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, 2,000 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 plasma MTX concentrations. Again, ”‘‘high-dose” leucovorin was followed before 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 × 10⁻⁵ 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 (di-I-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 × 10⁻⁵ 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**

<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>Total</td>
<td>40</td>
</tr>
</tbody>
</table>

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High-Dose Methotrexate with Minimal Leucovorin Rescue

MARCH 1979
Table 2

Methotrexate (MTX) pharmacology following high-dose methotrexate with minimal leucovorin rescue

<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></td>
<td></td>
</tr>
<tr>
<td>2.2             1.3- 5.0</td>
<td>8.0 0.9- 72</td>
<td></td>
</tr>
<tr>
<td>Delayed, additional leucovorin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.4             3.5-10.5</td>
<td>220 72 -261</td>
<td></td>
</tr>
<tr>
<td>Normal initially, 48 hr [MTX] &gt;1 (10^{-4}) M, additional leucovorin</td>
<td>2.6 2.5- 2.8</td>
<td>20.4 10.2- 35.4</td>
</tr>
</tbody>
</table>

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] < \(10^{-4}\) M or nadir of myelosuppression passed.

Chart 1. Three patterns of plasma methotrexate clearance following 18-hr high-dose infusion. A, normal methotrexate clearance. Cross-hatched areas, actual plasma methotrexate concentrations; hatched areas, expected methotrexate concentrations based upon actual 18- and 24-hr plasma determinations. Leucovorin rescue (LR) was limited to i.v. administration at Hr 30, 36, and 42 following the start of the methotrexate infusion. B, delayed methotrexate clearance. Five infusions wherein expected methotrexate clearance, based upon early (18- and 24-hr) plasma determinations, deviated from normal plasma methotrexate clearance. High-dose leucovorin rescue (50 to 100 mg/sq m i.v. every 6 hr) was begun at Hr 48 and continued until either the plasma methotrexate concentration fell below \(1 \times 10^{-8}\) M or the nadir of myelosuppression had passed. C, normal initial methotrexate clearance; 48-hr plasma methotrexate concentration \(>1 \times 10^{-4}\) 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.

(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 \(1 \times 10^{-8}\) 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 clearance (as measured by 30-, 36-, 42-, and 48-hr plasma methotrexate levels) closely approximated the projected methotrexate clearance (based upon early 18- and 24-hr plasma methotrexate levels). The cause of myelosuppression in these 3 infusions remains unclear. In only 1 of these 3 infusions was there a significant change in serum
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)</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] &gt;1 × 10^6 M, additional leucovorin</td>
<td>3/118 (2.5)</td>
<td>0/3 (0)</td>
</tr>
</tbody>
</table>

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

b 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 (14) 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 × 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, T2, 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 15 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.

DISCUSSION

A number of investigators have shown that leucovorin rescue after high-dose methotrexate therapy reduces host toxicity without impairing therapeutic response (2, 4, 7, 8). There is no convincing evidence, however, that high doses of methotrexate with leucovorin rescue are therapeutically more efficacious than are conventional doses of methotrexate. Leucovorin rescue regimens vary widely between protocols (11), and little effort has been made to limit pharmacologically the amount of leucovorin administered. Indeed, the thrust of presently used high-dose methotrexate-leucovorin rescue clinical protocols has been to give more, rather than less, leucovorin in an effort to prevent host toxicity. Recent data published by Sirotnak et al. (13) regarding ascitic L1210 leukemia- and Sarcoma 180-bearing mice suggest that efforts to minimize dosages of leucovorin can result in optimization of high-dose methotrexate-leucovorin rescue protocols and improved therapeutic effectiveness. The present study has demonstrated the feasibility of such an approach when applied to clinical chemotherapy. Institution of leucovorin rescue was delayed for 30 hr. Further delays in starting leucovorin therapy have been reported to be associated with a high incidence of clinical toxicity (3). The dosage of reduced folate was based upon individual patients’ plasma clearance of methotrexate. We attempted to achieve concentrations of leucovorin in the plasma which were 10-fold higher than the 36-hr methotrexate concentration. In the absence of a sensitive, reliable, and reproducible assay for the reduced folates, leucovorin doses were calculated by assuming that comparable doses of methotrexate and leucovorin result in similar plasma drug concentrations. This assumption is unproven, but it did allow us to minimize the dosages of leucovorin administered in 110 of the 118 infusions reported with acceptable toxicity. We are presently developing a high-pressure liquid chromatographic assay for both leucovorin and the other reduced folates in an effort to verify the assumed concentrations of reduced folates in this study.

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...
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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