

Concepts in use of high-dose methotrexate therapy

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In cancer chemotherapy, routine monitoring of drug concentrations has been practical only for methotrexate (MTX). The primary setting for pharmacokinetic monitoring of MTX is its use in high doses (HDMTX) for adjuvant therapy of osteosarcoma, for single-agent treatment of intracranial lymphomas, and in combination therapy of childhood leukemia as well as adult and pediatric non-Hodgkin lymphomas. Typically, HDMTX is infused in doses of 3–15 g/m² over a period of 6–24 h. Precautions must be taken to ensure a high urine flow and an alkaline urine pH, so as to prevent precipitation of MTX in urine. Patients with decreased renal function, advanced in age, and taking nonsteroidal anti-inflammatory drugs or nephrotoxic agents are at increased risk of developing renal dysfunction during MTX infusion, thus being placed at high risk for toxicity. At the end of HDMTX infusion, and periodically thereafter for 24–48 h, drug concentrations are measured to assure that the disappearance rate of MTX from plasma is occurring at a normal rate. Also, at the end of HDMTX infusion, the patient is given leucovorin (5-formyl-tetrahydrofolic acid; LV), which replenishes intracellular stores of reduced folate and attenuates the toxicity secondary to HDMTX. In the presence of inappropriately high concentrations of MTX, routine doses of LV will be ineffective; the dose of LV required must be increased in proportion to the MTX concentration it faces in plasma. In practice, routine monitoring of plasma MTX concentrations allows early detection of abnormal clearance, as well as institution of early and effective countermeasures, including the use of increased and prolonged LV rescue.

INDEXING TERMS: leukemia • osteosarcoma • monitoring therapy • pharmacokinetics • urine • leucovorin

The use of high-dose methotrexate (HDMTX) has shown benefit in the treatment of osteosarcomas, childhood lympho-

mas, and certain adult non-Hodgkin lymphomas, particularly those of the Burkitt type [1–4].¹ Although methotrexate (MTX) is used in the treatment of many malignancies, the benefit of using HDMTX (≥ 1 g/m²), as opposed to conventionally dosed MTX (< 1 g/m²), has been better appreciated in the treatment of childhood acute lymphocytic leukemia (ALL) and osteosarcoma [4–10]. Freeman et al. [5] reported that the systemic relapse rate was notably decreased in children with ALL treated with HDMTX compared with those receiving conventionally dosed MTX. Subsequently, Evans et al. [4] showed that a critical relationship existed between serum concentrations of MTX in children with ALL and the probability of remaining in remission. In the latter study, children with ALL who achieved a steady-state MTX concentration of ≥ 16 μ mol/L had a much better chance of remaining in remission vs those children with concentrations of ≤ 16 μ mol/L.

The optimal HDMTX dosing in childhood ALL, however, remains to be defined. In the studies by Evans et al. [4], although all children received the same dose of HDMTX (1 g/m²), there was considerable variation in the steady-state serum concentrations of MTX attained (9.3–25.4 μ mol/L). The authors concluded that variability in drug elimination between patients was responsible for the wide range in steady-state MTX concentrations and therefore in the patients' systemic exposure [4]. HDMTX also appears to be important in the treatment of central nervous system leukemia; although a head-to-head comparison of HDMTX with conventional-dose MTX in treating active CNS leukemia has not been performed, enough pilot studies experience exists to support a superior role for HDMTX in treating childhood CNS ALL [11]. MTX at conventional doses fails to adequately protect against CNS relapse [12]. This most likely reflects (as Shapiro et al. [13] have shown) the fact that barely cytotoxic concentrations of MTX (0.1 μ mol/L) are achieved in the cerebrospinal fluid (CSF) when conventional doses of MTX (500 mg/m²) are used. Conversely, HDMTX dosed at 33.6 g/m² was shown by Balis et al. [6] to achieve

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¹ Nonstandard abbreviations: MTX, methotrexate; HDMTX, high-dose methotrexate; LV, leucovorin; ALL, acute lymphocytic leukemia; CNS, central nervous system; CSF, cerebrospinal fluid; GFR, glomerular filtration rate; MTX-PG, polyglutamated methotrexate; DHFR, dihydrofolate reductase; and MTHE, N⁵-methyl-tetrahydrofolate.

100-fold higher CSF concentrations ($10 \mu\text{mol/L}$); lower-dosed HDMTX ($3\text{--}7.5 \text{ g/m}^2$) is also capable of reaching higher CSF concentrations ($1 \mu\text{mol/L}$), as Pitman et al. [14] have shown.

Coincident with the achievement of higher MTX concentrations in CSF, the use of HDMTX was associated with impressive responses (80% complete and 20% partial response rates) for subjects with active childhood CNS ALL [6]. Similarly, single-arm trials indicate surprising effectiveness of HDMTX for patients with primary non-AIDS-related CNS lymphoma [15].

Dose intensification of MTX also appears to be important in the treatment of osteosarcomas. HDMTX as part of adjuvant multidrug chemotherapy has helped decrease the 2-year relapse rate for osteosarcoma from 83% to 34% [7]. Delepine et al. [8] treated osteosarcoma patients with HDMTX by using a dose-escalation protocol, which permitted higher dosing of HDMTX based on serum pharmacokinetic monitoring, and compared this group with patients whose dosages were based on age and body surface area. These studies demonstrated that higher doses of MTX (up to 20 g/m^2), a regimen possible only with pharmacokinetic monitoring, resulted in a substantial increase in total MTX dose given and in mean peak serum concentrations achieved. Higher mean peak serum concentrations were in turn correlated with substantially higher rates of histologic response in surgically resected specimens (66% vs 45%) and greater disease-free survival at 5 years (92% vs 74%). Similar experiences have also been reported by others in support of HDMTX use and the existence a dose-response relationship for MTX in osteosarcoma [9, 10].

HDMTX has not conferred a survival advantage in head and neck cancer, despite the presence of a dose-response relation reported for patients with head and neck cancer treated with either 50 (low), 500 (medium) or 5000 mg/m^2 (high) doses of MTX [16]. Much higher responses were seen in patients who successfully completed all courses of HDMTX (90%) compared with those treated with medium (27.3%) and low doses of MTX (35.3%). However, the higher response rates observed with HDMTX in this study were negated by the unacceptably high toxicity (including several deaths), as well as the high dropout rate (55%) observed in the HDMTX group; more importantly, no survival advantage was conferred by HDMTX treatment. Similarly, added toxicity but no survival advantage was conferred to patients with small cell lung cancer who were treated with HDMTX, compared with patients treated with conventionally dosed MTX as part of a multidrug regimen [17].

Mechanism of MTX Action and Pharmacology

Knowledge of the folate metabolic pathways and the mechanism of action of MTX provides a basis for the biochemical argument for the use of HDMTX. (For a detailed overview of the cellular pharmacology of MTX, see the excellent review by Ackland and Schilsky [18]). Folate in the form of $N^{5,10}$ -methylene-tetrahydrofolate has a pivotal role as a cofactor in the synthesis of deoxythymidylate (dTTP); other reduced folates are also involved in the synthesis of purines (Fig. 1). Rapidly dividing cells require thymidine triphosphate (dTTP), as well as purines for DNA synthesis; MTX blocks cell growth by interfering with the

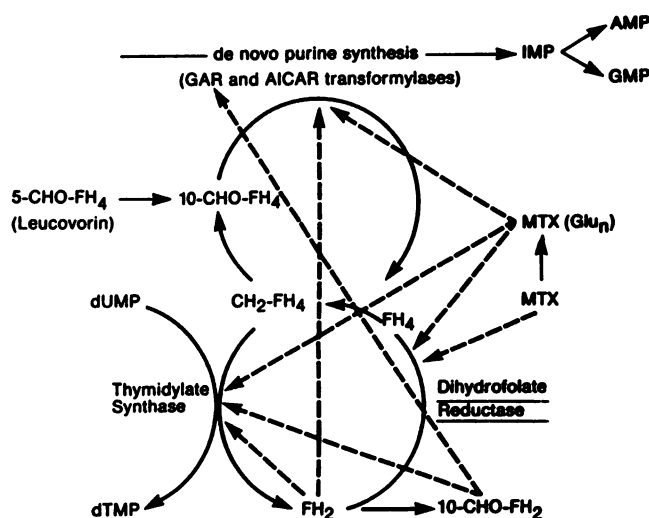


Fig. 1. Site of action of MTX and its polyglutamated derivatives, MTX-PG and MTX(Glu_n).

Also shown are the folate byproducts dihydrofolate (FH_2) and 10-formyldihydrofolate (10-CHO-FH_2), which accumulate after inhibition of dihydrofolate reductase (DHFR) and, together with MTX and MTX(Glu_n), inhibit the synthesis of thymidylate and purines [28]. GAR, glycineamide ribonucleotide; AICAR, aminoimidazole carboxamide ribonucleotide. Source: Chabner et al. In: DeVita VT, Hellman S, Rosenberg, SA, eds. Cancer principles and practice of oncology. Philadelphia: JB Lippincott, 1989:349. Reprinted by permission.

synthesis of these nucleotides (see Fig. 1). The concentration of MTX required to block cell growth has been studied by Chabner and Young [19] in healthy as well as in tumor-bearing mice by determining the *in vivo* incorporation of tritium-labeled deoxyuridine in bone marrow, intestinal mucosa, and ascitic L1210 leukemic cells. Their studies showed that DNA synthesis returned to 50% of pretreatment rates at MTX plasma concentrations $<10 \text{ nmol/L}$ for bone marrow and ascitic leukemic cells and at $<5 \text{ nmol/L}$ for intestinal mucosal cells.

MTX plasma concentrations also appear to influence selective nucleotide blockade. Zaharko et al. [20], evaluating the biochemical aspects of low- vs high-dose continuous MTX infusion in mice, noted that at the lower dose a thymine block was produced at sustained plasma MTX concentrations of 10 nmol/L ; at the higher dose, which produced a sustained plasma MTX concentration of 100 nmol/L , production of both purine and thymidylate was blocked.

Duration of exposure appears to be as critical as drug concentration exposure. Pinedo and Chabner [21] showed that the nadir for depletion of nucleated bone marrow cells depended on the drug concentration as well as the length of exposure in mice treated with constant-infusion MTX. A nadir decrease to 30% of control cells in bone marrow was reached with infusions that resulting in MTX plasma concentrations of $10\,000 \text{ nmol/L}$ for 12 h, 1000 nmol/L for 24 h, 100 nmol/L for 48 h, and 10 nmol/L for 72 h. Surprisingly in this study, comparison of the $C \times t$ (concentration $\times$ time) constants, which reflect drug exposure, yielded values within a markedly wide range. This finding indicated that duration of exposure was the predominant factor, once the threshold for cytotoxicity was exceeded [21]. Hence, achieving prolonged periods of exposure, even at lower plasma MTX concentrations can have serious implications for

tumor kill as well as host toxicity. The latter was demonstrated by Zaharko et al. [20] in mice treated with constant MTX infusions; LD₂₀ (20% lethality dose) was reached when mice were exposed to MTX at either 10 nmol/L for 80 h or 100 nmol/L for only 24 h.

Knowledge of the cellular pharmacology of MTX permits an appreciation of potential sites of resistance that may defeat MTX therapy and that may, in theory, be overcome with HDMTX. These areas of cellular MTX handling have the potential to serve as advantage points for HDMTX over conventionally dosed MTX. The first of these advantage points is MTX transport across the cell membrane. A saturable, energy-dependent transport system normally facilitates the transport of endogenous folates, such as N⁵-methyltetrahydrofolate (MTHF), the predominant folate form. MTHF competes with MTX for active transport, as does also leucovorin (N⁵-formyltetrahydrofolic acid; LV), discussed later. Also facilitating transport is passive diffusion, which is dependent on increasing extracellular concentrations of MTX; this form of cellular transport takes on a role of its own in cells that have become resistant to MTX by loss of an active transport mechanism [18]. The intracellular steady-state concentrations achievable in cells through passive diffusion alone, however, appear to be considerably less: In a study by Hill et al. [19], L5178Y lymphoblasts with an impaired active transporter for MTX demonstrated intracellular steady-state concentrations of MTX 6.3 times lower than the concentrations in wild-type cells at extracellular MTX concentrations of 10 μ mol/L; at 50 μ mol/L, however, the difference was 2.2 times lower. Hence, by achieving higher extracellular drug concentrations, HDMTX may facilitate entry of MTX into cells; this may be of particular importance in the eradication of clones resistant to MTX on the basis of cell membrane transport defects.

The second potential advantage point for HDMTX capitalizes on the finding that, once inside the cell, MTX is metabolized to polyglutamated (MTX-PG) derivatives akin to natural folates (Fig. 2 [22]). Formation of MTX-PG, particularly those with longer (four to five) glutamate chains, leads to two notable changes in the biochemical behavior of MTX: (a) reduced drug efflux, which results in sustained intracellular MTX concentrations for a prolonged period [23–25], and (b) enhanced binding and inhibition of the intracellular target enzymes dihydrofolate reductase (DHFR), thymidylate synthetase, and 5-aminoimidazole-4-carboxamide ribotide transformylase [23, 25–28]. Formation of MTX-PG is influenced by both extracellular MTX concentrations and duration of exposure [24, 25]. Synold et al. [29], in evaluating MTX-PG formation in bone marrow blasts taken from children with ALL who were treated with either low- or high-dose MTX, found that MTX-PG formation was substantially greater in blasts from children treated with HD-MTX. Enhanced MTX-PG formation with HDMTX therapy may be facilitated by exposure to higher extracellular MTX concentrations as well as prolonged exposure. The optimal extracellular MTX concentration and duration for MTX-PG formation in studies involving several breast cancer lines appears to be $\geq 2 \mu$ mol/L for 24 h [24, 30]; this is clinically achievable with HDMTX but not with conventional MTX therapy [5, 6].

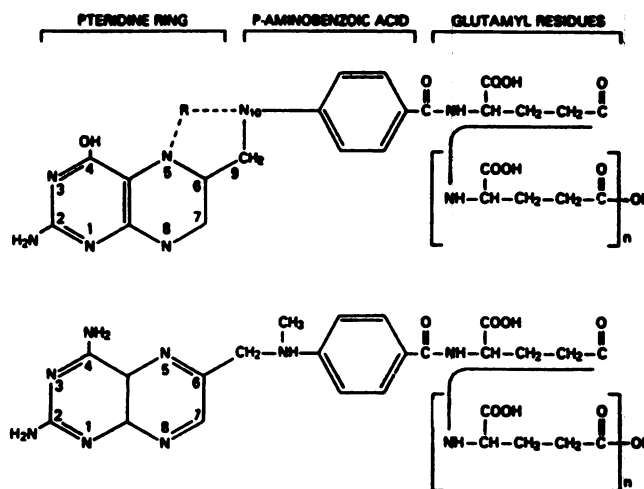


Fig. 2. Structure of tetrahydrofolate (top) and methotrexate (bottom) polyglutamates.

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Although HDMTX may offer an advantage for eradicating tumor cells by increasing intracellular MTX concentrations and formation of MTX-PG, the benefit of this therapy over conventional-dose MTX may lie in the ability to eradicate resistant clones that contain greater concentrations of DHFR as a result of enhanced transcription [31] or gene amplification [32]. Limitations to HDMTX therapy, in turn, may result from failure to eradicate resistant clones because of defective formation of MTX-PG [30] or qualitative changes to DHFR that result in diminished binding [33].

Toxicity with HDMTX Therapy

Before many preventative measures, including careful drug monitoring with supplemental LV rescue (see below), were incorporated into most HDMTX regimens, considerable toxicity was noted in connection with HDMTX therapy. Von Hoff et al. [34], reviewing the records of 498 patients treated with HDMTX before 1977, noted a 6% incidence of drug-related deaths. Of these deaths, 80% were attributed to severe myelosuppression, which resulted in either sepsis or hemorrhage; the remaining 20% were attributed to renal failure. More contemporary series—incorporating rigorous hydration, urine alkalinization, and careful drug monitoring with supplemental LV rescue—have shown a considerable variation in toxicities, apparently largely dependent on the age of the patients. Younger patients for the most part had mild, tolerable toxicities when treated with HDMTX [6, 10], whereas older patients exhibited significant toxicities, including drug-related deaths [16, 17]. In the studies by Saeter et al. [10], 376 preoperative courses of HDMTX (8 to 12 g/m²) were administered to 97 patients with osteosarcoma (median age 16 years). Mild gastrointestinal complaints (nausea, oral mucositis, diarrhea) were the most common adverse effects of HDMTX therapy in this young group of patients, and no deaths occurred. Severe bone marrow toxicity (WHO grade III/IV) complicated 0.5% of courses unaccompanied by life-threatening infections. Renal toxicity, as indicated

by transient increases in creatinine (including one episode up to 5 times normal values) complicated 1.4% of all courses. Interestingly, delayed MTX excretion (as assessed by serum MTX monitoring) was seen in 15% of HDMTX-treated patients. The vast majority of these patients responded with 24 h of additional hydration and supplemental LV coverage [10]. Transient liver function abnormalities occurred in 80% of these, but almost all were benign in consequence and reversible. Balis et al. [6] reported no deaths in 20 children with ALL (median age 6.5 years) treated with even higher doses of HDMTX (33.6 g/m²). The most serious adverse event was focal seizure activity and transient hemiparesis in one patient who received HDMTX [6]. One-third of the courses were marked by neutropenia; a few patients required hospitalization for treatment with antibiotics. No renal toxicity was seen; 60% of the courses, however, were accompanied with transient abnormalities in liver function, although the patients were asymptomatic.

In contrast to the relatively mild and tolerable untoward effects noted in the young patients treated with HDMTX, Woods et al. [16] studied of 22 patients with head and neck cancer (median age 61 years) and attributed 3 deaths to resulting myelosuppression from treatment with HDMTX (5 g/m²). Moreover, 6 of the 22 patients experienced severe renal toxicity, in contrast to the unimpressive renal toxicity encountered with the younger patients treated with HDMTX. All patients had to have a minimum creatinine clearance of 60 mL/min to enter the study. No significant correlation was seen between initial serum creatinine concentration or creatinine clearance and development of renal toxicity. Besides older age, poorer performance status may have been a determining factor for enhanced HDMTX-related toxicity in this study: One-third of the patients had an ECOG performance status of >1 [16].

Prevention of HDMTX-Related Toxicity

Careful patient selection, adequate hydration and urinary alkalization, avoidance of drug interactions, drainage of third-space fluids (when present), and pharmacodynamic monitoring with appropriate adjustments of LV doses have succeeded in making HDMTX, in general, well-tolerated chemotherapy.

ASSESSING RENAL FUNCTION

Because the kidneys are the principal route of excretion for MTX, determination of normal renal function is considered a prerequisite for HDMTX administration. Glomerular filtration, tubular secretion, and tubular reabsorption all play a role in the renal handling of MTX [18]. Stoller et al. [35] showed that as much as 80% of MTX appears in the urine unchanged within 12 h of MTX administration. Abelson et al. [36] showed that transient decreases in glomerular filtration rate (GFR) (mean decrease, 43%; mean post-HDMTX inulin clearance, 67 mL/min) were seen in nine children who were receiving HDMTX and had a nontoxic course. After completion of HDMTX treatment, no significant change in serum creatinine was seen in these patients [36]. Similar observations of transient decreases in creatinine clearance have also been reported in adult patients treated with HDMTX, who similarly experienced no toxicity [37]. In contrast, Abelson et al. [36] retrospectively found that

three patients who experienced toxicity from HDMTX had greater reductions in GFR (mean decrease, 61%; mean post-HDMTX inulin clearance, 33 mL/min) than the decreases identified in their prospective analysis of nontoxic patients. Toxicity secondary to HDMTX was also related to prolonged length of time for GFR reduction [36].

A normal serum creatinine concentration and a minimum GFR of 60 mL/min have generally been adopted as reasonable criteria for adequate renal function to ensure sufficient clearance of HDMTX [16, 17, 38]; however, these criteria do not entirely abrogate risk for HDMTX-related toxicity. Abelson et al. [36] showed that the serum creatinine was within normal values before the start of HDMTX therapy in the three patients who exhibited toxicity, as was also their creatinine clearance. The presence of an initial normal serum creatinine value failed also to identify 7 patients who experienced toxicity (including 1 death) in a series of 78 patients who received HDMTX [39]. Determination of pre-HDMTX treatment GFR does not predict MTX clearance and subsequent toxicity, as Kerr et al. [40] have shown, perhaps because tubular function contributes to MTX excretion. Age, and perhaps age-dependent changes in tubular function, play a substantial role in influencing MTX pharmacokinetics, including the rate of renal clearance [41]. Pharmacokinetic monitoring of a test dose of MTX to predict which patients are at high risk for HDMTX toxicity may be useful [40] and should be considered in elderly patients or in those with borderline renal function.

MAINTAINING ADEQUATE HYDRATION

Aggressive hydration is necessary along with urine alkalization (discussed later) to promote brisk diuresis and to prevent intratubular precipitation of MTX (Fig. 3), MTX-related renal failure, and subsequent toxicity secondary to delayed MTX clearance. In HDMTX therapy, hydration takes on a more significant role because of the production of 7-OH-MTX, a metabolite of MTX not appreciably produced at conventional doses of MTX [42, 43]. The limited aqueous solubility of 7-OH-MTX may contribute to HDMTX-related renal toxicity



Fig. 3. Kidney autograph (~475×) from a rhesus monkey 24 h after administration of [³H]MTX at a dose of 200 mg/kg (546 Ci), demonstrating intratubular MTX precipitation [42, 43].

[42, 43]. The effect of hydration on HDMTX pharmacokinetics was the subject of a study by Ferrari et al. [44], who examined the relation of higher (2 L/m²) vs lower dose (1.5 L/m²) hydration on MTX plasma concentrations and MTX elimination. Their studies showed that excess hydration decreased plasma MTX (427 vs 585 μ mol/L) when sampled at the end of the HDMTX infusion, whereas plasma concentrations 14 and 38 h later were not statistically different. Although excess hydration was noted to decrease peak MTX concentrations substantially, no additional toxicities were noted in the group receiving the lower amount of hydration. The optimal urine flow to ensure adequate renal excretion of MTX, as analyzed by Sasaki et al. [45] in children receiving HDMTX, should be 0.1–1.8 mL/m² per minute with a urine pH of 7.0 to ensure adequate MTX clearance. Interestingly, a considerably higher flow was needed with lower urine pH, because of the marked decrease in drug solubility at more acidic pH.

MAINTAINING ALKALINE URINE PH

MTX and its metabolite 7-OH-MTX, which is seen predominantly with HDMTX therapy, show respectively 20- and 12-fold increased solubility when pH increases from 5.0 to 7.0 [42]. Renal tubular precipitation of MTX and 7-OH-MTX (Fig. 3) occurs in an acidic urine environment (pH < 5.7) [38]; this likely contributes to renal failure and delayed MTX clearance [14, 42, 43]. Pitman and Frei [14] showed that urinary alkalinization achieved with oral sodium bicarbonate resulted in substantially less nephrotoxicity and myelotoxicity when historically compared with patients without urinary alkalization. A pH of >7.0 was maintained in this study, in addition to rigorous hydration (>3 L/day). Interestingly, Sand et al. [46] showed that maintaining high urinary flow was not as important as maintaining an alkaline urine pH in promoting MTX clearance.

AVOIDING DRUG INTERACTIONS

About 50% of MTX is bound to serum proteins, a fairly constant proportion irrespective of serum MTX concentration [46]. Toxicity may occur in HDMTX treatment if drugs having the potential to displace MTX from serum proteins are administered with HDMTX—e.g., salicylates, phenylbutazone, phenytoin, and sulfonamides [47]. Administration of HDMTX with nonsteroidal anti-inflammatory drugs is especially to be avoided because of the potential to inhibit MTX renal clearance and to displace serum-bound MTX, thereby creating higher and prolonged MTX concentrations [48]. Thyss et al. [49] reported 3 deaths in a series of 36 patients who inadvertently received the nonsteroidal anti-inflammatory ketoprofen during HDMTX administration. Use of concomitant probenecid should also be avoided with HDMTX because it inhibits renal tubular transport of MTX [50], as would potentially nephrotoxic drugs such as gentamicin and cisplatin [47].

DRAINAGE OF THIRD-SPACE FLUIDS

The presence of third-space fluids (e.g., ascites and pleural effusions) constitutes an important contraindication to the administration of HDMTX. Wan et al. [51] showed that the plasma half-life of MTX was prolonged in patients with third-

space fluids. Prolonged MTX exposure (with subsequent toxicity) reflects sustained back-diffusion into the intravascular compartment from accumulated MTX in third-space fluids, where high concentrations of MTX can accumulate. Drainage of third-space fluids before HDMTX administration has been recommended to prevent toxicity [38].

MONITORING SERUM MTX CONCENTRATIONS

Monitoring serum MTX is an essential part of HDMTX administration aimed at identifying patients at highest risk for HDMTX-related toxicity. In doing so, prompt action (see below) can be taken to avert or minimize subsequent toxicity. Several nomograms based on the disappearance of MTX from serum have been empirically constructed to identify those patients at highest risk for toxicity; they vary somewhat, depending on the HDMTX regimen used (a typical nomogram is shown in Fig. 4). Various cutoff points based on the half-life of MTX plasma or serum disappearance have been used for initiating action to prevent or minimize toxicity. Stoller et al. [39] measured MTX plasma clearance in 78 patients (395 treatment courses) who received HDMTX in a 6-h infusion. By 48 h after starting the infusion, an MTX plasma concentration of 0.9 μ mol/L was associated with a higher frequency of toxicity: About one-half of the patients who had a higher MTX concentration experienced severe myelotoxicity [39]. Other cutoff points have also been identified as signifying MTX concentrations that place patients at higher risk of toxicity; again, these vary with the HDMTX regimen used and are based on time points from 18 to 72 h post-HDMTX infusion start [52–55].

Management of HDMTX-Related Toxicity

LEUCOVORIN "RESCUE"

Use of HDMTX would be lethal were it not followed by the administration of reduced folates (such as LV) to circumvent the metabolic blockade imposed by MTX [18]. The use of LV rescue was first introduced by Goldin et al. [56], who showed that drug-related deaths in mice inoculated with L1210 leukemic cells were substantially less when LV was administered with MTX. However, compared with the animals that had received MTX alone, overall survival was improved only in animals that

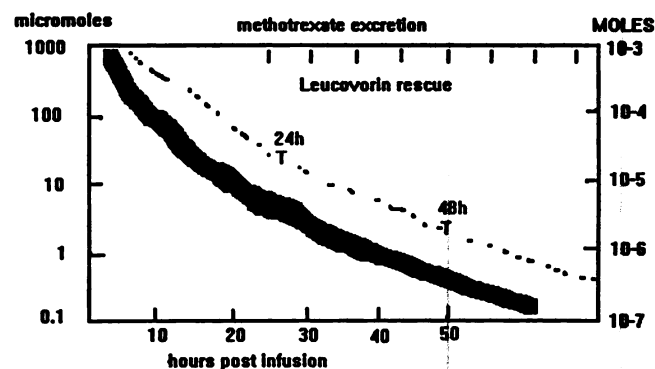


Fig. 4. Typical nomogram for serum methotrexate (MTX) clearance: Concentrations above T (toxicity line) indicate actual or possible imminent severe MTX-related toxicity.

Courtesy of David Harmon, Massachusetts General Hospital.

Table 1. Leucovorin dosimetry with high-dose methotrexate (MTX) treatment and extended "leucovorin rescue" schedules.

Ref	MTX dose	Leucovorin schedule ^a	MTX toxicity alert		Extended leucovorin schedule ^b
			Time, h	Conc, $\mu\text{mol/L}$	
52	50–300 mg/kg	Starting at 8 h, 40 mg/m ² IV, then 15 mg IM/po for 11 doses.	24	10	400 mg by infusion over 24 h, then 100 mg over next 24 h.
53	8–12 g/m ²	10 mg po every 6 h, starting at 20 h, for 10 doses.	24	20	Based on formula: 40 (MTX serum conc.) $\div$ normal MTX conc., upper limit.
			48	2	
			72	0.1–0.9	
54	12.5 g/m ²	Starting at 18 h, 15 mg IV every 3 h for 2 doses then po every 6 h for 48 h.	72	0.3	10–30 mg every 6 h. 100 mg IV every 3 h.
55	1.0 g/m ²	0.9 to 72 mg/m ² for 3 doses at 30, 36, and 42 h.	36	1	50–100 mg/m ² every 6 h.

^a Time points depicted for initial leucovorin schedule are from start of high-dose methotrexate infusion.

^b Until "rescue"; i.e., MTX $\leq$ 0.1 $\mu\text{mol/L}$.

IV, intravenously; IM, intramuscular injection; po, orally.

received delayed (12 h post-MTX injection), but not MTX-concurrent, LV. These results illustrated that LV rescue can indeed abrogate MTX-related toxicity. The timing of the "rescue," however, greatly influences overall tumor-free survival by improving the index of normal vs malignant cell rescue. In humans, LV administration can be delayed as long as 24 to 36 h from the start of HDMTX administration and still, in general, maintain fairly tolerable toxicity [6, 10, 57].

LV *in vivo* is converted to MTHF, which ordinarily serves as the major circulating reduced folate, and which acts to replete the reduced intracellular folate pool required for the production of thymidylate and the purines [18]. Rescue with MTHF has also been shown [58]; however, LV, a more stable form of reduced folate, is the preferred pharmacological agent for abrogating MTX-related toxicity. LV is a mixture of stereoisomers, of which only the L-isomer is metabolically active; thus only ~50% of a given dose is actually active drug [59].

The dose and frequency of LV rescue have been developed empirically and differ according to the regimen of HDMTX used (Table 1). Bertino [60] showed that DNA synthesis is effectively restored in healthy human marrow cells exposed to 2 $\mu\text{mol/L}$ MTX when LV is added at a 10-fold excess.

In addition to repleting reduced intracellular folate pools, excess extracellular concentrations of LV may promote "rescue" by competing with MTX for active transport into cells [61]. Duration of the LV rescue is also important and should be continued until serum MTX concentrations are <10 nmol/L; higher concentrations inhibit bone marrow proliferation [19]. LV rescue is logically continued until plasma MTX falls below 10 nmol/L, at which point circulating natural folates are believed to be sufficient to prevent cytotoxicity [62].

ALTERNATIVE "RESCUE"

HDMTX-related renal failure and accidental MTX overdoses (systemic as well as intrathecal) may lead to life-threatening complications in which LV rescue alone may not be sufficient. In these situations, where inordinately high MTX concentrations ($\geq$ 10 5 $\mu\text{mol/L}$) persist, a recombinant derivative of the bacterial enzyme carboxypeptidase-G2 has been used success-

fully in experimental [63, 64] and clinical settings [65, 66] to prevent life-threatening complications. The enzyme works by rapidly hydrolyzing MTX into the inactive metabolites 4-deoxy-4-amino-*N*¹⁰-methylpteroic acid and glutamate [64]; it is available upon request from the Cancer Therapy Evaluation Program of the National Cancer Institute.²

A role for HDMTX in the treatment of osteosarcoma, childhood lymphomas, and certain adult lymphomas, including those of the CNS, has been established. Despite earlier studies with HDMTX, which were complicated with substantial toxicities, contemporary series incorporating many safeguards have succeeded in making HDMTX, in general, a well-tolerated chemotherapy. These safeguards include careful patient selection, adequate hydration and urinary alkalization, avoidance of drug interactions, drainage of third-space fluids (when present), and pharmacodynamic monitoring with appropriate LV rescue.

² Phone: 301-496-6138.

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