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Potentiation of Domperidone-Induced Catalepsy by a P-glycoprotein Inhibitor, Cyclosporin A

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ABSTRACT: The distribution of domperidone (DOM), a peripheral dopamine D_2 receptor antagonist, to the brain is restricted by P-glycoprotein (P-gp) at the blood–brain barrier (BBB) and for this reason, DOM rarely causes parkinsonian symptoms, such as extrapyramidal side effects (EPS), unlike other dopamine D_2 antagonists. In this study, we aimed to investigate whether cyclosporin A (CsA), a P-gp inhibitor, potentiates EPS induced by DOM.

The intensity of EPS was assessed in terms of the duration of catalepsy in mice. D_1, D_2 and mACh receptor occupancies at the striatum were measured *in vivo* and *in vitro*. Moreover, the distribution of DOM to the brain was investigated by using an *in situ* brain perfusion technique. The intensity of DOM-induced catalepsy was significantly potentiated by the coadministration of CsA. The *in vivo* occupancies of D_1, D_2 and mACh receptors, as well as the brain distribution of DOM, were increased by CsA. These results suggest that CsA increases the brain distribution of DOM by inhibiting P-gp at the BBB, and potentiates catalepsy by increasing the occupancies of the D_1 and D_2 receptors. The risk of DOM-induced parkinsonism may be enhanced by the coadministration of CsA. Copyright © 2003 John Wiley & Sons, Ltd.

Key words: domperidone; cyclosporin A; catalepsy; dopamine receptor; receptor occupancies

Introduction

Parkinsonian symptoms, such as extrapyramidal side effects (EPS), are a major drawback in the use of antipsychotic drugs and are caused by blockade of dopamine D_1 and D_2 receptors in the striatum. The intensity of EPS correlates well with the extent of D_2 receptor occupancy assessed by positron emission tomography (PET) in humans [1,2]. On the other hand, a selective D_1 receptor antagonist, SCH23390, was also reported to induce EPS in humans [3]. Therefore, the extent of D_1 and D_2 receptor blockade is the determining factor for EPS.

Catalepsy is widely used as a model to assess the risk of drug-induced EPS [4]. Indeed, catalepsy is induced by the blockade of dopamine D_1 and D_2 receptors and attenuated by the blockade of mACh receptor in the same manner as drug-induced parkinsonism [4,5]. We have shown in mice that the intensity of catalepsy can be quantitatively predicted by the occupancies of D_1, D_2 and mACh receptors in the striatum [6].

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Drug-induced parkinsonism is induced not only by antipsychotic drugs, but also by anti-dopaminergic prokinetic drugs such as metoclopramide and sulpiride [7]. Domperidone (DOM), another prokinetic drug, is also a potent dopamine D_2 antagonist with the K_i value of 0.86 nM [8,9]. However, DOM scarcely causes drug-induced parkinsonism, unlike metoclopramide and sulpiride, because the transport of DOM across the blood–brain barrier (BBB) is quite limited despite DOM's high lipophilicity [10,11]. This may be a consequence of efflux transport via P-glycoprotein (P-gp) at the BBB, since DOM is a substrate of P-gp [12], and the brain distribution of DOM is increased by a P-gp inhibitor, verapamil [13]. Further, DOM has been reported to induce neuroleptic-like reactions (lack of spontaneous movement and crouching posture) in *mdr1a* gene knockout mice, which lack P-gp [12]. Moreover, it has been reported in humans that quinidine, a P-gp inhibitor, evoked a central nervous adverse reaction to loperamide, a P-gp substrate [14].

Therefore, it is quite feasible that DOM may also produce central nervous side effects when a P-gp inhibitor is concomitantly administered. CsA is a well-known potent P-gp inhibitor [15,16]. Therefore, coadministration of CsA may enhance the brain distribution of DOM, resulting in DOM-induced parkinsonism.

In this study, we aimed to investigate the effect of CsA on DOM-induced catalepsy in mice. We also investigated the effects of CsA on the *in vivo* and *in vitro* D_1, D_2 and mACh receptor occupancies in the striatum, and the brain distribution of DOM.

Experimental

Animals

Male ddY mice (24–32 g) were purchased from Nippon Medical Science Animal and Supplement Laboratory Co., Ltd. (Tokyo, Japan) or Seac Yoshitomi, Ltd. (Fukuoka, Japan). Animals had free access to food (Solid Feed MF, Oriental Yeast, Tokyo, Japan) and water.

Reagents

DOM, CsA and haloperidol were kind gifts from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan), Novartis Pharma K., K. (Basle, Switzerland) and Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan), respectively. Atropine sulfate and (R)-(+)-SCH23390 hydrochloride were purchased from Wako Pure Chemical (Osaka, Japan) and Funakoshi (Tokyo, Japan), respectively. Propranolol was provided by AstraZeneca (Osaka, Japan).

^{3}H-SCH23390 (89.0 Ci/mmol) and ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) (48.0 Ci/mmol) were obtained from Amersham International (Buckinghamshire, UK). ^{3}H-Raclopride (78.4 Ci/mmol) and ^{131}I-human serum albumin were obtained from NEN Research Products Co., Ltd. (Boston, MA) and Daiichi Radioisotope Laboratory Co. (Tokyo, Japan), respectively. All other chemicals used in the experiments were of analytical grade.

Preparation of drug solution

DOM was dissolved in 3% lactic acid and diluted with 5% D-glucose. CsA was dissolved in Cremophor EL (650 mg/ml) and ethanol. Haloperidol was dissolved in 0.3% tartaric acid and diluted with saline. Atropine sulfate was dissolved in saline.

Measurement of catalepsy

The test drug (DOM; 10, 20, 60 or 80 mg/kg, haloperidol; 0.5 mg/kg) was administered intraperitoneally. Haloperidol was employed as a positive control. CsA (100 mg/kg) was orally given at 120 min before the administration of DOM. Control animals were given the vehicle under the same conditions.

Catalepsy was assessed by means of the bar method at 30, 90, 180 and 270 min after the administration of the test drug [17]. If the duration of catalepsy exceeded 360 s, it was evaluated as 360 s [18].

Measurement of in vivo receptor occupancies

In vivo receptor occupancies were measured according to the method previously reported by us [6]. ^{3}H-SCH23390, ^{3}H-raclopride and ^{3}H-QNB (3 μCi/body) were used as receptor-specific radioligands for D_1, D_2 and mACh receptors, respectively.

DOM (60 mg/kg) was administered intraperitoneally at 120 min after oral administration of CsA (100 mg/kg) or vehicle. Haloperidol was intraperitoneally administered at a dose of 0.5 mg/kg. Receptor-specific radioligands (3 μCi/body) were administered intravenously at 1 75 min after administration of DOM or haloperidol, and the mice were decapitated at 10 min thereafter. The striatum and cerebellum were immediately dissected. Each sample was weighed, added to 1 ml of Solvable (Packard Bioscience B.V., Groningen, Netherlands) and incubated at 50°C until solubilized. Then 0.2 ml of 30% H_2O_2 was added, the vial was left to cool at ambient temperature for 60 min, and 10 ml of scintillation cocktail ATOMLIGHT (Packard Bioscience B.V., Groningen, Netherlands) was added. The radioactivity was measured with a liquid scintillation counter (LSC-3100, Aloka, Tokyo).

To measure the nonspecific binding of ^{3}H-QNB, atropine sulfate (50 ml/kg) was administered subcutaneously at 25 min before the administration of ^{3}H-QNB. The mice were decapitated at 10 min after administration of ^{3}H-QNB and submitted to the above procedure.

D_1 or D_2 receptor occupancy (Φ_D) was calculated according to the following equation:

$$\Phi_D = \left(1 - \frac{A-1}{B-1}\right) \times 100 \quad (1)$$

where A and B are the radioactivity ratios (striatum/cerebellum) in the presence and absence of drugs, respectively. The cerebellum was utilized as a dopamine receptor-free region to estimate nonspecific binding of ligands.

On the other hand, mACh receptor occupancy (Φ_{mACh}) was calculated according to the following equation:

$$\Phi_{mACh} = \left(1 - \frac{A-C}{B-C}\right) \times 100 \quad (2)$$

where A, B are the radioactivity ratios (striatum/cerebellum) in the presence and absence of drugs, respectively, and C is the nonspecific binding that was determined as described above.

In vitro receptor binding study

Preparation of the membrane sample was performed as described previously [6]. The striatum was rapidly dissected from mice, and homogenate of striatal tissue was prepared in 100 volumes (w/v) of ice-cold 50 mM Tris-HCl buffer (pH 7.4) with a Teflon-on-glass tissue homogenizer. The homogenate was centrifuged (20, 000g, 10 min, 4°C), resuspended in ice-cold 5 0 mM Tris–HCl buffer (pH 7.4), and centrifuged again. The final pellet was resuspended in 200 volumes (w/v) of the buffer for dopamine D_1 and D_2 receptors or in 300 volumes for mACh receptor.

For D_1 and D_2 receptors, aliquots of the membrane preparations were incubated with each drug and 1 nM ^{3}H-SCH23390 (for D_1 receptor) or ^{3}H-raclopride (for D_2 receptor) for 15 min at 37°C in the incubation buffer (pH 7.4) (50 mM Tris–HCl buffer containing the following salts: NaCl 120 mM, KCl 5 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM). For mACh receptor, aliquots of the membrane preparations were incubated with each drug and 0.2 nM ^{3}H-QNB for 30 min at 37°C in the incubation buffer. The final tissue concentrations were 1 mg of original wet weight tissue per 1 ml for D_1 or D_2 receptors, and 2 mg/3 ml for mACh receptor.

The reaction was terminated by rapid pouring of 700 μl of the contents over Whatman GF/C glass filters (Whatman Corporation, Maidstone, UK) under vacuum. The filters were rinsed twice with 3 ml of ice-cold incubation buffer and transferred into scintillation vials. After addition of 8 ml of ATOMLIGHT, radioactivity was measured with a liquid scintillation counter.

Nonspecific binding was measured in the presence of 10 μM SCH23390, 10 μM haloperidol and 1 μM atropine sulfate for D_1, D_2 and mACh receptors, respectively.

Pharmacokinetics of DOM and CsA

DOM (60 mg/kg) was administered intraperitoneally to mice at 120 min after the oral administration of CsA (100 mg/kg) or vehicle. The mice were decapitated at 30, 90, 180 or 270 min after the administration of DOM. The blood was collected and rapidly centrifuged (2000g, 5 min) to obtain plasma. The brain was excised, washed with ice-cold saline and weighed. The samples were stored at −20°C until analysis.

The concentrations of DOM in plasma and brain were measured by high-performance liquid chromatography (HPLC) with fluorescence detection by the reported method [19] with minor modifications. To 50 μl of each plasma sample, 10 μl of propranolol (3 μg/ml) as an internal standard and 200 μl of methanol were added. The mixture was shaken with a vortex mixer for 60 s and centrifuged at 13,000*g*, 4°C for 10 min. Then 50 μl of the supernatant was applied to the HPLC system. Homogenates of brain samples were prepared in 9 volumes (w/v) of ice-cold methanol with ultrasound. To 1 ml of each homogenate, 10 μl of propranolol (3 μg/ml) was added. The mixture was shaken with a vortex mixer for 60 s and centrifuged at 13,000*g*, 4°C for 10 min. The supernatant (600 μl) was transferred to another tube and evaporated to dryness under a nitrogen stream. The dried residue was dissolved in 100 μl of mobile phase and further centrifuged at 13000*g*, 4°C for 2 min. Then 25 μl of the supernatant was applied to the HPLC system.

The HPLC system consisted of a pump (LC-10AD; Shimadzu, Kyoto, Japan), a system controller (SCL-10A; Shimadzu), a degasser (DGU-4A; Shimadzu), an autoinjector (SIL-10AXL; Shimadzu), and a spectrofluorometric detector (RF-10A; Shimadzu), which was set at 282 nm for excitation and 328 nm for emission. The separation was performed with a reversed-phase column (YMC PACK ODC AP-313, 250 mm × 6 mm ID, 5 μm particle size; YMC, Tokyo, Japan), which was maintained at 25°C by a column oven (CTO-10 AC; Shimadzu). A guard column C-KGC-324APC-3 (23 mm × 4.0 mm ID, 5 μm particle size, YMC) was attached before the analytical column. The mobile phase consisted of 0.02 M phosphate buffer (pH 3.5) and methanol (60 : 40, v/v) and was pumped at a rate of 1.5 ml/min. The detection limit was 0.1 μg/ml and 0.1 μg/g in plasma and brain, respectively.

Concentration of CsA in the blood was measured with a fluorescence polarization immunoassay (FPIA) system (Dainabot, Osaka, Japan) according to the manufacturer's instructions. Homogenates of brain samples were prepared in 9 volumes (w/v) of ice-cold methanol with ultrasound. The homogenate was centrifuged at 13,000*g* for 4 min. The supernatant was applied to the FPIA system.

In situ brain perfusion

The right cerebral hemisphere of a mouse was perfused according to the method previously reported by us [20]. Krebs–Henseleit buffer, which consists of 118.0 mM NaCl, 4 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$ and 2.5 mM $CaCl_2$, was used as the perfusion fluid. Before the experiment, 10 mM glucose, 10 mg/ml DOM and 2.5 mM CsA were added to the perfusate, and oxygenated with 95% O_2–5% CO_2. The perfusate was adjusted to pH 7.4 with 1 M HCl and equilibrated at 37°C in a water bath.

Perfusion fluid was infused retrogradely into the external carotid artery at a constant rate of 1.0 ml/min [20] by a pump (55–1111, Harvard Apparatus, Natick, MA). The perfusion was continued for 35 s (including 5 s for to reach the brain) and terminated by decapitation of the mouse. The brain was removed from the skull. The brain concentration of DOM was measured as described above.

Measurement of brain capillary volume

Brain capillary volume was measured by using ^{131}I-human serum albumin, which was purified with an Amicon Centrifree (Millipore Corporation, Bedford, MA) until free iodine fell below 0.3%. ^{131}I-Human serum albumin (127 kBq/Body) was injected into the tail vein, and the mouse was decapitated at 2 min after the injection. Each sample was weighed, and the radioactivity was measured with a γ-counter. The vascular volume was calculated from the radioactivity ratio (brain radioactivity per unit weight/blood radioactivity per unit volume).

Measurement of the blood/plasma distribution of DOM

Mouse blood (390 μl) was incubated at 37°C for 5 min, then 10 μl of DOM solution was added and the mixture was further incubated for 10 min. The sample was centrifuged at 13,000*g*, 37°C for 10 min to obtain plasma, and

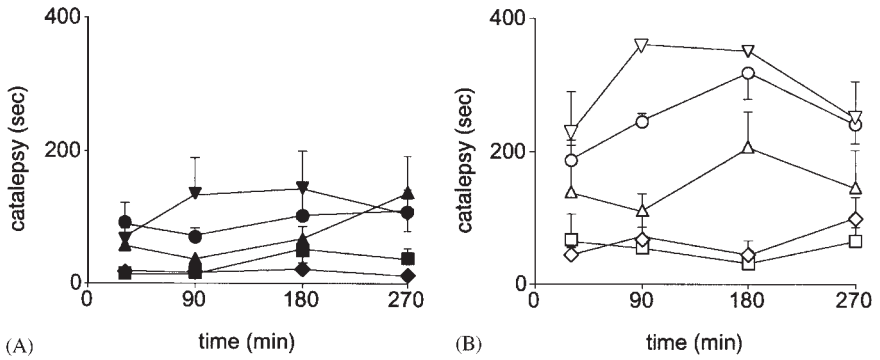

Figure 1. Time courses of catalepsy induced by DOM. The amplitude of catalepsy was assessed by the bar method [17] at 30, 90, 180 and 270 min after administration of DOM without (A; solid mark) or with CsA (B; opened mark). DOM (0 (◇), 10 (□), 20 (△), 60 (○) or 80 (▽) mg/kg i.p.) was administered and CsA (100 mg/kg p.o.) was given at 120 min before the administration of DOM. Control animals (A) were given only the vehicle for CsA. Each point indicates the mean ± SEM ($n = 5$–6)

the concentration of DOM was measured as above.

Correction of brain capillary volume

Brain concentration of DOM assessed by HPLC was corrected by applying Equation (3) to obtain the real concentration in the brain (C^*_{brain}):

$$C^*_{brain} = \frac{C_{brain} - rRBC_p}{1 - r} \quad (3)$$

where C_{brain} is the concentration of DOM in the brain assessed by HPLC (μg/g brain), r is the brain capillary volume (ml/g brain), RB is the ratio of concentration in blood and plasma and C_p is the concentration of DOM in plasma (μg/ml). Likewise, the real concentration of CsA was calculated as follows:

$$C^*_{brain} = \frac{C_{brain} - rC_b}{1 - r} \quad (4)$$

where C^*_{brain} is the real concentration of CsA in the brain (μg/g brain), C_{brain} is the concentration of CsA in the brain assessed by HPLC (μg/g brain), r is the brain capillary volume (ml/g brain) and C_b is the concentration of CsA in blood (μg/ml).

Statistical analysis

Statistical analysis was performed with the Mann–Whitney U-test. A p value of less than 0.05 was considered statistically significant.

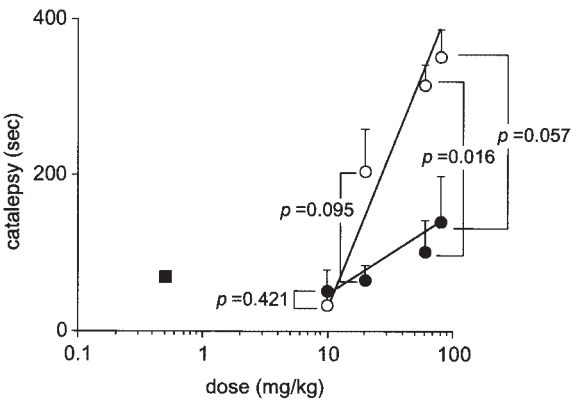

Figure 2. Dose-dependency of catalepsy at 180 min after intraperitoneal administration of DOM or haloperidol. DOM (10, 20, 60 or 80 mg/kg i.p.) was administered without (●) or with (○) CsA (100 mg/kg p.o.). Haloperidol (0.5 mg/kg i.p.; ■) was used as a positive control. Each point indicates the mean ± SEM ($n = 5$–6). $^*p < 0.05$ compared to the control

Results and Discussion

Catalepsy induced by DOM

Figure 1 shows the time courses of DOM-induced catalepsy in mice in the presence and absence of CsA. DOM evoked catalepsy in a dose-dependent manner. The intensity of catalepsy reached maximum at 180 min after administration of DOM, and was enhanced by pretreatment with CsA.

Figure 2 presents the dose-response relationships of catalepsy at 180 min after administration

Table 1. *In vivo* dopamine D_1, D_2 and mACh receptor occupancies[a]

Drugs	Receptor occupancy (%)		
	D_1	D_2	mACh
DOM (60 mg/kg i.p.) +CsA vehicle (p.o.)	29.3 ± 10.7	76.1 ± 1.4	11.2 ± 5.9
	$p = 0.051$		
DOM (60 mg/kg i.p.) +CsA vehicle (100 mg/kg p.o.)	60.8 ± 3.2	84.1 ± 1.0	42.6 ± 7.6
haloperidol (0.5 mg/kg i.p.)	28.0 ± 7.5	61.6 ± 8.2	31.3 ± 15.7

[a] Each value is the mean ± SEM (n = 3–7). Statistical analysis was performed with the Mann–Whitney U-test.

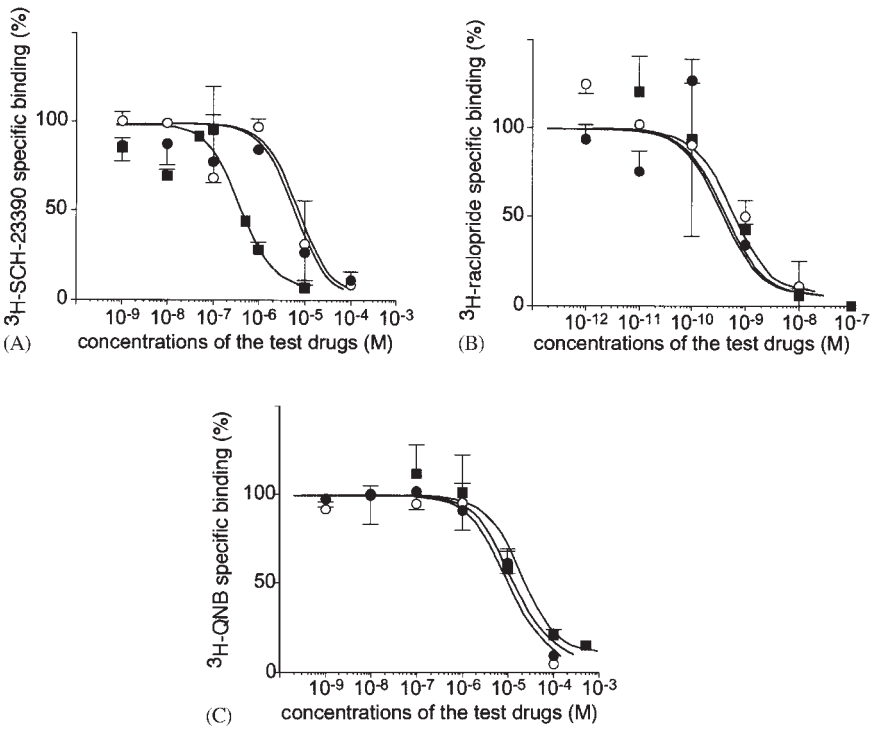

Figure 3. Inhibition curves for binding of ^{3}H-SCH23390 (for D_1 receptor) (A), ^{3}H-raclopride (for D_2 receptor) (B) and ^{3}H-QNB (for mACh receptor) (C). DOM was administered without (●) or with (○) CsA (100 mg/kg p.o.). Haloperidol (0.5 mg/kg i.p.; ■) was used as a positive control. The membrane samples were prepared according to the reported method [6]. Radioactivity was measured with a liquid scintillation counter. Each point represents the mean of three determinations ±SEM

of DOM in the presence and absence of CsA. CsA potentiated the catalepsy induced by DOM. The amount of DOM required to induce catalepsy for 100 s was 13.0 or 39.8 mg/kg in the presence or absence of CsA, respectively, indicating that CsA potentiated the extent of DOM-induced catalepsy by 3-fold. These results are consistent with the finding that DOM exhibited more potent beha-

Table 2. *In vitro* K_i values for D_1, D_2 and mACh receptor[a]

Drugs	K_i (nM)		
	D_1	D_2	mACh
DOM	825 ± 225	0.37 ± 0.10	3970 ± 351
DOM + CsA (0.5 μM)	1260 ± 334	0.52 ± 0.07	3850 ± 570
haloperidol	82 ± 8	0.46 ± 0.24	5200 ± 1472

[a] Each value is the mean ±SEM (n = 3).

Table 3. Receptor occupancies and comparison between the amplitudes of observed catalepsy and predicted catalepsy

Drugs	Receptor occupancy (%)			Catalepsy (sec)	
	D_1	D_2	mACh	Observed	Predicted
DOM (60 mg/kg i.p.) +CsA vehicle (p.o.)	29.3	76.1	11.2	102	106
DOM (60 mg/kg i.p.) +CsA vehicle (100 mg/kg p.o.)	60.8	84.1	42.6	> 317	121
haloperidol (0.5 mg/kg i.p.)	28.0	61.6	31.3	71	70

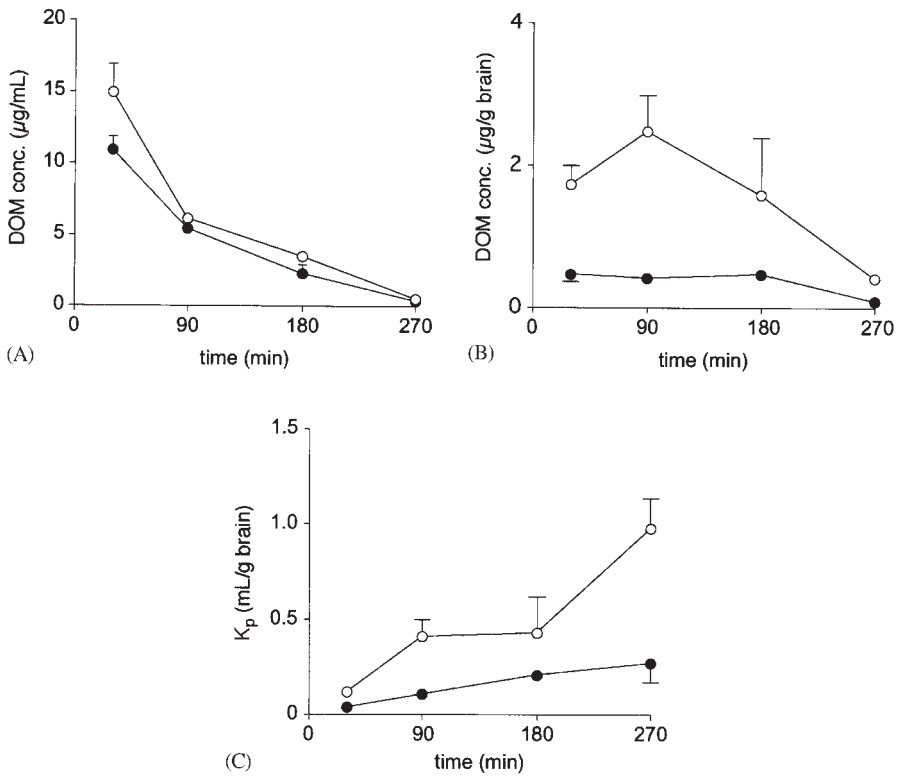

Figure 4. Time courses of plasma concentration (A), brain concentration (B) and K_p value (C) of DOM. DOM (60 mg/kg i.p.) was administered at 120 min after CsA (100 mg/kg p.o.; ○) or the vehicle for CsA (●). Plasma and brain concentrations were measured by the HPLC method. The K_p value represents the tissue-to-plasma concentration ratio. Each point indicates the mean ± SEM ($n = 3$–5)

vioral effects (crouched posture and lack of spontaneous movement) in *mdr1a* knockout mice than in wild-type mice [12].

Receptor occupancies

In vivo D_1, D_2 and mACh receptor occupancies are shown in Table 1. Both D_1 and D_2 receptor occupancies by DOM in the striatum were increased by coadministration of CsA.

The inhibition curves for the binding of receptor-selective radioligands to the striatal membranes by DOM alone, DOM with 0.5 μM CsA, and haloperidol are shown in Figure 3. The K_i values are listed in Table 2. The K_d value of each specific radioligand was taken from our

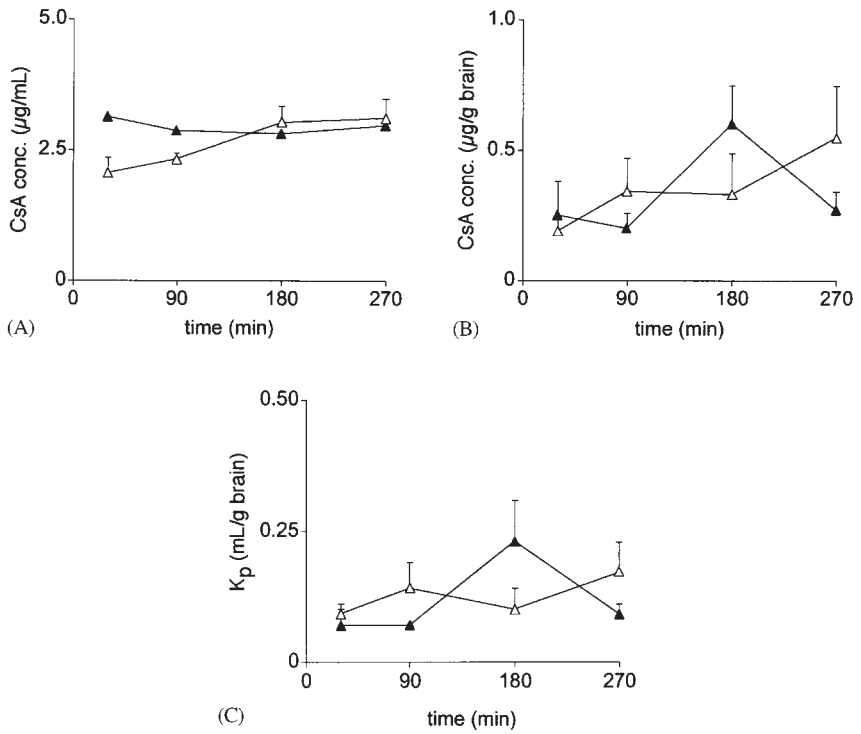

Figure 5. Time courses of blood concentration (A), brain concentration (B) and K_p value (C) of CsA. DOM (60 mg/kg i.p.; △) or the vehicle for DOM (▲) was administered at 120 min after the administration of CsA (100 mg/kg p.o.). The concentration of CsA was measured by fluorescence polarization immunoassay (FPIA). Each point indicates the mean ± SEM ($n = 3$–5)

previous report to calculate K_i values from IC_{50} values (^{3}H-SCH23390: 0.22 nM, ^{3}H-raclopride: 1.0 nM, ^{3}H-QNB: 0.075 nM) [21]. CsA did not affect the K_i values of DOM for D_1, D_2 and mACh receptors. Moreover, CsA did not bind to D_1, D_2 and mACh receptors over the range of 0.05–5 μM (data not shown). Therefore, the increase in receptor occupancies of DOM by CsA *in vivo* may not be attributable to direct effects of CsA on the receptors.

We have reported that the intensity of catalepsy in mice can be predicted from the occupancies of the D_1, D_2 and mACh receptors at the striatum based on a pharmacokinetic/pharmacodynamic model [6]. We applied this model to the present results to predict the intensity of catalepsy (Table 3). The predicted intensity of catalepsy after the administration of DOM or haloperidol was in agreement with the observed values. On the other hand, the intensity of catalepsy induced by DOM in the presence of CsA was higher than predicted by the model. This disagreement may be attributed to the properties of the model, in that the occupancy-response relationship is quite steep in the range of higher receptor occupancies.

Pharmacokinetics of DOM and CsA

The brain capillary volumes in the presence or absence of CsA were 0.012 ± 0.007 (ml/g tissue) and 0.011 ± 0.006 (ml/g tissue), respectively (mean ± SEM, $n = 3$) (Figure 6). As CsA did not affect the capillary volume, the mean value of the two groups (0.012 ml/g tissue) was used to assess the real brain concentration. Figure 4 presents the time courses of plasma concentration, brain concentration and K_p value (tissue-to-plasma concentration ratio) of DOM.

Brain concentration and K_p value were corrected for the brain capillary volume of 0.012 (ml/g tissue) and the blood/plasma distribution ratio of 0.978. The plasma concentration of DOM was slightly increased by coadministration of CsA. The brain concentration and K_p value of DOM were also increased by coadministration of CsA. Figure 5 shows the time courses of blood concentration, brain concentration and K_p value of CsA. The kinetics of CsA was not affected by coadministration of DOM.

These data suggest that the increase of receptor occupancies in the presence of CsA was caused by an increase in the brain concentration of DOM. Although the plasma concentration of DOM was slightly increased by CsA, the increase in the brain concentration was mainly attributed to the enhanced distribution of DOM to the brain (Figure 4). Therefore, increased brain distribution of DOM may be responsible for the potentiation of DOM-induced catalepsy by CsA.

In this study, blood CsA concentration ranged from 2.0 to 3.1 μM, which corresponds to the concentration used successfully in a clinical study to inhibit P-gp in humans in order to overcome multidrug resistance to VAD (vincristine, doxorubicin and dexamethasone) in he treatment of multiple myeloma (1.3 μM in blood) [22]. Therefore, the blood concentration of CsA in this study should be enough to inhibit P-gp. Accordingly, the increased brain distribution of DOM could well be due to the inhibition of P-gp at the BBB by CsA. In the clinical setting, the blood concentration of CsA at the end of intravenous infusion of CsA at a dose of 3.5 mg/kg has been reported to reach a peak of 0.63–1.94 μM in patients with renal failure [23]. Therefore, a clinically feasible concentration of CsA may inhibit P-gp, resulting in an increased brain distribution of DOM. We also confirmed by measuring the brain capillary volume that CsA did not affect the tight junctions. Thus, the elevated accumulation of DOM was not caused by disruption of the tight junctions at the BBB.

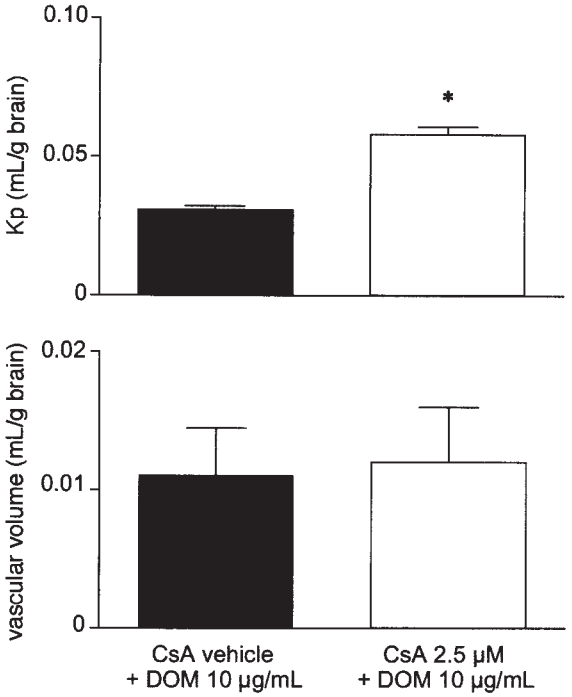

Figure 6. The effect of CsA on the brain distribution of DOM. Brain capillary volume was measured by using ^{131}I-human serum albumin. Mice ($n = 3$) were decapitated at 2 min after the injection of ^{131}I-human serum albumin into the tail vein. The vascular volume was calculated by use of the radioactivity ratio measured with a γ-counter. The brain distribution of DOM was investigated by using the *in situ* brain perfusion technique and measured by HPLC ($n = 4$). The K_p value was significantly increased by CsA ($^*p < 0.05$). Each column indicates the mean ± SEM

In situ brain perfusion

By using the brain perfusion technique, which is free of the effect of plasma protein binding and peripheral metabolism, we could investigate directly the changes of the BBB-permeability of DOM; the K_p values of DOM were obtained as 0.058 ± 0.013 (ml/g tissue) or 0.031 ± 0.007 (ml/g tissue), respectively, in the presence or absence of 2.5 μM CsA, (mean ± SEM.; $n = 4$) (Figure 6). Thus, the K_p value was significantly increased by 1.87-fold by concomitant perfusion of 2.5 μM CsA. Because the K_p value of DOM increased even after a short period of perfusion, a transient increase in the blood level of CsA after intravenous infusion may be sufficient to potentiate the brain distribution of DOM in the clinical setting.

In conclusion, CsA increased the brain distribution of DOM by inhibiting P-gp at the BBB. The elevation of the brain concentration of DOM leads to an increase in the occupancies of the D_1

and D_2 receptors and results in the potentiation of catalepsy. P-gp inhibitors such as CsA may enhance the risk of DOM-induced parkinsonism in the clinical setting.

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