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The effect of simple micellar systems on the solubility and intestinal absorption of clofazimine (B663) in the anaesthetised rat

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Abstract

Clofazimine (B663) is a highly lipophilic drug used in the treatment of leprosy. The solubility and gastrointestinal membrane permeability (P_{app}) of B663 in buffer and in micellar solutions were examined. Membrane permeability was determined using a rat gut perfusion model and, in addition, these studies incorporated the hydrophilic marker PEG 4000. The micellar systems included the bile salts; sodium deoxycholate, sodium cholate and sodium taurocholate, and the synthetic surfactants; sodium dodecyl sulphate (anionic) and cremophor EL (non-ionic). The low P_{app} of B663 in buffer (0.98×10^{-5} cm s^{-1}) was ascribed to a combination of the low flow rate used, and the high degree of ionisation of B663 at pH 7.2 which resulted in the production of an impermeable ion. Limited absorption of PEG 4000, in buffer, was also observed. All micellar systems investigated enhanced the solubility of B663. Maximum solubility (> 350 fold) was observed in the non-ionic surfactant. The P_{app} of B663 was unaffected by low concentrations of bile salts, however, at the higher concentration of sodium cholate (80 mM) an increase was observed. The P_{app} of PEG 4000 increased with increasing bile salt concentration. Both synthetic surfactants enhanced the P_{app} of PEG 4000, in contrast, B663 was enhanced only by the non-ionic surfactant. These results have shown that micellar systems can enhance the absorption of B663 from saturated solutions. Solubilisation did not inhibit absorption, rather the increase in solubility was reflected by increases in absorption rates.

Key words: Clofazimine; Micelle; Bile salt; Cremophor-EL; Sodium dodecyl sulfate; Solubility; Membrane permeability

1. Introduction

Clofazimine ($C_{27}H_{22}Cl_2N_4$) is a rimnophenazine derivative used in the treatment of dapsone resistant leprosy. It has been shown that clofazimine (B663) and its derivatives are active against *Mycobacterium avium* which can cause life threatening infections in AIDS patients (Lindholm-Levy and Heifets, 1988). B663 is practically insoluble in water and is reputedly the most lipophilic drug administered orally to man (Hooper and Purohit, 1983). Log P values in the range 4.39 (octanol:water), to 5.102 (isoctane:buffer) (Canavan et al., 1986) and 7.45 (Hooper and Purohit, 1983) have been reported.

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The absorption of clofazimine from the gastrointestinal tract is incomplete, however, it is enhanced by the co-administration of lipid vehicles. Following oral administration in the form of coarse crystals only 20% of the drug is absorbed, increasing to 85% when co-administered as a micro-crystalline oily suspension (Yawalker and Vischer, 1979). Consequently, the physiological processes involved in the digestion and absorption of the lipid vehicle may be relevant in the absorption of this drug. Bile salts and fatty acids are known to play a role in the absorption of fats by solubilizing the water insoluble products of fat digestion into water soluble aggregates or micelles (Carey and Small, 1972). Micellar systems have, therefore, been exploited as vehicles for oral drug delivery. However, the mechanisms by which micellar systems affect absorption are complex and conflicting results have been reported indicating that these systems may enhance (Muranishi, 1985), or retard transport (Amidon et al., 1982; Poelma et al., 1989, 1991).

The purpose of this study was to examine the effects of a range of simple micellar systems on the solubility and gastrointestinal absorption of clofazimine, using a rat gut perfusion model.

2. Theory

The apparent permeability coefficient (P_{app}) of the drug is an indication of the overall permeability of the gastrointestinal barrier to the drug and is defined in Eq. 1 (Ho et al., 1977, 1983).

$$P_{app} = 1/\left[(1/P_{aq}) + (1/P_m)\right] \quad (1)$$

where P_{aq} is the permeability coefficient of the aqueous boundary layer for drug molecules (cm s^{-1}) and P_m represents the membrane permeability coefficient (cm s^{-1}).

Values of P_{aq} and P_m may be calculated as follows:

$$P_{aq} = D_{aq}/h \quad (2)$$

where D_{aq} is the aqueous diffusion coefficient of all drug species (cm s^{-1}) and h denotes the effective thickness of the aqueous boundary layer.

$$P_m = P_o + P_p \quad (3)$$

where P_o is the permeability coefficient of the lipoidal pathway of the membrane for the non-dissociated drug species and P_p denotes the permeability coefficient of the aqueous pore pathway.

The experimental apparent permeability coefficient was calculated for each system using the following expression:

$$P_{app} = -(Q/2\pi rl) \cdot \ln(C(l)/C(0)) \quad (4)$$

where Q is the flow rate (ml s^{-1}) and $C(l)/C(0)$ represents the fraction of drug remaining in the intestinal lumen of length l and effective lumenal radius r.

The rate of drug absorption (R), mg s^{-1}, was also calculated for each system according to the following:

$$R = (C(0) - C(l)) \cdot Q \quad (5)$$

where $(C(0) - C(l))$ is the difference in concentration of drug entering and leaving the lumen.

3. Experimental

3.1. Materials

All bile salts of least 98% purity were obtained from Sigma Chemical Co. Sodium dodecyl sulphate, purity 99% or greater, was obtained from BDH Chemicals Ltd. Cremophor EL was kindly supplied by BASF. Clofazimine (B663) was obtained from the Health Research Board Laboratories, Trinity College, Dublin. All solvents used were of HPLC grade. Sorenson's phosphate buffer was prepared using Analar grade salts.

3.2. Determination of solubility

The saturated solubility of clofazimine in each of the systems under investigation was determined using the method of Hamlin and Higuchi (1966). Samples were assayed by UV at 283 nm.

3.3. Rat gut perfusion experiments

Absorption studies were conducted according to the method of Komiya et al. (1980) by perfus-

ing clofazimine, in the systems under investigation, through the cannulated upper intestine of the anaesthetised rat. Male Wistar rats were used throughout the study. All perfusate samples collected were weighed, and the results obtained indicated no significant change in flow output rate during perfusion. The P_{app} values were calculated using the actual fluid flow rate based on the weight of perfusate sample.

3.4. Analytical methods

Perfusion samples were assayed by HPLC using the following conditions: column type, Partisil 10 PAC (25 cm × 4.6 mm); mobile phase, ethanol : *n*-heptane (50 : 50); flow rate, 1 ml min^{-1}; detection by UV at 283 nm. To assess membrane integrity all systems incorporated a 'non-absorbable' marker, [^{14}C]PEG 4000. The [^{14}C]PEG 4000 had a specific activity of 15 mCi/g and was supplied as an aqueous solution containing 3% ethanol. The marker supplied was diluted 1 in 100. 1 ml of the dilution was added to 25 ml of the perfusing solution. Samples were analysed on a Tri-carb 4000 liquid scintillation counter using cocktail-T as scintillant.

3.5. Measurement of diffusion coefficients

Diffusion coefficients were measured by two separate techniques: (1) quasi-elastic light-scattering analysis; the values obtained were corrected for viscosity which was measured using a U-tube viscometer, and (2) diffusion cell method as described by Goldberg and Higuchi (1968). A silver membrane filter of diameter 25 mm and pore size 0.45 μm (Millipore AG4502500) was used. The cell was calibrated using benzoic acid as a standard substance of known diffusion coefficient.

4. Results and discussion

Initially the solubility and gastrointestinal absorption of B663 were determined in phosphate buffer solution, subsequently, similar studies were undertaken using a range of simple micellar systems incorporating natural (bile salts) and synthetic surfactants, both anionic and non-ionic.

4.1. The solubility and gastrointestinal absorption of clofazimine in aqueous solution

4.1.1. Solubility studies

The saturated solubility of clofazimine in isotonic buffer, pH 7.2, was determined as 0.49×10^{-3} mg ml^{-1} ($n = 3$). Using this measured solubility value and a pK_a of 8.51 (Fahelebom et al., 1993) a value for the intrinsic solubility of 2.283×10^{-5} mg ml^{-1} was obtained. The pH solubility profile of clofazimine, previously reported (O'Driscoll and Corrigan, 1992), illustrated that the solubility decreased from 5.68 to 0.278×10^{-3} mg ml^{-1} over the pH range 5.15–7.8. This is consistent with the basic nature of the compound.

4.1.2. Permeability studies

4.1.2.1. Clofazimine. The difference in the absorption of clofazimine, from both saturated and unsaturated (~ 70%) solutions, in isotonic buffer pH 7.2 was not statistically significant. The P_{app} of clofazimine, at a flow rate of 0.1 ml min^{-1}, was calculated as 0.98×10^{-5} cm s^{-1} (± 0.257). This value represents a mean percentage drug absorbed of 19.45%. Komiya et al. (1980), have determined the P_{app} of various compounds over a range of flow rates, using similar experimental techniques. Using the data published by Komiya et al. (1980) the thickness of the aqueous boundary layer (ABL), at the low flow rate, 0.1 ml min^{-1}, was estimated to be 853 μm. Based on the results of Komiya et al. (1980), an estimated P_{app} of approx. 6.62×10^{-5} cm s^{-1} was deemed reasonable for a highly lipophilic compound, at a flow rate of 0.1 ml min^{-1}. However, the experimentally obtained P_{app} for B663 was much lower.

Possible explanations for the overall low experimental permeability of clofazimine were considered. The pH solubility profile was not consistent with significant self-association or micelle formation (Roseman and Yalkowsky, 1973; Rowe, 1979; King et al., 1989). In addition, the solubility of B663 in the presence of the deaggregating agents, i.e., sodium salicylate and urea (King et al., 1989)

was determined. There was no difference between the solubilities measured in the presence and absence of the deaggregating agents. The presence of a deaggregating agent would break up any aggregates formed by self-association and would increase the solubility of the drug. Consequently, no evidence to support self-association of clofazimine could be obtained.

In contrast, the low P_{app} may be explained by the high degree of ionisation of the drug at the acidic microclimate pH (~ 6.3) in the GI tract. Due to the high degree of ionisation, it is proposed that negligible membrane permeability of the ionised form occurs and that the absorption of the drug is, therefore, membrane controlled. The absorption rate of B663 was also low, reflecting the poor aqueous solubility of B663 at pH 7.2.

The P_{aq} for clofazimine was determined from Eq. 2 as 8.697×10^{-5} cm s^{-1}. The aqueous diffusion coefficient of B663 was calculated as 7.419×10^{-5} cm s^{-1} according to the Stokes Einstein equation, using molecular weights and the published value for the diffusion coefficient of progesterone (Amidon et al., 1982).

Ho et al. (1977) have indicated that molecules with molecular weights in excess of 300 are not absorbed through the aqueous pore pathway, therefore in the case of B663, the permeability coefficient of the membrane, P_m, can be considered as the permeability coefficient of the drug through the lipoidal pathway of the membrane, P_o. By substituting the experimentally obtained P_{app} for B663 and the estimated value of P_{aq}, into Eq. 1 a P_m value of 192.7×10^{-5} cm s^{-1} was calculated (Table 1). This value of P_m represents the membrane permeability of the unionised fraction.

4.1.2.2. PEG 4000. Analysis of the [^{14}C]PEG 4000 showed that a fraction was absorbed, and a P_{app} of 0.447×10^{-5} cm s^{-1} (± 0.039) was calculated. Detailed examination of the sample weights indicated that these results were not due to a dilution effect. Limited absorption of PEG 4000 has previously been reported (Winne and Gorig, 1982).

The P_{aq} for PEG 4000 was calculated using Eq. 2, as described for B663. The value of D_{aq} for PEG 4000, calculated according to the Stokes Einstein equation, was 3.64×10^{-6} cm^2 s^{-1}. Substitution of both the value for P_{aq} and the experimental value of P_{app} into Eq. 1 allowed calculation of P_m (Table 1). Since PEG is a hydrophilic compound absorption is considered to be membrane controlled. In addition, absorption of PEG occurs mainly via the aqueous pore or paracellular pathways in the membrane (Katz and Hollander, 1989). Therefore, the value of P_m calculated for PEG can be considered as the permeability coefficient of the aqueous pore P_p, and can be used as an indication of paracellular absorption.

Table 1
In situ parameters for clofazimine and PEG 4000 in isotonic phosphate buffer, using the rat gut perfusion model

Parameter	Clofazimine	PEG 4000
P_{app} (cm s^{-1})	0.977×10^{-5}	0.447×10^{-5}
P_{aq} (cm s^{-1})	8.697×10^{-5}	4.27×10^{-5}
P_m (cm s^{-1}), unionised	192.7×10^{-5} (P_o)	0.471×10^{-5} (P_p)
Fraction unionised	0.0057	–
Flow rate (ml min^{-1})	0.1	0.1
ABL thickness (μm)	853	853

4.2. Solubility of clofazimine in simple micelles

4.2.1. Bile salts

The solubility of B663 in simple bile salt micelles was investigated. The three bile salts used were: sodium deoxycholate, a dihydroxy unconjugated bile salt, with a CMC between 2 and 5 mM; sodium cholate, a trihydroxy unconjugated bile salt, CMC 3–5 mM; and sodium taurocholate, a trihydroxy conjugated bile salt, CMC 8 mM.

All bile salts studied enhanced the solubility of the drug, the solubility increasing with an increase in bile salt concentration (Fig. 1). The increase in solubility was, however, not linear. The study using deoxycholate was conducted at pH 8.0 due to problems of gel formation at lower pH values. The results for the individual bile salts used did not differ greatly. The relative solubility enhancement observed in each case compared to the aqueous solubility of the drug at the corresponding pH is shown in Table 2. The maximum enhancement observed, within the concentrations used, was approx. 63-fold in the presence of 80

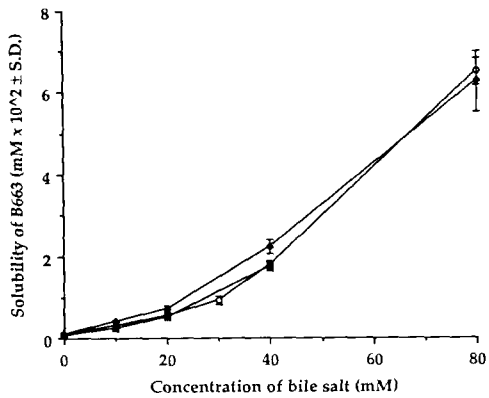

Fig. 1. The solubility of clofazimine (mean ± S.D.) in simple bile salt micellar systems; (○) sodium cholate (pH 7.2); (◆) sodium taurocholate (pH 7.2); (■) sodium deoxycholate (pH 8.0).

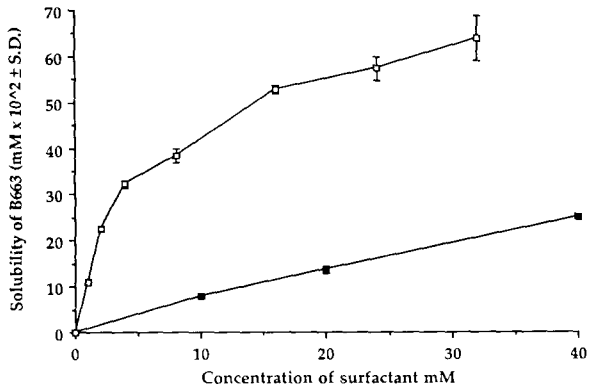

Fig. 2. The solubility of clofazimine (mean ± S.D.) in synthetic surfactants; (□) cremophor EL; (■) sodium dodecyl sulphate.

mM sodium cholate. The pH of each system was monitored and remained constant both with increasing concentration of bile salt, and over the entire time course of the solubility determinations.

With conventional micelles linear solubility plots would normally be obtained, indicative of a simple partition phenomenon (Fahelebom et al., 1993), however, bile salt micelles are more complex. In all three cases, the solubility profiles obtained curve upwards. The bile salt aggregates at low concentration of bile salt probably exist in the form of primary micelles with an aggregate number of 4–8. As the concentration of surfactant increases the primary aggregates associate through hydrogen bonding to form more complex secondary micelles (Small, 1971).

Table 2
Relative solubility enhancements for clofazimine (i.e., ratio of solubility in surfactant system compared to aqueous solubility at the relevant pH) in the presence of simple bile salt micellar systems

Bile salt	Concentration of bile salt (mM)				
	10	20	30	40	80
Sodium cholate (pH 7.2)	2.9	5.4	8.9	17.3	62.5
Sodium taurocholate (pH 7.2)	4.1	7.0	–	21.4	60.3
Sodium deoxycholate (pH 8.0) [a]	4.2	8.6	–	30.3	–

[a] Compared to solubility of clofazimine at pH 8.0.

4.2.2. Synthetic surfactants

The effects of sodium dodecyl sulphate (SDS), an anionic surfactant, and cremophor EL (polyoxyethylene glycerol triricinoleal 35, Polyoxyl 35 castor oil), a non-ionic surfactant, on the solubility of clofazimine were investigated. The increase in solubility of clofazimine in the presence of increasing concentrations of SDS is essentially linear (Fig. 2). The effect of increasing concentration of cremophor EL on the solubility of clofazimine is also shown in Fig. 2. This plot is non-linear and has a decreasing slope.

Both synthetic surfactants enhanced the solubility of the drug to a much greater extent than the corresponding bile salt systems. The non-ionic surfactant was a more effective solubiliser of clofazimine than the anionic. The solubility of clofazimine in the presence of 2% cremophor EL (approx. 8 mM) was enhanced by 365-fold, compared to 217-fold in the case of 40 mM SDS. The hydrophilic/lipophilic balance (HLB) of cremophor EL lies between 12 and 14 (Basf data sheet) while that of SDS is approx. 40 (Helenius and Simons, 1975). These values demonstrate that SDS has a greater hydrophilic character. The observation that non-ionic surfactants are greater solubilisers than anionic surfactants of the same hydrocarbon chain length, for solubilisates lo-

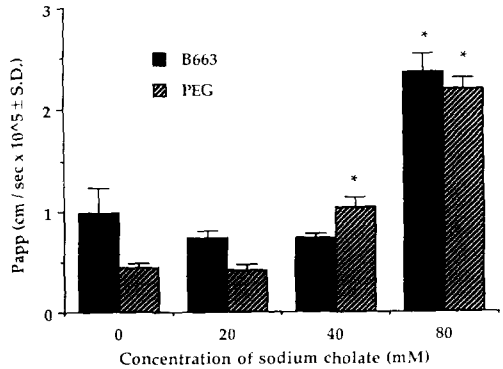

Fig. 3. The effect of sodium cholate concentration on the apparent permeability coefficients (mean ± S.D.) of clofazimine (B663) and PEG 4000 from solutions saturated with B663 ($n = 6$) (*statistically significant from the P_{app} in phosphate buffer at the 98% level).

cated in the micellar interior, has been reported previously (Klevens, 1950; Schott, 1967). In general, the solubilising capacity increases in the order anionic < cationic < non-ionic, the effect being attributed to the formation of micelles with a less dense hydrocarbon core which can accomodate more solubilisate. In addition, the non-ionic surfactants have a lower CMC than the ionic surfactants and are therefore more efficient solubilisers at lower concentrations.

4.3. Absorption of clofazimine from simple micellar systems

4.3.1. Bile salts

The effects of increasing concentration (0–80 mM) of sodium cholate on the P_{app} of both B663 and PEG are shown in Fig. 3.

4.3.1.1. Clofazimine. For concentrations up to 40 mM NaC, saturated with drug, no significant enhancements in the P_{app} of B663 relative to the saturated buffer solution were observed. At 80 mM NaC, however, a large increase in P_{app} occurred. The rate of drug absorption increased in the presence of all concentrations of surfactant, due to the increase in solubility.

The effects of NaC and its taurine conjugate bile salt NaTC on the P_{app} of both B663 and PEG are compared in Fig. 4. At a concentration of 40 mM, NaTC did not significantly enhance the P_{app} of B663 relative to NaC, the rate of drug absorption increasing with increasing surfactant concentration.

4.3.1.2. PEG 4000. The P_{app} of PEG 4000 increased significantly with increasing concentrations of both bile salts (Fig. 3 and 4). This result suggests that the mechanism whereby the bile salts alter the permeability of PEG, relative to that of the drug, may differ.

In the presence of micelles large amounts of unionised B663 become solubilised in the hydrophobic interior of the micelle. The proportion of free drug in solution, either ionised or unionised, is negligible. These micelles are transported across the ABL, consequently increased concentrations of drug are available at the absorption surface. Because B663 is so lipophilic the membrane permeability of the unionised drug is very large (Table 1) and, as a result, the rate limiting step in absorption in the case of micellar systems may shift from the membrane to the ABL.

The increase in P_{app} observed with the high concentration of bile salt (80 mM) could not be fully explained in terms of drug in the micelles diffusing across the ABL. It has been reported that bile salts alter the viscosity and structure of the mucus layer and that this effect is concentration dependent (Martin et al., 1978; McQueen et

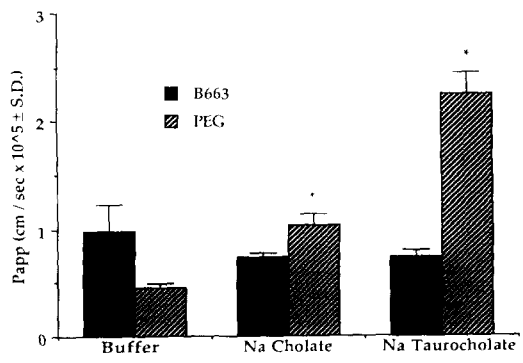

Fig. 4. A comparison of the apparent permeability coefficients (mean ± S.D.) of clofazimine and PEG 4000 from simple bile salt (40 mM) micellar systems ($n = 6$) (*statistically significant from the P_{app} in phosphate buffer at the 98% level).

Table 3
The apparent permeability coefficients (P_{app}) and the micellar aqueous diffusion coefficient (D^*_{aq}) of clofazimine (B663), in sodium cholate systems saturated and unsaturated with B663

System	P_{app} (cm s^{-1}) ($\times 10^5$) ($\pm$S.D.)	D^*_{aq} (cm^2 s^{-1}) ($\times 10^7$) ($\pm$S.D.)	
		Method A [a]	Method B [b]
40 mM sodium cholate no B663 present	–	9.67	–
40 mM sodium cholate unsaturated with B663	1.164 $\pm$ 0.150 (n = 6)	8.023	11.815 $\pm$ 1.38
40 mM sodium cholate saturated with B663	0.738 $\pm$ 0.041 (n = 6)	4.090	2.89 $\pm$ 0.17

[a] Quasi-elastic light-scattering data.
[b] Diffusion cell method.

al., 1983; Poelma, et al., 1990). Consequently, it is postulated that the enhancement effect observed in this study, at the higher bile salt concentration, may be due to a reduction in the thickness of the ABL. This erosion of the ABL may occur through breakdown of the mucus layer which, because of its viscous nature, may impose a significant resistance to absorption.

The significance of the resistance contributed by the ABL to the P_{app} is further illustrated by comparison of the P_{app} for B663 in NaC (40 mM) micelles saturated and unsaturated (53%) with drug (Table 3). There was a trend towards a higher P_{app} for B663 in the unsaturated system, although this effect was not statistically significant. The aqueous diffusion coefficient of B663 in (40 mM) NaC micelles (D^*_{aq}) was measured using both quasielastic light scattering data and a diffusion cell method, these results are also shown in Table 3. The D^*_{aq} of the NaC micelle is included for comparison. The D^*_{aq} of B663, measured by both methods, was lower in the saturated system. Increasing the degree of saturation of the NaC 40 mM micelles with B663 from 52% to 100% led to a reduction in the D^*_{aq}, as measured by QELS, from 8.02 to 4.09 $\times 10^{-7}$ cm^2 s^{-1}. Thus, this 2-fold decrease in diffusion in D^*_{aq} is the same order of magnitude as the decrease in P_{app}. Therefore, the lower P_{app} of micelles saturated with drug may be explained in terms of a lower diffusion coefficient of the micelle across the ABL.

PEG is a hydrophilic compound and its absorption is considered membrane rather than ABL controlled. As discussed previously, the value of P_m can be considered as the permeability of the aqueous pore pathway, i.e., P_p. Theoretical values for the P_p of PEG 4000, in the presence of bile salts, were calculated, as before, by substituting the estimated value of P_{aq} and the experimental values of P_{app} for PEG 4000, into Eq. 1. These values of P_p together with the P_{app} values of PEG 4000 in the presence of bile salts are shown in Table 4. The values of both the P_p and the P_{app} of PEG 4000 increased with increasing concentration of bile salts used. It is postu-

Table 4
Estimates of the permeability of the aqueous pore pathway (P_p) and the experimental permeability coefficients (P_{app}) for PEG 4000 in the presence of bile salts

Perfusate (n)	P_p (cm s^{-1}) ($\times 10^5$)	P_{app} (cm s^{-1}) ($\times 10^5$) ($\pm$S.D.)
Isotonic buffer pH 7.2 (12)	0.4707	0.447 $\pm$ 0.039
20 mM sodium cholate (6)	0.4646	0.421 $\pm$ 0.045
40 mM sodium cholate (6)	1.3570	1.030 $\pm$ 0.103 [a]
80 mM sodium cholate (6)	4.5130	2.194 $\pm$ 0.12 [a]
20 mM sodium taurocholate (4)	1.1240	0.890 $\pm$ 0.084
40 mM sodium taurocholate (6)	4.7610	2.251 $\pm$ 0.187 [a]

[a] Statistically significant from the P_{app} in isotonic phosphate buffer $\geq$ 98%.

lated therefore that the enhancement in P_{app} is due to a change in the paracellular permeability of the membrane caused by the effect of the bile salts on the tight junctions; such an effect has been suggested previously (Winne and Gorig, 1982; Yamashita et al., 1990).

4.3.2. Synthetic surfactants

The absorption of clofazimine from a saturated solution containing 2% cremophor EL system was measured. In addition, the absorption of both clofazimine and PEG from sodium dodecyl sulphate (SDS), 20 mM ($n = 2$) and 40 mM ($n = 6$) saturated with drug, was measured. The P_{app} of clofazimine from each of these systems along with the rate of absorption are shown in Table 5. The corresponding values of P_{app} for the absorption of clofazimine from isotonic phosphate buffer are included for comparison.

It can be seen that the P_{app} of clofazimine increased significantly in the presence of 2% cremophor EL. In contrast, at a concentration of 40 mM (1.15%), SDS had no effect on the P_{app} of clofazimine, however, the P_{app} in the presence of 20 mM SDS was significantly lower than the P_{app} in buffer alone (pH 7.2). The P_{app} of clofazimine from 40 mM sodium dodecyl sulphate is of the same order of magnitude as that from 40 mM sodium cholate.

The P_{app} values of PEG from the 2% cremophor EL system and the 20 mM and 40 mM sodium dodecyl sulphate system are significantly higher than the P_{app} of PEG from buffer alone (Table 5). In addition, however, for the 20 and 40 mM SDS system the P_{app} of PEG is significantly higher, 95%, than the corresponding P_{app} of clofazimine. The permeability coefficients of PEG 4000 at both concentrations of SDS are very similar. The P_{app} values of both clofazimine and PEG are very similar for the 2% cremophor EL system. A difference therefore appears to exist between the effect of the two surfactants on transport mechanisms, since the increased P_{app} of PEG in the presence of the non-ionic emulgent is accompanied by a similar increase in the P_{app} of drug, while the increased P_{app} of PEG in the presence of both 20 and 40 mM SDS is not.

Both surfactants exert an effect on the membrane as is illustrated by the increase in the P_{app} values of PEG, however, the effect of the anionic surfactant is greater. This observation is consistent with various studies indicating greater membrane effects with anionic compared to non-ionic surfactants (Penzotti and Mattocks, 1968; Attwood and Florence, 1983). However, monitoring the permeability of PEG 4000 allows only the effect on P_p (paracellular route) to be examined and does not provide information on the effects of the surfactants on P_o, which in combination with P_p determines the overall P_m.

The permeability of the drug was enhanced by the non-ionic surfactant but not by the anionic SDS. This result again suggests different absorption mechanisms. In the case of the membrane in addition to the effect on the P_p, already established, it is proposed that the non-ionic surfactant may alter the permeability of the lipoidal pathway (P_o), e.g., by increasing the fluidity of the membrane and thus facilitating transport of drug both free and solubilised.

The surfactants may also affect transport across the ABL (P_{aq}). The value of P_{aq} is influenced by the micellar aqueous diffusion coefficient of the molecule (D^*_{aq}) and the thickness of the ABL.

Table 5
The experimental permeability coefficients (P_{app}) of B663 and PEG 4000, and the rates of absorption (R) of B663 from simple synthetic micellar systems at pH 7.2

Perfusate (n)	P_{app} B663 (cm s^{-1}) ($\times 10^5$) ($\pm$S.D.)	R B663 (mg s^{-1}) ($\times 10^5$) ($\pm$S.D.)	P_{app} PEG (cm s^{-1}) ($\times 10^5$) ($\pm$S.D.)
Isotonic phosphate buffer (pH 7.2) (12)	0.977 $\pm$ 0.257	0.0132 $\pm$ 0.002	0.447 $\pm$ 0.039
2% Cremophor EL (6)	1.229 [a] $\pm$ 0.083	7.3300 $\pm$ 0.430	1.148 [a] $\pm$ 0.061
20 mM sodium dodecyl sulphate (2)	0.469 [a] $\pm$ 0.102	1.1565 $\pm$ 0.227	1.286 [a] $\pm$ 0.024
40 mM sodium dodecyl sulphate (6)	0.752 $\pm$ 0.064	3.0550 $\pm$ 0.241	1.247 [a] $\pm$ 0.193

[a] Statistically significant from the corresponding P_{app} in isotonic phosphate buffer $\geq 95\%$.

Table 6
The aqueous micellar diffusion coefficient of clofazimine (D^*_{aq}) from simple synthetic micellar systems, saturated with B663, measured by the diffusion cell method

System	D^*_{aq} ($cm^2\ s^{-1}$) ($\times 10^6$) ($\pm$S.D.)
B663 in 40 mM sodium cholate	0.288 $\pm$0.0173
B663 in 2% cremophor EL	0.2817 $\pm$0.159
B663 in 20 mM SDS	2.13
B663 in 40 mM SDS	1.435 $\pm$0.488

The apparent micellar aqueous diffusion coefficients (D^*_{aq}) of clofazimine in both the non-ionic emulgent cremophor EL system and the anionic sodium dodecyl sulphate system were measured using the diffusion cell system. The values of (D^*_{aq}) from each system are shown in Table 6. The aqueous micellar diffusion coefficient (D^*_{aq}) of clofazimine in 40 mM sodium cholate saturated with drug and measured by the same method is included in Table 6 for comparison. The aqueous micellar diffusion coefficient (D^*_{aq}) of clofazimine from the saturated 2% cremophor EL system is of the same order as that from a 40 mM sodium cholate system saturated with drug. The D^*_{aq} of clofazimine from the 20 mM sodium dodecyl sulphate was calculated as 2.13×10^{-6} $cm^2\ s^{-1}$ and for the 40 mM system as 1.435×10^{-6} $cm^2\ s^{-1}$ which indicates that the diffusion of the SDS micelles saturated with drug is faster than any other of the micellar systems studied. In spite of these high D^*_{aq} values the P_{app} of B663 was lower in the anionic compared to the non-ionic surfactant. The values of D^*_{aq} were measured in a simple aqueous media in vitro. In vivo, the ABL is a much more complex environment which includes mucus. Thus, the D^*_{aq} values measured by the diffusion cell method may be an overestimation of the true in vivo value. In contrast, the observed difference in P_{app} values of B663 in the two surfactants may be due to different effects on the thickness of the ABL.

In addition, it has been reported that adjuvants can affect charge-selective permeability and electrical resistance of rat jejunal membrane and thus influence absorption rates (Yamashita et al., 1990). Such effects may also contribute to the P_{app} differences observed in this work.

5. Conclusions

Micellar systems can enhance drug absorption. These results appear to be inconsistent with those of Poelma et al. (1989, 1991), who reported that micellar systems inhibited lipophilic drug absorption. However, it must be noted that in the current study saturated solutions of a highly insoluble compound (B663) were used, in contrast to the unsaturated systems used by Poelma et al. (1989, 1991).

The reported enhancements in absorption are due to a combination of factors including effects on the solubility of the drug, the ABL, and the membrane. The relative importance of these effects is dependent on the physicochemical properties of the drug and the micelle components.

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