International Journal of Pharmaceutics, 79 (1992) 135–140

IJP 02618

Absorption-partition relationships for true homologous series of xenobiotics as a possible approach to study mechanisms of surfactants in absorption. IV. Phenylacetic acid derivatives and anionic surfactants

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(Received 31 May 1991)
(Accepted 1 August 1991)

Key words: Colonic absorption; Phenylalkylcarboxylic acid; Lipophilicity

Summary

This paper – the latest in this series of reports – deals with the effects of the anionic surfactant sodium lauryl sulfate on the colonic absorption of acidic xenobiotics (phenylalkylcarboxylic acids), as compared with those exerted by the nonionic polysorbate 80. The effects of these surfactants are qualitatively identical but quantitatively different. Thus, the increase in polarity of the colonic absorbant membrane is greater with lauryl sulfate, whereas micellar solubilization is much higher with polysorbate. As for the rest (i.e. the elimination of the stagnant aqueous diffusion layer as a limiting step for absorption), the two surfactants behave in a similar way. The biopharmaceutical implications of this comparative behaviour are briefly discussed.

Introduction

In preceding papers (Plá-Delfina et al., 1987; Collado et al., 1988; Garrigues et al., 1989) mathematical relationships were established between the absorption rate constants found in rat colon and in rat small intestine, and lipophilicity indexes, in the absence and presence of surfactants (cationic and nonionic) for aromatic amines. Somewhat later, the above correlations were extended to phenylacetic acid derivatives in the presence of a nonionic surfactant (Bermejo et al., 1991; Fabra-Campos et al., 1991), and the significance and range of applicability of the proposed equations were discussed. In the present study, the latest of this report series, the influence of an anionic surfactant on these acidic xenobiotics has been examined in order to ascertain whether the reported mechanism is general in character and inherent to all synthetic surfactants or dependent on the polar head group of the amphiphile. Experiments were developed in the rat colon; gastric experiments confirming this behaviour have recently been reported (Garrigues et al., 1990).

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Materials and Methods

Xenobiotics and surfactant

Seven previously tested acidic compounds, phenylalkylcarboxylic acids, possessing from one to seven -CH_2- groups in the straight alkyl chain, were used as xenobiotics. For use as surfactant, sodium lauryl sulfate ($C_{12}H_{25}$-O-SO_3Na) was selected as the most genuine representative of the anionic sulfated amphiphiles.

Absorption technique

The in situ rat gut absorption technique (Doluisio et al., 1969) adapted as previously reported (Martín-Villodre et al., 1986), and using the colon as absorption site, was performed on male Wistar rats weighing 220–290 g. The preparation procedure and characteristics of the perfusion solutions were the same as those described earlier (Bermejo et al., 1991). Xenobiotic solutions were adjusted to pH 7.5. Perfusion concentration of the solutes ranged from 0.05 to 0.20 mg/ml, according to their solubilities. Five animals per compound and series were used.

Two series of absorption tests were carried out: in the presence of surfactant immediately below its critical micelle concentration, CMC (0.29 mg/ml), yielding k_0 values as absorption rate constants; and in the presence of the surfactant at a clearly supramicellar concentration, SMC (12.5 mg/ml), yielding k_s values. The results were compared, for each series, among themselves and with those reported for acidic xenobiotics in the absence of surfactant, i.e., in free solution, yielding k_a values (Bermejo et al., 1991). In order to avoid membrane adsorption (Doluisio et al., 1969) and dilution (Martín-Villodre et al., 1986) effects, the zero-time sample was not considered in the calculation of absorption rate constants.

Analytical methods

The HPLC procedure already described (Bermejo et al., 1991) was used to quantify solute concentration in biological samples. The composition of the particular solvent for each compound was the same as indicated. Calibration lines were linear over the sample concentration range ($r > 0.999$) and had coefficients of variation ranging from 1.82 to 3.75.

Lipophilicity constants

Since it was observed that lipophilicity constants, as far as correlation with absorption rate constants is concerned, lead to similar results irrespective of whether the surfactant is present in partition systems (Collado et al., 1988), HPLC capacity factors, K', and reversed-phase TLC constants, $(1/R_f) - 1$ (Bermejo et al., 1991), were used as lipophilicity indexes for correlation with colonic absorption rate constants. Moreover, since a true homology between all tested compounds exists, their molecular weights, M, treated as described later, were also used as lipophilicity constants.

Micellar partition constants

As described by Collet and Koo (1975) and Tomida et al. (1978), micellar solubilization is a partition process, which can be accurately measured by different direct methods (dialysis, for example) and via indirect procedures such as through absorption rate constants (Pérez-Buendía et al., 1989), leading to similar results. Micellar partition constants (P_a) determined by the latter method for the acids in polysorbate 80 and in sodium lauryl sulfate were calculated and compared using the expression $P_a = k_0/k_s - 1$ (Plá-Delfina et al., 1987).

Absorption-lipophilicity correlations

Absorption (k_a, k_0 or k_s) and lipophilicity or partition (K', $(1/R_f) - 1$ or 10^M, designated in general as P) data were fitted to previously established equations (Plá-Delfina et al., 1987). In brief:

In the absence of surfactant:

$$k_a = \frac{K_m P^a}{B + P^a} \qquad (1)$$

where K_m represents the limiting asymptotic value of the membrane absorption rate constant and the terms a and B are readily calculable constants arising from the technique used (Plá-

Delfina and Moreno, 1981; Martin-Villodre et al., 1986).

In the presence of surfactant:

At the CMC:

$$k_0 = CP^d \quad (2)$$

$$k_s = \frac{CP^d}{1 + EP^f} \quad (3)$$

where k_s is usually lower than k_0, since only the free, nonmicellized fraction is available for absorption.

In the above expressions, C, d, E and f are readily calculable constants as previously indicated (Plá-Delfina et al., 1987; Collado et al., 1988).

When correlations are established with respect to molecular weight, M, instead of P values and since a potential correlation exists between P and M for truly homologous compounds (see, for example, Plá-Delfina and Moreno, 1981), the term P in the above equations was substituted by 10^M, which can be considered as an 'ideal', error-free lipophilicity index for this type of series.

The fitting operations were developed in an IBM-PC computer; the MULTI program (Yamaoka et al., 1985) was applied to fit the interdependent equations, by means of the Marquardt algorithm without weighting the data.

To evaluate the goodness of fits, correlation coefficients between experimental and model-predicted absorption rate constants were calculated, as well as the AIC values (Akaike, 1976).

In order to establish the extent of membrane polarity modification by the different surfactants tested, a comparison of the slope of CMC correlations was conducted through the statistical t-test.

Results

The absorption rate constants found in the presence of the surfactant at the CMC (as k_0) and at the SMC (as k_s) are listed in Table 1. Previously determined absorption rate constants determined in the absence of surfactant (as k_a) are also given in Table 1 as reference points. Lipophilicity indexes, i.e., capacity factors K', and TLC constants $(1/R_f) - 1$, previously determined at pH 7.5 (Bermejo et al., 1991), as well as the molecular weights, M, of the acids have also been included. Internal partition coefficients, P_a, are also listed as lipophilicity indexes.

Each set of absorption rate constants was correlated with each of the partition constants through the above equations. The equation parameters found are given in Table 2, as well as

TABLE 1

First-order absorption rate constants (h^{-1}) found in rat colon for the tested acids under the different working conditions, and HPLC and TLC lipophilicity constants determined at pH 7.5 (molecular weights have also been included as error-free lipophilicity constants)

Tested acids	Lauryl sulfate concentration in perfusion fluids			Lipophilicity constants			
	None [a] (k_a) ± SD	0.018% (CMC) (k_0) ± SD	1.25% (SMC) (k_s) ± SD	K' [a]	M	$1/R_f - 1$ [a] ± SD	P_a
Phenylacetic [b]	0.730 ± 0.067	1.255 ± 0.224	1.843 ± 0.117	0.087	136.15	1.009 ± 0.076	–
Phenylpropionic	2.044 ± 0.164	1.962 ± 0.246	2.065 ± 0.133	0.165	150.18	1.406 ± 0.104	–
Phenylbutyric	2.541 ± 0.142	2.345 ± 0.217	2.193 ± 0.304	0.437	164.20	1.882 ± 0.142	0.069
Phenylvaleric	3.245 ± 0.217	2.479 ± 0.286	2.156 ± 0.390	0.976	178.23	2.615 ± 0.125	0.150
Phenylcaproic	3.629 ± 0.167	2.916 ± 0.395	1.921 ± 0.154	2.102	192.26	3.697 ± 0.148	0.518
Phenyloenanthic	4.510 ± 0.418	3.341 ± 0.370	1.701 ± 0.146	4.813	206.29	5.277 ± 0.296	0.964
Phenylcaprylic	4.691 ± 0.451	3.608 ± 0.520	1.473 ± 0.142	10.939	220.31	7.307 ± 0.422	1.449

[a] From Bermejo et al. (1991).

[b] Not included in absorption-partition correlations.

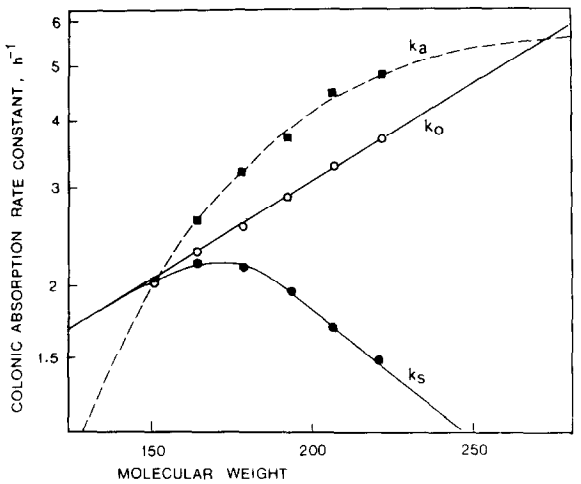

Fig. 1. Plot of absorption rate constants found for the tested compounds in rat colon in the absence of surfactant (■———■), in the presence of lauryl sulfate at CMC (○———○) and at SMC (●———●) according to Eqns 1, 2 and 3, respectively, against molecular weights as lipophilicity indexes. Parameter values are shown in Table 2.

statistical data indicative of the goodness of the fits.

The correlations observed for molecular weight have been graphically outlined in Fig. 1, as representative of the general behaviour of the tested compounds. It should be noted that phenylacetic acid, the first member of the series under test, was excluded from the correlation studies, since its behaviour was atypical relative to that of the remaining compounds; this was attributed, as pointed out earlier (Bermejo et al., 1991), to its proportionally greater degree of ionisation, which leads to a completely ionised species at the colonic pH.

Discussion

CMC experiments

The results obtained in rat colon in the presence of lauryl sulfate at the CMC (Table 2; Fig. 1, k_0 line) clearly show that the expected effects of the surfactant are actually elicited. The hyperbolic equation (Eqn 1) which fits the data obtained in free solution (Bermejo et al., 1991) becomes potential – i.e. linear and double-logarithmic – in nature (Eqn 2), thus indicating that the limiting effect on k_a values exerted by the aqueous stagnant water layer adjacent to the absorbing membrane when compounds are perfused in free solution, has been lost. That would account for the importance of the apolar group in that action, since it has been demonstrated that sodium taurocholate does not influence the layer constitution (Bermejo et al., 1991).

The actual slope of the k_0 correlation line (Table 2), which is much lower than expected from free solution correlation, indicates that the membrane polarity has been increased by surfactant molecules (Plá-Delfina et al., 1987). It should be noted that this latter effect is stronger with

TABLE 2

Equation parameters and statistical figures associated with the correlations between absorption rate constants and lipophilicity indexes

Surfactant	Lipophilicity	Equation parameters				Statistics	
		$C \pm SD$	$d \pm SD$	$E \pm SD$	$f \pm SD$	AIC	r
Polysorbate 80 [a]	M	$0.221 \pm 2.24 \times 10^{-2}$	$6.14 \times 10^{-3} \pm 2.20 \times 10^{-4}$	$6.46 \times 10^{-4} \pm 4.36 \times 10^{-4}$	$1.71 \times 10^{-2} \pm 1.44 \times 10^{-2}$	−24.848	0.998
Sodium lauryl sulfate	M	$0.622 \pm 6.66 \times 10^{-2}$	$3.48 \times 10^{-3} \pm 2.30 \times 10^{-4}$	$1.22 \times 10^{-4} \pm 2.16 \times 10^{-7}$	$1.86 \times 10^{-2} \pm 1.86 \times 10^{-2}$	−20.113	0.990
	K'	$2.624 \pm 4.28 \times 10^{-2}$	$1.36 \times 10^{-1} \pm 9.98 \times 10^{-3}$	$2.36 \times 10^{-1} \pm 4.12 \times 10^{-2}$	$8.04 \times 10^{-1} \pm 9.58 \times 10^{-2}$	−21.437	0.991
	$1/R_f - 1$	$1.869 \pm 6.52 \times 10^{-2}$	$3.35 \times 10^{-1} \pm 2.42 \times 10^{-2}$	$3.24 \times 10^{-2} \pm 1.28 \times 10^{-2}$	1.966 ± 0.232	−21.837	0.991

[a] From Bermejo et al. (1991).

lauryl sulfate than with polysorbate, as determined by Bermejo et al. (1991); this can be assessed from the comparison of the k_0/M correlation slopes, d, that are statistically different ($p < 0.001$) and would mean that the passage of lipophilic compounds across the modified membrane is more effectively reduced and that of hydrophilic compounds more effectively enhanced by lauryl sulfate than by polysorbate, as judged from the values in Table 2. As a consequence of this greater effect on membrane polarity, the k_0 line of lauryl sulfate intersects with the k_a hyperbola later than does that of polysorbate, as found by Bermejo et al. (1991). Moreover, in other nonspecialized absorption sites such as the stomach, the two surfactants show a similar comparative behaviour, with perhaps clearer differences, probably due to the sensitivity of the gastric mucosae (Garrigues et al., 1990), as shown in Fig. 2.

SMC experiments

At the SMC, the presumed surfactant behaviour, as pointed out by Plá-Delfina et al. (1987), is again confirmed as a general rule. The

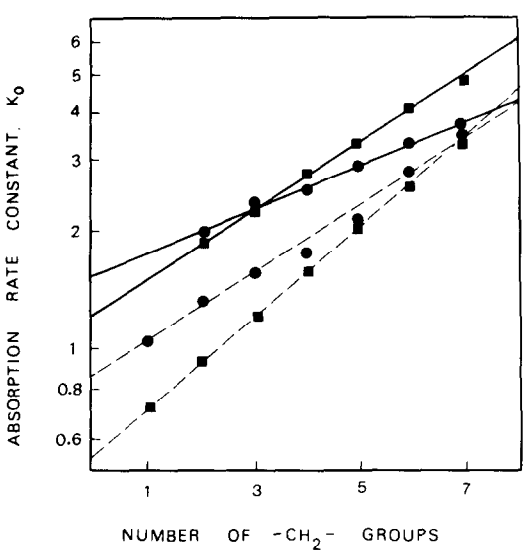

Fig. 2. Comparative behaviour at CMC of lauryl sulfate (●) and polysorbate (■) in colon (———) and stomach (------); the latter results are from Bermejo et al. (1991). The increase in membrane permeability is always higher for lauryl sulfate, as assessed from the lower slope of the curves.

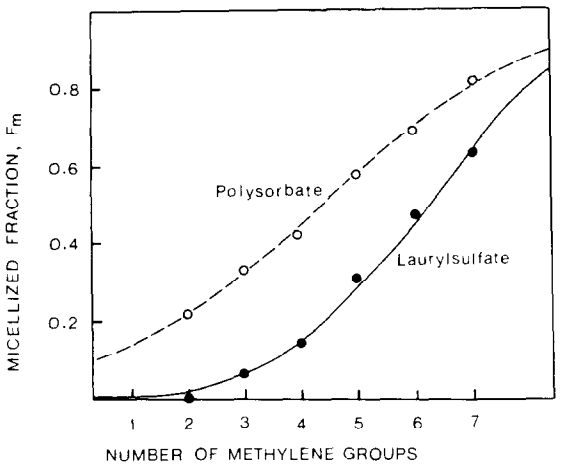

Fig. 3. Plot of solubilized fractions of the solutes in the colonic perfusate in the presence of 1.25% sodium lauryl sulfate (●———●) or 5.0% polysorbate (○- - -○) as determined by Bermejo et al. (1991), against solute lipophilicity (i.e. the number of straight-chain methylene groups in the molecule of the compound). Solubilization by the nonionic surfactant is shown to be more effective at the tested concentrations.

bilinear equation (Eqn 3) was found to fit k_s and lipophilicity data better, as a consequence of the multiple-phase equilibrium arising between compounds in free solution, micelle-solubilized compounds and the absorbing membrane, as shown in Table 3 and Fig. 1 (k_s line).

As comparative solubilizing features of polysorbate and lauryl sulfate, it should be pointed out that lauryl sulfate does not appear to be as potent as polysorbate as a compound solubilizer under the working conditions used for colon tests, as demonstrated by the micelle partition coefficient P_a, relative to solute lipophilicity (as judged by the number of -CH_2- groups in the aliphatic chain). As can be observed from Fig. 3, the solubilized fraction of the compounds, F_m, calculated as $1 - (k_s/k_0)$ (Plá-Delfina et al., 1987) is always lower in the presence of lauryl sulfate than with polysorbate, both at approximately equal molar concentrations. This can be due to an interaction between the anionically charged micelles and ionised (up to 99.9%) solutes, which could hinder or even prevent extensive trapping of the compounds within the amphiphile aggregates.

Biopharmaceutical implications

The observations reported on the different ability of surfactants to exert general influences on absorption should be considered when they are used as drug components. Only a careful study on drug bioavailability would demonstrate which solubilizer or wetting agent is the optimum; in a general approach, from our results, anionic surfactants would maximally improve the absorption of a hydrophilic compound as they produce the most significant change in permeability and some lipophilic drugs, if orally administered as solubilized solutions, due to the lower degree of trapping, and, consequently, reduction in the free fraction. If dilution is carried out, nonionic surfactants would give rise to the greatest improvement in the absorption of lipophilic drugs as they act as gentle membrane modifiers, while they completely avoid its limiting absorption step.

Acknowledgments

The present work is a part of an investigative project developed 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 a grant to one of us (M.V. B.). The authors are also indebted to Dr Francisco Comellas (Instituto de Quimica Textil; CSIC, Barcelona) for CMC determination of the surfactant used in this work.

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