Serum and Cerebrospinal Fluid Distribution of 5-Methyltetrahydrofolate after Intravenous Calcium Leucovorin and Intra-Ommaya Methotrexate Administration in Patients with Meningeal Carcinomatosis

B. M. Mehta, J. P. Glass, and W. R. Shapiro

ABSTRACT

Serum and cerebrospinal fluid (CSF) concentrations of citrovorum factor (CF) and 5-methyltetrahydrofolic acid (5-MTHFA) were measured after i.v. infusion of leucovorin (50 or 100 mg/sq m) in seven patients undergoing treatment for meningeal carcinomatosis by intra-Ommaya reservoir injection of methotrexate (MTX). Serum CF levels rapidly rose after leucovorin administration as did 5-MTHFA, its conversion product. A small amount of CF entered the CSF, but peak CSF 5-MTHFA increased about 10-fold. The concentration × time (C × t) of additional 5-MTHFA in the CSF was greater [114.4 ± 36.1 (S.E.) μg/ml × min] after 100-mg/sq m doses of leucovorin than after 50 mg/sq m [14.2 ± 4.3 μg/ml × min] (p < 0.05). The CSF MTX concentration exceeded 5-MTHFA by 2 to 3 logs throughout the 48 hr of observation, while serum 5-MTHFA and CF exceeded serum MTX by 0.5 to 2 logs. This study demonstrates that leucovorin administered i.v. to patients receiving intra-Ommaya MTX does not increase CSF concentrations of "rescue" folate above those of CSF MTX and are unlikely to interfere with MTX action against meningeal tumor. On the other hand, i.v. leucovorin does permit serum "rescue" folate to operate, thus reducing the systemic toxicity that may follow intraventricular administration of MTX.

INTRODUCTION

In previous studies, Shapiro et al. (20) determined that intraventricular administration of MTX via Ommaya reservoirs assured higher concentrations of the drug in the CSF than could be obtained when MTX was administered via lumbar puncture. This was confirmed by Bleyer et al. (4), who also demonstrated that the Ommaya reservoir route of administration was a more effective form of therapy for meningeal leukemia than was lumbar puncture administration. After i.v. administration of MTX, the plasma concentration of MTX rapidly falls, and its entry into the nervous system from the blood is limited by the blood-brain barrier (3, 20). However, once MTX has entered the brain, the central nervous system becomes a reservoir from which MTX may leak out continuously. The resulting low but persistent blood levels can prove to be damaging to bone marrow. Shapiro et al. (20) showed that MTX leaking from the CSF produced continuous low serum levels for 2 days. Bleyer et al. (4) found that MTX levels could persist in serum for up to 4 days. Prolonged low concentrations of MTX in the systemic circulation can be more toxic than are short-duration high concentrations (6).

In therapy with high-dose MTX administration, patients are usually given leucovorin (leucovorin, DL-5-formyltetrahydrofolate, calcium salt), the most stable form of the reduced folates (5, 11), in order to reverse the effect of systemic MTX (7, 12) and to "rescue normal cells." While leucovorin itself may reverse the MTX, it is also probable that the conversion product of leucovorin, 5-MTHFA, the naturally occurring serum folate, also contributes to the rescue. 5-MTHFA has been found to be the form in which folates enter the nervous system (9), probably via the choroid plexus (22).

We had considered administering leucovorin systemically to patients undergoing intra-Ommaya MTX treatment of meningeal carcinomatosis, in order to protect the bone marrow from the effects of the persistent low serum MTX concentrations. We were concerned, however, about its conversion into 5-MTHFA and the entry of 5-MTHFA back into the nervous system where it might block the effectiveness of the MTX. We therefore studied the distribution kinetics of MTX, CF, and 5-MTHFA in the serum and CSF of patients with meningeal carcinomatosis who received MTX via Ommaya reservoir and, simultaneously, leucovorin via i.v. infusion.

MATERIALS AND METHODS

Patients. Seven patients were studied (Table 1). All patients had meningeal carcinomatosis (23) and were undergoing therapy of this disease with combined whole-brain radiation therapy and chemotherapy via Ommaya reservoirs. Ommaya reservoirs were placed soon after diagnosis, and most patients received either MTX alone or MTX plus 1-β-D-arabinofuranosylcytosine by protocols published previously (23).

MTX and Leucovorin Administration. MTX (Lederle Laboratories, Pearl River, N. Y.) as the sodium salt in 0.9% sodium chloride solution (2.5 mg/ml) was administered as described previously (20). The drug was made up in a small amount of sterile water without preservative and placed in a 10-ml syringe. The Ommaya reservoir was entered via a 23-gauge scalp vein needle, and 3 ml of spinal fluid were withdrawn and set aside. The 10-ml syringe was connected and the plunger was gradually withdrawn, filling the syringe with the patient's own CSF. The MTX was then administered over a 5-min period. Leucovorin (Lederle Laboratories), available as the calcium salt without added preservative, was dissolved in 250 ml of 0.9% sodium chloride solution and administered i.v. at a dose of either 50 or 100 mg/sq m. The 50-mg/sq m dose approximates the average daily p.o. dose given to patients receiving high-dose i.v. MTX. The dose was doubled in a second group to determine serum and CSF folate concentrations at the higher dose.
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Table 1

Patient characteristics and MTX and leucovorin doses

All patients had meningeal neoplasm metastatic from the stated primary tumor. An Ommaya reservoir was placed in each patient. MTX was given into the reservoir (except Patient LG); leucovorin was infused i.v. over the indicated time.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (yrs)</th>
<th>Primary tumor</th>
<th>MTX mg/sq m</th>
<th>Total mg</th>
<th>Leucovorin mg/sq m</th>
<th>Total mg</th>
<th>Time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MG</td>
<td>F</td>
<td>66</td>
<td>Breast</td>
<td>6.25</td>
<td>10.0</td>
<td>50</td>
<td>72.5</td>
<td>5.25</td>
</tr>
<tr>
<td>LG</td>
<td>F</td>
<td>51</td>
<td>Breast (1)</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>63.0</td>
<td>4.0</td>
</tr>
<tr>
<td>WM</td>
<td>M</td>
<td>49</td>
<td>Mycosis fungoides</td>
<td>6.25</td>
<td>11.5</td>
<td>50</td>
<td>92.0</td>
<td>3.25</td>
</tr>
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<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT</td>
<td>F</td>
<td>60</td>
<td>Breast</td>
<td>7.0</td>
<td>11.9</td>
<td>100</td>
<td>170.0</td>
<td>4.5</td>
</tr>
<tr>
<td>BS</td>
<td>M</td>
<td>66</td>
<td>Melanoma</td>
<td>7.0</td>
<td>10.0</td>
<td>100</td>
<td>142.0</td>
<td>5.25</td>
</tr>
<tr>
<td>DD</td>
<td>F</td>
<td>59</td>
<td>Breast</td>
<td>7.0</td>
<td>12.0</td>
<td>100</td>
<td>170.0</td>
<td>4.25</td>
</tr>
<tr>
<td>EN</td>
<td>M</td>
<td>43</td>
<td>Lymphoma</td>
<td>7.0</td>
<td>12.4</td>
<td>100</td>
<td>175.0</td>
<td>5.0</td>
</tr>
</tbody>
</table>

The infusion time for the leucovorin varied from 3.25 to 5.25 hr, as shown in Table 1.

Sampling. Venous blood samples (5 ml) were drawn at 0, 1, 2, 4, 6, 8, 20, 24, and 48 hr for assay. Blood was allowed to clot at room temperature, and the serum was separated by centrifugation. Ascorbic acid (sodium salt) (1 mg/ml) was added to the serum, and the samples were immediately stored at −20°C. The ventricular CSF samples were obtained simultaneously with the blood samples by previously described procedures (20), sodium ascorbate (1 mg/ml) was added, and the samples were stored immediately at −20°C until assay.

Assay Procedures. Determination of MTX in the serum and in the CSF was carried out by an enzyme titration method using dihydrofolate reductase as described by Sirotnak and Donsbach (21). Determination of CF was carried out by the microbiological disc assay method described by Mehta and Hutchison (14) using MTX-resistant Pedicoccus cerevisiae as the assay organism. Determination of 5-MTHFA was carried out by a differential microbiological disc assay procedure (15) using MTX-resistant strains of Lactobacillus casei and Streptococcus faecium var. durans.

RESULTS

Table 1 presents the patient characteristics and MTX and leucovorin doses administered. In the first 3 patients (Group 1), MTX was given at 6.25 mg/sq m, and leucovorin was given at 50 mg/sq m. In the next 4 patients (Group 2), MTX was given at 7 mg/sq m, and leucovorin was given at 100 mg/sq m. Patient LG received an infusion of leucovorin without MTX and the next day was given MTX along with a second leucovorin infusion. By measuring CF and 5-MTHFA without and with MTX, we could determine whether the CF and 5-MTHFA assays were affected by the presence of MTX, either in CSF or in blood. Chart 1 displays decay curves from Patient LG who received leucovorin (50 mg/sq m). Shown are the CSF and serum MTX, CF, and 5-MTHFA concentrations. Chart 2 depicts similar curves from Patient DD who received leucovorin (100 mg/sq m). As noted previously (13, 16), leucovorin is converted to 5-MTHFA, and the serum concentrations of the 2 compounds approach each other rapidly. However, little CF entered CSF; the concentrations of CF in CSF usually represented less than 1% of the corresponding concentration in the serum.

The MTX concentrations in the CSF fell exponentially in each patient from approximately 250 µg/ml (5 × 10⁻⁴ M) to approximately 2 to 2.5 µg/ml (4 to 5 × 10⁻⁶ M) over a 48-hr period in a manner similar to that reported previously (4, 20). The 3-phase half-lives averaged 0.5, 2.5, and 6 hr, respectively. The serum MTX concentration generally rose to a maximum of approximately 0.05 µg/ml (1 × 10⁻⁷ M) where it remained for the duration of the experiment (48 hr). Serum CF concentrations rose to approximately 0.5 µg/ml (1 × 10⁻⁶ M) and then declined rapidly after the conclusion of the leucovorin infusion, falling below 0.005 µg/ml (1 × 10⁻⁸ M) within 24 hr. Serum 5-MTHFA levels increased pari passu with the CF levels and remained above normal levels for approximately 24 hr before declining toward normal. CSF concentrations of 5-MTHFA rose quickly in the first hours of the infusion from their preinfusion concentrations of 0.01 to 0.05 µg/ml (2 to 10 × 10⁻⁸ M) to reach a peak of 0.1 to 0.3 µg/ml (2 to 6 × 10⁻⁷ M) and then gradually fell over the next 12 to 15 hr to return to normal levels. In all cases, CSF MTX concentrations remained greater than those of CSF 5-MTHFA throughout the course of the experiment. At the same time, 5-MTHFA levels in serum remained above the serum MTX levels, and the combined CF plus 5-MTHFA levels in the serum well exceeded those of the serum MTX concentrations.

Table 2 lists the C x t determinations for MTX, CF, and 5-MTHFA in serum and CSF for the low-dose (Group 1) and the high-dose (Group 2) leucovorin study. In each patient, the C x t for MTX in the CSF exceeded that of the CSF 5-MTHFA. In contrast, the C x t of MTX in the serum was well below that of the C x t for CF plus the C x t for 5-MTHFA. Doubling the dose of leucovorin generally increased the serum and CSF CF C x t and 5-MTHFA C x t, although the large variation among the concentrations meant that only some of the increases were statistically significant (Table 2).

DISCUSSION

These results confirmed our original findings (20) that, following intra-Ommaya administration, MTX concentration within the CSF persists above the "therapeutic concentration" (1 × 10⁻⁶ M) for at least 36 hr. The results also supported the observation that leucovorin is rapidly and substantially metabolized into 5-MTHFA. Similar increases in serum 5-MTHFA have been reported by other investigators (2, 8, 13, 17). Nixon and Bertino (16) found that radiolabeled leucovorin administered p.o. was substantially metabolized during the transfer from the gastrointestinal tract to the systemic circulation, and 90% of the serum folate was identifiable by chromatography.
as 5-MTHFA. Mehta et al. (13) studied serum distribution of CF and 5-MTHFA after p.o. and i.m. administration of calcium leucovorin in normal adults and found 92 to 93% of the total reduced serum folate to be in the form of 5-MTHFA at 30 min. In the present study, our data demonstrate that, at the end of the i.v. infusion of leucovorin, about 50% of the total reduced folate in the serum assayed as 5-MTHFA. These findings confirm those of Nixon and Bertino (16), who observed that, 90 min after i.v. administration of leucovorin, about 40% of the serum folate was still in the form of the administered radiolabeled leucovorin.

It is also evident from our results not only that little leucovorin enters CSF but, more importantly, that its metabolite 5-MTHFA enters to only a modest degree after high-dose i.v. leucovorin administration. After the usual i.t. dosage, MTX concentrations remain 1 to 3 orders of magnitude higher than the highest 5-MTHFA level achieved in the CSF. While the relationships between CSF (and serum) concentrations of MTX and 5-MTHFA and their tissue concentrations have not been determined in vivo, systemic administration of the usual doses of leucovorin are unlikely to raise CSF 5-MTHFA levels enough to interfere with the action of MTX administered i.t. In contrast, the serum levels of both CF and 5-MTHFA exceeded the serum levels of MTX during the entire period. It has now become our practice to administer leucovorin p.o. (or sometimes parenterally) routinely in any patient who suffers mucositis or bone marrow depression following intra-Ommaya MTX administration. The present investigation indicates that such a procedure does not interfere with the effectiveness of MTX in the CSF, at least for doses of leucovorin not exceeding the equivalent of 100 mg/sq m i.v. over a 4- to 5-hr infusion. In unpublished observations, we have noted that leucovorin given p.o. or i.m. does not increase 5-MTHFA in the CSF of such patients. Furthermore, the study on Patient LG failed to show interference by MTX either in the metabolic conversion of CF to 5-MTHFA or in the cross-over of 5-MTHFA into the CSF.

Finally, a question about the possible effectiveness of leucovorin in the prevention of central nervous system toxicity must be raised in view of our findings that CF acutely increases CSF 5-MTHFA only modestly and then only transiently. Previous studies examined CSF folates in patients on anticonvulsant medications (18); such patients may have reduced serum and CSF folic acid concentrations. Adding folic acid p.o. increases serum folate but not CSF folate, since folic acid is not converted to 5-MTHFA, and therefore CSF folates do not increase (10). On the other hand, chronic administration of leucovorin does increase CSF total folates (10). It is possible that prolonged administration of leucovorin would increase CSF 5-MTHFA levels, although this has not actually been attempted. Long-term administration of leucovorin might protect the central nervous system against MTX toxicity, and such a procedure would be worth testing. In a recent review, Shapiro et al. (19) noted that there may be a relationship between folic acid metabolism in the brain and one or more of the several forms of MTX central nervous system toxicity. Abelson (1) has suggested that transient encephalopathy may be related to MTX inhibition of biopterin metabolism. Although this might be possible for the transient encephalopathy, it seems unlikely to
TABLE 2

MTX, CF, and 5-MTHFA concentrations in serum and CSF

<table>
<thead>
<tr>
<th>Patient</th>
<th>Total time of study (hr)</th>
<th>MTX C x t</th>
<th>CF C x t</th>
<th>Total 5-MTHFA C x t</th>
<th>MTX C x t x 10^7</th>
<th>CF C x t</th>
<th>Total 5-MTHFA C x t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MG</td>
<td>24.0</td>
<td>55.7</td>
<td>273</td>
<td>&lt;0.005</td>
<td>458</td>
<td>451</td>
<td>6.8</td>
</tr>
<tr>
<td>LG1</td>
<td>25.25</td>
<td>114</td>
<td></td>
<td>&lt;0.006</td>
<td>405</td>
<td>396</td>
<td>1.0</td>
</tr>
<tr>
<td>LG2</td>
<td>48.25</td>
<td>96.2</td>
<td></td>
<td>0.033^a</td>
<td>345</td>
<td>250</td>
<td>14.0</td>
</tr>
<tr>
<td>WM</td>
<td>47.25</td>
<td>40.3</td>
<td>264</td>
<td>0.015</td>
<td>408</td>
<td>364</td>
<td>2.7</td>
</tr>
<tr>
<td>Mean ± S.E.</td>
<td>186.6 ± 47.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

| Group 2 |                         |           |         |                     |                   |         |                     |
| MT      | 48.0                    | 58.0      | 991     | <0.005              | 1595              | 1581    | 4.6                 |
| BS      | 48.0                    | 62.7      | 441     | <0.005              | 344               | 379     | 3.4                 |
| DD      | 49.0                    | 74.7      | 462     | 0.060^d             | 964               | 788     | 6.8                 |
| EN      | 48.0                    | 69.6      | 507     | 0.024               | 1221              | 1152    | 5.2                 |
| Mean ± S.E. | 600 ± 131            |           |         |                     |                   |         |                     |

| t test ^b | p < 0.05 | NS^a | NS | p < 0.05 |

Notes:
- C x t is in μg/ml x min.
- The "total" 5-MTHFA was determined by measurement. The "additional" 5-MTHFA equaled the total 5-MTHFA minus the product of the average base-line value times the duration, i.e., the "normal" 5-MTHFA C x t.
- Average value: zero = 0.037; terminal = 0.028.
- Average value: zero = 0.018; terminal = 0.012.
- Average value: zero = 0.023; terminal = 0.015.
- Average value: zero = 0.070; terminal = 0.048.
- Average value: zero = 0.002; terminal = 0.005.
- Between means of each group.
- NS, not significant.

account for the chronic encephalopathy associated with long-term MTX administration.

ACKNOWLEDGMENTS

We wish to thank Dr. Dorris J. Hutchison for her helpful suggestions and Ann L. Gisolfi for technical assistance.

REFERENCES

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