Effects of Single-Dose and Fractionated Cranial Irradiation on Rat Brain Accumulation of Methotrexate

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ABSTRACT

The effects of single-dose and fractionated whole-brain irradiation on brain methotrexate (MTX) have been studied in a rat model. The amount of MTX present in the brain 24 hr after a single i.p. dose (100 mg/kg) was the same whether animals were sham irradiated or given a single dose of 2000 rads 6 or 48 hr prior to the drug (6.9, 8.3, and 6.8 pmol MTX/g, wet weight, respectively). Animals sham irradiated or given 2000 rads in 10 fractions over 11 days and treated with an average dose of 1.2 mg MTX/kg i.p. twice a week for 24 weeks did not differ significantly in their brain MTX concentration (7.9 and 8.3 pmol MTX/g, wet weight, respectively). Chronically MTX-treated animals became folate deficient whether they were irradiated or not (450 and 670 pmol folate/g, wet weight, brain in MTX-treated and control animals). Thus, MTX accumulates in the brain with acute or chronic administration, and this accumulation is not altered by this amount of brain irradiation.

INTRODUCTION

For the past 20 years, the antifol MTX3 has been a mainstay in the treatment of ALL of childhood (20). MTX is used as one of the 2 major systemic maintenance agents. It remains the drug of choice for intrathecal treatment and prophylaxis against CNS leukemia. The combined use of cranial irradiation and intrathecal MTX has lowered the incidence of CNS disease from approximately 80% to 5% in children with ALL. The difficulties of this regimen become apparent when one examines its potential morbidity. MTX alone can cause a degenerative leukoencephalopathy, and when combined with irradiation the incidence and severity of the encephalopathy may increase (1). In addition to its use in treatment of CNS leukemia, several groups have given high-dose systemic MTX for the treatment of intracranial neoplasms, without concomitant use of irradiation, in the hopes of achieving therapeutic successes without the encephalopathy (2, 13, 17). This treatment plan is based upon a report that mice receiving a single dose of 2000 rads and MTX (100 mg/kg i.p.) had brain MTX concentrations of 25 nm while mice receiving lower doses of radiation or not irradiated had no detectable drug in the brain (5). Thus, by giving the MTX first, it was hoped that CNS toxicity would be minimized. The assumption is that in the absence of irradiation there is less penetration of MTX into the brain.

The present report documents the lack of increase of intracranial MTX after fractionated cranial irradiation followed by low-dose chronic drug administration or after a single dose of irradiation followed by a single high dose (100 mg/kg) of drug. These findings, although different from those of Griffin et al. (5), are in keeping with the work of Levin et al. (3, 11) with respect to the general lack of an effect of irradiation on the net brain accumulation of other compounds such as urea and a number of anticancer drugs such as bleomycin and VM-26, each with a differing capacity to cross the blood-brain barrier.

MATERIALS AND METHODS

Rats were males from a WAG/RijMCW breeding colony of defined-flora animals as described by Moulder and Martin (14). Screening has shown the absence of Mycoplasma pulmonis and common viruses. All animal work, including irradiation and drug treatment, was performed in a barrier facility. To minimize activation of hepatic microosomal enzymes, all rats were housed on autoclaved hardwood bedding, and insecticides were not used in the facility. At the time of initiating our experiments, the animals weighed approximately 150 g and would be considered adolescent.

Cranial Irradiation. For fractionated cranial irradiation, animals were treated with parallel opposed lateral fields using a 250-kV beam with an half-value layer of 2 mm aluminum and a dose rate of 200 rads/min. Animals were tranquilized with diazepam kindly supplied by D. Petke, Roche Laboratories. The entire head was irradiated above the roof of the mouth and forward of a point 5 mm caudal of the base of the skull. The field provided shielding of the salivary glands. For single-dose irradiations, unsedated animals were confined to Plexiglas jigs and treated with a single posteroanterior field using a 270-kV beam with a half-value layer of 3 mm copper and a dose rate of 45 rads/min. The jigs restricted movement of the animals to less than 2 mm, and lead shielding on the jigs limited the body dose to less than 1% of the cranial dose. The irradiated field covered the entire head forward of a point 12 mm caudal of the base of the skull. Control animals were drugged or jigged (as appropriate) and placed in the treatment area. Radiation dosimetry was done in Plexiglas phantoms using a Keilby Model 135614 radiation dosimeter and a Farmer-type ionization chamber.

MTX and Folate Assay. The MTX content of the brain was determined using the radioligand-binding assay for MTX described previously (8) after the brain tissue was extracted as detailed below. The assay has an absolute sensitivity of 5 x 10⁻¹⁴ mol MTX (and polyglutamates) using [³H]MTX, 15 to 20 Ci/mmol, at a 35% counting efficiency and is essentially non-cross-reactive with significant endogenous folates (8–10). Folates were determined by radioassay using a folate binder present in β-lactoglobulin (Sigma Chemical Co., St. Louis, MO) in a sequential type of procedure (21). This assay yields total folates in tissues, plasma, and spinal fluid equivalent to the standard microbiological assay (Lactobacillus casei) and is unaffected by the presence of MTX.

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2 Supported by National Cancer Institute Grant CA 34840. Present address: Department of Pediatrics, University of Southern California, Los Angeles Children's Hospital, Los Angeles, CA.
3 The abbreviations used are: MTX, methotrexate; ALL, acute lymphoblastic leukemia; CNS, central nervous system.
4 B. A. Kamen, unpublished observation.

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In order to ensure that the MTX in the brain was not due to contaminating blood in the brain extract, the volume of blood in the brain (v/w) was determined by using the 51Cr-labeled erythrocytes (4). The amount of MTX in blood was determined, and this concentration was subtracted from that found in the brain extract.

**Tissue Extraction.** The brains were rapidly removed after the animals were anesthetized with CO2 and sacrificed by cardiac puncture exsanguination (in general, 50 to 60% of the estimated blood volume was removed by cardiac puncture). The brains were rinsed in cold 0.9% NaCl solution, blotted dry, weighed, and then homogenized in 3 volumes (w/v) of 0.05 mM Tris, pH 8.0, containing 0.01 M EDTA and 0.15 M mercaptoethanol using a Brinkman polytron (setting 5, 30 sec on ice). Aliquots of the homogenate were then counted to determine their 51Cr content or spun at 27,000 × g for 30 min at 4°. The supernatant was boiled for 10 min and centrifuged at 12,000 × g for 5 min to further deproteinize prior to assaying for MTX. Samples were refrigerated overnight and assayed the next day. Assays of spiked untreated brain homogenates show an extraction efficiency of 85%. It is important to note that, if the brains were not homogenized and extracted immediately, there was a high (2 to 5 pmol/g) and variable background in untreated animals. The nature of this material is not yet known, but it may be oxidized folate or pyridine nucleotide which could inhibit dihydrofolate reductase. When the tissue is extracted as described, the background (assay of untreated brain) does not account for more than 5% of the MTX found in the treated animals (see "Results").

**RESULTS**

In one series of experiments, the timing and dose of MTX as well as the irradiation was identical to those described by Griffin et al. (5). Specifically, the animals received 2000 rads to the whole cranium in a single dose and then were given MTX (100 mg/kg i.p.) 6 or 48 hr after the treatment. All animals were sacrificed 24 hr after the dose of MTX, and the brains were analyzed for drug (Table 1). There was detectable MTX in the brains of the control animals. Furthermore, these values are not significantly different from those for the irradiated animals (p > 0.1). The amount of MTX in the brain is the 0.5 to 1% of that found in the liver and kidneys of these animals. The amounts of MTX in the brain due to blood were 0.38, 0.72, and 0.28 pmol/g wet weight, brain in the control, 6-hr, and 48-hr animals, respectively. These values are less than 10% of the total brain MTX and reflect the 1% blood volume (v/w) of the brain as determined by the 51Cr labeling technique.

Clinically, most irradiation is given in fractionated doses. In the treatment of ALL, this is followed by chronic low-dose systemic MTX weekly for up to 30 months. Therefore, we also examined brain MTX of animals that received 2000 rads in 10 fractions over 11 days and were treated with 0.5 to 2 mg MTX/kg twice weekly for 24 weeks beginning on the first day of irradiation. At the starting dose of 2 mg/kg/dose, the animals became anemic (hematocrit decreased from 45 to 50% to 30 to 35%). MTX doses were decreased when anemia became severe and were increased when the hematocrit recovered. On 3 occasions (13.5, 20.5, and 21.5 weeks), no drug was given. The lowest 2-week cumulative MTX dose used was a total of 1 mg/kg given during Weeks 20 and 21. The mean dose of MTX during the 24 weeks of treatment was 1.2 mg/kg/dose.

The brain content of MTX is presented in Table 2. The amount of MTX in the brain is comparable to that found in animals given a single high dose of irradiation and one high dose of drug (Table 1). As in the previous experiment, there was no significant difference in the total amount of MTX found in the brain regardless of cranial irradiation (p > 0.1). As in the single-dose experiment, the blood MTX concentration did not appreciably alter the brain MTX content of the whole-brain extract. In a similar experiment, we saw also no significant difference in brain MTX between the irradiated and control animals following 13 rather than 24 weeks of MTX (data not presented).

In an attempt to more completely define a mechanism for MTX toxicity, we measured the folate content of the rat brains in the chronically treated group (Table 2). There was no difference in the brain folate levels in the radiation-plus-MTX and MTX-alone animals, but both groups had brain folate levels significantly lower than those of the control animals. The whole-blood folate concentration was also decreased in the MTX-treated animals, whether or not the animals received irradiation.

**DISCUSSION**

Combined modality therapy and prophylaxis of CNS leukemia using cranial irradiation and intrathecal MTX has dramatically lowered the incidence of CNS disease, but the morbidity of this therapy is also well acknowledged (1). The mechanism of the toxicity is less well understood. Clinical experience showing the generally low levels of MTX in the cerebrospinal fluid following systemic administration and the increased incidence of toxicity when irradiation and intrathecal or systemic MTX are used in combination has led to the commonly accepted notion that combined therapy carries a higher risk of morbidity than does single-agent therapy. Based upon clinical observations, the incidence of CNS disease is significantly lower when cranial irradiation and intrathecal MTX are used in combination than when either modality is used alone. The mechanism of this action of cranial irradiation is not clearly defined, but a decrease in the brain MTX content has been demonstrated (5). The objective of the present study was to determine whether or not cranial irradiation decreases the brain MTX content using both single high dose and fractionated dose schedules of irradiation and MTX.

**Table 1**

Effect of a single large dose of radiation on MTX accumulation in the brain

Animals received drug 6 or 24 hr postirradiation. Brain blood volume was determined using the 51Cr-labeled RBC.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Brain MTX (pmol/g, wet wt)</th>
<th>Fraction of brain volume that was blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTX only</td>
<td>6</td>
<td>6.9 (6.8-8.0)</td>
<td>0.010 (0.008-0.012)</td>
</tr>
<tr>
<td>RADS + 6 hr - MTX</td>
<td>6</td>
<td>8.3 (6.7-9.9)</td>
<td>0.010 (0.009-0.011)</td>
</tr>
<tr>
<td>RADS + 24 hr - MTX</td>
<td>6</td>
<td>6.8 (6.6-8.6)</td>
<td>0.009 (0.008-0.010)</td>
</tr>
<tr>
<td>Controls</td>
<td>6</td>
<td>-0.3 (-0.3)</td>
<td>0.009 (0.008-0.010)</td>
</tr>
</tbody>
</table>

*MTX, 100 mg/kg i.p.; RADS, 2000-rad single dose to entire head.

**Table 2**

Effect of fractionated irradiation (2000 rads in 10 fractions) on brain accumulation of MTX given on a twice-weekly schedule for 24 weeks

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Brain MTX (pmol/g, wet wt)</th>
<th>Brain folate (pmol/mg)</th>
<th>Blood folate (pmol/ml)</th>
<th>Fraction of brain volume that was blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTX only</td>
<td>6</td>
<td>7.9 (7.0-8.8)</td>
<td>450 (370-510)</td>
<td>890 (360-1780)</td>
<td>0.013 (0.009-0.017)</td>
</tr>
<tr>
<td>RADS and MTX</td>
<td>6</td>
<td>6.3 (7.0-9.6)</td>
<td>440 (390-500)</td>
<td>750 (430-1070)</td>
<td>0.011 (0.009-0.013)</td>
</tr>
<tr>
<td>Controls</td>
<td>7</td>
<td>0.2 (-1.7)</td>
<td>670 (800-740)</td>
<td>1800 (1550-2080)</td>
<td>0.013 (0.008-0.018)</td>
</tr>
</tbody>
</table>

*MTX, average dose 1.2 mg/kg i.p., twice weekly, 24 weeks; RADS, 2000 rads in 10 fractions.

b Numbers in parentheses, 95% confidence interval.
teraction of irradiation and MTX in causing a leukoencephalopathy has generally been thought to be related to the enhancement of MTX uptake by the brain as a consequence of the irradiation (12, 15, 16). The laboratory data for this observation were derived primarily from experiments in which mice received a single large dose of irradiation (2000 rads) and a single large i.p. dose of MTX (100 mg/kg). There was no detectable drug in the brain unless the animals had received cranial irradiation; then, the brain MTX content was approximately 25 pmol/g brain 24 hr after the dose of drug was given. These data (and the desire to develop more effective chemotherapy) have led to the use of systemic MTX in high doses prior to the use of irradiation under the assumption that there will be insignificant uptake of MTX by the brain, or at least no synergistic, toxic effect of drug and irradiation.

Recent work from our laboratory (9, 10) shows that MTX on a weekly or single-dose schedule results in the formation of MTX polyglutamates in the brain and that these derivatives have a half-life of greater than 1 month. These data tend to nullify the presumed safety of giving MTX prior to irradiation. The work presented here confirms the uptake of MTX by brain tissue in a second strain of rats and also demonstrates this uptake in 3 different drug and irradiation schedules. Whether the difference in these results from those of Griffin et al. (5) is due only to the species of animal tested cannot be said with certainty. Other relevant studies also show conflicting results. As noted, Levin et al. (3, 11) found little effect of cranial irradiation of Fischer rats on the transport of urea, galactitol, NaCl, glycosmin, or VM-26. Seshadri et al. (18) found that the presence of CNS disease (lymphoma and leukemia patients) rather than cranial irradiation altered the blood-brain barrier to MTX. Siemes et al. (19) showed a progressive 2-fold increase in total cerebrospinal fluid protein in a pattern that was suggestive of blood-brain barrier disruption in children with ALL treated with 1800 to 2400 rads and intrathecal MTX. These authors stated that the significance of the abnormal protein pattern and whether these changes could parallel a change in permeability to chemotherapeutic agents were not known.

In addition to emphasizing that irradiation does not significantly influence the brain content of MTX, it should be stressed that the effect of MTX polyglutamate tissue stores in brain (or other tissue) subsequently irradiated is not known. It is possible that pretreatment with MTX could increase or decrease sensitivity to ionizing irradiation.

In an effort to determine a mechanism for MTX damage to the CNS, we measured brain and blood folate. The chronically MTX-treated animals became folate deficient. These data are in keeping with our earlier clinical observations of low folate concentrations in liver and RBC from children receiving weekly MTX as part of continuation therapy for ALL (6, 7). Although it is taught that MTX induces a functional deficiency of reduced folates by blocking the reduction of oxidized forms in mitotically active cells, the data here suggest that MTX accumulation may be at the expense of cellular folate pools. Therefore, chronic drug administration could also result in an absolute folate deficiency in normal tissues. Since folate deficiency, whether caused by genetic or acquired mechanisms, is associated with neurological dysfunction, the vitamin deficiency resulting from MTX therapy may have a role in the leukoencephalopathy associated with its use.

REFERENCES

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