ELSEVIER

International Journal of Pharmaceutics 107 (1994) 159–166

international journal of pharmaceutics

Compared effects of synthetic and natural bile acid surfactants on xenobiotic absorption. III. studies with mixed micelles

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(Received 20 July 1993; Modified version received 6 December 1993; Accepted 20 December 1993)

Abstract

The present work deals with the effects of bile salts on absorption. It has previously been demonstrated that although they are surfactants, these salts are not able to disrupt the aqueous diffusion layer which effectively limits the absorption of the lipophilic compounds. They exhibit less ability to solubilize in their micelles when they are perfused only in the presence of the tested compounds. The present study was carried out with two types of bile salts – sodium taurocholate and sodium glycocholate – along with other compounds naturally occurring in vivo, such as lecithin and sodium oleate. In this way the hypothesis that an intrinsic mechanism is involved in promoting the effect of bile salts on absorption was assessed by simulating a physiological environment, which always shows mixed micelles of bile salts with phospholipids and fat. Correlations between absorption and lipophilicity parameters are useful for indirect quantification of this phenomenon. The enhancer effect of bile salts on the absorption of lipophilic compounds may be due to their effective absorption in the gut, which helps the disintegration of the micelles. Despite the fact that the characteristics of natural surfactants are not as good as those of synthetic surfactants for promoting absorption, a consideration of all their properties can explain all the features reported.

Key words: Mixed micelles; Bile salt; Surfactant; Absorption-partition correlation; Micellar solubilization; Stagnant diffusion layer

1. Introduction

The present work focuses on the effects of bile salts on absorption. As their property of major interest is the ability to lower surface tension, the study followed the design as in the previously reported studies on synthetic surfactants.

Natural surfactants – represented by bile salts – do not act as synthetics do, irrespective of the type we consider. This conclusion was clearly established in a previous study (Bermejo et al., 1991) and further tested in a subsequent investigation (Garrigues et al., 1994) in which the correlations found in the presence of these two types of surfactant at two levels – CMC and SMC – were compared. Briefly, the differences found were as follows:

(a) Natural surfactants at the CMC do not disrupt the aqueous boundary layer, so the

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SSDI 0378-5173(94)00009-T

representative biophysical model of the absorption process is the same as in free solution. Thus, natural surfactants do not act as promoters of the absorption of lipophilic compounds when they are perfused at the CMC. The opposite was demonstrated for synthetic surfactants.

(b) The ability to solubilize xenobiotics in their micelles is clearly lower than in the case of synthetics, at least for the tested series of compounds. Steric restrictions and a lower aggregation number are probably the reasons for this difference.

(c) Despite the fact that they increase membrane polarity as do all the surfactants assayed, there is a considerable difference in the extent of this phenomenon. Sodium taurocholate (STC) and sodium glycocholate (SGC), although less toxic to the biological system, cannot be used as absorption promoters for hydrophilic compounds, while synthetic surfactants are very effective.

We believe that, despite the work that has been done, the characterization of the effects of the bile salts is not complete. There is a point that cannot be explained in light of the theory: fat and cholesterol are absorbed in vivo only in the presence of bile salts. It has been stated that the solubilization process is responsible for this phenomenon (Simmonds, 1972; Feldman et al., 1975; Watt and Simmonds, 1984). The way to investigate the mechanism involved was, then, to simulate in vivo conditions by incorporating mixed micelles into the system. In the present study, this question is probed deeply by adding first lecithin, a representative phospholipid, to both bile salts – STC and SGC – at the SMC. On additional testing, sodium oleate (SO) was studied as a fat model only in the presence of SGC because this salt produces the strongest increase in membrane polarity.

2. Materials and methods

The methodology used in previous work (Bermejo et al., 1991) was maintained in the present study, in order to make comparisons.

Briefly, our aim was to establish correlations and comparisons between in vivo absorption parameters, i.e., absorption rate constants, and lipophilicity indexes, i.e., partition chromatographic constants.

2.1. Test compounds

As model compounds, a series of phenylalkylcarboxylic acids previously characterized under a wide range of conditions (Garrigues et al., 1990, 1992, 1994; Bermejo et al., 1991; Fabra Campos et al., 1991) were assayed.

As bile salts, the previously studied STC and SGC were used. They are model natural surfactants since they are conjugated species and are the major ones in rat and human bile, respectively (Hay and Carey, 1990). They were purchased from Sigma Co. with a purity of 98 and 99%, respectively.

To form mixed micelles, two types of compounds were tested separately. Firstly, lecithin (Lec) was used as a representative phospholipid since it is the major phospolipid in the bile of mammals. Lecithin was obtained from BDH Co. with a purity of 90%. It was assayed at 3.3 mM in the presence of 9 mM STC and SGC separately.

Sodium oleate (SO) was selected since it is a fatty acid usually present in the intestinal lumen as a result of fat digestion. In addition, it is an alkaline soap, therfore, it exhibits some characteristics of synthetic surfactants and has been used in several studies as an absorption promoter (Carey and Small, 1970, 1972; Shiau, 1981; Donovan and Carey, 1990). It was purchased from Sigma Co. with a purity of 98%. A concentration of 0.02 mM in the perfusion fluids was used as the CMC, after being experimentally determined by the ring method, and a concentration of 3.3 mM was used as the SMC, which is equivalent to the molar concentration of lecithin in the presence of 9 mM SGC.

2.2. Absorption parameters

As absorption parameters, we used the absorption rate constants determined by the in situ rat colon technique (Martín Villodre et al., 1986) on five Wistar rats per condition and acid. There-

fore, four series of experiments were compared: in the presence of STC + Lec; SGC + Lec; SGC + SO, and in the presence of sodium oleate at its CMC.

No experiments to determine the intrinsic colonic absorption rate of surfactants and modifiers were carried out, as it has been reported that it is always negligible (Kakemi et al., 1970).

2.3. Lipophilicity constants

Chromatographic partition constants determined by TLC ($1/R_f - 1$) and by HPLC (K') were used as experimentally determined partition indexes. In addition, the molecular weights of the compounds were used as an error-free scale of lipophilicity. These parameters have been widely validated in these type of studies as being genuine partitioning indexes. Values were taken from previous studies performed with these xenobiotics in the presence of different surfactants (Garrigues et al., 1990, 1994; Bermejo et al., 1991).

2.4. Analysis of the samples

Separation was achieved by HPLC, using a reverse-phase technique and quantification was performed by UV absorption at 258 nm, as previously reported (Garrigues et al., 1990). Since SO and Lec did not interfere with the analytical system, no change with respect to the previously validated technique was necessary.

2.5. Fitting of models to data

The fitting operations were carried out on a PC computer with PCNONLIN (3.0). Model-predicted equations for each condition (Plá Delfina et al., 1987; Bermejo et al., 1991) were used. Briefly:

(A) In the presence of synthetic surfactants and soaps at the CMC, a potential correlation is found and Eq. 1 is applied. If bile salt surfactants are used, a hyperbolic correlation (Eq. 2) is observed.

$$k_0 = C \cdot P^d \tag{1}$$

$$k_0 = \frac{k_m \cdot P^a}{B + P^a} \tag{2}$$

where P represents any lipophilicity parameter, C, d, a and B are constants, and k_m designates the limiting asymptotic constant for lipophilic acids.

(B) Whenever micelles are incorporated into the system – the SMC conditions – there is a partitioning process for the xenobiotics between the luminal fluid and the apolar micelle nuclei. If this is taken into consideration, the following equation describing the process of absorption is obtained:

$$k_s = \frac{k'_0}{1 + P_a} \tag{3}$$

P_a represents the partitioning process in the micelles and can be correlated with lipophilicity indexes (P) as follows:

$$P_a = E \cdot P^f \tag{4}$$

E and f being the constants of the equation.

k'_0 refers to the absorption constant of the free fraction (Eq. 1 or Eq. 2). It can equal k_0 values if micelles do not substantially interact with membrane (STC micelles (Bermejo et al., 1991), however, it should be slightly modified in the presence of micelles able to increase membrane polarity – SGC micelles (Garrigues et al., 1994). k_m is then the only parameter maintained in the simultaneous fit of SGC correlations. Thus, the following equation is used:

$$k_s = \frac{k_{m_n} \cdot P^{a_n}}{(B_n + P^{a_n}) \cdot (1 + E_n \cdot P^{f_n})} \tag{5}$$

($n = 1, 2$)

In order to facilitate identification of the different experiments, a subscript has been used: 1 refers to STC + Lec experiences, 2 to SGC + Lec series.

Concerning the correlation of SGC + SO, the equation used was as follows:

$$k_s = \frac{C_3 \cdot P^{d_3}}{(1 + E_3 \cdot P^{f_3})} \tag{6}$$

As can be seen, Eq. 6 differs in form from Eq. 5. The reasons for this will be discussed later.

3. Results

The absorption rate constants found in the presence of SO at the CMC (k_0), STC + Lec (k_{sm-1}) and SGC + SO (k_{sm-3}) are listed in Table 1. Previously determined absorption rate constants in the absence of surfactants (k_a) and with SGC + Lec (k_{sm-2}) and lipophilicity indexes are also given in Table 1 as reference points.

Equation parameters found after fitting the potential and hyperbolic models to k_0 in the presence of SO *versus* each lipophilicity index are given in Table 2, as well as statistical figures indicative of the goodness of the fits.

Equation parameters and statistical figures associated with the simultaneous fit of k_0, k_s and k_{sm} with each lipophilicity index are reported in Table 3. The correlations obtained for k_0 values (found at the CMC) vs $(1/R_f - 1)$ have been graphically outlined in Fig. 1 as representative of the general behaviour of the tested compounds; the correlation found for free solution k_a values has also been included. In Fig. 2 the correlations found under the different supramicellar conditions are plotted.

4. Discussion

4.1. Correlations found at the CMC

Despite the fact that our aim was to analyze the induced effects of the mixed micelles, the addition of sodium oleate to the perfusion fluid forced us to study its influence on absorption.

Table 1
Absorption rate constants obtained under the different conditions assayed

Tested acids	Absorption rate constants (h^{-1})					Lipophilicity indexes	
	k_a [a]	k_0	k_{sm-1}	k_{sm-2} [b]	k_{sm-3}	$1/R_f - 1$ [a]	K' [a]
Phenylpropionic	2.045 ± 0.164	2.080 ± 0.177	1.937 ± 0.244	1.677 ± 0.245	1.595 ± 0.293	1.406 ± 0.104	0.165 ± 0.01
Phenylbutyric	2.541 ± 0.142	2.275 ± 0.132	2.217 ± 0.243	2.001 ± 0.076	2.071 ± 0.250	1.882 ± 0.142	0.437 ± 0.01
Phenylvaleric	3.245 ± 0.217	2.537 ± 0.294	2.519 ± 0.368	2.203 ± 0.186	2.347 ± 0.071	2.615 ± 0.125	0.976 ± 0.01
Phenylcaproic	3.629 ± 0.150	2.867 ± 0.514	2.855 ± 0.187	2.461 ± 0.130	2.225 ± 0.148	3.697 ± 0.148	2.102 ± 0.01
Phenyloenanthic	4.141 ± 0.325	3.252 ± 0.201	2.518 ± 0.193	2.300 ± 0.226	2.117 ± 0.235	5.277 ± 0.296	4.813 ± 0.01
Phenylcaprylic	4.339 ± 0.188	3.584 ± 0.204	1.822 ± 0.129	2.147 ± 0.143	1.948 ± 0.080	7.307 ± 0.422	10.939 ± 0.01

[a] Taken from Bermejo et al. (1991).
[b] Taken from Garrigues et al. (1994).

Table 2
Equation parameters after fitting to potential or hyperbolic models for k_0 constants in the presence of SO at its CMC vs each lipophilicity index (statistical figures associated are also indicated)

Model equations		Equation parameters: statistical figures		
		$(1/R_f - 1)$	K'	M
Hyperbolic	K_m	40.20 ± 96.90	999 ± 151 168	612.61 ± 34,932
	a	0.361 ± 0.065	0.137 ± 0.058	$0.35 \times 10^{-2} \pm 0.92 \times 10^{-3}$
	B	20.86 ± 52.48	384.9 ± 5 840	999.44 ± 56 912
	r	0.999	0.998	0.999
	SSQ	0.113×10^{-2}	0.827×10^{-2}	0.369×10^{-2}
	AIC	−34.70	−22.77	−27.61
Potential	C	1.847 ± 0.012	2.591 ± 0.020	0.614 ± 0.023
	d	0.335 ± 0.004	0.137 ± 0.005	$0.321 \times 10^{-2} \pm 0.083 \times 10^{-3}$
	r	0.999	0.998	0.999
	SSQ	0.119×10^{-2}	0.825×10^{-2}	0.368×10^{-2}
	AIC	−36.40	−24.78	−29.62

Table 3
Equation parameters and associated statistical figures for the simultaneous fit of k_0, k_s and k_{sm} with each lipophilicity index

	Parameters	Partition constant		
		K'	$(1/R_f - 1)$	M
Taurocholate-lecithin mixed micelles	k_{m_1}	3.889 ± 0.270	3.658 ± 0.133	3.751 ± 0.158
	a_1	0.544 ± 0.098	1.716 ± 0.211	0.016 ± 0.002
	B_1	0.411 ± 0.115	1.701 ± 0.119	243.834 ± 164.765
	E_1	0.040 ± 0.017	0.002 ± 0.001	$0.732 \times 10^{-7} \pm 1.000 \times 10^{-7}$
	f_1	1.308 ± 0.199	3.034 ± 0.372	0.032 ± 0.004
	r	0.972	0.979	0.976
	SSQ	0.045	0.033	0.038
Glycocholate-lecithin mixed micelles	k_{m_2}	3.473 ± 0.196	3.332 ± 0.118	3.660 ± 0.305
	a_2	0.597 ± 0.091	1.867 ± 0.276	0.015 ± 0.003
	B_2	0.300 ± 0.075	1.472 ± 0.191	163.02 ± 158.18
	E_2	0.183 ± 0.034	0.075 ± 0.028	0.002 ± 0.003
	f_2	0.407 ± 0.089	0.934 ± 0.204	0.011 ± 0.002
	r	0.972	0.981	0.980
	SSQ	0.024	0.016	0.018
Glycocholate-sodium oleate mixed micelles	C_2	13.384 ± 9.296	1.160 ± 0.190	$0.263 \times 10^{-3} \pm 0.591 \times 10^{-3}$
	d_2	0.855 ± 0.291	3.112 ± 0.621	0.027 ± 0.007
	E_3	4.852 ± 4.072	0.351 ± 0.045	$0.282 \times 10^{-4} \pm 0.540 \times 10^{-4}$
	f_3	0.995 ± 0.239	3.377 ± 0.574	0.030 ± 0.006
	r	0.990	0.995	0.994
	SSQ	0.684×10^{-2}	0.345×10^{-2}	0.442×10^{-2}

The hyperbolic model has been assumed, except in the presence of SO. See text.

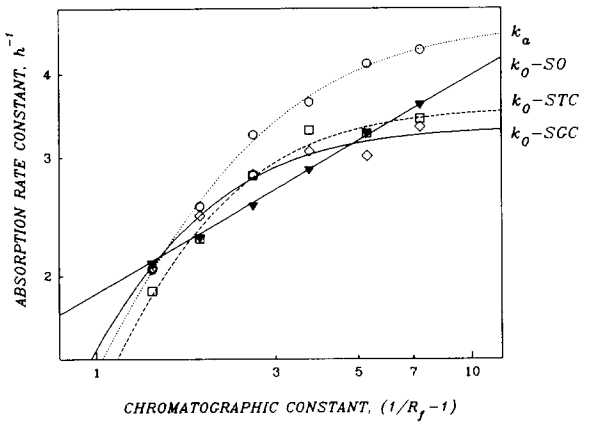

Fig. 1. Plot of absorption rate constants found for the tested compounds in rat colon in the presence of SO at the CMC (▼). k_0 for STC (□) and k_a (○) taken from Bermejo et al. (1991) and k_0 for SGC (◆) taken from Garrigues et al. (1994) are also graphically outlined.

Following our methodology, this implies the establishment of the model in the presence of its CMC.

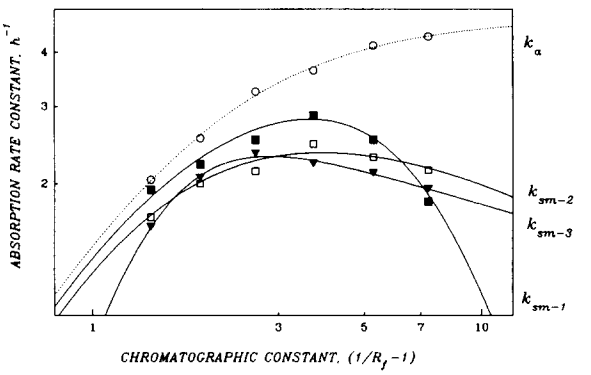

Fig. 2. Comparative behaviour of mixed micelles of bile salts with lecithin [$k_{sm\text{-}1}$ (■), $k_{sm\text{-}2}$ (□)] and with SO [$k_{sm\text{-}3}$ (▼)].

From the results, it is clear that the soap structure of the oleate enables to eliminate the limiting effect of the aqueous diffusion layer. That can be seen in the transformation of the hyperbolic correlation (Eq. 2) into a potential one (Eq. 1): not only is the AIC value lower for that fitting, but also the F test demonstrates that the introduction of another parameter – i.e., using Eq. 2 – does not produce significant differences in the SSQ ($F = 0.159$, 71.68%). On the other hand, SO acts as a potent modifier of the membrane polarity. This property has been widely reported in other assays (Muranushi et al., 1981) and can be proven by the slope of the correlation obtained in the present work. As can be seen in Fig. 1, the potential fitting applied is always lower than the hyperbola describing free solution conditions; i.e., every absorption rate constant is lower than the respective value of k_a for every acid, even the lipophilic species. The membrane becomes much more polar in the presence of SO and, therefore, less permeable, at least for our compounds. It would only have effects as an absorption promoter if very highly lipophilic compounds were assayed.

4.2. Mixed micelles

The effects of three types of mixed micelles were characterized: STC + Lec, SGC + Lec and SGC + SO.

As can be deduced from Fig. 2, STC + Lec micelles are the most effective solubilizers. They produce a significant decrease in the absorption rate constants of the acids ($k_{sm\text{-}1}$ values), becoming more marked as the lipophilicity increases, regardless of the values used for comparison (k_a or k_0). This ability makes it simple to establish the biophysical model describing those assay conditions, since the effect on membrane polarity is masked by the partitioning process; nevertheless, the presence of the aqueous stagnant layer should be borne in mind to fit accurately the data. As can be seen in Fig. 2 and Table 3, Eq. 5 is functionally and exactly applied. This type of micelle has the same electric charge as STC micelles, but since their volume is considerably increased, the charge per area unit is probably reduced. On the other hand, because of the larger volume, steric restrictions are not as strict as in the case of STC micelles.

When results from experiments with SGC are analyzed, a different picture is observed. In spite of being a similar conjugated bile salt, its effect on the membrane is more marked than that exerted by STC. This can be seen – as previously noted (Garrigues et al., 1994) – in the asymptote of the hyperbola describing the k_0 correlation (Fig. 1). As a result, when the SMC conditions are assayed the model needs to take that effect into account by modifying the equation global fit: k_m remains common whereas a and B are separately fitted. Concerning the effect of the incorporation of lecithin into the system ($k_{sm\text{-}2}$), a slight improvement in the solubilization ability of SGC alone (Garrigues et al., 1994) is seen. Nevertheless, the increase in the micellar volume is not as large as that observed with STC. Steric restrictions are present, at least for the compounds we are using, therefore, no significant change in absorption rate can be seen.

Finally, the introduction of a fatty acid ($k_{sm\text{-}3}$) does not produce a dramatic change in the system. Probably because of its effect on membrane polarity, always present at the SMC, SO does not affect absorption more than lecithin despite its ability to undergo dissolution in the system, as can be seen by the magnitude of the constants obtained. Concerning the model which best describes the situation, the SMC correlation type for synthetic surfactants (Eq. 6) has been used because this soap effectively removes the stagnant layer. The fit is really good and its interpretation leads to a conclusion: even sodium oleate is unable to promote the SGC solubilization ability of our compounds.

Thus, since the series of xenobiotics studied covers a wide range of lipophilicity, steric restrictions should be responsible for this bile salt-micelle behaviour.

4.3. Biopharmaceutical implications in vivo

After the work we have conducted with surfactants, there is a point to consider before using them: the concentration is critical. The two types

of surfactants (synthetic and natural) differ greatly in their effects, depending on the presence or absence of micelles. In this context, it should be noted that even if the surfactant is added at a high concentration, since amphiphiles have a tendency to cover interfaces, the micelles will probably disintegrate in the gut as they move to the surface of the absorbing membrane. In the case of oral absorption, another effect should be taken into account: a dilution in the liquid present will occur. The extent of this phenomenon may be different depending on the circumstances, such as the presence of food or the simultaneous ingestion of water. At any rate, as the human intestinal lumen usually contains a liquid portion, dilution can be also responsible for disintegration of micelles.

The ability of micelles to increase membrane polarity makes them a useful group of substances for promoting the absorption of hydrophilic compounds. To exert such an effect, the concentration in the lumen in vivo is not very important. In this respect, any type of surfactant, natural or synthetic, is adequate.

Concerning lipophilic compounds, another situation can be predicted. Our results show that natural surfactants, despite their physiological role in absorption, are not the most suitable. They show only one advantage: their relatively low toxicity. On the other hand, synthetic surfactants offer two characteristics of relevance: (1) they can solubilize such compounds more easily, thus yielding, after dilution of their micelles, a form that is ready to be dissolved and, thus, more rapidly absorbed; and (2) their ability to disrupt the limiting effect of the aqueous stagnant layer at premicellar concentrations makes them actual absorption enhancers under these conditions for lipophilic xenobiotics.

This can be deduced from our experiments using the colon as the absorption site, where no active absorption of the bile salts (leading to their effective reabsorption into the bloodstream) has been demonstrated. However, if the small intestine, particularly its distal fraction, were used for absorption tests, this general picture could be modified since, as has recently shown by Del Estal et al. (1991), sodium taurocholate at supramicellar concentrations (38 mM) acts as an effective absorption promoter for albendazole, a highly lipophilic drug, whereas the synthetic surfactant polysorbate 80 (38 mM) alone or in the presence of STC (from 5 to 57 mM) does not. This can be attributed to a progressive disappearance from the luminal solution of the bile salt (via active systemic absorption), with the subsequent breaking of the micelles and the release of the drug in a readily absorbable form. In contrast, the solubilizing capacity of the synthetic surfactant micelles would remain quite stable, since there is no systemic absorption of the surfactant.

All these ideas should be experimentally checked in vivo, in the intact animal through oral administration and analysis of the plasma level curves. We are presently engaged in this work.

Acknowledgements

The present work is part of a research project carried out with a grant from the Dirección General de Investigación Científica y Técnica (DGICYT, PB86-580), of the Ministry of Education and Science of Spain. The authors are indebted to the Conselleria de Cultura de la Generalitat Valenciana for M.V.B.'s grant and to the Ministry of Education and Science of Spain for V.M.'s grant. The authors are also indebted to Dr Francisco Comellas (Instituto de Química Textil, CSIC, Barcelona) for CMC determination of the surfactants used in this work.

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