A Primate Model for Study of Methotrexate Pharmacokinetics in the Central Nervous System

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SUMMARY

A new technique enabling repetitive sampling of cerebrospinal fluid (CSF) in unanesthetized rhesus monkeys was developed to study the pharmacokinetics of methotrexate (MTX) in the CSF. CSF and plasma MTX levels were monitored following intraventricular and intravenous MTX administration. CSF and plasma MTX disappearance curves in the monkey were virtually identical to curves generated in humans, suggesting that the mechanisms of transport between the CSF and plasma compartments are similar in both species. These observations validate this experimental primate model and indicate its potential application to the pharmacological study of CNS chemotherapeutic agents in man.

INTRODUCTION

We recently developed an experimental model that enables repetitive sampling of CSF1 over extended periods of time in unanesthetized rhesus monkeys (8). Our surgical technique involves the s.c. implantation of an Ommaya reservoir with the cannula inserted through the foramen of Magendie into the 4th ventricle, without tissue damage or breakdown of the blood-brain barrier.2 We used this system to study the pharmacokinetics of MTX in the CSF following intraventricular, i.l., and i.v. administration. The purpose of this report is to demonstrate that the CNS pharmacokinetics of MTX can be readily evaluated in this primate model, and that they are strikingly similar to those observed in humans.

MATERIALS AND METHODS

Animals. Adult male rhesus monkeys (Macaca mulatta) weighing between 2.9 and 8.1 kg were obtained from the NIH Primate Center. Each animal was kept in a separate cage and fed Purina Monkey Chow and water ad libitum. Values for body surface area used to calculate drug dosage were obtained from the formula: Surface area (sq m) = (1.18 \times 10^{-3}) \text{ (weight in g)}^{0.3} \text{ according to the method of Spector (7).}

Intraventricular Catheterization and Reservoir Implantation. A silicone Pudenz catheter was surgically placed into the 4th ventricle and attached to a s.c.-implanted Ommaya CSF reservoir in anesthetized monkeys (8). This system permits sterile ventricular cerebrospinal fluid sampling over extended periods of time without requiring chronic immobilization, and has been shown to provide mixing of injected drugs with lateral ventricular CSF.

Reservoir Sampling and Injection. Except for the i.v. MTX infusions and lumbar injections (see below), all sampling and injections were performed on conscious animals sitting in primate chairs. To ensure adequate mixing of the reservoir contents with ventricular CSF, the reservoir chamber was pumped 4 times before and after sampling or injection. The hair over the reservoir chamber was shaved, the reservoir injection site was prepped with 3% hexachlorophene, alcohol, and a 10% povidone:iodine solution, and a 25-gauge scalp-vein needle was inserted percutaneously into the reservoir chamber (8). MTX was mixed in Elliott's B solution and injected quantitatively following isovolumetric removal of CSF. In those animals receiving more than one MTX administration, a minimum of 10 days was allowed to elapse between experiments.

Infusions (i.v.). Infusions (i.v.) of MTX for 6 to 8 hr required prior sedation with i.m. ketamine (5 to 10 mg/kg) followed by i.v. pentobarbital (450 mg/kg). A constant-rate infusion was maintained by use of a motorized infusion pump (Sigmamotor, Inc., Middleport, N. Y.).

Injections (i.l.). Monkeys were anesthetized with pentobarbital, and MTX, 13.5 mg/sq m, in 1.0 ml Elliott's B solution was injected via lumbar puncture. The animals were placed in the lateral decubitus position, and samples of CSF were taken from the Ommaya reservoir. Ascent of drug into the 4th ventricle was studied by removing 0.5 ml CSF after pumping the reservoir once.

MTX Assay. Lyophilized, preservative-free MTX (Ben Venue Laboratories, Bedford, Ohio) was used for these experiments. The MTX concentration in biological specimens was measured by the dihydrofolate reductase enzyme inhibition assay with lower limits of sensitivity of 5 \times 10^{-10} \text{ M in CSF and } 1 \times 10^{-9} \text{ M in plasma (4).}

RESULTS

Intraventricular Administration. Fourteen animals re-
ceived intraventricular injections of MTX at 13.5 mg/sq m, a
dose comparable to that used in humans (2, 3). The mean
(± 1 S.D.) CSF and plasma concentrations during the 4 days
after injections are shown in Chart 1, together with corre-
sponding values observed in humans after i.l. injections of
12.0 mg/sq m. The human data in Chart 1 were derived from
a study of 76 patients with acute leukemia treated with
prophylactic i.t. MTX (1, 2). In the CSF, MTX concentrations
were initially higher in primates than in man. The initial half-
disappearance times in the CSF were 3.0 hr in primates and
5.0 hr in man. Thereafter, the CSF curves for the 2 species
were superimposable over a 10,000-fold range in values,
with identical half-disappearance times of 14.0 hr between
48 and 96 hr after injections. The apparent volumes of
distribution calculated by extrapolation from the initial half-
lives were 44 ml (147 ml/sq m) in monkeys and 80 ml (83 ml/
sq m) in humans.

In both species, the peak plasma level occurred 3 hr after
injection at 2 x 10⁻⁷ M (the ascending portion of the plasma
curve is not shown in Chart 1). Plasma clearance was ini-
tially more rapid in the monkey, such that the plasma level
fell below 10⁻⁸ M by 24 hr in the monkey and by 32 hr in man
(Chart 1).

Previous investigations in humans indicated that MTX
disappearance from CSF during the 1st 24 hr after injection
follows 1st-order kinetics (1). To determine whether 1st-
order kinetics could also be demonstrated with the primate
model, the influence of dose on the concentration of the
drug achieved in the CSF was examined in 3 monkeys.
Chart 2 shows that CSF MTX concentration 24 hr after
injection was directly and linearly proportional to dosage
over a range of 7.5 to 22.5 mg/sq m. Additional studies at
higher doses (not shown in Chart 2) demonstrated nonline-
arity of the concentration-dose curves, suggesting that the
kinetics were not 1st-order at higher doses (>40 mg/sq m).

Injections (i.l.). The results obtained when 2 monkeys
were given 13.5 mg MTX per sq m via lumbar puncture are
compared in Chart 3 with values in 2 patients treated simi-
larly with 12.5 mg/sq m by Shapiro et al. (6). The ventricular
MTX levels peaked earlier and at higher concentrations in
the monkeys than in the patients (Chart 3).
Infusions (i.v.). Four monkeys were infused i.v. with MTX. The infusion schedule was similar to a therapeutic infusion given to 4 patients. It was designed to provide relatively constant plasma concentrations of $5 \times 10^{-6}$ M. The desired steady-state level was achieved in humans with a priming dose of 75 mg/sq m over 1 hr, followed by a constant infusion of 15 mg/sq m/hr. In primates, the desired plasma profile was approximated with a priming dose of 54 mg/sq m over 1 hr, followed by a constant infusion of 14 to 33 mg/sq m/hr. MTX concentrations in serial samples obtained during the primate infusions were compared with a limited number of determinations performed in man. There was excellent agreement between the 2 species (Chart 4) with CSF:plasma MTX ratios of 0.02 to 0.05 12 hr after starting the infusions in man and 0.01 to 0.04 at 6 to 8 hr after starting the infusions in monkeys.

**DISCUSSION**

In this study, the pharmacokinetics of MTX administered into the CSF in Rhesus monkeys was compared with human data taken from the report of Bleyer and Dedrick (1). The latter study differs somewhat from the primate model used here, in that the dosage relative to body surface area was slightly lower in the patient study (12.0 versus 13.5 mg/sq m), and that the CSF sampling and drug administrations were performed by the lumbar route in humans and by the ventricular route in the primates.

In the human studies, the average patient was 11 years old, weighed 35 kg, and had a body surface area of 0.96 sq m and a CSF volume of 140 ml. In the primate studies, the average monkey weighed 5.6 kg and had a body surface area of 0.37 sq m and a CSF volume of 13 ml. Since the average absolute dose was 12.5 mg in man and 5.0 mg in monkeys, the initial MTX concentration in the CSF would have been expected to be slightly higher in the monkey: 385 μg/ml ($8.5 \times 10^{-4}$ M) in the monkey versus 89 μg/ml ($2.0 \times 10^{-4}$ M) in man. This expected difference is consistent with the higher values for CSF MTX observed in primates for the 1st 12 hr after injection (Chart 1). The fact that the CSF curves converge by 24 hr suggests that the initial clearance from the injection site is somewhat more rapid in monkeys than in man. One process that may account for a smaller initial half-life in the primate is a higher rate of CSF turnover in this species. Evidence from this study which supports this hypothesis is seen in Chart 3, which shows that the ascent of MTX from the lumbar space to the cerebral ventricles is more rapid in the primate than in the human. The slower ascent in man may also be due to the longer length of the spinal column and to the additional distance from the 4th ventricle to the right lateral ventricle where the cannula was positioned in man.

Translocation of MTX from the blood into the CSF can be quantitated most accurately if the plasma concentration is held constant. Our experience thus far with i.v. infusions in the rhesus monkey suggests that a steady-state plasma concentration can be achieved with a priming injection followed immediately by a constant infusion at an hourly rate equal to that of the priming dose. The priming and hourly infusion doses, which were derived empirically, can be described in terms of the following formula:

$$\text{MTX dose (mg/sq m)} = (5.4 \times 10^{6}) ([\text{MTX}]_0)$$

where $[\text{MTX}]_0$ is the desired plasma level in moles/liter. To maintain a plasma MTX level of $5 \times 10^{-6}$ M, for example, $(5.4 \times 10^{6})(5 \times 10^{-6}) = 27$ mg/sq m should be given rapidly i.v., followed immediately by a constant infusion of 27 mg/sq m/hr. If such an infusion is continued for more than 6 hr, citrovorum factor rescue is probably necessary to prevent serious MTX toxicity. We have not observed toxicity in animals administered 100 mg of citrovorum factor i.m. 24 and 48 hr after starting a high-dose MTX infusion.

Plasma clearance can be estimated from the infusion rate of MTX required to maintain a constant plasma concentration. If 27 mg/sq m/hr is required to maintain a plasma concentration of $5 \times 10^{-6}$ M, then the plasma clearance can be calculated to be 198 ml/sq m/min, which compares with a mean value of 110 ml/sq m/min for man (5). At steady state, the CSF:plasma MTX ratio has been observed to be 0.03 to 0.05 by Shapiro et al. (6) and 0.02 to 0.05 in our study (Chart 4). The human values compare well with primate values of 0.01 to 0.04 observed in this study 6 to 8 hr after the infusion was started.

The human and primate data were strikingly similar for both the plasma and CSF disappearance curves following either intraventricular or i.v. MTX administration. This observation would indicate that the kinetics and, presumably, the mechanisms of transport between the CSF and plasma compartments are similar in both species. Thus, detailed pharmacokinetic analyses derived from use of this primate model should help predict the distribution kinetics of cytotoxic agents in man as well as provide information regarding the basic nature of these processes. In addition, this model system offers the opportunity to study both blood-brain and CSF-brain pharmacokinetics and also allows neurotoxicological assessment of drugs used to treat CNS neoplasms.

**REFERENCES**

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