Treatment of Acute Lymphocytic Leukemia by High-Dose Intravenous Methotrexate

Jaw J. Wang, Arnold I. Freeman, and Lucius F. Sinks

Summary

A pharmacokinetic study of methotrexate levels in blood and cerebrospinal fluid was performed in 42 patients who received one or more courses of high-dose methotrexate at 500 mg/sq m infused i.v. over a 24-hr period. Methotrexate level in the lumbar cerebrospinal fluid reached $1.2 \times 10^{-10}$ M at 0.5 hr and remained constant at that level for the first 24 hr. Similar methotrexate levels were noted in the ventricular fluid obtained through an Ommaya device on three patients with brain tumors treated with high doses of methotrexate. Preliminary clinical results using high-dose methotrexate combined with simultaneous intrathecal methotrexate in 23 children with newly diagnosed acute lymphocytic leukemia indicate that this treatment program is safe to administer and to date appears effective in the prevention of central nervous system leukemia.

Introduction

Prophylactic treatment to the CNS for prevention of CNS leukemia has become a critical part of the treatment of children with ALL (8, 10). Several current regimens include prophylactic i.t. chemotherapy with or without cranial irradiation (1, 9, 14, 15). A different approach was initiated in the Pediatric Department at Roswell Park Memorial Institute in 1972 using i.v. MTX at 500 mg/sq m with concurrent i.t. MTX and given soon after bone marrow remission was achieved. Pharmacokinetic studies of MTX in blood and CSF were performed in order to determine whether the addition of this regimen has a pharmacological basis in preventing CNS leukemia.

Materials and Methods

Subjects. The patients that were studied included 23 newly diagnosed children with ALL, an additional 16 patients with ALL who had relapsed on 2 or more occasions, and 3 patients with brain tumors.

Drug Administration. MTX was obtained from the Cancer Therapy Evaluation Branch of the National Cancer Institute. MTX was given to patients at 500 mg/sq m body surface, one-third by i.v. push and the remaining two-thirds infused i.v. over 24 hr. Twenty-four hr after completion of i.v. MTX, a single dose of i.m. leucovorin was given at 12 mg/sq m.

Sample Collection. Concurrent CSF and blood samples from each patient were obtained from 0.5 to 72 hr after high-dose i.v. MTX was started.

Preparation of Dihydrofolate Reductase. L1210 ascitic tumor resistant to MTX was chosen as an enzyme source because of its high dihydrofolate reductase activity (3). The tumor was supplied by the Cancer Drug Center of this Institute and was maintained in DBA2/Ha female mice with weekly i.p. injections of MTX, 10 mg/kg. Tumor cells ($1 \times 10^6$) were injected into 10 to 15 mice, and 7 days later the ascites were removed and pooled for preparation of the enzyme. All procedures were performed at 0–4°C. Cells were sedimented at 1000 × g for 5 min and the supernatant was removed. RBC were removed by osmotic lysis, and the remaining tumor cells were diluted with 10 volumes of distilled H2O. The suspension was then homogenized in a Tri-R homogenizer until the cells were completely disrupted. The supernatant solution obtained after centrifuging at 10,000 × g for 30 min was adjusted to pH 5.1 with acetic acid (1 N) and dialyzed overnight against distilled H2O. The clear supernatant solution obtained after centrifugation at 10,000 × g for 10 min was divided into 3-mI aliquots and stored in liquid nitrogen. Under these conditions the enzyme was stable for at least 3 months.

Determination of MTX. MTX in serum and CSF was determined by the method of Werkheiser et al. (19). This involves the measurement of the effect of the aliquots of these fluids on the activity of dihydrofolate reductase. The amount of enzyme used was such that the net decrease in absorbance in 5 min measured at 340 nm was about 0.15 (2). As a standard, an identical amount of dihydrofolate reductase was titrated with the freshly prepared solution of MTX of known concentration. This assay allows detection of $1 \times 10^{-9}$ M MTX.

Results

The MTX levels were determined on 57 matched pairs of CSF, and simultaneous serum samples were obtained from 38 patients who received one or more courses of high-dose MTX (Chart 1). It shows that MTX levels in the CSF reached...
10⁻⁷ M within the 1st 30 min and remained constant at this level for 24 hr, during which time the patients were receiving i.v. infusion of MTX. Throughout the 24-hr infusion, MTX levels in blood were approximately 100-fold greater than that of the CSF (i.e., 10⁻³ M in serum versus 10⁻⁷ M in CSF).

Chart 2 shows the results obtained form 3 patients with brain tumors who had an Ommaya device implanted for intraventricular chemotherapy. Multiple samples of CSF were obtained from the Ommaya device in each individual at 1, 2, 4, 6, and 24 hr following institution of the i.v. infusion of MTX. The results indicate that MTX concentrations in ventricular fluid were between 10⁻⁷ and 10⁻⁶ M during the 1st 24 hr and the levels appeared similar to those or CSF obtained from lumbar punctures in the leukemic patients.

Chart 3 shows the results of serum MTX level following standard p.o. MTX therapy (15 mg/sq m). Serum MTX level reached 10⁻⁷ within 2 hr and then dropped to undetectable levels after 24 hr.

The treatment protocol for children with newly diagnosed ALL currently used at our Institute is shown in Chart 4. The initial induction treatment consists of steroids and vincristine for 4 weeks followed by a 10-day course of L-asparaginase. After patients have completed induction and the treatment with L-asparaginase, 3 courses of MTX at 500 mg/sq m at 3 weekly intervals are administered. One-third of this dose is given by i.v. push and the remaining two-thirds is infused i.v. over 24 hr. Twenty-four hr after completion of i.v. MTX, a single dose of leucovorin is given at 12 mg/sq m. In addition, 6 doses of i.t. MTX, 12 mg/sq m, are given, 3 during induction and 3 concomitantly with high-dose i.v. MTX. Following the courses of treatment with high-dose MTX, a single dose of leucovorin is given at 12 mg/sq m. In addition, 6 doses of i.t. MTX, 12 mg/sq m, are given, 3 during induction and 3 concomitantly with high-dose i.v. MTX. Following the courses of treatment with high-dose MTX, maintenance treatment is initiated consisting of daily p.o. 6-mercaptopurine and weekly p.o. MTX plus pulses of steroid and vincristine.

The clinical protocol was initiated in August 1972, and 23 previously untreated children (<17 years of age) with ALL have been entered into this program. All 23 children achieved an M-1 marrow and to date all remain in continuous sustained complete remission from 1 to 32 months. All patients remain free of CNS leukemia. Thirteen patients have now been on therapy for longer than 1 year (Chart 5).

Toxicity secondary to high-dose MTX was not a severe problem. In the 23 1st-line patients who were already in remission, toxicity was minimal, whereas in the children with far-advanced leukemia, toxicity was more notable. In the former group, only 3 children experienced mild stomatitis; no significant marrow depression, CNS, or renal toxicity was observed. Of the remaining 16 patients with far-advanced disease, 5 had mild leukopenia and stomatitis and 1 developed severe but reversible leukopenia, stomatitis, dermatitis, and renal dysfunction.

**DISCUSSION**

MTX is a potent inhibitor of dihydrofolate reductase. In mice it has been shown that MTX inhibition of DNA synthesis in leukemic cells, bone marrow, and intestinal epithelium depends on a plasma concentration of free (unbound) MTX specific for each tissue (6). Thus, there was incomplete recovery of DNA synthesis in ascitic L1210 leukemic cells at any time after MTX treatment, although there was partial recovery when the MTX concentration of ascitic fluid returned to 10⁻⁶ M. The bone marrow had a 50% recovery at concentrations of 10⁻⁸ M or less and the intestinal epithelium had a 50% recovery at concentrations of 5 × 10⁻⁶ M (6). Investigation using continuous MTX infusion to achieve constant plasma levels has confirmed that MTX inhibition of DNA synthesis on target tissues is primarily due to the duration of exposure of the tissue to suprathereshold concentrations of drug, rather than the peak levels (20).

The clinical application of these findings requires a detailed knowledge of MTX pharmacokinetics in man and a determination of the sensitivity of normal and malignant human tissue in vivo. There is scanty information on the latter. Hryniuk and Bertino (11) demonstrated a pretreatment in vitro correlation between MTX concentration and suppression of DNA synthesis in human leukemic blasts, but there was no correlation between the ability of MTX to suppress DNA synthesis in vitro and the subsequent clinical
response to MTX therapy. They treated 21 patients with acute lymphocytic and acute nonlymphocytic leukemia with MTX doses of 80 to 240 mg/sq m with 5 complete and 7 partial remissions and found the greatest suppression of DNA synthesis in patients who later went into remission, but this suppression did not correlate with that expected from the pretreatment in vitro tests.

Following an i.v. bolus, the plasma level of MTX followed a triphasic pattern of disappearance with the half-life of each phase being 0.8, 3.5, and 27 hr, respectively (12). The 3rd phase with prolonged low levels of MTX is probably the major cause of bone marrow toxicity. Therefore, the toxicity of high-dose infusions can be minimized or prevented if followed within 42 hr by leucovorin (7); and this may be explained by antagonism of the leucovorin to the toxic effects of the slow terminal phase of MTX disappearance in the plasma. Because of the ability of leucovorin to prevent toxicity secondary to high-dose MTX, there are ongoing clinical trials (13) using high-dose MTX. The rationale for such therapy is based on the possibility that, in poorly perfused or transport-resistant tumors, cytotoxic intracellular drug concentrations can be obtained without intolerable host toxicity (5). The apparent effect of this approach may have other explanations such as a unique biochemical effect of high drug concentrations.

The rationale for using high-dose MTX (500 mg/sq m) with concurrent i.t. MTX for the prevention of CNS leukemia was based on the concept that by giving high-dose i.v. MTX and simultaneous i.t. MTX, higher MTX levels than i.t. MTX alone could be achieved and in all regions of CNS, since MTX would not be pumped out of CNS as quickly as when only i.t. MTX was given, thus producing more effective CNS prophylaxis. Support for this concept comes from the experiments of Oldendorf and Danson (16) who have clearly shown an increase in [14C]sucrose in the rabbit brain following combined i.v. and ventricular perfusions of this substance. The higher level achieved by combined infusion over either i.v. or ventricular perfusion was believed to be due to the prevention of [14C]sucrose loss from extracellular space of the brain into blood or into the CSF. Furthermore, following 3 hr of perfusion, [14C]sucrose in the brain appeared to be in true equilibrium with both plasma and CSF. Other experiments by Bourke et al. (4) have shown that i.v. infusion with simultaneous ventricular perfusion of 5-[14C]-fluorouracil achieved increased levels of this drug in all regions of the brain in the monkey.

In a recent report, Shapiro et al. (17) showed CSF levels of $6 \times 10^{-7}$ M following i.v. infusion of MTX at 500 mg/sq m.
over 24 hr given to patients with meningeal leukemia and meningeal carcinomatosis. They also showed negligible ventricular fluid concentrations following a single dose of 50 mg MTX i.v. Also of interest was the fact that MTX given intraventricularly (via an Ommaya reservoir) was distributed rapidly and evenly in the subarachnoid space, whereas MTX given via lumbar puncture showed considerable variation from patient to patient in ventricular fluid concentration.

High-dose i.v. MTX has the additional theoretical advantage of also "hitting" leukemic cells in other pharmacological sanctuaries such as the testis, ovaries, eyes, spleen, etc. which may be the initial site of relapse (18).

Our preliminary clinical data suggest that this treatment program is safe to administer with minimal toxicity and to date has been effective in preventing or delaying CNS leukemia. In addition to the theoretically CNS prophylaxis of high dose MTX, i.t. MTX was also given in the 23 patients with untreated ALL and MTX i.t. has proven efficacy in CNS prophylaxis (14); in addition, dexamethasone was used in place of prednisone and dexamethasone appears to be more effective than prednisone in preventing CNS disease. However, of prime importance is the fact that the results reported here provide a solid pharmacological basis on which to proceed with a controlled clinical trial in ALL.

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