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Compared effects of synthetic and natural bile acid surfactant on xenobiotic absorption. II. Studies with sodium glycocholate to confirm a hypothesis

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Summary

The effects of sodium glycocholate (SGC) on the intestinal absorption of drug-related xenobiotics are investigated, on the basis of previously established absorption/partition relationships. Six phenylalkylcarboxylic acids, closely related to nonsteroid anti-inflammatory drugs in structure and constituting a true homologous series, were used as test compounds through an in situ rat gut technique, using the whole colon as nonspecialized absorption membrane model. Whereas the synthetic surfactants (i.e., polysorbates and laurylsulphates) at the critical micelle concentration have been shown to disrupt the aqueous boundary layer adjacent to the membrane, SGC does not; in contrast, it reinforces its limiting effect on solute diffusion, thus leading to a poorer absorption of the compounds as their lipophilicity increases. On the other hand, at supramicellar concentrations, the micelle solubilizing effect of SGC for the compounds is incomparably lower than that found for synthetics, even in the presence of mixed micelles with lecithin. These results, in conjunction with previous observations, seem to indicate that as far as xenobiotic absorption is concerned, synthetics and natural bile acid surfactants behave as entirely different biopharmaceutical species.

Introduction

The introduction of a strict methodology based on lipophilicity/absorption correlations for homologous series of compounds, has led to a functional theory about the effects of surfactants on absorption, which can explain all the features previously observed (Plá-Delfina et al., 1987). Concerning the absorption of xenobiotics in solution, three main effects were postulated: (a) synthetic surfactants at their critical micelle concentration (CMC) disrupt the limiting effect that the aqueous diffusion layer exerts on the lipophilic compounds of the series, thus promoting their absorption; (b) synthetic surfactants have an effect on the polarity of the lipoidal membrane, rendering it more permeable for highly hydrophilic substances; and (c) when perfused at supramicellar concentrations (SMC), the solubi-

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lizing ability of the aggregates formed apparently masks all these effects and produces a decrease in the absorption rate of the compounds that becomes more important as their lipophilicity increases.

It is clear then that this category of pharmaceutical excipients can be manipulated to promote bioavailability of poorly absorbable drugs under certain conditions. On the other hand, such studies stimulated interest in another group of substances occurring naturally in the gastrointestinal tract, that are considered part of the broad class of surfactants, i.e., the bile salts.

A thorough review of the data published on this matter made it possible to postulate a particular behaviour of this group of substances. The main differences that interest us here can be summarized in two points: they are effectively absorbed by the bowel in a passive and an active fashion (Dietschy, 1968) and they give rise to the formation of micelles with either a very low number of molecules in aggregation or do not form them at all (Hofmann and Small, 1967).

On the basis of these considerations, a study designed with the same methodology was carried out using taurocholate as the model compound (Bermejo et al., 1991). The conclusions of this work were, briefly stated, that this amphiphile is not able to remove the stagnant diffusion layer that limits lipidic absorption in its absence and that by itself its ability to solubilize this kind of compounds is significantly diminished as compared to synthetic surfactants.

Nevertheless, the intrinsic interest of bile salts because of their role in physiologic absorption conditions and their low toxicity to the membrane encouraged us to confirm this hypothesis by assaying the other major bile salt, i.e., sodium glycocholate.

Materials and Methods

Test compounds

A homologous series formed by six phenylalkylcarboxylic acids, ranging from two to seven methyl groups in the straight alkyl chain, was used. This series was previously assayed under different conditions (Garrigues et al., 1990, 1992; Bermejo et al., 1991; Fabra-Campos et al., 1991), which provides a good basis for interpreting any possible modification exerted by the bile salt. Their pK_a values range from 4.5 to 4.7, implying that at the working pH (7.5) they are practically fully ionized species (about 99.9%). They were supplied as reactive grade products (Merck A.G., Janssen Co., Lancaster Co.), and their purity was confirmed by HPLC analysis. The concentration of xenobiotics in the perfusion solutions ranged from 0.05 to 1 mg/ml, according to their solubilities.

The naturally occurring surfactant selected was sodium glycocholate (SGC), supplied by Sigma Co., with a purity of 98%. It is one of the most genuine bile salts as it is a conjugated species, thus being difficult to precipitate in the gut; it is a trihydroxylated salt which reduces its intrinsic toxicity to the absorbing membrane (Gullickson et al., 1977; Kimura et al., 1985) and it is the major bile salt in man. Two concentrations were employed in two different series of experiments: (a) the critical micelle concentration (CMC) in perfusion fluids, which was experimentally determined as 2 mM; and (b) a supramicellar concentration (SMC), which was fixed at 9 mM, in order to avoid the damage to the membrane that can occur at higher concentrations (Saunders et al., 1975) and to approximate physiological conditions after dilution in the gut (Dietschy, 1968).

In order to assess the influence of mixed micelles, lecithin was assayed in a further series of experiments (c). This compound has the advantage of being insoluble, which prevents its having any effect by itself on absorption. On the other hand, it is the major phospholipid of rat bile (Hay and Carey, 1990). Lecithin was supplied as egg lecithin by BDH Co., with a purity of 90%. A concentration of 3.3 mM was incorporated into the supramicellar solutions of SGC, which represents an intermediate bile salt/phospholipid ratio between in vivo conditions in man and in rat (Coleman et al., 1979).

Absorption experiments

The study was carried out on male Wistar rats, grown in our laboratory in standard stabling con-

ditions. The selected animals ranged from 2 to 3 months of age and weighed from 200 to 300 g after being fasted for 20 h.

The in situ rat gut technique using the whole colon was employed, as in previous studies (Doluisio et al., 1969; Martín-Villodre et al., 1986).

Isotonic perfusion solutions were prepared, buffered with phosphate solution 1/15 M (pH 7.5) and the corresponding amount of surfactant was added. Subsequently, lecithin, dispersed by means of an ultrasound bath, was added to the solutions belonging to the mixed micelle series. In the case of sodium glycocholate tests, the plain vehicle was used as the perfusion solution; otherwise, an aliquot was used to prepare the different xenobiotic solutions of a determined concentration. After dissolving the compounds, the pH was again adjusted and the osmolarity controlled with a Kyoto-Daiichi Osmostat OM-6020 osmometer.

The absorption rate constant was characterized in the usual manner, i.e., as the slope of the regression line which describes the natural logarithm of the remaining xenobiotic concentration vs time. Initial non-perfused samples, at zero time, were not used in the regression (Martín-Villodre et al., 1986). The concentrations in the samples were treated as actual values at the sampling time, since very little reduction in volume was observed at the end of the experiment (30 min, less than 5%). An average of five determinations was considered to be representative of the process for every compound and series. The rate constants determined at the CMC, SMC and in the presence of mixed micelles were conventionally noted as k_0, k_s and k_{sm}, respectively.

Analysis of the samples

Samples were analyzed by a reversed-phase HPLC technique, as previously described (Garrigues et al., 1990).

The equipment consisted of a Waters 590 Model pump, a U6K injector, a Lambda-Max detector, set at 258 nm and a Model 730 Data Module.

The technique was performed on analytical Novapak C18 columns (150 × 3.9 mm) with 5 mm GuardPak precolumns. Mobile phases were adjusted for every acid; in essence, they were prepared as mixtures of acetonitrile and aqueous 0.1 N acetic acid (pH 3.0). Elution was always carried out at room temperature and at a flow rate of 1 ml/min. Validation of the procedure was achieved both for intra-day and inter-day analysis (Shah et al., 1992); coefficients of variation ranged from 1.85 to 5.14% and accuracy was always between −6.5 and 9.7%.

Sodium glycocholate was also analyzed by a reverse-phase HPLC technique. Separation was achieved in a Spherisorb ODS2 column (150 × 4.6 mm) with a mobile phase of methanol/phosphate buffer 4.3 mM (pH 2.5)/acetonitrile (65:70:20), at a flow rate of 1 ml/min. Quantification was performed by UV detection at 205 nm. Linearity was established for the concentration range ($r > 0.999$), and coefficients of variation were between 0.25 and 1.89%.

Lipophilicity constants

To characterize the lipophilicity of the compounds, two previously determined chromatographic constants (Bermejo et al., 1991) were used: TLC constants ($1/R_f - 1$) and capacity factors K'. Since genuine partition conditions were demonstrated for both systems, the corresponding data have been used without further considerations.

Briefly, TLC was developed simultaneously for all the acids on RP8 Merck chromatoplates with the aid of a mixture of phosphate buffer 1/30 M (pH 7.5) and acetone (45:55, v/v). The R_f was measured under UV light (254 nm) and the constant ($1/R_f - 1$) was calculated; the average value of six chromatograms was used to establish correlations.

Capacity factors were obtained on a Novapak C18 (150 × 39 mm) column with a mixture of acetonitrile and phosphate buffer 1/30 M (pH 7.5) (25:75, v/v) as mobile phase. The retention times of the acids and dead time of the column were measured on the above-mentioned equipment. Four chromatograms were developed to calculate the K' constants.

In addition, molecular weights were used, since they represent an error-free lipophilicity index provided there is perfect homology between the

compound series. It has been demonstrated that in this particular case, this parameter is equivalent to every experimentally determined lipophilicity constant (Garrigues et al., 1990).

Fitting of models to data

In order to correlate absorption rate constants (k_0, k_s, k_{sm}) with lipophilicity indexes (represented, in general, by P), equations based on the two biophysical models described previously were used.

Briefly, concerning the assays in the presence of SGC at the CMC, the generally applied theory of surfactants (Plá-Delfina et al., 1987) predicts a potential correlation as shown in Eqn 1:

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

where C and d are parameters depending on the technique used.

However, experiments carried out with sodium taurocholate showed a different behaviour, which led to correlations with an asymptote (Bermejo et al., 1991). Thus, the correlation was hyperbolic in nature, as it was when the compounds in free solution were assayed. Eqn 2 is, therefore, applied:

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

where a and B are constants and K_m is a parameter that represents the asymptote, i.e., the maximum absorption rate constant in that condition for the series.

Experiments with micelles create multiphasic equilibria due to the apolar nature of the micelle nuclei. Therefore, a bilinear equation is the most suitable kind to describe the correlation, as previously established (Plá-Delfina et al., 1987). Eqn 3 represents the general model in this group of experiments:

$$k_s,\ k_{sm} = \frac{k_0'}{1 + P_a} \tag{3}$$

where P_a represents the partition process of the xenobiotics in the considered micelles and k_0' is the absorption rate constant of the free fraction of the xenobiotic. Although k_0' approaches k_0 (Eqn 2) and can be used as such in several cases (Plá-Delfina et al., 1987; Garrigues et al., 1990, 1992; Bermejo et al., 1991; Fabra-Campos et al., 1991), strictly speaking it does not exactly correspond to k_0 because the SMC has a slightly cumulative effect on the membrane polarity as the concentration of surfactant increases; it is obviously related to k_0 and can be quantitated as a function of xenobiotic lipophilicity by a hyperbolic equation ($k_0' = (K_m \cdot P^{a'})/(B' + P^{a'})$) where K_m remains constant and a' and B' are freely fitted.

In every micellar phase, the value of P_a is a function of lipophilicity (Plá-Delfina et al., 1987) that can be described as $P_a = E \cdot P^f$, in which E and f are constants depending on the micelle solubilization capacity of the surfactant and the lipophilicity of the tested series. In order to clarify the nomenclature, we changed the symbol parameter for mixed micelle correlation to G and h. So that:

$$k_s = \frac{(K_m \cdot P^{a'})/(B' + P^{a'})}{1 + E \cdot P^f} \tag{4}$$

$$k_{sm} = \frac{(K_m \cdot P^{a'})/(B' + P^{a'})}{1 + G \cdot P^h} \tag{5}$$

Fitting was carried out by means of PCNONLIN (3.0), without a weighting function. When possible, a simultaneous fit was developed to estimate common parameters (i.e., Eqns 2 and 3). To choose the best model, the two different fits for CMC correlations were compared by means of the Akaike criterion (Akaike, 1976). To evaluate the goodness of the following fits, squared sums of residuals (SSQ) and correlation coefficients (r) between experimental and model-predicted values were calculated.

Results

Tests carried out with SGC at the SMC indicated that in rat colon, the absorption rate constant of the process is negligible. At the end of

TABLE 1

Absorption rate constants obtained under the different conditions assayed

Tested acids	Absorption rate constants (h^{-1})			
	k_a ($n = 5$) [a] Free solution	k_0 ($n = 5$) SGC, CMC	k_s ($n = 5$) SGC, SMC	k_{sm} ($n = 5$) Mixed micelles
Phenylpropionic	2.045 ± 0.164	2.058 ± 0.289	1.901 ± 0.265	1.677 ± 0.245
Phenylbutyric	2.541 ± 0.142	2.467 ± 0.575	2.312 ± 0.135	2.017 ± 0.076
Phenylvaleric	3.245 ± 0.217	2.830 ± 0.250	2.676 ± 0.268	2.232 ± 0.186
Phenylcaproic	3.629 ± 0.150	3.063 ± 0.255	2.871 ± 0.422	2.464 ± 0.130
Phenyloenanthic	4.141 ± 0.325	3.015 ± 0.258	3.058 ± 0.277	2.298 ± 0.226
Phenylcaprylic	4.339 ± 0.188	3.334 ± 0.328	2.965 ± 0.167	2.147 ± 0.143

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

the experiment (30 min) there was a remaining percentage of 95.95(±3.58)%; no correction for water reabsorption was needed (Doluisio et al., 1969). Moreover, the process could not be accurately fitted to a first-order kinetics unless a longer sampling time was used. Nevertheless, this conclusion made it possible to carry out the rest of the experiments, since we can affirm that a significant change in SGC concentration does not occur during the sampling period.

The absorption rate constants found in the different series of experiments for every acid have been listed in Table 1.

Table 2 shows the results of the potential and the hyperbolic fitting to k_0 data vs every lipophilicity index. Statistical figures associated are also noted in order to facilitate comparison.

The equation parameters of the simultaneous fit carried out using the best model and associated statistical figures are indicated in Table 3. A representative plot of the absorption-partition correlation is given in Fig. 1.

TABLE 2

Equation parameters after fitting to potential or hyperbolic models for k_0 constants 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	3.336 ± 0.117	3.458 ± 0.182	3.548 ± 0.206
a	1.929 ± 0.408	0.586 ± 0.143	0.013 ± 0.003
B	1.508 ± 0.199	0.237 ± 0.081	73.512 ± 78.819
r	0.984	0.985	0.982
SSQ	0.035	0.032	0.038
AIC	−14.15	−14.60	−13.62
Potential			
C	2.097 ± 0.141	2.676 ± 0.072	0.939 ± 0.193
d	0.242 ± 0.047	0.100 ± 0.017	$0.253 \cdot 10^{-2} \pm 0.046 \cdot 10^{-2}$
r	0.935	0.948	0.943
SSQ	0.135	0.107	0.119
AIC	−8.02	−9.39	−8.79

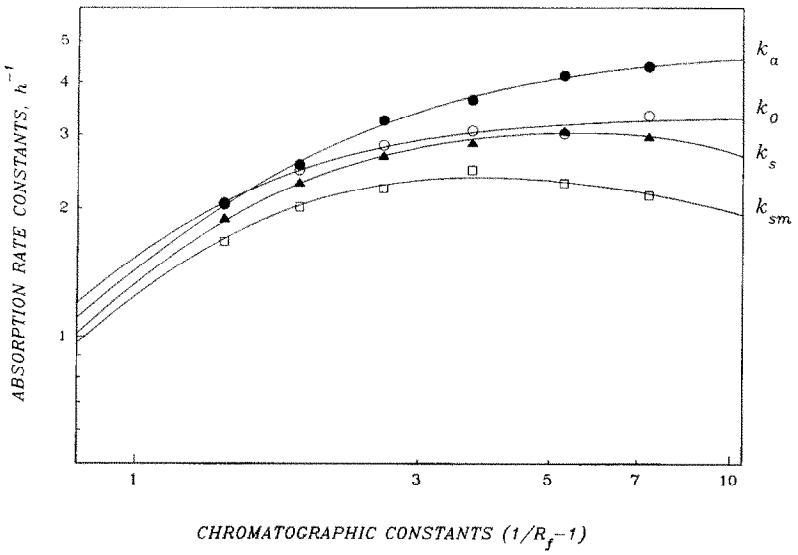

Fig. 1. Representative plot of the correlation obtained from the simultaneous fit, using the chromatographic constants $(1/R_f - 1)$ as the lipophilicity index. Results found with the remaining lipophilicity indexes are very similar.

Discussion

CMC experiments

The results obtained confirmed again the model hypothesis predictions (Bermejo et al., 1991). Since an asymptotic value of k_0 is derived from the inefficiency of potential fitting vs the hyperbolic fitting – which is demonstrated by the lower AIC for the more sophisticated model – addition of SGC at its CMC obviously does not disrupt the limiting effect of the aqueous boundary layer. Nevertheless, since there is a K_m that is different from the asymptote predicted in free solution experiments (4.9 h^{-1} vs 3.4 h^{-1}), it should be borne in mind that SGC affects to some extent the absorption of the more lipophilic

TABLE 3

Equation parameters and associated statistical figures for the simultaneous fit of k_0, k_s and k_{sm} with each lipophilicity index (the hyperbolic model for k_0 has been assumed)

Parameters	Partition constant		
	K'	$(1/R_f - 1)$	M
K_m	3.458 ± 0.182	3.336 ± 0.117	3.548 ± 0.206
a	0.586 ± 0.143	1.858 ± 0.408	0.013 ± 0.003
B	0.237 ± 0.081	1.151 ± 0.174	73.512 ± 78.819
a'	0.606 ± 0.089	1.929 ± 0.317	0.015 ± 0.002
B'	0.294 ± 0.071	1.508 ± 0.199	148.22 ± 109.76
E	$0.985 \cdot 10^{-3} \pm 0.588 \cdot 10^{-2}$	$0.125 \cdot 10^{-2} \pm 0.691 \cdot 10^{-2}$	$0.151 \cdot 10^{-7} \pm 0.1 \cdot 10^{-6}$
f	1.889 ± 2.388	2.126 ± 2.703	0.031 ± 0.030
G	0.175 ± 0.033	0.074 ± 0.032	0.003 ± 0.003
h	0.429 ± 0.089	0.954 ± 0.224	0.010 ± 0.002
AIC	−33.55	−34.92	−32.05
r	0.992	0.993	0.992
SSQ	0.0570	0.0529	0.0620

compounds. This effect can be explained in light of the experiments carried out by Poelma et al. (1990), where an increase in mucus viscosity was found in the presence of sodium taurocholate at its CMC. Another bile salt would probably produce the same effect and the biophysical modelling would account for that as a part of the aqueous boundary layer resistance.

On the other hand, SGC produces an increase in membrane polarity that has been widely reported (Gullickson et al., 1977; Poelma et al., 1990). Its effects can be seen from our experimental data if we consider that lipophilic xenobiotics of the series are not in the asymptote of free solution. Thus, the significant reduction in k_0 with respect to k_a can also be explained as a consequence of the above-mentioned effect as absorption of all the products is partially affected by the partitioning process in the lipoidal membrane.

SMC experiments

Micellar solubilization by SGC is almost negligible under the test conditions, as can be seen by the magnitude of the constants obtained with respect to those found from CMC experiments. The lack of solubilization ability of SGC constitutes an important difference with respect to previously tested synthetic surfactants, i.e., nonionic polysorbate 80 at 5% w/v reduces the absorption rate constant of phenylcaprylic acid (the most lipophilic compound of the series) to 18% (82% solubilized). Two reasons for this behaviour have been explored: ionisation of the micelles and the intrinsic ability of the amphiphile to incorporate lipophilic compounds in its micelles. The first idea is not very relevant because a clear difference is seen when comparing the percentage solubilized by the anionic synthetic surfactant sodium lauryl sulphate (Garrigues et al., 1992) and SGC (59.17 vs 11.07% for phenylcaprylic acid, respectively). This indicates that the micellar structure is partially responsible for that effect. This finds support in the very low aggregation number of the bile salt (Hofmann and Small, 1967) and the steric restriction during incorporation that is even more evident than for sodium taurocholate micelles. In fact, sodium taurocholate micelles solubilize at 15.77% of phenylcaprylic acid. This is significantly lower than in the case of the synthetic surfactants assayed but greater than the percentage solubilized with SGC (Bermejo et al., 1991).

Nevertheless, the introduction of an apolar phase into the system produces a change in the model that describes the process (from homogeneous to heterogeneous). In this way, a bilinear function is the best to predict absorption rate constants (see Fig. 1).

On the other hand, as the only parameter that can be maintained constant in a simultaneous fit of k_0, k_s and k_{sm} with each lipophilicity index is K_m, it would seem that the micelles exert an effect on membrane polarity. The mechanism of this effect cannot be explained at this stage of the study, but it has been noted by several authors (Feldman and Gibaldi, 1969; Kakemi et al., 1970; Amidon et al., 1982). At present, some research on the matter is being carried out, which appears to indicate that the increase in polarity is slightly cumulative as surfactant concentration increases, even when the CMC is surpassed (unpublised data). This circumstance, which is irrelevant when micelle solubilization is significant, as occurs with many synthetic surfactants and even with taurocholate (Plá-Delfina, 1987; Bermejo et al., 1991; Garrigues et al., 1992), makes a further correction of k_0 values to k_0' necessary as was pointed out above. Nevertheless, we have achieved its functional quantification, as the parameters a' and B' have been freely fitted. It should be noted that the symbol of K_m is maintained in order to facilitate comprehension, but under these circumstances, it does not apply to the asymptote since it does not exist. In fact, an increase in lipophilicity always represents a facilitated partitioning process in the micelles, which is a limiting step for the absorption. K_m would only represent the maximum constant rate of absorption of the free fraction of the xenobiotic and the apparent absorption rate constant (k_s, k_{sm}) is calculated on the basis of the total amount of compound present in the lumen (Plá-Delfina et al. 1987).

Concerning the more realistic conditions of solutions with mixed micelles formed by SGC and lecithin, an improved ability to solubilize can be

observed in the absolute value of the constants as well as in the correlation tendency. The SGC micelle volume is therefore responsible for poor solubilization: as lecithin is included in its core, leading to the expansion of micelles, steric hindrance diminishes. In this way, the generally accepted idea of bile salts as absorption enhancers of lipophilic compounds can be explained in light of this feature. Its mechanism usually involves a solubilization effect that, after dilution in the gut, produces a higher dissolution percentage or rate for lipophilic compounds.

Concluding remarks

The present work, then, allows us to extend the previous hypothesis concerning the role played by bile salts in absorption as stated. If there is any reason to consider these substances as enhancers, their solubilization ability under in vivo conditions – i.e., as a part of mixed micelles – for very insoluble drugs should be claimed.

On the other hand, this further evidence reasonably indicates that synthetic and natural bile salt surfactants behave as completely different biopharmaceutic species.

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