Clinical and Pharmacological Studies of Methotrexate–Minimal Leucovorin Rescue plus Fluorouracil

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ABSTRACT

The sequential combination of methotrexate (MTX) followed by 5-fluorouracil (5-FUra) was evaluated. We treated 26 patients with 101 courses of high-dose MTX and minimal leucovorin rescue plus 5-FUra. MTX, 1.0 g/sq m, was administered as an 18-hr infusion. Three doses of leucovorin were given i.v. at Hours 30, 36, and 42 following the start of the MTX infusion. Leucovorin dosages were individualized based upon the plasma MTX clearance of each patient. 5-FUra, 600 mg/sq m, was given as a rapid i.v. bolus at Hour 9 after the start of the MTX infusion. The 5-FUra administration time was later changed to Hour 20, 2 hr following the completion of the MTX infusion, in order to more closely approximate effective preclinical sequencing schedules. The 5-FUra was escalated to 1000 mg/sq m but was subsequently reduced to 800 mg/sq m because of unacceptable toxicity at the highest dose.

The addition of 5-FUra to this high-dose MTX regimen did not alter MTX pharmacokinetics or leucovorin requirements. Median values for peak plasma MTX concentration, plasma MTX half-life, and leucovorin dose did not differ significantly for each 5-FUra schedule. Ninety-four (93%) infusions were associated with rapid clearance, and seven (7.0%) were associated with delayed MTX clearance.

The incidence and severity of toxicity, particularly myelosuppression, were best related to the 5-FUra dose rather than to MTX pharmacokinetics. Seven (58.3%) infusions at a 5-FUra dose of 1000 mg/sq m resulted in severe toxicity. When the 5-FUra dose was decreased to 800 mg/sq m, severe toxicity occurred with only 2 of 11 infusions (18.1%).

One complete response and one partial response were observed. There were two treatment-related deaths.

The clinical feasibility of incorporating 5-FUra in doses ≤800 mg/sq m into a sequential regimen with high-dose MTX and minimal leucovorin rescue has been demonstrated. Further clinical trials to determine the optimum MTX dose and timing of 5-FUra administration are warranted.

INTRODUCTION

The folate antagonist MTX3 and the antipyrimidine 5-FUra are frequently used in combination in the therapy of a variety of advanced tumors (8, 20, 22, 29) and in the adjuvant therapy of operable breast carcinoma (4). Despite widespread use of this combination, relatively little attention has been directed to the clinical consequences of the schedule of drug administration in terms of both tumor response and host toxicity. Preclinical studies examining the effects of various administration schedules on the potential synergy of this combination have yielded conflicting results (1, 3, 5, 9, 14, 24, 28).

Schedule-dependent synergy when MTX preceded 5-FUra was observed in 1974 by investigators in our laboratories who demonstrated inhibition of the immune response in mice (1, 9). Synergy occurred only when MTX preceded 5-FUra, and it was maximal with a time interval of at least 30 min between drugs. Simultaneous and 5-FUra-preceding-MTX schedules produced nonadditive and antagonistic effects. Antitumor schedule-dependent synergy was subsequently observed against the Sarcoma 180 mouse tumor and a mouse mammary tumor (3, 14) with the sequence MTX before 5-FUra, compared to simultaneous and 5-FUra-before-MTX administrations. Conversely, others have shown a reduction in 5-FUra antitumor activity with MTX pretreatment (28).

The use of high doses of MTX followed by the administration of a reduced folate, calcium leucovorin, has undergone extensive clinical trials (2, 10, 11, 13). We have previously reported our clinical and pharmacological studies using high-dose MTX, 1.0 g/sq m, as an 18-hr infusion, followed by minimal leucovorin rescue (27). With this approach, we were able to achieve plasma MTX concentrations of 5 × 10^{-5} M and to minimize the amount of leucovorin administered without excessive myelosuppression or other severe toxicity. The rate of MTX plasma clearance was shown to be a reliable predictor of drug toxicity.

We now report our results observed in patients treated with a similar MTX-minimal leucovorin rescue regimen plus 5-FUra administered either during or subsequent to the MTX infusion.

MATERIALS AND METHODS

Patients. Twenty-six patients received 101 courses of MTX-minimal leucovorin rescue plus 5-FUra. Twenty-four of these had advanced neoplasms, one was treated preoperatively, and another was treated following total resection and local-regional radiation of a soft-tissue sarcoma. The diagnoses are listed in Table 1. The patients ranged in age from 32 to 72 years with a median age of 56. There were 19 males and 7 females. Nine patients had received prior chemotherapy, 2 received prior radiation, 2 received chemotherapy and radiation, and 13 had had no prior therapy.

Pretreatment evaluation included complete blood count and platelet count, electrolytes, creatinine, blood urea nitrogen, liver function tests, urinalysis, 24-hr urine creatinine clearance, and chest roentgenogram. All patients had total leukocyte counts >4,000/cu mm, platelet counts >100,000/cu mm, normal serum creatinine, and a creatinine clearance >60 ml/min prior to each course. Physical examination, body weight, total fluid intake and output, renal function, and blood counts were...
monitored for 48 hr after each infusion. Patients were instructed to return for examination if fever, bleeding, or mucositis developed after discharge.

Toxicity Criteria. Toxicity was considered “mild” in the presence of one or more of the following: total leukocyte count, <4,000/cu mm; platelet count, <100,000/cu mm; stomatitis with ulceration, <25% of the oral mucosa; nausea and vomiting controlled by antiemetics; diarrhea not resulting in dehydration; alopecia; conjunctivitis; or serum creatinine, >2.0 mg/100 ml. Toxicity was graded as “severe” with one or more of the following: total leukocyte count, <2,000/cu mm; platelet count, <50,000/cu mm; stomatitis with ulceration, >25% of the oral mucosa; intractable vomiting; serum creatinine, >4.0 mg/100 ml.

Response Criteria. Complete response was defined as the disappearance of all measurable lesions for at least 4 weeks. Partial response required a ≥50% reduction in the product of the largest perpendicular diameters of one or more measurable lesions lasting for at least 4 weeks without progression in any other lesions present. Progressive disease was defined as a >25% increase in the product of the perpendicular diameters of any measurable lesion or the appearance of a new lesion. Stable disease occurred when there was less than a 50% reduction or less than a 25% increase in measurable disease.

Hydration Program. Hydration (i.v.) with 1 liter 5% dextrose in water containing 100 mg sodium bicarbonate was given every 6 hr starting 12 hr prior to the MTX infusion and was continued until the completion of leucovorin rescue at Hour 42. Urinary pH was maintained at ≥7.0 throughout this period.

Methotrexate. The total MTX dose was 1.0 g/sq m. Ten % of the dose was infused over the first 30 min, and the remainder was infused over the next 17.5 hr. Blood specimens for plasma MTX levels were obtained at 1, 9, 18, 24, 30, 36, 42, and 48 hr following the start of the 18-hr infusion. Plasma levels were determined using a competitive protein-binding assay (19).

Leucovorin Rescue. Only 3 doses of leucovorin (lo-leucovarin; calcium salt; Lederle Laboratories, Pearl River, N. Y.) were administered i.v. at Hours 30, 36, and 42 following the start of the MTX infusion. The leucovorin dose was individualized according to the plasma MTX clearance of each patient. The plasma MTX half-life was calculated from the plasma MTX concentrations at Hours 18 and 24. From these levels, an expected 36-hr MTX concentration was projected. Assuming that comparable MTX and leucovorin doses would yield equivalent peak plasma levels, the leucovorin dose was calculated to give a theoretical plasma concentration 10-fold higher than the projected 36-hr MTX concentration. The projected plasma clearance of MTX was later confirmed by measurement of plasma MTX concentrations at Hours 30, 36, 42, and 48. If actual measured plasma MTX concentrations exceeded the predicted values, indicating delayed excretion, “high-dose” leucovorin (100 mg/sq m i.v. every 6 hr) was indicated. Leucovorin i.v. hydration and urine alkalinization were then continued until the actual plasma MTX concentration fell below 1 x 10^-5 m (7) and the nadir of myelosuppression had passed.

5-FUra. 5-FUra, 600 mg/sq m, was given initially as a rapid i.v. bolus at Hour 9 after the start of the MTX infusion. Hour 9 was selected in order that peak plasma MTX levels would be present at the time of 5-FUra administration (27). The time of 5-FUra administration was later changed to Hour 20, 2 hr following the completion of the MTX infusion, in order to more closely approximate the sequencing schedules in preclinical studies. The 5-FUra dose was then escalated to 1000 mg/sq m but was subsequently reduced to 800 mg/sq m because of unacceptable severe toxicity. The 5-FUra administration schedules are listed in Table 2.

Therapy was repeated at 28-day intervals and was continued until clinical progression of disease. The one patient treated following surgery and radiation completed 12 courses of therapy.

RESULTS

MTX Pharmacokinetics. Rapid drug excretion based upon actual 36- and 48-hr plasma MTX levels was seen in 94 of 101 infusions. Peak plasma concentrations ranged from 1.4 to 8.9 x 10^-5 m, and occurred between Hours 9 and 18. Leucovorin doses ranged from 0.42 to 75.0 mg/sq m.

Median values for peak plasma MTX concentration, plasma MTX half-life, and leucovorin dose did not differ significantly for each 5-FUra administration schedule in this group of 94 infusions with rapid MTX excretion (Table 3). Neither did these values differ from those obtained when a similar MTX schedule was administered alone (27).

In 7 infusions (7.0%), actual 36- and 48-hr plasma MTX levels indicated delayed drug clearance. The median peak MTX concentration, 4.8 x 10^-5 m, was similar to peak values of infusions having normal drug clearance. The projected median MTX plasma half-life was 2.7 hr. The median leucovorin dose based upon projected normal drug clearance was 40 mg/sq m. When actual 30-, 36-, 42-, and 48-hr MTX levels indicated the delay in clearance, high-dose leucovorin was initiated. No cause for delayed MTX clearance was identified after analysis of renal and hepatic function. The frequency with which each 5-FUra dosage schedule was associated with delayed MTX clearance approximates the frequency of utilization of that schedule (Table 6). This suggests that the dose and time of 5-FUra administration did not influence MTX clearance.

Comparison of MTX pharmacokinetic data obtained in this

### Table 1

<table>
<thead>
<tr>
<th>Primary site</th>
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<td>Lung</td>
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<tr>
<td>Gastrointestinal</td>
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</tr>
<tr>
<td>Head and neck</td>
<td>4</td>
</tr>
<tr>
<td>Melanoma</td>
<td>3</td>
</tr>
<tr>
<td>Unknown primary</td>
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</tr>
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</tr>
<tr>
<td>Prostate</td>
<td>1</td>
</tr>
<tr>
<td>Malignant fibrous histiocytoma</td>
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</tr>
<tr>
<td>Total</td>
<td>26</td>
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### Table 2

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<th>5-FUra administration schedules</th>
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<tr>
<td>5-FUra dose (mg/sq m)</td>
</tr>
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</tr>
<tr>
<td>600</td>
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<td>600</td>
</tr>
<tr>
<td>800</td>
</tr>
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</tbody>
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### Table 3

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<tr>
<th>5-FUra pharmacokinetic parameters with different 5-FUra schedules (94 infusions with rapid MTX clearance)</th>
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</thead>
<tbody>
<tr>
<td>5-FUra dose (mg/sq m)</td>
</tr>
<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>600</td>
</tr>
<tr>
<td>600</td>
</tr>
<tr>
<td>800</td>
</tr>
<tr>
<td>1000</td>
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</table>

^a Number of hr after initiation of 18-hr MTX infusion at 1000 mg/sq m.

^b Median.

^c Numbers in parentheses, range.
study with values recorded when the same MTX dosage schedule was administered as a single agent (27) indicates that the addition of 5-FUra does not alter MTX kinetics or leucovorin requirements. In both studies, 93% of infusions were associated with rapid clearance, and 7% were associated with delayed drug clearance. The mean plasma MTX half-life, 2.2 hr, and the mean leucovorin dose, 8.0 mg/sq m, seen when this dosage schedule of MTX is given alone (27), are similar to the values observed when 5-FUra was added to the regimen (Table 3).

Toxicity. No unusual manifestations of toxicity were observed, but toxicities unique and unexpected were frontal headache and alopecia. The remainder consisted of mucositis; a patchy, erythematous, macular rash; conjunctivitis; nausea; vomiting; diarrhea; and myelosuppression (Table 4). The overall incidence and severity of toxicity related more closely to the 5-FUra dose than to the pattern of MTX clearance. When 5-FUra (600 mg/sq m) was given at either Hour 9 or Hour 20, the incidence of drug toxicity was similar to that seen when high-dose MTX was administered alone (27) (Table 5).

The incidence of severe toxicity rose sharply when the 5-FUra dose was escalated to 1000 mg/sq m. Seven of 12 infusions (58.3%) at this 5-FUra dose resulted in severe toxicity. This was due primarily to more frequent leukopenia, WBC <2,000 cells/cu mm (41.7%), and severe stomatitis (50%). When the 5-FUra dose was decreased to 800 mg/sq m, severe toxicity occurred with only 2 of 11 infusions (18.1%). The increased toxicity at the highest 5-FUra dose was statistically significant (compared to 600 mg/sq m, p < 0.005; compared to 800 mg/sq m, p < 0.05).

The increased severe toxicity seen when 5-FUra was given at a dose of 1000 mg/sq m was not due to any alterations in MTX pharmacokinetics. Median values for plasma MTX half-life, peak concentration, and leucovorin dose showed only minor variability with changes in the 5-FUra dosage schedules (Table 3). The 5-FUra dose could not be correlated with the incidence of either delayed MTX clearance or increased leucovorin requirements.

All 7 of the infusions associated with delayed MTX clearance resulted in toxicity, 3 severe and 4 mild (Table 6). Two (28%) of these infusions were associated with severe marrow suppression, WBC <2000 cells/cu mm and platelets <50,000 cells/cu mm.

Only 2 (18%) of the 11 infusions where a nadir WBC <2000 cells/cu mm and/or platelet count <50,000 cells/cu mm occurred with associated toxicity of delayed MTX clearance. The 5-FUra dose in these 11 instances was: 600 mg/sq m, 3; 800 mg/sq m, 2; and 1,000 mg/sq m, 6 (p < 0.005 for 600 versus 1,000 mg/sq m). This suggests that, when 5-FUra is added to this dosage schedule of MTX, the dose of 5-FUra is a more important determinant of myelosuppression than is plasma MTX clearance.

There were 2 treatment-related deaths. A patient with widely metastatic melanoma expired with neutropenia and septicemia. The 5-FUra dose was 600 mg/sq m given at Hour 9, and the plasma MTX clearance was normal. A patient with gastric carcinoma became neutropenic and expired 24 hr after becoming febrile. The 5-FUra dose was 800 mg/sq m. This infusion, however, was associated with delayed MTX clearance (36-hr MTX level, 2.8 μM), and the patient required high-dose leucovorin. Postmortem examination revealed extensive local growth of his gastric carcinoma and fibrous pericarditis.

Response. Twenty-one of the 26 patients treated were considered evaluable for response. Two died of progressive disease after only one infusion. One patient refused further therapy after one course. One patient with locally advanced epidermoid head and neck carcinoma received a single preoperative infusion and died of progressive disease postoperatively without further therapy. One patient with a soft-tissue sarcoma received 12 infusions following total resection and local-regional radiation. This patient remains disease free 28+ months following cessation of therapy. Of the remaining 21 evaluable patients, there were 1 complete and 1 partial response. A 44-

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**Table 5**

<table>
<thead>
<tr>
<th>5-FUra dose (mg/sq m)</th>
<th>Administration time (hr)a</th>
<th>No. of courses</th>
<th>No toxicity</th>
<th>Mild toxicity</th>
<th>Severe toxicity</th>
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<tr>
<td>600</td>
<td>9</td>
<td>65</td>
<td>29 (44.8)c</td>
<td>30 (46.1)</td>
<td>6 (9.2)</td>
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<tr>
<td>600</td>
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<td>13</td>
<td>4 (6.9)</td>
<td>10 (15.3)</td>
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<tr>
<td>800</td>
<td>20</td>
<td>11</td>
<td>4 (6.1)</td>
<td>5 (7.6)</td>
<td>1 (1.5)</td>
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<tr>
<td>1000</td>
<td>20</td>
<td>12</td>
<td>5 (8.3)</td>
<td>7 (11.2)c</td>
<td>1 (1.5)</td>
</tr>
</tbody>
</table>

a See text for definition.

b Numbers in parentheses, percentage.

c Significantly increased over 600 mg/sq m, p < 0.005; significantly increased over 800 mg/sq m, p < 0.05.

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**Table 6**

<table>
<thead>
<tr>
<th>5-FUra dose (mg/sq m)</th>
<th>Administration time (hr)a</th>
<th>No. of courses</th>
<th>Infusions with delayed MTX clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>600</td>
<td>9</td>
<td>65</td>
<td>4/65 (6.1)c</td>
</tr>
<tr>
<td>600</td>
<td>20</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>800</td>
<td>20</td>
<td>11</td>
<td>1/11 (9.1)</td>
</tr>
<tr>
<td>1000</td>
<td>20</td>
<td>12</td>
<td>2/12 (16.8)c</td>
</tr>
</tbody>
</table>

a Number of hr after initiation of MTX infusion.

b Numbers in parentheses, percentage.

c Not significantly increased over 600 mg/sq m, p > 0.1.
year-old man with melanoma metastatic to the liver and extending to involve the duodenal bulb had complete resolution of all measurable and evaluable disease at a 5-FUra dose of 600 mg/sq m given at Hour 9 for 18 courses, after which a different sequential MTX → 5-FUra program was used in the ambulatory setting. A 68-year-old woman with colon carcinoma metastatic to the liver had a partial response for 7 months, as documented by serial liver scans, at a 5-FUra dose of 600 mg/sq m given at Hour 9.

**DISCUSSION**

The demonstrated antitumor activity of MTX and 5-FUra when each is given as a single agent has led to the empiric use of this combination, despite the lack of definitive proof that an additive or synergistic effect occurs.

A number of biochemical mechanisms have been proposed to explain the schedule-dependent effects of synergy or antagonism seen with the combination of MTX and 5-FUra (3, 5). There is also a biochemical rationale for evaluation of sequential combination MTX preceding 5-FUra using higher than conventional MTX doses (6). This MTX dosage schedule was chosen to achieve a prolonged exposure of cells to a plasma MTX concentration in the range of 5 \times 10^{-5} \text{M}, a plasma MTX concentration approximately 1 log higher than the peak level achievable with conventional doses of MTX.

The results of this study suggest that the incidence and severity of toxicity, particularly myelosuppression, is best related to the amount of 5-FUra administered with this MTX dosage schedule rather than to MTX kinetics. Previous studies demonstrated that the incidence of myelosuppression following high-dose MTX related most closely to MTX plasma clearance (26, 27). Thus, either or both agents may determine myelosuppression, depending upon the relative molar concentrations of each. Toxicity with MTX, 1000 mg/sq m, over 18 hr followed by 5-FUra, 1000 mg/sq m, and minimal leucovorin rescue was unacceptable and exceeded that anticipated with these drugs given either as single agents or together at lower 5-FUra doses.

The toxicity reported by others utilizing sequential MTX preceding 5-FUra regimens has been variable (17, 21, 25). Differences in patient populations may in part account for the results noted in these studies. The marked increase in toxicity we observed with escalation of the 5-FUra dose above 800 mg/sq m suggests that there may be a threshold 5-FUra dose above which synergistic toxicity to host tissues with MTX exists. These results are in marked contrast to the minimal toxicity that we reported with this dose and schedule of MTX administered alone (27). In addition, MTX-preceding-5-FUra regimens which result in a high plasma MTX concentration over a prolonged period might be expected to be more toxic than those utilizing bolus administration of lower MTX doses (15).

The addition of 5-FUra to the MTX-minimal leucovorin rescue regimen did not alter MTX pharmacokinetics. Values for median peak plasma MTX concentration and half-life and the incidence of infusions with delayed MTX clearance were equivalent to data obtained when this dosage schedule of MTX was administered alone over 18 hr (27) and were not affected by changes in the dose and schedule of 5-FUra.

The administration of leucovorin may alter the interaction between MTX and 5-FUra (12). Preclinical studies suggest that, in the presence of 5-FUra, the amount of leucovorin required to rescue cells from high-dose MTX may be reduced and that leucovorin administration may in fact enhance 5-FUra toxicity (18, 23). In this study, the median leucovorin dose was equivalent to that required with the administration of 18-hr MTX alone and did not vary significantly with changes in 5-FUra dose and schedule. Empiric reductions of leucovorin below that predicted by plasma MTX clearance were not attempted.

The small number of patients with any one disease site treated in this study precludes any definitive conclusions regarding antitumor synergy of this combination. Complete responses in patients with melanoma to high-dose MTX alone have been reported (16). Other investigators have suggested that a sequential MTX-preceding-5-FUra combination is useful in the therapy of certain solid tumors (17, 21, 25). Thus, further clinical trials to determine the optimum MTX dose and timing of 5-FUra administration are warranted. The results of this study illustrate that 5-FUra in doses \(\leq 800 \text{ mg/sq m}\) can be incorporated safely into a sequential regimen with MTX and minimal leucovorin rescue.

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**REFERENCES**


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