A Clinical and Pharmacological Study of High-Dose Methotrexate with Minimal Leucovorin Rescue

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ABSTRACT

Forty patients with advanced neoplasms received 118 infusions of high-dose methotrexate (MTX) for 18 hr. The dosage of MTX was varied between 800 and 1350 mg/sq m in an effort to achieve a standard peak plasma concentration of $5 \times 10^{-3}$ M. Three doses of leucovorin were given i.v. at Hr 30, 36, and 42 following the start of the MTX infusion. Leucovorin dosages were individualized based upon each patient's plasma MTX clearance. Early (18- and 24-hr) plasma MTX determinations were used to project a 36-hr concentration; then, assuming that equivalent doses of MTX and leucovorin give rise to comparable peak plasma levels, the leucovorin dose was calculated so as to give a plasma concentration of reduced folate 10-fold higher than the projected 36-hr plasma MTX concentration. Late (30-, 36-, 42-, and 48-hr) plasma MTX levels were measured in order to confirm expected concentrations based upon 18- and 24-hr determinations. Three general patterns of MTX plasma clearance were noted. In 110 of 118 infusions, plasma MTX clearance was rapid (mean $t_{1/2}$, 2.2 hr), leucovorin doses were minimized (median dose, 8.0 mg/sq m), and the incidence of significant myelosuppression (WBC, 2000 and/or platelets, 50,000) was 2.7%. In five infusions, plasma MTX clearance was delayed from the onset (mean $t_{1/2}$, 6.4 hr), calculated leucovorin doses were high (median dose, 220 mg/sq m), and myelosuppression was seen in one of five infusions despite the initiation of prolonged administration of "high-dose" leucovorin rescue (50 to 100 mg/sq m i.v. every 6 hr). In the remaining three infusions, late MTX levels, when measured experimentally, deviated from projected concentrations based upon actual early plasma MTX determinations. Again, "high-dose" leucovorin was begun following the usual three protocol leucovorin doses, and myelosuppression did not occur.

The clinical feasibility of limiting doses of leucovorin rescue based upon plasma MTX clearance, without resulting excessive toxicity, has been demonstrated. The majority of patients (seven of eight) with delayed clearance of antifol were rescued clinically with "high-dose" leucovorin. Minimization of leucovorin rescue doses in an attempt to improve the therapeutic effectiveness of "high-dose" MTX chemotherapy should be further tested in future clinical protocols.

INTRODUCTION

The antifol, methotrexate, has a wide range of activity in the treatment of neoplastic diseases (9). Early preclinical studies by Goldin et al. (4) demonstrated that the toxicity of high doses of methotrexate could be minimized without loss of therapeutic activity by the administration of the reduced folate, calcium leucovorin. Clinical studies in the treatment of metastatic osteogenic sarcoma (7) and other solid tumors (2, 8) have since demonstrated the activity of such high-dose methotrexate-leucovorin rescue regimens. To date, the dosage of methotrexate used, the length of the methotrexate infusion, and the scheme for leucovorin rescue vary widely among protocols and have been established largely on an empirical basis. Studies by Pinedo et al. (12) have clearly shown a relationship between extracellular concentrations of methotrexate and leucovorin and the effectiveness of "rescue" in tissue culture and laboratory animals. Furthermore, recent studies by Sirotnak, et al. (13) suggest that the clinical efficacy of high-dose methotrexate-leucovorin rescue therapy in both L1210 leukemia- and Sarcoma 180-bearing mice can be optimized by minimizing the dose of reduced folate. A Phase 1 clinical trial by Jacobs and Santicky (6) using 3 fixed-dosage regimens of leucovorin demonstrated that, indeed, leucovorin dosages could be reduced without undue host toxicity. The aim of this investigation was to confirm the clinical relationship between plasma methotrexate concentration and dosage of leucovorin needed to prevent toxicity. The dose of leucovorin was individualized based upon each patient's plasma methotrexate clearance in an effort to minimize the dose of leucovorin administered, avoid host toxicity, and maximize therapeutic efficacy.

MATERIALS AND METHODS

Patients. Forty patients with advanced neoplasms received 118 infusions of high-dose methotrexate. The patients' diagnoses are listed in Table 1. The patients ranged in age from 23 to 77 years with a median age of 56. There were 31 males and 9 females. All patients had total leukocyte counts $>4,000$ cells/cu mm, platelet counts $>100,000$/cu mm, and normal serum creatinines and creatinine clearances $>60$ ml/min prior to each high-dose methotrexate infusion. Renal function, serial complete blood counts, and physical examinations were monitored for 48 hr after each drug infusion. The patients were instructed to return for examination in the event that fever, bleeding, or mucositis developed. Mucositis was graded according to Eastern Cooperative Oncology Group Toxicity Criteria. For the purposes of this study, myelosuppression was defined as a reduction of the total leukocyte count to $<2,000$ cells/cu mm and/or a reduction in platelet count to $<50,000$ cells/cu mm.

Methotrexate Infusion. The methotrexate used in this
study was commercially supplied by Lederle Laboratories, Pearl River, N.Y. Hydration i.v. with 1 liter 5% dextrose in water containing 100 mg sodium bicarbonate every 6 hr was started 12 hr prior to the methotrexate infusion and continued until completion of leucovorin rescue at Hr 42. The infusion of methotrexate was not begun unless the patient's urinary pH was ≥7.0. The methotrexate dosage for each patient's initial infusion was 1.0 g/sq m, with 10% of the drug infused over the first 0.5 hr, and the remainder was infused over 17.5 hr. In subsequent infusions, methotrexate dosage was adjusted in an effort to achieve a standard peak plasma level of $5 \times 10^{-6}$ M. This peak plasma level is approximately 1 log higher than peak level achievable with conventional doses of methotrexate. Blood specimens were obtained at 1-, 9-, 18-, 24-, 30-, 36-, 42-, and 48-hr following the start of the 18-hr infusion for determination of plasma methotrexate concentration. Plasma levels were determined using the competitive protein binding assay developed by Myers et al. (10). Methotrexate infusions were repeated at 28-day intervals, and therapy was continued until clinical progression of disease was noted.

**Leucovorin Rescue.** Only 3 doses of leucovorin (dl-leucovorin, calcium salt from Lederle Laboratories, Pearl River, N. Y.) were given, and these were administered i.v. at Hr 30, 36, and 42 following the start of methotrexate infusion. As previously mentioned, the dosage of leucovorin was individualized, based upon each patient’s plasma methotrexate clearance. Methotrexate levels drawn at Hr 18 and 24 after the initiation of the 18-hr methotrexate infusion allowed us to calculate the plasma methotrexate half-life; these actual levels were then used to project an expected 36-hr methotrexate concentration. It was assumed that comparable methotrexate and leucovorin doses yield equivalent peak plasma levels. Data from our laboratory and others (5) have shown methotrexate doses of 30 mg/sq m to result in peak plasma levels of approximately $5 \times 10^{-6}$ M. Using this ratio of dosage to plasma concentration, the leucovorin dose was calculated to give a theoretical plasma concentration of leucovorin 10-fold higher than the projected 36-hr methotrexate concentration. The projected plasma clearance of methotrexate based upon actual measurement of 18- and 24-hr plasma concentrations was later confirmed by actual measurement of plasma methotrexate concentration at Hr 30, 36, 42, and 48.

**RESULTS**

In an effort to achieve a standard peak plasma concentra-

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**Table 1**

**Patient diagnoses**

<table>
<thead>
<tr>
<th>Primary site</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>16</td>
</tr>
<tr>
<td>Head and neck</td>
<td>10</td>
</tr>
<tr>
<td>Genitourinary</td>
<td>4</td>
</tr>
<tr>
<td>Leukemia-lymphoma</td>
<td>4</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>2</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>2</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>40</strong></td>
</tr>
</tbody>
</table>

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High-Dose Methotrexate with Minimal Leucovorin Rescue

condition of $5 \times 10^{-5}$ M, methotrexate doses varied from 800 to 1350 mg/sq m. Actual peak methotrexate concentrations ranged from 1.4 to $15 \times 10^{-5}$ M. The projected 36-hr methotrexate level fell below $1 \times 10^{-6}$ M in 110 of 118 infusions, as noted in Chart 1A. In this group of patients, the mean plasma methotrexate half-life was 2.2 hr, and the median leucovorin dose was only 8.0 mg/sq m (Table 2). The leucovorin doses ranged from 0.9 to 72 mg/sq m. No additional leucovorin, other than the 3 protocol doses, was administered with any of these 110 infusions.

The projected 36-hr methotrexate level was $1 \times 10^{-6}$ M or greater in 5 infusions (Chart 1B). The mean plasma half-life was 6.4 hr, and the median leucovorin dose was 220 mg/sq m, with a range of 72 to 261 mg/sq m (Table 2). In view of the delayed drug clearance noted in these 5 infusions, high-dose leucovorin rescue, either 50 or 100 mg/sq m i.v. every 6 hr, was continued until either the actual plasma methotrexate concentration fell below $1 \times 10^{-6}$ M (1) or the nadir of myelosuppression had been passed. The mean peak methotrexate concentration of $8.0 \times 10^{-6}$ M in this group of patients was somewhat higher than the level of $4.5 \times 10^{-6}$ M noted in the infusions having normal clearance. No cause for these delayed methotrexate clearances could be identified on retrospective analyses of renal and liver function and urine output.

In the 115 infusions noted above, the actual late (i.e., drawn after Hr 24) plasma methotrexate clearance followed closely the projected clearance based upon the 18- and 24-hr plasma methotrexate measurements.

Finally, there were 3 infusions in which actual clearance after the 24-hr methotrexate level deviated considerably from the expected clearance. Whereas the projected 36-hr methotrexate level was below $1 \times 10^{-6}$ M, the actual clearance showed that even at Hr 48 the methotrexate level remained greater than $1 \times 10^{-6}$ M (Chart 1C). In this small group of patients, the projected mean methotrexate plasma half-life was 2.6 hr. Based upon this normal initial drug clearance, the median leucovorin dose was calculated to be 20.4 mg/sq m. However, when actual 30-, 36-, 42-, and 48-hr methotrexate levels were measured, a delay in plasma clearance was noted, and again high-dose leucovorin rescue (50 to 100 mg/sq m i.v. every 6 hr) was instituted. Again, there was no clear reason for the delayed methotrexate excretion in these patients. Two patients with delayed clearance of drug have since been retreated in the same manner without delayed clearance or toxicity.

Toxicity consisted primarily of mucositis, a generalized macular rash, conjunctivitis, and myelosuppression. Stomatitis, ranging from mild erythema to frank ulceration of >25% of the oral mucosa, was noted in 25 of 118 infusions (21.2%). Ulceration of >25% of the oral mucosa was seen, however, in only 5 infusions (4.2%). The incidence of stomatitis seemed to be unrelated to the pharmacological clearance of methotrexate, as clinically significant stomatitis was seen in only 1 of the 8 infusions showing delayed drug clearance.

A patchy erythematous macular rash was temporally related to methotrexate administration in 17 of 118 infusions (14.4%). This rash was seen in 2 of the 8 infusions associated with delayed methotrexate clearance.

Transient conjunctivitis was seen in 5 of 118 infusions.
(4.2%) and seemed to be unrelated to the occurrence of stomatitis or rash. Likewise, there was no association between the incidence of conjunctivitis and the occurrence of delayed methotrexate clearance or myelosuppression.

The incidence of myelosuppression following high-dose methotrexate infusion has been clearly shown to be related to the plasma clearance of the antifol (14). In the 110 infusions where the projected and actual 36-hr methotrexate level fell below $1 \times 10^{-8} \text{M}$, the overall incidence of myelosuppression was only 2.7% (3 of 110) (Table 3). In each of these 3 instances, the actual late plasma methotrexate concentration ($1 \times 10^{-8} \text{M}$). Three infusions where the expected methotrexate clearance fell within the normal range but where actual measurement of 30-, 36-, and 42-hr plasma methotrexate concentrations deviated from expected values. Again, high-dose leucovorin rescue was initiated following the 3 protocol leucovorin doses, as outlined in B.

### Table 2

<table>
<thead>
<tr>
<th>MTX clearance</th>
<th>MTX half-life (hr)</th>
<th>Leucovorin dose (mg/sq m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, no additional leucovorin</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Normal, no additional leucovorin</td>
<td>2.2</td>
<td>1.3-5.0</td>
</tr>
<tr>
<td>Delayed, additional leucovorin</td>
<td>6.4</td>
<td>3.5-10.5</td>
</tr>
<tr>
<td>Normal initially, 48 hr [MTX] &gt; 1 \times 10^{-8} \text{M}, additional leucovorin</td>
<td>2.6</td>
<td>2.5-2.8</td>
</tr>
</tbody>
</table>

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*a* Leucovorin i.v. only at Hr 30, 36, and 42 following the start of the MTX infusion.

*b* Leucovorin (50 to 100 mg/sq m i.v.) every 6 hr following the above noted 3 protocol doses and continued until plasma [MTX] < $10^{-8} \text{M}$ or nadir of myelosuppression passed.
There has been no evidence of recurrent disease 13, 14, and the lung, however, there were no partial or complete me
trexate. These data support previously published reports to high-dose methotrexate with "minimal" leucovorin rescue.
In view of the recent animal studies by Sirotnak et al. (13) and the clinical feasibility of such an approach as reported in this study, we think that attempts to minimize leucovorin dosages should be incorporated into future high-dose methotrexate-leucovorin rescue protocols. The low occurrence of myelosuppression observed with the high-dose methotrexate-minimal leucovorin rescue regimen described in this study should also make it possible to incorporate this approach into combination chemotherapy regimens that include other myelosuppressive agents, although it must be stressed that close clinical and pharmacological follow-up of patients treated in the manner described here must be carried out in order to avoid serious and sometimes fatal drug toxicity. Further elucidation of the pharmacology and pharmacokinetics of the reduced folates will allow for

**Table 3**

<table>
<thead>
<tr>
<th>MTX clearance</th>
<th>No. of infusions</th>
<th>Myelosuppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, no additional leucovorin</td>
<td>110/118 (93.2)b</td>
<td>3/110 (2.7)</td>
</tr>
<tr>
<td>Delayed, additional leucovorin</td>
<td>5/118 (4.2)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>Normal initially, 48 hr [MTX]</td>
<td>&gt;1 x 10^6 M, additional leucovorin</td>
<td>3/118 (2.5)</td>
</tr>
</tbody>
</table>

WBC <2,000 and/or platelets <50,000.

Numbers in parentheses, percentage.

creatinine, with a rise in the serum value from 1.1 to 2.0 mg/100 ml on Day 9 following the drug infusion.

In the 5 infusions in which there was markedly delayed projected and actual plasma clearance of methotrexate, high-dose leucovorin rescue (50 to 100 mg/sq m i.v. every 6 hr) was instituted immediately following the protocol doses at Hr 30, 36, and 42. The overall incidence of myelosuppression in this group was 20% (1 of 5). In 2 of these 5 infusions, there was no change in serum creatinine. In the remaining 3 infusions, there was a significant rise in serum creatinine (2.1, 5.0, and 2.8 mg/100 ml) above normal base line values (0.6, 1.1, and 1.0 mg/100 ml, respectively). However, this rise in serum creatinine above a normal upper limit of 1.5 mg/100 ml was not noted until several days (Days 8, 7, and 4, respectively) following the infusion of high-dose methotrexate. These data support previously published reports showing that plasma methotrexate levels provide an earlier and more reliable guide to the possible onset of myelosuppression after high-dose methotrexate therapy than does serum creatinine.

There were 3 infusions in which the actual clearance of methotrexate deviated markedly from the projected drug clearance. In each of these 3 infusions, the actual 48-hr plasma methotrexate concentration remained greater than 1 x 10^-6 M; in each, additional high-dose leucovorin rescue was instituted. Myelosuppression did not occur following any of these 3 infusions. In one of the 3, serum creatinine rose from a base line value of 0.8 mg/100 ml to a value of 1.7 mg/100 ml on Day 4 following the infusion of methotrexate. In the other 2 instances, there were no changes in serum creatinine.

The number of patients entered on this study is too small to make any definitive comment regarding response rates to high-dose methotrexate with "minimal" leucovorin rescue. In the 13 evaluable cases of non-oat cell carcinoma of the lung, however, there were no partial or complete responses. Nine of the 13 patients had stable disease for a median duration of 3 months. There was one objective partial response (>50% shrinkage of all measurable lesions) among the 4 patients with measurable advanced head and neck carcinoma. In the remaining 3 patients, the disease progressed. In addition, this regimen has been used as monthly adjuvant chemotherapy for patients with poor prognostic head and neck primaries (T1, T4, or N+), after adequate regional control with either surgery or radiation. Among 3 patients entered into this adjuvant study, to date there has been no evidence of recurrent disease 13, 14, and 14 months, respectively, following institution of monthly high-dose methotrexate with "minimal" leucovorin rescue. The one patient with non-Hodgkin's (diffuse histiocytic) lymphoma treated with this regimen has had an objective partial response lasting 9 plus months.
a more rational approach to leucovorin dosage reduction. The present study also confirmed that early recognition (within 24 hr) of delayed plasma methotrexate clearance allows for institution of high-dose leucovorin therapy and effective clinical rescue in the majority of infusions with delayed clearance of the antifol.

ACKNOWLEDGMENTS

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REFERENCES

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