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# Achievement of Long Duration Methotrexate Exposure with Concurrent Low Dose Thymidine Protection: Influence on Methotrexate Pharmacokinetics*

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**Abstract**—*Thymidine protection was evaluated as a technique for increasing the concentration × time exposure with long duration–high dose MTX infusions. Thymidine was infused at* 2.0 g/m$^2$/day *concurrently with MTX* 3.375 g/m$^2$/day. *The elimination half-life of MTX from the serum* ($T_{\frac{1}{2}}\beta$) *increased linearly with duration of MTX infusion, suggesting that long duration exposure to* $5 \times 10^{-5}$M *MTX resulted in storage of a pharmacokinetically significant amount of MTX in tissue reservoirs. Seventy-two-hr infusions were tolerated without major toxicity, despite the* 75% *reduction in thymidine dose rate and* 76% *increase in MTX dose compared to previous studies. The results indicate that even low doses of thymidine partially protect normal tissues* in vivo, *and suggest that differences in uptake and release of MTX from tissue deposits must be considered as a source of variation in MTX pharmacokinetics.*

## INTRODUCTION

THE OPTIMAL dose-schedule for cell cycle phase specific drugs, such as methotrexate (MTX) [1], is one that maintains cytotoxic concentrations in the environment of the tumor cell for long enough to permit a significant proportion of the tumor cells to enter the sensitive phase of the cell cycle and be damaged [2]. The cell cycle generation times of most human solid tumors are quite long relative to the duration of MTX exposure achievable in man with or without leucovorin rescue [3–6]. In addition many tumors are partially resistant to MTX and very high extracellular concentrations of MTX are required to produce cytotoxic amounts of free intracellular MTX [7]. Although current high dose MTX programs achieve very high serum MTX concentrations, the duration of exposure must be limited to 36–42 hr to avoid life-threatening toxicity [3, 4]. Thymidine partially protects normal human tissues against MTX [8–10], and this protection may be selective for marrow and gastrointestinal epithelial cells due either to a differential antipurine action of MTX, or differential ability of malignant and normal tissues to utilize exogenous thymidine [11, 12].

We have performed a phase I trial to determine how long $5 \times 10^{-5}$M MTX can be maintained in the systemic circulation of patients when thymidine is administered concurrently in a dose just sufficient to protect normal tissues [10]. Exposure durations of 72 hr are attained without serious toxicity, but because the elimination half-life of MTX increased linearly with the length of exposure longer duration exposures were impractical.

## MATERIALS AND METHODS

### *Patients*

All of the 10 patients who gave their informed consent to enter this study had biopsy proven malignancies not responsive to thera-

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*Table 1. Characteristics of 10 patients treated with MTX–dThd*

| Characteristic | No. of patients |
|---|---|
| Age range 4–67 yr, median 47 yr | |
| Sex: | |
| male | 7 |
| female | 3 |
| Prior treatment | |
| Chemotherapy only | 5 |
| Radiation only | 0 |
| Chemotherapy and radiotherapy | 4 |
| No prior treatment | 1 |

pies of established merit. Entrance criteria included life expectancy >2 months, creatinine <1.2 mg/dl, white blood count (WBC) >4000/mm$^3$, platelet count >150,000/mm$^3$, modified Karnofsky performance status of >50%, and age <70 yr. Some characteristics of the patient population are outlined in Table 1. Histologic types of cancer included: lung (non-oat cell) 1, leiomyosarcoma 2, pancreatic 1, bladder 1, breast 1, uterine cervix 1, acute lymphoblastic leukemia 2, lymphoma 1.

*Study design and treatment plan*

A total of 16 courses were given to 10 patients. Since our previous studies [9] had established the safety of 24-hr infusions, this trial was initiated using 48-hr infusions, and the duration of infusion was increased on subsequent courses to 72 hr. A MTX serum concentration of approximately $5 \times 10^{-5}$M was established with a bolus injection of 0.42 g/m$^2$, and then maintained by continuous infusion of 3.375 g/m$^2$/day for the duration of the infusion. A constant i.v. infusion of thymidine was started simultaneously and continued until the serum MTX concentration was $<5 \times 10^{-8}$ M. A thymidine dose of 1 g/m$^2$/day was used for the first 3 courses, but because of early toxicity a thymidine dose of 2 g/m$^2$/day was used all subsequent courses. All patients received $NaHCO_3$ 3.0 g p.o. every 3 hr to alkalinize the urine. $NaHCO_3$ was started 12 hr before the MTX infusion and was continued for 24 hr after the end MTX administration. In addition just before the start of the MTX infusion each patient was given 25 mEq of $NaHCO_3$ i.v. and then urine output was maintained at >3 l/day during the infusion. MTX and thymidine were obtained from the Division of Cancer Treatment National Cancer Institute. Thymidine was supplied as a 3% solution in 0.6% sodium chloride.

*Treatment parameters*

Measurements of patient performance and disease status, hemogram, and liver and kidney function were obtained prior to each course of therapy. In addition, blood urea nitrogen and serum creatinine were measured daily during the treatment period, and WBC and platelet counts were repeated at least weekly. Mucositis was graded on a scale of 1–4 with grade 1 being subjective symptoms only, grade 2 moderate and grade 3 severe limitation of p.o. intake, and grade 4 being complete cessation of p.o. intake. MTX serum concentrations were measured by radioimmunoassay using an antibody supplied by Diagnostic Biochemicals, Inc., San Diego, California.

## RESULTS

Sixteen courses of treatment were given to 10 patients; all courses were evaluable for toxicity, and 8 of the 10 patients were evaluable for tumor response. The geometric mean steady-state concentration of MTX in patients receiving 3.375 g/m$^2$/day ranged from 4.7 to

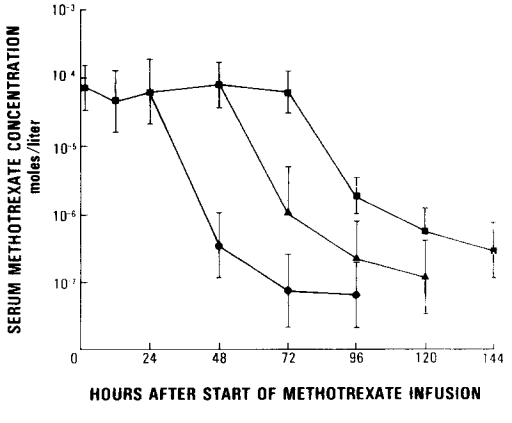


*Fig. 1. Geometric mean (±S.D.) serum MTX concentrations in patients receiving 3.375 g/m$^2$/day by continuous i.v. infusion for 24 hr (●); 48 hr (▲); 72 hr (■).*

$8.2 \times 10^{-5}$M. Figure 1 shows the pharmacokinetic profile of serum MTX concentrations for 24-, 48-, and 72-hr infusions; the data for the 24-hr infusion were taken from another recent drug trial performed at this institution with an identical MTX dose rate [9]. As plotted in Fig. 2 the elimination half-life ($T_{\frac{1}{2}}\beta$) during

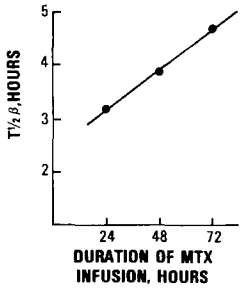


*Fig. 2. Variation of serum MTX elimination half life* ($T_{\frac{1}{2}}\beta$) *as a function of duration of MTX infusion.*

the first 24 hr after the end of drug infusion increased linearly from 3.2 hr for the 24-hr infusion, to 3.9 hr for the 48-hr infusion, and 4.7 hr for the 72-hr infusion. The mean MTX concentration 24 hr after a 2-day infusion was 3.3-fold higher than that after a 1-day infusion. Following a 3-day infusion, the mean MTX concentration 24 hr after the drug was stopped was 5.8-fold higher than after a 1-day infusion. There was little or no effect of infusion duration on the rate of drug level decay after the first 24 hr. None of these patients had prolongation of MTX excretion due to nephrotoxicity.

These results suggest that MTX was being stored in body reservoirs in amounts proportional to the duration of infusion, and then slowly released into the circulation after drug infusion was stopped. Such a reservoir effect has been observed in patients with large third space fluid collections such as pleural or peritoneal effusions [13, 14]. However, none of the patients in this study had effusions of any kind. Two patients were studied in detail to determine whether administration of a large dose of a closely related compound, leucovorin, could displace MTX from tissue stores. In neither case did the i.v. bolus injection of 100 mg/m$^2$ of leucovorin alter the rate of serum MTX decay.

Table 2 shows the incidence of toxicity as a function of duration MTX infusion. The initial courses were administered using a thymidine dose of 1 g/m$^2$/day, but because of early severe myelosuppression the remainder of the study was accomplished using 2 g/m$^2$/day of thymidine. Both 48- and 72-hr infusions produced mild to moderate myelosuppression and mucositis. In all cases complete recovery occurred by day 21, and neither the median WBC or platelet nadir, nor the day on which the nadir occurred, nor the grade of mucositis was clearly related to duration of MTX infusion. No other form of MTX-induced toxicity occurred during this trial. The total duration of MTX infusion was not limited by toxicity, but rather by the fact, as discussed above, that as the duration of infusion was increased the decrement in serum MTX concentration became slower and slower, often requiring patients to receive continuous i.v. infusion of thymidine for 9 out of every 21 days.

Figure 3 compares the areas under the concentration × time curve for the 72-hr infusion and an equitoxic "high-dose" MTX dose schedule consisting of bolus i.v. injection of 3.375 g/m$^2$ of MTX followed by thymidine rescue starting 24 hr later. This figure makes the point that the long duration infusion schedule allowed an increase in the concentration × time exposure to the tumor by a factor of >5-fold while producing approximately the same degree of toxicity to normal tissues. Despite this achievement no significant tumor responses were observed in this

*Table 2. Toxicity of MTX–dThd as a function of dThd dose and duration of infusion*

| Duration of MTX infusion | dThd dose (g/m$^2$/day) | No. of patients | No. of courses | WBC count nadir | | | Platelet count nadir | | | Mucositis incidence/ course |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Median count* | Range* | Median day | Median count* | Range* | Median nadir | |
| 48 hr | 1 | 3 | 3 | 2.9 | 0.7–3.2 | 7 | 52 | 7–218 | 13 | Grade I–1 |
| | 2 | 7 | 8 | 3.7 | 1.0–7.6 | 11 | 161 | 29–354 | 11 | Grade I–2<br>II–2 |
| 72 hr | 2 | 5 | 5 | 4.1 | 2.4–5.2 | 10 | 254 | 182–358 | 10 | Grade I–1<br>II–3<br>III–1 |

*Counts in thousands/mm$^3$.

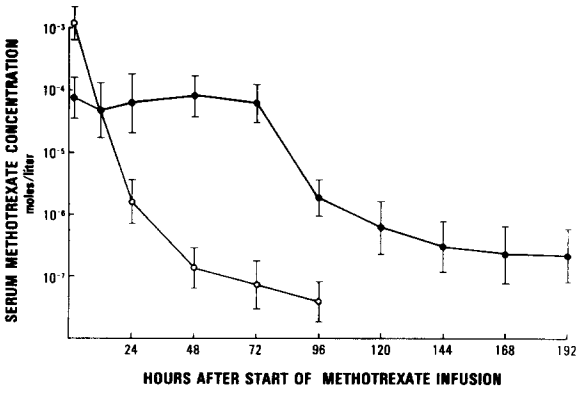


*Fig. 3. Comparison of area under serum MTX concentration curves for 2 equitoxic high dose MTX dose schedules: MTX* 3.0 g/m$^2$ *i.v. bolus with thymidine rescue* (○); *MTX* 3.375 g/m$^2$/day *i.v. constant infusion for* 72 hr *with concurrent thymidine protection* (●). *Each point represents the geometric mean* (±S.D.).

group of extensively pretreated patients with very advanced disease.

## DISCUSSION

This phase 1 trial yielded two noteworthy pieces of information: first, that during long duration high level infusions, MTX appears to exchange into body compartments, other than third space fluid collections, from which it is subsequently only slowly released; second, that it was possible to achieve very long duration high level exposures to MTX using much smaller doses of thymidine to protect normal tissues that had previously been demonstrated [8].

The results of this study strongly suggest that very high concentration–long duration exposure to MTX causes storage of sufficient drug in tissue reservoirs to alter the *in vivo* pharmacokinetics. This phenomenon has not been observed with bolus infusion schedules of doses even up to 200 mg/kg [15] except in patients with third space fluid collections [13, 14]. MTX may be stored in the form of polyglutamates. Many tissues and cells, including liver and kidney [16–19], red blood cells [17] and fibroblasts can synthesize polyglutamates of MTX which are then poorly diffusable and only slowly released from the cell [16]. The extent of polyglutamate formation is proportional to the concentration and duration of MTX exposure [16, 20]. Further studies will be required to determine whether polyglutamates of MTX constitute the deep compartment detected in this study, or whether the change in pharmacokinetics with duration of exposure is accounted for by diffusion of MTX into and out of pharmacologic sanctuaries such as the brain substance. Nevertheless, differences in uptake and release of MTX from tissue deposits must be considered an important source of patient to patient variation in MTX pharmacokinetics, and may account in part for the characteristic unpredictability of MTX toxicity [21].

MTX is a cell cycle phase specific agent [1], and thus its toxicity is a function of duration of exposure relative to the cell cycle time of the target cells once the MTX concentration is high enough to produce inhibitory quantities of free intracellular drug [22]. Human marrow can tolerate only 36–42 hr of unopposed MTX exposure [3] even when rescue agents are used following drug infusion [4, 9], before serious myelosuppression becomes evident. This is short in relation to the cell cycle generation time of most human solid tumors [5, 6], and has severly limited exploration of the potential for improvement in the therapeutic ratio with longer duration infusions, such as is evident with other cell cycle phase specific agents, particularly cytosine arabinoside [23]. The results of this study indicate that a program of concurrent infusion of high dose MTX and low dose thymidine allowed exposure to an average concentration of $6.6 \times 10^{-5}$M MTX for at least 72 hr, and maintained potentially cytotoxic concentrations of MTX ($>5 \times 10^{-8}$M) in the serum for a mean of 9 days. This approaches a long enough period of time that a significant proportion of the cells in even a slowly growing tumor might be expected to have entered S phase [5, 6], and exceeds by >5-fold the concentration × time exposure achievable with high dose MTX/leucovorin rescue programs. Despite the substantial increase in concentration × time exposure, the MTX/thymidine program can only be expected to improve response rates for those tumors in which a concentration of $6.6 \times 10^{-5}$M MTX is sufficient to inhibit dihydrofolate reductase in the malignant tissues. In the case of highly resistant tumors, a program of bolus infusion of MTX that produces very high peak serum concentrations of the drug, might be more effective since the MTX threshold of the tumor may be exceeded for at least some period of time even though the total concentration × time exposure is much smaller.

The ability of thymidine protection to permit 72-hr exposure to MTX was previously demonstrated by Ensminger and Frei [8] using thymidine at 8 g/m$^2$/day. The current study differs from the former in two important

regards. First, a 75% smaller dose rate of thymidine was used in the current study; second, in this study a total of 10.5 g/m$^2$ of MTX was successfully administered over 72 hr, which is 176% of the largest dose used in the previous trial. The much lower thymidine dose rate is of particular importance because we have demonstrated, both *in vivo* [10] and *in vitro* [24], that there is a very steep dose–response relation for thymidine protection and rescue of normal human tissues in the thymidine dose range of 0.3–3.0 g/m$^2$/day. Preliminary studies* indicate that the same is true for many malignant tissues. If there is any selectivity of thymidine protection, it may be due in part to differences in the ability of normal and malignant tissues to utilize thymidine via the salvage pathway. Where this difference is small, and the dose–response curve steep, the degree of selectivity may vary widely with slight changes in serum thymidine concentration. Thus the aim of this study was to use just enough thymidine to protect normal tissues, since excess thymidine may obliterate any therapeutic advantage of the MTX/thymidine combination [11].

*S. B. Howell, unpublished results.

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