ABSTRACT

A total of 15 patients with advanced neoplastic disease, 13 with different solid tumors, one with lymphoma, and one with acute lymphocytic leukemia, underwent treatment consisting of continuous infusion of methotrexate (2 g/sq m/day) with concomitant thymidine (8 g/sq m/day) and leucovorin (1 mg/sq m/day). The dose of methotrexate was increased progressively by lengthening the methotrexate infusion from 2 to 7 days. After cessation of methotrexate infusion, thymidine and leucovorin were continued until the plasma level of methotrexate decreased to $2 \times 10^{-8}$ M. Toxicity was mucositis (23 of 27 evaluable courses), leukopenia (15 of 26 evaluable courses), thrombocytopenia (10 of 26 evaluable courses), renal and hepatic toxicity and diarrhea. Plateau levels of plasma methotrexate or methotrexate plasma half-life did not correlate with toxicity.

INTRODUCTION

The clinical use of high-dose MTX with CF rescue is well established (5, 13). CF by supplying a preformed reduced folate cofactor circumvents all the major metabolic effects of MTX. The important deficiencies in growing mammalian cells resulting from a reduction of available reduced folate are the inhibition of thymidylate and of de novo purine biosynthesis (15). Supplying mammalian cells with dThd and a source of preformed purine will abolish the toxicity of MTX (9–11). Cells in which dThd can partially reverse the toxicity of MTX, the reversal of MTX toxicity by CF in the presence of dThd remains competitive. These observations form the basis of the present protocol. If the effect of MTX in tumor cells is not reversed by dThd, then the reversal of MTX toxicity by CF in these cells should remain competitive, and low levels of CF should have no effect in reversing the effect of high levels of MTX. On the other hand, since dThd partially reverses the toxicity of MTX in the bone marrow and the gut, a very low dose of CF (1 mg/sq m/day) should be sufficient to reverse the effect of high-dose MTX (2 g/sq m/day) in these tissues and thus prevent the major manifestations of MTX toxicity. Preliminary reports of this study have been published (3, 4).

MATERIALS AND METHODS

Chemicals. The MTX, CF, and dThd [formulated as 15 g dThd in 500 ml of 0.6% (v/v) sodium chloride solution] were obtained from the Investigational Drug Branch, Division of Cancer Treatment, National Cancer Institute.

Patients. A total of 15 patients, 8 women and 7 men, with a median age of 52 years (range, 28 to 72 years) were entered into the study. All had had prior chemotherapy (13 patients with polychemotherapy) and 4 patients had had radiotherapy. Diagnoses consisted of adenocarcinoma of the colon, 4 patients; osteosarcoma, 2 patients; and one patient each with acute lymphocytic leukemia, histiocytic lymphoma, and a variety of solid tumors. To be eligible for entry into the study, all patients were required to have a confirmed histological diagnosis of cancer, a minimum life expectancy of 2 months, a WBC of >4,000/cu mm, and a variety of solid tumors. To be eligible for entry into the study, all patients were required to have a confirmed histological diagnosis of cancer, a minimum life expectancy of 2 months, a WBC of >4,000/cu mm, and a platelet count of >100,000/cu mm, a total bilirubin of <1.2 mg/dl, aspartate aminotransferase of <50 IU, serum creatinine of <1.3 mg/dl, and creatinine clearance of at least 80 ml/min prior to initiation of therapy. Minimum half-life of MTX for up to 3 days with the concomitant administration of dThd. MTX toxicity was controlled, and antitumor activity was retained (6). There are data that indicate that the effect of dThd can be enhanced by low-dose CF. The reversal of MTX toxicity by CF is normally competitive, but in cells in which dThd can partially reverse the toxicity of MTX, CF reversal of MTX toxicity becomes noncompetitive in the presence of dThd (2). Thus, small amounts of CF are as effective in preventing toxicity to high levels of MTX as they are to low levels if dThd is added to the system. On the other hand, in cells in which dThd does not reverse the toxicity of MTX, the reversal of MTX toxicity by CF in the presence of dThd remains competitive. These observations form the basis of the present protocol. If the effect of MTX in tumor cells is not reversed by dThd, then the reversal of MTX toxicity by CF in these cells should remain competitive, and low levels of CF should have no effect in reversing the effect of high levels of MTX. On the other hand, since dThd partially reverses the toxicity of MTX in the bone marrow and the gut, a very low dose of CF (1 mg/sq m/day) should be sufficient to reverse the effect of high-dose MTX (2 g/sq m/day) in these tissues and thus prevent the major manifestations of MTX toxicity. Preliminary reports of this study have been published (3, 4).
**Phase I Study of High-Dose MTX/dThd/Low-Dose CF**

**Table 1**

**Toxicity and plasma levels of high dose continuous infusion MTX**

<table>
<thead>
<tr>
<th>Patient</th>
<th>MTX infusion (days)</th>
<th>CF (mg/day)</th>
<th>WBC nadir (cu/mm x 10^3)</th>
<th>Platelet nadir (cu/mm x 10^3)</th>
<th>Pretreatment</th>
<th>Maximum</th>
<th>Plasma MTX plateau level (x 10^-5 M)</th>
<th>Plasma MTX terminal phase t1/2 (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0.9</td>
<td>1.1</td>
<td>4.02 ± 0.75&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43.2</td>
</tr>
<tr>
<td>2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3.7</td>
<td>0.8</td>
<td>0.9</td>
<td>4.2 ± 0.6</td>
<td>45.3</td>
</tr>
<tr>
<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>4.02 ± 0.75&lt;sup&gt;d&lt;/sup&gt;</td>
<td>43.2</td>
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<tr>
<td>2</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>2.6</td>
<td>0.7</td>
<td>1.3</td>
<td>2.2 ± 0.48</td>
<td>45.3</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>3.8</td>
<td>0.9</td>
<td>1.0</td>
<td>2.99 ± 0.36</td>
<td>45.3</td>
</tr>
<tr>
<td>5</td>
<td>4</td>
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<td>1</td>
<td>0.7</td>
<td>0.8</td>
<td>1.1</td>
<td>2.99 ± 0.76</td>
<td>45.3</td>
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<tr>
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<td>4</td>
<td>2</td>
<td>1</td>
<td>1.4</td>
<td>0.9</td>
<td>0.9</td>
<td>3.98 ± 0.49</td>
<td>45.3</td>
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<tr>
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<td>0.7</td>
<td>2.4</td>
<td>1.4 ± 0.32</td>
<td>45.3</td>
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<tr>
<td>7</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>1.1</td>
<td>0.8</td>
<td>1.1</td>
<td>1.81 ± 0.49</td>
<td>45.3</td>
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<tr>
<td>8&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>2</td>
<td>1</td>
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<td>0.9</td>
<td>0.9</td>
<td>3.64 ± 0.63</td>
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<tr>
<td>5&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>4.57 ± 0.58</td>
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<tr>
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<td>3.65 ± 0.76</td>
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<tr>
<td>2&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>1</td>
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<td>0.6</td>
<td>0.6</td>
<td>3.65 ± 0.76</td>
<td>45.3</td>
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<tr>
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<td>1</td>
<td>0.7</td>
<td>1.0</td>
<td>1.0</td>
<td>3.65 ± 0.76</td>
<td>45.3</td>
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<tr>
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<td>1</td>
<td>1</td>
<td>0.7</td>
<td>0.9</td>
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<td>3.65 ± 0.76</td>
<td>45.3</td>
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<td>7</td>
<td>1</td>
<td>2.7</td>
<td>1.4</td>
<td>1.4</td>
<td>5.27 ± 1.11</td>
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<tr>
<td>13&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>7</td>
<td>1</td>
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<td>0.8</td>
<td>0.8</td>
<td>3.2 ± 0.92</td>
<td>45.3</td>
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<tr>
<td>15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>2.7</td>
<td>0.9</td>
<td>0.9</td>
<td>3.2 ± 0.92</td>
<td>45.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> Grading of mucositis: 1. redness only or mild ulceration, no impairment in food intake; 2. redness and moderate ulcerations, food intake slightly impaired; 3. food intake markedly impaired, parenteral feeding and/or analgesics required.

<sup>b</sup> Determined by fitting of the data to a 2- or 3-compartment open model by nonlinear least squares regression analysis using the program NONLIN (14) on a UNIVAC 90/60 computer. Choice of model was based on statistical evaluations of both models of the data for each patient.

<sup>c</sup> Leucovorin was given after discontinuation of dThd infusion.

<sup>d</sup> dThd infusion was interrupted for 12 hr on Day 7.

<sup>e</sup> Twenty-four-hr value not included because plateau value not reached at 24 hr.

<sup>f</sup> Platelet count, <100,000/cu mm)

**RESULTS**

A total of 27 courses were administered to 15 patients. Oral mucositis was noted in 23 of 27 courses. Mucositis was always present when the MTX infusion lasted over 4 days. Leukopenia (WBC <4000/cu mm) was the major toxicity occurring in 15 of 26 evaluable courses (Table 1) (one patient with acute lymphocytic leukemia was not evaluable for bone marrow toxicity). WBC below 2000/cu mm were noted in 4 courses. The duration of leukopenia was 1 to 18 days with a median of 6 days. Thrombocytopenia (platelet count, <100,000/cu mm) was less severe being noted in 10 of 26 evaluable courses with a median duration of 5 days (Table 1). There were no hemorrhagic complications of thrombocytopenia.

Five patients developed transient rises in serum creatinine (serum creatinine >1.5 mg/dl) which returned quickly to normal. Other toxicities included mild nausea and/or vomiting in 6 of 27 courses and diarrhea in 18 of 27 courses, in some patients persisting for up to 1 week after MTX administration. Elevation of aspartate aminotransferase up to twice the pretreatment level was noted in 2 patients. The toxic manifestations appeared usually in a sequential and overlapping pattern with mucositis from Day 4 through Day 16 and bone marrow depression from Day 9 through Day 22. Renal toxicity appeared at random from as early as Day 2 to a few days after the MTX administration was completed. An early hemoglobin drop (>2 g/dl) occurred in 6 of 26 courses.

Plateau plasma levels of MTX were reached in 17 of 19 courses in which it was evaluable by 24 hr after the start of the infusion. The mean plateau was 3.42 ± 1.18 (S.D.) x 10^-5 M. The postinfusion MTX decay in plasma was rapid at first, falling with a half-life of 3 to 9 hr. In courses in which sufficient data points were available to evaluate terminal phase half-life, this was prolonged in all cases with a range of 38.4 to 582 hr with a median value of 71.4 hr. The most important metabolite of MTX, 7-OHMTX, was measured in the urine of 2 patients (Patient 2 and Patient 10) on Days 1 and 7 of a 7-day infusion by the method of Jacobs et al. (12). The metabolite represented

10^-4 M. The rate of infusion was regulated with a constant speed IVAC infusion pump. Doses were modified as follows: MTX dose was increased progressively by lengthening the MTX infusion by daily increments from 2 days up to 7 days. When the infusion duration of MTX reached 7 days, the CF daily dose was reduced (Table 1) and in 3 patients was omitted (one of these, Patient 14, received only 5 days of a planned 7-day course because of renal function impairment).

Two or more patients were treated at each dose level. If only moderate toxicity was noted, these 2 patients were placed at the next escalation dose. Treatment courses were repeated every 3 weeks or after recovery from toxicity. Patients were followed with daily WBC, creatinine, blood urea nitrogen, and liver function tests. Daily MTX serum levels, measured by the enzymatic method of Zakrzewski and Nichol (19) as modified by Berlino (1), were obtained in all patients. Urine pH was checked every time the patient voided and was maintained at 7 or greater by administering additional sodium bicarbonate i.v. as needed.

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3.5 and 1.2%, respectively, of the urinary concentration of MTX on Day 1 and 2.8 and 5.3% on Day 7. In one patient receiving [3H]MTX, the concentration of 7-OHMTX rose from 6.4% of the total radioactivity 0 to 12 hr after the end of infusion to 30% in the 24 to 30 hr specimen. It is noteworthy, however, that by this time total content of radioactivity was only 16% of that of the 0- to 12-hr specimen. Thus, the relatively high percentage of 7-OHMTX in late urine samples reflects merely low solubility and thus delayed excretion of 7-OHMTX.

**Antitumor Effect.** Although designed as a Phase I study, overall antitumor activity was noted in 4 of 15 (26%) patients. In 2 patients with metastatic colon adenocarcinoma, one (Patient 3) with metastatic liver disease had a 30% reduction in liver size (using the sum of liver measurements below each costal margin at the midclavicular line and xiphoid process) and a 20% decrease in pelvic mass (product of perpendicular diameters), and the other (Patient 11) had a 30% reduction of a metastatic sternal mass (product of perpendicular diameters). A patient with breast adenocarcinoma (Patient 9), metastatic to the chest wall, had a 50% reduction in tumor size, and another patient with Stage IV histiocytic diffuse lymphoma (Patient 10) had over 50% reduction of a neck mass.

Responses were short lived, lasting 8, 4, 12, and 2 weeks, respectively. In addition, one patient with colon cancer metastatic to liver (Patient 2) remained stable for 7 months, and a patient with complete bowel obstruction due to ovarian cancer was relieved of obstruction but only for 10 days (Patient 8). This was documented by flat and upright abdominal films and also by contrast studies.

**DISCUSSION**

This study represented an attempt to demonstrate the possibility in humans of giving prolonged high-dose continuous infusion in an attempt to reverse toxicity without reversing antitumor effect of MTX. The prolonged exposure was thought to be advantageous to allow more of the cells of tumors with low-growth fractions to come into cycle and be exposed to the lethal effects of the drug. The study demonstrated the possibility of doing this and gave hints of activity against tumors not normally responsive to MTX. However, this was achieved at the expense of considerable logistic difficulty. The patients had to be monitored very closely to prevent the urine from becoming acidic, the dTTh continuous infusion had to be maintained in some cases for more than 2 weeks, and because of its very short half-life it could not be safely interrupted for longer than a brief period.

Toxicity on this regimen was tolerable when the fact that these patients had had in most cases very extensive prior treatment is considered. However, it is clear that, at this dose, MTX is toxic even in the presence of dTTh and low-dose CF when infused for more than 4 days. The effect of omitting CF is difficult to evaluate because of the small numbers of patients. Neither plateau levels of plasma MTX during infusion nor plasma half-life of MTX predicted for toxicity. The reason for this is probably the fact that the study was designed for maximum patient safety and called for dTTh infusion to be continued until plasma MTX was below $2 \times 10^{-8}$ M. Thus, prolonged half-lives were compensated for by longer dTTh infusions. Probably for the same reason, renal function impairment did not cause any apparent increase in MTX toxicity.

**REFERENCES**

Phase I Study of High-Dose Methotrexate with Thymidine and Low-Dose Leucovorin

Salvador Bruno, Gerald Grindey, Sigmund Zakrzewski, et al.


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