Active Transport of Methotrexate from Cerebrospinal Fluid in Humans

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ABSTRACT

The cerebrospinal fluid (CSF) efflux kinetics of methotrexate (MTX) were studied in three patients with indwelling Ommaya reservoirs. A small dose of MTX was injected intraventricularly several hr after the start of a high-dose continuous i.v. infusion of MTX. In all patients, the CSF antifolate concentration returned to the preinjection level before the end of the i.v. infusion. This result indicated that the efflux of MTX from CSF in humans is independent of plasma drug concentrations. Efflux kinetics were further characterized in one patient. Serially obtained CSF samples after intraventricular injections demonstrated a biphasic disappearance curve with α- and β-phase half-disappearance times of 1.7 and 6.6 hr, respectively. Prolongation of the β-phase half-time was associated with oral acetazolamide medication and with increased intracranial pressure, indicating that inhibition of CSF production slows MTX clearance. CSF MTX concentration, however, declined more rapidly than that of simultaneously administered diethylenetriaminepentaacetic acid, an extracellular marker substance excreted by bulk flow, indicating that bulk flow excretion alone is insufficient to account for MTX efflux from human CSF. Evidence that there is an active transport component was provided by probenecid pretreatment which also prolonged the CSF MTX half-life. These findings suggest that both passive and active mechanisms govern MTX efflux from the CSF in humans and that they can be inhibited by acetazolamide and probenecid, respectively.

INTRODUCTION

Effective chemotherapeutic treatment of neoplastic meningeal disease is dependent upon the achievement of sufficient drug concentrations for an appropriate period of time. Inadequate permeability of the blood-CSF barrier is frequently an obstacle to successful therapy with i.v. drug administration. In addition, with both i.v. and intrathecal drug administration, the peak CSF concentration is dependent upon the kinetics of efflux from the CSF of the agent in question. In the case of MTX, the CSF concentration is more than 20-fold lower than the systemic concentration during i.v. therapy (2). Since it is known that rapid decrease of CSF concentration is observed after intrathecal medication (9), we decided to study the influence of both concomitant systemic MTX infusion and certain drugs on the efflux of intraventricularly administered MTX.

MATERIALS AND METHODS

Three patients, aged 8, 12, and 17, were treated for recurrent meningeal infiltration with leukemia or lymphoma. They had previously received intrathecal MTX and cranial irradiation. Systemic chemotherapy consisted of vincristine, prednisone, Adriamycin, cyclophosphamide, and 42-hr i.v. MTX infusions. At the time of these studies, they had mild malignant pleocytosis (<100 WBC/μl) but normal CSF pressure and chemistry (glucose, protein, and LDH). Each patient had an Ommaya reservoir in place, and informed consent had been obtained. MTX, 0.5 or 1 mg, was injected intraventricularly several hr after the commencement of an i.v. MTX infusion, consisting of 300 mg/sq m during the first and 60 mg/sq m for each of the following 41 hr. Serial plasma and CSF (1 ml) samples were obtained before and after the injection, and the MTX concentrations were determined by the dihydrofolate reductase inhibition assay (1). In order to elucidate the influence of drugs on the efflux rate of MTX from the CSF, several studies were carried out in each of which 0.5 mg of MTX in 1 ml of Elliott B solution was injected into the reservoir of one patient. In these studies, the CSF cell count, sugar, protein, LDH, and pressure (160 to 270 mm H2O) did not change throughout each 48-hr study period. The studies at increased intracranial pressure were performed during the terminal phase of this patient when brain edema developed. Prior to each study, CSF MTX was undetectable (i.e., 1.0 × 10^-8 M). Starting with the lowest MTX concentration, the MTX exponential disappearance slopes were calculated using linear regression analysis and a least-squares fitting technique (r > 0.95) developed for the IBM 370/168 digital computer (7).

In one study, 100 μCi of 111In-labeled DTPA were injected simultaneously with MTX to evaluate CSF flow and to have an extracellular marker substance for kinetic comparisons. Radiouclide imaging was performed 4 and 24 hr after injection. In addition, DTPA efflux was measured by counting 0.1 ml of each CSF sample in a Packard Auto Gamma scintillation spectrometer. The CSF distribution of intraventricularly injected substances was also examined by performing lumbar punctures 6 and 48 hr after injection and assaying for MTX and radioactivity as above. The contribution of CSF turnover was studied by measuring MTX efflux (a) during p.o. acetazolamide administration (625 mg/sq m/day from 6 hr before to 30 hr after an intraventricular MTX injection) and (b) in the presence of intracranial pressure above 570 mm CSF. Probenecid, an inhibitor of renal MTX transport (3), was used in 3 different studies. Doses of 1250 and 2500 mg/sq m/day p.o. were given from 3 hr before to 39 hr after and from 8 hr before to 16 hr after a MTX injection. Under the conditions of increased intracranial pressure and premedication with 2500 mg/sq m/
day from 18 hr before to 36 hr after, the injection, MTX efflux was studied. The concentrations of probenecid in CSF and serum were determined by gas chromatography (6).

RESULTS

The MTX concentrations in plasma and CSF were measured before and after intraventricular MTX injections during 5 i.v. MTX infusions in 3 patients (Table 1). Before MTX was injected, CSF MTX concentration was 3 to 5% of the concomitant plasma concentration. After MTX was injected into the reservoir, the CSF MTX concentration promptly increased 100-fold and declined rapidly despite high plasma MTX levels. Preinjection CSF MTX values were reached in less than 15 hr (Chart 1). In 2 trials, single half-disappearance times ($t_1^+$) for MTX were 2.4 and 2.5 hr.

Single intraventricular injections of 0.5 mg MTX resulted in concentrations between $1.0 \times 10^{-5} \text{ M}$ and $4.0 \times 10^{-5} \text{ M}$ MTX in the ventricular CSF 30 min later, and MTX concentrations declined in a biexponential manner over the subsequent 48 hr. Having calculated a $\beta$ phase of MTX efflux (14 to 48 hr), the initial ($\alpha$) phase of MTX disappearance (0 to 14 hr) was obtained using the stripping technique (11). Mean calculated intercepts and $t_1^+$ of MTX were derived for the various conditions (Table 2). MTX disappearance showed $\alpha$ and $t_1^+$'s of 1.7 and 6.6 hr, respectively. The $\beta$ $t_2^+$ was prolonged to 7.3 hr (12%) with acetazolamide mediation and to 7.9 hr (20%) in the presence of increased intracranial pressure. The $\alpha$ and $t_1^+$'s for DTPA were 1.7 and 8.9 hr, the latter being 34% longer than the $\beta$ $t_2^+$ of MTX measured simultaneously. Lumbar CSF obtained 6 and 48 hr after injection showed slightly higher concentrations than in ventricular CSF at both time points (Chart 2), and radionuclide scan confirmed the absence of mechanical obstruction to CSF flow.

A daily dose of probenecid, 1250 mg/sq m, did not change the efflux kinetics of intraventricularly injected MTX. Administration of 2500 mg/sq m for a short time interval resulted in a longer $\beta$ $t_2^+$ of MTX of 7.7 hr (16%) (Chart 3). Under these conditions, probenecid was undetectable in the CSF sample taken at the time of MTX injection, whereas CSF samples obtained 9 and 17 hr after MTX administration contained 3.4 $\mu$g of probenecid per ml. The same dose of probenecid was administered for an extended period of time during the phase of raised intracranial pressure and resulted in CSF probenecid concentrations of 2.4 to 3.3 $\mu$g/ml. Under these conditions, there was an even more protracted disappearance of MTX with an $\alpha$ $t_2^+$ of 2.7 and a $\beta$ $t_2^+$ of 12.6 hr.

DISCUSSION

The observation that during a constant i.v. infusion of MTX the concentration achieved in the CSF is only 3 to 5% of the plasma level can be explained by either poor penetration of MTX across the blood-CSF barrier, or MTX efflux from the CSF being considerably more rapid than influx, or both. Our finding that MTX injected into the lateral ventricle leaves the CSF compartment rapidly even in the presence of a high plasma concentration of MTX supports the latter possibility and excludes simple diffusion as the major transport mechanism.

To confirm that bulk flow excretion is one component of MTX efflux from the CSF, we studied MTX kinetics under conditions...
Table 2
Disappearance $t_{1/2}$ for MTX from ventricular CSF after intraventricular injection

<table>
<thead>
<tr>
<th>Intracranial pressure</th>
<th>Drug injected</th>
<th>Concomitant medication</th>
<th>$t_{1/2}$ (hr)</th>
<th>Intercept</th>
<th>$t_{1/2}$ (hr)</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF (180–270 mm)</td>
<td>MTX DTPA</td>
<td>Probenecid 1250 mg/sq m</td>
<td>1.7</td>
<td>2.2 x 10^{-5} M</td>
<td>6.6</td>
<td>1.4 x 10^{-5} M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Acetazolamide (625 mg/sq m)</td>
<td>1.7</td>
<td>2.4 x 10^2 cpm</td>
<td>8.9</td>
<td>2.7 x 10^2 cpm</td>
</tr>
<tr>
<td>CSF (&gt;570 mm)</td>
<td>MTX</td>
<td>Probenecid (2500 mg/sq m)</td>
<td>1.6</td>
<td>2.8 x 10^{-5} M</td>
<td>7.9</td>
<td>1.2 x 10^{-5} M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.7</td>
<td>1.9 x 10^{-5} M</td>
<td>12.1</td>
<td>6.0 x 10^{-6} M</td>
</tr>
</tbody>
</table>

of altered bulk flow. Acetazolamide medication and raised intracranial pressure decrease CSF production (5) and therefore bulk flow excretion. In both cases, the half-life of MTX disappearance from the CSF was prolonged. Thus, excretion via the arachnoid granulations, as anticipated, is one component of MTX efflux.

The distribution of DTPA in the CSF is similar to that of MTX as indicated by identical $t_{1/2}$'s and slightly higher lumbar than ventricular concentrations (Chart 2) of both drugs. Since, however, the $t_{1/2}$ of MTX is shorter than that of DTPA, MTX excretion from the CSF cannot be accounted for by bulk flow alone.

Direct confirmation of an active transport mechanism was obtained by showing a prolonged $t_{1/2}$ for MTX efflux in the presence of probenecid. This is consistent with animal studies indicating that MTX efflux from the CSF can be reduced with probenecid (8, 10). The studies with more prolonged probenecid administration during the period of increased intracranial pressure showed greater delay of MTX clearance, suggesting that high p.o. doses of probenecid for a prolonged period of time are needed to affect the MTX efflux significantly and that the active transport mechanism is not obliterated by raised intracranial pressure.

Although the patients studied had central nervous system disease and also had received intrathecal therapy and cranial irradiation which might influence transport phenomena, MTX excretion kinetics in our patients when unperturbed by pharmacological intervention were similar to those reported by others (9).

These data indicate that the efflux of MTX from human CSF is the combined result of bulk flow excretion and an active transport mechanism. The observation of delayed drug excretion with raised intracranial pressure stresses the need for the reappraisal of the MTX dosage in patients with this finding. The fact that intraventricularly administered MTX is rapidly eliminated from the CSF in the presence of high systemic concent-
trations shows the independence of MTX CSF efflux pharmacokinetics from systemic drug concentrations. The potential therapeutic advantage of delaying MTX efflux from the CSF by inhibitors of CSF secretion (acetazolamide) and/or active transport (probenecid) is worthy of further exploration.

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REFERENCES

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