for cyclosporin was linear in the range 0.001–10 and 10–100 μg mL^{-1}. The standard curves for probucol and ubiquinone were linear in the range 0.5–50 ng mL^{-1} for ubiquinone and 0.5–250 ng mL^{-1} for probucol. Above these concentrations the standard curves followed a quadratic fitting. The CV was below 20% for low, medium, and high concentrations (n = 10 for each concentration).

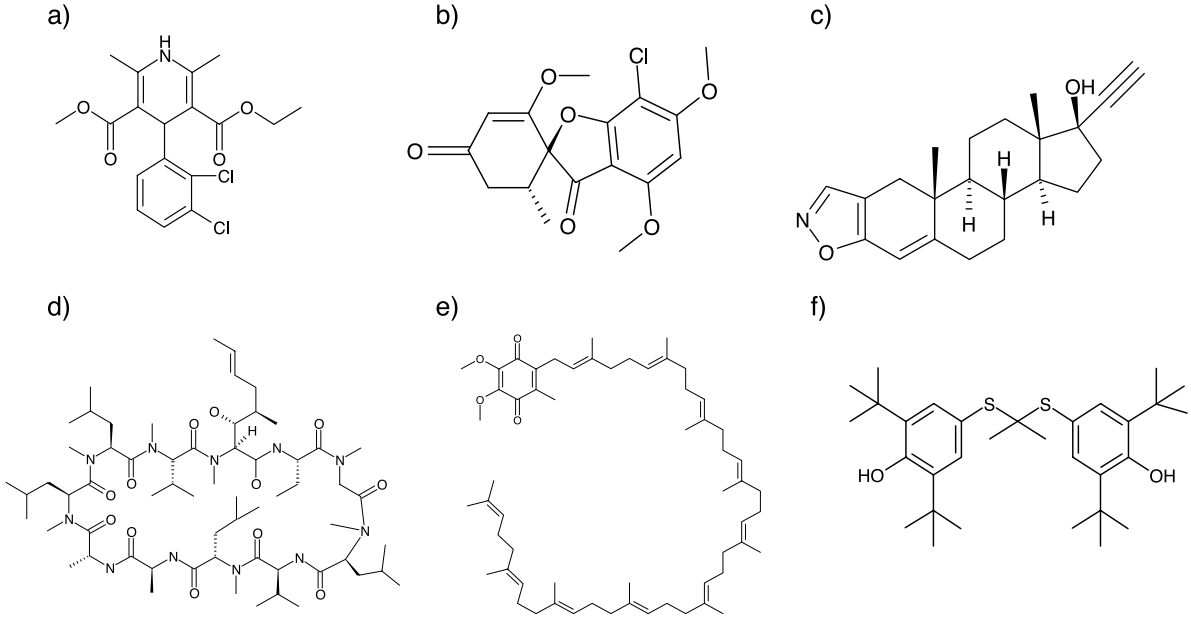

Fig. 1. Chemical structure of the model substances: (a) felodipine, (b) griseofulvin, (c) danazol, (d) cyclosporine, (e) ubiquinone, and (f) probucol.

Table II. HPLC Conditions including Separation Columns, the Mobile Phase and the Detection Wavelength for Felodipine, Danazol, and Griseofulvin

Media	Substance	Column	Mobile phase	λ (nm)
FeSSIF and HIF fasted	Felodipine	Waters Xterra C18 (4.6 × 150 mm, 5 μm)	Acetonitrile/methanol/phosphate buffer, pH = 3 (4:2:4, v/v)	220
	Danazol	Zorbax eclipse XDB C18 (4.6 × 150 mm, 5 μm)	Acetonitrile/acetic acid 50 mM, pH = 3 (60:40, v/v)	270
	Griseofulvin	Waters Xterra C18 (4.6 × 150 mm, 5 μm)	Acetonitrile/phosphate buffer, pH = 3 (47.5:52.5, v/v)	254
HIF fed and DIF fed	Felodipine	Waters Xterra C18 (4.6 × 150 mm, 5 μm)	Acetonitrile/acetic acid 50 mM, pH = 3 (60:40, v/v)	254
	Danazol	Zorbax eclipse XDB C18 (4.6 times; 150 mm, 5 μm)	Acetonitrile/methanol/phosphate buffer, pH = 3 (4:2:4, v/v)	270
	Griseofulvin	Waters Xterra C18 (4.6 × 150 mm, 5 μm)	Acetonitrile/acetic acid 50 mM, pH = 3 (37:63, v/v)	254

Data Analysis

Differences in solubility and dissolution rate for all the substances in fed compared to fasted HIF and in fed HIF compared to FeSSIF and fed DIF were statistically investigated by a *t* test, where a *p* value of <0.05 was considered to be significant.

The partial least squares (PLS) method was used to establish the relationship between the data for physicochemical descriptors (x) and the solubility ratio between fed and fasted HIF (y). The PLS calculation was performed using the software SIMCA-P version 10.5 (Umetrics AB, Umeå, Sweden). Prior to PLS modeling, all variables were scaled to unit variance and mean-centered (by subtraction of the variable average). The result of multivariate analysis of the 6×6 chemical data matrix and the y response gave a PLS model with one component, which was significant based on cross-validation.

RESULTS

Characterization of Intestinal Fluids

Characterization of fasting and fed HIF and DIF was performed to determine the pH, total protein concentration, bile secretion components, surface tension, buffer capacity, and total nutritional lipid content (Table IV). pH was lower in the fed HIF than in the fasted state, whereas the protein content and buffer capacity were higher. The values obtained for pH corresponded well between fed HIF and DIF, whereas the pH of FeSSIF was lower. No difference was seen in the surface tension between fasted HIF, fed HIF, and fed DIF. The bile acid concentration in fed state HIF was approximately four times higher than in fasted state HIF, but it corresponded well to the concentration in fed DIF. The concentration of bile acids in FeSSIF was two times higher than that found in fed HIF. The relative proportion of the different bile acids was quite similar in fasted and fed HIF, with glycocholic acid being the most abundant, whereas taurocholic acid was the major component in fed DIF and FeSSIF (Fig. 2). The concentration of phospholipids in fed HIF was much higher (3.2 mM) than in the fasted state HIF (0.2 mM); the other fed state media provided similar values to that obtained for fed HIF. Lysophosphatidylcholine was the dominant phospholipid in both the fed and fasted state HIF (Fig. 2), whereas phosphatidylcholine, which was added to FeSSIF, was found in a higher concentration in fed DIF. The relationship between bile acids and phospholipids was 9:1 and 2.5:1 in fasted and fed state HIF, respectively, and 4:1 in both fed DIF and FeSSIF. The concentration of neutral lipids including free fatty acids in fed

Table III. HPLC Conditions Including Separation Columns, Mobile Phases, Gradients and Detection Mass-to-Charge (m/z) for Cyclosporine, Probucol, and Ubiquinone

Substance	Column	Mobile Phase	Gradient	m/z (mg mol^{-1})
Cyclosporine	Waters Xterra C18 (2.1 × 50 mm)	(A) Methanol/H_2O (70:30, v/v) (B) Methanol	0 min: 100% A 2 min: 100% B 6 min: 100% B 7 min: 100% A	1203, 1225, 1241
Probucol	Zorbax Extend C18 (2.1 × 50 mm, 3.5 μm)	(A) Methanol/H_2O (90:10, v/v) (B) Butanol/hexane (70:30, v/v)	0: 100% A 3.5 min: 100% B 3.6 min: 100% A 5 min: 100% A	Parent: 279.3 Product: 223.3
Ubiquinone	Zorbax Extend C18 (2.1 × 50 mm, 3.5 μm)	(A) Methanol/H_2O (90:10, v/v) (B) Butanol/hexane (70:30, v/v)	0: 100% A 3.5 min: 100% B 3.6 min: 100% A 5 min: 100% A	Parent: 863.5 Product: 197.2

Table IV. Characterization of Fasted HIF, Fed HIF, Fed DIF, and NuTRIflex

	Fasted HIF	Fed HIF	Fed DIF	NuTRIflex
pH	7.5	6.1	5.8	5.4
Total protein conc. ± SD (mg mL^{-1})	1 ± 0.1	5 ± 0.1	5 ± 0.2	19 ± 1
Total bile salt conc. (mM)	2 ± 0.2	8 ± 0.1	8 ± 0.2	0
Total neutral lipid conc.[a] (mM)	0.1 ± 0.01	22 ± 1	12 ± 1	12 ± 3
Total phospholipid conc. (mM)	0.2 ± 0.07	3 ± 0.3	2 ± 0.2	3 ± 0.3
Surface tension ± SD (mN m^{-1})	28 ± 1	27 ± 1	27 ± 1	29 ± 1
Buffer capacity, base (mmol L^{-1} pH $unit^{-1}$)	2.8	13.2	14.1	11.8
Buffer capacity, acid (mmol L^{-1} pH $unit^{-1}$)	2.4	14.6	14.7	27.4

Results shown are the mean ± standard deviation (SD) when multiple tests have been performed (n = 3).
[a] Including fatty acids.

HIF was 21.9 mM. This was higher than the concentration in fasted HIF (0.1 mM) and fed DIF (12.2 mM). FeSSIF lacks the addition of neutral lipids. Only free fatty acids and cholesterol were present in the fasted HIF, whereas in fed HIF, in addition to these, both tri-, di-, and monoglycerides were present as a result of the nutritional drink being administered (Fig. 3). The composition of neutral lipids in fed DIF was similar to the one in fed HIF.

Solubility

Solubility of the model substances in all media is shown in Figs. 3 and 4. All the substances had a significantly higher solubility in the fed state HIF than in the fasted fluid (Fig. 3). The increase in solubility varied between a factor of 3.5 and 30. The solubility in fed HIF was higher for all the substances (2–5 times) compared to FeSSIF, whereas the values for HIF and DIF corresponded well, as indicated by differences of less than 30% (Fig. 4).

The relationship between the substances' physicochemical descriptors (x data) and the increase in solubility induced by food (y data) was quite good, as indicated by a regression coefficient (r^2) of 0.86. The coefficient plot in Fig. 5 shows the relative influence of the different physicochemical descriptors on the solubility enhancement brought about by food in comparison to the fasted state. The log P value was the most important predictor; the higher the log P value, the more pronounced was the increase in solubility brought about by the food. A weak but significant positive correlation was obtained for the melting point. The aqueous solubility showed the strongest negative correlation, that is, the effect food has on the solubility was increased as the aqueous solubility decreased, and that this effect was more marked than for the other values. H_{acc} also correlated quite strongly in a negative manner. The other factors did not provide any statistically significant effects.

Dissolution Rate

The dissolution rate of the model substances in all the media used is shown in Figs. 3 and 4. All the substances had a significantly higher dissolution rate in the fed compared to the fasted state human intestinal fluid (Fig. 3). The dissolution rate for cyclosporine, danazol, and felodipine increased to a similar extent, by 7, 5, and 6.5 times, respectively, whereas the increase for griseofulvin was much lower (a factor of 2). The dissolution rate in fed HIF was well predicted by both the DIF and the FeSSIF, as shown by the differences between them and HIF being less than 30% (Fig. 4).

DISCUSSION

This is the first study to investigate drug solubility and dissolution in real human intestinal fluid obtained under fed conditions. After intake of food, the grinding motion of the stomach along with enzymatic degradation creates a fat emulsion, which is secreted to the intestine at a constant rate. The intestinal fluid in this paper was sampled by use of the Loc-I-Gut method. A nutritional drink, containing partly degraded triglycerides and proteins, was administered directly to the jejunum by continuous perfusion. The single balloon technique used prevented the intestinal fluid from continuing further down the intestinal tract and allowed complete sampling of intestinal fluid from the jejunum (Persson *et al.*, unpublished data). Thus, the experimentally fed state created in this study is believed to closely resemble the conditions in the upper intestinal tract after intake of food. The effects of changes in composition of the intestinal fluid as it proceeds along the intestinal tract were not studied in this paper, since absorption of drugs is believed to primarily occur in the upper part of the intestine. Also, the effect of individual variations in lipid content was not determined, because of the large volumes of intestinal fluid required for this process. Instead, all the results obtained are derived from pooled intestinal fluid and the importance of individual variations remains to be elucidated.

The solubility of the investigated drugs was substantially increased after intake of food compared to fasting conditions. This is not surprising considering the 4× higher bile acid concentrations and the 14-fold increase in phospholipid levels in the fed compared to the fasted state, leading to an increased micellar solubilization capacity. In addition, lipidic components of nutritional origin are only present in the fed state. The latter components contribute to an increased solubility via monoglycerides and free fatty acids forming mixed micelles when present with the bile acids, as well as by dissolution of the drugs in lipidic emulsion droplets. Thus, the present study supports the importance of these factors for drug solubilization in the intestinal tract, and it also confirms that increased intestinal drug concentrations can be achieved for low solubility, nonionic drugs after intake of food.

The degree of solubility enhancement brought about by food varied for the different drugs. For example, there was a

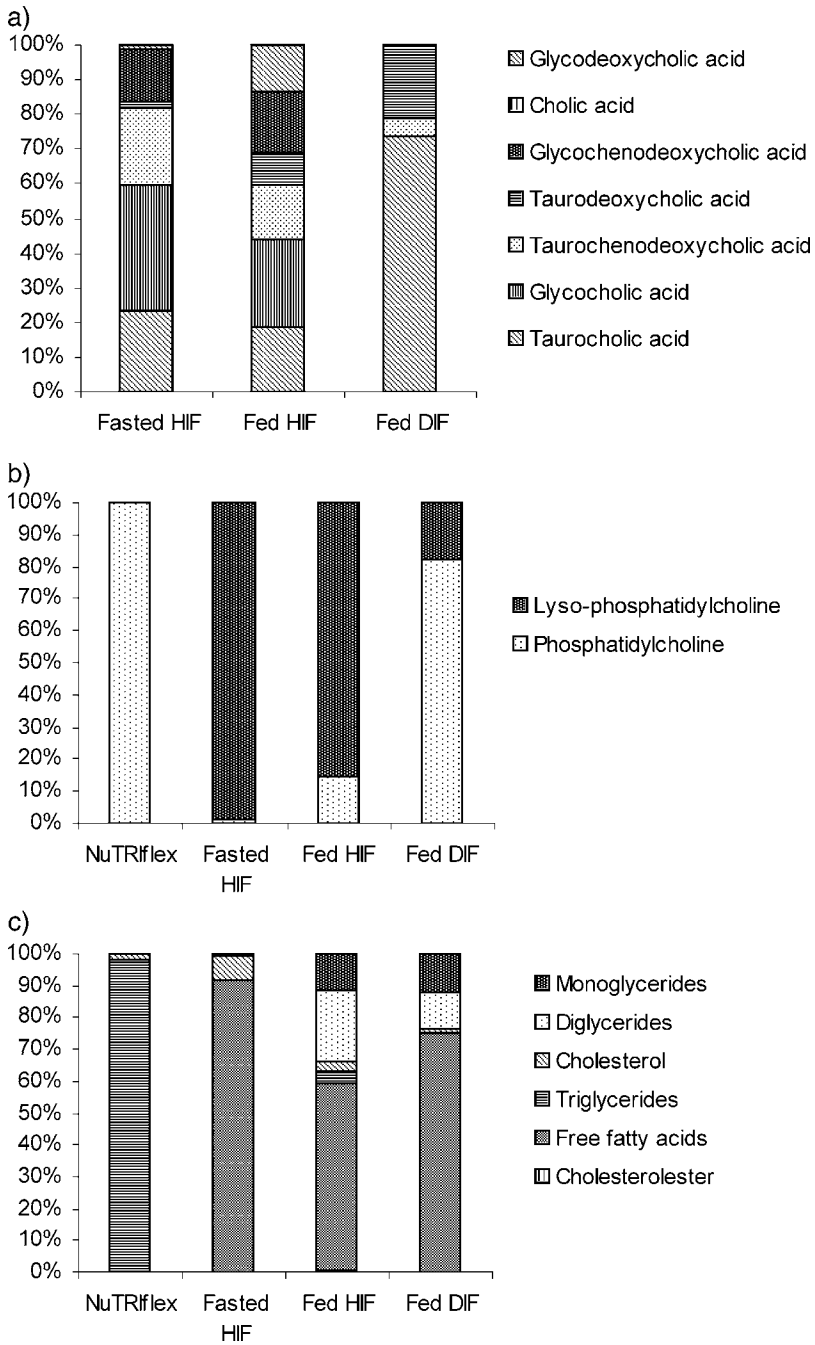

Fig. 2. Percentage of individual (a) bile acids, (b) phospholipids, and (c) neutral lipids in fed and fasted HIF, fed DIF, and NuTRIflex.

positive correlation in this study between the difference in solubility in fed compared to fasted state HIF and the lipophilicity of the drug. A higher log *P* value resulted in a higher increase in solubility from the fed to the fasted state. This is in accordance with earlier data obtained in simulated intestinal media where no solubilization was obtained for drugs with log $P < 2$, whereas more lipophilic compounds were increasingly solubilized by increasing lipophilicity (28). The increase in solubility between fed and fasted HIF was also correlated to the aqueous solubility of the compounds such that the solubility effect brought about by food will increase with decreased water solubility. This indicates that food-induced solubility enhancement should be expected for all low solubility drugs even if they are modestly lipophilic. The degree of solubility enhancement was clearly also influenced by proton acceptor properties in addition to lipophilicity and water solubility. This type of more extensive modeling of *in vivo* solubility effects compared to previous single parameter correlations should probably be expanded to include more compounds before definitive conclusions can be drawn regarding the influence on the various substance characteristics.

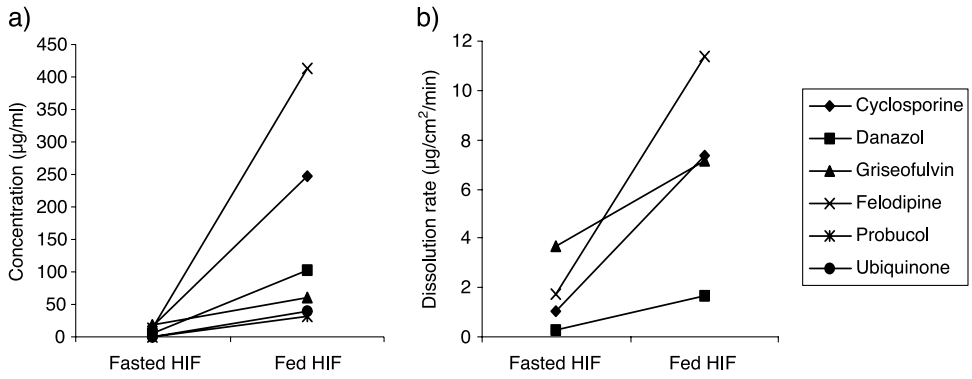

Fig. 3. (a) Solubilities (µg mL^{-1}) and (b) dissolution rates (µg cm^{-2} min^{-1}) for the model substances in fasted and fed HIF (mean ± SD).

The dissolution rate was also increased in fed compared to fasted state HIF for all the substances, but not to the same extent as the solubility rate (Fig. 6). A similar finding was reported in the study of Hörter and Dressmann (35), where the solubility of lipophilic compounds increased in simulated intestinal media with an increased content of surfactants, whereas the dissolution rates decreased. In addition, further studies in simulated intestinal fluids have shown that the dissolution rate of cholesterol could be decreased in the presence of mixed micelles containing phosphatidylcholine in spite of the improved solubility (20). The more pronounced increase that food exerts on solubility compared to its effect on the dissolution rate may be caused by slower diffusion of the larger micelles and a slow transfer of the drug into emulsion lipid droplets formed in the fed state intestinal fluids. Kossena *et al.* (16) studied the partitioning of poorly soluble drugs between the different colloidal phases formed in water containing bile acids and phosphatidylcholine with the addition of medium- and long-chained monoglycerides and free fatty acids. The colloidal species formed consisted primarily of mixed micelles and vesicles. In the long-chained digests, the mixed micellar phase was the predominant drug-solubilizing form, whereas in the medium-chained solution it was the vesicles that contributed most. The more lipophilic substances partitioned to the vesicles to a greater extent than to the mixed micelles (16). The monoglycerides formed in fed human intestinal fluid upon enzymatic degradation of triglycerides are probably a mixture of long- and medium-chained monoglycerides. The substances in this study would, therefore, probably partition equally between the mixed micelles and vesicles formed. As the radius of the vesicles is approximately 10 times that of the mixed micelles formed in plain bile salt/phospholipid systems, the diffusion coefficient should be lower. Another contributing factor is the possibility of drug dissolution in the emulsified lipids. This could provide a large capacity for dissolving drugs. However, owing to the small surface area of the lipid droplets compared to that of the micellar and vesicular phases, it could be expected that this will be a slow process, which would only affect the equilibrium solubility significantly; very little impact would be observed on the dissolution rate. Thus, the present data, showing a relatively small increase in the dissolution rate compared to that of the saturation solubility, confirm the *in vivo* relevance of the previous findings obtained in artificial media.

Drug dissolution can be calculated if solubility is determined according to the Noyes–Whitney equation, as this gives a linear relationship between the solubility and the dissolution rate of a substance under "sink conditions" (36). However, results in this study support the idea that the Noyes–Whitney equation might have to be modified when more complex fluids are used. In the Noyes–Whitney equation, the difference between the free drug and the solubilized drug diffusion coefficients is ignored. Theories exist for the dissolution rates of poorly soluble drugs in the presence of a solubilizing agent; these are based on different hydrodynamic models. For example, Singh *et al.* (37) studied the influence of micellar solubilization on dissolution rate in a system containing benzocaine-polysorbate 80 and found that, if surfactants in the

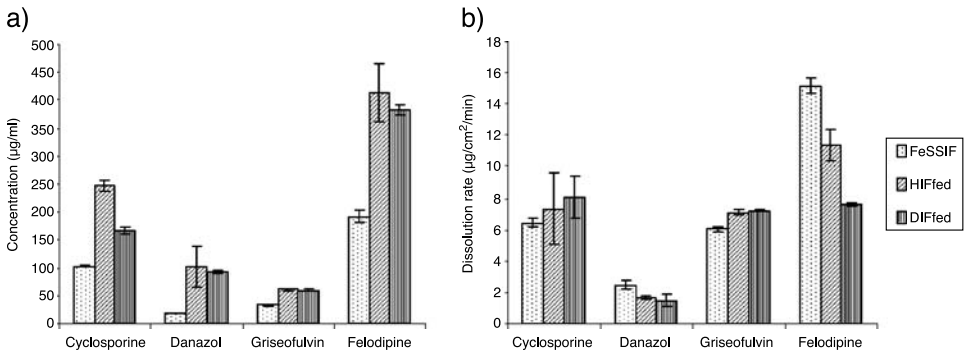

Fig. 4. (a) Solubilities (µg mL^{-1}) and (b) dissolution rates (µg cm^{-2} min^{-1}) for the model substances in fed state HIF, DIF, and FeSSIF (mean ± SD).

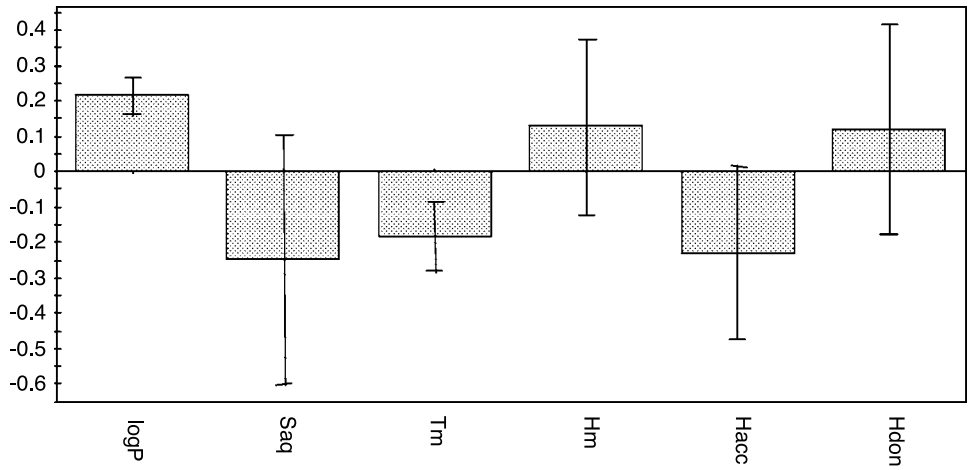

Fig. 5. Regression coefficients of the PLS model showing the effect of the physical–chemical descriptors and their interaction on the solubility enhancement brought about by food. The different descriptors were lipophilicity (log P), aqueous solubility (S_{aq}), melting point (T_m), the change in melting entrophy (ΔH_m), and the number of hydrogen acceptors (H_{acc}) and donors (H_{don}). The response coefficients are expressed as scaled and centered regression coefficients.

dissolution media were taken into consideration, the values calculated with these theories corresponded to the experimental data. The relative difference in the impact of food on solubility and dissolution verified in the present study suggests that dissolution rate should not be estimated from solubility data based on the assumption of a linear relation—for example, when used in advanced absorption computer simulations (38). Instead, experimental values of the dissolution rate determined in physiologically relevant media, or more sophisticated dissolution theories than the standard Noyes–Whitney equation should be used.

Naturally, HIF is the most relevant of media for *in vitro* dissolution or solubility studies; however, its use is limited by the fact that it is not easily accessible. It is therefore of interest to use alternatives to HIF, such as DIF and simulated intestinal fluids. Studies have been performed in the fasted state to evaluate simulated and dog intestinal fluids. The conclusion drawn from those studies was that both fasted simulated intestinal fluid and fasted dog intestinal fluid overestimate the solubility for poorly soluble drugs (10,22). In this study, we looked at the fed state and found that solubility was similar in fed HIF and DIF, but much lower in FeSSIF. This is attributable to the presence of dietary lipids in the real intestinal fluids, which are totally lacking in FeSSIF. The other main factor for intestinal solubilization, bile acids, was even higher in simulated compared to real fluids, but the compensatory effect of the bile acids was insufficient to make up for the absence of lipids. It should also be noted that a rather low fat meal was used in the present study and that even more pronounced differences between FeSSIF and real fluids are expected for meals with a higher fat content, such as the standard FDA breakfast (39). Consequently, improved FeSSIFs should be developed including lipids of dietary origin to obtain more realistic *in vivo* solubility predictions. The relative partitioning of nutritional lipids into mixed micelles *vs.* presence in oil droplets and the relative

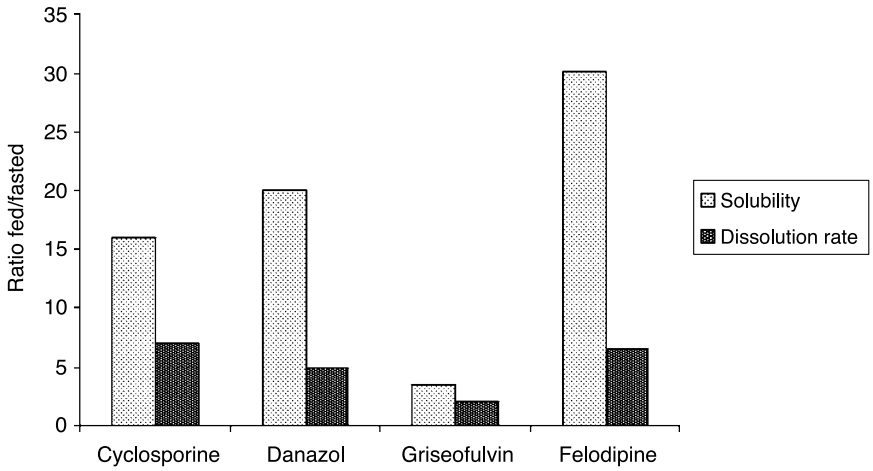

Fig. 6. The fed/fasted ratio for the solubility (S) and the dissolution rate (DR) for the four model substances.

importance of the different phases for drug solubility should be further investigated before defining an improved FeSSIF.

No significant difference could be observed in the dissolution rate between the three media despite the difference in solubility between real and simulated or dog intestinal fluids. This can be explained by the discussion above regarding the mechanisms for dissolution in media of various complexities. Thus, FeSSIF should be able to provide a relevant media for the dissolution rate in fed state studies, e.g., in testing during product development, when drug levels in the intestine are well below the saturation solubility. In addition, based on the good correspondence in both the solubility and the dissolution rate in fed conditions, the dog should be a reasonably good model for *in vivo* studies under fed conditions.

The relatively smaller increase in the dissolution rate compared to the solubility in fed intestinal fluids will also have consequences in the prediction of the effect of food on drug bioavailability. The most pronounced effect would be expected for drugs where absorption is limited by their saturation solubility in the GI tract, i.e., drugs with high dose numbers [$D_o = (M_0 / V_0) / C_s$, where M_0 is the dose of the drug administered, V_0 is the initial gastric volume, and C_s is the saturation solubility] (32). Danazol, with a D_o of 400 based on the solubility in fasted HIF, provides an example of such a drug; an increase in bioavailability after intake with food of a factor of 4 has been reported (4,18). For other low solubility drugs where "sink conditions" are maintained in the GI tract, the dissolution rate will limit the absorption. This concept is illustrated by the dissolution number ($D_n = t_{res} / t_{diss}$, where t_{res} is the mean residence time and t_{diss} is the time required for a particle of the drug to dissolve), where a value of greater than 1 for D_n indicates a dissolution-rate-limited absorption (32). For these kinds of drugs, a minor increase in bioavailability with food should be expected because the increase in dissolution rate is less pronounced than the increase in drug solubility. Felodipine is an example of such a drug, the bioavailability of which, despite a 30-fold increased solubility in the fed state, is only very marginally affected by food (40).

CONCLUSION

The intestinal solubility of aprotic drugs with a low water solubility is higher in the fed compared to the fasted state, as expected from increased levels of bile secretion and lipids. Although this is also true for the dissolution rate, it does not increase to the same extent. These findings need to be considered in the design of *in vitro* models and in the prediction of food effects on oral bioavailability of poorly soluble drugs.

The dog seems to be a good model for man with respect to dissolution in the small intestine after intake of a meal. FeSSIF offers a good means of performing dissolution studies, whereas it is less feasible for prediction of intestinal saturation solubility in the fed state.

ACKNOWLEDGMENTS

We are thankful to Dr. Ralf Nilsson, Dr. Lars Löfgren, and Dr. Göran Hansson for their excellent technical assistance in the lipid analysis, and Angela Ku for the PLS analysis.

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