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Original Articles

Pharmacokinetic Monitoring of High-dose Methotrexate

Early Recognition of High-Risk Patients

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Summary. *The administration of high-dose methotrexate (HDMTX) with leucovorin rescue carries with it a risk of severe toxicity which may be fatal. In the present study, patients with a 24-h serum concentration of* $< 5 \times 10^{-6}$ *M and an elimination half-life* ($T_{1/2}$) *of* < 3.5 *h during the first 24 h after the infusion were considered at low risk for toxicity and received conventional low-dose leucovorin rescue. Patients not meeting these criteria were considered at high risk for toxicity and received an escalated and extended course of leucovorin. The low-risk criteria were met following 109 of 114 HDMTX infusions administered to 30 patients. None of these patients developed toxicity with low-dose leucovorin. The 24-h serum concentration and the* $t_{1/2}$ *exceeded the low-risk criteria following five HDMTX infusions administered to three patients. In two of these three patients leucovorin was continued until the MTX concentration was* $< 10^{-8}$ *M (168–265 h) and no toxicity developed. The third high-risk patient discontinued his leucovorin 11 days prior to a MTX serum concentration* $< 10^{-8}$ *M and developed moderate toxicity. Clinical features present in the three high-risk patients, which were not present in the low-risk group, included a pleural effusion in one patient and gastrointestinal obstruction in the other two patients. The identification of 3/30 high-risk patients in the present study was consistent with a historical control group in which 6/65 patients developed severe toxicity. These data indicate that patients meeting the criteria described herein are at low risk to develop toxicity with conventional leucovorin rescue and that high-risk patients may be identified early enough to reduce or prevent toxicity.*

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Introduction

High-dose methotrexate (HDMTX) with leucovorin 'rescue' is widely used as adjuvant therapy for osteosarcoma, and in the treatment of non-Hodgkin's lymphoma, head and neck carcinoma, and other solid tumors [3, 23]. However, the administration of HDMTX with leucovorin carries with it the risk of severe toxicity which may be fatal [5, 10, 22]. A nationwide survey conducted prior to 1977 revealed a 6% incidence of mortality attributed to HDMTX [22]. For this reason, efforts have been made to identify factors which predispose to toxicity [5, 10] and to establish guidelines which reduce the risk of morbidity and mortality associated with HDMTX therapy.

Pharmacokinetic monitoring of methotrexate (MTX) has become a standard approach for prospective identification of patients at high risk for toxicity. However, there is no consensus among investigators regarding the exact criteria to be used to identify high-risk patients. Stoller et al. [20] suggest that patients with a 48-h serum concentration $> 9 \times 10^{-7}$ M and evidence of delayed clearance beyond 48 h are at high risk for toxicity with conventional rescue. Nirenberg et al. [13] reported that patients with a 24-h serum concentration $> 10^{-5}$ M, a 48-h level $> 10^{-6}$ M, and a 72-h concentration $> 10^{-7}$ M were at high risk for toxicity. However, continued low-dose leucovorin rescue did not prevent clinical toxicity in patients identified at high risk. Tattersall et al. [21] reported that patients with a serum concentration $> 5 \times 10^{-7}$ M at 48 h are likely to encounter severe myelosuppression. Isacoff et al. [9] initially reported that patients with serum levels $> 5 \times 10^{-6}$ M at 24 h were at increased risk for toxicity. More recently, these investigators have reported [9] that patients with a 24-h level $> 10^{-5}$ M or a 48-h concentration $> 5 \times 10^{-7}$ M are at increased risk for toxicity.

To be clinically useful, the criteria for prospectively monitoring HDMTX therapy should identify high-risk

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patients early enough to permit the initiation of a modified leucovorin rescue which would prevent toxicity. Since the effects of MTX may not be readily reversible if adequate leucovorin rescue is delayed for more than 42–48 h [1, 7], identification of high-risk patients at 24–36 h post-infusion would be advantageous. Because the cytotoxic effects of MTX appear to be a function of both concentration and duration of exposure, the rate of elimination of MTX from plasma may also be a useful criterion for identifying high-risk patients. In the present study, patients with a 24-h serum concentration $\leqq 5 \times 10^{-6}$ M and an elimination half-life ($t_{1/2}$) $\leqq$ 3.5 h during the first 24 h after the infusion were considered at low risk for toxicity and received conventional low-dose leucovorin rescue. The selection of 5×10^{-6} M as the maximum 24-h concentration was based on criteria published prior to the initiation of this study by Isacoff et al. [9]. The selection of 3.5 h as the maximum half-life for decline in serum concentrations was based on a previous study [18] at this institution, which reported a serum half-life of 2–3.5 h in children administered HDMTX. Patients not meeting these criteria were considered at higher risk for toxicity and received an escalated and extended course of leucovorin.

Materials and Methods

From December 1976 to January 1978, 30 patients ranging in age from 15 months to 25 years (median: 14 years) received 114 infusions of HDMTX at St. Jude Children's Research Hospital. Eighteen patients had osteosarcoma (12 adjuvant therapy), five colorectal carcinoma, four hepatocellular carcinoma, one testicular embryonal carcinoma, one chondrosarcoma, and one fibrosarcoma. All patients had a serum creatinine of < 1.2 mg/dl and a creatinine clearance of $\geqq$ 85 ml/min/1.73 m^2 prior to HDMTX. The dosage of MTX ranged from 725 mg/m^2 to 15,000 mg/m^2. All doses were administered as a constant rate intravenous infusion over six hours. The leucovorin rescue for all patients was begun three hours after the end of the MTX infusion. The dosage of leucovorin was 5% of the total MTX dosage administered in eight equal intravenous doses every three hours, followed by eight oral or intramuscular doses of 12 mg/m^2 every six hours. All patients received intravenous hydration and urinary alkalinization as previously recommended [5]. Patients with MTX serum concentrations > 5×10^{-6} M at 24 h following the end of the infusion and/or an elimination $t_{1/2}$ > 3.5 h were considered at increased risk to develop toxicity and were administered an escalated dose and duration of leucovorin rescue. The escalated dose of leucovorin was calculated to yield serum concentrations of leucovorin one log greater than MTX when MTX concentrations were > 10^{-7} M, based on previously published data describing the concentrations of leucovorin required to rescue given concentrations of MTX [17]. The dose of leucovorin was calculated to yield serum concentrations equimolar to MTX when MTX serum concentrations dropped below 10^{-7} M. Leucovorin dosage calculations were based upon available pharmacokinetic parameters for leucovorin [14], consistent with data reported by Mehta et al. [12].

MTX serum concentrations were measured in duplicate at 0, 6, 12, and 24 h after the end of the infusion by radioimmunoassay (Diagnostic Biochemistry, San Diego, California) and enzyme immunoassay (Syva Co., Palo Alto, California). Samples obtained later than 24 h after the infusion containing < 10^{-7} M MTX were measured by radioimmunoassay and by a radio-enzymatic ligand binding assay (New England Enzyme, Boston, Massachussets). The enzyme immunoassay and the radio-enzymatic assays consistently gave results lower than the radioimmunoassay. Preliminary results of a comparative evaluation in our laboratory of these three assays and analysis by high-pressure liquid chromatography suggest that the radioimmune assay is less specific for the unmetabolized MTX compound. However, results produced by any of the three methods used in the present study produced the same interpretations regarding the low-risk criteria. The (ln) serum concentration versus time data during the first 24 h after the infusion were fitted to a single exponential term by use of a linear least-squares regression computer program. The elimination half-life ($t_{1/2}$) during the first 24 h after the infusion was calculated as

$$t_{1/2} = 0.693/K$$

where K = -slope of the least squares regression line of the serum concentration-time data during the 24 h post-infusion period.

Results

Following 109 of 114 HDMTX infusions, the 24-h serum concentration was less than 5×10^{-6} M and the elimination half-life was less than 3.5 h. All of these patients received only low-dose leucovorin rescue for 72 h and none developed any evidence of MTX toxicity. The 24-h serum concentration was greater than 5×10^{-6} M following five infusions administered to three patients (Fig. 1). The elimination half-life was greater than 3.5 h following all five of these HDMTX infusions. The half-life for the remaining (low-risk) patients ranged from 1.7 h to 3.4 h with a median of 2.8 h. The elimination half-life was independent of MTX dosage or patient age (Fig. 2).

One of the high-risk patients was a 12-year-old boy with metastatic osteosarcoma, who had a left pleural effusion. This patient received 12 000 mg/m^2 of MTX with leucovorin rescue until his MTX serum concentration was < 10^{-8} M (168 h) and developed no MTX toxicity (Fig. 3). The second patient was an 18-year-old boy who had testicular embryonal carcinoma with retroperitoneal and hepatic metastases. This patient had failed prior therapy with cis-platinum and had developed right hydronephrosis and gastrointestinal obstruction secondary to tumor compression. Following his first dose of MTX (5000 mg/m^2) he had elevated serum levels and delayed MTX excretion. Leucovorin was therefore administered until his MTX serum concentration was < 10^{-8} M (265 h) and no MTX toxicity was observed. No subsequent doses of HDMTX were given. The third high risk patient was a 14-year-old boy with adenocarcinoma of the rectosigmoid colon and hepatic metastases. This patient also developed bilateral hydronephrosis and gastrointestinal (distal jejunal) obstruc-

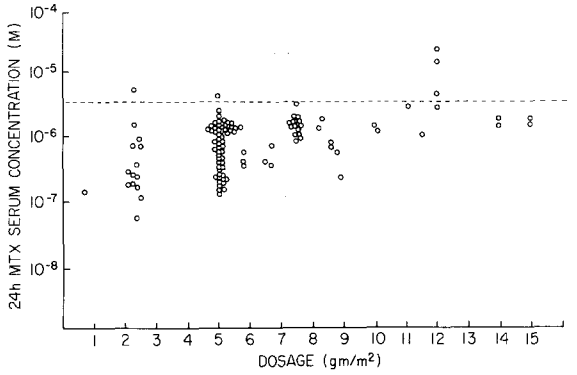

Fig. 1. The serum concentration of methotrexate (O) 24 h after the end of 114 six-hour high-dose infusion administered to 30 patients. Concentrations displayed against methotrexate dosage

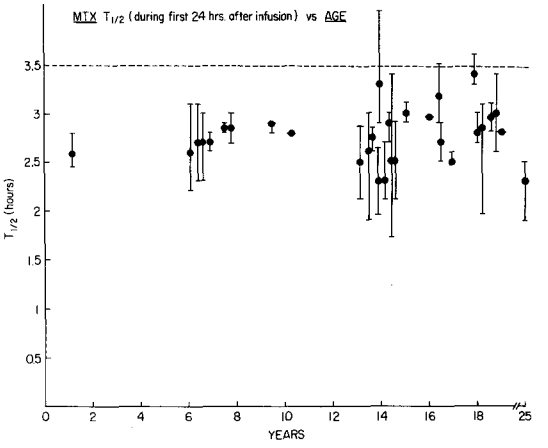

Fig. 2. The half-life for decline in methotrexate serum concentrations during the first 24 h after the end of a six-hour intravenous infusion. Mean and range (●) are shown for individual patients, displayed against patient age in years

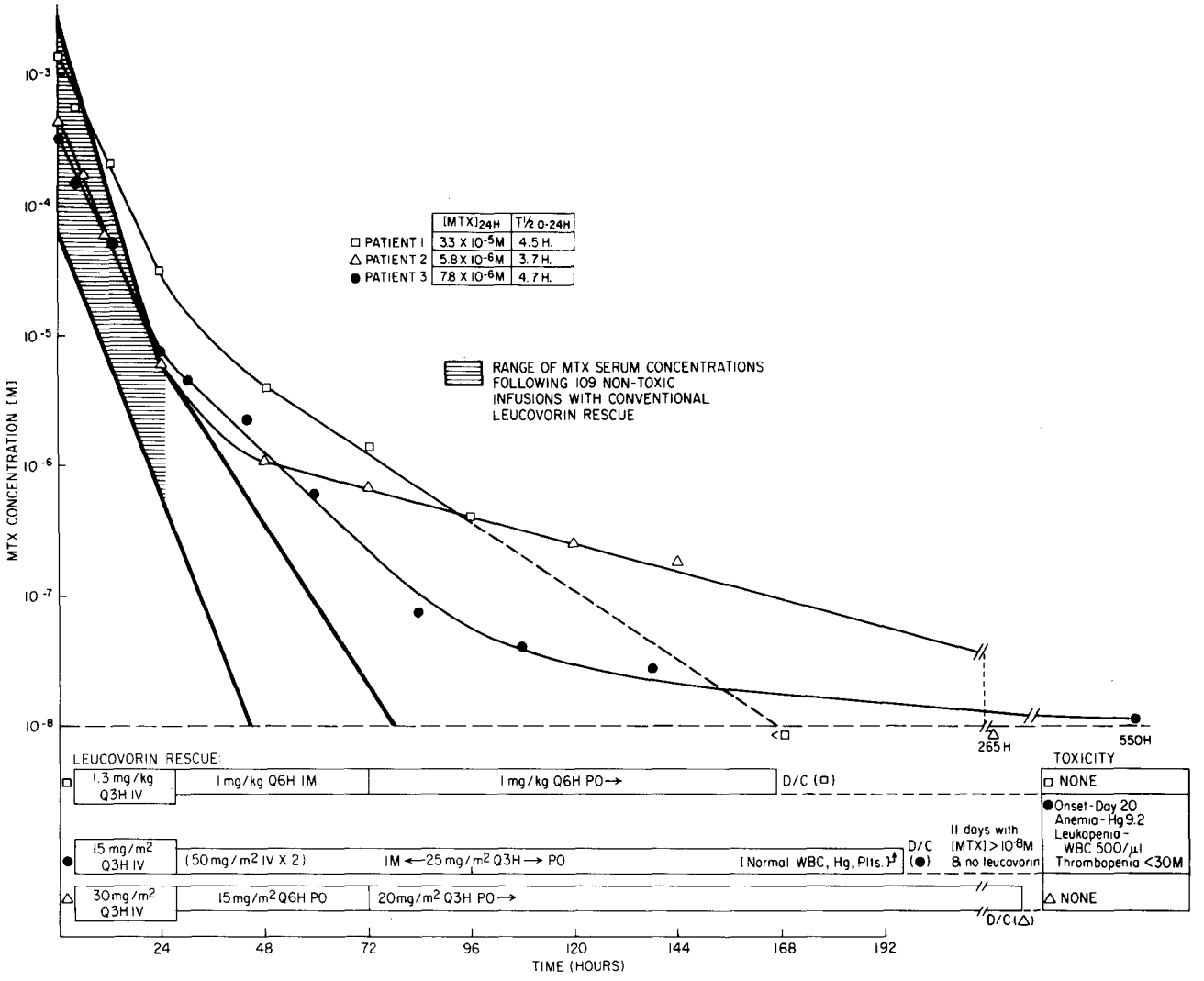

Fig. 3. Serum concentration versus time curves following 114 high-dose methotrexate infusions. Striped area represents range of serum concentrations following 109 infusions administered to 27 patients which met the low risk criteria. Serum concentration versus time plot beyond 24 h for the striped area was derived from 14 patients monitored for up to 76 h. Horizontal bars describe the dose and duration of leucovorin rescue for the three high-risk patients. Leucovorin rescue was discontinued at 72 h for all low risk patients

tion secondary to tumor compression prior to HDMTX. He was given 2500 mg/m^2 of HDMTX and received leucovorin rescue for approximately 200 h at which time he failed to return to the clinic to obtain additional leucovorin. Twenty days after the HDMTX and approximately 11 days after leucovorin was stopped because of non-compliance, anemia (Hg 9.2), leukopenia (500/μl), and thrombocytopenia (< 30 000/μl) were documented at another hospital. The patient was transferred back to this institution where the MTX serum concentration on the day of return was 1.5×10^{-8} M (550 h), indicating that at least 11 days had elapsed during which the MTX serum concentration was $> 10^{-8}$ M and no leucovorin was given. No other evidence of MTX toxicity developed (renal, hepatic, mucosal) and hematological recovery was evident within seven days.

All patients administered HDMTX had normal renal function prior to the MTX infusion. None of the low-risk patients had a pleural effusion, hydronephrosis, or gastrointestinal obstruction; clinical features which were present in the three high-risk patients.

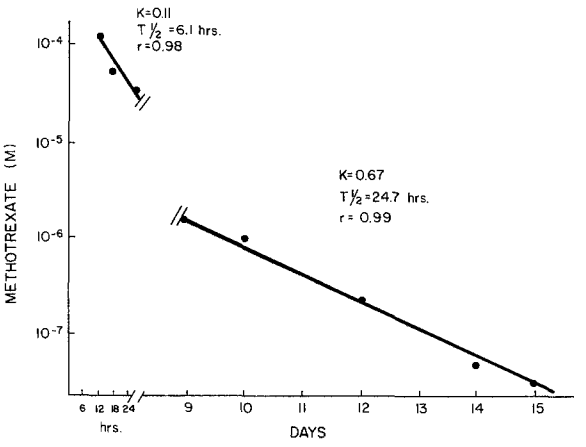

Fig. 4. Serum concentration versus time curve for one patient in historical control group who developed fatal methotrexate (MTX) toxicity prior to the initiation of prospective monitoring of all high-dose MTX patients

Discussion

The present study indicates that patients with a 24-h MTX serum concentration $< 5 \times 10^{-6}$ M, a $t_{1/2}$ (0—24 h) < 3.5 h and no evidence of clinical features associated with delayed MTX clearance (renal dysfunction, pleural effusion, ascites, other 'third spaces', or possibly GI obstruction) are at low risk for toxicity with conventional low-dose leucovorin rescue. Using these criteria for prospective monitoring of HDMTX patients, no drug related deaths have occurred. Hematopoietic toxicity occurred in one of three high-risk patients, probably because leucovorin rescue was not continued until the MTX serum concentration was $< 10^{-8}$ M. Pinedo and Chabner [15] have reported that, at MTX concentrations $> 10^{-8}$ M, the cytotoxic effects are a function of both drug concentration and duration of exposure. Their data demonstrated that exposure to 5×10^{-8} M for 72 h produces the same effect as exposure to 10^{-5} M for 12 h. Previous animal studies have indicated that the inhibition of DNA synthesis in tumor cells, bone marrow, and intestinal epithelium requires the presence of a serum concentration of free MTX specific for each tissue [4]. The inhibition of DNA synthesis in mouse bone marrow is virtually complete with plasma MTX concentrations above 10^{-8} M, whereas intestinal epithelium show similar inhibition at MTX levels above 5×10^{-9} M. Subsequent studies using an infusion device to maintain constant serum concentrations demonstrated the partial inhibition of DNA synthesis at levels of 2×10^{-8} M and more complete inhibition of intestinal mucosa at this concentration [26, 27]. Similar findings have been reported in humans where resumption of DNA synthesis did not occur until serum concentrations were 2×10^{-8} M or below [24]. Although the absolute threshold appears to be organ dependent and remains to be precisely quantitated in human experiments, we currently recommend that leucovorin be continued in *high-risk* patients until the MTX concentration is $< 10^{-8}$ M.

We have previously reported [5] that prior to the initiation of prospective monitoring of all HDMTX infusions at this institution, severe toxicity occurred following 6/307 infusions administered to 65 patients. Using the criteria described in the current study, the number of high-risk patients identified (3/30) is consistent with our previous experience of 6/65 patients developing severe toxicity. As previously reported [5], three of these six cases of severe toxicity prior to 1976 had a fatal outcome. Serum MTX concentrations and elimination $t_{1/2}$ values were available retrospectively in only one of these six cases of severe toxicity. As shown in Fig. 4, the 24-h MTX concentration of 3×10^{-5} M and the $t_{1/2}$ (0—24 h) of 6.0 h would have prospectively identified this patient as being at high risk for toxicity.

It must be stressed that some clinical features associated with delayed MTX clearance may not always affect the elimination $t_{1/2}$ during the first 24 h after HDMTX administration. As previously reported in detail [6], the presence of a pleural effusion may prolong the terminal phase $t_{1/2}$ of MTX (> 30 h post-infusion) while not significantly delaying excretion during the first 24 h post-infusion. Therefore, patients with pleural effusions, ascites, or other 'third spaces' should be considered high-risk patients regardless of their 24-h serum concentration or $t_{1/2}$ (0—24 h) and should have serum levels monitored until the MTX concentration is $< 10^{-8}$ M. Conversely, delayed MTX excretion and severe tox-

icity may also occur in patients with no clinical features currently associated with delayed MTX clearance [5]. The importance of gastrointestinal obstruction as a clinical feature associated with delayed MTX clearance is unclear. Two of the three high-risk patients described herein had a small bowel obstruction. Both of these patients also had hepatic metastases of their primary disease with minimally abnormal liver function (total bilirubin 1.1–2.5 mg/dl, prothrombin time 3–6 s > control). Previously reported [2, 11, 25] kinetic models describing MTX disposition have identified the gastrointestinal tract as a major site of distribution and metabolism of MTX. Zaharko [25] and Bischoff [2], in studies of MTX disposition in lower animals and man, reported the persistence of higher MTX concentrations in gut lumen of the small intestine when compared to liver, kidney, muscle, or plasma. Their model predictions in mouse and man indicated that higher plasma levels in man are due to less rapid clearances by the kidney and bile and a longer residence time in the small intestine of man. Subsequent kinetic models [19] for MTX have included multiple compartments to describe the disposition of MTX in the GI tract of man. Methotrexate in the gastrointestinal tract may either be reabsorbed by a saturable process, excreted in the feces, or taken up and metabolized by bacteria in the large intestine. The differences between man and smaller animals in the persistence of MTX in the gastrointestinal tract and the rate and extent of metabolism by gut bacteria is due in part to differences in transit time between the small and large intestine [25]. Whether a partial GI obstruction in man could sustain serum concentrations by preventing fecal excretion and bacterial metabolism and thereby serving as a resource (i.e., 'third space') for slow reabsorption of MTX is conceivable, but remains to be conclusively documented.

Criteria for identification of high-risk patients is important since an escalated dose and duration of leucovorin rescue should be reserved for only those patients at high risk for toxicity. Sirotnak et al. [19] have demonstrated that progressive increases in the leucovorin dosage on any schedule reduces both the toxicity and antitumor effects of MTX. The present study indicates that patients meeeting all the criteria described herein are at low risk to develop toxicity with conventional low-dose leucovorin rescue. Conversely, these criteria apparently permit identification of potentially high-risk patients early enough to reduce or prevent toxicity by modifying the dose and duration of leucovorin rescue. Application of these criteria in an expanded patient population will determine whether these guidelines retain their validity as an early indicator of impending toxicity.

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References

1. Bertino, J. R.: Techniques in cancer chemotherapy. Use of leucovorin and other rescue agents after methotrexate treatment. Semin. Oncol. **4,** 203–216 (1977)
2. Bischoff, K. B., Dedrick, R. L., Zeharko, D. S., Longsheth, J. A.: Methotrexate pharmacokinetics. J. Pharm. Sci. **60,** 1128–1133 (1971)
3. Catane, R., Bono, V. H., Louie, A. C., Muggia, F. M.: High dose methotrexate, not a conventional treatment. Cancer Treat. Rep. **62,** 178–180 (1978)
4. Chabner, B. A., Young, R. C.: Threshold methotrexate concentrations for in-vivo inhibition of DNA synthesis in normal and tumorous target tissues. J. Clin. Invest. **52,** 1804–1811 (1973)
5. Chan, H., Evans, W. E., Pratt, C. B.: Recovery from toxicity associated with high-dose methotrexate: Prognostic factors. Cancer Treat. Rep. **61,** 797–804 (1977)
6. Evans, W. E., Pratt, C. B.: Effect of pleural effusion on high-dose methotrexate kinetics. Clin. Pharmacol. Ther. **23,** 68–72 (1978)
7. Goldie, J. H., Price, L. A., Harrap, K. R.: Methotrexate toxicity: Correlation with duration of administration, plasma levels, dose and excretion pattern. Eur. J. Cancer. **8,** 409–414 (1972)
8. Isacoff, W. H., Townsend, C. T., Eilber, F. R., et al.: High-dose methotrexate therapy of solid tumors: observations relating to clinical toxicity. Med. Pediatr. Oncol. **2,** 319–325 (1976)
9. Isacoff, W. H., Morrison, P. F., Aroesty, J., et al.: Pharmacokinetics of high-dose methotrexate with citrovorum factor rescue. Cancer Treat. Rep. **61,** 1665–1674 (1977)
10. Jaffe, N., Traggis, D.: Toxicity of high-dose methotrexate (NSC-740) and citrovorum factor (NSC-3590) in osteogenic sarcoma. Cancer Chemother. Rep. Part 3. **6** (1), 31–36 (1975)
11. Leme, P. R., Creaven, P. J., Allen, L. M., Berman, M.: Kinetic model for the disposition and metabolism of moderate and high-dose methotrexate in man. Cancer Chemother. Rep. **59** (1), 811–817 (1975)
12. Mehta, B. M., Gisolfi, A. L., Hutchinson, D. J., Nirenberg, A., Kellick, M. G., Rosen, G.: Serum distributions of citrovorum factor and 5-methyltetrahydrofolate following oral and IM administrations of calcium leucovorin in normal adults. Cancer Treat. Rep. **62,** 345–350 (1978)
13. Nirenberg, A., Mosende, C., Mehta, B. M., Gisolfi, A. L., Rosen, G.: High-dose methotrexate with CF rescue: Predictive value of serum methotrexate concentrations and corrective measures to avert toxicity. Cancer Treat. Rep. **61,** 779–783 (1977)
14. Nixon, P. F., Bertino, J. R.: Effective absorption and utilization of oral formyltetrahydrofolate in mass. New Engl. J. Med. **286,** 175–179 (1976)
15. Pinedo, H. M., Chabner, B. A.: Role of drug concentration, duration of exposure and endogenous metabolites in determining methotrexate cytotoxicity. Cancer Treat. Rep. **61,** 709–715 (1977)
16. Pinedo, H. M., Zaharko, D. S., Bull, J., Chabner, B. A.: The relative concentration of drug concentration and duration of exposure to mouse bone marrow toxicity during continuous methotrexate infusion. Cancer Res. **37,** 445–450 (1977)
17. Pinedo, H. M., Zaharko, D. S., Bull, J. M., Chabner, B. A.: The reversal of methotrexate cytotoxicity to mouse bone marrow

cells by leucovorin and nucleosides. Cancer Res. **36,** 4418–4478 (1978)
18. Pratt, C. B., Roberts, D., Shanks, E., et al.: Clinical trials and pharmacokinetics of intermittent high-dose methotrexate-leucovorin rescue for children with malignant tumors. Cancer Res. **34,** 3326–3331 (1974)
19. Sirotnak, F. M., Moccio, D. M., Dorick, D. M.: Optimization of high-dose methotrexate with leucovorin rescue therapy in L1210 leukemia and sarcoma 180 murine tumor models. Cancer Res. **38,** 345–353 (1978)
20. Stoller, R. C., Hande, K. R., Jacobs, S. A., Rosenberg, S. A., Chabner, B. A.: Use of plasma pharmacokinetics to predict and prevent methotrexate toxicity. N. Engl. J. Med. **297,** 630–634 (1977)
21. Tattersall, M. H. N., Parker, L. M., Pitman, S. W., Frei, E.: Clinical pharmacology of high-dose methotrexate. Cancer Treat. Rep. **6** (3), 25–29 (1975)
22. Von Hoff, D. D., Penta, J. S., Helman, L. J., et al.: Incidence of drug-related deaths secondary to high-dose methotrexate and citrovorum factor administration. Cancer Treat. Rep. **61,** 745–748 (1977)
23. Von Hoff, D. D., Rozencweig, M., Louie, A. C., Bender, R. A., Franco, M.: "Single"-agent activity of high-dose methotrexate with citrovorum factor rescue. Cancer Treat. Rep. **62,** 233–235 (1978)
24. Young, R. C., Chabner, B. A.: An in-vivo method for monitoring differential effects of chemotherapy on target tissue in animals and man: Correlation with plasma pharmacokinetics. Cancer Res. **52,** 92a (1973)
25. Zaharko, D. S., Dedrick, R. L., Bischoff, K. B., et al.: Methotrexate tissue distribution: Prediction by a mathematical model. J. Natl. Cancer Inst. **46,** 775–784 (1971)
26. Zaharko, D. S., Dedrick, R. L., Peale, A. L., Drake, J. C., Lutz, R. J.: Relative toxicity of methotrexate in several tissues of mice bearing Lewis lung carcinoma. J. Pharm. Exp. Ther. **189,** 585–592 (1974)
27. Zaharko, D. S., Fung, W. P., Yang, K. H.: Relative biochemical aspects of low and high doses of methotrexate in mice. Cancer Res. **37,** 1602, 1607 (1977)

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Note Added in Proof. Since completion of this manuscript, 176 additional HDMTX infusions have been given to 35 patients. The 24 h MTX concentration and half-life exceeded the low-risk criteria following six doses given to six patients. These six patients were given an escalated and extended course of leucovorin rescue. Toxicity was not observed following these six doses or the remaining 170 HDMTX infusions given conventional leucovorin rescue.