Surface Activity and Concentration Dependent Intestinal Permeability in the Rat

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Purpose. To investigate the relation between intestinal effective permeability (P_{eff}) and surface activity of fluvastatin and verapamil.

Methods. P_{eff}-values were determined for fluvastatin, antipyrine and D-glucose following colon perfusions in the rat *in situ*. The perfusion solutions differed regarding concentrations of fluvastatin (0–2500 μM) and surface tension (58.9–43.7 mN/m). A cellulose derivative, ethyl-(hydroxyethyl) cellulose (EHEC), was added to lower the surface tension of one of the perfusion solutions. The surface tension of perfusion solutions containing R/S-verapamil (8–814 μM) and R/S-verapamil + chlorpromazine (814 μM + 10 mM) were related to the corresponding P_{eff}-values from the literature.

Results. The P_{eff} of fluvastatin correlated inversely ($r^2 = 0.985$, $p < 0.05$) with the surface tension of the perfusion solutions below the critical micelle concentration (CMC, 1 mM). Decreasing the surface tension with EHEC increased the P_{eff} of fluvastatin by 36% ($p < 0.001$), but not to the extent anticipated from the correlation between the P_{eff} and the surface tension. EHEC also increased the P_{eff} of antipyrine by 49% ($p < 0.01$) but not for D-glucose. The P_{eff} of R/S-verapamil correlated inversely with the surface tension ($r^2 = 0.980$, $p < 0.001$).

Conclusions. The ability of fluvastatin to decrease the surface tension at the membrane surface can partly explain the concentration dependent colonic P_{eff} of fluvastatin. This study shows that the surface activity of the drug molecule itself is an important physicochemical factor that should be taken into consideration when evaluating drug absorption studies performed *in vitro* or *in situ*.

KEY WORDS: surface activity; surface tension; drug absorption; intestinal permeability; P-glycoprotein; fluvastatin; verapamil; chlorpromazine.

INTRODUCTION

The intestinal effective permeability coefficient (P_{eff}) of a drug in various regions of the gastrointestinal tract is one of the fundamental factors determining the extent of oral absorption. Fluvastatin, a synthetic inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, exhibits a regional as well as a concentration dependent P_{eff} in the rat intestine (1). The colonic P_{eff} of fluvastatin in the rat increased from 0.6 to 1.0×10^{-4} cm/s ($p < 0.05$) when the concentration in the intestinal lumen was raised from 1.6 to 160 μM (1). Although the exact mechanisms behind this concentration dependency are not known, it is consistent with saturation of any efflux system (e.g., P-glycoprotein) located in the colonic membrane (1–2). Two other inhibitors of HMG-CoA reductase, lovastatin acid and atorvastatin, have recently been reported to interact with the P-glycoprotein system (3–5). In our own intestinal perfusion experiments in rats, we observed an increased P_{eff} of fluvastatin in the ileum when lovastatin acid was present in excess (1). However, when lovastatin acid was added to the lumenal side during a single pass perfusion of the rat ileum and an intravenous infusion of fluvastatin was given simultaneously, the exsorption of fluvastatin (transport from blood to lumen) increased surprisingly (1). This suggests that fluvastatin is not a substrate for P-glycoproteins, but that instead lovastatin affects the physical barrier-properties of the intestinal membrane, leading to increased passive transport of fluvastatin and other molecules. Apparently, fluvastatin is a drug with a complex intestinal absorption that might involve parallel transport mechanisms.

Fluvastatin is a surface active molecule since it consists of one lipophilic part and one more hydrophilic part (Fig. 1). It is well known that surface active compounds (e.g., sodium dodecyl sulfate, polysorbates, medium chain fatty acids and bile acids) can be used as permeation-enhancers (6–9). The mechanisms by which surface active compounds enhance intestinal permeation have been shown to involve both paracellular and transcellular routes. For instance, it has been suggested that sodium caprate influences the paracellular route through phospholipase C-dependent inositol triphosphate and diacylglycerol pathways, while the transcellular route is affected by membrane perturbation caused by the interaction between sodium caprate and membrane proteins or lipids (10–11). Furthermore, the transport-enhancing effect of medium chain fatty acids and their acylglycerols on penicillin V *in vitro*, has recently been shown to correlate well with the compounds ability to reduce the surface tension (12). Shima and coworkers also suggested that to a great extent the permeation-enhancer effect of diacylglycerol-caproic acid arises from a direct physicochemical effect, and not from an effect on tight junction integrity *via* protein kinase C (12).

The aim of this study was to investigate whether the concentration dependent permeability of fluvastatin in the rat colon could be related to its surface activity. The absorption of various transport and viability markers were assessed in parallel. Finally, we examined whether the permeability of a known substrate for the P-glycoprotein efflux system, R/S-verapamil, also could be related to its surface activity.

MATERIALS AND METHODS

Study Design

The study involved four groups of rats (I–IV) each of which was perfused in the colon with a different perfusion solution. Each solution was perfused in the colon of 4–5 rats, and 19 rats were used in total in this study. The perfusion solutions differed in their concentrations of fluvastatin: (I) 0 μM

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ABBREVIATIONS: P_{eff}, effective permeability; EHEC, ethyl(hydroxyethyl)cellulose; LDH, lactate dehydrogenase; NWF, net water flux.

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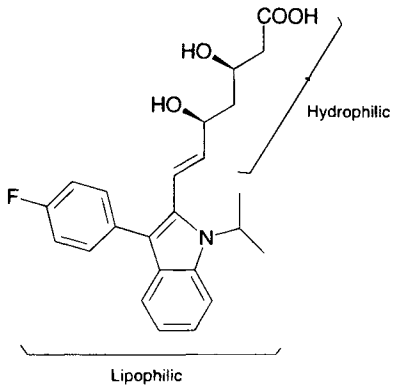

Fig. 1. Fluvastatin is a carboxylic acid with a pKa of 4.6, a log P (octanol/water) of 3.8, a predicted log D (pH 6.5) of 1.8 and a molecular weight of 411 (27). The water solubility of fluvastatin sodium is approximately 80 g/l and the drug is usually administered orally in 20–40 mg doses. Fluvastatin is amphiphilic, consisting of one lipophilic part and one more hydrophilic part.

(control), (II) 1.6 μM + 10 ppm ethyl(hydroxyethyl)cellulose (EHEC), (III) 800 μM and (IV) 2500 μM of fluvastatin. The EHEC was included in perfusion solution II to lower the surface tension. All solutions had the same pH (6.5) and osmolality (278 ± 3 mOsm/kg). Antipyrine (1.1 mM, Sigma Chemical Co., USA) and D-glucose (10 mM) were included in all perfusion solutions as markers for passive diffusion and viability, respectively. We have previously measured the rat colonic P_{eff} of fluvastatin at 1.6, 16 and 160 μM under the same conditions, so these values were used as a comparison (1).

Single Pass Perfusion Experiments in Rats

Male Sprague-Dawley rats (Crl:CD(SD)BR, Charles River, Uppsala, Sweden) weighing 277 ± 17 g (mean ± S.D.), were housed under constant and controlled conditions (22.5°C, 50% air humidity, 12 hours light cycle) in the animal facilities at the Biomedical Center, Uppsala. The animals had free access to tap water and regular rat chow (R36 Lactamin AB, Stockholm, Sweden) until 14–18 h prior to the experiment, when the food was withdrawn. Anesthesia was induced by an i.p. injection of 135 mg/kg of Inactin®-Byk (thiobutabarbital sodium), and the rats were placed on a heating pad (CMA-150, Carnegie Medicine AB, Stockholm, Sweden) to maintain the body temperature at 37 ± 1°C. Surgery was performed as described previously (1). Briefly, after onset of deep anesthesia, laparotomy was performed, and the proximal end of the colon was cannulated with plastic tubing (4 mm o.d.) and connected to a syringe on an infusion pump (Model 22, Harvard Apparatus Company, USA). An outlet tube was introduced through the anus and ligated 5–6 cm proximally the anus. The segment was cleaned by perfusing it with normal saline at 37°C until a clear outlet perfusate was obtained; it was then perfused with one of the four solutions at 0.2 ml/min for 105 min.

The outlet perfusate samples were collected into preweighed vials at 45, 60, 75, 90 and 105 min. Each vial was re-weighed after collection, and then the sample was frozen and stored at −20°C pending analysis. The segment was completely rinsed with 20 ml of normal saline at 37°C after the 105 min perfusion. The length of the segment was measured with a thread at time 110 min. Approval for this study was given by the Animal Ethics Committee, Uppsala University (approval number C246/95).

Intestinal Perfusion Solutions

The perfusion solutions consisted of 5.4 mM potassium chloride, 48 mM sodium chloride, 35 mM mannitol, 10 mM D-glucose, and 1 g/l polyethylene glycol 4000 (PEG 4000), all in a phosphate buffer pH 6.5. Trace amounts of ^{14}C-PEG 4000 and ^{3}H-D-glucose were added to the solutions as markers for water flux and viability, respectively (2.5 and 10 μCi/l, respectively. Amersham Labs., Buckinghamshire, England). Fluvastatin sodium was obtained from Astra Hässle AB, Sweden. The ethyl(hydroxyethyl)cellulose (EHEC) that was used for the lowering of the surface tension of the perfusion solution II was kindly provided by Akzo Nobel AB, Stenungsund, Sweden. EHEC is a nonionic and nontoxic water soluble cellulose derivative, substituted with a mixture of hydrophobic alkyl and hydrophilic alkylene oxide groups (13). 10 ppm of a high-molecular fraction of EHEC (PR) with an average molecular weight of 1.24×10^6 was used. The critical polymer concentration and the cloud point for EHEC (PR) are 3.6 ppm and 48°C, respectively (unpublished result).

Viability of the Colon Membrane

The viability of the colon membrane during the *in situ* perfusions was assessed by monitoring the total recovery of ^{14}C-PEG 4000, the P_{eff} of D-glucose and the appearance of lactate dehydrogenase (LDH) in the outlet perfusate (8,14). LDH was assayed in the 45 min fraction, in a pooled 60 and 75 min fraction and in a pooled 90 and 105 min fraction, after less than 2 hours storage at room temperature.

Stability and Adsorption Test

The possible interaction of fluvastatin, antipyrine and D-glucose with EHEC was investigated using equilibrium dialysis at 37°C with a membrane cut-off at 12 000 Da. None of the compounds under study were found to interact with EHEC at the concentrations used in the perfusion experiments. Colonic fluid was collected from two separate rats by gently rinsing the cannulated colon segment (as described above) with blank perfusion solution. The first 2.5 ml of fluid that left the segment was used for stability testing. Both antipyrine and fluvastatin were found to be stable in this fluid. The surface tension remained stable for 30 min when one part of the colon fluid was added to nine parts of the perfusion solution containing EHEC, indicating that EHEC was stable too. The fluvastatin was protected from light throughout the study.

Determination of Surface Tension

A duNoüy interfacial tensiometer (A. Krüss Optisch-Mechanische Werkstätten, Hamburg, Germany) was used to measure the surface tension (at 37°C, liquid/air) of the perfusion solutions containing 0, 1.6, 16, 160, 800 and 2500 μM fluvastatin, and 1.6 μM fluvastatin with 10 ppm EHEC. The surface tension was also measured of perfusion solutions containing verapamil at 8.1, 81 or 814 μM, and verapamil + chlorpromazine at 814 μM + 10 mM. The corresponding intestinal P_{eff} values of R/S verapamil have previously been measured in

the rat under identical conditions (15). The critical micelle concentration (CMC) of fluvastatin was obtained from plots of surface tension versus the logarithm of fluvastatin concentration.

Analytical Methods

Fluvastatin was assayed with a slightly modified reversed-phase HPLC method with fluorescence detection, as described previously (1,16). The mobile phase consisted of methanol, acetonitrile and a 17 mM ammonium-dihydrogen-phosphate solution, pH 3.5 (50:29:21). The samples and the standards were diluted with the mobile phase and 50 μl injected on the column (Hypersil 5 ODS, 250 × 4.6 mm). The flow rate was 1.0 ml/min, the detection wavelengths were 305 and 380 nm (excitation and emission). Fluvastatin was eluted after 5.1 min and the limit of quantification was 8 ng/ml (CV 7.8%). Antipyrine was assayed with a previously used and validated HPLC method, with the detection limit at 1.0 μg/ml (17).

The concentrations (dpm/ml) of ^{14}C-labeled PEG 4000 and ^{3}H-D-glucose were determined by liquid scintillation counting for 2 × 10 min (Mark III, Searle Analytical Inc., USA) after the addition of 8 ml of Beckman Ready Safe. The average value of these two scintillation measurements was used in the calculations. The concentration of LDH in the outlet perfusate was assessed with a colorimetric method (LDH, procedure # 500, Sigma). The pH and the osmolality were measured with a pH-meter (Metrohm 632) and an osmometer (Vescor 5500), respectively.

Data Analysis

Data analysis was performed as described previously (1). Briefly, the effective permeability, P_{eff}, across the intestinal mucosa was calculated according to a tube model (18):

$$P_{eff} = \frac{Q_{in} \times \ln (C_{in}/C_{out})}{2 \pi rL} \quad (1)$$

where Q_{in} is the inlet flow rate (0.2 ml/min), r and L are the radius and length of the segment, C_{in} and C_{out} are the inlet and outlet concentrations, respectively. The outlet concentration was corrected for fluid flux across the colonic mucosa.

The possible influence of the different perfusion solutions on the radius, recovery of PEG 4000, net water flux and the P_{eff} of the study compounds was tested with analysis of variance, followed by Fisher's protected least square difference to identify significantly different groups (StatView, Abacus Concepts, Inc., USA). A probability value less than 0.05 was considered to be significant. The correlations between P_{eff} and the surface tension and between P_{eff} and the concentration were tested by linear regression. All data are presented as the mean values from 4–5 rats and their standard deviations.

RESULTS

The critical micelle concentration (CMC) of fluvastatin was estimated to be 1 mM in the perfusion solution and 10 mM in pure water. All estimated variables from the colon perfusion experiments are presented in Table I. The colonic P_{eff} of fluvastatin increased with increasing concentration of fluvastatin up to the sub-CMC concentration (800 μM). At a higher perfusate concentration (2500 μM), i.e., in the presence of micelles, the P_{eff} of fluvastatin decreased ($p < 0.05$, Table I). The P_{eff} of antipyrine also increased at higher concentrations of fluvastatin, and decreased in the presence of fluvastatin micelles (Table I). The colonic P_{eff} of antipyrine was 49% higher in the EHEC group compared to the control group ($p < 0.01$). The P_{eff} of D-glucose was found to be low (0.08×10^{-4} cm/s) and neither affected by the presence of EHEC nor fluvastatin up to 0.8 mM (Table I). However, in the presence of fluvastatin micelles, the P_{eff} of D-glucose increased by 184% (0.08 to 0.22×10^{-4} cm/s) compared to the control group ($p < 0.0001$).

We used four different perfusion solutions in the in situ experiments: (I) no fluvastatin, (II) 1.6 μM fluvastatin with 10 ppm EHEC, (III) 800 and (IV) 2 500 μM fluvastatin. The surface tension of each solution was 58.9 ± 0.2, 46.3 ± 0.1, 45.0 ± 0.6 and 43.7 ± 0.4 mN/m, respectively. The surface tension of the perfusion solution containing 1.6, 16 and 160 μM fluvastatin (these concentrations were used in a previous report (1)) was 57.3 ± 0.1, 54.9 ± 0.2 and 52.1 ± 0.4 mN/m, respectively. The concentrations of fluvastatin in the perfusion solutions and the surface tensions of the solutions both correlated to the P_{eff} of fluvastatin at perfusate concentrations up to the sub-CMC concentration of 800 μM (Fig. 2). However, no correlations were found when the P_{eff}-values from the micelles group and the EHEC group were included too (Fig. 2). The P_{eff} of fluvastatin increased by 57% ($p < 0.001$) when the surface tension was decreased by the addition of EHEC, although this was not as much as expected from the correlation between P_{eff} and surface tension (Fig. 2).

Table I. Perfusion Variables Obtained During Single-Pass Perfusions in the Rat Colon at Different Concentrations of Fluvastatin

	0 μM, Control	1.6 μM + EHEC	800 μM	2500 μM
n	4	5	5	5
Radius (cm)	0.23 ± 0.02	0.23 ± 0.03**	0.30 ± 0.05*	0.29 ± 0.04*
PEG 4000 recovery (%)	99 ± 1	99 ± 1**	96 ± 1*	94 ± 2*
NWF (μL/h/cm)	−75 ± 59	−111 ± 38**	−121 ± 32**	136 ± 35*
P_{eff} D-glucose ($\times 10^{-4}$ cm/s)	0.08 ± 0.04	0.07 ± 0.03**	0.09 ± 0.01**	0.22 ± 0.05*
P_{eff} antipyrine ($\times 10^{-4}$ cm/s)	1.06 ± 0.34	1.58 ± 0.24**	1.48 ± 0.04**	0.82 ± 0.11
P_{eff} fluvastatin ($\times 10^{-4}$ cm/s)	NA	0.96 ± 0.09**	1.38 ± 0.04**	0.60 ± 0.12

Note: All results are calculated under steady state conditions, with the exception of the PEG 4000 recovery, which is calculated for the whole experiment (mean ± S.D.). EHEC: Ethyl(hydroxyethyl)cellulose 10 ppm was used to lower the surface tension. NWF: net water flux, a negative value indicates absorption of fluid. P_{eff}: Effective permeability. NA: not applicable.

* Significantly different ($p < 0.05$) from the control group.

** Significantly different ($p < 0.05$) from the 2500 μM group.

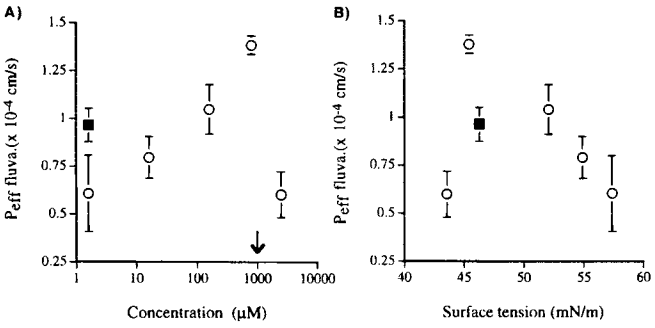

Fig. 2. The effective permeability (P_{eff}) of fluvastatin (○) correlates with the concentration (A, $r^2 = 0.958$, $p < 0.05$) and correlates inversely with the surface tension (B, $r^2 = 0.981$, $p < 0.01$) at concentrations up to the critical micelle concentration (arrow) of fluvastatin. No correlations were found when data from the EHEC (■) and the micelles groups were included in the correlations. Data are presented as the mean values ± S.D., n = 4–5.

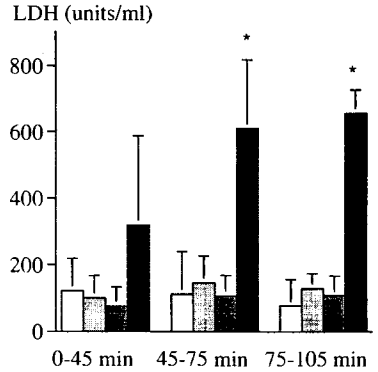

Fig. 4. Mean (± S.D.) concentrations of the viability marker lactate dehydrogenase (LDH) in the outlet perfusate during the *in situ* colon perfusions with the four different solutions: No fluvastatin (control, white, n = 4), 1.6 μM fluvastatin with 10 ppm EHEC (light gray, n = 5), 800 μM fluvastatin (dark gray, n = 5) and 2 500 μM fluvastatin (black, n = 5). The CMC of fluvastatin was approximately 1 000 μM. * significantly higher ($p < 0.0001$) than the other solutions in the same time interval.

The measured surface tension of the perfusion solutions containing R/S-verapamil (8.1, 81 and 814 μM) was 59.0 ± 0.1, 56.5 ± 0.6 and 53.0 ± 0.3 mN/m, respectively. When 10 mM of chlorpromazine was added to the solution containing 814 μM of verapamil, the surface tension decreased significantly (53.0 ± 0.3 to 44.4 ± 0.3 mN/m, $p < 0.001$). The corresponding P_{eff}-values of R- and S-verapamil, in the same perfusion solution and concentrations as used for the determinations of the surface tensions, have been determined previously in the rat jejunum (15). The rat jejunal P_{eff} of verapamil, with and without chlorpromazine, correlated inversely ($r^2 = 0.980$, $p < 0.001$) to the surface tensions of the solutions used in the experiment (Fig. 3).

The recovery of ^{14}C-PEG 4000 was lower ($p < 0.05$) at the fluvastatin concentrations 800 and 2 500 μM (96 ± 1% and 94 ± 2%) than for the control group (99 ± 1%). In addition, there was a higher concentration of LDH in the outlet colonic perfusate during steady state (45–105 min) in the presence of micelles (Fig. 4). Furthermore, there was a net secretion of fluid from the colon segment when it was perfused with the solution containing fluvastatin in micelles. In all other groups, a net absorption of fluid was observed (Table I). The inner radius of the colon was larger at the two highest perfusate concentrations of fluvastatin compared to the control group ($p < 0.05$) (Table I).

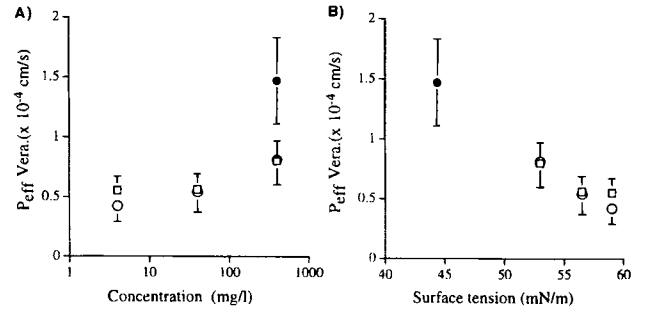

Fig. 3. Effective jejunal permeability (P_{eff}) in rats of S-verapamil (○) and R-verapamil (□) at different concentrations of R/S-verapamil (A) and at the corresponding surface tensions (B). In the presence of 10 mM chlorpromazine (●), the surface tension decreased and the P_{eff} increased (R- and S-verapamil overlap). The P_{eff} correlated inversely with the surface tension ($r^2 = 0.980$, $p < 0.001$).

DISCUSSION

The aim of this work was to study whether the decreased surface tension with increasing concentrations of fluvastatin in the perfusate could affect the P_{eff} across the colonic mucosa. The surface tension of a solution of a surface active compound is often linearly related to the logarithmic concentration of the compound (19). Thus, any concentration dependent effect exhibited by a surface active compound could indirectly be correlated with the surface tension. However, the surface activity of a compound *per se* might contribute to its biological effect (such as membrane transport and protein interactions). For instance, the surface activity of a series of neuroleptic phenothiazines (e.g., chlorpromazine) has been related to their clinical pharmacological potencies (20). Moreover, it has been suggested that the surface active properties of verapamil may contribute to its biological activity, since the drug appears to act at the level of the cell membrane (21).

The tendency for surface-active molecules to adsorb into an interface decreases the interfacial tension and favors expansion of the interface (19). Computer simulations have shown that interfacial interactions can lead to larger partitioning into the bilayer membrane for those molecules that prefer the interface to either the aqueous or the hydrocarbon environment (22). Thus, the surface activity is an important property of a molecule as it will affect its partition into biological membranes. An optimal balance between polar and non-polar parts of a molecule would improve the absorption of the molecule, since it has to partition into and diffuse across both hydrophilic and lipophilic layers during the overall absorption process.

We found a good inverse correlation between the P_{eff} of fluvastatin and the surface tension at perfusate concentrations of fluvastatin between 1.6–800 μM. Thus, when the surface tension was decreased by the addition of EHEC, a higher P_{eff} was expected for fluvastatin. Indeed, the P_{eff} of fluvastatin increased (by 36%), but it was less than expected from the

correlation between the colonic P_{eff} of fluvastatin and the surface tension (Fig. 2). The colonic P_{eff} of antipyrine, a marker for passive transcellular diffusion, simultaneously increased by 49% in the EHEC group when compared to the control group. Because of its large molecular weight (1.24×10^6), EHEC is presumed not to be absorbed into the colonic mucosa. This suggests that the observed increase in the P_{eff} of fluvastatin and antipyrine in the presence of EHEC arises from the lowering of the surface tension *per se*, and not from any direct effects within the apical membrane of the colonocytes. A possible mechanism for these observations is that the decreased surface tension favors an increased wetting of the hydrophobic colonic surface, and thereby increases the effective membrane area available for drug partitioning. These results also suggest that the previously reported concentration dependent P_{eff} of fluvastatin cannot be explained solely by the ability of fluvastatin to decrease the surface tension at the membrane surface (1). The concentration dependent P_{eff} of fluvastatin is probably also influenced by effects within the colonic membrane, such as interactions with lipids that could result in an altered fluidity of the membrane. However, we can not completely rule out the hypothesis that fluvastatin also might be a substrate for any efflux system present in the intestinal mucosa. We are therefore currently investigating the possible involvement of multidrug resistance-associated protein (MRP), in order to better understand the complex intestinal absorption mechanisms for fluvastatin.

Several surfactants, e.g., Cremophor EL, Solutol HS 15 and Tween 80, have been evaluated as potential reversals of multidrug resistance, MDR, (23). In resistant human leukemic cells *in vitro*, the surfactants caused a dose dependent increase in intracellular daunorubicin, a substrate of P-glycoproteins, and simultaneously an increase in membrane fluidity (23). These effects were observed at concentrations below the CMC of the compounds, indicating that the monomers are the active species. Furthermore, various structurally and chemically unrelated drugs known to be inhibitors of the P-glycoprotein system (e.g., verapamil, chlorpromazine, reserpin and cyclosporin A) have been reported to increase the fluidity of the cell membrane and the permeability of various lipophilic chromophores, both in normal and MDR cells *in vitro* (24). In fact, it has been suggested that the effectiveness of MDR reversal by various modulators correlates favorably with their ability to increase membrane permeability (24). Increased fluidity of the intestinal membrane could be a possible mechanism behind the observed increase in P_{eff} of fluvastatin and antipyrine at increasing concentrations of fluvastatin in the present study. These effects were observed at concentrations below the CMC of fluvastatin, indicating that the monomer is the active species. Furthermore, as both verapamil and chlorpromazine are surface active compounds, an increase in membrane fluidity and/or an alteration in the transport kinetics of the efflux protein may explain the increased P_{eff} of each enantiomer of verapamil in the presence of chlorpromazine, and the good inverse correlation found between the rat jejunal P_{eff} of verapamil and the surface tension. However, the concentration dependence of fluvastatin has been reported to be negligible in the rat jejunum, indicating that the concentration dependent P_{eff} of verapamil is indeed due to the involvement of P-glycoprotein, rather than just being a physicochemical effect (1,15).

At concentrations above the CMC of fluvastatin, a decreased P_{eff} of fluvastatin and antipyrine was observed. This suggests that antipyrine interacts with the fluvastatin micelles. Thus, the lower P_{eff} is probably caused by a loss of thermodynamic activity of the two drugs. On the other hand, the P_{eff} of D-glucose and the absorption of ^{14}C-PEG 4000 increased in the presence of the micelles. This might be from an increased paracellular absorption since the lowest concentrations of various surface active compounds that enhance the paracellular absorption of mannitol and ^{14}C-PEG 4000 have been reported to be close to their CMC-values (7,9). As we also found higher concentrations of the viability marker LDH in the outlet perfusate when the micelles were present, loss of membrane integrity at the apical cell membrane of the colonocytes is a more plausible explanation for the increase in absorption of D-glucose and ^{14}C-PEG 4000. This is probably mediated by extraction of membrane proteins and lipids into the micelles, which results in a more leaky colonic barrier (25). Our results are in line with those of Gullikson and coworkers who reported that perfusions with deoxycholate above its CMC caused shortened villi, epithelial cell lysis, net water secretion and an increase in the intestinal permeability of inulin, dextran and albumin in the hamster small intestine *in situ* (26). As fluvastatin is administered to humans in doses of 20–40 mg, the highest concentration expected in the upper gastrointestinal tract is approximately 200–400 μM, which is below the CMC.

To summarize, it appears that the surface activity of fluvastatin generates a non-toxic permeation-promoting effect for already highly permeable compounds in the rat colon, at concentrations lower than the CMC. This effect can partly be explained by the lowering of the surface tension of the perfusion solution. At these concentrations, no effect was observed on the P_{eff} of D-glucose, suggesting that the permeation enhancement was not the result of increased paracellular permeability. At concentrations of fluvastatin above the CMC, we observed an increased permeation for D-glucose and PEG 4000 and an increased leakage of the intracellular enzyme LDH, suggesting impaired integrity of the colonic membrane. The surface activity is probably not the major reason behind the observed concentration dependent P_{eff} in rat jejunum for R/S-verapamil. Instead, the involvement of P-glycoproteins is probably the most important mechanism for R/S-verapamil. Altogether, this study shows that the surface tension is an important physicochemical factor that should be taken into consideration when evaluating drug absorption studies performed *in vitro* or *in situ*.

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