The biliary excretion of pravastatin in normal rats and Eisai hyperbiliruminemic rats (EHBRs) was examined in vivo and in vitro using bile canalicular membrane vesicles (CMVs). In vivo, the total body clearances at steady-state (\(C_{L_{\text{tot}}}\)) for both rat strains decreased as the infusion rate increased. At the lowest infusion rate, \(C_{L_{\text{tot}}}\) for normal rats was 1.6 times higher than that for EHBRs. Under this set of conditions, the biliary excretion clearance (\(C_{L_{\text{ bile}}}\)), defined as the biliary excretion rate at steady-state divided by the concentration in the liver (\(C_{\text{liver}}\)), for normal rats was 3-fold higher than that for EHBRs. The \(C_{L_{\text{ bile}}}\) fell markedly with increasing \(C_{\text{liver}}\) for normal rats and the Michaelis constant (\(K_{\text{M}}\)) for \(C_{\text{liver}}\) was 180 \(\mu M\); in contrast, the degree of saturation was slight if any in EHBRs. In vitro, the uptake of pravastatin by CMVs prepared from normal rats exhibited clear ATP-dependence, whereas only a minimal effect of ATP was observed on the uptake by CMVs from EHBRs. Transport kinetic studies were performed over a wide range of pravastatin concentration (0.2–10,000 \(\mu M\)) with a tracer tritium-labeled pravastatin. Saturation was observed both in the ATP-dependent (\(K_{\text{M}}\) 220 \(\mu M\)) and ATP-independent (\(K_{\text{M}}\) 480 \(\mu M\)) uptake by CMVs prepared from normal rats. ATP-dependent uptake of 2,4-dinitrophenyl glutathione, a typical substrate for the canalicular multispecific organic anion transporter (cMOAT), was inhibited by pravastatin in a concentration-dependent manner and the resultant inhibitory constant of pravastatin (170 \(\mu M\)) was comparable with the \(K_{\text{M}}\) value of ATP-dependent pravastatin uptake itself. In conclusion, biliary excretion of pravastatin is mediated mainly by cMOAT in normal rats. This can explain the decrease in the biliary excretion of pravastatin in EHBRs.

The HMG-CoA\(^1\) reductase inhibitor, pravastatin, as far as its pharmacological effect (decrease in the plasma cholesterol level) is concerned, is relatively liver specific (1). As one of the reasons for this tissue-specific activity, we have previously demonstrated that after iv administration of pravastatin, liver accounted for most of the uptake compared with other tissues (2). Based on the results obtained with in vitro isolated rat hepatocytes, pravastatin was taken up actively by a so-called “multispecific anion transporter (3)” (4). In addition, the permeability surface area products for the influx of unbound pravastatin evaluated in four different experimental systems (in vivo, in vitro liver perfusion, isolated cells, and primary culture cells) were in good agreement (5), indicating that the active transport mechanism on the liver surface for this drug is entirely responsible for its initial distribution into the liver. As described above, regarding the hepatic uptake of pravastatin, we and others (4, 6, 7) have identified a number of characteristics. After oral administration, pravastatin was well absorbed from the gastrointestinal tract (70% in rats and more than 50% in dogs) (8). Regarding transport across the intestinal brush-border membrane, a proton-gradient dependent carrier-mediated mechanism is involved (9). Pravastatin was taken up efficiently by the liver as described above, and enterohepatic circulation has been suggested to take place (8). These carrier-mediated transport mechanisms contribute to the efficient exposure of the target enzyme in the liver to the drug administered orally; distribution to other tissues is restricted because of its high hydrophilicity. Consequently, these features of pravastatin, in the light of its disposition in the body, lead to liver-selective inhibition of cholesterol biosynthesis.

As for the biliary excretion of pravastatin, studies have been directed at investigating whether it is a substrate for aprimary active transport system, termed the “canalicular multispecific organic anion transporter” (cMOAT), cloned recently (10–12). Interest has arisen in this topic as it has become clear that most classes of organic anionic compounds and their conjugates are excreted in bile via this transport system (13, 14). For this reason, studies with mutant rats, such as TR\(^-\) and Eisai hyperbiliruminemic rats (EHBRs), in which this transport system is defective, are extremely useful (13–16). We demonstrated that the steady-state biliary excretion of pravastatin fell to 40% in EHBRs compared with that in normal rats in an in vitro single-pass liver perfusion study (17). The bile to liver cytosol unbound drug concentration ratio, however, was approximately 90 for both rat strains. Furthermore, ATP-dependent uptake by bile canalicular membrane vesicles (CMVs) was observed not only for normal rats but even

\(^{1}\)Abbreviations used are: HMG-CoA; hydroxymethylglutaryl CoA, EHBRs; Eisai hyperbiliruminemic rats, cMOAT; canalicular multispecific organic anion transporter, CMVs; bile canalicular membrane vesicles, \(C_{L_{\text{tot}}}\); total body clearance, \(I_{\text{infusion}}\); infusion rate, \(C_{\text{p}}\); plasma concentration of pravastatin at steady-state, \(V_{\text{max}}\); biliary excretion rate, \(K_{\text{M}}\); Michaelis constant, \(V_{\text{max}}\); maximum transport velocity, \(P_{\text{diff}}\); nonspecific diffusion clearance, AIC; Akaike’s information criterion, \(K_{\text{I}}\); inhibitory constant, \(C_{\text{p}}\); biliary excretion clearance, \(C_{\text{liver}}\); pravastatin concentration in the liver, DNP-SG; 2,4-dinitrophenyl glutathione, GSH; glutathione, \(T_{\text{m}}\); maximal secretory rate.

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for EHBRs, although the degree of stimulation by ATP was slight in EHBRs. Adachi et al. (18) have demonstrated that uptake of pravastatin by CMVs from normal rats and EHBRs was dependent on both ATP and the pH gradient. They also showed that there was a reduction in the biliary excretion of pravastatin after iv administration to EHBRs compared with that in normal rats. The experimental evidence shown above suggested the possibility of the presence of multiple transport systems for pravastatin. There still remains a possibility, however, that both transport mechanisms, that is cMOAT-mediated (deficient in EHBRs) and a novel ATP-dependent transporter-mediated (maintained in EHBRs), are involved in biliary excretion of pravastatin. So far, only $[^{14}C]$ labeled pravastatin has been available with a relatively low specific activity; thus it was impossible to examine the transport kinetics of this compound at low concentrations, e.g. to clarify the contribution of cMOAT-mediated transport across the bile canalicular membrane quantitatively.

The purpose of this study is to gain a better understanding of the mechanism of pravastatin excretion into bile and the defect in pravastatin excretion in EHBRs. We carried out a series of in vivo studies involving continuous infusion at different infusion rates over a wide range and compared the biliary excretion at steady state in normal rats and EHBRs. Furthermore, we performed a transport study by CMVs to see if pravastatin is a substrate for cMOAT using newly synthesized $[^{3}H]$ labeled pravastatin with a specific activity three orders of magnitude higher than that of the $[^{14}C]$ labeled material. For this purpose, we performed an uptake study with $[^{3}H]$ labeled pravastatin using CMVs from both normal and EHBR livers, and an inhibition study of pravastatin on ATP-dependent uptake of $[^{3}H]$ 2,4-dinitrophenyl glutathione (DNP-SG), a typical substrate for cMOAT, by CMVs from normal rat liver.

Materials and Methods

Chemicals. $[^{14}C]$ Pravastatin, $[^{3}H]$ pravastatin, and unlabeled pravastatin were kindly donated by Sankyo Co. Ltd. (Tokyo, Japan). Glutathione, [glycine-2-$^{3}H$] (44.8 Ci/mmol) was purchased from New England Nuclear Corp. (Boston, MA). 1-Chloro-2,4-dinitrobenzen, glutathione S-transferase (from rat liver), ATP (disodium salt), creatine phosphate, and creatine phosphokinase (Type I: from rabbit muscle) were from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade.

The radiochemical purity of $[^{14}C]$ pravastatin synthesized as described previously (8), determined by HPLC, was 98.8%, and the specific activity was 11.3 mCi/mmol. $[^{14}C]$ labeled positions were shown in (8). $[^{3}H]$ Pravastatin previously (8), determined by HPLC, was 98.8%, and the specific activity was 1124 YAMAZAKI

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Pravastatin was infused at 0.021, 0.50, 2.5, 4.9, 9.8, and 22 nmol/min/kg for normal rats (open) or 0.024, 2.5, 4.6, 10 and 24 nmol/min/kg for EHBRs (closed). Each symbol and vertical bar represent the mean ± SE of 3 rats. The horizontal bars were within the limits of the symbols. (a) CL_{tot} vs. Infusion rate (I), (b) CL_{tot} vs. plasma concentration of pravastatin at steady-state (C_{p,ss}).

as the amount of pravastatin in the liver (nmol/g liver) assuming that 1 g liver = 1 ml, and K_{d, in vivo} (µM), V_{max, in vivo} (nmol/min/kg) and P_{dif, in vivo} (ml/min/kg) represent the Michaelis constant as for CL_{liver}, maximum excretory velocity and nonspecific diffusion clearance, respectively. The input data were weighted as the reciprocal of the observed values, and the algorithm used for the fitting was the Damping Gauss Newton Method (25). AIC values were used to assess the suitability of the models (26).

In vitro. The kinetic parameters for pravastatin uptake by CMVs were estimated according to the following equation:

\[ v_s = V_{max, in vivo} S/(K_{d, in vivo} + S) + P_{dif, in vivo} S \]  

where \( v_s \) is the uptake rate of the ligand by CMVs, assessed by the uptake amount at 2 min (nmol/min/mg protein), \( S \) is pravastatin concentration (µM), \( V_{max, in vivo} \) is the maximum uptake rate by CMVs (nmol/min/mg protein), \( K_{d, in vivo} \) is the Michaelis constant (µM) and \( P_{dif, in vivo} \) is the nonspecific uptake clearance (µl/min/mg protein).

The inhibitory constant (\( K_i \)) value of pravastatin on the ATP-dependent uptake (obtained by subtracting the uptake in the absence of ATP from that in the presence of ATP) of [3H] DNP-SG (1 µM) was calculated according to the following equation, from the inhibition data obtained by varying the pravastatin concentration (1 - 100,000 µM):

\[ v_s = V_{max} S/(K_d(1 + i/K_i) + S) + P_{dif} S \]  

where \( K_i \) is the inhibitory constant (µM) of pravastatin, \( i \) is the pravastatin concentration (1 - 100,000 µM), \( S \) is the DNP-SG concentration (1 µM). The \( K_d \) (15.8 µM) and \( P_{dif} \) (1.30 µl/min/mg) values obtained by a different experiment were used, and the parameters \( K_i \) and \( V_{max} \) were obtained by the fitting.

Results

Disposition of Pravastatin in vivo. The plasma concentrations of pravastatin for both rat strains reached a plateau approximately 40 min after the beginning of the iv infusion, 0.02 - 24.0 µmol/min/kg, i.e. a steady-state had been attained (data not shown). Fig. 2(a) shows the relationship between the infusion rate (I) and the total body clearance (CL_{tot}), defined as the infusion rate divided by the plasma concentration of pravastatin at steady-state (60 min after the beginning of infusion; C_{p,ss}) (eq. 1). The values of CL_{tot} for each rat declined with increasing infusion rate. At the lowest infusion rate, the values of C_{p,ss} were less than 1 µM for each rat (0.44 ± 0.06 µM for normal rats and 0.81 ± 0.09 µM for EHBRs, respectively), and the CL_{tot} (in ml/min/kg) for normal rats (48.0 ± 5.5) was 1.6 times higher than that for EHBRs (29.8 ± 3.1) (fig. 2(b)).

Fig. 3 shows the concentration-dependence of the biliary excretion of pravastatin at steady-state. At any infusion rate, the biliary excretion for normal rats was higher than for EHBRs. For normal rats, saturation was exhibited clearly, whereas the biliary excretion for EHBRs was almost linear. For normal rats, the biliary excretion clearance (CL_{bile, in vivo}) was calculated as V_{bile} divided by C_{liver} decreased with the increase in C_{liver} (from 14.2 ± 3.2 at C_{liver} = 0.73 ± 0.09 µM to 8.70 ± 1.26 at C_{liver} = 793 ± 33 µM). For EHBRs, the values fell from 4.00 ± 0.88 at C_{liver} = 0.91 ± 0.17 µM to 1.44 ± 0.35 at C_{liver} = 2110 ± 250 µM. To obtain the kinetic parameters for the biliary excretion of pravastatin, three different models described in eqs. 2-4 were fit to the data. Model 1 fit the data for normal rats best (the AIC values were as follows: 26.1 for model 1, 22.5 for model 2, and 23.4 for model 3). The kinetic parameters obtained are listed in table 1. For EHBRs, the AIC values were comparable among the 3 models (23.4 for model 1, 22.5 for model 2, and 2110 ± 22.6 for model 3); however, in practical terms reliable values for parameters were obtained only for model 3 (for model 1, the computer calculated SD values were >10-fold larger than the fit values; for model 2, the K_m value calculated was more than twice the highest value for C_{liver}, observed experimentally). The P_{dif, in vivo} value in model 3 was calculated as 1.45 ± 0.07 ml/min/kg (table 1).

Transport Study in Canicular Membrane Vesicles (CMVs). As shown in fig. 4(a), the uptake of [3H] pravastatin by CMVs prepared from normal rats exhibited ATP-dependence at 0.2 µM (tracer only), whereas no stimulatory effect of ATP was observed in the presence of 2 mM of unlabeled pravastatin. For CMVs from EHBRs, a minimal effect of ATP was observed in the uptake of pravastatin (fig. 4(b)); however, concentration-dependence was observed in CMVs from both rat strains. The kinetic parameters (mean ± computer calculated SD) for the uptake of pravastatin by CMVs from normal rats using the data shown in fig. 5 were obtained as listed in table 1.

The inhibitory profiles of pravastatin on the ATP-dependent uptake

\[ K_i = \frac{1}{K_M + [S]} \]  

\[ V_{max} = \frac{K_M [S]}{1 + [S]} \]  

\[ P_{dif} = \frac{C_{liver}}{CL_{bile, in vivo}} \]
CMVs (10 µg protein) were incubated with (closed) or without (open) ATP (5 mM) and ATP-regenerating system (10 mM creatine phosphate and 100 µg/ml of creatine phosphokinase) in the medium. Circles represent the tracer uptake. Each point and vertical bar represent the mean ± SE of three determinations. Significantly higher than the control (p<0.05).

of [3H] DNP-SG (1 µM) were almost identical with those of the self-inhibition of pravastatin itself (fig. 6). The resultant K_i value of pravastatin was 172 ± 38 µM (mean ± computer calculated SD), comparable with the K_M value (223 ± 29) µM (mean ± computer calculated SD) for the ATP-dependent uptake itself (table 1).

**Discussion**

We have previously suggested that a primary active transport mechanism which is maintained in EHBRs contributes at least partly to the biliary excretion of pravastatin. However, the decrease in the biliary excretion of pravastatin in EHBRs suggested that this drug is also a substrate for cMOAT (17). So far, only the [14C] labeled radioisotope of this compound with a relatively low specific activity has been available, and this has prevented us carrying out a detailed transport study, especially the uptake by CMVs. For example, we could not reduce the radiolabeled pravastatin concentration below 200 µM to obtain reliable counts in the CMV uptake study (17). Also, the lowest concentration in the study of Adachi et al. with CMVs was 100 µM (18). With [14C] pravastatin, we were unable to examine the possibility that other transport system(s) with higher affinity might be involved. In general, transport studies using CMVs were performed at a ligand concentration around 10^{-8} ~ 10^{-5} M range (19, 22, 24). In

![Fig. 4. Time profiles for the uptake of [3H] pravastatin by the CMVs prepared from normal rats (a) and EHBR (b).](image)

![Fig. 6. Effect of pravastatin on the ATP-dependent uptake of [3H] DNP-SG (1 µM) by CMVs from normal rats.](image)
addition, we observed a clear decrease in the biliary excretion of pravastatin in EHBRs in perfused liver at 10⁻⁶ M for C_{liver} with the infusate concentration of 1 µM (17), which was slightly higher than the therapeutic concentration of this drug (< 0.3 µM) (1). In the present study, we have been able to examine the biliary excretion of pravastatin in normal rats and EHBRs in in vitro CMV studies at much lower concentrations using newly synthesized [³H] labeled pravastatin with a specific activity three orders of magnitude higher than that of the [¹⁴C] labeled material.

Furthermore, although membrane vesicles are an artificial experimental system, they are a useful tool for investigating the precise mechanism of transport. However, a quantitative assessment based on such results alone may not reflect accurately what is happening in vivo. Consequently, to examine the contribution of any transport mechanism to the biliary excretion of pravastatin, studies should be performed with other experimental systems that are more physiological than membrane vesicles. Therefore, we also examined the biliary excretion of pravastatin in an in vivo constant infusion study.

We found that the biliary excretion of pravastatin is actually reduced in EHBRs compared with that in normal rats in vivo under a wide range of plasma and/or liver concentrations (fig. 3). These results suggest that cMOAT mediated transport is involved in the biliary excretion of pravastatin. In vitro, the uptake of pravastatin by CMVs prepared from normal rats exhibited clear ATP-dependence, whereas a minimal ATP-dependence was observed in the uptake by CMVs from EHBRs (fig. 4), which is consistent with our previous study (17). Saturation was observed both in ATP-dependent (K_M, 220 µM) and ATP-independent (K_M, 480 µM) uptake by CMVs prepared from normal rats (fig. 5). ATP-dependent uptake of DNP-SG was inhibited by pravastatin in a concentration-dependent manner and the resultant K_M of pravastatin (170 µM) was comparable to the K_M value of ATP-dependent pravastatin uptake itself (fig. 6). Furthermore, those values (K_M and K_I) obtained with in vitro CMVs were comparable with that (K_M) in vivo (table 1). These results indicate that pravastatin shares the same transporter on the bile canalicular membrane as DNP-SG, a typical substrate for cMOAT, and this causes the decrease in biliary excretion in EHBRs in vivo.

To demonstrate directly that cMOAT-mediated transport is actually involved in the biliary excretion of pravastatin in vivo is quite difficult without using cMOAT knock-out animals or specific inhibitors of cMOAT that are effective under in vivo conditions. So far, a cMOAT knock-out mouse has not been developed, and a cMOAT-specific inhibitor has yet to be discovered. We consider that our approach in this study, i.e. quantitative comparison of the kinetic parameters obtained for both in vivo and in vitro experimental systems, is presently the most reliable and practical way to evaluate the contribution of the cMOAT-mediated transport to biliary excretion under in vivo conditions.

The K_{M, in vivo} defined as a total concentration in the liver, for normal rats was around 180 µM (table 1). It may be appropriate to compare the K_{M, in vivo} value corrected for intracellular binding (K_{M,d}) for unbound drug concentration in the liver; K_{M, u. in vivo} with K_M obtained by in vitro CMV study, since it is a general concept in the pharmacokinetics that only the unbound drug can penetrate the membrane. Previously we evaluated the unbound fraction of pravastatin in liver cytosol at steady-state in the single-pass liver perfusion study (17). The resultant unbound fraction (the unbound drug concentration in the cytosol vs. total drug concentration in liver homogenate) was 0.54 in rats at C_{liver} (µM); around 5 µM. According to the previous result, the K_{M, u. in vivo} was calculated to be around 100 µM, assuming that the intracellular binding was linear within the concentration range in the present study. The likelihood that a remarkable nonlinear tissue binding did occur is low since previously we did not observe any significant difference in plasma unbound fraction (f_u = 0.67) for normal rats over a wide range of concentration (0.5~1000 µM) (27). Even if saturable binding was involved, the K_M for the unbound drug was estimated within the range of 100~200 µM, a consideration that would lead one to conclude the K_M values for biliary excretion of pravastatin obtained for in vivo and in vitro experimental systems are comparable regardless of the correction of intracellular binding.

The exact reason for the three times difference in P_{diff, in vivo} values between the two-rat strains are not clear. It is probable, however, that the nonspecific diffusion can be dependent on the bile flow rate. It is well known that bile flow is two to three times lower in EHBRs by nature, mainly because of its deficiency in the excretion of organic anions and their conjugates (28). In addition to this fact, we found infusion rate-dependent (i.e. C_{liver}-dependent) increase in bile flow rate at steady-state for both rat-strains (for normal rats; from 44 to 145 µl/min/kg, for EHBRs; from 27 to 54 µl/min/kg, respectively). It is difficult to argue in a precise manner about the difference in P_{diff, in vivo} values determined in vivo using CMVs. However, the absolute values for the uptake of pravastatin by CMVs from EHBRs were minute (fig. 4(b)). Furthermore, a direct comparison of the P_{diff, in vitro} values between the two-rat strains is inappropriate in this case since there may be nonspecific adsorption of the ligand to the outer/inner membrane of the leaky vesicles. Further study is required to investigate the reason for the difference in P_{diff, in vivo} values between the two-rat strains.

We cannot rule out the possibility that the putative primary active transport system we suggested previously is also involved in the biliary excretion of pravastatin. This is because a small, but significant, stimulation of uptake by ATP is exhibited in CMVs from EHBRs (fig. 4) (17). Considering the results obtained in the present study, it seems likely that biliary excretion of pravastatin via bile canalicular membrane is mediated mainly by cMOAT and the contribution from other primary active transport system is rather small. Saturation in ATP-independent uptake by CMVs was clearly observed in normal rats (fig. 5(a)), indicating that a certain carrier-mediated ATP-independent transport system may be involved in pravastatin excretion. A small, but distinct, saturable contribution in the biliary excretion in EHBRs was also detected (fig. 3(b)). There has also been a report of clear saturation of uptake by CMVs in the absence of ATP for glutathione (GSH) (29). Uptake of GSH by CMVs from both normal rats and EHBRs does not show any ATP-dependence although the biliary excretion decreased clearly in EHBRs. These authors hypothesized that, for the biliary excretion of GSH, there was almost no GSH in the bile of mutant rats and this could be best explained as a secondary defect owing to cis-inhibition by retained endogenous organic anions for the defective carrier and/or loss of trans-stimulation by these same substrates which are normally concentratively transported into bile (29). Furthermore, studies using CMVs have demonstrated that both ATP- and membrane potential-dependent transport mechanisms are responsible for the biliary excretion of bilirubin glucuronide (30) and DNP-SG (31), and that a membrane potential-dependent transport system is maintained in mutant rats (30). Tamai et al. (32) also demonstrated that a positive inner membrane potential stimulates the uptake of the cephalosporin antibiotic, cephradime (monovalent anion), by CMVs. We have already demonstrated the multiplicity in the transport systems for organic anions which are deficient in mutant rats (14, 22, 24, 33). At present, we
cannot argue for a saturable transport of pravastatin which we detected in our in vitro (fig. 4) and/or in vivo studies in EHBRS (fig. 3 (b)). A number of questions need to be answered, e.g. are these two saturable systems identical or not and what is the driving force(s)? Further studies are required to clarify the mechanism in detail using CMVs as well as molecular biological techniques.

Measurement of biliary output at various plasma concentrations has been used frequently, although it is a classical approach, to characterize the biliary excretion of ligands in vivo. This was particularly the case before the development of the in vitro techniques (34). One criteria, although an indirect one, suggesting that a carrier-mediated excretion mechanism actually exists involving the bile canalicular membranes in vivo is to demonstrate a definable maximum secretory rate (T\textsubscript{m}). As far as organic anions are concerned, T\textsubscript{m} values for several ligands such as bilirubin, indocyanine green, bromosulphthalein, and dibromosulphophthalein etc. have been reported (24, 34).

For pravastatin, no similar study has been conducted. We also measured the hepatic uptake of pravastatin and assessed CL\textsubscript{m,iv}, as it may provide additional insight even though it represents a relatively crude approach to intracellular localization and quantitation. Regarding T\textsubscript{m}, pravastatin exhibits a substantially higher value (6800 nmol/min/kg; fig. 3) than other ligands which are organic anions such as bilirubin, indocyanine green, bromosulphthalein and dibromosulphophthalein (24, 34). This might be explained not only by the intrinsic transport mechanism in the liver, e.g. the hepatic transport capacity for pravastatin is high, but also by the physicochemical characteristics of the ligand itself, e.g. pravastatin is very hydrophilic compared with the other amphipathic organic anions described above. Thus, T\textsubscript{m} may reflect an ephemeral balance between the intrinsic excretory capacity of the liver and the toxicity of the ligand (34).

The therapeutic plasma concentration of pravastatin is less than 0.3 \mu M (1), which is close to the C\textsubscript{p,0} (0.44 ± 0.06 \mu M) obtained at the lowest infusion rate for normal rats in the present study (fig. 2(a)). Assuming that there is no significant species difference in the disposition of pravastatin in the body between rat and human, we can roughly estimate the contribution of the carrier-mediated transport in the biliary excretion under clinical conditions. The resultant K\textsubscript{S,iv} (181 \mu M) is considerably higher than C\textsubscript{live} (0.73 ± 0.09 \mu M) at the lowest infusion rate. Therefore, we can evaluate the contribution of the saturable transport to total biliary excretion as follows:

\[
(\frac{V_{\text{max,iv}}}{K_{\text{S,iv}}})(\frac{V_{\text{max,iv}}}{K_{\text{S,iv}}} + P_{\text{diff, iv}}) \times 100 \approx 89\%
\]

This calculation, combined with the result that indicates that the K\textsubscript{S} and K\textsubscript{M} values obtained in vitro with CMVs are comparable with that (K\textsubscript{S}) in vivo, allows us to state that the biliary excretion of pravastatin is governed mainly by a cMOAT-mediated transport mechanism at therapeutic doses. Previously, we clarified that the hepatic uptake of pravastatin occurs > 90% by a carrier-mediated transport mechanism that we identified using isolated hepatocytes (4).

This is the first case in which both the hepatic uptake and the biliary excretion of one drug can be explained mainly by carrier-mediated transport mechanisms at therapeutic concentrations.

In conclusion, biliary excretion of pravastatin was mediated mainly by cMOAT in normal rats. This can explain the decline in the biliary excretion of pravastatin in EHBRS in vivo. Further studies are required to clarify the nature of the transport mechanism exhibiting saturation without ATP-dependence.

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References


