In Vivo and *in Vitro* Blood–Brain Barrier Transport of 3-Hydroxy-3-Methylglutaryl Coenzyme A (HMG-CoA) Reductase Inhibitors

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Among the HMG-CoA reductase inhibitors, lovastatin and simvastatin have central nervous system (CNS) side effects, such as sleep disturbance, whereas pravastatin does not. This difference in CNS side effects may be due to a difference in blood–brain barrier (BBB) permeability among these inhibitors. To test this hypothesis, we compared the BBB transport ability of HMG-CoA reductase inhibitors by using an *in vivo* brain perfusion technique in rats and an *in vitro* culture system of bovine brain capillary endothelial cells. The *in vivo* BBB permeability coefficients of the lipophilic inhibitors, [^{14}C]lovastatin and [^{14}C]simvastatin, were high. In contrast, that of the hydrophilic inhibitor, [^{14}C]pravastatin, was low and not significantly different from that of [^{14}C]sucrose, an extracellular space marker. Similarly, the *in vitro* BBB permeability coefficients of [^{14}C]lovastatin and [^{1}C]simvastatin were high, while that of [^{14}C]-pravastatin was low. The *in vivo* and *in vitro* transcellular permeabilities obtained for HMG-CoA reductase inhibitors were comparable. This study shows that the BBB permeability correlates with the CNS side effects of the HMG-CoA reductase inhibitors.

KEY WORDS: pravastatin; lovastatin; simvastatin; 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor; blood–brain barrier (BBB) transport; brain perfusion; cultured brain capillary endothelial cell; central nervous system (CNS) side effect; lipophilicity.

INTRODUCTION

Drugs acting peripherally may have undesirable side effects on the central nervous system (CNS) if they can enter the brain. The blood–brain barrier (BBB) consists of brain capillary endothelial cells (BCEC), and it restricts and regulates the movement of molecules between the blood and the brain. Therefore, the BBB permeability affects the ability of drugs to act on the CNS, and it has been shown to depend on their lipophilicity (1).

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, e.g., pravastatin, lovastatin, and simvastatin, have recently been developed for the therapy of hypercholesterolemia. The microsomal enzyme HMG-CoA reductase is a rate-limiting enzyme in cholesterol biosynthesis (2). The target organ for HMG-CoA reductase inhibitors is the liver, as the major site of cholesterol synthesis.

Lovastatin and simvastatin, but not pravastatin, have been reported to cause CNS side effects such as sleep disturbance (3,4), even though all three have similar chemical structures (Fig. 1). Different CNS side effects might result from different abilities to permeate through the BBB, because lovastatin and simvastatin are highly lipophilic, while pravastatin is hydrophilic.

Simvastatin and lovastatin are inactive lactone prodrugs that are expected to be converted *in vivo* to the active open-ring forms, lovastatin acid and simvastatin acid, respectively. Following oral administration of lovastatin and simvastatin, their acid forms as well as the lactone prodrugs are found in plasma (5). Thus, either one or both of these forms may enter the brain.

The purpose of this study was to examine whether the difference of CNS side effect among HMG-CoA reductase inhibitors does indeed correlate with their different abilities to enter the brain. To evaluate BBB permeability, we employed the *in situ* rat brain perfusion technique (6) as an *in vivo* model system and primary cultures of BCEC (7,8) as an *in vitro* model system.

MATERIALS AND METHODS

Chemicals

[^{14}C]Pravastatin (9.57 mCi/mmol), [^{14}C]lovastatin (6.57 mCi/mmol), and [^{14}C]simvastatin (7.16 mCi/mmol) were kindly supplied by Sankyo Co., Ltd. (Tokyo). [^{14}C(U)]Sucrose (3.7 mCi/mmol) was purchased from New England Nuclear (Boston, MA). All isotopes were stored at −20°C until use. Horse serum was purchased from GIBCO (Grand Island, NY), rat tail collagen (type I) was from Collaborative Research Inc. (Bedford, MA), collagenase/dispase, dispase, and human fibronectin were from Boehringer Mannheim GmbH (Mannheim, Germany), amphotericin B, dextran (industrial grade, MW 87,000), gentamicin sulfate, heparin, and Percoll were from Sigma Chemical Co. (St. Louis, MO), polymyxin B sulfate was from Wako Pure Industries Ltd. (Osaka, Japan), Protosol (tissue solubilizer) was from New England Nuclear Corp., Clear-sol I (xylene-based liquid scintillation cocktail) was from Nacalai Tesque Inc. (Kyoto, Japan), and Ketaral 50 (ketamine hydrochloride) was from Sankyo Co., Ltd. (Tokyo). All other chemicals were commercial products of reagent grade.

Preparation of [^{14}C]Lovastatin Acid and [^{14}C]Simvastatin Acid

[^{14}C]Lovastatin acid and [^{14}C]simvastatin acid (1 mg/mL, 2.5 m*M*) were obtained from [^{14}C]lovastatin and [^{14}C]simvastatin, respectively, by hydrolysis in 0.05 *N* NaOH solution at 20°C for 30 min. The hydrolyzed solutions were adjusted to pH 7.4 with 0.2 *N* HCl and stored at 4°C until use.

In Vivo Brain Transport Study

The *in vivo* transport experiments were carried out by the *in situ* brain perfusion technique using the methods reported previously (6). Male Wistar rats weighing 200–250 g (Sankyo Laboratory Co., Toyama, Japan) were anesthetized

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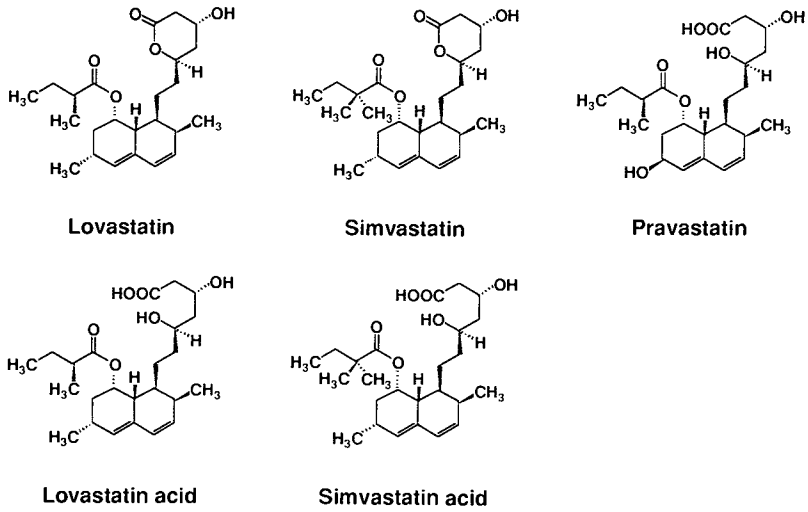

Fig. 1. The structures of the HMG-CoA reductase inhibitors.

with intramuscular ketamine hydrochloride, 235 mg/kg, and xylazine, 2.3 mg/kg. After exposure of the right carotid artery, the occipital and superior thyroid arteries were ligated and cut, and the right pterygopalatine artery was ligated. The right external carotid artery was catheterized for the perfusion to the internal carotid artery with polyethylene tubing (SP-10, Natume Seisakusho Co., Tokyo) filled with sodium heparin (100 IU/mL). An aliquot (0.050 μCi/mL) of [^{14}C]substrate was dissolved in perfusion medium containing 142 m*M* NaCl, 28 m*M* $NaHCO_3$, 4.2 m*M* KH_2PO_4, 1.0 m*M* $MgSO_4$, 1.7 m*M* $CaSO_4$, 6.0 m*M* D-glucose, 0.01% bovine serum albumin (BSA), and 0.2% dimethyl sulfoxide (DMSO) (pH 7.4). The perfusion medium was freshly prepared, oxygenated with 95% O_2/5% CO_2, and heated to 37°C by a temperature-regulating circulator (Type FF, HAAKE, Berlin, Germany).

Perfusions were initiated by starting the infusion pump (Model 22, Harvard Apparatus, South Natick, MA) at a perfusion rate of 4.98 mL/min, with simultaneous ligation of the right common carotid artery to prevent mixing with the systemic blood. At the designated time, the rat was decapitated and the perfused brain was removed from the skull. The arachnoid membrane and meningeal vessels were carefully removed. The right cerebral hemisphere was dissected from the perfused brain and weighed. In addition, samples of perfusion solution were collected from the syringe in duplicate to determine the actual drug concentration perfused. The right cerebral hemisphere was solubilized in a sample vial containing 3 mL of Protosol at 60°C for 3 hr. Then 0.6 mL of 30% of hydrogen peroxide was added to the dissolved solution, and after standing at room temperature for 15 min, the solution was incubated at 60°C for 30 min. After neutralization with 128 μL of glacial acetic acid and addition of Clearsol I, the radioactivity was measured in a liquid scintillation counter (LSC-1000; Aloka Co., Ltd., Tokyo).

The apparent cerebrovascular permeability coefficient, $P_{app,vivo}$, was determined from the following equation:

$$P_{app,vivo} = -F \cdot \ln[1 - (q_{br}/C_{pf} \cdot T \cdot F)]/S \quad (1)$$

where F is the perfusion rate, q_{br} is the amount of ^{14}C-substrate in the cerebral hemisphere at the time of decapitation, C_{pf} is the concentration of ^{14}C-substrate in the perfusate, T is the net perfusion time of cerebral perfusion fluid (lag time, 5 sec), and S is the surface area of brain capillaries, i.e., 100 cm^2/g brain, reported previously (9).

Isolation and Culture of BCEC

BCEC were isolated from cerebral gray matter of bovine brains as described previously (7) with minor modifications (8). The isolated BCEC were stored at −100°C in culture medium containing 20% horse serum and 10% DMSO until use for cell culture. Isolated BCEC were grown on Transwells (polycarbonate membrane; pore size, 12 μm; diameter, 12 mm; surface area, 1 cm^2; Costar, Cambridge, MA) in 12-well cluster dishes (Costar). Prior to seeding, the polycarbonate filter was coated with rat tail collagen under UV light and then with human fibronectin. Isolated BCEC were seeded at 50,000 cells/cm^2 on Transwells and cultured at 37°C under 95% air and 5% CO_2. Transport experiments were performed when the cells reached confluence, in 10–12 days. These primary cultured cells have been shown to retain the morphological properties typical of brain capillary endothelial cells observed *in vivo*, i.e., tight junctions (data not shown). Furthermore, the cells were confirmed to be capillary endothelial cells by the immunostaining method using Factor VIII-related antigen (10) (data not shown).

In Vitro BCEC Transport Study

Primary cultured monolayers of BCEC on Transwells were washed three times with 1 mL of incubation solution (141 m*M* NaCl, 4 m*M* KCl, 2.8 m*M* $CaCl_2$, 0.5 m*M* $MgSO_4$, 1.72 m*M* NaH_2PO_4, 10 m*M* D-glucose, 10 m*M* HEPES, 0.01% BSA, and 0.2% DMSO, pH 7.4 adjusted with 1 *N* NaOH) at 37°C and placed in a 12-well cluster dish, which was maintained at 37°C. Transport was initiated by adding 500 μL of the incubation solution containing [^{14}C]pravastatin at 75 μ*M* (0.35 μCi), [^{14}C]lovastatin acid at 75 μ*M* (0.24 μCi), [^{14}C]simvastatin acid at 75 μ*M* (0.25 μCi), [^{14}C]lovastatin at 25 μ*M* (0.080 μCi), [^{14}C]simvastatin at 25 μ*M* (0.090 μCi), or [^{14}C]sucrose at 430 μ*M* (0.80 μCi) to the donor chamber and

1.5 mL of the incubation solution to the receiver chamber (Fig. 2). At designated times, 300-μL aliquots were sampled from the receiver chamber and replaced with the same volume of the incubation solution. The radioactivity of the collected solution was measured by using a liquid scintillation counter.

The amount of substrate that had permeated through the monolayer of BCEC at each time point was calculated, with correction for the amount withdrawn from the receiver chamber at the preceding time points and then standardized by dividing by the substrate concentration in the donor chamber. Four or five wells containing primary cultured monolayers of BCEC and four or five blank wells containing only collagen- and fibronectin-coated polycarbonate membrane, were used to determine the permeability.

As shown in Fig. 2, the overall permeation process of a drug through a monolayer of BCEC consists of the diffusion process at the unstirred water layer of donor and receiver chambers, the permeation through the monolayer of BCEC, which involves transcellular and paracellular processes, and permeation through the polycarbonate filter. The following equations can be obtained on the basis of previous treatments (11):

$$\frac{1}{P_{tot}} = \frac{1}{P_{uns,d}} + \frac{1}{P_{trans,vitro} + P_{para}} + \frac{1}{P_{filt}} + \frac{1}{P_{uns,r}} = \frac{1}{P_{trans,vitro} + P_{para}} + \frac{1}{P_{filt,app}} \quad (2)$$

where P_{tot} is the apparent permeability coefficient, $P_{uns,d}$ is the diffusion rate in the unstirred water layer at the donor side, $P_{trans,vitro}$ is the transcellular permeability coefficient, P_{para} is the paracellular permeability coefficient, P_{filt} is the permeability coefficient of the filter, $P_{uns,r}$ is the diffusion rate in the unstirred water layer at the receiver side, and $P_{filt,app}$ is the apparent permeability coefficient of the filter.

The apparent permeability coefficient, P_{tot}, was obtained from the slope of a plot of the permeated amount vs time by linear regression analysis. The slope was linear up to 10 min for all substrates examined.

$P_{filt,app}$ was obtained from the permeation of the drug through the collagen/fibronectin-coated polycarbonate membrane of the Transwell as the permeability coefficient including the diffusion rates in the unstirred water layer at the donor side ($P_{uns,d}$) and receiver side ($P_{uns,r}$) of the BCEC monolayer.

$$\frac{1}{P_{tot,pm}} = \frac{1}{P_{para}} + \frac{1}{P_{filt,app,pm}} \quad (3)$$

The permeability coefficient of [^{14}C]sucrose measured with the BCEC monolayer is denoted $P_{tot,pm}$, and that measured with the collagen/fibronectin-coated polycarbonate membrane of the Transwell is denoted $P_{filt,app,pm}$ as the permeability coefficient including the diffusion rates in the unstirred water layer (P_{uns}) at the donor side ($P_{uns,d}$) and receiver side ($P_{uns,r}$) of the BCEC monolayer.

From Eqs. (2) and (3), the transcellular permeability coefficient is given by Eq. (4).

$$P_{trans,vitro} = \frac{P_{tot} \cdot P_{filt,app}}{P_{filt,app} - P_{tot}} - \frac{P_{tot,pm} \cdot P_{filt,app,pm}}{P_{filt,app,pm} - P_{tot,pm}} \quad (4)$$

The permeability coefficient is expressed as microliters per minute per surface area (1 cm^2/well) of the Transwell (μL/min/cm^2).

Accumulation Study in the BCEC

At the end of the *in vitro* BCEC transport study, the polycarbonate membrane of the Transwell with or without the cell monolayer was washed five times with 1 mL of ice-cold incubation solution. The polycarbonate membrane was cut away from the Transwell and solubilized in a sample vial containing 1.5 mL of Protosol at 60°C for 3 hr. After neu-

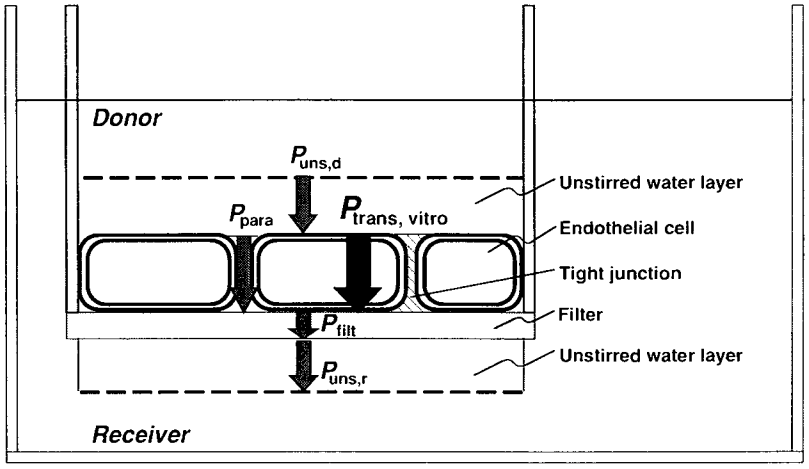

Fig. 2. Schematic diagram for the analysis of drug transport through a monolayer of primary cultured brain capillary endothelial cells. The permeability coefficient of each drug through the BCEC monolayer denoted P_{tot}. P_{tot} indicates the apparent permeability coefficient including diffusion rates in the unstirred water layer at the donor side ($P_{uns,d}$) and receiver side ($P_{uns,r}$), the transcellular permeability coefficient ($P_{trans,vitro}$), the paracellular permeability coefficient (P_{para}), and the permeability coefficient in the filter (P_{filt}).

tralization with 64 μL of glacial acetic acid and addition of Clear-sol I, the radioactivity was measured in a liquid scintillation counter. The protein content of cultured cells per Transwell was determined by the method of Lowry *et al.* (12), using bovine serum albumin as a standard. Net accumulation, expressed as the cell-to-medium (cell/medium) ratio (μL/mg protein), was obtained by dividing the apparent accumulated amount per milligram protein by the ^{14}C-substrate concentration in the incubation medium.

RESULTS

Time Course of *in Vivo* [^{14}C]Lovastatin Uptake into the Rat Brain

The time course of [^{14}C]lovastatin uptake at 37°C into the rat brain is illustrated in Fig. 3. The data show that the *in vivo* uptake of [^{14}C]lovastatin was linear over a 30-sec perfusion period, and the value of uptake extrapolated to zero time was close to zero. The permeability coefficients were evaluated from the uptake in the 30-sec perfusion period.

In Vivo Cerebrovascular Permeability Coefficient (P_{vivo}) in Rat

The apparent cerebrovascular permeability coefficients, $P_{app,vivo}$, for [^{14}C]HMG-CoA reductase inhibitors and [^{14}C]sucrose are shown in Table I. The highest permeability coefficient compared to that of [^{14}C]sucrose, an extracellular space marker, was observed for [^{14}C]lovastatin, followed by [^{14}C]simvastatin, [^{14}C]simvastatin acid, and [^{14}C]lovastatin acid, in that order. No significant difference of permeability coefficient was observed between [^{14}C]pravastatin and [^{14}C]sucrose.

Time Course of Permeability Through Monolayers of BCEC

The time courses of apparent permeability through monolayers of BCEC for [^{14}C]HMG-CoA reductase inhibitors and [^{14}C]sucrose are illustrated in Fig. 4. The slope, i.e., total permeability coefficient (P_{tot}), of [^{14}C]lovastatin was greatest among [^{14}C]HMG-CoA reductase inhibitors and [^{14}C]sucrose. Among the acid forms, the slope was in the order of [^{14}C]lovastatin acid > [^{14}C]simvastatin acid and the slope of [^{14}C]pravastatin was close to that of [^{14}C]sucrose. Regarding the reproducibility of the results, the [^{14}C]sucrose permeability coefficient was essentially constant among cultured monolayers (10–12 days old) from different preparations of BCEC (mean ± SE; n = 9; 1.29 ± 0.083 μL/min/cm^2).

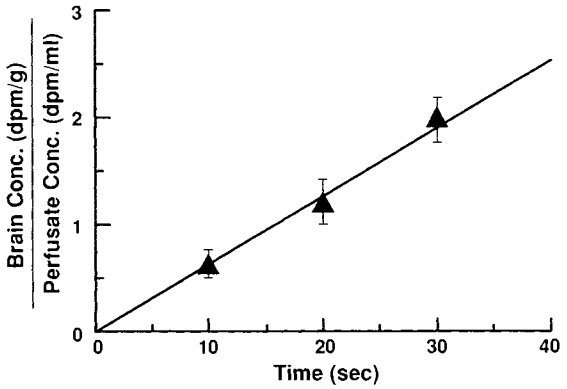

Fig. 3. Time course of [^{14}C]lovastatin uptake into the rat brain determined by the *in situ* brain perfusion method. Uptake of [^{14}C]-lovastatin was measured at 37°C by infusing the rat brain in perfusion medium (pH 7.4) containing 142 m*M* NaCl, 4.2 m*M* KH_2PO_4, 1 m*M* $MgSO_4$, 1.7 m*M* $CaSO_4$, 6 m*M* D-glucose, 0.01% BSA, 0.2% DMSO, and 28 m*M* $NaHCO_3$. The perfusion rate was 4.98 mL/min. Each point represents the mean ± SE of five experiments.

Transcellular Permeability Coefficient Through Monolayers of BCEC

The apparent permeability coefficients of [^{14}C]HMG-CoA reductase inhibitors, P_{tot}, and that of [^{14}C]sucrose, $P_{tot,pm}$, were estimated from the slopes in Fig. 4. The permeability coefficients through the collagen/fibronectin-coated membrane for [^{14}C]HMG-CoA reductase inhibitors, $P_{filt,app}$, and that for [^{14}C]sucrose, $P_{filt,app,pm}$, were also determined. Using these values of P_{tot}, $P_{tot,pm}$, $P_{filt,app}$, and $P_{filt,app,pm}$ and Eqs. (2)–(4), the transcellular permeability coefficients, $P_{trans,vitro}$, were estimated for [^{14}C]HMG-CoA reductase inhibitors and are shown in Table II. The $P_{trans,vitro}$ of [^{14}C]lovastatin was greatest among [^{14}C]HMG-CoA reductase inhibitors, while that of [^{14}C]pravastatin was smallest.

Drug Accumulation in the BCEC Monolayers During the *in Vitro* BCEC Transport Study

The accumulations of [^{14}C]HMG-CoA reductase inhibitors and [^{14}C]sucrose in the BCEC monolayer during the *in vitro* BCEC transport study are shown in Table III. The accumulation of [^{14}C]simvastatin was greatest among [^{14}C]HMG-CoA reductase inhibitors and [^{14}C]sucrose. That of [^{14}C]pravastatin was smallest among them, with no significant difference between the values observed for [^{14}C]-pravastatin and [^{14}C]sucrose.

Comparison of *in Vivo* and *in Vitro* Transendothelial Permeability Coefficient

Figure 5 shows the relation between transendothelial permeability coefficients of [^{14}C]HMG-CoA reductase inhibitors obtained in the *in vivo* and *in vitro* studies. The *in vivo* transendothelial permeability coefficient ($P_{trans,vivo}$) was obtained from the apparent cerebrovascular permeability coefficients of [^{14}C]HMG-CoA reductase inhibitors corrected for the extracellular amount estimated from the permeability coefficient of [^{14}C]sucrose. Fairly good coincidence between *in vivo* and *in vitro* permeabilities was observed for [^{14}C]lovastatin acid. The value of $P_{trans,vivo}$ observed for [^{14}C]pravastatin was relatively smaller than the *in vitro* value, while for the other [^{14}C]HMG-CoA reductase inhibitors in *in vitro* values were smaller than the *in vivo* values.

DISCUSSION

It has been proposed that CNS side effects of drugs, such as β-blockers, are a consequence of transport of the drugs into the brain (13,14). However, the relation between CNS side effects and the BBB permeability to drugs remains unclear. In the present study we have demonstrated that the

Table I. The Cerebrovascular Permeability Coefficients Determined by the Brain Perfusion Method for [^{14}C]HMG-CoA Reductase Inhibitors and [^{14}C]Sucrose in Rats

Compound	Permeability coefficient (μL/min/cm^2)[a] $P_{app,vivo}$	$P_{trans,vivo}$[b]	PC[c] (pH 7.0)	n
[^{14}C]Sucrose	0.175 ± 0.011	—	—	5
[^{14}C]Pravastatin	0.178 ± 0.021	0.00322 ± 0.024	0.59	5
[^{14}C]Lovastatin acid	0.442 ± 0.024*	0.268 ± 0.024*	50.0	5
[^{14}C]Simvastatin acid	0.653 ± 0.058*	0.478 ± 0.058*	115	5
[^{14}C]Simvastatin	27.7 ± 3.9*	27.5 ± 3.9*	48,400	5
[^{14}C]Lovastatin	57.9 ± 9.6*	57.7 ± 9.6*	18,700	5

[a] The permeability coefficient of each [^{14}C]substrate was measured at 37°C for 30 sec by infusing the rat brain with perfusion medium (pH 7.4) containing 142 m*M* NaCl, 4.2 m*M* KH_2PO_4, 1 m*M* $MgSO_4$, 1.7 m*M* $CaSO_4$, 6 m*M* D-glucose, 0.01% BSA, 0.2% DMSO, and 28 m*M* $NaHCO_3$. The perfusion rate was 4.98 mL/min. Each value represents the mean ± SE.

[b] The *in vivo* transendothelial permeability coefficient obtained after correction for the extracellular amount of [^{14}C]HMG-CoA reductase inhibitors estimated from the permeability coefficient of [^{14}C]sucrose.

[c] Octanol/buffer partition coefficients (PC) were obtained from Ref. 16.

* Significantly different from the pravastatin value by Student's t test ($P < 0.001$).

CNS side effects of HMG-CoA reductase inhibitors are correlated with their ability to permeate through the BBB.

The *in vivo* uptake of [^{14}C]lovastatin by the rat brain increased linearly with brain perfusion time and the extrapolated value was close to zero at zero time (Fig. 3). The results suggest no significant adsorption of [^{14}C]lovastatin on the surface of the BCEC and no back-flux of [^{14}C]lovastatin from the brain to the blood. Therefore, the values shown in Table I reflect unidirectional permeability coefficients from the blood to the brain.

The apparent *in vivo* cerebrovascular permeability coefficients ranged from 0.175 to 57.9 μL/min/cm^2 (Table I). The highly lipophilic inhibitors, [^{14}C]lovastatin and [^{14}C]simvastatin, show high permeability coefficients. On the other hand, the hydrophilic inhibitor, [^{14}C]pravastatin, shows a low permeability coefficient. Moreover, no significant difference in the apparent cerebrovascular permeability coefficient was observed between [^{14}C]pravastatin and [^{14}C]sucrose. These results suggest that lovastatin and simvastatin penetrate into the brain, while pravastatin does not. Interestingly, the values of [^{14}C]simvastatin acid and [^{14}C]lovastatin acid, the open-ring forms, were significantly greater than that of [^{14}C]pravastatin. This result suggests that simvastatin acid and lovastatin acid also penetrate into the

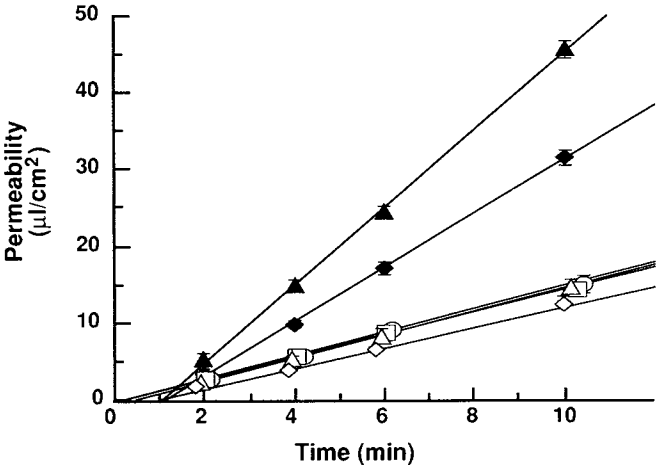

Fig. 4. Time courses of apparent permeability through a BCEC monolayer for [^{14}C]HMG-CoA reductase inhibitors and [^{14}C]sucrose. Transport was initiated by adding incubation solution (pH 7.4) containing 141 m*M* NaCl, 4 m*M* KCl, 2.8 m*M* $CaCl_2$, 0.5 m*M* $MgSO_4$, 1.72 m*M* NaH_2PO_4, 10 m*M* D-glucose, 10 m*M* HEPES, 0.01% BSA, and 0.2% DMSO to the donor chamber. At designated times, a sample was taken from the receiver chamber, and the radioactivity permeated from the donor to the receiver chamber was converted into the volume permeated from the donor chamber to the receiver chamber. The average permeability was plotted vs time, and the slope (equal to the permeability coefficient) was estimated by a linear regression analysis. [^{14}C]Lovastatin (▲), [^{14}C]simvastatin (◆), [^{14}C]simvastatin acid (◇), [^{14}C]lovastatin acid (△), [^{14}C]-pravastatin (□), and [^{14}C]sucrose (○). Each point represents the mean ± SE of four or five experiments.

Table II. Permeability Coefficients Through Cultured Monolayers of BCEC for [^{14}C]HMG-CoA Reductase Inhibitors

Compound	Permeability coefficient $P_{trans,vitro}$ (μL/min/cm^2)[a]
[^{14}C]Pravastatin	0.0755 ± 0.15
[^{14}C]Simvastatin acid	0.193 ± 0.064
[^{14}C]Lovastatin acid	0.420 ± 0.16
[^{14}C]Simvastatin	4.76 ± 0.32
[^{14}C]Lovastatin	8.32 ± 0.47

[a] Incubation solution containing each ^{14}C-substrate was added to the donor side chamber of the Transwell. Methods for determination and calculation of the permeability coefficient across the cultured monolayers of BCEC are described under Materials and Methods. Each value was estimated from the results for four or five Transwells containing cultured monolayers of BCEC and four or five Transwells alone.

Table III. The Accumulation in Cultured Monolayers of BCEC During Transendothelial Transport Study of [^{14}C]HMG-CoA Reductase Inhibitors and [^{14}C]Sucrose

Compound	Cell/medium ratio (μL/mg protein)[a]
[^{14}C]Sucrose	2.41 ± 0.59
[^{14}C]Pravastatin	1.33 ± 0.63
[^{14}C]Lovastatin acid	26.4 ± 3.0
[^{14}C]Simvastatin acid	53.5 ± 6.0
[^{14}C]Lovastatin	290 ± 12
[^{14}C]Simvastatin	522 ± 61

[a] Accumulation of each ^{14}C-substrate was measured at the end of the 10-min transcellular transport study. The cells on the polycarbonate membrane of the Transwell were solubilized and the radioactivity was measured in a liquid scintillation counter. Each value represents the mean ± SE of four or five experiments.

brain. Pravastatin and lovastatin acid differ only at the 6 position of the decalin system (Fig. 1). Thus, significant differences in permeability through the BBB among these acid-form analogues, pravastatin, lovastatin acid, and simvastatin acid, arise from minor structural differences.

Although the cerebrovascular permeability coefficient determined by the brain perfusion technique does reflect the ability of the drug to pass from the blood to the brain, the value obtained is not the BBB permeability itself. To estimate the BBB permeability to HMG-CoA reductase inhibitors, we employed primary cultured monolayers of BCEC on Transwell. However, when using cultured monolayers of BCEC, there can be a problem with leakiness of endothelial tight junctions (15). The *in vitro* paracellular permeability coefficient is not negligible because of the leakiness of the tight junctions compared with that *in vivo*. Therefore, the permeability coefficient across the BCEC monolayer should be corrected for the paracellular permeability coefficient measured with an extracellular marker such as sucrose. The corrected permeability coefficient, obtained by subtracting the transcellular permeability coefficient ($P_{trans,vitro}$), gives the net BBB permeability.

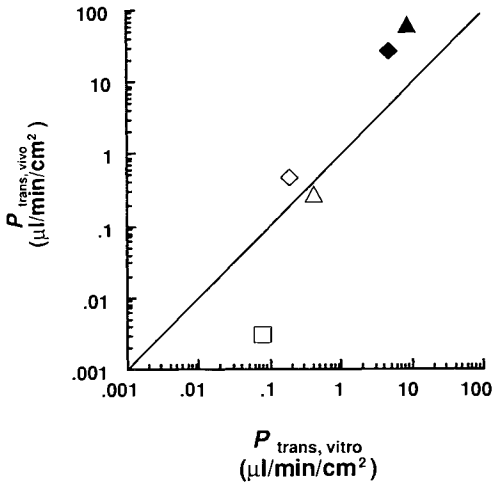

Fig. 5. Comparison of *in vivo* and *in vitro* permeability coefficients at the BBB. The values on the horizontal axis are taken from Table II, and the values on the vertical axis are taken from Table I. [^{14}C]Lovastatin (▲), [^{14}C]simvastatin (◆), [^{14}C]simvastatin acid (◇), [^{14}C]lovastatin acid (△), and [^{14}C]pravastatin (□). The line represents a 1:1 relationship.

[^{14}C]Lovastatin shows the greatest transcellular permeability coefficient among the HMG-CoA reductase inhibitors examined, and [^{14}C]pravastatin shows the smallest value (Table II). The difference of transcellular permeability coefficients was 110-fold and the accumulation in BCEC again depends on the lipophilicity of the HMG-CoA reductase inhibitors (Table III). However, simvastatin is more lipophilic than lovastatin (Table I), but the BBB permeability of [^{14}C]simvastatin is lower than that of [^{14}C]lovastatin (Tables I and II). The intracellular accumulation data (Table III) suggest that the discrepancy between lipophilicity and transcellular permeability could arise from the efflux rate of simvastatin at the abluminal side of BCEC being smaller than that of lovastatin.

Significant accumulation in the BCEC was observed for [^{14}C]simvastatin acid and [^{14}C]lovastatin acid. These results support the *in vivo* evidence of significant uptake of [^{14}C]simvastatin acid and [^{14}C]lovastatin acid into the rat brain. These accumulations might be relevant to the CNS side effects.

Finally, the *in vivo* and *in vitro* transcellular permeabilities agreed well for the HMG-CoA reductase inhibitors tested (Fig. 5). These results suggest that the *in vitro* transcellular permeability coefficient reflects the *in vivo* BBB permeability and that primary cultured bovine brain capillary endothelial cells are suitable for characterizing the BBB permeability to various drugs. The design of hydrophilic peripherally acting drugs with restricted BBB permeability appears to be an effective strategy for minimizing CNS side effects.

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