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Concentration- and Region-dependent Intestinal Permeability of Fluvastatin in the Rat

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Abstract

The purpose of this study was to investigate the mechanisms of transport of fluvastatin across the intestinal mucosa in various regions of the intestine in the rat. In-situ single-pass perfusions of the jejunum, ileum and colon were performed and the effective permeability (P_{eff}) of fluvastatin, antipyrine and D-glucose were assessed in each region, at three different perfusate fluvastatin concentrations (1·6, 16 and 160 μM). The effect of lovastatin acid on the bi-directional transport of fluvastatin across the ileal mucosa was also studied.

The P_{eff} of fluvastatin was found to be dependent both on the intestinal region and on the concentration in the intestinal lumen ($P < 0{\cdot}001$). Fluvastatin had the lowest P_{eff} ($0{\cdot}55 \pm 0{\cdot}10 \times 10^{-4}$ cm s^{-1}) in the jejunum at 1·6 μM, and the highest P_{eff} ($1{\cdot}0 \pm 0{\cdot}16 \times 10^{-4}$ cm s^{-1}) in the colon at 160 μM. The highest concentration of fluvastatin increased the average absorption of water from the intestine by 209% ($P < 0{\cdot}05$), and the average P_{eff} of D-glucose by 29% ($P < 0{\cdot}05$). The presence of excess lovastatin acid (100 μM, compared with fluvastatin 1·6 μM) at the luminal side increased the average absorption of water by 218% ($P < 0{\cdot}001$), and the P_{eff} of fluvastatin in the ileum and the colon by 44 and 50%, respectively ($P < 0{\cdot}05$). The presence of lovastatin acid on the luminal side in the ileum also increased the blood-to-lumen transport (exsorption) of fluvastatin by 43% ($P < 0{\cdot}001$).

The increased intestinal absorption of fluvastatin at higher concentrations does not suggest that substantial absorption occurs by any carrier-mediated process in the absorptive direction. The increased bi-directional transport when lovastatin acid was added to the lumen suggests that fluvastatin is not a P-glycoprotein substrate. Instead, the concentration-dependent increase in the absorption of fluvastatin, water and D-glucose suggests a direct effect of fluvastatin on the transcellular passive transport.

Fluvastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase which is rapidly and completely (> 90%) absorbed in the intestine in man in the fasting state (Tse et al 1992). This is a consequence of its relatively high water-solubility and effective permeability (P_{eff}) in the proximal small intestine. The P_{eff} of fluvastatin has recently been measured in the jejunum in man by a regional perfusion technique (Lindahl et al 1996). It was found to be $2{\cdot}4 \times 10^{-4}$ cm s^{-1}, which indicates fluvastatin to be a highly permeable compound (Amidon et al 1995; Lindahl et al 1996). The extent of absorption is complete for fluvastatin and simvastatin, but incomplete for pravastatin (34%) and lovastatin acid (31%) (Lennernäs & Fager 1997).

The mechanisms of transport of these four HMG-CoA reductase inhibitors across the intestinal mucosa are still unclear. The incomplete absorption of pravastatin is in accordance with its physicochemical properties (logD pH 7 = −0·23, MW = 405), which predict low passive membrane diffusion. However, it has been suggested that pravastatin is transported across brush-border membrane vesicles in the rabbit jejunum by a monocarboxylic acid transport system, rather than by simple diffusion (Tamai et al 1995). Furthermore, pravastatin, lovastatin and simvastatin, have all been shown to have affinity for the multispecific anion transporter in the rat liver (Yamazaki et al 1993). In-vitro

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studies of neuroblastoma cells have suggested that lovastatin acid interacts with the P-glycoprotein efflux system (Dimitroulakos & Yeger 1996). This transport protein is also located in the apical membrane of the enterocyte (Thiebaut et al 1987). Whether or not the incomplete intestinal absorption of lovastatin acid is a consequence of this efflux mechanism is still unclear. For fluvastatin, however, no evidence of any carrier-mediated intestinal transport has been reported.

Our main aim in this study was to investigate the bi-directional membrane transport of fluvastatin to enable better understanding of the mechanisms involved in the rapid and complete intestinal absorption of fluvastatin. The second purpose was to investigate whether there is any regional dependency in the intestinal permeability of fluvastatin. In parallel, the permeability of antipyrine and D-glucose were assessed as markers for passive and active intestinal membrane transport, respectively. It is our view that better understanding of the mechanisms that lead to a high intestinal permeability is important because this new information could be used in the design of new drugs for oral administration.

Materials and Methods

Single-pass-perfusion experiments

Male Sprague–Dawley rats (Crl : CD(SD)BR; Charles River, Uppsala, Sweden), 236–315 g, were housed under constant and controlled conditions (22·5°C, 50% air humidity, 12-h light–dark cycle) in the animal facilities at the Biomedical Centre, Uppsala. The animals had free access to tap water and regular rat chow (56% carbohydrates, 19% proteins, 12% water, 4% fat, 12·6 kJ g^{-1}; R36; Lactamin AB, Stockholm, Sweden) until 14–20 h before the experiment, when the food was withdrawn. Anaesthesia was induced by intraperitoneal injection of 120–150 mg kg^{-1} 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. To facilitate normal breathing a plastic tube was introduced into the trachea. The abdomen was opened with a midline longitudinal incision and the intestinal segment (jejunum, ileum or colon) was located. Approximately 10 cm of the intestine was isolated and cannulated with plastic tubing (4 mm o.d., U-74; Codan Triplus AB, Kungsbacka, Sweden). A loop of the inlet tube of approximately 10-cm length was placed inside the abdominal cavity so that the perfusion solution entered the isolated segment at body temperature. Only one intestinal segment was used in each perfusion experiment and animal. The jejunal and ileal segments were placed outside the abdominal cavity; the colonic segment was left inside the abdominal cavity.

The segment was initially rinsed with 10–20 mL saline (37°C) until a clear perfusate was obtained. The inlet tube was then filled with the perfusion solution and connected to a syringe placed in a Harvard infusion pump (Model 22; Harvard Apparatus Company, USA). The perfusion rate was 0·2 mL min^{-1}. To avoid fluid and heat loss from the animals the surgical area was covered with a thin plastic sheet and aluminium foil. Each perfusion experiment lasted for 105 min and the outlet perfusate was quantitatively collected on ice at 45, 60, 75, 90 and 105 min. The lengths of jejunal or ileal segments were measured with thread after 45 min; colonic segments were measured upon completion of the experiment. When perfusion was complete, segments were rinsed with saline (20 mL) to collect the remaining amounts of substances of interest in the perfusion system. All the perfusion syringes and the samples collected were weighed and the samples were frozen immediately and stored at −20°C pending analysis.

Approval of this study was given by the Animal Ethics Committee, Uppsala University (C246/95).

Intestinal perfusion solutions

The perfusion solution consisted of 5·4 mM potassium chloride, 48 mM sodium chloride, 35 mM mannitol, 10 mM D-glucose, and 1 g L^{-1} polyethylene glycol 4000 (PEG 4000), all in 70 mM phosphate buffer. The pH and the osmolality were 6·5 and approximately 290 mOsm kg^{-1}, respectively. Trace amounts of [^{14}C]PEG 4000 and [^{3}H]D-glucose (2·5 and 10 μCi L^{-1}, respectively; Amersham Laboratories, Buckinghamshire, UK) were added to the solution as markers for water flux and carrier-mediated transport, respectively. Antipyrine (1·1 mM) was included as a marker for passive diffusion across the intestinal barrier. Finally, fluvastatin sodium was added to give an inlet perfusate concentration of 1·6, 16 or 160 μM.

In a few experiments lovastatin acid was added in excess (100 μM lovastatin, 1·6 μM fluvastatin) to the perfusion solution. A stock solution of lovastatin acid was prepared according to the method of Keyomarsi et al (1991) in which lovastatin is converted from its inactive lactone prodrug form to its active dihydroxy open-acid form (lovastatin acid, Figure 1) by first dissolving it in ethanol (95%) and then adding NaOH (1 M). The resulting solution was neutralized with HCl (1 M) to pH 7·2 and then diluted with plain perfusion solution. This stock solution of lovastatin acid (10 mM) was then

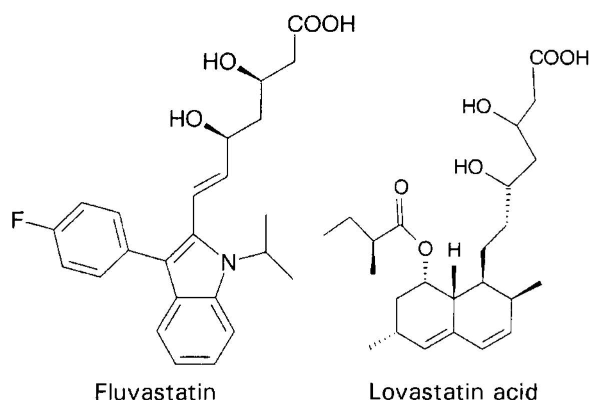

Figure 1. The molecular structures of fluvastatin and lovastatin acid.

used for the preparation of the perfusion solution. The final concentration of ethanol in the intestinal perfusion solution was less than 0·1% (v/v).

Intestinal exsorption of fluvastatin

The transport of fluvastatin from the serosal to the mucosal side of the intestinal wall was studied in-situ in rats. Fluvastatin was dissolved in saline (766 μg mL^{-1}) and given to eight rats by an intravenous infusion into the jugular vein. The infusion rate was 28 μg fluvastatin min^{-1} for 5 min then 14 μg min^{-1} for 100 min, aiming at a steady state plasma fluvastatin concentration of 1·6 μM. For four of the rats perfusion of the ileum was performed as described above with a solution containing lovastatin acid (100 μM); for a separate control group of four rats perfusion of the ileum was performed with the same solution without lovastatin acid.

Stability and adsorption test

There was no precipitation, chemical instability or adsorption of fluvastatin by the plastic materials during a 150 min in-vitro perfusion (37°C). Furthermore, in-vitro incubations (60 min) of fluvastatin in intestinal fluids from rats revealed that fluvastatin was stable in fluids from the jejunum, ileum and colon. Fluvastatin was protected from light throughout the study. The stability of antipyrine has already been demonstrated (Lennernäs et al 1994).

Analytical methods

Fluvastatin was assayed in the intestinal perfusate with HPLC; the method used was a slight modification of the reversed-phase HPLC method with fluorescence detection developed by Toreson & Eriksson (1996). The mobile phase was a 50:29:21 mixture of methanol, acetonitrile and 17 mM ammonium dihydrogen phosphate solution, pH 3·5. The samples and the standards were diluted with the mobile phase and 50 μL was injected on the column (Hypersil 5 ODS, 250 mm × 4·6 mm). The flow rate was 1·0 mL min^{-1}, the excitation and emission wavelengths were 305 and 380 nm. The variabilities, expressed as the coefficients of variation at the concentrations of the quality controls, were 1·2, 1·5 and 2·5% at the concentrations 245, 123 and 11 ng mL^{-1}, respectively. Fluvastatin was eluted after 5·1 min and the limit of quantitation was 8 ng mL^{-1} (CV 7·8%). Antipyrine was assayed with a previously used and validated HPLC method (Lennernäs et al 1992, 1995); the limit of detection was 1·0 μg mL^{-1}.

The concentrations (disintegrations min^{-1} mL^{-1}) of [^{14}C]PEG 4000 and [^{3}H]D-glucose were determined by liquid scintillation counting for 2 × 10 min (Mark III; Searle Analytical, USA) after the addition of Beckman Ready Safe (8 mL). The average value of these two scintillation measurements was used in the calculations.

The pH and the osmolality were measured with a pH-meter (Metrohm 632) and an osmometer (Vescor 5500), respectively.

Data analysis

All calculations were performed with steady-state concentrations of the outlet intestinal perfusate, which was considered to have been achieved when the concentration of [^{14}C]PEG 4000 was stable. The net water flux, NWF, cm^{-1} intestinal segment was calculated from:

$$NWF = (1 - ([PEG]_{out}/[PEG]_{in}))Q_{in}/L \quad (1)$$

where $[PEG]_{in}$ and $[PEG]_{out}$ are the inlet and outlet concentrations, respectively, of [^{14}C]PEG 4000 and Q_{in} is the inlet flow rate (0·2 mL min^{-1}). L is the length of the intestinal segment. The volume, V, of the intestinal segment during each sampling interval at steady state was estimated by use of the equation (Lennernäs et al 1992):

$$V = ((\Sigma PEG_{in} - \Sigma PEG_{out})/[PEG]_{out}) - \text{tube volume} \quad (2)$$

i.e. the total amount of [^{14}C]PEG 4000 that had entered the system at a certain time (PEG_{in}) minus the total amount that had left the system at that time (PEG_{out}) represents the amount of [^{14}C]PEG 4000 in the system. This amount is then divided by the outlet concentration of [^{14}C]PEG 4000 in the actual sampling interval to estimate the volume of the whole perfusion system. Because the inlet tube is filled with the perfusion solution at the start of the perfusion, only the volume of the outlet tube is subtracted from the volume of the whole system to estimate the segment volume (Lennernäs et al

1992). Knowing the length, L (measured at time 45 min), of the perfused segment and assuming it to have the shape of a cylinder, the inner radius, r, was readily estimated:

$$r = (V/\pi L)^{1/2} \quad (3)$$

The effective permeability, P_{eff}, across the intestinal mucosa was calculated according to a tube model (Komiya et al 1980):

$$P_{eff} = (Q_{in} \ln(C_{in}/C_{out}))/2\pi rL \quad (4)$$

where C_{in} and C_{out} are the inlet and outlet concentrations, respectively. The outlet concentrations of the drug were corrected for the fluid flux.

The possible influence of concentration and intestinal region on the P_{eff} of fluvastatin was tested by two-way analysis of variance, and correlation between the P_{eff} and the NWF was tested by linear regression (StatView, Abacus Concepts, USA). The effects of the presence of lovastatin in the perfusion solution were tested with an unpaired *t*-test. $P < 0.05$ was considered significant. All data are presented as the mean values and their 95% confidence intervals.

Results

Absorption studies

The P_{eff} of fluvastatin was dependent both on the intestinal region and on the concentration of fluvastatin present in the perfusion solution (Tables 1 and 2). The highest P_{eff} of fluvastatin ($1.0 \pm 0.16 \times 10^{-4}$ cm s^{-1}) was obtained in the colon region and with the highest concentration (160 μM) of fluvastatin. The P_{eff} values of fluvastatin, antipyrine and D-glucose for the three different concentrations of fluvastatin and in the three different regions of the intestine are given in Table 1. The P_{eff} of antipyrine was significantly higher in the colon than in the jejunum or the ileum. Furthermore, the P_{eff} of antipyrine was slightly higher, although the difference was not significant, at the highest perfusate concentration of fluvastatin in both the jejunum and the ileum (Table 1). The P_{eff} for D-glucose was significantly lower in the colon than in the jejunum or ileum. In the ileum the P_{eff} of D-glucose was significantly affected by the concentration of fluvastatin, with a higher P_{eff} value at the two highest perfusate concentrations of fluvastatin (Table 1).

In all three regions of the intestine absorption of water was achieved, with the highest rate of absorption being in the colon segment (Table 3). The absorptive flux of water also increased in parallel with the increased luminal concentration of fluvastatin (Table 4). The absorption of water, fluvastatin and D-glucose was affected by the luminal concentration of fluvastatin, and their values for P_{eff} were correlated with the NWF in the different regions of the intestine (Figure 2).

The presence of excess lovastatin acid (62 times the molar concentration of fluvastatin) in the perfusion solution increased the P_{eff} of fluvastatin in all three regions of the intestine (Table 5). This effect was statistically significant in both the ileum and the colon. In addition, the jejunal P_{eff} of D-glucose increased from 0.61 ± 0.28 to $0.92 \pm 0.19 \times 10^{-4}$ cm s^{-1} ($P = 0.013$) when lovastatin acid was added to the intestinal perfusate. Furthermore, lovastatin acid significantly increased the absorption of fluid from all the regions of the

Table 2. The effective permeabilities of different regions of the intestine to different concentrations of fluvastatin.

	Jejunum	Ileum	Colon
Fluvastatin 160 μM	0·6±0·2	0·8±0·2	1·0±0·2
Fluvastatin 16 μM	0·6±0·2	0·6±0·2	0·8±0·2
Fluvastatin 1·6 μM	0·55±0·1	0·55±0·1	0·6±0·3

Values ($\times 10^{-4}$ cm s^{-1}) are means ± 95% confidence intervals. The P_{eff} was dependent both on intestinal region and on perfusate concentration ($P < 0.001$).

Table 1. Effective permeability of the compounds studied in different regions of the rat intestine and at different concentrations of fluvastatin.

	Jejunum			Ileum			Colon		
	Fluvastatin	Antipyrine	D-Glucose	Fluvastatin	Antipyrine	D-Glucose	Fluvastatin	Antipyrine	D-Glucose
Fluvastatin 160 μM	0·62±0·16 n=4	0·80±0·28 n=4	0·80±0·30 n=4	0·82±0·21 n=4	0·89±0·43 n=4	0·97±0·28 n=4	1·00±0·16 n=4	1·10±0·49 n=4	0·04±0·05 n=4
Fluvastatin 16 μM	0·60±0·20 n=6	0·74±0·18 n=6	0·80±0·16 n=6	0·60±0·15 n=5	0·79±0·19 n=5	0·79±0·15 n=5	0·80±0·15 n=4	1·20±0·16 n=4	0·05±0·06 n=4
Fluvastatin 1·6 μM	0·55±0·10 n=4	0·61±0·10 n=4	0·61±0·13 n=4	0·53±0·05 n=5	0·67±0·11 n=5	0·73±0·08 n=5	0·61±0·28 n=4	1·20±0·40 n=4	0·02±0·05 n=4

The effective permeabilities are presented as mean values ($\times 10^{-4}$ cm s^{-1}) from n experiments with 95% confidence intervals.

Table 3. Regional technical variables obtained during intestinal perfusion in rats.

	Jejunum n = 20	Ileum n = 19	Colon n = 17
Recovery of polyethylene glycol 4000 (%)	97 ± 1·7	96 ± 1·8	96 ± 3·7
Net water flux (mL h^{-1} cm^{-1})*	−0·005 ± 0·013	−0·034 ± 0·024	−0·091 ± 0·040
Radius (cm)	0·23 ± 0·03	0·24 ± 0·02	0·26 ± 0·03

All variables (means ± 95% confidence intervals) were calculated at steady state in the outlet perfusate. The rate of absorption of water was greater in the colon than in the jejunum and the ileum (a larger negative value of net water flux; $P < 0.01$). *A negative value indicates absorption of fluid.

Table 4. The net water flux of different concentrations of fluvastatin across the intestinal mucosa in different regions of the intestine.

	Jejunum	Ileum	Colon
Fluvastatin 160 μM	−0·025 ± 0·03	−0·06 ± 0·07	−0·13 ± 0·15
Fluvastatin 16 μM	0·0025 ± 0·02	−0·035 ± 0·07	−0·08 ± 0·04
Fluvastatin 1·6 μM	0·005 ± 0·025	−0·02 ± 0·035	−0·05 ± 0·08

Values (mL h^{-1} cm^{-1}) are means ± 95% confidence intervals. The net water flux was dependent both on the region of the intestine ($P < 0.001$) and on the luminal concentration of fluvastatin ($P < 0.05$). A negative net water flux indicates net fluid movement in the absorptive direction.

intestine (data not shown). Antipyrine was not significantly affected by the presence of lovastatin. The mean recovery of the non-absorbable water flux marker [^{14}C]PEG 4000 during the in-situ perfusion experiments was more than 95% in all three regions of the intestine (Table 3). The calculated mean intestinal radius (eq 3) under these experimental conditions was estimated to be 0·23, 0·24 and 0·26 cm in jejunum, ileum and colon, respectively (Table 3)·

Exsorption studies

During intravenous infusion (105 min) of fluvastatin and simultaneous ileal perfusion (105 min), addition of lovastatin acid (100 μM) to the intestinal perfusion solution increased the absorption of fluid (a negative NWF) from the ileal segment (Table 6). However, the presence of lovastatin acid at the luminal side also increased the exsorption of fluvastatin from the blood side to the lumen side of the ileal segment (Table 6). The mean P_{eff} values for D-glucose and antipyrine increased slightly, by 5 and 22%, respectively, in the presence of lovastatin acid but the increases were not significantly different statistically.

Discussion

In this study we investigated whether the intestinal P_{eff} of fluvastatin was concentration-dependent in the different regions of the intestine as a consequence of any carrier-mediated transport of the compound. We found significant concentration-dependence, with the highest P_{eff} value at the highest perfusate concentration of fluvastatin (Table 2). Although the exact mechanism(s) responsible for this is (are) not clear, there are several possible explanations. One of the most likely would be the P-glycoprotein system, or another efflux system, located in the intestinal brush-border membrane.

The higher P_{eff} of fluvastatin at higher concentrations of fluvastatin in the perfusate might be a result of saturation of any efflux system(s) involved. It has been suggested that substrates for the P-glycoprotein efflux system are cationic, hydrophobic molecules with at least two planar rings and molecular weights of 400–1500 (Leveille-Webster & Arias 1995). Fluvastatin has three planar rings, a molecular weight of 411 and is hydrophobic (log P octanol–water = 3·8) (Figure 1). It is, however, an acid with a pK_a of 4·6 and will, therefore, be negatively charged to a very large extent in the intestinal lumen (pH 6·5). Although an acid, another HMG-CoA inhibitor with a similar molecular structure, lovastatin acid (see Figure 1), has recently been suggested as interacting with the P-glycoprotein efflux system in neuroblastoma cells in-vitro (Dimitroulakos & Yeger 1996). The presence of lovastatin acid in excess in the perfusion solution increased the P_{eff} of fluvastatin in all regions of the intestine, suggesting

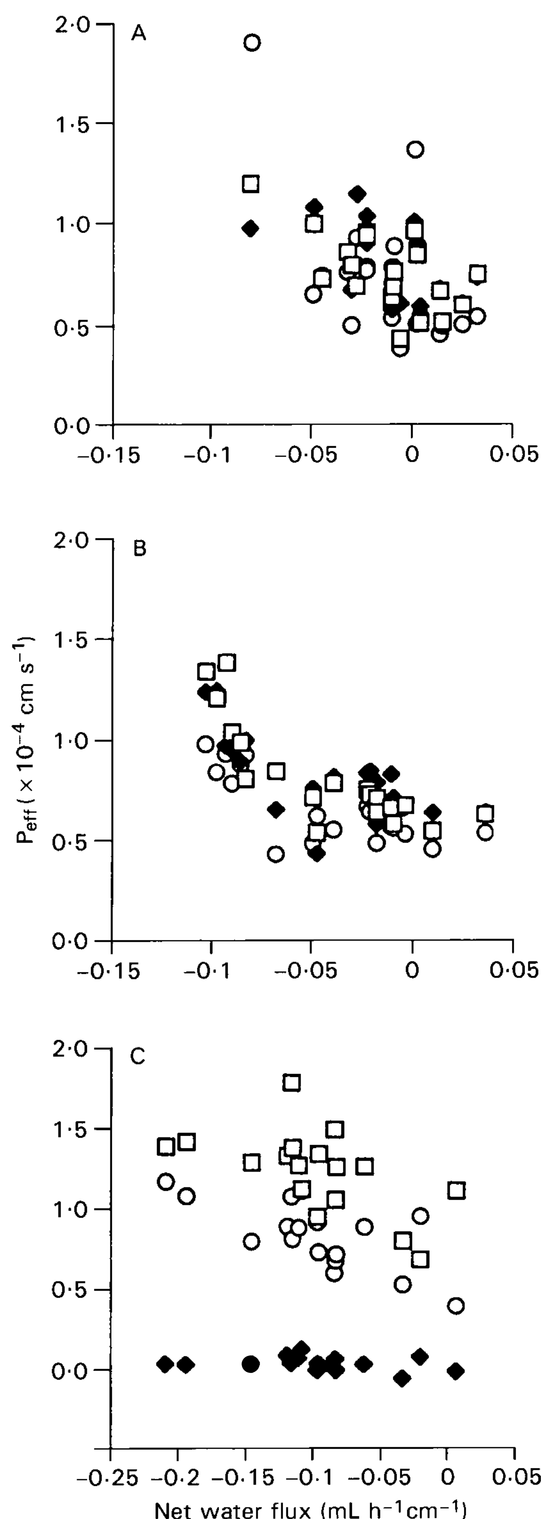

Figure 2. The correlations between the effective permeability, P_{eff}, for the compounds studied and the net water flux in the three regions of the intestine (A. jejunum; B. ileum; C. colon). A negative net water flux indicates absorption of fluid. The best correlations were obtained in the ileum, with correlation coefficients (r^2) of 0·67, 0·46 and 0·56 for antipyrine (□), D-glucose (◆) and fluvastatin (○), respectively. In the jejunum, the corresponding r^2 values were 0·39, 0·28 and 0·36, and in the colon 0·32, 0·05 and 0·47, respectively. The corresponding *P* values (*t*-test) were < 0·05 for all compounds in all regions, except for D-glucose in the colon region.

that fluvastatin might be a substrate for P-glycoproteins. When fluvastatin was given as an intravenous infusion it was therefore to be expected that a lower blood-to-lumen transport rate of fluvastatin would arise in the presence of excess lovastatin in the intestinal perfusion solution. Instead we observed a higher blood-to-lumen transport of fluvastatin in the presence of lovastatin acid at the luminal side. This suggests that fluvastatin is not a substrate for P-glycoproteins.

Carrier-mediated transport of fluvastatin in the absorptive direction could theoretically occur by means of the monocarboxylic carrier. It has been suggested that this absorption mechanism is important for the absorption of pravastatin (Tamai et al 1995). However, as the P_{eff} of fluvastatin increased at higher concentrations, we conclude that fluvastatin is not absorbed to any major extent by the monocarboxylic carrier.

Fluvastatin was found to induce increased absorption of water from all regions of the intestine in a concentration-dependent manner. Water absorption was more pronounced in the colon than in the small intestine, which suggests that solvent-drag might have contributed to the increased P_{eff} of the study compounds. The water absorption correlated ($P < 0{\cdot}05$) with the P_{eff} of antipyrine and fluvastatin in all the regions of the intestine, although the correlation coefficients (r^2) were not high. For D-glucose correlation was very poor in the large intestine ($P > 0{\cdot}05$). When water absorption was induced by the presence of lovastatin in the perfusion solution and fluvastatin was given as an intravenous infusion, a higher blood-to-lumen transport rate was observed for fluvastatin. This indicates that water and fluvastatin do not have to be transported across the intestinal mucosa by the same route or mechanism(s). Thus, we suggest that solvent-drag is not the major mechanism behind the concentration-dependent P_{eff} observed for fluvastatin. This is also in line with previous work from our laboratory which showed that solvent-drag, induced by a hypoosmotic solution, did not influence the P_{eff} of compounds of this molecular size (> 200 Da) in the current rat model (Fagerholm 1997).

Fluvastatin is an amphiphilic molecule with one relatively lipophilic region (the planar rings) and one more hydrophilic part (the side chain, see Figure 1); it can thus be regarded as a surface-active molecule. Surfactants, especially anionic, have been reported to increase the permeability of hydrophilic marker molecules (mannitol and PEG 4000) across monolayers of intestinal epithelia (Caco-2) cells from man in a concentration-dependent fashion (Anderberg et al 1992). It was

Table 5. Effect of excess lovastatin acid in the perfusion solution on the effective permeability of different regions of the intestine to fluvastatin.

	Jejunum	Ileum	Colon
Fluvastatin 1·6 μM	0·55±0·1	0·55±0·05	0·60±0·30
Fluvastatin 1·6 μM + lovastatin acid 100 μM	1·10±0·5	0·75±0·25*	0·90±0·20*

Values ($\times 10^{-4}$ cm s^{-1}) are means±95% confidence intervals. The presence of excess lovastatin acid in the perfusion solution increased the effective permeability of all regions of the intestine to fluvastatin. *The effect was significant in the ileum and the colon ($P < 0.05$), but not in the jejunum ($P = 0.05$).

Table 6. Effect of excess lovastatin acid in the perfusion solution on the lumen-to-blood fluid flux in the ileum.

	Net water flux (mL h^{-1} cm^{-1})	Blood-to-lumen transport of fluvastatin (ng h^{-1} cm^{-1})
Control	−0·02±0·03	50±4
Lovastatin acid 100 μM	−0·05±0·01*	70±4*

Values are means±95% confidence intervals. The lumen-to-blood fluid flux increased in parallel with the blood-to-lumen transport of fluvastatin and when lovastatin acid was added to the intestinal perfusion solution. Fluvastatin was administered as an intravenous infusion, aiming at a steady state concentration of 1·6 μM. *$P < 0.05$, significantly different from result for control group.

suggested that the mechanism behind this observation was increased paracellular diffusion, although increased permeation through more leaky cell membranes could not be excluded (Anderberg et al 1992). Because fluvastatin not only increased absorption of water, but also the P_{eff} of the lipophilic molecules antipyrine and fluvastatin, a physicochemical interaction between fluvastatin and the apical cell membrane of the intestinal cells is a possible mechanism.

For antipyrine and fluvastatin, both of which are thought to be transported across the intestinal mucosa by passive diffusion, significantly higher P_{eff} values were obtained in the colon than in the jejunum and the ileum. Antipyrine and fluvastatin are moderately to highly lipophilic drugs, with log D values (octanol/water, pH 6·5) of 0·38 and 1·89, respectively (Lindahl et al 1996). Previously, other groups have observed in animals in-vitro that the colon is more permeable to lipophilic molecules than is the small intestine (Jezyk et al 1992; Ungell et al 1996, 1998). For more hydrophilic molecules, on the other hand, the small intestine seems to be more permeable (Chadwick et al 1977; Ungell et al 1996), although the literature contains conflicting reports (Ma et al 1995).

It has been suggested that the reason for the different permeability patterns for lipophilic and hydrophilic molecules along the intestine is that the lipid composition of the colonocytes favours the partitioning of lipophilic molecules (Ungell et al 1996). Another plausible explanation is the influence of the unstirred water layer immediately adjacent to the intestinal wall; this diffusion barrier has been shown to have a substantial influence on the P_{eff} of lipophilic (highly permeable) molecules in the in-situ rat model (Levitt et al 1984, 1992; Winne et al 1987). This layer has been reported to be thinner in the rat colon than in the small intestine (Levitt et al 1984).

Regional P_{eff}-data for fluvastatin (160 μM) and antipyrine in the in-situ rat model have recently been estimated by our laboratory (Fagerholm et al 1997). In that study, the P_{eff} values of fluvastatin and antipyrine in the small intestine were approximately 20–60% higher than in the current study. The P_{eff} values obtained in the colon were, however, not different from the colon values obtained here (approx. 1×10^{-4} cm s^{-1}, Table 1). Although the reason for this discrepancy is not clear, one explanation might be that the Sprague–Dawley rats used in the two studies came from different breeders. Whether rats from different breeders have different intestinal permeability has, however, not been well addressed in the literature.

In man the jejunal P_{eff} of fluvastatin was 3·9-times higher in the jejunum under in-vivo conditions at the same perfusate concentration (160 μM), which agrees with our previously reported correlation for permeability in man and rat (Fagerholm et al 1996). That intestinal barrier function (viability) was maintained in all experiments was shown by the almost complete recovery of the marker molecule [^{14}C]PEG 4000 (Lundin et al 1997).

To summarize, we have shown that the intestinal P_{eff} of fluvastatin is both concentration- and region-dependent in the in-situ rat model. The highest P_{eff} of fluvastatin was obtained in the colon and at the highest perfusate concentration of fluvastatin. Fluvastatin is probably not a substrate for the

P-glycoprotein efflux system, because lovastatin acid increased both the intestinal P_{eff} and the blood-to-lumen transport of fluvastatin, and because fluvastatin also affected the absorption of water, antipyrine and D-glucose. Instead, changes in the intestinal barrier homeostasis and thermodynamics, as a consequence of a pharmacological effect or physicochemical interaction between fluvastatin and the apical cell membrane of the intestinal cells, or both, might be more plausible mechanisms explaining the concentration-dependent permeability of fluvastatin.

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