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Evaluation of 24-Hour Infusion of High-Dose Methotrexate – Pharmacokinetics and Toxicity

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Summary. *High-dose methotrexate was administered by constant infusion over 24 h to children with various malignancies. Citrovorum factor was given at the completion of methotrexate infusion and continued for 72 h. Serum concentration of 10^{-4} M could be sustained for 24 h with doses of methotrexate over 100 mg/kg. This infusion regimen was well tolerated and only mild neutropenia and mouth sores were seen in most patients. Severe toxicity was seen in one patient and was related to prolonged retention of methotrexate in the circulation. Careful monitoring of serum drug level is mandatory in the use of any high-dose methotrexate regimen.*

Introduction

Some malignant tumors are 'naturally' resistant or slightly sensitive to methotrexate. This drug resistance is dependent on the active transport of the drug into the cell, the availability of binding sites for methotrexate, and the activity of dihydrofolate reductase within the cell [4]. Recent experimental observations suggest that the cytotoxic determinant for a cell is the 'free' intracellular methotrexate – the amount of drug in excess of the fraction that is tightly bound to dihydrofolate reductase [1, 3, 7]. The use of high-dose methotrexate with citrovorum factor rescue attempts to overcome this drug resistance by overwhelming the carrier-mediated cell membrane transport mechanism and calling upon other passive mechanisms to achieve significant intracellular methotrexate concentration. The dosage, duration, and frequency of administration of high-dose methotrexate are highly variable in reported studies [6]. It is not known whether a very high serum methotrexate concentration such as 10^{-3} M for 4–6 h is superior to a moderately high serum level of 10^{-5} M to 10^{-4} M of the drug over a more prolonged period of time in terms of therapeutic efficacy and toxicity. There may be an advantage in prolonging the duration of exposure of tumor cells to the drug if this can be done without undue risk and complications. This study is to evaluate the pharmacokinetics and toxicity of 24 h infusions of high-dose methotrexate with citrovorum factor rescue.

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Patients and Methods

Since 1974, twenty-four patients aged 16 months to 20 years with various malignancies (osteogenic sarcoma, lymphoma, acute lymphoblastic leukemia, and other miscellaneous tumors) were treated with high-dose methotrexate by 24-h infusion. The infusions were given every 2–3 weeks, or every 5–6 weeks if other forms of therapy were used. The initial dose of methotrexate was usually 25 mg/kg except on a few occasions when, due to other forms of therapy, a lower starting dose was used. The dosage was increased by 25 mg/kg with each subsequent dose until a dose of 150 mg/kg was reached. Citrovorum factor was started immediately at the end of the methotrexate infusion and continued for 72 h. Patients weighing less than 50 kg received citrovorum factor 9 mg intravenously every hour for 6 doses followed by 3 mg every 3 h. Those who weighed more than 50 kg received 15 mg intravenously hourly for 6 doses and then 6 mg every 3 h. These doses of citrovorum factor and the schedule of doses were chosen because of the prolonged period of infusion. Extra doses of citrovorum factor were given for those patients who had clinical toxicity. Sodium bicarbonate was given orally or intravenously to maintain the urinary pH above 7. Intravenous fluids were maintained until oral intake was adequate.

Serum samples were obtained at 12, 24, 48, and 72 h for determination of methotrexate concentration by radioimmunoassay method[1]. These were stored at −70° C if not tested on the same day. Complete blood counts and liver and renal function tests were determined before and at 24 h and 7–14 days after methotrexate infusions.

1 Diagnostic Biochemistry, Inc., San Diego, California

0344-5704/79/0003/0177/$ 01.00

Table 1. Serum concentrations following 24-h infusion of methotrexate

Dosage (mg/kg)	Mean methotrexate concentration			
	12 h	24 h	48 h	72h
25	2.1×10^{-5} M (7)[a] (1.1–3.0)[b]	2.5×10^{-5} M (8) (1.1–6.2)	8.0×10^{-7} M (9) (1.1–23.5)	8.0×10^{-8} M (7) (1.8–18.7)
50	3.3×10^{-5} M (12) (1.4–4.8)	4.1×10^{-5} M (14) (1.7–8.9)	9.4×10^{-7} M (11) (1.5–23.9)	1.9×10^{-7} M (10) (0.8–5.0)
75	5.9×10^{-5} M (13) (2.5–10.6)	6.2×10^{-5} M (13) (1.9–11.3)	2.2×10^{-7} M (10) (0.7–5.0)	3.6×10^{-7} M (8) (1.1–11.0)
100	9.3×10^{-5} M (12) (4.8–16.6)	9.3×10^{-5} M (10) (2.8–26.0)	1.8×10^{-6} M (11) (0.2–6.2)	1.1×10^{-7} M (4) (1.0–1.4)
125	9.9×10^{-5} M (8) (7.1–12.7)	1.1×10^{-4} M (7) (0.5–1.7)	1.1×10^{-6} M (9)[c] (0.3–2.8)	1.7×10^{-7} M (6)[c] (0.7–3.4)
150	1.2×10^{-4} M (4) (0.5–1.9)	1.2×10^{-4} M (10) (0.9–1.7)	1.3×10^{-6} M (3) (0.8–1.9)	4.2×10^{-7} M (2) (1.0–7.4)

[a] Number of determinations
[b] Range
[c] Excluding one patients with severe toxicity

Results

A total of 180 infusions were given to these 24 patients. Serum samples were obtained during 66 infusions at various drug dosages and the mean serum methotrexate concentrations are shown in Table 1 and Fig. 1. With each given dose of methotrexate, the serum drug levels at 12 and 24 h were similar. There was an increase in the serum drug concentration during infusion with an increase in the dose of methotrexate. Serum levels of 10^{-4} M were achieved with doses of methotrexate over 100 mg/kg. There was a rapid decrease of the serum drug levels in the first 24 h after drug administration, followed by a slower decrease in the next 24 h (Fig. 1).

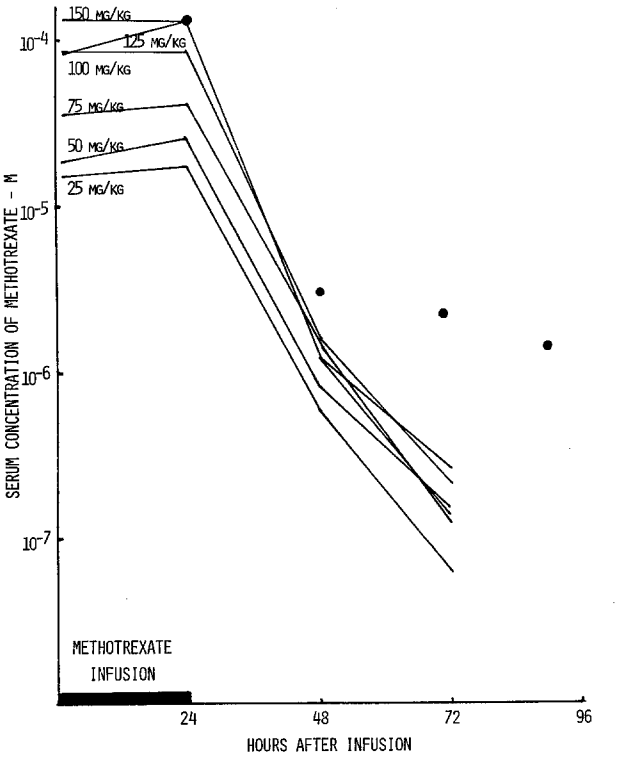

Fig. 1. Serum concentrations of methotrexate. ● denotes serum levels in the patient with severe toxicity

Twenty-two of the 180 infusions were associated with one or more toxicities (Table 2). Mild to moderate neutropenia (absolute neutrophil count of 500–1500/μl) was observed 4 to 7 days after methotrexate infusion in 12 patients, all of whom were asymptomatic. Only one patient developed mild thrombocytopenia. One patient had a life-threatening hematologic complication after an infusion of methotrexate at 125 mg/kg. At 24 h, there was an increase of the serum uric acid to 7.9 mg/dl, although the serum creatinine and creatinine clearance were normal. He was treated with continuous intravenous fluids with supplemental bicarbonate to maintain an alkaline urinary pH. He was discharged with citrovorum factor three days after the methotrexate infusion. Toxic symptoms started the day after discharge from the hospital, with mouth sores and pruritis. He did not take citrovorum factor and was admitted three days later with severe diarrhea and exfoliating dermatitis. Severe neutropenia (< 500/μl) and thrombocytopenia were present and there was ulceration in the perianal region. His serum methotrexate concentration at that time was 5.2×10^{-8} M. There were mild elevations of serum transaminases, but the renal function remained normal. He was treated with antimicrobials and granu-

Table 2. Toxicity associated with 24-h infusion of methotrexate

Dosage of MTX mg/kg	Infusions with toxicity	↓ WBC	↓ Platelet	Mouth ulcer	Skin	Renal	Liver
25	2/28	1	0	1	0	0	1
50	5/34	4	0	0	0	1	1
75	5/29	3	1	2	0	0	0
100	3/34	1	0	2	0	0	0
125	4/28[a]	3[a]	1[a]	1[a]	1[a]	1	1[a]
150	3/19	1	0	3	0	0	0

[a] Including one patient with severe toxicity

locyte transfusions and recovered completely in two weeks. His serum methotrexate concentrations were abnormally high at 72 and 90 h after the infusion (Fig. 1).

Mucosal ulceration in the mouth occurred in seven patients at varying doses of methotrexate infusion. All but one had mild symptoms and did not require extra doses of citrovorum factor.

Two patients had abnormal creatinine clearance after 7 and 16 infusions of methotrexate, respectively, and no further treatment with high-dose methotrexate was given to these patients.

Liver enzyme changes were seen at the end of the infusion of methotrexate on two occasions but were normal within one week, except in one patient. This patient had persistent abnormal liver function after an infusion of methotrexate at 25 mg/kg for metastatic synovial sarcoma. He had had prior radiotherapy and chemotherapy with dactinomycin, vincristine sulfate, cyclophosphamide, and doxorubicin. Serum transaminases were normal before the administration of methotrexate. It was not certain whether the abnormal enzyme levels represented drug toxicity or involvement of the liver by tumor. He received another infusion of methotrexate at 50 mg/kg. The abnormal liver enzyme levels persisted for three months until the time of his death due to progression of his tumor. At autopsy, histologic changes in the liver were consistent with chronic drug toxicity. Although the serum drug concentrations during the two infusions were within the values given in Figure 1, he did have a pleural effusion and this might have had an important role in a sustained release of methotrexate from this third space.

Discussion

The serum concentrations of methotrexate in our patients were all over 10^{-6} M, a level at which all high-affinity binding sites in human acute lymphoblastic leukemia are sufficiently saturated in vitro and appreciable levels of exchangeable drug begins to accumulate [1, 5]. Higher serum concentrations may be necessary to sustain the presence of 'free' intracellular methotrexate for tumor cells that are 'naturally' resistant or slightly sensitive to methotrexate. Serum concentration of 10^{-4} M can be maintained for 24 h in our patients with dosages of methotrexate above 100 mg/kg. This is comparable to the findings of Cohen and Jaffe [2].

Toxicity with 24-h methotrexate infusion was mild and well tolerated by our patients. Neutropenia was most common but life-threatening infection as a result of severe neutropenia occurred only in one patient. Mouth ulcers were mild and this usually led to temporary reduction of the subsequent dose of methotrexate infusion. Nausea and vomiting were common during the infusion but it was difficult to evaluate since some of our patients began vomiting even before the drug was started. Abnormal liver function as judged by elevation of serum transaminases was uncommon in our patients. The mild toxicity in our patients might be related to the intense citrovorum factor rescue after the methotrexate infusions. Only one patient required extra doses of citrovorum factor because of severe toxicity. Unfortunately, due to lack of compliance by this patient, it is not known whether extra doses of citrovorum factor could have prevented the complications.

The abnormally high serum drug concentrations after methotrexate infusion in the patient with severe toxicity may have been due to an altered metabolism of the drug. Although renal function was normal as indicated by normal blood urea nitrogen, serum creatinine, and creatinine clearance in the patient, a temporary renal dysfunction after methotrexate infusion could not be completely ruled out because of an unexplained high serum uric acid level in the absence of active tumor. It appears that the serum drug levels are reliable in predicting severe toxicity and should be carefully monitored with each infusion of high-dose methotrexate.

In a small number of our patients with osteogenic sarcoma, our 24-h infusion regimen appeared to be as effective as other high-dose methotrexate regimens.

They were in complete remission from 18+ to 50+ months. Being able to maintain a good serum drug concentration (10^{-5} to 10^{-4} M) for 24 h, further studies with prolonged infusion of 'lower' dose methotrexate will enable us to test the role of duration of drug exposure [3, 7]. Moreover, there would be a considerable saving in the cost of such treatment regimens.

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