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ORIGINAL ARTICLE

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Effect of probenecid on ventricular cerebrospinal fluid methotrexate pharmacokinetics after intralumbar administration in nonhuman primates

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Abstract *Purpose*: Intrathecal methotrexate (MTX) achieves high concentrations in the cerebrospinal fluid (CSF) following intralumbar administration. However, peak ventricular CSF MTX concentrations are highly variable and are < 10% of those achieved with intraventricular dosing. The objectives of this study were to evaluate the effect of intralumbar and intravenous probenecid on ventricular CSF MTX concentrations after intralumbar administration of MTX, and to compare the pharmacokinetics of MTX after intralumbar and intraventricular administration. *Methods*: Nonhuman primates (*Macaca mulatta*) with permanently implanted catheters in the lateral and fourth ventricles received 0.5 mg intraventricular (lateral ventricle) MTX, or 0.5 mg intralumbar MTX with and without intralumbar or intravenous probenecid. Animals were kept prone for 1 h after MTX administration, and ventricular CSF was sampled up to 48 h from a fourth ventricular Ommaya reservoir. MTX concentrations were measured using the dihydrofolate reductase enzyme inhibition assay. Area under the ventricular CSF MTX concentration-time curve (AUC) was used as a measure of MTX exposure. *Results*: Peak ventricular CSF MTX concentrations and AUCs were highly variable after intralumbar MTX administration. Ventricular CSF MTX AUCs increased by a mean of 3.2-fold after the addition of intralumbar probenecid. Intravenous administration of probenecid did not result in an increase in ventricular CSF MTX AUCs. Asymptomatic pleocytosis was observed in all animals after intralumbar probenecid administration. Ventricular CSF MTX concentrations and AUCs were less variable after intraventricular administration of MTX. *Conclusion*: The administration of intralumbar but not intravenous probenecid increases the ventricular CSF MTX exposure after intralumbar MTX administration.

Keywords Methotrexate · Cerebrospinal fluid · Intrathecal · Probenecid · Nonhuman primate model · Pharmacokinetics

Abbreviations *AUC:* area under the concentration-time curve · *CSF:* cerebrospinal fluid · *CNS:* central nervous system · *i.t.:* intrathecal · *i.v.:* intravenous · *MTX:* methotrexate

Introduction

Intrathecal (i.t.) methotrexate (MTX) is a cornerstone of prevention and treatment of leptomeningeal leukemia. Because of the small volume of cerebrospinal fluid (CSF), MTX concentrations exceeding 100 μM can be achieved with a dose of 12 mg i.t., resulting in a substantial pharmacokinetic advantage for this route of administration [5, 16, 18]. MTX administered i.t. will induce a remission in 80% to 90% of children with acute lymphoblastic leukemia who experience a meningeal relapse, but few of these patients are cured with i.t. therapy alone [1]. As adjuvant or preventive therapy in children with newly diagnosed acute lymphoblastic leukemia, i.t. MTX alone or in combination with i.t. cytarabine or cranial radiation significantly reduces the meningeal relapse rate [13].

A major limitation of i.t. drug administration is nonuniform distribution of drug throughout the subarachnoid space. Ventricular CSF MTX concentrations after an i.t. dose are < 10% of the

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concentrations achieved with an intraventricular dose and have a high interpatient variability. After intralumbar injection of 6.25 or 12.5 mg/m^2 in humans, peak ventricular CSF MTX concentrations range from 0.6 to 22 μM, which is substantially lower than the 200 μM peak concentration achieved after intraventricular administration of 6.25 mg/m^2 [18]. Lower CSF MTX concentrations at sites distant from the site of injection substantially reduce the pharmacokinetic advantage and efficacy of i.t. MTX. The MTX concentration within the CSF after i.t. administration is dependent on the site and mode of administration [2], body position after an intralumbar dose [3], bulk CSF flow and absorption, choroidal drug uptake and clearance, and diffusion or transport of drug across the CSF-brain interface [4].

Surgically implanted ventricular access devices, such as the Ommaya reservoir, were developed to provide a convenient and reliable route for delivering drugs directly into the ventricular CSF [15]. Although there are no large, prospective comparative trials testing the efficacy of this route of administration, retrospective studies suggest that the use of these devices is more efficacious and less toxic than the traditional intralumbar route [6, 7, 12]. In addition, intraventricular MTX injection achieves higher and less-variable drug concentrations in the ventricular CSF and better distribution of drug throughout the subarachnoid space [18].

MTX is a weak acid, and probenecid can inhibit renal tubular transport of MTX [9]. A probenecid-sensitive transport pump is also present in the choroid plexus of rabbits [19]. In rabbits, intraperitoneal or intraventricular administration of probenecid concurrently with i.t. MTX has been shown to result in higher MTX CSF concentrations, slower clearance of MTX from the CSF, and lower plasma MTX concentrations [10, 17, 19]. In humans, oral probenecid (1.7 g/m^2) increases CSF MTX concentrations 2.8- to 4.2-fold after systemic administration of high-dose MTX [11], and at a dose of 2.5 g/m^2 probenecid prolongs the terminal half-life of MTX in CSF after intraventricular MTX administration [8]. Intraventricular probenecid administered concurrently with intraventricular MTX enhances MTX distribution to the lumbar space in Rhesus monkeys, suggesting that the probenecid-sensitive transport system may be widely distributed in the meninges [2].

The present study, which was performed in nonhuman primates, was designed to assess (1) the effect of concurrent intralumbar probenecid on the ventricular CSF distribution of intralumbar MTX, (2) the toxicities of i.t. administered probenecid, (3) the effect of intravenous (i.v.) probenecid on the ventricular CSF distribution of intralumbar MTX, and (4) the inter-animal variability of ventricular CSF MTX exposure after intralumbar and intraventricular MTX administration.

Materials and methods

Drugs and chemicals

MTX without preservative and leucovorin were obtained from Immunex (Seattle, Wash.). Dihydrofolate reductase was obtained from Biopure Corporation (Cambridge, Mass.). Probenecid and other chemicals used in preparation of drug and for the dihydrofolate reductase inhibition assay were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Animals

Six adult Rhesus monkeys (*Macaca mulatta*), each with an indwelling lateral ventricular catheter attached to a subcutaneous access port for drug administration and a fourth ventricular catheter attached to an Ommaya reservoir for CSF sampling as previously described [14], were used in this study (Fig. 1). MTX was administered via direct intralumbar injection in four animals, and through a lumbar catheter attached to a subcutaneous access port in two animals (R838A and R895). The monkeys ranged in weight from 9.4 to 12.1 kg. They were fed Purina Monkey Chow twice daily and were group-housed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, National Academy Press, Washington DC, 1996).

Experiments

When administered alone by the intralumbar or intraventricular route, MTX was diluted in 0.9% sodium chloride to a final concentration of 0.5 mg/ml. When administered via the intralumbar route in combination with probenecid, MTX and probenecid were solubilized in 0.1 *N* NaOH, and the pH was adjusted to 7.5–8.5 with 1 *N* HCl, to give a final concentration of 0.5 mg MTX/ml and 5 or 24 mg probenecid/ml. For i.v. administration, probenecid was

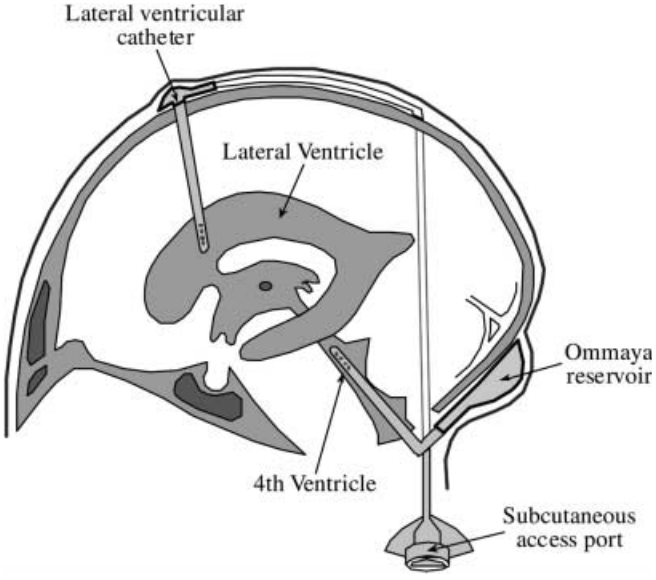


Fig. 1 Diagram of nonhuman primate model. CSF samples were obtained from the fourth ventricular catheter that is attached to a subcutaneous Ommaya reservoir. MTX was administered into the lumbar space via intralumbar injection or intraventricularly through the access port that is attached to the lateral ventricular catheter

solubilized in 0.1 *N* NaOH, and the pH was adjusted to 7.5–8.5 with 1 *N* HCl, to give a final concentration of 10 mg/ml. All drugs were sterilized by filtration through a 0.22 μm filter prior to administration.

A 0.5 mg dose of MTX was used in all experiments. The CSF volume in the Rhesus monkey (13 to 15 ml) is approximately one-tenth of that in humans, so that a 0.5 mg dose in the animal model is equivalent to a 5 mg dose in humans. MTX was administered as a bolus injection into the lumbar space alone or in combination with probenecid (24 mg, $n=1$; 5 mg, $n=5$). In separate experiments, the effect of i.v. probenecid (250 mg) was studied by administering probenecid as a 10 min i.v. infusion 15 and 0.5 h before and 8 h after an intralumbar dose of MTX ($n=2$). MTX was also administered alone into the lateral ventricle ($n=4$). Each animal served as its own control, and the order of administration was randomized within each animal. MTX administrations were separated by at least 1 week. Animals were kept prone for the first hour after administration to increase ventricular CSF MTX distribution [3]. Leucovorin (10 mg) was administered intramuscularly 24 h after the administration of MTX to prevent potential MTX-associated systemic toxicities. Animals were assessed daily for the development of drug-related toxicities.

CSF sampling and MTX analysis

CSF, 0.2 ml, was sampled from the fourth ventricular Ommaya reservoir, which was pumped four times prior to each sample, at 0.5, 1, 4, 8, 12, 24, 32, and 48 h after MTX administration. Ventricular CSF MTX concentrations were measured with the dihydrofolate reductase enzyme inhibition assay adapted for a 96-well microplate reader [20]. This assay is linear from 0.01 to 0.1 μ*M*, and has a lower limit of quantification of 0.01 μ*M*. The within-run coefficients of variation (CVs) at 0.03 μ*M* and 0.08 μ*M* were 4.0% and 2.7%, respectively, and the interday (total) CVs were 7.6% and 1.8%.

Pharmacokinetic analysis

Ventricular CSF MTX AUC was used as a measure of MTX exposure and derived by the linear trapezoidal method. Half-life was estimated by linear regression of the log-transformed CSF MTX concentration-time data. The peak CSF MTX and all data points measured afterwards were included for the calculation of the half-life.

Statistical analysis

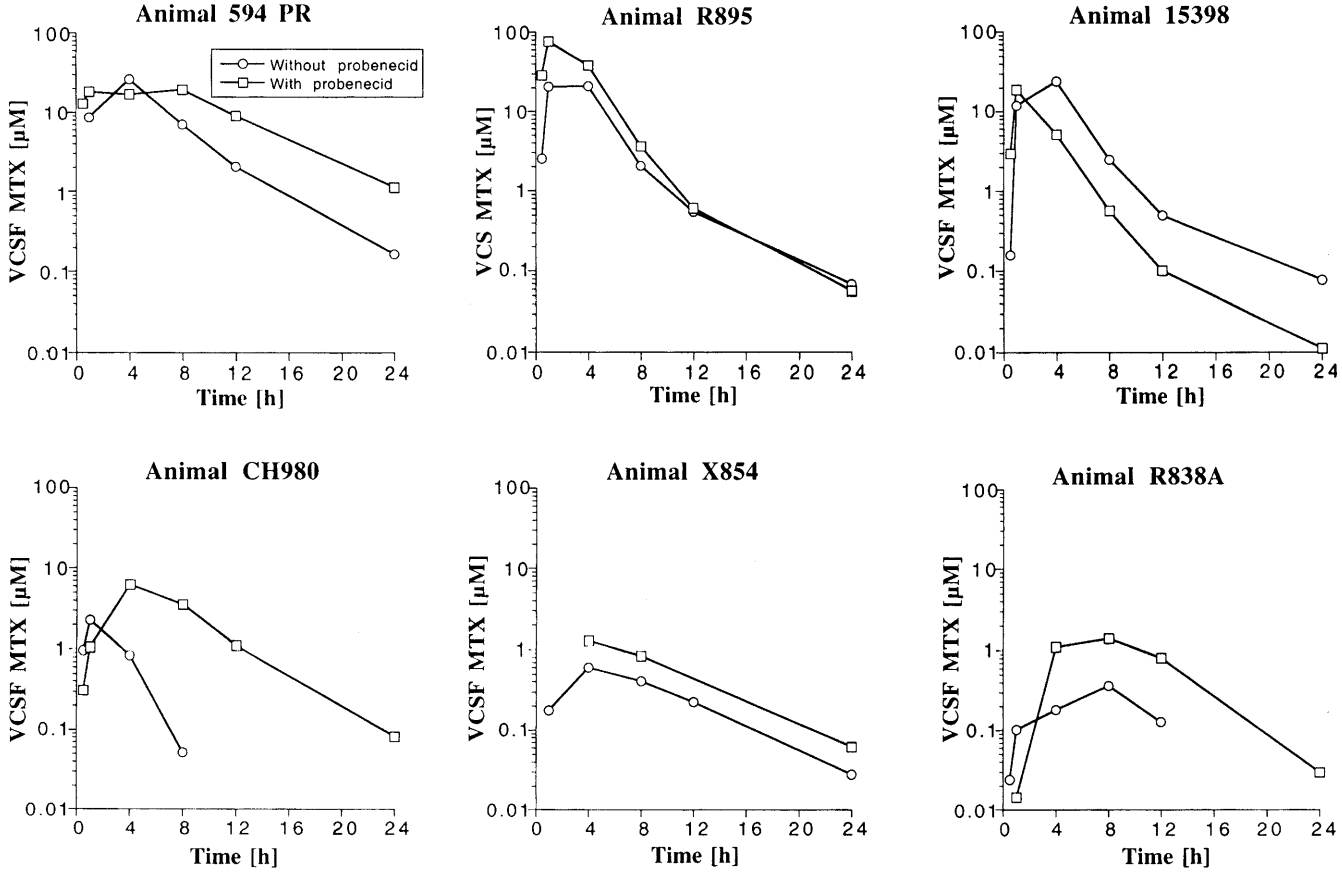
The Wilcoxon signed rank's test was used to determine if differences in the pharmacokinetic parameters obtained from animals that received intralumbar MTX or intralumbar MTX plus intralumbar probenecid or from animals that received intralumbar or intraventricular MTX were statistically significant.

Results

Effect of intralumbar probenecid

Fourth ventricular CSF MTX concentrations after intralumbar MTX and intralumbar MTX in combination with intralumbar probenecid were highly variable among animals (Fig. 2). Peak ventricular CSF MTX concentrations without probenecid ranged from 0.4 to

Fig. 2 Ventricular CSF concentration-time profiles of MTX after administration of intralumbar MTX (0.5 mg) without (○) and with (□) intralumbar probenecid (5 mg). Animal 594 received 24 mg probenecid



26 μ*M* (mean 12 μ*M*), and with probenecid ranged from 1.3 to 77 μ*M* (mean 21 μ*M*). With the addition of probenecid the MTX AUC increased in all but one animal by a mean of 3.2-fold (range 0.5- to 6.9-fold). The observed increase in AUC after the addition of intralumbar probenecid did not reach statistical significance ($P = 0.173$). The animals with the lowest ventricular CSF MTX AUCs after i.t. MTX alone had the greatest relative increase in the AUC with the combination of i.t. MTX and probenecid.

The half-life of MTX was a mean of 1.4-fold (range 0.7- to 2.9-fold) longer when probenecid was administered with the i.t. MTX (Table 1). Ventricular CSF MTX concentrations of ≥1.0 μ*M* were observed in all six animals after the addition of probenecid compared to four of six animals without the addition of probenecid. Ventricular CSF MTX concentrations were maintained at ≥1.0 μ*M* for 5.9 h (range 0 to 15 h) when MTX was given alone and 12 h (range 6 to 24 h) when MTX was given in combination with probenecid.

Toxicity of intralumbar probenecid

A transient, asymptomatic CSF pleocytosis (1000 WBC/μl 24 h after probenecid) was noted in the first animal that received 24 mg i.t. probenecid, and had resolved completely within 7 days of probenecid administration. All animals subsequently received probenecid at a reduced dose of 5 mg i.t. and peak CSF cell counts after the combination of i.t. MTX and probenecid at 24 h ranged from 104 to 1150 WBC/μl (mean 548 WBC/μl). At 48 h after i.t. administration of probenecid and MTX, CSF cell counts had decreased and ranged from 60 to 334 WBC/μl (mean 173 WBC/μl).

Effect of i.v. probenecid

Administration of 250 mg probenecid by i.v. infusion 15 h and 0.5 h prior to the intralumbar administration of MTX, and 8 h after i.t. MTX in two animals did not effect ventricular CSF MTX concentrations, as shown in Fig. 3.

Table 1 Ventricular CSF MTX AUCs and MTX half-lives after intralumbar administration of intralumbar MTX (0.5 mg) with (+) and without (–) probenecid. Wilcoxon rank test showed no significant differences in ventricular CSF MTX AUCs ($P = 0.173$) with and without probenecid

Animal	Ventricular CSF MTX AUC (μ*M*·h)		MTX half-life (h)	
	–	+	–	+
594 PR	154	254	2.8	3.9
R 895	124	302	3.2	2.6
15398	119	56	3.2	2.2
CH 980	7	48	1.3	3.8
X 854	6	14	4.4	6.1
R 838A	3	16	2.7	3.3
Mean ± SD	69 ± 71	115 ± 128	2.9 ± 1.0	3.7 ± 1.4

Comparison of intraventricular and intralumbar MTX administration

Peak ventricular CSF MTX concentrations (fourth ventricle) after administration of MTX into the lateral ventricle ($n = 4$) ranged from 120 to 294 μ*M* (mean 216 μ*M*) (Fig. 4), and were much higher and less variable than ventricular CSF MTX concentrations after intralumbar administration. Fourth ventricular CSF MTX AUCs were also higher after lateral ventricular MTX administration and less variable compared to ventricular CSF concentrations after intralumbar MTX administration (Table 2). The observed differences in AUCs did not reach statistical significance ($P = 0.068$).

Discussion

Preventive i.t. MTX has substantially lowered the incidence of leptomeningeal relapse in acute lymphoblastic leukemia. However, the CNS still accounts for a significant proportion of the relapses that occur in this disease. Nonuniform distribution of MTX within the CSF space after intralumbar injection may limit the efficacy of this form of regional chemotherapy.

Improving the distribution of MTX within the CSF space could enhance its therapeutic effect. In nonhuman primates maintaining a prone body position for 1 h after intralumbar MTX administration resulted in a >15-fold increase in the mean peak ventricular CSF concentrations and AUCs compared with immediate placement in an upright position, and represents an effective, safe and simple way to increase MTX distribution throughout the CSF [3]. Intraventricular administration of MTX via a surgically implanted access device provides more uniform CSF MTX distribution compared to intralumbar MTX administration [2, 18], but MTX CSF distribution remains uneven after intraventricular injection, and MTX concentrations are lower at sites more distant from the injection site, even during continuous intraventricular MTX infusions [2]. In addition, the use of these devices is usually restricted to patients with overt leptomeningeal disease. A probenecid-sensitive MTX transport pump is present in the choroid plexus of rabbits [19], and several studies have demonstrated that intraventricular, intraperitoneal and systemic administration of probenecid can increase CSF MTX concentrations [2, 8, 10, 11, 17, 19].

Administration of probenecid via the i.t. route could have the advantages of overcoming the need for probenecid to cross the blood-CSF barrier, and allowing direct access to the probenecid-sensitive transport system.

In the present study the addition of 5 or 24 mg intralumbar probenecid to intralumbar MTX resulted in a 3.2-fold increase in ventricular CSF MTX AUCs, and the animals with the lowest ventricular CSF MTX AUCs after intralumbar MTX alone had the most substantial relative increases in ventricular drug expo-

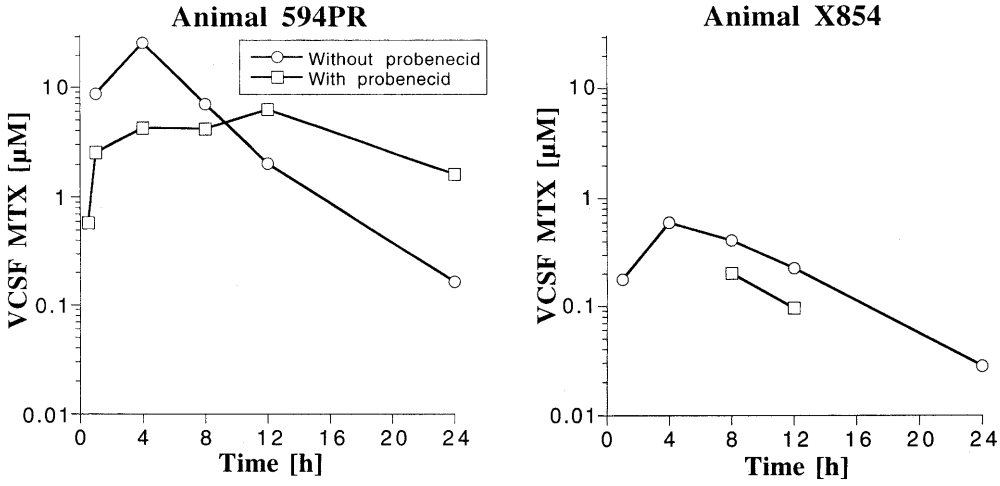


Fig. 3 Ventricular CSF concentration-time profiles of 0.5 mg intralumbar MTX without (○) and with (□) i.v. probenecid (250 mg, three doses)

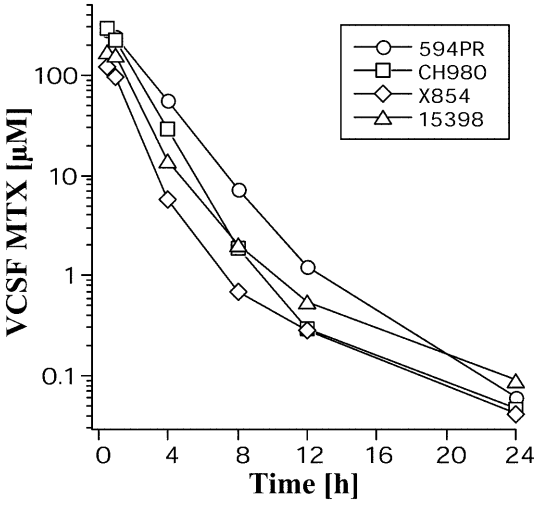


Fig. 4 Ventricular CSF MTX concentration-time profile of MTX after administration of intraventricular MTX (0.5 mg)

sure with the combination of intralumbar MTX and probenecid. As previously reported, this study demonstrated significant variability in ventricular CSF MTX concentrations among animals after intralumbar administration of MTX [18]. However, despite this variability, only one of six animals failed to show an increase in MTX AUC after intralumbar probenecid administration. Cytotoxic ventricular CSF MTX concentrations of ≥1.0 μ*M* [18] were achieved in all animals after the addition of probenecid compared to four of six animals without probenecid, and ventricular CSF MTX concentrations were maintained at ≥1.0 μ*M* for twofold longer with the addition of intralumbar probenecid.

The increase in ventricular CSF MTX AUC observed in our study was not statistically significant, and is comparable to the 2.5-fold increase in the lumbar steady-state MTX concentration after intraventricular administration of probenecid with MTX in the same animal model [2], and to the 2.8- to 4.2-fold increase in ventricular CSF MTX concentrations after systemic administration of probenecid with high-dose i.v. MTX [11]. The increase in MTX ventricular CSF exposure with the addition of intralumbar probenecid was observed in animals kept prone for 1 h after intralumbar MTX, and therefore has an additive effect with positioning in improving drug distribution to the ventricular CSF.

Even though intralumbar probenecid was clinically well tolerated, all animals developed a transient, asymptomatic pleocytosis, even after a dose reduction from 24 to 5 mg. There did not appear to be a relationship between the degree of enhancement of ventricular CSF MTX distribution and probenecid dose. Therefore, intralumbar probenecid doses < 5 mg may also be as effective.

Systemic administration of probenecid could have the advantage of avoiding potential probenecid-associated CNS toxicities from i.t. injection. In our model i.v. probenecid did not affect ventricular CSF MTX AUCs. However, only two animals were studied, and an effect of systemic probenecid on ventricular CSF MTX exposure can therefore not be ruled out with certainty. The probenecid dose used in our study was 250 mg i.v. 15 and 0.5 h prior to MTX administration, and at 8 h after MTX administration. This dose corresponds approximately to a dose of 0.5 g/m^2 in humans. In humans, Bode et al. demonstrated an increase in the CSF MTX half-life only after systemic administration of probenecid at a dose of 2.5 g/m^2 per day, but not after a dose of 1.25 g/m^2 per day [8]. Howell et al. observed an increase in CSF MTX concentrations after systemic administration of 1.7 g/m^2 probenecid together with high-dose systemic MTX administration [11]. The systemic probenecid dose administered in our experiments was therefore lower, possibly contributing to a lack of efficacy. However, the probenecid dose used in our experiments is more consistent with the oral probenecid dose used for the treatment of gout, which ranges between 200 and 500 mg twice daily. Systemic administration of probenecid to increase CSF MTX exposure after intralumbar administration may therefore not be practicable.

Table 2 Ventricular CSF MTX AUC after intraventricular versus intralumbar administration of MTX (0.5 mg). Wilcoxon rank test showed no significant differences in ventricular CSF MTX AUCs ($P = 0.068$) after ventricular versus lumbar MTX administration

Animal	Ventricular CSF MTX AUC (μ*M*·h)	
	Ventricular	Lumbar
594 PR	795	154
CH 980	650	7
15398	421	119
X 854	254	6
Mean ± SD	530 ± 240	72 ± 76
CV (%)	45	107

In summary, in nonhuman primates intralumbar but not i.v. administration of probenecid with MTX increased ventricular CSF MTX exposure. Further studies are required to evaluate the potential clinical benefits and safety of this approach.

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