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FULL PAPER

Pharmacology

CAN a P-gp modulator assist in the control of methotrexate concentrations in the rat brain? –inhibitory effects of rhodamine 123, a specific substrate for P-gp, on methotrexate excretion from the rat brain and its optimal route of administration

Naofumi OGUSHI[1,2], Kazuaki SASAKI[1] and Minoru SHIMODA[1]*

[1] The Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan

[2] Ogushi Animal Hospital, 3-2556-5 Wakasa Tokorozawa-shi, Saitama 359-1151, Japan

ABSTRACT. Although methotrexate (MTX) is mainly transported by reduced folate carrier, P-gp and MRP1 may also be involved in its transport. In our previous study, a potent P-gp and MRP1 modulator, Cyclosporine A, potentiated MTX concentration in rat brain. Since it is important for MTX therapy for brain tumor to clarify which transporter is dominant, we herein determined whether the specific P-gp substrate, rhodamine123 (Rho123), potentiates the transport and retention of MTX in the brain. Rho123 was injected intravenously or intrathecally into rats immediately after injection of MTX. 6 or 12 hr after the MTX injection, brains were isolated just after the sampling of cerebrospinal fluid (CSF). Blood was also collected intermittently. MTX concentrations were determined in plasma, CSF and the brain using high-performance liquid chromatography with UV detection. When MTX was intravenously injected, Rho123 didn't affect MTX concentrations in the brain. However, Rho123 resulted in significantly higher MTX concentrations in the brain at 12 hr after injection when MTX was intrathecally injected. It is suggested that Rho123 inhibits the excretion of MTX from the brain, but does not potentiate its distribution from the blood into the brain. This reveals that P-gp can be one of the major transporters of MTX in rat brain. Therefore, treatments with P-gp modulators may contribute to intrathecal MTX therapy for brain tumor. Since plasma concentration-time curves of MTX were not affected by Rho123, treatments with P-gp modulators may not potentiate the adverse effects of MTX.

KEY WORDS: blood-brain barrier, intrathecal administration, methotrexate, p-glycoprotein, rhodamine 123

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P-glycoprotein (P-gp) and multidrug resistance-associated protein 1 (MRP1) are the major organic transporters responsible for the excretion of xenobiotics from the body. P-gp and MRP1 play important roles in the blood-brain barrier (BBB) and blood-Cerebrospinal fluid (CSF) barrier [6, 9, 10, 12, 21, 26–29, 31–33].

Although methotrexate (MTX) is one of the anticancer drugs prescribed for central nervous system (CNS) tumors, its entry into the CNS is strongly restricted because of its water solubility. In order to achieve effective concentrations in humans and animals, MTX has been administered intravenously at a high dose or intrathecally at a low dose. However, its effects have been limited [1, 15, 25].

A folate analog, MTX, is basically transported by folate transporters; reduced folate carrier (RFC) and proton-coupled folate transporter (PCFT). RFC and PCFT are also found at the blood-brain barrier [36].

MTX is not likely to be a substrate of P-gp or MRP1, because it does not share the following common characteristics of P-gp substrates: (1) a planar structure, (2) high lipophilicity and (3) neutral or positive charge. However, Roninson and his collaborators

*Correspondence to: Shimoda, M., The Department of Veterinary Medicine, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan. e-mail: ms@cc.tuat.ac.jp

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Table 1. Definition of administration groups

Group	MTX	Rho123
Miv	i.v.	
Mit	i.t.	
Miv+Riv	i.v.	i.v.
Mit+Riv	i.t.	i.v.
Mit+Rit	i.t.	i.t.

This table shows 5 administration groups defined by the combinations of the drugs and their administration routes as follows. Miv: MTX (i.v.) +saline (i.t.), Mit: MTX (i.t.), Miv+Riv: MTX (i.v.) +Rho123 (i.v.) +saline (i.t.), Mit+Riv: MTX (i.t.) +Rho123 (i.v.), Mit+Rit: MTX (i.t.) +Rho123 (i.t.). Each value is the mean ± S.D. (n=5). The doses of MTX and Rho123 administered were 2 and 0.2 mg/body, respectively.

reported that MTX may be a substrate for P-gp and MRP1 in RFC deficient cells *in vitro* [8, 22, 35].

We previously demonstrated that cyclosporine A (CysA) potentiated the distribution of intrathecally administered MTX into the rat brain [23]. This resulted from that MTX transport to the brain was inhibited by CysA, which is a potent P-gp and MRP1 modulator [13, 29]. It is, therefore, suggested that MTX is transported by P-gp or MRP1. In the present study, we examined effects of co-medicated rhodamine123 (Rho123), a specific P-gp substrate, on distribution of MTX into brain using different combinations of administration routes, in order to clarify the main transporter of MTX in blood-brain barrior.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (9 weeks old, weighing between 286 and 326 g) were obtained from CLEA Japan Inc. (Tokyo, Japan) and utilized in all experiments. Male Sprague-Dawley rats were maintained under a 12:12-hr light-dark cycle and had free access to food and water prior to experimentation. Experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and approved by the Animal Experiment Committee, Tokyo University of Agriculture and Technology.

Chemicals

MTX and its polyglutamates were purchased from Schircks Laboratories (Jona, Switzerland). MTX solution was prepared at 20 mg/m*l* by diluting a commercially available injectable formulation (Methotrexate® Injection, Takeda Pharmaceutical Co., Ltd., Osaka, Japan) with sterilized saline. Rho123 was purchased as a hydrochloride salt (Wako Pure Chemicals, Osaka, Japan). Rho123 solution was prepared at 2 mg/m*l* by dissolving Rho123 in sterilized saline.

Drug administration and sampling protocol

All administration was conducted under anesthesia with pentobarbital (50 mg/kg, intraperitoneally). MTX (2 mg/body) and Rho123 (0.2 mg/body) were injected into animals via an intravenous (i.v.) or intrathecal (i.t.) route at the same time. In order to avoid increases in intracranial pressure, i.t. injections were performed after removing CSF as much as possible. We defined 5 groups as follows; group Miv: MTX (i.v.) +saline (i.t.), group Mit: MTX (i.t.), group Miv+Riv: MTX (i.v.) +Rho123 (i.v.) +saline (i.t.), group Mit+Riv: MTX (i.t.) +Rho123 (i.v.) and group Mit+Rit: MTX (i.t.) +Rho123 (i.t.). (Table 1)

In rich Rho123 and in poor Rho123; we compared MTX concentrations in brain at 6 hr with at 12 hr [30].

Blood (0.2 m*l*) was sampled from the caudal vein 1, 2, 3, 4, 5, 6, 9 and 12 hr after drug administration. In our preliminary study, the half-lives of intravenously and intrathecally administered MTX were 35 ± 3.2 and 84 ± 20 min, respectively.

Then, rats in each group were euthanized 6 or 12 hr after drug administration following the sampling of CSF (0.1 m*l*) from the cisterna, and the brain was collected (n=5, respectively). In order to prevent brains from contamination with MTX in blood as long as possible, the brains were isolated after removing whole blood from the bodies. The isolated brain was sagittally divided into two pieces at the median line for the analysis of MTX and Rho123 concentrations and stored at −80°C until used.

Sample preparation

One piece of the brain was homogenized with methanol (20 m*l*) to extract MTX and MTX polyglutamates. The homogenate was centrifuged at 3,000 × *g* for 20 min in order to separate the clear liquid layer and residue. The clear layer was evaporated, and the residue was mixed with 10 mM sodium phosphate buffer (pH 1.6, 50 m*l*) in order to dissolve the water-soluble polyglutamates of MTX. The mixture was then centrifuged at 3,000 × *g* for 20 min. The clear liquid layer obtained was mixed with the layer that was evaporated to dryness.

In order to purify and concentrate MTX and its polyglutamates, the mixture was subjected to solid phase extraction (Sep-Pak® Plus C18 cartridge, Waters Corporation, MI, U.S.A.). MTX and its polyglutamates were eluted with 2 m*l* of 50% methanol solution

(pH 7.0), and the elas then subjected to a HPLC analysis of MTX.

The other piece of the brain was homogenized with methanol (20 m*l*) to extract Rho123. The homogenate was centrifuged at 3,000 ×*g* for 20 min to obtain the supernatant. The supernatant was subjected to a HPLC analysis of Rho123.

Plasma and CSF samples (0.1 m*l*) were added $HClO_4$ (0.2 m*l*) to remove plasma proteins and were then centrifuged at 12,000 ×*g* for 2 min to divide the clear liquid layer (A) and residue (B). (A) was subjected to a HPLC analysis of MTX. (B) was mixed with acetonitrile (0.3 m*l*) to dissolve the remaining Rho123 and was then centrifuged at 12,000 ×*g* to obtain the supernatant (C). A mixture of the same volumes of (A) and (C) was subjected to a HPLC analysis of Rho123.

HPLC analysis

MTX and its polyglutamates were analyzed using a HPLC system. The mobile phase consisted of 10 mM phosphate buffer (pH 1.6) and acetonitrile (90:10, v/v), and the effluent was monitored by a UV detector (SPD-6A®, Shimadzu, Kyoto, Japan) at a wavelength of 313 nm. The analytical column was an ion-exchange column (PARTICIL® 10 SCX, 4.6 × 250 mm, Whatman, part of GE Healthcare, Tokyo, Japan). Plasma samples were monitored for MTX (monoglutamate) only, because MTX polyglutamates are typically found in red blood cells. MTX and its polyglutamates were measured in CSF and brain samples. And, sum of MTX and its polyglutamates concentrations were expressed as total MTX. However, MTX pentaglutamate (-glu5) and hexaglutamate (MTX-glu6) were not analyzed in brain samples, because negligible amounts were obtained in a preliminary study. The recoveries of MTX monoglutamate, diglutamate, triglutamate and tetraglutamate were 93.2 ± 4.1, 79.2 ± 5.5%, 72.7 ± 4.0 and 70.2 ± 1.7%, respectively (n=5) at 1 μg/m*l*. Their coefficients of variation (CV) were 4.4, 6.9, 5.5 and 2.5%, respectively. Interday CV values in the assay ranged between 1.6 and 5.8% with a limit of quantification of 3.5 *n*g/m*l* at a signal-to-noise ratio of 3 (n=5).

Rho123 was analyzed by HPLC with fluorometric detection. The mobile phase consisted of 50 mM phosphate buffer (pH 4.0) and acetonitrile (60:40, v/v), and the effluent was monitored by a fluorometric detector (RF-10AXL®, Shimadzu) at excitation and emission wavelengths of 490 and 550 nm, respectively. The C18 column (RP-18 GP 250–3.0, 5 μm®, Kanto Chemical) was used as an analytical column. The recovery of Rho123 was 100.4 ± 2.7% (CV=2.7%) at 2 μg/m*l* (n=5). The interday CV values in the assay ranged between 1.9 and 4.8% with a limit of quantification of 5.5 *n*g/m*l* at a signal-to-noise ratio of 3 (n=5).

Pharmacokinetic analysis

A one compartment open model was used to analyze the pharmacokinetics of MTX. The plasma concentration at time 0 hr (C_0) and elimination rate constant (k_{el}) in the following equation were calculated using the nonlinear least-squares fitting.

$$C_P = C_0 \, e^{-kel \, t}$$

where Cp and t represent the plasma concentration and time after the administration of MTX, respectively.

The area under the plasma concentration-time curve (AUC) was obtained as a sum of the area from 0 to the last sampling time by the trapezoidal method. The elimination half-life ($t_{1/2}$), apparent volume of distribution (V_d) and total body clearance (Cl_{tot}) were calculated using the following equations,

$t_{1/2}=0.693/k_{el}$

$V_d=Dose/C_0$

$Cl_{tot}=Dose/AUC$

If Rho123 completely inhibits the excretion of other molecules, the elimination of MTX from the body may delay, and the AUC of MTX from 0 to the last sampling time may increase. As a result, distribution of MTX into the brain and CSF may be affected.

In order to compare the efficiency of Rho123, we excluded the effects of AUC. Therefore, we defined the brain-plasma AUC ratio (BBR) and CSF-plasma AUC ratio (CBR) as indices of distribution as follows:

BBR=brain MTX concentration / plasma MTX AUC,

CBR=CSF MTX concentration / plasma MTX AUC

Statistical analysis

Data are displayed as means ± SD. Differences in mean values between the groups were analyzed by Scheffé's multiple comparison test after a one-way ANOVA. Equal variances among the groups were confirmed by Bartlett's test. Comparisons between two groups were confirmed by Student's *t*-test. Differences were considered significant at $P<0.05$.

RESULTS

Although there is a tendency that slopes after intravenous injections ($t_{1/2}$=35 ± 3.2) were steeper than those after intrathecal injections ($t_{1/2}$=84 ± 20), the slope was similar between Rho123 treated and not treated groups. No significant differences were observed in pharmacokinetic parameters, including kel, Vd, AUC, CLtot and $t_{1/2}$ between Rho123 treated and not treated groups (Fig.1, Table 2). This result indicates that Rho123 did not have a significant pharmacokinetic interaction with MTX (2 mg/body) at the dose administered (0.2 mg/body).

Figure 2 shows MTX concentrations in brains and CSF after MTX administartion. At 6 hr post administration, CSF concentrations tended to be higher than brain concentration in intrathecally administered groups (Mit, Mit+Riv and Mit+Rit groups). Especially, CSF significantly involved more MTX than brain in Mit ($P<0.05$). On the other hand, CSF concentrations were almost the same as brain concentrations when MTX was intravenously injected (Miv and Miv+Riv groups). At 12 hr post administration, CSF concentrations showed a remarkable decrease in all of the groups. In contrast with 6 hr, brain concentrations

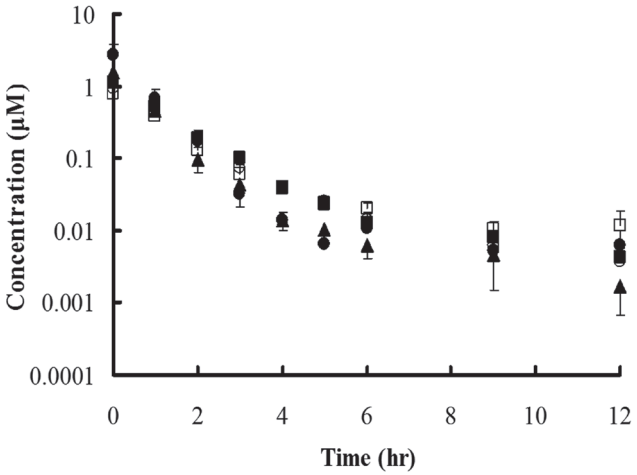

Fig. 1. MTX concentrations in plasma after the administration of MTX with or without Rho123 to rats. ●; MTX (i.v.) +saline (i.t.), ■; MTX (i.t.), ▲; MTX (i.v.) +Rho123 (i.v.) +saline (i.t.), □; MTX (i.t.) +Rho123 (i.v.), ○; MTX (i.t.) +Rho123 (i.t.). Each value is the mean ± S.D. (n=5). The doses of MTX and Rho123 administered were 2 and 0.2 mg/body, respectively. No significant differences were observed in pharmacokinetic parameters, including kel, Vd, AUC, CLtot and t1/2 between Rho123 treated and not treated groups.

Table 2. Pharmacokinetic parameters of MTX after administrations of MTX with or without Rho123 to rats

Group	C_0 (μM)	k_{el} (1/hr)	AUC (μM·hr)	V_d (l/body)	Cl_{tot} (l/hr)	$t_{1/2}$ (min)
Miv	1.3 ± 0.28	1.2 ± 0.17	1.25 ± 0.27	10 ± 2.2	12 ± 3.1	35 ± 3.2
Mit	0.51 ± 0.19	0.49 ± 0.22	1.02 ± 0.28	–	–	84 ± 20
Miv+Riv	1.3 ± 0.20	0.96 ± 0.10	1.41 ± 0.23	12 ± 1.4	12 ± 2.2	43 ± 2.9
Mit+Riv	0.58 ± 0.12	0.72 ± 0.26	0.86 ± 0.052	–	–	58 ± 9.0
Mit+Rit	1.3 ± 0.53	0.97 ± 0.32	1.45 ± 0.24	–	–	43 ± 8.3

Abbreviations stand for as follows: C_0; initial concentration, k_{el}; elimination rate constant, AUC; area under the plasma concentration-time curves, V_d; volume of distribution, Cl_{tot}; total body clearance, $t_{1/2}$; elimination half-life, Miv: MTX (i.v.) +saline (i.t.), Mit: MTX (i.t.), Miv+Riv: MTX (i.v.) +Rho123 (i.v.) +saline (i.t.), Mit+Riv: MTX (i.t.) +Rho123 (i.v.), Mit+Rit: MTX (i.t.) +Rho123 (i.t.). Each value is a mean ± S.D. (n=5). Doses of MTX and Rho123 were 2 and 0.2 mg/body, respectively. Each parameter was not significantly different among these groups.

tended to be higher than CSF in the groups administered MTX intrathecally with Rho123 (Mit+Riv and Mit+Rit groups). Especially, Mit+Rit significantly revealed higher MTX concentration than the others at 12 hr post administration.

Figure 3 shows BBR (ratio of MTX concentration in brain against plasma AUC) and CBR (ratio of MTX concentration in CSF against plasma ACU) after the administration of MTX. Both BBR and CBR indicated similar tendency to MTX concentration in brain and CSF, respectively.

At 6 hr post administration, CBR was significantly higher than BBR when MTX was intrathecally injected (Mit, $P<0.05$). However, CBR became quite small at 12 hr after the administration. On the other hand, BBR became significantly higher than CBR 12 hr after the administration when MTX and Rho123 were intrathecally administered (group Mit+Rit, $P<0.05$).

Significantly higher concentrations of Rho123 were observed in brain from the group injected intrathecacally at both 6 and 12 hr after the injection (group Mit+Rit, $P<0.05$) (Fig. 4).

DISCUSSION

In our previous study, we administered MTX intravenously or intrathecally to rats with or without CysA, which was also intravenously or intrathecally injected. After 6 hr, the brain and CSF were sampled, and their MTX concentrations were compared. CysA did not significantly affect MTX concentrations in the brain or CSF when MTX was intravenously injected. In contrast, when MTX was intrathecally administered, intravenously administered CysA was found to have a more prominent effect on MTX concentrations in the brain than in the CSF [23].

In the present study, a specific P-gp substrate, Rho123, increased brain MTX concentrations when MTX was intrathecally administered, which is consistent with our previous findings. This result suggests that this P-gp modulator competitively inhibited

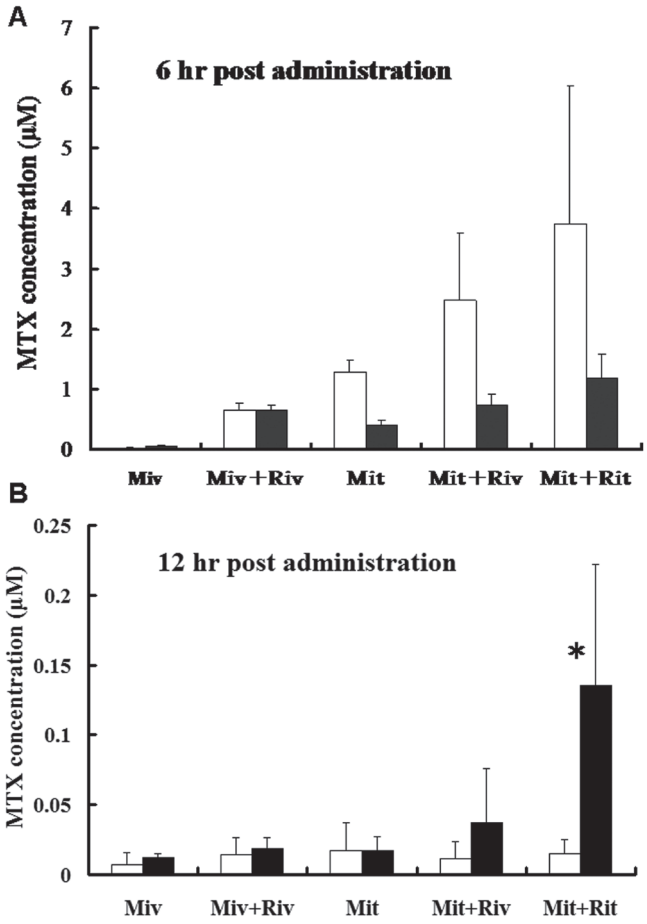

Fig. 2. Total MTX concentrations in the brain and CSF after the administration of MTX with or without Rho123 to rats. This figure is composed of charts A and B, which show MTX concentrations at 6 and 12 hr post administration, respectively. The abbreviations used are as follows: Miv: MTX (i.v.) +saline (i.t.), Mit: MTX (i.t.), Miv+Riv: MTX (i.v.) +Rho123 (i.v.) +saline (i.t.), Mit+Riv: MTX (i.t.) +Rho123 (i.v.), Mit+Rit: MTX (i.t.) +Rho123 (i.t.). White and black bars represent CSF and brain concentrations, respectively. Each value is the mean ± S.D. (n=5). The doses of MTX and Rho123 administered were 2 and 0.2 mg/body, respectively. *Mit+Rit revealed significantly higher MTX concentration in brain, compared with the other groups (P<0.05, Scheffe's multiple comparison test).

the excretion of MTX from the brain because both CysA and Rho123 are P-gp substrate and not RFC and PCFT substrate; folate analog [36].

Another possibility is that Rho123 potentiated the distribution of MTX from the blood into the brain. However, this is unlikely, because Rho123 did not significantly increase brain MTX concentrations when MTX was intravenously administered.

MTX may easily diffuse from CSF into the brain through supraependymal cells, because significantly higher MTX concentrations were observed in the brain 6 hr after its intrathecal injection, compared with intravenous injection. In addition, MTX may be easily excreted from CSF and the brain into the blood, because its concentrations were markedly lower 12 hr than 6 hr after drug administration. Pacchionian granulation, the BBB or CSF-brain barrier have been suggested as excretion routes.

In order to compare the MTX decrease in CSF and brain, we calculated the MTX concentration ratio 12 hr to 6 hr after drug administration (Table 3). While there was no significant difference in the CSF, Mit+Rit indicated significantly higher rate than Mit and Mit+Riv in the brain. This suggested that of these, the BBB may be inhibited the most by the Rho123 treatment, which is supported by the rapid decrease observed in MTX concentrations in CSF at 12 hr, even when Rho123 was co-administered and the slower decrease in MTX concentrations in the brain at 12 hr when Rho123 was co-administered.

However, the effects of Rho123 on MTX concentrations in the brain were weaker when it was intravenously rather than intrathecally injected. In contrast, CysA potentiated the distribution of MTX in the brain after it was intravenously rather than intrathecally injected. Thus, Cys A and Rho123 may have the different affinities to the brain or different permeabilities through the BBB. As shown in Fig. 4, Rho123 concentrations in the brain and CSF were significantly higher in Mit+Rit than in Mit+Riv 6 and 12 hr after drug administration. We speculate that although intravenously administered Rho123 poorly penetrates into brain, its

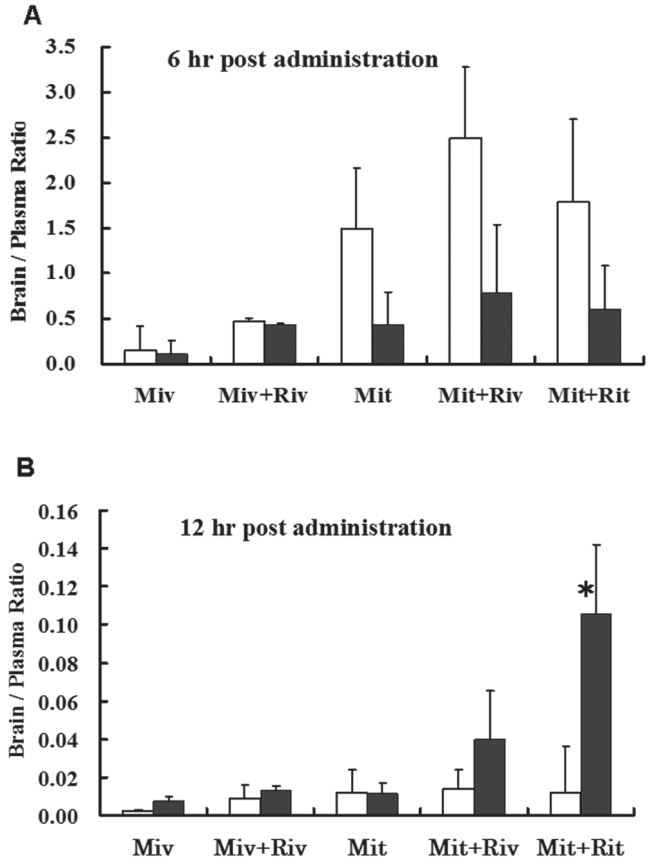

Fig. 3. Ratios of MTX concentration in the brain to plasma AUC (BBR) and in CSF to plasma AUC (CBR) after the administration of MTX with or without Rho123 to rats**.** This figure is composed of charts A and B, which show MTX concentrations at 6 and 12 hr post administration, respectively. The abbreviations used are as follows: Miv: MTX (i.v.)+ saline (i.t.), Mit: MTX (i.t.), Miv+Riv: MTX (i.v.) +Rho123 (i.v.)+ saline (i.t.), Mit+Riv: MTX (i.t.) +Rho123 (i.v.), Mit+Rit: MTX (i.t.)+Rho123 (i.t.). The doses of MTX and Rho123 administered were 2 and 0.2 mg/body, respectively. White and black bars represent CSF and brain concentrations, respectively. Each value is the mean ± S.D. (n=5). *Mit+Rit revealed significantly higher BBR, compared with the other groups (P<0.05, Scheffe's multiple comparison test).

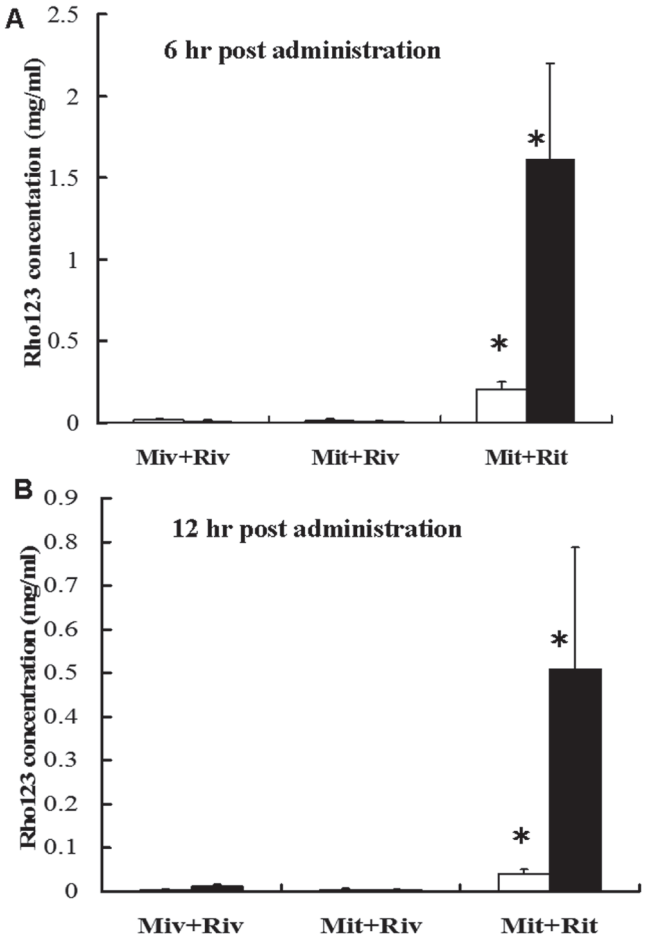

Fig. 4. Rho123 concentrations in the brain and CSF at 6 and 12 hr after the administration of MTX with Rho123 to rats. This figure is composed of charts A and B, which show Rho123 concentrations at 6 and 12 hr post administration, respectively. The abbreviations used are as follows: Miv+Riv; MTX (i.v.) +Rho123 (i.v.)+ saline (i.t.), Mit+Riv; MTX (i.t.) +Rho123 (i.v.), Mit+Rit; MTX (i.t.)+Rho123 (i.t.). The doses of MTX and Rho123 were 2 and 0.2 mg/body, respectively. Black and white bars represent brain and CSF concentrations, respectively. Each value is the mean ± S.D. (n=5). *Mit+Rit revealed significantly higher concentration in both CSF and brain, compared with the other groups (P<0.05, Scheffe's multiple comparison test).

affinity to brain tissues is high. This affinity may owe to its lipophilicity or transporters involved in brain tissue, such as glial cells.

Although MTX does not appear to be a substrate for P-gp because of its negative charge, the results of the present study suggest that it is a P-gp substrate, even under *in vivo* conditions. Therefore, the co-administration of P-gp modulators with MTX may be effective, even for MTX-resistant tumors, because MTX resistant tumors have RFC functional disorders [16, 24]. As such, combined cancer chemotherapy involving MTX with P-gp modulators may be effective for many CNS tumors.

Since P-gp acts as a transporter not only in the brain, but also in other tissues, including the kidney, liver and intestine, P-gp modulators may alter the pharmacokinetics of co-medicated drugs that are P-gp substrates, such as doxorubicin and etoposide [4, 5, 7, 11, 18–20, 34]. However, in the present study, the plasma concentration-time profiles of MTX were not affected by the treatment with Rho123 at the dose, as shown in Fig. 1 and Table 2. This result suggests that the adverse effects associated with MTX chemotherapy are not potentiated by treatments with P-gp modulators. In addition, an i.t. injection of MTX may result in markedly higher MTX concentrations in the brain and markedly lower concentrations in other tissues than those after an i.v. injection because the i.t. dose is markedly lower than the i.v. dose. This may result in less adverse effects. Therefore, an i.t. injection of MTX combined with an i.v. or i.t. injection of P-gp modulators, such as CysA and Rho123, may be an effective therapy for CNS tumors.

In conclusion, P-gp appears to play an important role in the excretion of MTX from the brain. The i.t. administration of MTX

Table 3. Rate of MTX concentration 12 hr after administration to 6 hr

12 hr / 6 hr	Mit	Mit+Riv	Mit+Rit
CSF	1.2 ± 0.43 (%)	0.79 ± 0.38 (%)	2.35 ± 1.27 (%)
Brain	4.2 ± 0.73 (%)	0.66 ± 0.30 (%)	*18.54 ± 6.87 (%)

Abbreviations stand for as follows: Mit: MTX (i.t.), Miv+Riv: MTX (i.v.) +Rho123 (i.v.) +saline (i.t.), Mit+Riv: MTX (i.t.) +Rho123 (i.v.), Mit+Rit: MTX (i.t.) +Rho123 (i.t.). Each value is a mean ± S.D. (n=5). *Brains of Mit+Riv indicated significantly larger ratio than the others.

with a P-gp modulator may maintain a sufficient MTX concentration in the brain for a longer period of time without increasing systemic body exposure. If P-gp modulators appropriately control MTX concentrations in the brain, this combined chemotherapy may lead to promising outcomes for patients with CNS tumors.

Another advantage may exist for i.t. MTX therapy with P-gp modulators, such as CysA, P-gp and MRP1, are known to be crucially involved in the development of intrinsic and acquired multidrug resistance (MDR) by many cancers. The overexpression of P-gp and MRP1 on the surface of cancer cells has been shown to contribute to the MDR phenotype [2, 3, 14, 17, 29].

The aim of cancer chemotherapy with a P-gp or MRP1 modulator for MDR tumors is to maintain anticancer drugs at adequate concentrations in tumor cells for a longer period of time. If CysA effectively functions as a P-gp or MRP1 modulator in CNS tumor cells, it may modulate MDR.

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