Neuropharmacological Effects of Methotrexate Perfused through the Cerebrospinal Fluid System of the Rhesus Monkey

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SUMMARY

Thirteen adult Rhesus monkeys were repeatedly perfused through the ventriculocisternal or ventriculolumbar spaces with Elliott’s B solution containing various concentrations of methotrexate (MTX) and trace amounts of [3H]MTX and [carboxy-14C]inulin. The concentrations of MTX ranged from 4.8 to 0.15 mg/ml representing perfusion dosages of 551 mg/sq m to 16 mg/sq m. The average steady-state concentration out-concentration in (C0/C1) value for MTX was 0.78 ± 0.04 for the ventriculocisternal and 0.66 ± 0.01 for the ventriculolumbar routes.

MTX treatments did not significantly affect mean inulin steady-state C1/C0 values or CSF formation rate.

With the exception of a monkey perfused with MTX at an inflow concentration of 4.8 mg/ml, body weight, food intake, and urine output, analyzed at weekly intervals, generally were not remarkably affected by MTX perfusions. In five monkeys perfused with MTX in concentrations of 4.8 to 0.6 mg/ml, gross neurological toxicity was observed, principally in the form of seizures and hypokinesia during perfusion series with occasional residual motor deficit.

Significant cerebral damage was associated with the brains of two monkeys perfused with MTX at concentrations of 2.4 and 0.6 mg/ml and two monkeys perfused at concentrations of 1.2 and 0.3 mg/ml; three of the four animals displayed signs of gross neurotoxicity, and two animals developed permanent motor deficits. However, the extent to which neurotoxic signs could be attributed solely to MTX was difficult to judge because some changes in central nervous system morphology were associated with the mechanical aspects of the procedure.

Overall behavioral performance as measured by a visual pattern discrimination reinforced by avoidance or escape from an electric shock was not significantly affected by repeated perfusions of MTX (0.6 mg/ml) in two monkeys not otherwise studied in detail.

INTRODUCTION

Anticancer agents are introduced directly into the CSF in order to achieve therapeutic doses in the proximity of cancer cells located on the brain side of the blood-brain barrier. Injection i.t. of MTX, which has now been widely used in the treatment of meningeal leukemia, was first described by Hyman et al. (10) and Whiteside et al. (33). Livingston and Carter (14) have recently concluded that in most cases rather short remissions were reported that may have been related to inadequate therapy. While by-passing the barrier, i.t. injection does not provide relatively long-term bathing of the affected tissue at a controlled concentration level. In studies of injection into subarachnoid fluid, there is evidence that the material does not readily enter the ventricular system (16, 21) unless, as reported by Rieselbach et al. (21), the volume of solution injected into the CSF space is adequately adjusted.

In order to provide maximum bathing of the ventricular and subarachnoid pathways for an extended period of time in the treatment of various neoplastic diseases of the central nervous system, Rubin et al. (24) used an inflow-outflow (perfusion) technique similar to that previously used for other therapeutic maneuvers in man and for animal studies (6). This procedure and the results obtained have also been discussed by Ratcheson and Ommaya (20) and Bering et al. (2). Recently, Tarsy et al. (30) suggested that the perfusion procedure described by these workers may be more efficient and might have avoided the observed toxic reactions of patients given intraventricular infusions of 5-ido-2'-deoxyuridine.

The study reported here was undertaken to obtain neuropharmacological data for MTX perfused through either the VL or VC route of Rhesus monkeys.

MATERIALS AND METHODS

The methods and procedures used in these studies were generally those previously described by Merker et al. (17).

Animals. A total of 13 Rhesus monkeys (Macaca mulatta), 7 males and 6 females, were perfused with MTX for this study.
study. The mean weight of these animals during the experiments was 3.1 kg with a range of 2.4 to 4.3 kg. In addition, the brains from 6 other animals, perfused with Elliott's B solution not containing drug, were used for neuropathological examination. All monkeys were maintained in individual cages with free access to water and Purina food (150 to 200 g/day).

Cannulation Site. The cannulation site was the left lateral ventricle at the anteroposterior plane of the anterior commissure and foramen of Monro (A.17.0, L1.0-2.0, H12.0) according to the work of Snider and Lee (27). The ventricular cannula was a 1.5 inch, 23-gauge regular-wall needle with a rounded closed point and 2 lateral holes drilled 180° apart 1 and 2 mm from the tip. A modified and miniaturized version of a stereotaxic device originally designed by Dr. B. Silverstone (New England Medical Center) was implanted s.c. and extradurally in the skull and used to guide the cannula to the ventricular location.

Perfusion. Both VC and VL perfusion routes were used. In each case, the animal was seated in a primate chair. For VC perfusion, a 22-gauge, thin-walled, short-bevel needle was placed in the cisterna magna for collection of cisternal outflow. Ear bars were used to immobilize the head and to produce the proper degree of flexure. Phencyclidine hydrochloride (Sernylan; Parke-Davis, Detroit, Mich.) was administered i.m. before and during VC perfusions, starting with an anesthetic dose of 3 mg/kg and continuing with maintenance doses at approximately 1-hr intervals in an amount equivalent to 0.8 mg/kg/hr. For VL perfusion, ear bars were not used, but the head was generally immobilized by pressure pads and the animal was generally given an initial phencyclidine dosage of 1 mg/kg followed by maintenance doses equivalent (by temporal averaging) to about 0.3 mg/kg/hr. A 0.020- x 0.037-inch tubing (Silastic; Dow Corning, Midland, Mich.) was threaded into the lumbar sac for collection of lumbar outflow.

A Harvard syringe pump was used to introduce fluid at a calibrated flow rate of 129 to 130 μl/min, about 3 times the normal rate of CSF formation. A Statham Model P23Db pressure transducer set at the height of the external auditory meatus as reference, was used to monitor CSF pressure continuously. The average height of the orifice of the outflow tubing relative to the auditory meatus was adjusted to −2 cm for VC perfusion and −8 cm for VL perfusions. Effluent was collected in graduated cylinders for volumetric measurement and, since rather long lengths of tubing were required, all calculations were corrected for the resulting dead space.

Perfusions were usually, but not always, carried out at weekly intervals, with a duration of 190 to 200 min unless terminated earlier due to a rise in CSF pressure above 30 to 50 cm H₂O and/or a drop in out-flow rate below 50 to 100 μl/min. Prior to perfusing with drug, monkeys were given at least 2 perfusions with Elliott’s B solution containing radio-labeled inulin in order to make certain that the animal was a good candidate in terms of pressure flow characteristics of the CSF system and to obtain base-line information.

Immediately following these initial perfusions, drug perfusions were begun at weekly intervals for up to a maximum of 10 treatments unless they were terminated earlier for reasons of technical breakdown or severe morbidity or death of the animal.

Perfusion Fluid. The perfusion fluid was Elliott’s B solution, originally described by Elliott et al. (7), manufactured sterile and pyrogen free by Baxter Laboratories, Inc., Morton Grove, Ill., and obtained from them. In all cases, [carboxy-¹⁴C]inulin, obtained as a sterile aqueous solution (New England Nuclear, Boston, Mass.; NEC-164P, Lots 96-192-1A, 246-128-38, 261-145, 334-088), was added to the Elliott’s solution in a concentration of 5 nCi [¹⁴C]inulin per ml perfusion fluid; at the specific activity supplied, this was equivalent to approximately 2 μg perfusion fluid. In the case of control perfusions, the pH was then adjusted to pH 7.3 to 7.4 and the solution was passed through a 0.22-μm Millipore filter. The inulin served as a marker substance for measuring CSF formation rate. CSF formation rate was calculated as described by Heisey et al. (8) using equations of Pappenheimer et al. (19).

MTX. MTX in the form of 4-amino-N¹⁴- methylpteroylglutamic acid sodium sterile in 50-mg multiple-dose vials (Ledderle Laboratories, Pearl River, N. Y.; Lots 105-311, 090-299, 102-304, 138-282) was dissolved with Elliott’s B solution. The resulting MTX solution was then added to a larger quantity of perfusion fluid in an appropriate amount to make up a given concentration of MTX. In experiments designed with radioactive MTX, tracer amounts of [3',5'-T]MTX sodium salt was used (Nuclear Chicago Corp., Des Plaines, Ill.; TRK 224, Batch 2, with a nominal specific activity of 2520 mCi/m mole, or Batch 5, with a nominal specific activity of 3000 mCi/m mole). A small amount of this material was purified before each perfusion by thin-layer chromatography on cellulose in 0.1 M sodium phosphate buffer at pH 7, followed by thin-layer chromatography on DEAE-cellulose in 1 M ammonium bicarbonate. It was added to the perfusion fluid containing the [¹⁴C]inulin and MTX in an amount to provide 64 to 250 nCi (not over 0.040 μg/ml) perfusion fluid. Finally, the pH was adjusted to 7.3 to 7.4 and the solution filtered through a 0.22-μm Millipore filter. The final ionic concentration of the MTX perfusion solution contained at least an additional Na⁺ content of 16 mEq/liter because the clinical dosage form of MTX contained Na⁺ in the form of Na₂MTX [Na⁺ (4 mEq/liter) in Na₂MTX (1 mg/ml)] and NaCl (12 mEq/liter) and an unspecified amount of NaOH. In addition, the clinical dosage form of MTX used in these experiments contained 64 μg methylparaben and 16 μg propylparaben per mg MTX as preservatives.

Scintillation Counting. Aliquots of the collected effluent were counted as doubly labeled samples in a Nuclear-Chicago Model 6860 Mark I liquid scintillation counter. For reduction of sources of counting error in inulin measurement caused by insolubility of inulin in the scintillation solvent, samples were treated in 0.1 ml, 1% sodium peridate in 1 M formic acid. Phenethylamine (0.2% by volume) was added prior to the scintillation fluid to keep the MTX in solution.

Calculation of Drug Dosage. The total quantity of drug introduced into the CSF was determined by taking into account perfusate concentration, inflow rate, and perfusion duration. Drug dosage is designated in terms of mg/kg (6) and mg/sq m based on body surface area values for the
monkey given by Spector (28).

Pharmacological Recording. An 8-channel Schwarzer polygraph Physioscript was used to monitor a number of pharmacological responses: electroencephalograms were recorded in a number of experiments by bipolar recording from scalp needle electrodes over the left and right parietal areas; surface electrodes (Beckman) were used to monitor the electrocardiogram; respiratory rate was measured by a Schwarzer displacement transducer strapped to the chest or abdomen. Rectal temperature was obtained before and after perfusion by a thermistor and Waters electronic thermometer.

Behavioral Analysis. Full-scale behavioral studies were carried out with 2 monkeys. The animals were trained in a visual pattern discrimination task reinforced by the avoidance or escape of an electric shock. For each session, the monkeys were seated in a Foringer primate chair in a sound-reducing enclosure in the presence of white noise.

Stimulus and response arrangements consisted of the display of a visual pattern behind each of 2 response panels located to the left and right at the monkey’s eye level and reached by its left or right hand; the program presented occasions for a reinforced response on a trial-by-trial basis.

The program was divided into several stages reflective of the level of training: Stage 1, acquisition of panel response; Stage 2, the acquisition of trial and avoidance responding; and Stage 3, discrimination. Discrimination was considered learned when the monkey reached 36 of 40 trials in a single session.

Events were controlled and recorded by means of solid-state switching equipment (BRS-Foringer System 7). Counts of principal events were displayed on counters.

An effort was made to test behaviorally those animals being perfused both on the day before and the day after each perfusion. In addition, the animals were tested during 1 session a day as often as possible on other interperfusion days.

Morphological Studies. Experimental animals that were moribund or had lived approximately 6 months from the last CSF perfusion were killed by intracardiac perfusion with 10% buffered formalin. The brain and spinal cord were removed and fixed in formalin. For the gross examination the brains were sliced coronally at 0.5-cm intervals utilizing standard basal landmarks where appropriate. After embedding in paraffin, 10-nm sections were cut and stained with hematoxylin and eosin, hematoxylin and eosin with Luxol fast blue, cresyl violet with Luxol fast blue, and phosphotungstic acid-hematoxylin. Abnormalities exclusive of those related to puncture site were evaluated for site and degree.

RESULTS

General Health Status and Vital Sign Data

During the course of these experiments, perfused monkeys maintained good health status. In general, body weight, food intake, and urine output data, analyzed at weekly intervals, revealed no remarkable fluctuations. An exception was monkey 50-S, perfused with MTX (4.8 mg/ml) which in the course of the perfusion series became anorexic and suffered marked weight loss.

For control perfusions with Elliott’s B solution, final heart rate was increased or decreased by, at most, 18% and respiratory rates varied from 5% increase to a 14% decrease. At the highest concentration of MTX perfused via the VC route, 4.8 mg/ml, final heart rate and respiration were not remarkably affected. Rectal temperature also was not adversely affected during perfusions of either Elliott’s B solution alone or with MTX.

Inulin and MTX Steady-State Outflow-Inflow Concentrations (C1/C0), Retention of MTX, and CSF Formation Rates

A representative VC experiment is presented in Chart 1 to illustrate the time course of events associated with the outflow to inflow concentrations (C1/C0) of MTX and inulin. The mean time for MTX and inulin to reach steady-state conditions of perfusion was approximately 60 min.

During steady-state conditions of perfusion, the average C1/C0 values for MTX were 0.75 ± 0.04 for the VC and 0.66 ± 0.01 for the VL routes; MTX C1/C0 values for the VC and VL routes were significantly different (Mann-Whitney U test, p < 0.05). The average inulin C1/C0 values during MTX perfusions were 0.75 ± 0.02 for each route. The C1/C0 values for MTX and inulin, during perfusion with MTX through the VL route, were significantly different (Wilcoxon matched-pairs signed-rank tests).

The percentage of MTX equivalents recovered per perfusion ranged from 61 to 91% for the VC route; with the exception of 2 monkeys, values ranged from 64 to 73% for the VL route (Table 1). The 2 animals that were exceptions (monkeys 42-P and 53-S) displayed recoveries of 17 and 35% of the perfused MTX.

Average CSF formation rates for the VC space were 29.8 ± 2.0 μl/min during Elliott’s B perfusions without drug and 49.9 ± 5.3 μl/min during MTX perfusions; these rates were significantly different (Mann-Whitney U test, p < 0.05). The average CSF formation rates for the VL space were 45.1 ± 2.5 μl/min during Elliott’s B perfusions without drug and 44.9 ± 2.0 μl/min during MTX perfusions; these values were not significantly different. CSF formation rates obtained
Table 1

Recovery of MTX equivalents during VC and VL space perfusions of monkeys

The amount of MTX perfusion was calculated from the MTX concentration in the perfusion fluid, duration of perfusion, and inflow rate. MTX equivalents were determined using [3H]MTX as a tracer in the perfusion fluid. Percentage of recovery was calculated from the amount of MTX perfused and the amount of MTX equivalents recovered.

<table>
<thead>
<tr>
<th>Route</th>
<th>Monkey</th>
<th>Sex</th>
<th>MTX concentration (mg/ml)</th>
<th>No. of perfusions</th>
<th>Av. Amount of MTX perfused (mg)</th>
<th>Av. MTX equivalents recovered (mg)</th>
<th>% recovery</th>
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<td>50-S</td>
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<td>44-Q</td>
<td>F</td>
<td>2.4</td>
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<td>49-R</td>
<td>M</td>
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<td>24.2</td>
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<td>10</td>
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<td>4</td>
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<td>2.2</td>
<td>35</td>
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<td>59-T</td>
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<td>0.3</td>
<td>10</td>
<td>7.1</td>
<td>4.6</td>
<td>65</td>
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<tr>
<td></td>
<td>56-T</td>
<td>M</td>
<td>0.15</td>
<td>9</td>
<td>3.7</td>
<td>2.7</td>
<td>73</td>
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</table>

During drug perfusions of the VC and VL spaces did not differ significantly.

Gross Central Nervous System Symptomology

Prior to the start of the MTX perfusion series, monkeys were given at least 2 Elliott's B control perfusions. During or following these Elliott's B perfusions, convulsions, tremors, or significant neurotoxic signs were not observed to occur. Tables 2 and 3 contain data pertaining to convulsions, tremors, and motor deficits associated with perfusions of MTX.

Exploratory experiments were done to establish a suitable initial concentration of MTX for use in repeated perfusion trials; data on 3 monkeys, not presented in this report, indicated that convulsions and death resulted when MTX was perfused under pentobarbital anesthesia via the VL route in a concentration of 16 to 8 mg/ml, at a rate of 0.064 μl/min for periods ranging from 140 to 235 min. A 4th monkey, while showing severe convulsions during perfusion at 8.0 mg/ml at the same rate for 163 min, survived.

Table 2

Neuropharmacological responses of monkeys to MTX perfused VC

<table>
<thead>
<tr>
<th>Concentration (mg/ml)</th>
<th>Av. dose*/perfusion</th>
<th>No. of perfusions</th>
<th>Convulsions</th>
<th>Tremors</th>
<th>Motor deficits</th>
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<tr>
<td>4.8</td>
<td>50-S M</td>
<td>47 551</td>
<td>3 0</td>
<td>P&lt;sub&gt;12&lt;/sub&gt;, P&lt;sub&gt;12&lt;/sub&gt; bilateral hypokinesia in arms and legs</td>
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<td>37-O F</td>
<td>2 24</td>
<td>6 0</td>
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</table>

* MTX solutions were freshly prepared in Elliott's B solution; for details of preparation and analysis see "Materials and Methods."

Based on total quantity of drug introduced calculated from perfusate concentration (C<sub>d</sub>), inflow rate, and perfusion duration; body surface area was based on values given by Spector (28).

P<sub>s</sub>, drug perfusion number during or after which the response was observed.
Neuropharmacology of MTX via CSF Space of Monkey

Table 3

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<th>Concentration* (mg/ml)</th>
<th>Av. dose*/perfusion</th>
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<th>mg/kg</th>
<th>mg/sq m</th>
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* MTX solutions were freshly prepared in Elliott's B solution; for details of preparation and analysis see "Materials and Methods.”

* Based on total quantity of drug introduced calculated from perfusate concentration (C<sub>D</sub>), inflow rate, and perfusion duration; body surface area was based on values given by Spector (28).

without residual motor impairment. At the next lowest concentration tested, 4.8 mg/ml, 8 VC perfusions were carried out at the 130-μl/min rate in Monkey 50-S without causing death, but convulsions and motor deficits were observed. According to combined data from VC and VL perfusions, 4 of 11 monkeys convulsed at least once during perfusions with MTX within the concentration ranges of 4.8 mg/ml (~550 mg/sq m) and 0.6 mg/ml (~60 mg/sq m). These neurotoxic signs did not occur during the period in which the animals had been given control perfusions of Elliott’s B solution.

At the highest concentration administered repeatedly via the VC route (4.8 mg/ml), a variety of severe central nervous system symptoms appeared in Monkey 50-S. These will be briefly described in order to establish the extent of neurological disturbances encountered. Fine tremor (10 to 12/sec) was seen during the 1st drug perfusion, and partial paralysis of the hind legs was noted following it, but this symptom had disappeared the following day. Relatively few trains of rapid muscular contractions were observed during the 3rd drug perfusion, but the animal was unable to stand on its legs the following day, with considerable right-sided weakness. During the 4th perfusion, twitching of the left side of the face and left leg was seen and the symptoms continued beyond the end of the perfusion. Spastic quadriplegia followed the 5th perfusion, and this condition gave way to bilateral unsteadiness on the following day which continued throughout the interperfusion period. During the 8th perfusion, spikes appeared on the electroencephalograph record about 12 min from the start, they rapidly became more pronounced, and convulsive motor activity became manifest; the electroencephalograph became flat except for very-low-frequency, low-voltage changes and, appearing moribund, the monkey was killed by intracardiac perfusion with formalin.

In animals that convulsed during or after a perfusion, CSF pressures ranged from 10 to 19 cm H<sub>2</sub>O for the VC route and ~3 to 7 cm H<sub>2</sub>O for the VL route. Average pressures for the same animals when perfusions did not result in convulsions were: VC route, ~6 to 13 cm H<sub>2</sub>O; VL route, ~5 to 15 cm H<sub>2</sub>O. Apparently, convulsions were not necessarily associated with perfusions in which average perfusion pressure was significantly altered.

Residual motor deficits were encountered in 2 monkeys (45-Q, 53-S) perfused with MTX within a concentration range of 1.2 to 0.6 mg/ml. In 1 of these monkeys (53-S), only 1 of 8 perfusions were maintained below a pressure of 30 cm H<sub>2</sub>O, and the average amount of MTX equivalents recovered was considerably less than other animals (Table 1). Development of a residual motor deficit and associated severe leukoencephalopathy noted for this animal may be related to this combination of high perfusion pressure and low MTX recovery.

At concentrations of 0.15 and 0.3 mg/ml, VC- and VL-perfused monkeys did not convulse or develop motor deficits.
Morphological Studies

The brains of 12 monkeys given MTX perfusions and 6 monkeys given 9 to 18 VL or VC perfusions of Elliott’s B solution not containing drug were examined, grossly and histologically, for morphological changes. The brains of 4 unperfused monkeys were similarly prepared and used as a comparative standard. Results were classified with respect to 3 main headings: leukoencephalopathy (absent, mild, moderate, severe), ependymal cell loss, and transventricular fusion. Examination and classification were carried out by F. H. Gilles without knowledge of the dosage schedule.

Unperfused. None of these brains showed leukoencephalopathy or transventricular fusion, but 3 were classified as showing slight ependymal cell loss.

Elliott’s B Solution Perfusion. One brain showed mild white matter degeneration and deep focal gliosis; all had extensive ependymal cell loss. Five (including the one with leukoencephalopathy) showed transventricular fusion; in these 5 cases, the ipsilateral ventricle was stripped of its ependymal surface, and there was widespread transventricular fusion of the layers of subependymal astrocytes. In 1 of these monkeys perfused VC, there was evidence of a focal lesion in the medulla.

Drug-Perfusion Series. Five of these brains (56-T[0.15], 37-O[0.3], 59-T[0.3], 52-S[0.6], 57-T[1.2]) showed no leukoencephalopathy, but each had mild patchy ependymal cell loss, and (37-O, 52-S, 57-T) showed transventricular fusion. Monkey 37-O showed evidence of medulla damage.

Two (49-R[1.2], 50-S[4.8]) showed mild to moderate leukoencephalopathy in the form of white matter damage with astrogliosis and macrophage formation focally dispersed in ipsi- and contralateral hemispheres. These 2 also showed ependymal cell loss and transventricular fusion similar to that seen in the controls. Both showed evidence of medulla damage.

In addition to ependymal cell loss and transventricular fusion, 4 brains (42-P[0.3], 45-Q[0.6], 53-S[1.2], 44-Q[2.4]) showed severe necrotizing leukoencephalopathy with white matter destruction present asymmetrically, periventricularly in both hemispheres. Two of these (42-P, 44-Q) had collapse of the ipsilateral ventricle, but the abnormality extended far into the white matter of the opposite hemisphere. The opposite lateral ventricle was not dilated. The 3rd (53-S) had extensive destruction of white matter particularly severe in the hemisphere ipsilateral to the puncture and, while maximal near the puncture site, extended both frontally and occipitally into neighboring gyri and out to the cortex as well as into the anterior and posterior limbs of the internal capsule and across the corpus callosum. The 4th (45-Q) showed marked thinning of the corpus callosum (particularly the splenium) and bilateral dilation of the occipital horns. There were also multiple islands of macrophages surrounding the trigone. This animal also showed evidence of medulla damage.

The brain of the monkey surviving the single MTX perfusion of 8.0 mg/ml described above showed prominent thinning of the corpus callosum and deep hemispheral white matter damage with mild astrogliosis bilaterally.

Ependymal cell loss and transventricular fusion are clearly associated with the perfusion procedure itself, appearing in 5 of the 6 control brains examined as well as the treated brains, but leukoencephalopathy, especially in severe form, seems to be associated with the additional presence of drug. However, there is little relationship between dose level (shown in mg/ml following the monkey number) and severity of the morphological changes found.

All 6 of the Elliott’s B solution-perfused and 4 of the 5 MTX-perfused monkeys that showed no leukoencephalopathy also displayed no gross symptoms (Tables 2 and 3). However, there was not a clear relationship between the extent of white matter destruction and the development of grossly detectable impairment, monkeys 42-P and 44-Q remaining free of lasting motor deficit and monkey 50-S showing the most severe motor symptoms of all.

Behavioral Response. Behavioral data for 2 additional monkeys not included above (128-FF and 129-FF) perfused with MTX (0.6 mg/ml) are presented in Chart 2. The performance of Monkey 128-FF was at about the 50% level prior to, during, and following drug perfusions. The performance of Monkey 129-FF improved during the treatment period suggesting that the drug did not substantially impair the acquisition of the discrimination. However, this monkey convulsed during the 6th drug perfusion.

Behavioral response of monkeys to a visual pattern discrimination program reinforced by the avoidance or escape of an electric shock. Animals were perfused VL with MTX at a concentration of 0.6 mg/ml. Dosage (mg/kg) was calculated from the total amount of drug administered [concentration of perfusate (mg/ml) x duration of perfusion (min) x inflow rate (ml/min)]. See Table 3 for other neuropharmacological responses.

Chart 2. Behavioral response of monkeys to a visual pattern discrimination program reinforced by the avoidance or escape of an electric shock. Animals were perfused VL with MTX at a concentration of 0.6 mg/ml. Dosage (mg/kg) was calculated from the total amount of drug administered [concentration of perfusate (mg/ml) x duration of perfusion (min) x inflow rate (ml/min)]. See Table 3 for other neuropharmacological responses.
DISCUSSION

In the studies reported in this paper, steady-state levels of MTX were obtained in the CSF space of the monkey approximately 60 min after starting VC or VL perfusions. Within a reasonable time limit, artificial CSF fluid containing relatively high concentrations of MTX were perfused through the CSF space. Perfusions of MTX (0.15 mg/ml; 3.3\times10^{-4} \text{ M}) did not cause gross symptoms of neurotoxicity or severe neurological lesioning; this concentration of MTX is significantly higher by several orders of magnitude than that required effectively to sterilize L1210 leukemia and KB cells in vitro (31) and, based on data of Bleyer et al. (3), it is significantly greater than the geometric mean concentration of MTX found in the CSF of nontoxic patients treated i.t. with 12 to 15 mg/sq m.

The mean steady-state $C_\text{V}/C_\text{T}$ value of 0.66 for MTX perfused via the VL route differed significantly from the value of 0.75 for inulin. This is in agreement with the findings of Rubin et al. (24) and suggests that a small fraction of the MTX was leaving the VL space by 1 or more pathways different from inulin due presumably to the larger molecular weight and the inherent greater negativity of the [carboxy-14C]inulin molecule (15). Rubin et al. (23) have suggested that [3H]MTX is actively transported from CSF to blood, but they inferred from the similarity of the MTX and inulin curves that active transport may be of minor clinical significance at such levels (24).

The somewhat larger $C_\text{V}/C_\text{T}$ values for MTX in the monkey in contrast to the rabbit reported by Rubin et al. (23) may reflect a species difference, although perfusion speed and MTX concentration differences are also possible causes for these discrepancies. The average $C_\text{V}/C_\text{T}$ value of 0.75 for inulin during MTX perfusions of monkeys is in agreement with the values reported by Rubin et al. (23) for rabbits during VC perfusions with MTX. The percentage of MTX equivalents recovered during VC and VL drug perfusions is in accord with the data on 1 patient (M. B.) presented by Rubin et al. (24).

Extreme alterations in vital signs, i.e., heart rate, respiration, and body temperature, were not observed to occur during perfusions. A number of chemical, biophysical, biochemical, and physiological factors may be responsible for this lack of effect, e.g., inability of MTX to interact with specific receptors located within regulatory sites; however, it is also reasonable to suppose, based on the findings of Levin et al. (13) and Altman and Chorover (1) that MTX did not diffuse from the ventricular spaces into regulatory centers in sufficient depth and/or concentration adversely to affect respiration, cardiac activity, or body temperature.

It is difficult to interpret the precise role of the MTX formulation used in producing damage to brain tissue and the toxicological symptoms observed. Marked white matter destruction and associated symptomatology seem to be related to the drug or the drug plus perfusion rather than the perfusions alone, since they do not appear singly or in combination in the control and lowest-dose animals. The greater effect of MTX compared with mechanical aspects of multiple perfusions is also suggested by the significant white matter destruction found in the brain of the animal surviving a single perfusion at a dose level of 8.0 mg/ml. The occurrence of severe motor symptoms in the animal receiving the highest dose with multiple perfusions and the absence of extensive brain damage suggest the possibility that MTX can have a direct effect not mediated by structural change. The pattern of development of symptoms in this animal argues against attributing them to medulla damage.

On the other hand, morphological changes were found that can be attributed to mechanical aspects of the perfusions. The occurrence of transventricular fusion in both control and treated animals suggests the possibility that the perfusion inflow rate, selected to maximize dosage without greatly exceeding the formation rate, may still have been too high, and a better compromise might be achieved with a lower rate. Extensive white matter damage was found in the brain of 1 monkey perfused with the relatively low concentration of 0.3 mg/ml and in another perfused at 0.6 mg/ml. In assessing the neurotoxicity of ventriculally perfused MTX, morphological changes already cited, as well as such lesions as subarachnoid hemorrhage and intracerebral hematoma found in some of the animals, caution should be exercised against ruling out the possible contribution of mechanical aspects of the perfusions.

Weiss and Raskind (32) reported damage in human brains after local treatment with MTX. Evidence that brain lesioning is associated with intraventricular instillation of MTX has also been presented by Shapiro et al. (26) and Bresnan et al. (4). On the basis of the topographic pattern of the lesions observed in their group of patients, Shapiro et al. (26) suggested that toxic concentrations of MTX may have accumulated in the perriventricular tissue as a result of the presence of a preexisting ventricular obstruction. The topographically irregular distribution of the non-dose-dependent leukoencephalopathy seen in some of the MTX-perfused monkey brains in the present study might be related to the regional loss of ependymal cells and transventricular fusion resulting from the perfusion procedure itself. Bleyer et al. (3) encountered severe neurotoxicity in 5 of 25 patients, but elevated levels of MTX in the CSF could not be accounted for by subarachnoid blockage.

Recently, Spector (29) reported that MTX decreased rather than increased the incidence of chemically induced seizures in rats by possibly causing a deficiency of some metabolic products of folic acid. Hommes and Obbens (9) showed that sodium folate is a strong convulsant in normal and epileptic rats, and Roberts (22) found that MTX had an effect similar to that of folate in inhibiting the uptake of glutamate by rat dorsal root ganglia. While it would seem likely on the basis of present evidence that such mechanisms as these may underlie the seizures seen in MTX perfusions, other possibilities to account for such convulsions may be considered. It is possible that the excess of Na\(^{+}\) in the clinical formulation of MTX may be a contributory factor, since the perfusion fluid containing this formulation is nonphysiological in ionic composition, especially at high dose levels.

Phencyclidine, when used i.v. in relatively large doses of 15 mg/kg, can cause convulsions in nonhuman primates (5).
as well as skeletal muscle hypertonicity and burst discharges from muscle tremors (12). However, in our experiments the dose of phencyclidine was administered i.m. and was significantly below the convulsive threshold. In other studies, MIX has been observed to cause convulsions in man (3, 11) as well as laboratory animals (32) in the absence of phencyclidine. Saiki et al. (25) suggested that the preservatives methylparaben and propylparaben in clinical samples of MTX may be the causative agents in the development of motor deficits in man. This suggestion is based somewhat on the findings of Nathan and Sears (18) that methyl hydroxybenzoate can block nerve conduction in concentrations of 0.1 and 0.2%. However, Bleyer et al. (3) have reported neurotoxicity in patients treated with preservative-free MTX, and in our laboratories 4 monkeys did not develop symptoms of neurotoxicity following perfusions of Elliott's B solution containing methylparaben and propylparaben in concentrations ranging from 1.9 to 3.8 mg/ml and 0.5 to 1.0 mg/ml, respectively.

Behavioral performance of monkeys was not adversely affected by MTX perfusions at 0.6 mg/ml. One monkey showed continuing improvement in a visual pattern discrimination task reinforced by electric shock. Successful performance of this task presumably requires intact sensory, motor, integrative, memory, and other higher systems.

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Neuropharmacological Effects of Methotrexate Perfused through the Cerebrospinal Fluid System of the Rhesus Monkey


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