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Pharmacokinetic and Clinical Studies of 24-h Infusions of High-Dose Methotrexate

Harvey J. Cohen[1] and Norman Jaffe

Children's Hospital Medical Center, Sidney Farber Cancer Institute, and Harvard Medical School, Boston, Massachusetts, USA

Summary. *Cytocidal activity of a drug is dependent on both drug dosage and duration of exposure. In contrast to the 'conventional' 6-h infusion and in an attempt to improve its efficacy, the high-dose methotrexate therapeutic regimen was given over a 24-h period with 10% of the dose administered in the first hour. Citrovorum factor was initiated at hour 24 and continued for 72 h. Treatment was administered every 2–3 weeks. 57 infusions were performed in twelve patients aged 7–20 years (six with osteogenic sarcoma and six with acute lymphoblastic leukemia). Determinations of serum methotrexate levels revealed that the levels were dependent on the dose. Levels assayed at 24 h revealed the following results: 4.4 ± 1.4 × 10^{-5} molar with 4.5 g/m², 2.04 ± 0.34 × 10^{-4} molar with 7.5 g/m² and 4.59 ± 0.80 ×10^{-4} molar with 12.5 g/m². Major toxicity was myelosuppression in 12 of 57 patients. There were no responses. The study demonstrates that 24-h infusions of high-dose methotrexate can be tolerated every 2–3 weeks in patients without bone marrow involvement and levels of at least 10^{-4} molar can be maintained during the infusion.*

Introduction

High-dose methotrexate with citrovorum factor ('citrovorum factor rescue') has been utilized extensively in the treatment of osteogenic sarcoma. In most therapeutic regimens, a modification of the 'pulse' method originally described by Djerassi [2] is employed. In this regimen, the methotrexate is administered intravenously over 4–6 h and citrovorum factor rescue is initiated several hours later [7, 12–14]. Occasionally, 'pulse' methotrexate infusions have been of even shorter duration with rescue commencing 24 h later [11]. In many instances, pre-treatment with vincristine is administered on the basis that it will enhance the uptake of methotrexate by tumor cells [3].

In contrast to the 'pulse' infusions, 20–24-h infusions with high-dose methotrexate have also been employed [2, 5, 11]. In Djerassi's experience, the procedure was associated with an increased incidence of toxicity. The latter, however, could be averted by prolonging the duration of citrovorum factor rescue, and with this approach responses were achieved in patients who had apparently developed resistance to the 6-h 'pulse' infusion [5].

The foregoing experiences prompted a study to determine the clinical and kinetic relationships of 24-h infusions of high-dose methotrexate in patients who appeared resistant to 'conventional' high-dose 'pulse' treatment. The objectives of the study were threefold:

1. To adjust the dosage of methotrexate in order to maintain a serum level of 10^{-4} molar or greater for 24 h.
2. To evaluate the toxicity while maintaining this level in children with and without bone marrow dysfunction.
3. To determine if the regimen had any effect on tumors which appeared unresponsive to the 6-h infusion.

Materials and Methods

The protocol for 24-h infusions of methotrexate is outlined in Figure 1. Treatment comprised vincristine, 2 mg/m² (maximum 2 mg), administered intravenously. This was followed ½ h later by escalating

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Reprint requests should be addressed to: Norman Jaffe, Sidney Farber Cancer Institute, 44 Binney Street, Boston MA 02115, USA

[1] Charles A. Janeway Scholar and American Cancer Society Fellow

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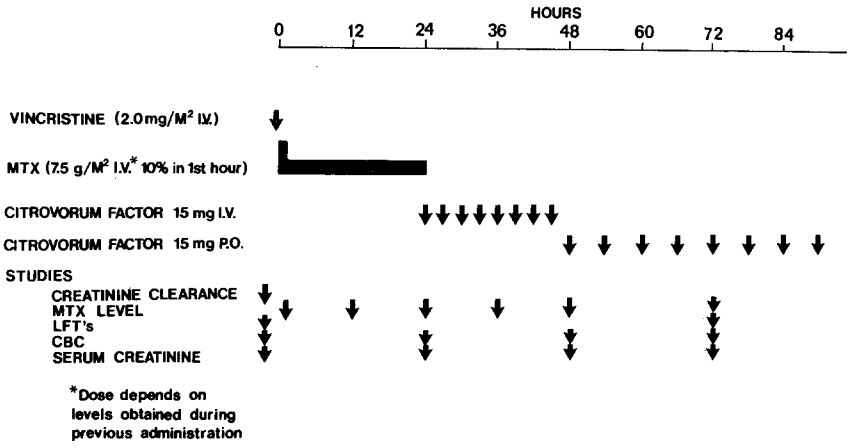

Fig. 1. Protocol for 24-h infusion of high-dose MTX. Regimen for 24-h infusion of high-dose methotrexate. 10% of the 24-h dose was administered intravenously in 1 h and the remaining 90% in 23 h. Citrovorum factor rescue commenced immediately after completion of the infusion. Treatment was preceded by vincristine, 2 mg/m², administered ½ h before initiation of the methotrexate infusion

Table 1. Results of therapy

Patient	Disease	Results
B. P.	Osteogenic sarcoma (adjuvant)	Relapsed after 9 months
R. P.	Osteogenic sarcoma (adjuvant)	Relapsed after 6 months
C. A.	Osteogenic sarcoma	Progressive disease after 2 months
C. N.	Osteogenic sarcoma	No response
D. L.	Osteogenic sarcoma	No response
M. S.	Osteogenic sarcoma	No response
J. T.	ALL (remission)	Relapsed after 3 months
L. V.	ALL (relapse)	Partial response for 1 month
T. D.	ALL (remission)	Continues in remission on MTX and Ara C 11^{+} months
R. M.	ALL (remission)	Relapsed after 2 months
J. E.	ALL (relapse)	No response
J. C.	ALL (relapse)	No response

doses of methotrexate: 10% of the total dose was administered intravenously in 1 h and the remaining 90% in 23 h. Citrovorum factor rescue commenced immediately after completion of the infusion. 15 mg was administered every 3 h intravenously for the first 24 h (8 doses). This was followed by 15 mg administered by the oral route every 6 h for 48 h (8 doses). The total duration of citrovorum factor was, therefore, similar to that administered in the 6-h infusion [7] but it extended an extra 16 h since rescue commenced at hour 24 rather than hour 8, as in the 'pulse' infusion.

The dose of methotrexate in each infusion was adjusted so as to achieve and maintain a level of 10^{-4} molar for the first 24 h during its administration. Serum methotrexate levels were obtained at intervals during this period (generally hour 1, 12, and 24). The dose was dependent upon the levels obtained during the previous administration and was increased by 25% until the 24-h level was greater than 10^{-4} molar. Treatment with doses producing this concentration was administered every 2–3 weeks.

Pre-treatment requisites comprised a normal creatinine clearance, serum methotrexate level, liver function studies, hemogram and absence of toxicity. Studies obtained during methotrexate treatment comprised a minimum estimation of three methotrexate levels during the first 24 h and subsequently at 36, 48, and 72 h. Liver function studies, hemograms and serum creatinine levels were obtained at 24, 48, and 72 h. Other studies were obtained as indicated. Adequate facilities for supportive care in the event of toxicity were available. All patients received a minimum of 3 l/m²/24 h of fluid by the intravenous or oral route throughout the duration of treatment.

Patients (Table 1). Six patients with osteogenic sarcoma who had previously developed metastases on 6-h infusions of high-dose methotrexate were entered into the study. These patients had also been treated with four weekly courses of the 6-h 'pulse' infusions [8]. In two patients, the metastases had been surgically removed and in four, pulmonary disease was still evident.

Concurrently, six patients with advanced acute lymphoblastic leukemia who had received conventional forms of treatment were also admitted for investigation. They had previously also received methotrexate by the intrathecal or oral route. Three were in remission and three in relapse. The intent was to induce remission in the patients who had relapsed and maintain remission in those who had already achieved this status.

Results

The initial levels of the serum methotrexate at 24 h were found to be related to the dose of the methotrexate infused (Table 2). Thus, a dose of 4.5 g/m² produced a level of $4.4 \pm 1.4 \times 10^{-5}$ molar, 7.5 g/m² $2.04 \pm 0.34 \times 10^{-4}$ molar, and 12.5 g/m² $4.59 \pm 0.80 \times 10^{-4}$ molar. With 7.5 g/m², levels in excess of 10^{-4} molar within the first 24 h were achieved in over 80% of patients. During the ensuing 72 h, lower levels were detected. At 48 h, the

Table 2. Concentrations of methotrexate detected at various times following infusions of 7.5 g/m²

Time[a]	Concentration ± S.E.M.[b]	Number of determinations
1	$1.96 \pm 0.27 \times 10^{-4}$ molar	12
12	$3.15 \pm 0.83 \times 10^{-4}$ molar	9
24	$2.04 \pm 0.34 \times 10^{-4}$ molar	22
48	$2.35 \pm 0.44 \times 10^{-6}$ molar	25
72	$4.60 \pm 1.2 \times 10^{-7}$ molar	19

[a] Hours post infusion
[b] Standard error of the mean

decay curves revealed a mean value of approximately 2.35×10^{-6} molar and at 72 h approximately 4.6×10^{-7} molar.

A comparison of the decay curve between a 6-h infusion and a 24-h infusion of methotrexate administered as 7.5 g/m² is illustrated in Figure 2. The 6-h infusion demonstrates an initial high level of 1×10^{-3} molar at 12 h with a rapid decay. At 24 h, the level is 1×10^{-5} molar, at 48 h, 1×10^{-6}, and at 72 h, 5×10^{-7}. In contrast, the initial serum methotrexate level of the 24-h infusion is not as high as the 6-h infusion, but at 24 h the serum methotrexate level is at least 1½ logs higher and then falls at the same rate of decay as the 6-h infusion.

Therapeutic Response. The 24-h infusion of methotrexate failed to achieve any major effects in patients with osteogenic sarcoma. The two patients receiving the 24-h infusions as adjuvant therapy relapsed after 6 and 9 months, respectively. One patient demonstrated progressive disease after 2 months and in the remaining three, disease appeared stationary for variable periods of time but was not eradicated.

Two patients with acute lymphoblastic leukemia in remission relapsed after 2 and 3 months, respectively. A partial response lasting 1 month was achieved in one patient. Two other patients in relapse failed to achieve a response. When the results of these investigations became apparent, it was elected to add arabinosyl cytosine to the last patient in remission. This status has been maintained utilizing high-dose methotrexate and arabinosyl cytosine for 11^+ months.

Toxicity. Toxicity comprised myelosuppression, mouth sores and an elevated serum glutamic oxalacetic transaminase level. The manifestations were not severe. In a total of 57 treatments, the incidence was approximately 15% (12 patients). Most of the toxicity occurred in patients with relapsed leukemia.

Pharmacokinetically, toxicity could be correlated with a prolonged elevation of serum methotrexate levels at 48 and 72 h. Thus, as illustrated in Figure 3, two patients with serum methotrexate levels in excess of 1×10^{-5} at 48 h developed toxicity 5 days after initiation of the infusion. This manifested as mucositis and an eleva-

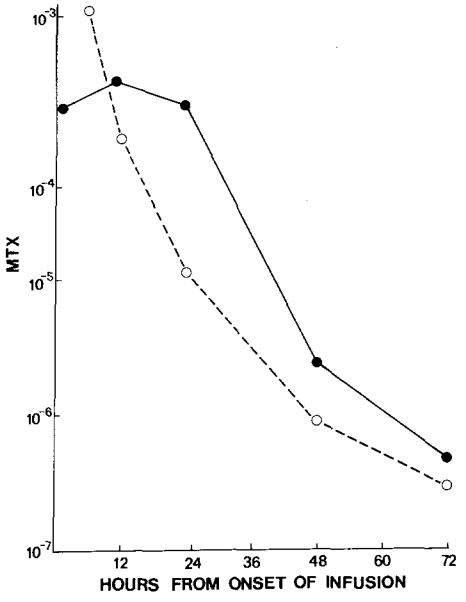

Fig. 2. Comparison of 6-h and 24-h infusion of MTX (7.5 g/m²). ●—● 24-h infusion; ○ - - - ○ 6-h infusion

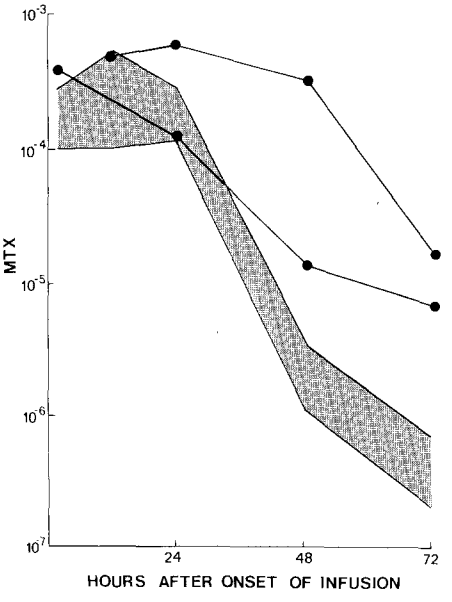

Fig. 3. Kinetics of two toxic courses of 24-h infusions of methotrexate. The shaded black area refers to the normal decay curve observed in 6-h and 24-h methotrexate infusions. Delay in the excretion of methotrexate was followed by toxicity

tion in the serum glutamic oxalacetic transaminase. One of these patients also demonstrated an elevation of the serum creatinine level at 48 h (above 50% of the pretreatment level). This phenomenon was also noted in approximately 25% of the other cases of toxicity. When the elevations in the serum methotrexate level were reported, the fluid intake was increased to approximately 4 $l/m^2/24$ h and citrovorum factor rescue was extended to 96 h. Excessive toxicity was consequently not encountered.

Discussion

In the initial Phase I–Phase II studies with high-dose methotrexate and citrovorum factor rescue in osteogenic sarcoma, serum methotrexate assays were not available. However, studies indicate that drug concentration, duration of exposure and drug metabolism are major determinants of methotrexate effect [4, 10]. The immediate goal of the 24-h infusion, consequently, was to attain levels similar to the 6-h infusion. An initial level of 1×10^{-3} molar was not achieved with the 10% bolus infusion. However, throughout the 24-h period, levels of 1×10^{-4} molar were present. These are similar to those which may be achieved with a 6-h infusion but failed to produce a response.

This investigation also failed to substantiate the results achieved in the previous study. The patients had obviously developed resistance to the methotrexate regimen. This could have been induced by prior exposure to weekly treatments which had not been employed in earlier patients.

The serum methotrexate levels in the 24-h infusions were related to the dose of methotrexate administered. Satisfactory levels could only be achieved by increasing the methotrexate dose from 4.5 g/m^2. With this regimen, toxicity was only slightly in excess of that reported with the tri-weekly schedule (approximately 10%) [6]. Alkalinization, which is utilized by others, also, was not employed [1, 9].

In previous studies, improvements in the therapeutic efficacy of methotrexate were obtained by increasing the dose and frequency of methotrexate administration. This appears rational since methotrexate primarily affects cells that are mitotically active during the S phase (DNA synthesis) [10]. Whether responses will similarly be observed by utilizing weekly 24-h infusions or by escalating the dose of the 24-h regimens remains to be determined. With the availability of pharmacologic methods to monitor serum methotrexate, the safety of methotrexate administrations has improved. Future studies could be directed along these lines.

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