The Prevention of Methotrexate Toxicity by Thymidine Infusions in Humans

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SUMMARY

Continuous i.v. thymidine (TdR) was given to 12 patients with metastatic cancer in an attempt to prevent methotrexate (MTX) toxicity. MTX was infused in 27 courses with progressive dose increase from 80 mg/sq m for 24 hr to 6 g/sq m for 72 hr. TdR at 8 g/sq m/day was infused concurrently and continued 24 to 48 hr beyond MTX infusion. The median pretreatment serum TdR level was 0.19 @M. With TdR infusion, the median level was 1.5 @M. Serum TdR fell with a half-time of 8 to 10 min after a pulse dose or cessation of infusion. Spinal fluid TdR equaled serum TdR levels after 2 hr of infusion. Less than 2% of administered TdR appeared in urine. MTX serum levels were proportional to dose infused, ranging from 80 to 100 @M with 2 g/sq m/day. The half-time for MTX clearance from serum was 4 to 8 hr. Spinal fluid MTX reached equilibrium at 3 to 12% of serum levels by 4 hr.

Bone marrow dysfunction during MTX infusion was prevented by TdR as determined by labeling indices and cyt fluorographic analyses. Toxicity was not seen in patients with normal MTX clearance using 48-hr infusions of MTX where TdR was continued for an additional 48 hr after the MTX infusion had ended. However, 3 of 6 courses of MTX at 6 g/sq m over 72 hr led to toxicity. Toxicity was reversible in 2 patients, 1 of whom was retreated with a similar dose duration of MTX without toxicity when TdR was continued beyond the end of the MTX infusion for 48 hr instead of the usual 24 hr. The 3rd patient with toxicity died of progressive disease and thrombocytopenia 19 days after treatment. No TdR-related toxicity or unusual MTX toxicity was detected. Antitumor effects were noted in 4 patients. TdR offers significant protection against MTX toxicity and deserves further clinical study.

INTRODUCTION

MTX binds to dihydrofolate reductase, thus interfering with the regeneration of the tetrahydrofolate necessary for 1-carbon transfer in de novo purine and TdR synthesis. The inhibitory effects of MTX on nucleic acid synthesis and cell growth in vitro can be largely circumvented by the addition of TdR alone in some tumor cell systems. In other systems, however, a purine such as hypoxanthine or deoxyadenosine in addition to TdR is required to prevent the inhibitory effects of MTX. It has been previously observed that TdR can prevent MTX toxicity in normal mice (27).

There is evidence, on the other hand, that MTX antitumor effect may be preserved despite TdR administration. When 5 tumor cell lines were treated in vitro with MTX plus TdR, purine deoxyribonucleic triphosphate (dATP, dGTP) pools were selectively and markedly decreased to less than 7% of pretreatment values with the development of cytotoxicity (34). In other studies designed to demonstrate a potentially specific antitumor effect, mice bearing the L1210 leukemia were treated with MTX plus TdR and significant antitumor effect was found (27, 33).

This study was designed to determine whether TdR could prevent the toxicity of MTX in man. Cytokinetic studies were performed on bone marrow cells to assess the impact of TdR on the MTX-induced inhibition of cellular DNA synthesis. In addition, the repeated administration of large doses of TdR allowed for simultaneous pharmacokinetic studies of this agent in serum, urine, and CSF.

MATERIALS AND METHODS

Patients. Twelve adult patients with advanced disease refractory to conventional therapy were entered in the study. There were 7 women and 5 men with a median age of 50 (range, 27 to 71 years). The distribution of patients by tumor type included: 7 patients with adenocarcinoma (colon, 3; renal, 2; ovary, 1; breast, 1); 2 with acute myeloblastic leukemia; and 1 each with fibrosarcoma, melanoma, and squamous carcinoma.

All patients fulfilled the following criteria: (a) an estimated life expectancy of at least 2 months, (b) a WBC greater than 4,000/cu mm, with platelet count greater than 150,000/cu mm, (c) blood urea nitrogen less than 20 mg/100 ml, a serum creatinine less than 1.5 mg/100 ml, and a creatinine clearance of at least 50 ml/min. Patients with abdominal or pleural effusions and patients with pyelographic evidence of impending renal outflow obstruction were excluded. Written informed consent was obtained from all patients.

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Patients were hospitalized for all courses either in the Clinical Center of the Peter Bent Brigham Hospital or in the Dana Cancer Center Hospital of the Sidney Farber Cancer Institute.

Drugs and Treatment Program. MTX and TdR were obtained from the National Cancer Institute. The daily dose of MTX was placed in 500 ml of 5% dextrose in water and administered over a 24-hr period with a constant-infusion pump. A loading pulse of MTX equal to one-eighth of the 24-hr dose was given to establish early equilibrium. TdR was similarly dissolved in 500 ml of 5% dextrose in water and concurrently was infused with another constant-infusion pump through the same scalp vein needle as that for the MTX. TdR is stable in solution for periods exceeding 6 to 12 months and is now formulated as 15 g TdR in 500 ml of 0.6% (w/v) sodium chloride solution by the National Cancer Institute.

At first, loading doses of TdR were given, but they were subsequently discontinued when pharmacokinetic results (see below) indicated that this was unnecessary. To decrease the likelihood of nephrotoxicity, patients were given p.o. or i.v. sodium bicarbonate as required to maintain urine pH greater than 7 (24).

For determination of the pharmacokinetics of TdR, 2 patients were given an i.v. pulse dose of 2 g/sq m of TdR with subsequent determination of serum TdR levels. The serum disappearance of TdR was also determined in 4 patients (3 of whom also had received MTX) from blood samples drawn following the cessation of a 3-day infusion of TdR, 8 g/sq m/day.

The experimental design for MTX-TdR treatment is schematically presented in Chart 1. It has previously been demonstrated (1, 11) that MTX toxicity relates more to duration of inhibitory effect (as obtained with prolonged infusions) than to dose administered (if given as a pulse). Increasingly prolonged MTX infusions were therefore used in order to demonstrate, more conclusively, the ability of concurrent TdR to prevent the toxic effects of constant, high levels of MTX. MTX was first given at 80 mg/sq m over 24 hr with dose escalation to 2 g/sq m over 24 hr. After it became evident that concurrent TdR was protective, the duration of the MTX infusion was progressively lengthened to 48 and 72 hr with subsequent dosage escalation as shown in Table 3. TdR was administered throughout at 8 g/sq m/day, a dosage extrapolated from the amount of TdR necessary for protection in the mouse (10, 27, 33).

Either 3 or 4 days of TdR infusion were used as shown in Table 3. TdR treatment was initiated concurrently with MTX in all instances.

Clinical Laboratory Studies. Complete blood counts were obtained prior to treatment and twice weekly during treatment. Renal function was monitored by a daily serum creatinine and blood urea nitrogen determination while infusions were being given and by a weekly determination thereafter. Liver function tests including bilirubin, serum glutamate-oxalate transaminase, alkaline phosphatase, and lactate dehydrogenase were evaluated weekly.

MTX and TdR were measured using previously described radioimmunoassays (18, 25). Both assays are sensitive to 0.05 µM levels of drug with maximum variability of about ±10% on duplicate samples. The binding affinity of TdR in the radioimmunoassay is at least 200-fold greater than the binding affinities of thymine and potentially interfering nucleosides (18). MTX levels were determined daily during all courses but were not used to guide therapy inasmuch as the duration of TdR protective effects was unknown. Spinal fluid for MTX and TdR levels was obtained by lumbar puncture using a 22-gauge spinal needle in 4 patients.

Bone marrow aspirations for cytokinetic studies were performed prior to and at 24, 48, or 72 hr after start of treatment during 12 courses in 8 of the 12 patients. Cytokinetic studies on bone marrow aspirates consisted of labeling indices determined after incorporation of [3H]deoxycytidine (15) using a 1-hr incubation in vitro and autoradiography on smears using Kodak NTB2 emulsion. Flow cytfluoro-tagic analyses were performed to determine the distribution of DNA content in marrow cells and to allow quantification of the percentage of cells with S- and G2-phase DNA content (22, 23).

RESULTS

TdR and MTX Pharmacokinetics. TdR was administered at 8 g/sq m/day by constant infusion, and serum levels were monitored by radioimmunoassay. The median pretreatment TdR level was 0.19 µM (Table 1). With TdR infusions serum TdR levels rose approximately 8-fold to 1.5 µM (median level). The serum clearance of TdR after an i.v. 2-g/sq m pulse dose was approximately 8 to 10 min (Chart 2). In patients receiving constant TdR infusions for 3 days, serum TdR levels fell after cessation of the infusion with a t½ also of 8 to 10 min, returning to pretreatment levels by 45 min in all instances (Chart 2).

TdR in CSF reached equilibrium with serum levels in 2 hr (Chart 3). Serum and CSF TdR levels were essentially equal thereafter throughout the infusion (Table 2).

Urine specimens were collected, and unmetabolized TdR measured by radioimmunoassay in 4 patients whose creatinine clearance ranged from 50 to 120 ml/min. In each case, the simultaneous clearance of TdR measured over a 24-hr collection period was approximately 4 times creatinine clearance. The actual amounts of TdR recovered in urine ranged from 200 to 300 mg, less than 2% of administered dose. Catabolic products of TdR such as β-aminoisobutyric acid were not measured.

Serum levels of MTX varied linearly with dose infused, ranging from 1 to 2 µM with 160 mg/sq m/day to 80 to 100
Serum levels of thymidine during continuous infusion at 8 g/sq m/day

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pretreatment</th>
<th>With infusion</th>
<th>Increase (treated/basal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. S. (1)</td>
<td>0.1</td>
<td>0.9 ± 0.2</td>
<td>9</td>
</tr>
<tr>
<td>M. S. (2)</td>
<td>0.14</td>
<td>0.9 ± 0.1</td>
<td>6</td>
</tr>
<tr>
<td>R. F. (1)</td>
<td>0.25</td>
<td>1.5 ± 0.3</td>
<td>6</td>
</tr>
<tr>
<td>R. F. (2)</td>
<td>0.18</td>
<td>1.0 ± 0.05</td>
<td>6</td>
</tr>
<tr>
<td>R. F. (3)</td>
<td>0.16</td>
<td>3.0 ± 0.5</td>
<td>19</td>
</tr>
<tr>
<td>L. S. (1)</td>
<td>0.20</td>
<td>1.4 ± 0.1</td>
<td>7</td>
</tr>
<tr>
<td>L. S. (2)</td>
<td>0.30</td>
<td>1.9 ± 0.1</td>
<td>6</td>
</tr>
<tr>
<td>E. M.</td>
<td>0.10</td>
<td>0.58 ± 0.03</td>
<td>6</td>
</tr>
<tr>
<td>R. D.</td>
<td>0.11</td>
<td>1.1 ± 0.1</td>
<td>10</td>
</tr>
<tr>
<td>K. K.</td>
<td>0.50</td>
<td>7.0 ± 0.1</td>
<td>14</td>
</tr>
<tr>
<td>J. N.</td>
<td>0.22</td>
<td>1.6 ± 0.2</td>
<td>7</td>
</tr>
<tr>
<td>E. D. M.</td>
<td>0.76</td>
<td>6.4 ± 0.3</td>
<td>8</td>
</tr>
<tr>
<td>I. Y.</td>
<td>0.25</td>
<td>0.8 ± 0.1</td>
<td>3</td>
</tr>
<tr>
<td>P. M.</td>
<td>0.12</td>
<td>3.0 ± 0.2</td>
<td>25</td>
</tr>
<tr>
<td>Median:</td>
<td>0.19</td>
<td>1.5</td>
<td>8</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, course sequence in multiple courses for indicated patient.
  * Mean ± S.D. of the daily determinations on given course.

MTX with Thymidine in Humans

Courses of Treatment and Toxicity. Although similar doses of TdR had been previously administered to patients with pernicious anemia without toxic effects (21), initially in this study 2 patients were given TdR alone at 8 g/sq m/day for 3 days. Peripheral blood counts were not changed, nor was any toxicity noted during a 3-week follow-up period. Bone marrow cytokinetic studies were not performed, however.

Twenty-seven courses of treatment with MTX-TdR were given with only 5 toxic courses (Table 3). Toxicity in all cases followed a pattern clearly recognized as due to MTX (11, 14). One patient developed MTX-related nephrotoxicity subsequent to inadequate fluid intake at a MTX dose of 640 mg/sq m over 24 hr. The blood urea nitrogen and serum creatinine had doubled by the end of the MTX infusion and the t1/2 for serum clearance of MTX was prolonged to 20 hr (compared to a t1/2 of 8 hr on previous and subsequent nontoxic courses). This patient developed moderate mucositis and platelet and WBC nadirs of 80,000 and 1800/cu mm, respectively. This patient was subsequently retreated at the same MTX dose, 640 mg/sq m over 24 hr with vigorous i.v. hydration, and had no toxicity. Another patient developed renal toxicity with MTX, 1300 mg/sq m, given over 48 hr with subsequent moderate mucositis and thrombocytopenia to 61,000/cu mm. This patient was felt to have developed nephropathy due to inadequate alkalinization of urine early in the MTX infusion. No toxicity ensued on subsequent courses with adequate alkalinization (i.e., maintenance of urine pH greater than 7).

At the highest dose duration, 6 g of MTX over 72 hr, 3 of 5 patients treated had mucositis and myelosuppression. Although not all became toxic, all 5 patients had relatively high serum MTX levels of 2 to 6 μM when the TdR was stopped (Table 4). Of the 3 patients becoming toxic, 2 recovered completely. Although their MTX clearance was normal (with serum t1/2 less than 8 hr), both patients had prior radiotherapy to marrow areas that may have predisposed to myelosuppression. One of the 2 patients who recovered was retreated and had no toxicity when the TdR infusion was continued 48 hr beyond cessation of MTX administration. The 3rd patient had extensive liver metastases from colon cancer. MTX clearance was normal while the patient remained in the hospital and received i.v. fluids (i.e., TdR-containing solution). Rapid clinical deterioration with development of gross ascites occurred within 3 days after discharge from the hospital. Renal function and clear-
Table 2

Simultaneous CSF and serum levels of MTX and TdR during continuous infusions

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time (hr)</th>
<th>TdR concentration (µM)</th>
<th>MTX concentration (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. N.</td>
<td>24</td>
<td>Serum 1.1</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>CSF 0.91</td>
<td></td>
</tr>
<tr>
<td>R. F.</td>
<td>24</td>
<td>Serum 1.8</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>CSF 0.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. M.</td>
<td>48</td>
<td>Serum 0.63</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CSF 0.70</td>
<td></td>
</tr>
<tr>
<td>L. S.</td>
<td>48</td>
<td>Serum 1.5</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CSF 1.7</td>
<td></td>
</tr>
</tbody>
</table>

* 8 g/sq m/day.

b 1 g/sq m/day.

c 2 g/sq m/day.

d 0.32 g/sq m/day.

Table 3

Courses of treatment and toxicity with MTX-TdR

<table>
<thead>
<tr>
<th>MTX</th>
<th>mg/sq m</th>
<th>Duration (hr)</th>
<th>No. of patients</th>
<th>No. of courses</th>
<th>Toxic courses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>80</td>
<td>24</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>24</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>320</td>
<td>24</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>640</td>
<td>24</td>
<td>4</td>
<td>5</td>
<td>1*</td>
</tr>
<tr>
<td></td>
<td>1300</td>
<td>24</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1300</td>
<td>48</td>
<td>2</td>
<td>2</td>
<td>1*</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>48</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>3000</td>
<td>48</td>
<td>1</td>
<td>1</td>
<td>0</td>
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<tr>
<td></td>
<td>3000</td>
<td>72</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>6000</td>
<td>72</td>
<td>5</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

* Nephrotoxicity with decreased renal clearance of MTX.

Table 4

MTX levels and clearance in 72-hr infusions

<table>
<thead>
<tr>
<th>Patient</th>
<th>MTX dose (g/sq m)</th>
<th>Level (µM) at cessation of TdR*</th>
<th>Serum clearance (t1/2) (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. S.</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>E. M.</td>
<td>6</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>R. F.</td>
<td>6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>K. K.</td>
<td>6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>J. N.</td>
<td>6</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>C. Y.</td>
<td>6</td>
<td>7.5</td>
<td></td>
</tr>
</tbody>
</table>

* 24 hr after end of MTX infusion.

b Determined in 48-hr period after end of MTX infusion.

c Toxic patients (see text).

d Died 19 days after treatment (see text).

Table 5

Bone marrow function with MTX-TdR treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pretreatment</th>
<th>At end of MTX infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Labeling indicesa (mean % labeled nuclei)</td>
<td>Cytofluorography analysisb (mean % cells G1 phase DNA content)</td>
</tr>
<tr>
<td></td>
<td>Pretreatment</td>
<td>At end of MTX infusion</td>
</tr>
<tr>
<td>L. S.</td>
<td>13 (8-15)</td>
<td>17 (10-25)</td>
</tr>
<tr>
<td>E. M.</td>
<td>15 (10-22)</td>
<td>17 (10-25)</td>
</tr>
<tr>
<td>R. F.</td>
<td>12 (10-20)</td>
<td>17 (10-25)</td>
</tr>
<tr>
<td>K. K.</td>
<td>14 (10-22)</td>
<td>17 (10-25)</td>
</tr>
<tr>
<td>J. N.</td>
<td>13 (8-15)</td>
<td>17 (10-25)</td>
</tr>
<tr>
<td>C. Y.</td>
<td>15 (10-22)</td>
<td>17 (10-25)</td>
</tr>
</tbody>
</table>

a Determined by 1 hr incubation with [3H]deoxycytidine.

b See Refs. 22 and 23.

c Numbers in parentheses, range.

d Four courses each at 24-, 48-, and 72-hr MTX infusions.

Bone Marrow Cytokinetics. Bone marrow aspirations were performed before and at the end of the MTX infusions for cytokinetic studies. As shown in Table 5, there were no significant changes in labeling indices as determined with [3H]deoxycytidine. The percentage of cells with greater than G1-phase DNA content was unchanged (Table 5). In no instance did treatment lead to a significant change in labeling indices or in the percentage of S- and G2-phase cells as determined by cytofluorography. This is further evidence that thymidine protects against the marrow toxicity of MTX and contrasts with the cell-cycle block at the G1-S interface seen with MTX alone (23). Determinations of bone marrow function at the end of the MTX infusion were normal in 2 of the patients who subsequently became toxic. Unfortunately, follow-up bone marrow cytokinetic studies were not performed prior to onset of peripheral WBC and platelet count depression in those 2 patients. However, the normal cytokinetic determinations during the TdR infusion and the 2- to 3-day delay in toxicity suggest that toxicity was due to the effect of elevated MTX levels continuing beyond the time when TdR levels had returned to pretreatment values.

Antitumor Effect. Although this was a pilot trial in patients with advanced disease who had failed conventional ther-
apy, there was suggestive evidence of antitumor activity in 4 of the 12 patients treated. Two patients with metastatic colon cancer to liver had a 30% reduction in liver size by palpation. Liver-related enzyme and bilirubin levels returned to normal from 4-fold elevated pretreatment values in 1 of the 2 patients. The carcinomaembryonic antigen levels decreased by 75% for 1 patient and 50% for the other. Response lasted for 9 months and 1 month, respectively. One patient with en cuirasse breast cancer had a greater than 50% reduction in skin involvement lasting for 3 months. Another patient with pain requiring constant narcotics due to extensive bone involvement with renal carcinoma had complete pain relief requiring no narcotics for over 3 months.

**DISCUSSION**

Although some pharmacokinetic information is available from the study of tracer doses of TdR in man and rodents (18, 26), there has been no previous clinical pharmacological study of large doses in man. In prior human studies using pulse-radiolabeled tracer doses of TdR, there was a very rapid early phase of serum clearance with a $t_{1/2}$ of 1 to 2 minutes and a more prolonged 2nd phase with a $t_{1/2}$ of 20 to 25 min (26). The serum clearance of TdR in mice given pulse i.v. doses follows slightly different kinetics as determined by radioimmunoassay (18). After injection, there is an early phase with a $t_{1/2}$ of 1 min and a rapid transition at 5 min to a 2nd phase with a $t_{1/2}$ of 8 min. The $t_{1/2}$ for TdR clearance from patients’ serum as determined in our study was found to be approximately 8 to 10 min. Since samples were usually not obtained until 10 min after the TdR administration was ended, a rapid early 1st phase may not have been detected.

Studies in cell culture systems have shown that extracellular TdR concentration changes lead to intracellular pool changes within 1 to 2 min (2). The size of the intracellular TTP pool is small under most conditions and may turn over every 5 to 10 min (7). Easily detectable incorporation of [3H]TdR into newly synthesized DNA occurs within seconds in cultured cells (2, 7). Moreover, [3H]TdR is incorporated into human bone marrow cell DNA in vitro by less than 1 min after injection (26). Fluorodeoxyuridine, which appears to be transported into cells, phosphorylated, and catabolized much like TdR, has a similarly short serum half-life (5–7). There is potentiation of fluorodeoxyuridine pharmacological effect on bone marrow function, and perhaps also on tumor, when a prolonged continuous infusion is used (32). The rapid serum clearance of TdR, certainly leading quickly to evoked changes in intracellular TdR pools, supports continuous infusion of TdR, as we have done, as the method most likely to give maximum protection against the inhibitory effects of MTX on endogenous TMP production.

The catabolism of the TdR not incorporated into nucleic acid yields β-aminoisobutyric acid and CO₂ (7, 26, 28). In man, 30 to 80% of label in tracer doses of [3H]TdR ends up as titratable water, with much less, perhaps 5%, as urinary β-aminoisobutyric acid (7, 26). The remaining label is incorporated into DNA thymine. In this study, the catabolic products of TdR were not measured. However, the renal excretion of unchanged TdR was measured and amounted to less than 2% of the administered dose.

The dosage of TdR chosen for this study, 8 g/sq m/day, was chosen by extrapolation, based on body surface area, from the minimal dose of TdR found to prevent MTX toxicity in mice (10, 27, 33). A dose-response curve was not attempted for TdR protection. However, a previous study on the effect of TdR on pernicious anemia showed reversal of megaloblastic changes in the peripheral blood and bone marrow at total daily i.v. doses of 2 to 4 g given over 5 days (21). On the other hand, similar studies using lower i.m. doses, 250 to 500 mg/day, had no effect (4). Thymine p.o. at 3.4 g also appears to be more effective than a lower dose of 1.5 g (both doses given 3 times per day) in treating pernicious anemia (29).

The levels of TdR in patients were increased 8-fold on the average by TdR infusions at 8 g/sq m/day. However, the levels reached with TdR treatment never exceeded 10 μM. Thus it is extremely unlikely that TdR administration in manageable doses will ever lead to the considerably higher (i.e., μM) TdR levels necessary to inhibit DNA synthesis in in vitro cell culture systems (7).

We have shown that thymidine offers significant protection against MTX toxicity in man. The MTD of MTX given in a pulse is approximately 330 mg/sq m in man (8, 14). The MTD decreases to 100 mg/sq m when MTX is infused over a 24- to 30-hr period (1). In MTX-leucovorin rescue studies, it has been observed that leucovorin instituted more than 36 hr after the initiation of MTX infusion does not prevent toxicity (1, 11). By contrast, in this study, we were able to give up to 6 g of MTX per sq m in a 72-hr infusion when TdR was administered concurrently. However, 3 of the 5 patients receiving 6 g/sq m in 72 hr became toxic; the extension of TdR infusions for only 24 hr beyond the end of the MTX infusion does not appear to be adequate. Toxicity was not observed as long as renal clearance of MTX remained normal (serum $t_{1/2}$ less than 8 hr) and TdR was administered 48 hr beyond the termination of the MTX infusion. Thus, in man, TdR can protect against MTX toxicity and provide an estimated 20- to 60-fold increase in the MTD of MTX. This protective effect exceeds the 4-fold increase in the MTD of MTX with TdR that has been shown in mouse studies (27, 31).

When TdR is given concurrently with MTX to mice, toxicity can be blocked with maintenance of antitumor effect (27, 33). Since MTX inhibits purine (and perhaps protein) synthesis as well as thymidine synthesis, this raises the possibility that host tissues may differ from certain tumors with respect to various macromolecular biosynthetic processes. Cell culture studies have shown that some cells require a preformed purine, such as hypoxanthine, plus TdR to survive MTX treatment, whereas others survive with TdR alone (34). There is evidence from earlier studies on [14C]formate incorporation in vitro into bone marrow cells that preexisting purines can be utilized while thymidine is rapidly synthesized de novo for DNA synthesis (36, 37). These in vitro studies, the major effect of aminopterin, when it was used, appeared to be inhibition of formate incorporation into DNA thymine (37). When in vivo [14C]formate incorporation studies were done on rat intestinal cells, label was found equally distributed in the thymine of DNA and in the adenine and guanine of both RNA and DNA (13). Moreover, thymine and purine synthesis were nearly equally and totally inhibited by
aminopterin as determined by [14C]formate incorporation.

In a study in mice, TdR alone was able to prevent the toxic effects of MTX on bone marrow cells (31). However, a pu-
rine source, hypoxanthine (plus allopurinol), was required to pre-
vent gut toxicity. This would support the suggestion
derived from the formate incorporation studies above that MTX has an inhibitory effect on purine as well as TdR
synthesis in gut mucosal cells. On the other hand, signif-
icant gastrointestinal toxicity was not seen in our patients
-treated with MTX-TdR. The differences in gut toxicity be-
tween mouse and man may relate to interspecies differ-
ences in dietary content of purines as well as in the local
salvage of purines from the gut intraluminal contents. Alter-
natively, it is possible, but not yet directly shown, that there
is sufficient hypoxanthine present in human serum to pro-
tect gut mucosal cells (20).

Further analysis of purine and TdR biosynthesis in human
mucosa and tumor samples may elucidate differences be-
tween requirements for de novo purine synthesis as com-
pared to utilization (by salvage pathways) of preexisting
purines. The occurrence of antitumor effect in several of the
patients treated in this program indicates that a differential
is likely to exist for some tumors.

Although MTX toxicity in man has been shown by this
study to be largely due to inhibition of TMP synthesis and
reversible with TdR administration, the therapeutic advan-
tage of this maneuver will require further investigation. The
different transport properties of TdR as compared to leuco-
vorin may make TdR more effective should some tumors
transport MTX (and leucovorin) well but TdR less effectively
than do normal tissues (12, 19). In addition, whereas leuco-
vorin competes with MTX for transport, TdR is transported
between mouse and man may relate to interspecies differ-
ences in requirements for de novo purine synthesis as corn-
plifying the potentially selective antitumom effects of the MTX
clinical studies are warranted to attempt to define and am-

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