No Evidence for the Involvement of the Multidrug Resistance-Associated Protein and/or the Monocarboxylic Acid Transporter in the Intestinal Transport of Fluvastatin in Rats

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ABSTRACT Fluvastatin, an amphiphilic anion, shows a nonlinear increase in effective intestinal permeability (P_{eff}) with increasing luminal concentrations in rats. The main objective of this study was to investigate whether or not this observation could be attributed to an efflux-mediated transport by the multidrug resistance-associated protein (MRP). In parallel, we investigated the possible involvement of the monocarboxylic acid transporter (MCT) in the rapid intestinal absorption of fluvastatin. Single-pass perfusions were performed in the ileum and colon of the rat, with and without the presence of well-established inhibitors/substrates for the MRP (probenecid) and the MCT (nicotinic acid). The results suggest that neither the MRP nor the MCT are involved to any significant extent in the absorption process of fluvastatin in the rat intestine. Thus, the previously reported concentration-dependent P_{eff} of fluvastatin in these intestinal regions of the rat is probably not attributable to saturation of any efflux mediated by MRP.

INTRODUCTION

Fluvastatin (carboxylic acid; pKa 4.6, log D octanol/buffer pH 6.5 = 1.9) is an inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. We have previously reported that fluvastatin has a high, but concentration-dependent, effective permeability (P_{eff}) in both the small and the large intestine of the rat (1). The P_{eff} was higher at higher intestinal concentrations, suggesting an efflux-mediated transport of fluvastatin by P-glycoprotein (Pgp) and/or multidrug-associated protein (MRP) (1). This nonlinear P_{eff} was most pronounced in the ileum and the colon (1). Other inhibitors of HMG-CoA reductase, such as lovastatin acid and atorvastatin, have been reported to be substrates for or inhibitors of Pgp (2-4). However, we found that fluvastatin was not transported by Pgp to any significant extent in rats intestine, and that other mechanism(s) have to be involved instead (1). For instance, because fluvastatin is surface active, it decreases the surface tension between the luminal aqueous phase and the intestinal membrane, which may facilitate the absorption at increasing luminal concentrations. We have previously reported that the colonic P_{eff} of fluvastatin correlates inversely with the surface tension ($r^2 = 0.980$, $P < .05$) at luminal concentrations below the critical micelle concentration of fluvastatin (1 mmol/L) (5). However, this physicochemical effect could only partially explain the observed concentration-dependent P_{eff} of fluvastatin in rats (5).

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Therefore, to further investigate the mechanism behind the concentration-dependent intestinal P_{eff} of fluvastatin, we wanted to investigate the possible involvement of efflux proteins other than Pgp.

MRPs are a family of 190-kDa large -efflux proteins present in many tissues, where they probably function as active transporters of various amphiphilic anions (6). The MRP family contains at least 6 homologous members, MRP1-6, with partly different tissue distribution in the body. MRP1, MRP2 (cMOAT), and MRP5 are reported to be expressed in the small and/or the large intestine in humans (7,8). Based on in vitro experiments with various tissues from rats and humans, MRP1, MRP3, and MRP5 seem to be expressed at the basolateral membrane of cells only (eg, enterocytes) and are transporting in the absorptive direction, whereas MRP2 is expressed at the apical (brush border) membrane and is transporting in the secretive direction (9). Therefore, of these transporters, only MRP2 could be involved in a secretory efflux of drugs into the intestinal lumen. The regional intestinal distribution of MRP2 in rats is contradictory in literature. Gotoh et al reported that the highest expression (mRNA) of MRP2 in the rat intestine is found in the proximal small intestine and the lowest expression in the colon (17). Makhey et al, on the other hand, reported that the functional intestinal expression (efflux of known substrates) of MRP2 in rats is highest in the colon and the ileum and lower in the jejunum (10). The latter results are in line with our previous findings that the concentration-dependent permeability of fluvastatin is most pronounced in the ileum and the colon of rats (1). Therefore, our hypothesis is that a member of the MRP family, probably the MRP2, is responsible for the concentration-dependent intestinal P_{eff} of fluvastatin. This hypothesis is also supported by the fact that another inhibitor of HMG-CoA reductase, pravastatin, is transported by the MRP2 in the liver (11,12).

The monocarboxylic acid transporter (MCT) in the intestine has been reported to be the predominant mechanism for the uptake of the HMG-CoA reductase inhibitor pravastatin (13). This transport route was inhibited by several monocarboxylic acids such as acetic acid, benzoic acid, nicotinic acid,

lovastatin acid, and simvastatin acid (13). This process suggests that HMG-CoA reductase inhibitors might also be transported by the MCT. Furthermore, it was recently reported that another HMG-CoA reductase inhibitor, atorvastatin, is transported across Caco-2 cell monolayers by the MCT (14). Thus, together with passive diffusion, the observed high intestinal P_{eff} of fluvastatin in rats might include active transport across the intestinal mucosa through the MCT. Apparently, fluvastatin belongs to a class of drugs that exhibit complex intestinal absorption mechanisms, involving both passive and active transport in each direction.

The purpose of this study was to investigate the possible involvement of the 2 counteracting transporters, MRP2 and MCT, in the intestinal transport of fluvastatin in rats.

EXPERIMENTAL PROCEDURES

Chemicals

Fluvastatin sodium was provided by AstraZeneca (Mölnådal, Sweden). Antipyrine, nicotinic acid, and probenecid were obtained from Sigma Chemicals (St. Louis, MO). ^{14}C -labelled PEG 4000 and ^3H -labelled D-glucose (15 mCi/g and 26 Ci/mmol, respectively) were purchased from Amersham Labs, (Buckinghamshire, UK). The intestinal perfusion solution consisted of 5.4 mmol/L potassium chloride, 48 mmol/L sodium chloride, 35 mmol/L mannitol, 10 mmol/L D-glucose, and 1 g/L polyethylene glycol 4000 (PEG 4000), all in a 50-mmol/L phosphate buffer at pH 6.5.

Study Design

Six groups of rats ($n = 4-6$, 30 animals in total) were used in this study, which was approved by the Animal Ethics Committee, Uppsala University. The rats were perfused in the ileum or the colon with a perfusion solution containing either 1.6 $\mu\text{mol/L}$ fluvastatin (control), 1.6 $\mu\text{mol/L}$ fluvastatin + 1 $\mu\text{mol/L}$ probenecid, or 1.6 $\mu\text{mol/L}$ fluvastatin + 10 mmol/L nicotinic acid in random order. Only 1 intestinal segment was used in each perfusion experiment and animal. The concentrations of the

study compounds/transport inhibitors and physicochemical properties of the 3 perfusion solutions are presented in Table 1. The molecular

structures of fluvastatin, probenecid, and nicotinic acid are shown in Figure 1.

Table I. Concentrations of compounds entering the two intestinal segments and physicochemical properties of the perfusion solutions* used for the three different treatment groups.

Compound/physicochem.	Treatment		
	Control	Probenecid	Nicotinic acid
Fluvastatin (μM)	1.6	1.6	1.6
Antipyrine (μM)	53	53	53
D-glucose (mM)	10	10	10
Probenecid (mM)	-	1.0	-
Nicotinic Acid (mM)	-	-	10
pH	6.53 ± 0.02	6.53 ± 0.02	6.32 ± 0.02
Osmolality (mmol/kg)	284 ± 2	283 ± 3	298 ± 6
Surface Tension (mN/m)	58.5 ± 0.6	58.9 ± 0.8	58.8 ± 2.0

* 5.4 mM KCl; 48 mM NaCl; 35 mM mannitol; 1 g/l PEG 4000 in a 50 mM phosphate buffer pH 6.5. ^{14}C -PEG 4000 ($2.5 \mu\text{Ci/l}$) and ^3H -D-glucose ($10 \mu\text{Ci/l}$) were included as markers for water flux, active transport and viability. Antipyrine was included as a marker for passive transcellular diffusion. Mean $\pm$ S.D.

Fig. 1. Nicotinic acid (10 mM) and probenecid (1 mM) were employed as inhibitors of the monocarboxylic acid transporter (MCT) and the multidrug resistance-associated protein (MRP), respectively, to study the possible involvement of these carriers in the intestinal transport of fluvastatin (1.6 μM) in the rat.

Intestinal Single-Pass Perfusions

Male Sprague-Dawley rats, 275-310 g (CrI:CD[SD]BR, Charles River, Uppsala, Sweden) were used. The rats were acclimatized for at least 1 week under controlled conditions (22 to 23°C, 50% air humidity, 12 hours light cycle) before the

experiments. They had access to tap water and regular rat chow (R36; Lactamin AB, Stockholm, Sweden) until 14 to 20 hours prior to the experiment, when the chow was withdrawn. The surgery and intestinal perfusions were performed as described previously (1). In brief, anaesthesia was induced by an intraperitoneal injection of 135 to 150 mg/kg body weight of Inactin-Byk (thiobutabarbital sodium), and the rats were placed on a heating pad to maintain the body temperature at $37 \pm 1^\circ\text{C}$. The abdomen was opened by a midline longitudinal incision and a segment of the ileum (10 cm) or the colon (8 cm) was cannulated with plastic tubing (4 mm outer diameter) and perfused (0.2 mL/minute) for 105 minutes.

Chemical Assays

The concentrations of fluvastatin and antipyrine in the outlet intestinal perfusate were assayed with high-performance liquid chromatography, as described earlier (1,15). The perfusate concentrations of ^{14}C -labelled PEG 4000 and ^3H -labelled D-glucose were determined by liquid scintillation counting. The pH, osmolality, and surface tension were measured with a pH-meter, an osmometer (Wescor 5500; Wescor,

Logan, UT), and a duNoüy interfacial tensiometer (Krüss, Hamburg, Germany), respectively.

Data Analysis

The net water flux (NWF), effective permeability (P_{eff}), and intestinal radius were calculated from steady-state concentrations in the outlet perfusate as described previously (1). In brief, the NWF was calculated from the following:

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

where $[\text{PEG}_{\text{in}}]$ and $[\text{PEG}_{\text{out}}]$ are the inlet and outlet concentrations of ^{14}C -PEG 4000, respectively, and Q_{in} is the inlet flow rate (0.2 mL/minute). The P_{eff} across the intestinal mucosa was calculated according to the following:

$$P_{\text{eff}} = \frac{Q_{\text{in}} \times \ln(C_{\text{in}}/C_{\text{out}})}{2 \pi r L} \quad (\text{Eq. 2})$$

where C_{in} and C_{out} are the inlet and outlet (fluid flux corrected) concentrations of the compound investigated, respectively (16). The intestinal radius was calculated from the length (measured with a thread) and the volume of the segment (amount PEG 4000 in the segment/concentration, PEG 4000 exiting the segment), assuming the segment to have the shape of a cylinder.

The influence of nicotinic acid and probenecid on these transport variables was tested with one-way analysis of variance (StatView; Abacus Concepts, Berkeley, CA). A probability value less than .05 was considered significant. Fisher's least square difference was used to identify significantly different groups. All results are presented as mean values $\pm$ SD for 4 to 6 rats.

RESULTS AND DISCUSSION

The possible involvement of an anionic efflux protein (eg, MRP2) in the nonlinear intestinal absorption of fluvastatin in rats was investigated using a single-pass perfusion technique in the ileum and the colon. These 2 intestinal regions have been reported to

exhibit the most pronounced concentration-dependent permeability to fluvastatin (1). The MRP2 has been reported to be expressed in both the ileum and the colon of the rat, but at a lower concentration than in the duodenum and the jejunum (17). On the other hand, Makhey et al reported that the functional expression of MRP2 (ability to efflux known substrates) is highest in the ileum and the colon in rats (10).

Probenecid, an organic anion transport inhibitor, was employed as a substrate/inhibitor of the MRP2. Probenecid has previously been shown to interact directly with, and to inhibit, MRP-mediated transport in *in vitro* studies with membrane vesicles of MRP-overexpressing cancer cells (18,19). In addition, probenecid has been reported to inhibit the intestinal efflux of several organic anions such as cefazolin, phenol red, cephaloridine, and calcein in rats (20-23). This inhibitory function suggests that probenecid interacts with the MRP2, which seems to be the only known member of the MRP family that effluxes its substrates into the intestinal lumen (9,17). However, in the present study, probenecid (1 mmol/L) had no effect on the transport of fluvastatin (1.6 $\mu\text{mol/L}$) in the ileum or the colon (Figure 2). This lack of effect suggests that fluvastatin is not effluxed to any significant extent by an organic anionic efflux protein, such as the MRP2, in rats *in situ*. Also, the ileal and colonic P_{eff} of antipyrine and D-glucose were unaffected by the presence of probenecid (Figure 2). Thus, the previously reported concentration-dependent P_{eff} of fluvastatin in rats cannot be explained by a saturated efflux-mediated transport by MRP2.

Fig. 2. The effect of probenecid (1 mM, hatched bars) and nicotinic acid (10 mM, shaded bars) on the P_{eff} of: a) fluvastatin, b) antipyrine and c) D-glucose, in the ileum and the colon of the rat. * $p = 0.005$ compared to the control group (open bars). Mean $\pm$ S.D., $n = 4-6$.

To our knowledge, no inhibitors of HMG-CoA reductase have previously been investigated as potential substrates for an intestinal anionic efflux system. In the rat liver, on the other hand, pravastatin is excreted into the bile via the canalicular multispecific organic anionic transporter (cMOAT, MRP2) (11,12).

The second aim of this study was to investigate whether or not the MCT is involved in the intestinal absorption of fluvastatin. This transporter is expressed both in the small and in the large intestine (13,24). Two inhibitors of HMG-CoA reductase, pravastatin and atorvastatin, are known to be transported across the small intestinal mucosa by this carrier, rather than by simple diffusion (13,14). Tamai et al reported that nicotinic acid (10 mmol/L) reduced the uptake of pravastatin across rabbit brush border membrane vesicles by more than 40%; this reduction was attributed to competitive inhibition of the H⁺-dependent MCT (13).

In the present study, however, nicotinic acid (10 mmol/L) did not affect the P_{eff} of fluvastatin (1.6 μmol/L) in the ileum (Figure 2). In the colon, on the other hand, the P_{eff} of fluvastatin increased by 37% ($P < .05$) in the presence of nicotinic acid at the same concentrations as in the ileum (Figure 2). A possible explanation is that the lower pH of the perfusion solution in the presence of nicotinic acid (pH 6.3 vs 6.5) (Table 1) increased the driving force for the H⁺-dependent MCT. This hypothesis is supported by data showing that the uptake of pravastatin in rabbit ileal brush border vesicles increased by roughly 30% when the extravesicular pH was decreased from 6.5 to 6.3 (13). In opposition to this hypothesis is the observation that nicotinic acid did not affect the ileal transport of fluvastatin in the present study. Thus, the involvement of the MCT in the intestinal transport of

fluvastatin seems less probable. This lack of involvement is in accordance with the previously reported increase in the ileal and colonic P_{eff} to fluvastatin in the presence of excess lovastatin, a drug that has been shown to inhibit the MCT (1,13). Furthermore, the previously reported nonlinear increase in the P_{eff} of fluvastatin at higher luminal concentrations is not in accordance with a carrier saturated in the absorptive direction (1).

A more feasible explanation for the higher P_{eff} of fluvastatin in the colon at the lower pH, when nicotinic acid was added, is the higher fraction of non-ionized drug at that pH. On the basis of the Henderson-Hasselbalch equation, the fraction of non-ionized fluvastatin (acid, pK_a 4.6) increased by 58% at pH 6.3 compared to the fraction at pH 6.5. Thus, the increased P_{eff} of fluvastatin at the lower pH might be explained by the increased fraction of non-ionized fluvastatin, according to the pH-partition hypothesis (25). It is possible that we did not observe this pH effect in the ileum because of the thicker protective mucus and unstirred water layer adjacent to the intestinal wall in this region (26). This layer might have kept the ileal microclimate pH unaffected, despite the slight decrease in the bulk pH. The intestinal P_{eff} of antipyrine and D-glucose was not affected by nicotinic acid (Figure 2).

The recovery of the water flux marker ¹⁴C-PEG 4000, the intestinal radius, and the net water flux were unaffected by probenecid or nicotinic acid (Table 2). A high recovery of ¹⁴C-PEG 4000 in the outlet perfusate, stable net water fluxes over time, a high P_{eff} of D-glucose in the ileum, and a low P_{eff} of D-glucose in the colon all indicate that the viability of the intestinal mucosa was maintained in all experiments (Table 2 and Figure 2).

Table II. Absorption variables obtained during single-pass perfusions in the six different groups of rats.

Region:	Ileum			Colon		
Treatment:	Control	P-cid	N-acid	Control	P-cid	N-acid
n:	5	4	5	5	5	6
PEG 4000 recovery (%):	95 ± 1	96 ± 2	95 ± 2	98 ± 2	96 ± 1	97 ± 1
Net Water Flux (μl/h/cm):	-4 ± 38	-20 ± 47	-24 ± 18	-105 ± 52	-110 ± 41	-103 ± 38
Radius (cm):	0.23 ± 0.03	0.22 ± 0.05	0.25 ± 0.06	0.24 ± 0.07	0.26 ± 0.02	0.24 ± 0.04

P-cid: probenecid; N-acid: nicotinic acid. The results are presented as mean values ± S.D. from n perfusion experiments. A negative value of the net water flux denotes absorption of fluid. Each individual value of the intestinal radius was used in the calculation of the effective permeability.

The results of the present study show that no significant transport interactions between fluvastatin and probenecid and nicotinic acid exist in the rat intestine. Thus, the previously reported concentration-dependent P_{eff} of fluvastatin in these intestinal regions in rats is probably not attributable to saturation of the MRP2 at higher luminal concentrations of fluvastatin. Because previous results have suggested that the Pgp is not involved in the transport process, the nonlinear P_{eff} of fluvastatin might be caused by an as-yet unknown transport protein in the intestinal epithelium. It is also possible that the previously reported physicochemical interaction between fluvastatin and the intestinal membrane is the major mechanism behind the observed concentration-dependent P_{eff} of fluvastatin in rats (5). Finally, the rapid intestinal absorption of fluvastatin is not mediated by active transport by the MCT to any significant extent. Instead, passive diffusion is probably the dominating absorption mechanism for fluvastatin, which also agrees with its physicochemical properties (27).

REFERENCES

- Lindh A, Sandstrom R, Ungell AL, Lennernas H. Concentration- and region-dependent intestinal permeability of fluvastatin in the rat. *J Pharm Pharmacol*. 1998;50:737-744.
- Dimitroulakos J, Yeager H. HMG-CoA reductase mediates the biological effects of retinoic acid on human neuroblastoma cells: lovastatin specifically targets P-glycoprotein-expressing cells. *Nat Med*. 1996;2:326-333.
- Holmberg M, Sandberg C, Nygren P, Larsson R. Effects of lovastatin on a human myeloma cell line: increased sensitivity of a multidrug-resistant subline that expresses the 170 kDa P-glycoprotein. *Anti-Cancer Drugs*. 1994;5:598-600.
- Wu X, Whitfield LR, Stewart BH. P-glycoprotein-mediated apical secretion of atorvastatin in Caco-2 cell monolayers. *PharmSci*. 1998;1:S-450.
- Lindh A, Persson B, Ungell AL, Lennernas H. Surface activity and concentration dependent intestinal permeability in the rat. *Pharm Res*. 1999;16:97-102.
- Deeley RG, Cole SP. Function, evolution and structure of multidrug resistance protein (MRP). *Semin Cancer Biol*. 1997;8:193-204.
- Kool M, de Haas M, Scheffer GL, et al. Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologues of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. *Cancer Res*. 1997;57:3537-3547.
- Sugawara I, Akiyama S, Scheper RJ, Itoyama S. Lung resistance protein (LRP) expression in human normal tissues in comparison with that of MDR1 and MRP. *Cancer Lett*. 1997;112:23-31.
- Borst P, Evers R, Kool M, Wijnholds J. The multidrug resistance family. *Biochim Biophys Acta*. 1999;1461:347-357.
- Makhey VD, Guo A, Norris DA, Hu P, Yan J, Sinko PJ. Characterization of the regional intestinal kinetics of drug efflux in rat and human intestine and in Caco-2 cells. *Pharm Res*. 1998;15:1160-1167.
- Fukumura S, Takikawa H, Yamanaka M. Effects of organic anions and bile acid conjugates on biliary excretion of pravastatin in the rat. *Pharm Res*. 1998;15:72-76.
- Yamazaki M, Akiyama S, Niinuma K, Nishigaki R, Sugiyama Y. Biliary excretion of pravastatin in rats: contribution of the excretion pathway mediated by canalicular multispecific organic anion transporter. *Drug Met Dispos*. 1997;25:1123-1129.
- Tamai I, Takanaga H, Maeda H, Ogihara T, Yoneda M, Tsuji A. Proton-cotransport of pravastatin across intestinal brush-border membrane. *Pharm Res*. 1995;12:1727-1732.
- Wu X, Whitfield LR, Stewart BH. pH-dependent transport of atorvastatin across the apical membrane of Caco-2 cells. *PharmSci*. 1998;1:S-449.
- Lennernas H, Ahrenstedt O, Hallgren R, Knutson L, Ryde M, Paalzow LK. Regional jejunal perfusion, a new in vivo approach to study oral drug absorption in man. *Pharm Res*. 1992;9:1243-1251.
- Fagerholm U, Johansson M, Lennernas H. Comparison between permeability coefficients in rat and human jejunum. *Pharm Res*. 1996;13:1336-1342.
- Gotoh Y, Suzuki H, Kinoshita S, Hirohashi T, Kato Y, Sugiyama Y. Involvement of an organic anion transporter (canalicular multispecific organic anion transporter/multidrug resistance-associated protein 2) in gastrointestinal secretion of glutathione conjugates in rats. *J Pharmacol Exp Ther*. 2000;292:433-439.
- Lam BK, Xu K, Atkins MB, Austen KF. Leukotriene C4 uses a probenecid-sensitive export carrier that does not recognize leukotriene B4. *Proc Natl Acad Sci U S A*. 1992;89:11598-11602.
- Nakamura T, Oka M, Aizawa K, et al. Direct interaction between a quinoline derivative, MS-209, and multidrug resistance protein (MRP) in human gastric cancer cells. *Biochem Biophys Res Commun*. 1999;255:618-624.
- Fujita T, Yamada H, Fukuzumi M, Nishimaki A, Yamamoto A, Muranishi S. Calcein is excreted from the intestinal mucosal cell membrane by the active transport system. *Life Sci*. 1997;60:307-13.
- Saitoh H, Gerard C, Aungst BJ. The secretory intestinal transport of some beta-lactam antibiotics and anionic compounds: a mechanism contributing to poor oral absorption. *J Pharmacol Exp Ther*. 1996;278:205-211.
- Saitoh H, Fujisaki H, Aungst BJ, Miyazaki K. Restricted intestinal absorption of some beta-lactam antibiotics by an energy-dependent efflux system in rat intestine. *Pharm Res*. 1997;14:645-649.
- Sarkadi B, Muller M, Hollo Z. The multidrug transporters—proteins of an ancient immune system. *Immunol Lett*. 1996;54:215-219.

24. Ritzhaupt A, Wood IS, Ellis A, Hosie KB, Shirazi-Beechey SP. Identification of a monocarboxylate transporter isoform type 1 (MCT1) on the luminal membrane of human and pig colon. *Biochem Soc Trans.* 1998;26:120.
25. Hogben CAM, Tocco DJ, Brodie BB, Schanker LS. On the mechanism of intestinal absorption of drugs. *J Pharmacol Exp Ther.* 1959;125:275-282.
26. Levitt MD, Aufderheide T, Fetzter CA, Bond JH, Levitt DG. Use of carbon monoxide to measure luminal stirring in the rat gut. *J Clin Invest.* 1984;74:2056-2064.
27. Winiwarter S, Bonham NM, Ax F, Hallberg A, Lennernas H, Karlen A. Correlation of human jejunal permeability (in vivo) of drugs with experimentally and theoretically derived parameters. A multivariate data analysis approach. *J Med Chem.* 1998;41:4939-4949.