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Bile salt composition is secondary to bile salt concentration in determining hydrocortisone and progesterone solubility in intestinal mimetic fluids

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ABSTRACT

Simulated intestinal fluids (SIFs) used to assay the solubility of orally administered drugs are typically based on a single bile salt; sodium taurocholate (STC). The aim of this study was to develop mimetic intestinal fluids with a closer similarity to physiological fluids than those reported to date by developing a mixed bile salt (MBS) system (STC, sodium glycodeoxycholate, sodium deoxycholate; 60:39:1) with different concentrations of lecithin, the preponderant intestinal phospholipid. Hydrocortisone and progesterone were used as model drugs to evaluate systematically the influence of SIF composition on solubility. Increasing total bile salt concentration from 0 to 30 mM increased hydrocortisone and progesterone solubility by 2- and ~25-fold, respectively. Accordingly, higher solubilities were measured in the fed-state compared to the fasted-state SIFs. Progesterone showed the greatest increases in solubility in STC and MBS systems (2–7-fold) compared to hydrocortisone (no significant change; $P > 0.05$) as lecithin concentration was increased. Overall, MBS systems gave similar solubility profiles to STC. In conclusion, the addenda of MBS and lecithin were found to be secondary to the influence of BS concentration. These data provide a foundation for the design of more bio-similar media for pivotal decision-guiding assays in drug development and quality control settings.

1. Introduction

A key tenet of the biopharmaceutical classification system is drug solubility (Amidon et al., 1995). It is well recognised, however, that the use of simple "aqueous solubility" to predict solubility in the intestine has limitations when poorly water-soluble drugs are considered (Dressman et al., 2007). Similar issues arise in dissolution testing, which is a core methodology in the development and compendial assessment of solid oral dosage forms. Dissolution testing provides an *in vitro* measure of the release of drugs from their dosage forms and typically employs pH 6.8 phosphate buffer to represent intestinal secretions. In contrast, intestinal fluid comprises a complex mixture of components, including bile (containing bile salts, enzymes and lipids), carbohydrates, fats, proteins and any ingested substances (Dressman et al., 1998).

Human bile salts can be classified into three groups according to their conjugation with amino acids and their degree of hydroxylation (Hoffman and Mysels, 1987). These classes are the trihydroxy conjugated (predominately taurocholate and glycocholate), dihydroxy conjugated (e.g., glycodeoxycholate (GDC), glycochenodeoxycholate, taurochenodeoxycholate and taurodeoxycholate) and unconjugated forms (e.g., deoxycholate (DC), cholate, and chenodeoxycholate). Bile salts are surface active and form mixed micelles and other colloidal phases with phospholipids in the intestinal fluid. Simulated intestinal fluids (SIFs) have been used to attempt to reproduce this environment *in vitro* and such studies have demonstrated the influence of bile salts on drug solubility (Mithani et al., 1996; Wiedmann and Kamel, 2002; Dressman et al., 2007) and dissolution (Galia et al., 1998; Dressman et al., 1998; Luner and Kamp, 2001).

The first SIFs were proposed by Dressman et al. (1998) as biorelevant media to represent the fasted (Fa) and fed (Fe) states; these contained the preponderant bile salt in human bile, sodium taurocholate (STC), combined with the major phospholipid, lecithin (Galia et al., 1998). The composition of the fasted-state SIF (FaSSIF) was based upon *in vivo* conditions in the fasted human intestine where the concentration of bile salt ranges from 3 to 5 mM and the ratio for bile salt:phospholipid ranges between 2:1 and 5:1 with an average of 3:1. The FaSSIF combines STC and lecithin (L) at concentrations of 3 mM and 0.75 mM, respectively, in phosphate buffer (pH 6.8). STC and L were used at higher concentrations to represent the Fe state (15 mM and 3.75–4 mM, respectively) with an acetate buffer used to control the pH of the medium (FeSSIF). Since these basic SIF compositions were first established, a number of modifications have been advocated (Table 1), the most extensively used of which were made by Jantratid et al. (2008). These authors

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Table 1
Original simulated intestinal fluids devised Dressman et al. (1998) (Galia et al., 1998) and the major variations in compositions proposed subsequently.

Development of SIF	Purpose	Composition	Reference
Original simulated intestinal fluid	Physiological media for drug dissolution purposes	Fasted- and fed-state SIFs based upon sodium taurocholate and lecithin	Galia et al. (1998), Dressman et al. (1998)
More biosimilar intestinal mimetic fluids	More representative bile salt composition	The substitution of the major bile salt, taurocholate, with the other bile salts such as glycocholate or taurodeoxycholate. Although in the pH range of the small intestine only the taurocholate is fully ionised.	Pedersen et al. (2000a,b), Luner and Kamp (2001), Sunesen et al. (2005), Ilardia-Arana et al. (2006), Boni et al. (2007), Jantratid et al. (2008), Soderling et al. (2010)
	The inclusion of lipid digestion products	Addition of lipids and lipid digestion products (medium and long chain triglycerides, fatty acids and monoglycerides; oleic acid, sodium oleate, glycerol monooleate, monoolein) or lysophosphatidylcholine.	Pedersen et al. (2000b), Ilardia-Arana et al. (2006), Sunesen et al. (2005), Jantratid et al. (2008), Kleberg et al. (2010), Lind et al. (2007)
	A different buffer system	The substitution of the phosphate buffer with the physiological bicarbonate buffer.	Boni et al. (2007)
		The use of maleate instead of phosphate buffer	Jantratid et al. (2008), Kleberg et al. (2010)
	SIFs representing temporal postprandial (early, middle and late) changes in the small intestine	STC concentration range from 4.5 to 10 mM, lecithin concentration range from 0.5 to 3 mM, addition of glycerol monooleate and sodium oleate.	Jantratid et al. (2008)
More convenient or more economic compositions	Use of crude bile salt extracts	The use of bile salt extracts or the substitution of pure sodium taurocholate with sodium taurocholate from animal origin. Problems with batch to batch variability in types of bile salts and relative composition.	Vertzoni et al. (2004), Sunesen et al. (2005)
	Use of alternative phosphatidylcholine sources	The use of partially hydrolysed soybean phosphatidylcholine (less saturated fatty acids) as a substitute for egg phosphatidylcholine.	Vertzoni et al. (2004), Sunesen et al. (2005)
SIFs that are biocompatible with intestinal cells or tissue	For use with 'absorption models' in permeability evaluations	Substitution of acetate and phosphate buffers with Hanks Balanced Salt solution or Leibovitz's L-15 nutritional medium, addition of varying bile salt concentrations (5–15 mM), addition of lysophosphatidylcholine, oleic acid and monoolein	Patel et al. (2006), Lind et al. (2007)

updated the FaSSIF by decreasing the phospholipid concentration from 0.75 mM to 0.2 mM, causing the bile salt:phospholipid ratio to increase to 15 (FaSSIF-V1). The buffer was also changed from phosphate to maleate reducing the osmolality closer to those of *in vivo* values (180 mOsm/kg). The FeSSIF was also updated in a similar fashion with the addition of lipid digestion products (Jantratid et al., 2008). More recently Kleberg et al. (2010) have advocated the use of a fasted SIF with a bile salt concentration of 2.5 mM, bile salt to lecithin ratio of 4 dissolved in a Trizma maleate buffer, termed Copenhagen fasted. The Copenhagen fed SIF uses bile salt concentrations between 5 and 20 mM and also incorporates lipid digestion products. Despite attempts to design some of these fluids to be more physiologically relevant, all of them have been based on the use of a single bile salt, or a crude mixture of bile salts that show batch variability in bile salt concentration and composition (Sunesen et al., 2005) and there has been no attempt to employ a SIF containing the three main classes of bile salts in a ratio representative of human bile.

The aim of this study was to investigate whether SIFs with closer similarity to physiological fluids than those devised previously, i.e. with more physiologically relevant bile salt composition, would impact on drug solubility. A mixed bile salt (MBS) system containing TC, GDC and DC as representative of the predominant bile salts in each of the major bile salt classes at a ratio of 60:39:1, respectively, was selected to represent the composition of bile salts measured in intestinal fluid, typical of that sampled from six fasted healthy volunteers (Perez de la Cruz Moreno et al., 2006). Bile salt concentrations were fixed at 3 mM for the fasted-state and 15 mM for the fed-state, as employed in the earlier studies (Dressman et al., 1998; Galia et al., 1998). The solubility of two non-ionisable steroid drugs of different lipophilicity (hydrocortisone and progesterone) was measured in STC and MBS systems. The role of bile salt concentration, pH and bile salt:lecithin ratio on drug solubility was investigated systematically to study a pertinent range of compositions and allow the relative impacts of these variables to be evaluated.

2. Materials and methods

2.1. Materials

Hydrocortisone and progesterone were purchased from Fluka Riedel-deHaen, Germany with a purity of ≥97.0% (HPLC grade). Sodium taurocholate (STC), sodium glycodeoxycholate (SGDC), sodium deoxycholate (SDC) and 2-(N-morpholino)ethane-sulfonic acid (MES) (1 M) were all purchased from Sigma Aldrich, UK. Lecithin (Epikuron 200, phosphatidylcholine enriched fraction of soybean lecithin) was purchased from Cargill Europe Limited, UK. Sodium hydroxide (NaOH) and potassium chloride (KCl), both analytical grades, were obtained from BDH Chemicals, Poole, UK. Potassium phosphate monobasic (KH_2PO_4, analytical grade) was obtained from Acros Organics, USA. Acetic acid (HPLC grade) and methanol (HPLC grade) were obtained from Rathburn Chemicals Limited, UK and Fisher Scientific, UK, respectively.

2.2. Preparation of SIFs and bile salt solutions

Since there is no strict definition of what a simulated intestinal fluid is, for the purposes of this study all systems containing bile salt(s) and lecithin are termed 'SIFs'. Those systems without lecithin will be termed as 'bile salt solutions' implicit in the knowledge that *in vivo* the intestinal composition is more complex than just bile salts alone. The latter solutions were used to clarify the

Table 2
Composition of the bile salt solutions and simulated intestinal fluids (SIFs) used for the saturation solubility studies.

SIF code	pH	STC (mM)	SGDC (mM)	SDC (mM)	Lecithin (mM)
MBS-Fe	5.0	9.0	5.85	0.15	–
MBS-Fe2	5.0	9.0	5.85	0.15	7.5
MBS-Fe3	5.0	9.0	5.85	0.15	5
MBS-Fe4	5.0	9.0	5.85	0.15	3.75
MBS-Fe5	5.0	9.0	5.85	0.15	3
MBS-Fa	6.5	1.8	1.17	0.03	–
MBS-Fa2	6.5	1.8	1.17	0.03	1.5
MBS-Fa3	6.5	1.8	1.17	0.03	1
MBS-Fa4	6.5	1.8	1.17	0.03	0.75
MBS-Fa5	6.5	1.8	1.17	0.03	0.6
Va	7.2	3.19	0.96	0.02	–
Vb	7.0	1.64	1.44	0.02	–
Vc	7.4	2.85	0.97	0.03	–
Vd	6.9	0.99	1.01	0.02	–
Ve	6.8	2.02	0.95	0.01	–
Vf	6.4	1.48	0.61	0.01	–
STC-Fa	6.5	3.0	–	–	–
STC-Fa5	6.5	3.0	–	–	0.6
STC-Fa4[a]	6.5	3.0	–	–	0.75
STC-Fa3	6.5	3.0	–	–	1
STC-Fa2	6.5	3.0	–	–	1.5
STC-Fe	5.0	15.0	–	–	–
STC-Fe5	5.0	15.0	–	–	3
STC-Fe4[a]	5.0	15.0	–	–	3.75
STC-Fe3	5.0	15.0	–	–	5
STC-Fe2	5.0	15.0	–	–	7.5
P-Fa4[b]	6.5	3.0	–	–	0.75
P-Fe2[b]	6.0	15.0	–	–	7.5

SGDC, sodium glycodeoxycholate; SDC, sodium deoxycholate; MBS, mixed bile salt; STC, sodium taurocholate; Fa2, Fa3, Fa4 & Fa5, fasted SIFs with bile salts:lecithin ratios of 2:1, 3:1, 4:1 & 5:1, respectively; Fe2, Fe3, Fe4 & Fe5, fed SIFs with bile salts:lecithin ratios of 2:1, 3:1, 4:1 & 5:1, respectively; Fa & Fe, fasted and fed SIFs without lecithin; Va–Vf, volunteers solutions, based on human intestinal fluids of fasted volunteers (Perez de la Cruz Moreno et al., 2006); Fed systems, contain 144 mM acetic acid, 203.89 mM KCl and made to pH with NaOH; Fasted systems, contain 28.66 mM KH_2PO_4, 103.29 mM KCl and made up to pH with NaOH. Solutions/SIFs made up to 1 l with deionised water. Patel SIFs, contain no acetic acid or KH_2PO_4 but made up to 1 l with HBSS buffer and pH adjusted with MES (1 M) solution.

[a] Equivalent to Galia-fed & fasted (Galia et al., 1998).
[b] Patel-fed & fasted (Patel et al., 2006).

function of bile salt(s) present within what are now termed as SIFs or biorelevant fluids.

Bile salt solutions, containing either STC or MBS, were prepared by dissolving the appropriate bile salt(s) in either acetate buffer (pH 5.0) or phosphate buffer (pH 6.5) to produce final concentrations ranging from 0 to 30 mM. A series of solutions were also prepared as above but with inclusion of lecithin at a fixed ratio of BS:L of 4:1 across this bile salt concentration range. SIFs (STC as lone bile salt and MBS) were prepared and their effect on drug solubility was compared against reference SIFs or human bile salt solutions reported in the literature, namely the fasted (Fa) and fed (Fe) SIFs of Dressman et al. (1998) and Patel et al. (2006), and the human intestinal fluids of Perez de la Cruz Moreno et al. (2006), respectively. These systems were prepared according to the formulae in Table 2 with the numbers following the Fa or Fe designation indicating the BS:L ratio. In general all SIFs and solutions were prepared by adding BS(s) to a small amount of aqueous phase and the required final amounts of L (if present) and inorganic salts (if present) added. The mixtures were stirred until complete dissolution of all ingredients had occurred. The fluid pH was adjusted by the addition of NaOH or MES according to the required composition before making up to volume. These were then stored at −20 °C until required for use.

2.3. Solubility studies

The saturated solubility of hydrocortisone and progesterone was performed by adding excess drug (5 mg) to a 1.5 ml volume of the SIF or solution to be tested. Vials were shaken at 150 rpm and at 37 °C in an orbital incubator (Stuart Scientific Co. Ltd., UK) for 48 h. At the end of the incubation time the drug suspension was transferred into microcentrifuge vials and centrifuged at 2000 × *g* for 20 min at 37 °C (Spectrafuge Mini Centrifuge, Labnet, UK). The supernatant was then diluted with the corresponding pre-warmed solution at 37 °C followed by injection onto the HPLC column.

2.4. HPLC analysis of hydrocortisone and progesterone

The HPLC method for the analysis of hydrocortisone and progesterone was a modification of a method described previously (Pedersen et al., 2000a). The mobile phase was composed of methanol:deionised water (60:40, v/v) for hydrocortisone and (80:20, v/v) progesterone, respectively. A Spectraphysics HPLC with UV detector set at a wavelength of 248 nm for hydrocortisone and 254 nm for progesterone (Spectra-Physics, USA) with an analytical column (Luna C18 (2) 100A (5 μm) 150 mm × 4.6 mm) fitted with a guard column (Luna 4 mm × 3 mm (Phenomenex, UK)) was employed. A flow rate of 1 ml/min, with injection volume of 10 μl and an elution time of 10 min, was used throughout the study. Hydrocortisone was eluted with a retention time of 5.6 ± 0.01 min and its limits of quantification (LOQ) and detection (LOD) were 4.5 ± 1.7 and 1.5 ± 0.6 μg/ml, respectively. The retention time for progesterone was 4.9 ± 0.01 min and the corresponding LOD and LOQ values were 0.9 ± 0.6 and 2.8 ± 1.9 μg/ml, respectively. A calibration curve was constructed for both drugs, using one specified SIF (Dressman et al., 1998) and the calibration curves of both drugs were shown to be linear ($r^2 > 0.999$).

2.5. Data analysis

All values were expressed as mean ± SD. Statistical evaluation of data was performed with SPSS® (version 15.0, SPSS Inc., Chicago, IL, USA). Solubility data were compared using either *t*-test or one-way analysis of variance (ANOVA) followed, where appropriate, by the Tukey's *post hoc* test. In all cases, a difference was considered significant at $P \leq 0.05$.

3. Results

3.1. Effect of reference fluids on drug solubility

The solubility of hydrocortisone and progesterone was assessed in SIFs developed by previous workers containing a single bile salt i.e. STC (Galia et al., 1998; Patel et al., 2006) and in solutions based on *in vivo* data from human volunteers (Perez de la Cruz Moreno et al., 2006).

The solubility of hydrocortisone and progesterone in SIFs representing the Fe state were significantly higher than those representing the Fa state (Fig. 1). This effect was more apparent with progesterone than hydrocortisone. Moreover, there was a significant difference between the solubility in the two reference Fe SIFs (STC-Fe4 and P-Fe2) for both drugs.

For the human volunteer data the total bile salt concentration did not exceed 4.17 mM, which is equivalent to fasted-state conditions (Table 2). Further examination of this data (Table 3) showed significant differences ($P < 0.05$) between the solubility of hydrocortisone in Vb and the Va, Vc and Vf solutions. For progesterone, there was a significant difference between its solubility in Vd, in which progesterone solubility was lowest, and all of the other volunteer bile salt solutions ($P < 0.05$). However, there was only a 4.6%

Table 3
Saturation solubility (mg/ml) values of hydrocortisone and progesterone in volunteers simulated intestinal fluids (data represent mean ± SD, $n = 5$).

SIF	Hydrocortisone	Progesterone
Va	450.88 ± 11.67	4.43 ± 0.17
Vb	466.23 ± 3.31	4.31 ± 0.38
Vc	451.72 ± 5.53	4.20 ± 0.23
Vd	461.42 ± 3.60	3.19 ± 0.15
Ve	455.69 ± 10.30	3.78 ± 0.08
Vf	445.62 ± 4.25	3.80 ± 0.31

variation in the solubility of hydrocortisone in the bile salt solutions corresponding to the composition from the six volunteers, and virtually no change in solubility as the total bile salt concentration in the six samples was varied. In contrast there was a 38.9% variation in progesterone solubility, with a greater correlation between solubility as total bile salt concentration was increased, compared to hydrocortisone ($R^2 = 0.0146$ *vs.* 0.6812 for hydrocortisone and progesterone, respectively).

3.2. Effect of pH, lecithin and bile salt concentration on drug solubility

The solubility of hydrocortisone and progesterone in STC and in MBS solutions was determined in the presence (BS:L ratio 4:1) and also in the absence of lecithin at two different pH values, 6.5 and 5.0 (data not shown), which represent the pH of fasted and fed state, respectively.

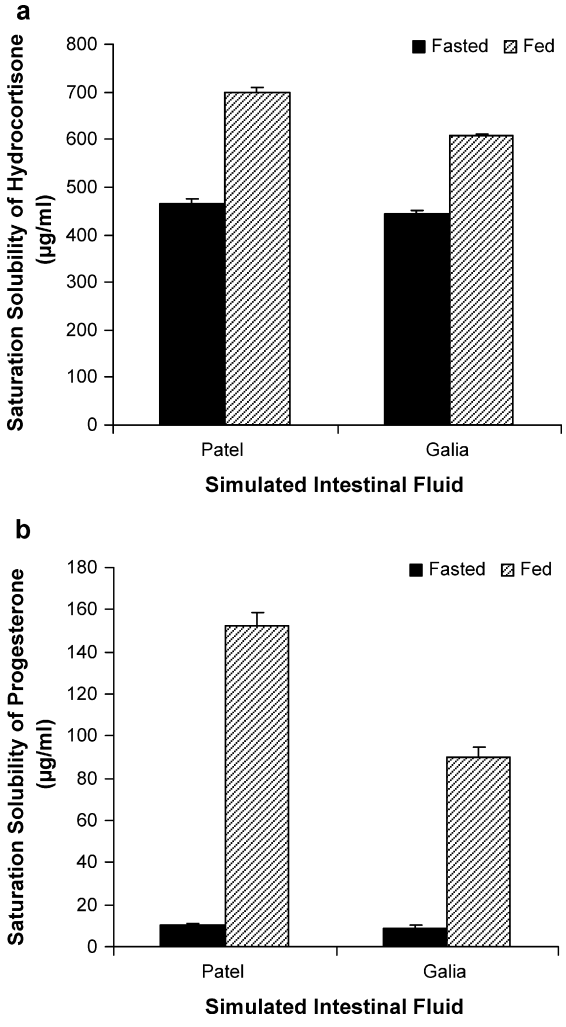

Fig. 1. Saturation solubility of (a) hydrocortisone and (b) progesterone in the reference simulated intestinal fluids (SIFs) Dressman (STC-Fa4 and STC-Fe4) and Patel (P-Fa4 and P-Fe2) representing fasted and fed states (data represent mean ± SD, $n = 5$).

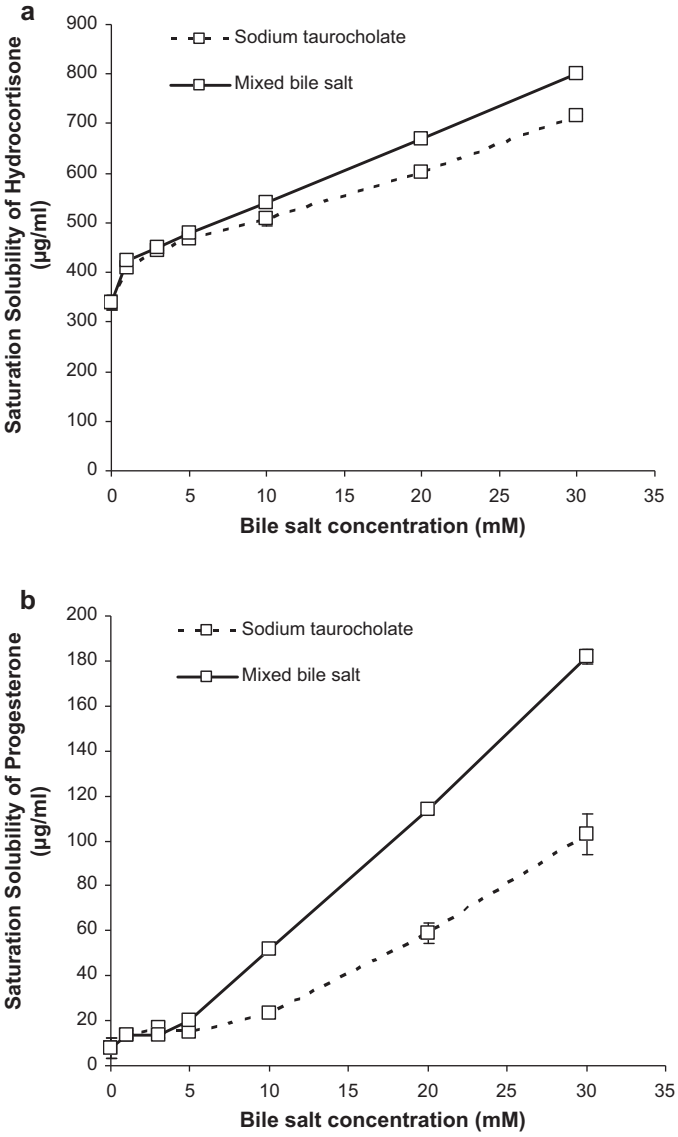

Fig. 2. Saturation solubility of (a) hydrocortisone and (b) progesterone in sodium taurocholate (STC) and MBS solutions at pH 6.5 plotted as a function of bile salt concentration, in the absence of lecithin (data represent mean ± SD, $n = 5$).

3.2.1. Effect of bile salt concentration

In the bile salt solutions, the solubility of hydrocortisone increased almost 2-fold as the STC concentration increased from 0 to 30 mM and 2.4-fold as the MBS concentration was increased (Fig. 2a). The solubility of progesterone in the STC solutions was increased up to 19-fold and approximately 33-fold in the MBS solutions (Fig. 2b) Changing the pH (data not shown) did not have a major effect on the solubility of both drugs.

3.2.2. Effect of addition of lecithin to bile salt solutions

The incorporation of lecithin at a bile salt:lecithin ratio of 4:1 generally did not greatly change the solubility of hydrocortisone (Fig. 3a) compared to that obtained without lecithin over the concentration range of 0–30 mM bile salt in both STC and MBS systems. The hydrocortisone solubility again increased approximately up to 2-fold in STC systems and ~2.4-fold in the MBS system as the bile

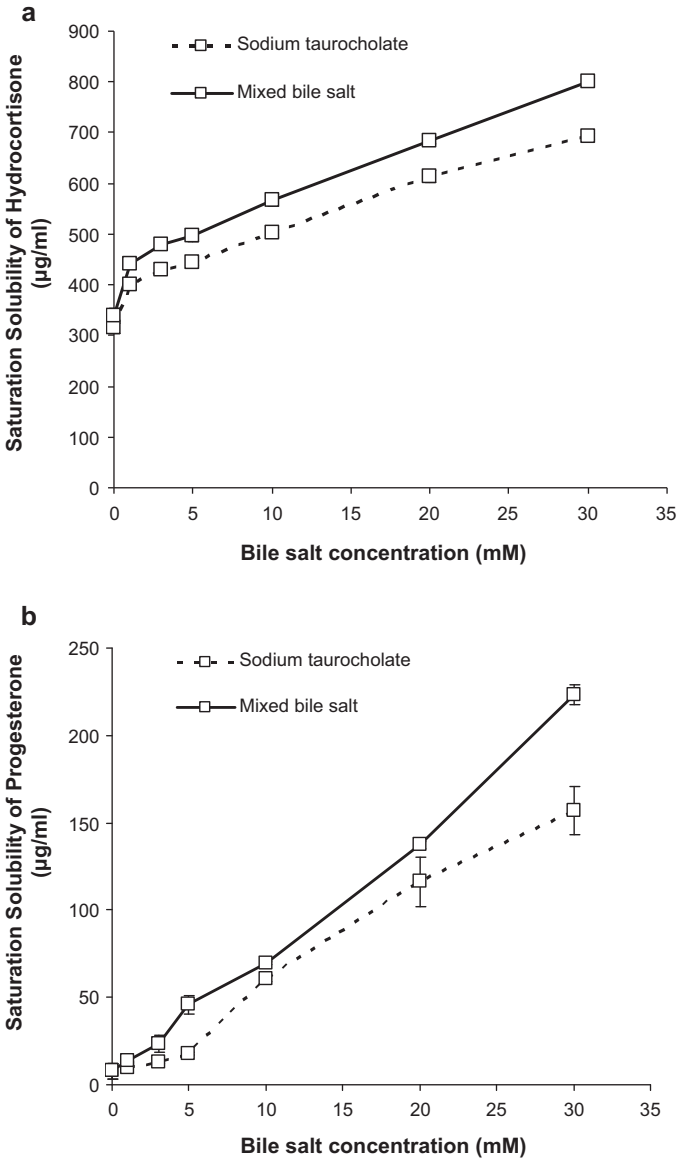

Fig. 3. Saturation solubility of (a) hydrocortisone and (b) progesterone in sodium taurocholate (STC) and MBS SIFs at pH 6.5 plotted as a function of bile salt concentration, in the presence of lecithin at a ratio of 4:1 bile salt:lecithin (data represent mean ± SD, $n = 5$).

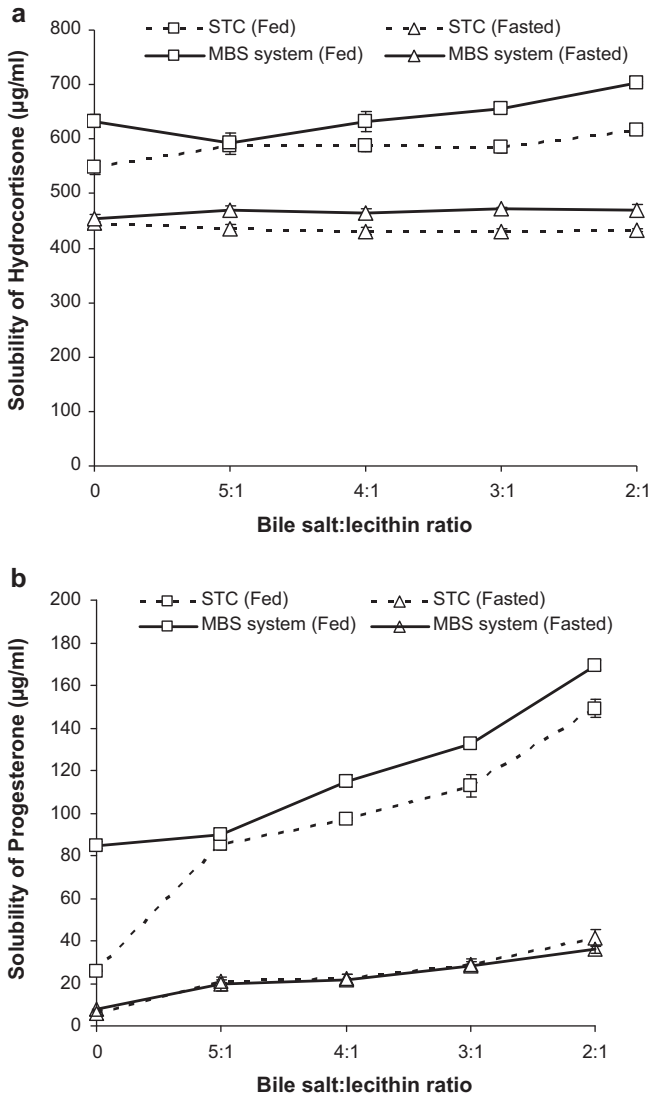

Fig. 4. Saturation solubility of (a) hydrocortisone and (b) progesterone in sodium taurocholate solutions (STC) and MBS SIFs at different ratios of bile salt:lecithin under fed and fasted conditions (data represent mean ± SD, $n = 5$).

salt concentration increased to 30 mM. The solubility of progesterone (Fig. 3b) increased approximately up to 29-fold in the STC systems and up to 38-fold in the MBS systems over the concentration range. Changing the pH value from 5.0 to 6.5, in accordance with previously reported pH changes between the Fe and Fa states, in either the MBS or STC systems had a minor effect on the solubility of both drugs (results not shown).

3.3. Effect of increasing L concentration under simulated fasted and fed conditions

The effect of varying lecithin concentration on the solubility of hydrocortisone and progesterone in STC and MBS systems was studied under conditions simulating Fe (pH 5.0, 15 mM total bile salt) and Fa (pH 6.5, 3 mM total bile salt) states. There was no significant change in the solubility of hydrocortisone as the lecithin concentration increased in the Fa STC and MBS SIFs ($P > 0.05$), whilst in the Fe SIFs only a slight increase in solubility was observed (Fig. 4a). However, progesterone showed an appreciable increase in solubility (up to 7-fold) in the Fa STC SIF and up to 5-fold in the Fa MBS SIF, a 6-fold increase in the Fe STC SIF and a 2-fold increase in the Fe MBS SIF as the lecithin concentration increased (Fig. 4b).

4. Discussion

Most utilized SIFs contain a single BS and these are used mainly for solubility, dissolution or permeability studies. It is known that the human bile is composed of a mixture of different bile salts, which can be grouped into three main classes; tri- and dihydroxy conjugated and unconjugated BSs. Therefore, the design of the new SIFs was based on the incorporation of TC, GDC and DC as the representative or the most predominant BS of each of these groups in one SIF, which was based on clinical data of HIFs (Perez de la Cruz Moreno et al., 2006).

The aqueous solubility for hydrocortisone is reported as 326 µg/ml (Mithani et al., 1996) and that for progesterone as 11.4 µg/ml (Roseman, 1972), both at 37 °C. In this study the respective buffer solubilities were determined to be 314 and 338 µg/ml for hydrocortisone and 8.1 and 9.8 µg/ml for progesterone in acetate

and phosphate buffer, respectively. The log P of hydrocortisone and progesterone are 1.6 and 3.87, respectively (Memisoglu-Bilensoy et al., 2006). These physicochemical properties of the two compounds suggest that hydrocortisone and progesterone have moderately and highly lipophilic properties, respectively. The two neutral steroids were thus chosen as model compounds to be representative of poorly water soluble drugs to investigate whether any differences in drug solubility might be apparent in the newly designed SIFs in comparison to SIFs containing STC alone.

4.1. Effect of SIFs on solubility

The solubility of hydrocortisone and progesterone was determined in SIFs used previously in either dissolution studies (Galia et al., 1998) or *in vitro* drug absorption experiments (Patel et al., 2006). The observed increase in solubility of both drugs in the Fe SIFs compared to the Fa SIFs was not unexpected since the amount of BSs in the Fe state (15 mM) was higher than in the Fa state (3 mM). Similar results have been published by other authors for a range of drugs (Pedersen et al., 2000a; Edmund et al., 2002; Levis et al., 2003; Kalantzi et al., 2006; Schamp et al., 2006; Bard et al., 2008). Moreover, the Fe state Patel SIF containing a 2:1 BS:L ratio, which was shown to be physiologically relevant as well as compatible with Caco-2 cells, induced a higher solubility of both drugs in comparison with Galia SIF (4:1). A higher L content therefore appeared to increase the solubility of the two steroids in the BS solution, although the increase was greater for progesterone than hydrocortisone.

The solubility of hydrocortisone and progesterone in the volunteer compositions was not increased markedly compared to that attainable in buffer solutions. This may have been due to the low total bile salt concentration (<5 mM) present in these solutions and the absence of lecithin, the importance of which is discussed later. The more positive correlation seen between the progesterone solubility and the total bile salt concentration compared to hydrocortisone might indicate that the concentration of bile salt affects the solubility of the more lipophilic progesterone compared to the less lipophilic hydrocortisone, and that composition is less important. However, drug solubility is dependent on the type of bile salt used. A study by Martin et al. (1978) showed that the solubility of progesterone in different solutions of STC, SGDC, SDC and STDC increased at concentrations up to 128 mM and that the solubilisation of progesterone was in the order of SDC > SGDC ≈ STDC > STC. This according to the authors might be due to the increased hydroxylation of the STC and the conjugation of the SGDC and STDC. However, there has been no definitive study examining the role of different mixtures of bile salts on drug solubility. Nevertheless, it can be inferred that the behaviour of a bile salt mixture is dependent upon bile salt structure as recognised by the development of a hydrophobic index (HI) of the mixture of bile salts (HI*m*), which is a quantitative measure of the hydrophilic–hydrophobic balance of bile salt(s) (Heuman, 1989):

$$\mathrm{HI}m = \sum_{x=1}^{n} \mathrm{HI}x\mathrm{F}x$$

where HI*x* is the hydrophobic index of the pure bile salts and F*x* is the molar fraction of bile salt *x* in a mixture of *n* different bile salts.

The determination of HI is based around the bile salt taurocholate because the behaviour of this conjugate is not affected by pH. Thus taurocholate has an HI of zero and any bile salt more hydrophobic than taurocholate will have an HI > 0. For example, lithocholate is the most hydrophobic of the major bile salts in human bile and has an HI of 1.00. Less hydrophobic bile salts have HI values <0 (negative values) an example being ursodeoxycholate (HI = −0.47) (Heuman, 1989). The HI of SDC, SGDC and STC are 0.72, 0.65 and 0.00, respectively (Heuman, 1989). The HI of the volunteer solutions, Va, Vb, Vc, Vd, Ve and Vf were calculated to be 0.15, 0.31, 0.17, 0.34, 0.21 and 0.19, respectively indicating that compared to taurocholate, these mixtures were more hydrophobic and were expected to solubilise lipophilic drugs better. However, for both hydrocortisone and progesterone there was poor correlation between these HIs and solubility suggesting that other factors may account for the differences in drug solubility between these mixtures, for example the bile salt concentration or solution osmolality.

4.2. Effect of pH, L and BS concentration

Changing the pH value from 5.0 (data not shown) to 6.5, in accordance with previously reported pH changes between the Fe and Fa states in either the MBS or STC systems did not have a significant effect on the solubility of both drugs. These findings concur with previous data in which the solubility of hydrocortisone was reported as not being influenced by pH (Pedersen et al., 2000a). Both hydrocortisone and progesterone are uncharged, and it can be concluded that the change in pH had no influence upon the solubilisation capacity of either system.

Measuring the solubility of a drug in the STC system and at higher concentrations of BS may provide an appropriate simple model system which might be predictive of the extent of solubilisation of drug in the intestine. However, the addition of L might better reflect the solubility in the intestinal composition, since mixed micelles will be formed with the BS (Wiedmann and Kamel, 2002). In general the solubility of hydrocortisone and progesterone in STC solutions increased beyond a concentration of 5 mM of STC, which is considered to be the critical micelle concentration (CMC) of STC (Wiedmann and Kamel, 2002). In the case of hydrocortisone, there was a modest increase in the solubility in both single and mixed bile salt systems when L was included with STC. In contrast, the effect of both BS concentration as well as addition of L on progesterone solubility was greater compared to hydrocortisone. This is likely to be attributable to the higher lipophilicity of progesterone, the expected strong hydrophobic interactions of this drug with the lipophilic interior of the mixed BS:L micelles, and also the increased size of such micelles compared to simple micelles (de Castro et al., 2001). Moreover, it is likely that due to the similarity in steroidal structure with BSs that the progesterone molecules could substitute with BS molecules with only slight modification to the structure of the micelle, due to the presence of hydrophilic groups at both ends of the progesterone molecule (Martin et al., 1978). This is likely to promote the solubility of progesterone to a greater extent than the inherently more soluble hydrocortisone.

The presence of more than one BS as in the MBS system enhanced the solubility of progesterone to a greater extent than the SBS system, and this could be due to a lowering of the CMC, which can be inferred from the data in Figs. 2b and 3b. This lowered CMC is likely to be due to the substitution of some of the STC with more hydrophobic BSs, the hydrophobic index of SDC, SGDC and STC being 0.72, 0.65 and 0.00, respectively (Heuman, 1989). The altered BS composition was more apparent for progesterone compared to hydrocortisone solubility (33- and 38-fold increase *vs.* 2.4-fold increase in the absence and presence of lecithin for progesterone *vs.* hydrocortisone, respectively).

4.3. Effect of varying L concentration

The solubility of a range of drugs is reported to be increased as a function of increasing L content (Luner et al. 1994). However this effect was not found to be marked in the case of hydrocortisone (Fig. 4) which might be a consequence of its relative hydrophilicity in comparison to progesterone. The effect of L content was clearly more pronounced with progesterone in the Fe state of both

single and MBS systems than with hydrocortisone. Pedersen et al. (2000a) showed that the increase in solubility of hydrocortisone in Fe state SIFs containing L was more than the Fa state, and this was attributed to the greater amount of BS in Fe SIFs acting to solubilise a greater amount of the L within mixed micelles compared to that achievable by concentrations of BS more representative of the Fa state, where less number of such micelles exist in equilibrium with other, simpler aggregates. A small increase in solubility of the more lipophilic progesterone was also obtained in the more complex systems containing MBS and lecithin in comparison to the equivalent STC systems.

On the basis of these results there would be no strong reason to advocate increasing the range of bile salts as components of SIFs from that of STC alone. This suggests that the increased complexity conferred by generating a more complex fluid with different bile salts as opposed to just one bile salt system might not lead to a marked increase in drug solubility. A study by Soderling et al. (2010) suggested from their studies that for neutral compounds one type of bile acid appeared to be enough to represent *in vivo* conditions when compared to using multiple bile salts. Soderling et al. (2010) further went on to suggest that bile salt structure had little impact on the solubilisation of poorly soluble compounds. Nevertheless these presently investigated systems and SIFs are still relatively simplistic in nature and the effect of BS composition on drug solubility could be different when more components of the digestion process are incorporated.

5. Conclusion

The present investigation has shown that it is the amount of BS as well as the presence of L in the SIF that are the major determinants of solubility in the case of the two steroidal drugs investigated (hydrocortisone and progesterone). The pH had little influence on the solubility of these drugs in SIFs tested, but this may be of importance for ionisable drugs. The presence of mixed bile salt solutions as opposed to STC alone did not have a marked effect on the solubility of either drug under the present conditions. Investigations are still continuing in order to add more physiological additives to those SIFs tested which may highlight the importance of using more complex SIFs in solubility and dissolution studies.

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