Dissolution of Hydrocortisone in Human and Simulated Intestinal Fluids

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Purpose. To compare solubility and dissolution rate of hydrocortisone in aspirated human intestinal fluids (HIFs) with simulated intestinal fluids (SIFs) and buffer.

Methods. Solubility and flux from a rotating disk of hydrocortisone were measured. The bile salt content, pH and osmotic pressure were determined in HIFs.

Results. In fasted state the solubility of hydrocortisone was higher in HIFs than in the buffer and SIFs. The flux of hydrocortisone in HIFs was similar to the flux in the buffer but lower than the flux in SIFs at fasted state. Addition of intestinal surfactants in SIFs increased solubility and flux at both fasted and fed state. The increase in solubility was caused by micelle formation in SIFs. The increase in flux may partly be explained by increased solubility. The bile salt content of the HIFs did not correlate with the solubility or the flux but pH in the HIFs seems to have some effect on the components of the HIFs resulting in increased solubility.

Conclusions. It is possible to perform comparable dissolution tests in HIFs and SIFs. The lack of correlation between the results in HIFs and the bile salt content may be explained by the relatively low lipofilicity of the model drug.

KEY WORDS: poorly soluble drugs; hydrocortisone; dissolution rate; solubility; intestinal fluids; bile salts.

INTRODUCTION

The bioavailability of poorly water-soluble drugs may be increased when taken with food (1–3). The mechanism of this food-effect is dependent on the physicochemical properties of the drug, interactions with food components, and the physicochemical and physiological changes of the conditions in the gastrointestinal (GI) tract associated with the change from fasted to fed state (4). In the fasted state concentrations between 0.1 and 13.3 mM bile salts have been reported showing a considerable inter- and intraindividual variability in the concentrations of bile components in the human small intestines (5). Bile salts and lecithin are the two major components in bile and the molar ratio is about 2:1 to 5:1 (6). The CMC of one major bile salt, sodium taurocholate (TC), was measured to be 4.7 mM, and in the presence of lecithin (4:1) the CMC dropped to 0.25 mM (7). Thus, it is possible that in the fasted state the composition of intestinal fluid might influence the dissolution rate of poorly water-soluble drugs not only by enhanced wetting but also through solubilization effects.

Different dissolution media representing the conditions in the proximal small intestine have frequently been used in dissolution studies of poorly water-soluble drugs (4). There is a need to evaluate the *in vivo* relevance of the composition of simulated intestinal fluids used in *in vitro* dissolution testing today. The literature reports little on dissolution studies in human GI fluids. Gastric juice from fasting subjects has been employed in contact angle measurements and powder dissolution studies (8,9) and studies of tablet processing and formulation factors on the dissolution rate in human gastric fluids have been reported (10). Furthermore, an *in vivo* dissolution technique has been developed for dissolution studies in human jejunum where the dissolution occurs in diluted intestinal fluids at fasted state (11,12).

The purpose of the present work was to develop methods for dissolution studies in aspirated human intestinal fluids (HIFs) as well as simulated intestinal fluids (SIFs) and to investigate the effects of gastrointestinal surfactants representing the fasted and the fed state on the dissolution rate and solubility of hydrocortisone as a model of a poorly water-soluble drug. Two intestinal surfactants were chosen to represent the bile surfactants: one bile salt and one phospholipid in a ratio of 4:1. Further two intestinal surfactants represented the major lipolytic product: one fatty acid and one monoglyceride. The concentration levels selected were based on measured data in the aqueous phase of human postprandial duodenal content (13).

MATERIALS AND METHODS

Materials

Micronized hydrocortisone was purchased from Nycomed, Denmark (log P: 1.6 (14); aqueous solubility: 0.33 mg/ml (7); MW: 362.47 g/mol (15) and MP: 217–220°C (15)). Sodium glycocholate 99% pure (GC), sodium taurocholate 98% pure (TC) and oleic acid 99% pure were obtained from Sigma (St. Louis, MO, USA). Samples of L-α-egg lecithin 98% pure (MW of 780.2 g/mol) and GC 99% pure were from Larodan (Malmö, Sweden). Emulsifier TS-ED 231 as glycerol monooleate with a MW of 356.5 g/mol was donated by Danisco Ingredients (Brabrand, Denmark). Emulsifier TS-ED 231 had a monoester content of at least 90%, a fatty acid content of more than 78% oleic acid, max. 15% linoleic and linolenic acids, and max. 10% palmitic, stearic and arachidic acids.

Dissolution Media

Human intestinal fluids (HIFs) aspirated from fasting subjects and 5 different SIFs were used. Sodium phosphate buffer (50 mM, pH = 6.5 ± 0.1) was included in the study as a reference dissolution medium. The HIFs were collected by vacuum pump in the proximal jejunum approximately 60 cm

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ABBREVIATIONS: GC, sodium glycocholate; GC/L, sodium glycocholate and lecithin; GC/L/O/M, sodium glycocholate, lecithin, oleic acid and glycerol monooleate; TC, sodium taurocholate; TC/L, sodium taurocholate and lecithin; HIF, human intestinal fluid; SIF, simulated intestinal fluid.

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distally of the pylorus (during an intestinal perfusion) from 9 healthy subjects having fasted for 10 hours (16). The perfusion study was approved by the Ethics Committee of the Medical Faculty, Uppsala University, Sweden.

To prevent microbial growth during the dissolution studies 6 mM NaN_3 and 0.01 mM chloramphenicol were added. The volume available from each person varied from 35 to 180 ml which limited the number of rotating disk experiments performed in each fluid.

The SIFs represent the upper intestinal fluid in the fasted and fed state conditions. The combination and concentration levels are presented in Table 1. The SIFs were produced in 50 mM phosphate buffer with a pH of 6.5 (±0.1). To prevent microbial growth, 3 mM NaN_3 was added and NaCl was added to give a total Na^+ concentration of 150 mM and an ionic strength of 166 mM.

Solubility Studies

Solubility studies were performed at 37°C. Excess of hydrocortisone and the dissolution medium were added to a 15 ml glass flask with a teflon screw stopper. Samples were taken at intervals of approximately 1, 2, 4 and 20 hours ($n \geq 4$, except for the HIFs where $n = 2$). Studies of the stability of hydrocortisone in aqueous solutions (17) indicated that hydrocortisone is stable during the solubility measurements. The samples were filtered through syringe filters 0.45 μm (CA membrane, PP housing, MFS, USA) discarding the first 0.5 ml. Samples from HIFs were centrifugated for 15 min. at 10,000 rpm (Hettich EBA 30) before filtration. The concentration of hydrocortisone in samples from the SIFs and buffer was determined by UV. The samples from HIFs were frozen and later analyzed by HPLC.

Solubility studies were further performed at pH 1.2 (USP 23; HCl-buffer), 3.5 (diluted acetic acid), and 6.5 (phosphate-buffer) to investigate whether the solubility of hydrocortisone is dependant on pH.

Rotating Disk Dissolution Studies

Dissolution experiments with the rotating disk method were addressed in both collected HIFs and in SIFs. The rotating disk apparatus consisted of a rotor motor, a stainless steel stirrer shaft connecting the motor to a stainless die (a tube) encasing the drug disk, and a jacketed 50 ml beaker containing 25 ml of the dissolution medium (37°C). The diameters of the beaker, disk, and die were 37, 10, and 15 mm, respectively. The rotating speed (RS) was controlled manually as well as the deviation from centered rotation which never exceeded 0.5 mm. The RS was set at 100 rpm and samples were taken until approximate 10% of saturation concentration and the sample volume of 1.00 ml was replaced by new dissolution medium ($n \geq 3$ for the SIFs and buffer; $n = 1$ for the jejunal fluid from subjects 1, 5, and 8; and $n = 2$ in the fluid from subject 2, 3, 4, and 6). The samples from HIFs were frozen (−20°C) for later HPLC analysis. The samples from the various SIFs and the buffer were analyzed by UV.

Rotating disk tests were made in a 0.5 mM GC solution at 100 rpm to investigate whether the disks were susceptible to wetting effects ($n = 3$). The concentration of 0.5 mM GC is below the CMC and has a lower surface tension than buffer (see Figure 4).

Since the jacketed vessel applied in these studies had a low volume compared with the traditionally used vessel, rotating disk tests were made in a larger flat-bottomed vessel with a diameter of 85 mm containing 200 ml dissolution media. Studies were made at 175 rpm in three dissolution media: buffer, GC/L(h) and a fluid containing 15 mM TC and 3.8 mM lecithin (TC/L(h)). This was done to assure that the hydrodynamics in the small vessel were comparable with the hydrodynamics in a larger vessel ($n \geq 2$).

Table 1. Concentrations of Surfactants in the Simulated Intestinal Fluids

Surfactants	Abbrev.	Concentration fasted (mM)	Concentration fed (mM)
Sodium glycocholate	GC(l) or GC(h)[a]	3.7	15
Sodium glycocholate	GC/L(l) or	3.7	15.0
+Lecithin	GC/L(h)[a]	0.9	3.8
Sodium glycocholate	GC/L/O/M		15.0
+Lecithin			3.8
+Oleic acid			4.0
+Glycerol monooleate			1.0

[a] (l) and (h) indicating low and high concentration levels, respectively.

Surface Tension

Surface tension was measured in GC solutions saturated with and without hydrocortisone to determine whether there were micelles in the SIFs. Surface tension was measured at 37°C by the Wilhelmy plate method (Krüss digital-tensiometer K10T) ($n \geq 3$). Bile salts form micelles over a concentration range (18), and CMC was therefore determined as the intersection of two linear portions from the concentration intervals of 0–0.4 mM and 5.3–15.0 mM.

Analytical Methods

Hydrocortisone in samples from HIFs were quantified by a modified HPLC method developed by Hansen et al. (19). As analytical column a 12.5 × 0.4 cm LiChrospher®100 RP-18e (5 μm) was used and the guard column was LiChrospher®100 RP-18 (5 μm) 4 × 4 mm (Merck, Germany). The mobile phase consisted of demineralized water and methanol (53:47% v/v). The flow rate of 1 ml/min (Merck Hitachi Pump L-7100, LaChrom) resulted in a retention time of 5 min. The samples (400 μl) were mixed with acetonitrile (400 μl) in a polypropylene microtube, vortex mixed for 10 sec and centrifuged for 2 min at 9500 g (Biofuge 15, Heraeus Sepatech). The supernatant was transferred to a similar microtube and centrifuged for another 30 sec. The second supernatant was injected (Merck Hitachi Autosampler L-7200, LaChrom) and the absorbance measured at 248 nm (Merck Hitachi UV Detector L-7400, LaChrom). The peak area were recorded and analyzed by Hitachi D-7000 HPLC System Manager (HSM) programme, and converted to concentrations by comparison with external standards. The limit of detection (LOD) was 0.3 μg/ml.

The concentration of hydrocortisone in samples from SIFs and the buffer was determined by UV at 248 nm (Perkin Elmer, Lambda 14P UV/VIS). LOD was 3.4 μg/ml.

The concentration of bile salts in the HIFs was determined by colorimetric determination of total 3α-hydroxy bile acids using the kit Enzabile® (Nycomed, Norway). The pH (632 pH-meter, Methrom, Switzerland) and the osmotic pressure (Vapor Pressure Osmometer 5500, Wescor Inc., USA) were determined in the HIFs.

Dynamic Light Scattering

Dynamic light scattering (DLS) measurements were made with GC(h), GC/L(h) and GC/L/O/M solutions without and saturated with hydrocortisone (λ = 780 nm, 90°, DynaPro-801™, ProteinSolutions, USA). These measurements yield translational diffusion coefficients from an autocorrelation of the collected photons. The hydrodynamic radius (R_h) was then calculated from the Stoke-Einstein equation. The solutions were filtered through 20 nm filters except the GC/L/O/M solutions being filtered through 100 nm filters.

Disk Preparation

The disks were compressed in the die on a plane surface (specially designed for disk preparation) by a hydraulic press at 3 tons for 1 min. The surface was checked for cracks and other irregularities before use.

Data Analysis

Analysis of variance (95% confidence level) was used to compare the differences in solubility and in flux at 100 rpm between the dissolution media, and a stepwise multiple regression was done on the solubility and flux in the HIFs versus the bile salt content, pH, and the osmolality (Statgraphics 7.0). The initial rotating disk dissolution rate was calculated by least-square regression analysis (Quattro Pro 6.01) of the drug concentration versus time profile. Levich (20) developed equation 1 that can be applied for the mass flux from a rotating disk surface when the dissolution process is convection/diffusion controlled:

$$J = 0.62\ D^{2/3}\ \nu^{-1/6}\ \omega^{1/2}\ C_s \qquad (1)$$

where J is the flux (mg/cm^2/sec), D the diffusion coefficient (cm^2/sec), ν the kinematic viscosity, ω the rotational speed of the disk, and C_s is the concentration at the surface of the disk (i.e. the saturation solubility). The equilibrium in the diffusion layer between the free drug and the drug associated with the surfactants was assumed to be rapid. The diffusion coefficient was therefore replaced by the experimentally measured diffusion coefficient, D_{exp}. The diffusion coefficient of the drug associated with the surfactants, D_{mic} was calculated from equation 2 (21)

$$D = D_{\exp} = \frac{(D_{mic}C_{mic}) + (D_0C_0)}{C_s} \qquad (2)$$

where C_s is the saturation solubility, C_0 the solubility in buffer, C_{mic} equal to C_s-C_0, and D_0 is the diffusion coefficient in buffer. Assuming a dilute system with no micelle-micelle interactions, the kinematic viscosity was assumed to be that of water. To test whether the dissolution of hydrocortisone was convection/diffusion or reaction controlled in the SIFs, the dissolution rate of hydrocortisone was investigated as a function of RS at 50, 100, 175, 200 and 250 rpm. Linear regression and test for lack of fit (Statgraphics 7.0) were performed on the dissolution rate versus the square root of the RS ($n \geq 2$).

RESULTS

Human vs. Simulated Intestinal Fluids

The solubility of hydrocortisone and flux from a rotating disk in the HIFs and the SIFs at fasted state levels are compared in Fig. 1(a). The solubility of hydrocortisone in the HIFs was slightly though significantly higher than in the fasted SIFs and the buffer. The solubility in buffer was significantly lower than in any other media. The flux of hydrocortisone in the HIFs was not significantly different from buffer but was significantly lower than in all the SIFs.

Human Intestinal Fluids

Comparisons of bile salt content, solubility, and flux in the individual HIFs are shown in Fig. 2. The bile salt content, pH and osmolality in the HIFs are shown in Table 2. The characterization of the human fluids agreed with earlier results with the same perfusion technique (5). The stepwise multiple regression showed that there was no correlation between the bile salt content and solubility nor flux. However, it showed that the solubility of hydrocortisone could be correlated with the pH in the HIFs (r^2 = 0.870). Fig. 3 depicts the increased solubility at lower pH and was in the interval 0.45–0.59 mg/ml. Measurements of the solubility in aqueous solutions with pH at 6.5, 3.5, and 1.2 gave solubilities of 0.35, 0.38, and 0.37 mg/ml, respectively. Furthermore, no degradation of hydrocortisone during the solubility measurements was seen.

Fasted vs. Fed State

The effects of fasted and fed state concentration levels of the intestinal surfactants in the SIFs are illustrated in Fig. 1 (a and b). A significant increase in solubility and flux was found comparing the fed state level SIFs.

Simulated Intestinal Fluids vs. Buffer

An increase in hydrocortisone solubility in the fasted state SIFs was observed when compared with buffer of 24 and 23% in the GC(1) and GC/L(1) fluids, respectively (see Fig. 1). Adding lecithin and the lipolytic metabolites at fed state concentrations in an attempt to simulate the human postprandial intestinal fluid, even higher increase in the solubility was found. The increase in solubility in the fed state SIFs in relation to buffer was 48% for the GC(h), 62% for the GC/L(h), and 81% for the GC/L/O/M fluid. Furthermore, there was an increase in the flux from the rotating disk in the fasted state SIFs when compared with buffer of 38 and 61% for GC(l) and GC/L(l), respectively. For the fed state SIFs, the increase in flux in relation to buffer was 90, 130, and 103% for GC(h), GC/L(h) and GC/L/O/M, respectively.

Dynamic Light Scattering

DLS measurements of the micellar size in the GC(h) fluid without hydrocortisone gave a hydrodynamic radius (R_h) of

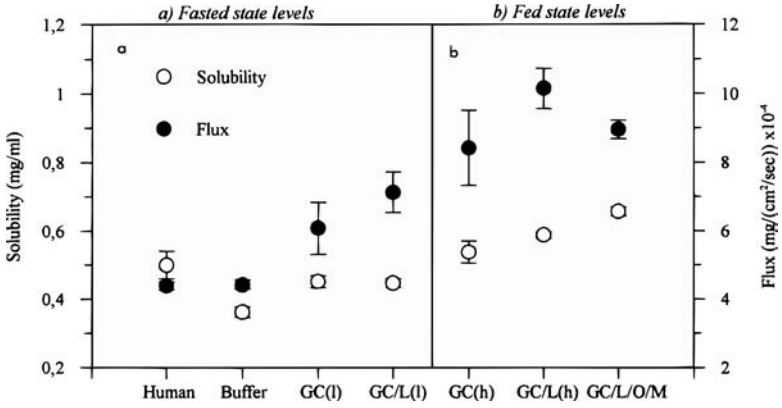

Fig. 1. Comparison of the solubility and rotating disk dissolution rate (as flux) of hydrocortisone in buffer, human and simulated intestinal fluids (HIFs and SIFs); n ≥ 3, mean ± s.d. *a*) Human: HIFs, GC (1): 3,7 mM sodium glycocholate, and GC/L (1): 3,7 mM sodium glycocholate + 0.9 mM lecithin. *b*) GC (h): 15.0 mM sodium glycocholate, GC/L (h): 15.0 mM sodium glycocholate + 3.8 mM lecithin, and GC/L/O/M: 15.0 mM sodium glycocholate + 3.8 mM lecithin + 4.0 oleic acid + 1.0 mM glycerol monooleate; n ≥ 3, ± s.d.

1.21 ± 0.08 nm and a R_h of 1.22 ± 0.07 nm when GC(h) was saturated with hydrocortisone. The measurements in the GC/L(h) fluid without and saturated with hydrocortisone gave R_h of 3.05 ± 0.08 and 3.02 ± 0.04 nm, respectively, but the measurements of the micelles in the GC/L/O/M fluid gave a wide distribution of micellar size. The R_h were 15 ± 8 and 36

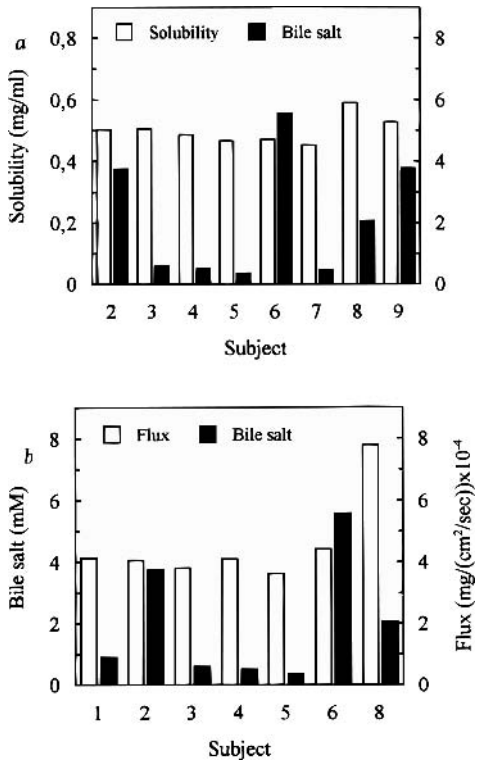

Fig. 2. Comparison of bile salt content in the human intestinal fluids (HIFs) and *a*) the solubility and *b*) the initial rotating disk dissolution rate (as flux) at 100 rpm of hydrocortisone in the fluids. For the solubility tests n = 2. For the rotating disk tests n = 2 except for subjects 1, 5, and 8 where n = 1.

Table 2. Concentrations of Bile Salts, pH, and Osmotic Pressure in the Intestinal Fluids Collected in the Proximal Jejunum in Fasting Human Subjects

Subject	Bile salts (mM)[a]	pH	Osmotic pressure (mmol/kg)
1	0.90 ± 0.10	4.3	248
2	3.75 ± 0.17	6.8	290
3	0.61 ± 0.08	6.6	284
4	0.52 ± 0.10	6.8	296
5	0.36 ± 0.03	6.7	282
6	5.57 ± 0.12	6.6	283
7	0.46 ± 0.24	7.4	288
8	2.06 ± 0.57	4.0	236
9	3.78 ± 0.20	6.0	238
Mean ± s.d.	2.00 ± 1.92	6.1 ± 1.2	272 ± 24

[a] Subjects 1, 3, 4, and 8, n = 3. For the others n = 2.

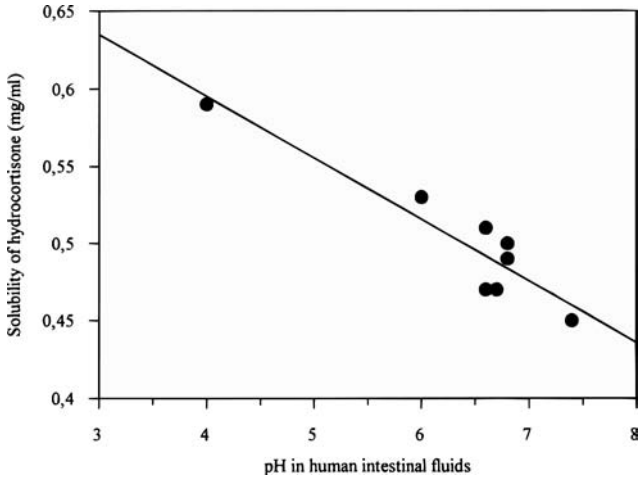

Fig. 3. Solubility of hydrocortisone in the human intestinal fluids (HIFs) versus pH. The line is representing the linear regression: Solubility = −0.0398*pH + 0.7546 (r^2 = 0.870).

Table 3. Micellar Diffusion Coefficients Determined by Dynamic Light Scattering, D_{DLS}, and Calculated from Rotating Disk Experiments, D_{mic}. The Experimentally Measured Diffusion Coefficient, D_{exp}, of Hydrocortisone in the Simulated Intestinal Fluids, and Buffer Are Included

Fluid (saturated with hydrocortisone)	$D_{DLS} \pm$ s.d. (cm^2/sec) $\times 10^{-6}$	D_{mic} (cm^2/sec) $\times 10^{-6}$	$D_{exp} \pm$ s.d. (cm^2/sec) $\times 10^{-6}$
Buffer	n.a.*	n.a.*	4.30 ± 0.35
GC(1): Sodium glycocholate 3.7 mM	n.d.**	6.91	4.81 ± 0.62
GC/L(1): Sodium glycocholate 3.7 mM + Lecithin 0.93 mM	n.d.**	13.03	5.94 ± 0.63
GC(h): Sodium glycocholate 15.0 mM	2.01 ± 0.12	10.88	6.44 ± 0.86
Without hydrocortisone	1.93 ± 0.13		
GC/L(h): Sodium glycocholate 15.0 mM + Lecithin 3.8 mM	0.85 ± 0.01	11.43	7.03 ± 0.41
Without hydrocortisone	0.83 ± 0.02		
GC/L/O/M: Sodium glycocholate 15.0 mM + Lecithin 3.8 mM + oleic acid 4.0 mM + glycerol monooleate 1.0 mM	0.13 ± 0.09	5.8	4.97 ± 0.27
Without hydrocortisone	0.23 ± 0.12		

* n.a. = not applicable.
** n.d. = not determined.

± 24 nm in the GC/L/O/M fluid without and saturated with hydrocortisone, respectively. The micellar diffusion coefficients obtained from the DLS measurements are shown in Table 3.

Convection/Diffusion Controlled Dissolution

The effect of the RS on the flux from the rotating disk is depicted in Fig. 4. The intercept was not significantly different from zero, and the test for lack of fit did not give a significant difference from linearity, suggesting a convection/diffusion controlled dissolution process (20). The flux from hydrocortisone disks in a 0.5 mM GC solution were the same as the flux in buffer suggesting that no wetting effect took place. The experimentally measured diffusion coefficient of hydrocortisone, D_{exp}, and the micellar diffusion coefficient, D_{mic}, calculated from the rotating disk results are shown in Table 3.

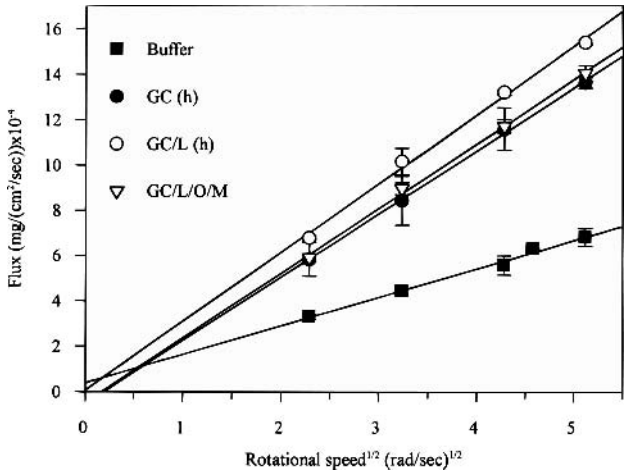

Fig. 4. Effect of rotational speed (RS) on the initial disk dissolution rate of hydrocortisone in buffer (■), in GC(h): 15.0 mM sodium glycocholate (●), in GC/L(h): 15.0 mM sodium glycocholate + 3.8 mM lecithin (○), and in GC/L/O/M: 15.0 mM sodium glycocholate + 3.8 mM lecithin + 4.0 oleic acid + 1.0 mM glycerol monooleate (▽). Linear regression gives Flux = $1.24 \times 10^{-4}(RS^{1/2}) + 0.41 \times 10^{-4}$ ($r^2 = 0.963$), Flux = 2.79×10^{-4} ($RS^{1/2}$) − 0.55×10^{-4} ($r^2 = 0.935$), Flux = 3.02×10^{-4} ($RS^{1/2}$) + 0.15×10^{-4} ($r^2 = 0.986$), and Flux = 2.84×10^{-4} ($RS^{1/2}$) − 0.42×10^{-4} ($r^2 = 0.992$), respectively; $n \geq 2$, ± s.d.

Hydrodynamics

Comparison of the flux in the 25 ml and 200 ml vessels suggested similar hydrodynamics in the two types of vessels. The flux with buffer in the 25 ml and 200 ml vessels were 5.56×10^{-4} and 5.06×10^{-4} mg/(cm^2*sec), respectively, and with the GC/L(h) fluid 13.20×10^{-4} and 13.00×10^{-4} mg/(cm^2*sec), respectively. The flux with the TC/L(h) fluid in the 200 ml vessel was 13.81×10^{-4} mg/(cm^2*sec) which is slightly higher than the flux in the GC/L(h) fluid. The solubility of hydrocortisone was 0.59 ± 0.01 mg/ml in the TC/L(h) fluid.

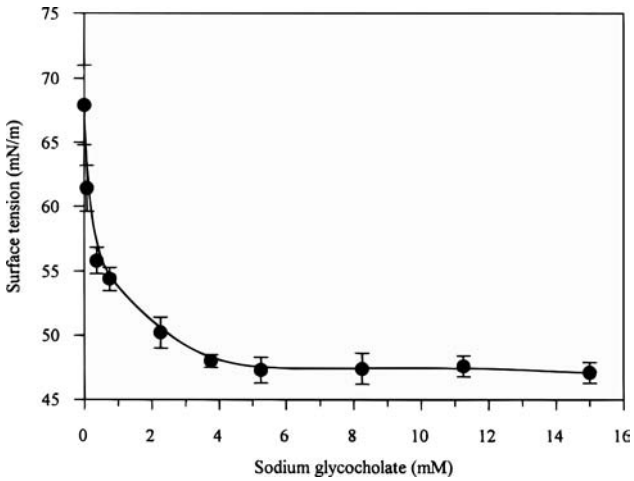

Fig. 5. Dependence of surface tension of sodium glycocholate solutions with concentrations from 0 to 15 mM. This curve includes both the results from the fluids without hydrocortisone and from the fluids saturated with hydrocortisone; $n \geq 6$, ± s.d.

Surface Tension

The surface tensions of the simulated fluid containing 0–15 mM GC with and without saturation of hydrocortisone are illustrated in Fig. 4. There was no difference between the solutions with and without hydrocortisone. The CMC was calculated to be 0.6 mM.

DISCUSSION

Human vs. Simulated Fluids

One purpose of *in-vitro* dissolution testing of pure drug substances is to predict *in-vivo* performance. The results obtained in the HIFs indicate that the solubility of hydrocortisone measured in one of the fasted state SIFs is closer to the solubility in the HIFs than that in buffer, i.e. they are better media for evaluating the intestinal solubility. The disk dissolution rate results suggest, however, that the buffer is an appropriate dissolution medium.

Bønløkke et al. (12) found that the *in vivo* intestinal dissolution of carbamazepine particles (log P 2.45) was faster than the *in vitro* dissolution in a SIF containing 3 mM bile salts. Furthermore, the lack of correlation between the bile salt content in the human perfusates and the dissolution rate suggested that the dissolution of carbamazepine was affected by other factors.

Solubility

Micelles are present in the fasted state SIFs since the concentration of GC is above the measured CMC. This is supported by the increase in hydrocortisone solubility when compared with buffer. When lecithin was added to a bile salt solution, the CMC decreases supporting our suggestion that micelles are present in the fasted state simulated fluid containing both GC and lecithin (7).

The solubility of hydrocortisone was 0.54 $\pm$ 0.03 mg/ml in the GC(h) fluid which is consistent with the literature (7,22). The solubility of hydrocortisone in both GC/L(h) and TC/L(h) fluids were 0.59 $\pm$ 0.01 mg/ml which also agrees with previously reported data (7). These solubility results together with data from rotating disk experiments clearly showed that there was no difference between the two bile salts.

The observed correlation between pH and the solubility of hydrocortisone in the HIFs lead to solubility measurements at three different pH levels. The results, however, did not demonstrate that the solubility is influenced by the pH in an aqueous solution. Hence, the effect seen in the HIFs could be due to a pH effect on the unknown intestinal surfactants and/or other components of the human fluids resulting in the increased hydrocortisone solubility.

Flux and Diffusion Coefficients

The micellar diffusion coefficients D_{DLS} of 0.83–2.01 $\times$ 10^{-6} cm^2/sec for GC(h) and GC/L(h) solutions are in agreement with the D_{mic} of 1.38 $\times$ 10^{-6} cm^2/sec obtained by Naylor et al. in rotating disk experiments with TC/L solutions (7). The increasing solubilities shown in Fig. 1 cannot account for the increasing flux values. Since the flux from hydrocortisone disks was the same in buffer and a 0.5 mM GC solution, wetting effects can neither explain the increasing flux values in the SIFs in Fig. 1.

While the D_{exp} of 4.30 $\pm$ 0.35 $\times$ 10^{-6} cm^2/sec for hydrocortisone is in accordance with the findings of Bakatselou et al. (23) of 4.73 $\times$ 10^{-6} cm^2/sec in 0.1 N NaCl, the values of D_{mic} found for the SIFs are much higher than the corresponding values of D_{DLS} and significantly higher than the D_{exp} value for hydrocortisone in buffer. It is unlikely that this discrepancy can be explained by a high payload of hydrocortisone in each micelle because the aggregation number is 3–4 for GC and TC micelles and 48 for GC/L (3:1) mixed micelles (24–26). The ratio of the concentration of intestinal surfactant molecules to micellar concentration of hydrocortisone is 15 for the GC(1), 20 for the GC/L(1) and approximately 30 for all the fed state SIFs. Furthermore, the DLS measurements showed the same size of micelles with and without hydrocortisone. The size of the micelles were in accordance with the literature (25,26).

It is more likely that the flux from the hydrocortisone disks is influenced by a failure in meeting the fluid-flow and mass-transport requirements for a rotating disk system (27). It was ensured that the diameter of the vessel containing the dissolution medium had no effect upon the observed flux and diffusivity. It might therefore be the design of the rotating disk assembly, in particular the diameter of the shaft and the height of the diskholder that causes the error. According to Fig. 1 and Table 3 the effects of the design contributes to the flux together with the solubility effects of hydrocortisone. These aspects of estimating the micellar diffusivity are subject for further investigations.

Studies of the dissolution behaviour of more lipophilic drugs than hydrocortisone in human and simulated intestinal fluids are presently conducted in order to gain more information about the desired properties of media for in vitro dissolution testing.

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