Thymidine Rescue of High-Dose Methotrexate in Humans

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ABSTRACT

Thymidine rescue was administered following 63 courses of high-dose methotrexate in 20 patients. In the first part of this study, the methotrexate was given as a 24-hr infusion and the dose was escalated from 0.14 to 8.54 g/sq m; in the second part, methotrexate was infused to maintain a serum concentration of 15 μM for 30, 36, or 40 hr. Thymidine rescue was started immediately after the end of the methotrexate infusions, and consisted of 8 g/sq m/day for 3 days or until serum methotrexate was below a toxic level. Mucositis and myelosuppression were the major toxicities. Neither was dose related. Serum methotrexate levels were proportional to the logarithm of the methotrexate dose. There was a mean 6-fold increase in thymidine concentration during rescue. However, thymidine levels prior to and during rescue were not related to the incidence of subsequent toxicity. Recovery of DNA synthesis in bone marrow cells was evident by nucleoside precursor incorporation at 24 hr after the start of rescue. Two of 16 evaluable patients achieved partial responses. This study indicates that thymidine is an effective rescue agent for high-dose methotrexate in humans.

INTRODUCTION

MTX is thought to exert cytotoxicity by causing depletion of intracellular reduced folates required as cofactors for the de novo synthesis of thymidylate and purines (18, 27). The biochemical action of MTX can be reversed either by restoring reduced cofactor pools with leucovorin, or by providing the end products of the cofactor-dependent reactions, dThd and purines (10, 11). In murine systems, normal marrow and gut cells in vivo can generally be protected by dThd alone (22, 25), whereas some tumor cells require a source of purines in addition to dThd (3, 13, 26). In this situation, rescue with dThd alone may be able to completely protect normal host tissues while leaving tumor cells partially unrescued (25). This may result in selective tumor cell kill and further improvement in the therapeutic ratio of MTX (5, 25).

A previous investigation at this institution demonstrated that, in humans, continuous infusions of dThd given simultaneously with continuous infusions of MTX blocked the toxicity of up to 2 g/sq m/day of MTX for 3 days (5). The current study was undertaken to determine whether dThd could be used as a rescue agent in humans, starting 24 hr or more after the beginning of MTX exposure. The results indicate that dThd can rescue doses of MTX as high as 8.54 g/sq m given over 24 hr and that exposures to cytotoxic MTX concentrations for up to 36 hr can be rescued with dThd.

MATERIALS AND METHODS

Patients. Patients with histologically proven cancer who had received and failed all therapies of proven merit, and who had recovered from the toxicity associated with any prior therapy were eligible for this study. The patient had to be capable of giving informed consent, and had to have a life expectancy in excess of 2 months. Normal renal function (serum creatinine <1.2 mg/dl, creatinine clearance >60 ml/min) and adequate bone marrow function (WBC >4,000/cu mm, platelets >150,000/cu mm) were required. Mild abnormalities of hepatic function, or prior therapy with MTX did not exclude a patient.

Study Design and Treatment Plan. This study was divided into 2 parts. In the first part, MTX was given as a 24-hr infusion at doses of 0.14 to 8.54 g/sq m. Each course of therapy was initiated by rapidly injecting one-eighth of the MTX dose i.v. and then infusing the remaining MTX by constant infusion pump over a 24-hr period. In the second part, a bolus injection of 0.125 g/sq m was given, followed by a constant infusion of 1.0 g/sq m/day for progressively longer periods. In all cases, dThd rescue was initiated with a 1.0-g bolus i.v. injection per sq m, followed by a constant infusion of 8 g/sq m/day, diluted to 1000 ml with 5% dextrose-water until the serum MTX concentration was <5 × 10^-8 or for a minimum of 72 hr. All patients received 3.0 g p.o. of NaHCO3 every 3 hr to alkalize the urine. NaHCO3 was started 12 hr before MTX infusion, and was continued during and for 24 hr after the end of the MTX infusion. MTX was not administered unless the urine pH was greater than 7.0. Urine output was maintained at greater than 3 liters/day during MTX infusions. Most patients were retreated every 3 weeks; several patients received weekly therapy. MTX and dThd were obtained from the Division of Cancer Treatment, National Cancer Institute. dThd was supplied as a 3% solution in 0.6% sodium chloride.

Treatment Parameters. The extent of measurable disease and performance status were assessed before each course of therapy. Complete blood counts with differential and platelet count were obtained weekly. Serum blood urea nitrogen and creatinine were measured before each course of treatment and at the end of the MTX infusion. In all patients, liver function tests were obtained at the beginning of each course, and in selected patients at weekly intervals. Mucositis was graded on a scale of I to IV with...
Grade I defined as minimal erythema not impairing p.o. intake and Grade IV as confluent ulceration preventing any p.o. intake. A partial response was defined as a greater than 50% decrease in the product of perpendicular diameters of measurable lesions. Stable disease was considered to be present when measurable lesions either diminished by less than 50% or increased by less than 25% in the absence of new lesions. Duration of response was considered from the time response was achieved.

**Bone Marrow Cytokinetics.** One ml of bone marrow was immediately anticoagulated with 15 mg of EDTA, and the sample was split and incubated at 37° with 1.0 μCi of [6-3H]Urd or [5-3H]Cyd for 1 hr (New England Nuclear, Boston, Mass.; Urd specific activity, 27.8 Ci/mmole; Cyd specific activity, 60 Ci/mmole). Samples were diluted with cold Hanks' balanced salt solution, placed on a Ficoll-Hypaque gradient, and centrifuged at 750 x g for 20 min. Cells recovered from the gradient were washed once in Hanks' balanced salt solution, and counted with a Model ZF Coulter counter. Smear preparations were coated with Kodak NTB-3 nuclear emulsion and were developed in D-19. Cells showing more than 5 grains/nucleus (background less than 5 grains/100 oil immersion fields) were counted as labeled. Approximately 90% of the labeled cells had more than 25 grains/nucleus. A minimum of 200 cells was counted for each patient, showing more than 5 grains/nucleus (background less than 10 grains/nucleus).

**Measurement of Plasma MTX, dThd, and Purine Nucleosides.** MTX and dThd levels were measured by radioimmunoassay as previously described (14, 20). Hypoxanthine, inosine, and adenosine were measured by high-pressure liquid chromatography after acid extraction of plasma in the laboratory of Dr. Yousef Rustum at the Roswell Park Memorial Institute, Buffalo, N. Y., as described elsewhere (21).

**RESULTS**

Twenty patients were entered on this study between July 1976 and April 1977. In Part 1, fifty 24-hr infusions of MTX were given at escalating doses. In Part 2, 13 infusions of 1 g/sq m/day were given, 4 for 30 hr, 5 for 36 hr, and 4 for 40 hr. In all cases, dThd rescue was started immediately after the end of the MTX infusion. All courses are evaluable for toxicity, and all patients are evaluable for response.

The 20 patients ranged in age from 30 to 67, with a median age of 52. Six were female and 14 were male. The histological types of cancer are listed in Table 1. Twelve patients had received prior chemotherapy and 12 patients had received prior radiation therapy; 5 patients had received neither form of treatment.

The most common toxicity was mucositis, which occurred on 22 of 63 courses (Tables 2 and 3). Mucositis was Grade I on 13 courses, Grade II on 8 courses, and Grade III on 1 course. The episode of Grade III mucositis occurred following the third course of MTX (3.80 g/sq m) in a patient who received treatment weekly rather than on the usual 21-day schedule. Mucositis became apparent on Days 5 to 7, was maximal between Days 8 and 11, and had completely cleared on all courses by Day 18. The occurrence of mucositis was quite variable and was not clearly related to the dose or duration of MTX. Two patients who developed mucositis were later treated with larger doses of MTX without recurrence of oral inflammation.

Myelosuppression occurred on 13 of the 63 courses. In 9 courses, there was leukopenia alone (WBC <2,000/sq mm), and in 4 courses thrombocytopenia alone (platelets <100,000/sq mm). In no case was the WBC below 1,400/cu mm; in only 2 of the 4 episodes of the thrombocytopenia was the platelet count less than 50,000 platelets/cu mm. Two of these episodes of thrombocytopenia occurred in a patient treated on a weekly rather than a 3-week schedule. The WBC nadir occurred on Days 5 to 7 and the platelet nadir on Days 10 to 14 at various dose levels and durations of MTX exposure, as shown in Tables 4 and 5. At doses below 0.56 g/sq m, there was no well-defined nadir. Recovery of blood counts to base line occurred in all patients prior to Day 22 of each course, and in no patient was subsequent therapy delayed because of leukopenia or...
thrombocytopenia. No megaloblastic changes were apparent on serial bone marrow examination and the myeloid to erythroid ratio remained stable, even with multiple courses of therapy. There were no infections or episodes of hemorrhage attributable to drug-induced leukopenia or thrombocytopenia. The incidence of myelosuppression was related to bone marrow reserve rather than to dose or duration of MTX exposure; in all but one situation, myelosuppression occurred only in patients who had received both prior chemotherapy and radiation therapy.

MTX-induced anorexia, nausea, and vomiting were minimal at doses of 1.0 g/sq m or less. Vomiting occurred in over one-half of the patients only at the 2 highest dose levels of 5.70 and 8.54 g/sq m. Renal toxicity consisting of a greater than 50% rise in serum creatinine occurred only twice; 1 of these 2 episodes was attributable to inadequate hydration. The creatinine returned to normal within 14 days, and this patient was subsequently treated with higher doses of MTX without difficulty. Renal function in the second patient required 24 days to return to normal. Dermatitis did not occur, and there were no rises in hepatic enzyme associated with MTX-dThd rescue.

The average serum MTX concentration was determined from measurements made at 1, 12, and 24 hr after the start of the MTX infusion on each course. The coefficient of variation of the MTX level was not more than 12% in any course. The geometric mean of the MTX levels ranged from $2.2 \times 10^{-8}$ M at a dose of 0.14 g/sq m to $2.6 \times 10^{-6}$ M at 8.54 g/sq m (Chart 1). The MTX level maintained by a dose of 1.0 g/sq m/day after a loading dose of 0.125 g/sq m in Part 2 of this study was $1.5 \times 10^{-8}$, averaged over all courses. The endogenous dThd concentration measured prior to therapy was $1.6 \times 10^{-7}$ M. Infusion of 8 g/sq m/day produced an average 6-fold increase in this level to $1.0 \times 10^{-5}$ M as shown in Chart 2. There was no correlation between the dThd level prior to treatment, or that attained during dThd rescue, and subsequent occurrence of toxicity.

Cytokinetic studies were performed on serial bone marrow samples from patients receiving 24-hr infusions of MTX followed by dThd rescue. The changes in thymidylate synthesis were estimated by incorporation of $[^{3}H]$Urd, and changes in DNA synthesis by incorporation of $[^{3}H]$Cyd. Two or more marrow samples were obtained either prior to therapy or at 24, 48, or 72 hr after the beginning of a 24-hr MTX infusion. In a random group of 15 patients receiving 1.0 g or more of MTX per sq m, the median $[^{3}H]$Urd labeling index was 14% and the median $[^{3}H]$Cyd labeling index was 10% prior to treatment. The median $[^{3}H]$Urd labeling index dropped to zero and remained markedly depressed until serum levels of MTX began to fall below $10^{-7}$ M. All patients with a labeling index of greater than 2% at 72 hr had serum MTX concentrations below $10^{-7}$, whereas patients with labeling indices below 2% had MTX concentrations above this level. In contrast, the median $[^{3}H]$Cyd labeling index was 2% at the end of the 24-hr MTX infusion, and had already begun to return toward base line by 48 hr.

Chart 3 shows the quantitative incorporation of $[^{3}H]$Urd and $[^{3}H]$Cyd into bone marrow cells of 5 patients, all of whom received 3.78 g of MTX per sq m over a 24-hr period and underwent 4 serial marrow aspirations. The $[^{3}H]$Urd incorporation was reduced to 8% of the pretreatment level by the end of the 24-hr infusion, and remained in this range up to 72 hr, consistent with the presence of inhibitory levels of MTX during this period. In contrast, the $[^{3}H]$Cyd incorporation was reduced to only 28% of its pretreatment level.
level by 24 hr, and by 48 hr, 1 day after the start of dThd rescue, had already exceeded control levels. Taken together, these cytokinetic data suggest that dThd rescue results in prompt resumption of DNA synthesis and progression of cells through the cell cycle, despite the continued inhibition of thymidylate synthesis.

The ability of dThd to rescue bone marrow may be closely tied to the availability of preformed purines. Plasma levels of hypoxanthine, inosine, and adenosine were measured by high-pressure liquid chromatography during a single course of therapy in 6 of the patients in this study who received more than 1.0 g/sq m over a 24-hr period. The level of all 3 compounds remained below 0.5 µM, the lower limit of sensitivity of the assay system, throughout all of these courses.

Although this was a toxicity-seeking trial and all but 5 patients had received extensive therapy prior to this trial, all patients were evaluable for response by virtue of one or more measurable lesions, or an elevated carcinoembryonic antigen. No objective responses occurred in patients with prior therapy; 1 patient with squamous cell carcinoma of the tongue previously treated with high-dose MTX had stable disease for 5 months. Among 5 patients without prior therapy, there was 1 partial response in a patient with head and neck cancer lasting 2+ months, and 1 partial response in a patient with squamous cell carcinoma of the lung lasting 2+ months. The remaining 3 patients, 2 with adenocarcinoma and 1 with large cell carcinoma of the lung, all had stable disease for 3, 3+, and 3+ months. Two of these patients continued to have some shrinkage of the tumor, although less than 50%.

**DISCUSSION**

The ability of dThd to reverse the impairment of DNA synthesis resulting from a deficiency of thymidylate synthesis was first demonstrated by Killman (16), who found that continuous infusions of 4.5 to 9.0 g over 48 to 72 hr produced a reticulocyte response and reduction of megaloblastic changes in patients with pernicious anemia. Later, tissue culture studies disclosed that dThd could reverse the toxicity of MTX, although in some cases, a source of purine was also required (3, 4, 19, 26). Tattersall et al. (25) and subsequently others (22), showed that coadministration of dThd alone with MTX could protect normal mouse tissues from MTX toxicity in vivo. Ensminger and Frei (5) extended this observation to humans by demonstrating that dThd alone completely protected marrow from as much as 6 g/sq m given over a 72-hr period.

In the use of leucovorin to improve the therapeutic ratio of high-dose MTX, delay in the leucovorin administration is an important feature (6). The results of this study indicate that the administration of dThd can also be delayed and yet still effectively block MTX toxicity to normal tissues. The maximum tolerated dose of MTX given over a 24-hr period without rescue is in the range of 80 to 120 mg/sq m (2). In this study, dThd was capable of adequately rescuing exposure to greater than 8.54 g/sq m given over a 24-hr period, or exposure to 10⁻⁸ M MTX for up to 40 hr, when started at the end of the MTX infusion. Thus, dThd rescue in humans increases the maximum tolerated dose by greater than 70-fold.

dThd rescue is of interest for 2 major reasons. The first reason is that it has the potential of exploiting differences in purine metabolism and nucleoside transport between normal and malignant cells to improve the selectivity of rescue. The ability of dThd or preformed purines to reverse the action of MTX differs in various cell systems (2, 9, 12, 26). In some animal systems, coadministration of MTX and dThd, while protecting normal tissues, still permits expression of the antitumorous action of MTX (22, 25). There are several possible explanations for this: MTX may alter deoxypurine pool sizes more drastically in the tumor cells; tumor cells may be less capable of utilizing intracellular or extracellular salvage pathway metabolites, or the malignant cells may be less able to transport dThd intracellularly or to phosphorylate it to TMP. To the extent that any of these constitute significant differences between normal and malignant cells in humans, dThd may be a more selective rescue agent than leucovorin, which circumvents the action of MTX on both thymidylate and de novo purine synthesis.

A second reason for interest in dThd as a rescue agent is that, in contrast to leucovorin, dThd does not compete with MTX for entrance into cells (2, 19). Leucovorin is...
transported by the same facilitated transport system that carries MTX, and the 2 compounds have approximately equal coefficients of transport (7, 8). Direct competition between leucovorin and MTX for entry has been demonstrated in vitro for both normal and malignant murine tissues, and for human cell lines in culture (1, 7, 23). On the other hand, dThd is transported by an entirely separate system (15). The cyto kinetic studies reported here demonstrate that dThd is able to initiate recovery of DNA synthesis in human bone marrow cells in vivo in the presence of high serum MTX concentrations. The ability to abruptly limit the duration of exposure to high concentrations of MTX may permit improvement in the therapeutic ratio through the use of long-term high-dose infusions.

Working with mouse bone marrow in vitro, Pinedo et al. (19) found that dThd alone was inadequate at any concentration to protect mouse colony-forming units-culture from MTX. However, dThd in combination with a preformed purine (hypoxanthine, inosine, or adenosine) was effective at purine concentrations above 1.0 μM. In contrast, our data demonstrate that in tumor-bearing humans, administration of dThd alone is adequate to effect rescue of both marrow and gut cells from the cytotoxic effect of high-dose MTX. The implication of this finding is that either MTX does not severely impair de novo purine synthesis in these normal human cells, or that when de novo synthesis is inhibited they can utilize purine salvage pathways, and that there are adequate levels of salvage pathway metabolites in plasma or intracellular sites to sustain DNA synthesis. Serial measurement of plasma hypoxanthine, inosine, and adenosine concentrations in a group of 6 patients successfully rescued with dThd disclosed that the levels were below 0.5 μM, the lower limit of the assay, both before treatment, during MTX infusion, and during dThd infusion. This suggests that in comparison to mouse marrow cells in culture, human marrow cells in vivo may require lower concentrations of these materials for rescue.

In a group of 5 patients, administration of 3.78 g of MTX per sq m over a 24-hr period failed to completely inhibit the quantitative incorporation of [3H]Cyd into marrow cells, reducing it to only 28% of the pretreatment value. This may be due to the inability of even high levels of MTX to completely inhibit DNA synthesis of marrow cells in vivo. Pinedo et al. (19) found that MTX did not produce consistent blockade of mouse colony-forming units-culture proliferation in the presence of undialyzed fetal calf serum or L-cell supernatant, and attributed this to the presence of high enough levels of salvage pathway metabolites to protect against the effect of MTX. If this same situation holds for human cells in vivo, and there are adequate salvage pathway metabolites present to partially block the action of MTX on bone marrow, then higher than normal local concentrations of salvage pathway metabolites in tumors may completely block MTX. This may be a major mechanism of tumor resistance to antifolies. Preliminary information from this laboratory suggests that in fact the dThd level in large necrotic rat tumors is 5- to 10-fold greater than plasma levels.

None of the toxicities encountered in this clinical study of MTX-dThd rescue were dose related or dose limiting. The occurrence of myelosuppression was closely related to the extent of prior chemotherapy and radiation therapy. Mucositis emerged as the major toxicity. This is significant in that some animal studies suggest that dThd is less able to protect gut cells from marrow (24). Mucositis was also clearly schedule dependent; 2 patients, who did not suffer mucositis when treated on a 3-week schedule, developed oral ulcerations when treated at the same or a lower dose on a weekly interval.

Other investigators using high-dose MTX with leucovorin rescue have found that toxicity was independent of dose but closely related to duration of exposure beyond 36 to 42 hr (6). The results of this study confirm this observation with respect to dose; with respect to duration, although 40-hr infusions were tolerated in a small group of patients, it is quite probable that the incidence of toxicity would increase rapidly with further increments in the total time of exposure.

Many important questions remain concerning dThd rescue. The optimal dose and duration of dThd rescue have not been defined. The dThd dose used in the studies reported here was empirically extrapolated from the minimal effective protective dose in rodents that was previously demonstrated to confer protection against MTX in humans (5). These same animal studies demonstrate that enhancement of the therapeutic ratio of MTX is a function of the dThd dose (22), and this principle may extend to humans. Most importantly, although this study has demonstrated that dThd is an effective rescue agent for high-dose MTX in humans, the question remains whether there are any tumors for which dThd rescue offers an advantage over leucovorin.

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