Potentiation of the Antitumor Activity of Methotrexate by Concurrent Infusion of Thymidine

J. Hoglind Semon and G. B. Grindey

Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York 14263

ABSTRACT

The effects of normal metabolites on the toxicity and antitumor activity of methotrexate against leukemia L1210 in DBA/2J mice were evaluated by a system that allows for long-term continuous i.v. infusion of unrestrained mice. In normal female DBA/2J mice, the infusion of methotrexate alone for 48 hr produced a 50% lethal dose of 6 mg/kg/day. Coadministration of thymidine (5 g/kg/day) and methotrexate, followed by an additional 48 hr of thymidine alone, dramatically reduced toxicity, resulting in a 50% lethal dose of about 45 mg/kg/day. A higher concentration of thymidine (18 g/kg/day), which was only marginally toxic alone, was even more effective and reduced the toxicity of methotrexate more than 35-fold.

Against leukemia L1210 in female DBA/2J mice, a 48-hr infusion of methotrexate produced a 33% increase in life span at the optimum dose of 1 mg/kg/day. The addition of thymidine (5 g/kg/day) to the methotrexate for 48 hr potentiated antitumor activity and resulted in a maximum 68% increase in life span with methotrexate at 4 mg/kg/day. At higher concentrations of methotrexate, two additional days of thymidine infusion were required for prevention of toxicity while maintaining antitumor activity. Maximum therapeutic selectivity and a 125% increase in life span were obtained with methotrexate at 16 mg/kg/day, infused for 48 hr concurrently with thymidine at 5 g/kg/day for 96 hr. A higher concentration of thymidine (15 g/kg/day), although affording a greater than 35-fold reduction in toxicity, also prevented antitumor activity.

The infusion of inosine alone or in combination with thymidine blocked both the toxicity and antitumor activity of methotrexate. These results indicate that the increase in the therapeutic selectivity achieved with the simultaneous infusion of methotrexate and thymidine may result from a complex modulation of cellular metabolism rather than simple end product reversal by the provision of thymidylate.

INTRODUCTION

Hakala (9) and Hakala and Taylor (10) using Sarcoma 180 in cell culture were the first to demonstrate that the lethal effects of MTX could be prevented by the simultaneous provision of the end products of folate metabolism: dThd, a purine such as hypoxanthine, and glycine. In these cells the addition of purine alone to the culture medium afforded a 2-fold decrease in the toxicity of MTX, whereas the addition of dThd alone was ineffective. In a subline of Sarcoma 180 selected for resistance to MTX, the addition of hypoxanthine to the unsupplemented medium had no MTX-sparing effect. However, the addition of dThd alone allowed a 4-fold decrease in toxicity (11). More recently, a similar dThd-sparing effect was observed by Borsa and Whitmore (3) using a subline of L-cells (L60T). In contrast, in L5178Y cells hypoxanthine but not dThd yielded partial protection from MTX toxicity (13). Thus, various investigators have confirmed that in some cells inhibition of purine nucleotide synthesis is most critical, whereas other investigators have attributed MTX growth inhibition primarily to a thymidylate deprivation.

Grindey and Moran (7) demonstrated in vivo that the antitumor activity but not the toxicity of MTX was reversed by coadministration of allopurinol. Previous work by Pomales et al. (29) had shown that the incorporation of purines into cellular nucleic acids in the mouse could be dramatically increased by this agent via inhibition of xanthine oxidase, a purine-degradative enzyme. Therefore, the antagonism by allopurinol of the antitumor effect of MTX may have been due to increased utilization of salvage purines such as hypoxanthine by the tumor. Preliminary studies involving injection of dThd phosphorylase into mice revealed that depletion of salvageable dThd had no effect on the therapeutic efficacy of MTX against leukemia L1210 (8).

The enzyme treatment did, however, increase the toxicity of MTX to the host. Further in vivo experiments by Tatterstall et al. (35) evaluated the effects of dThd on the antitumor activity of MTX. The injected dThd allowed some protection from MTX toxicity in normal mice and afforded a 44% increase in life span to L1210-bearing animals. However, the MTX alone on the same schedule was therapeutically ineffective (35). Thus, the antileukemic activity of MTX in vivo may be more related to a purineless death, whereas toxicity to normal target tissues may be attributed to a thymidylate limitation. Based on these in vivo and cell culture results, this study sought to investigate the effects of salvage metabolites administered by continuous i.v. infusion on the toxicity and antitumor activity of MTX in the mouse. The administration of normal metabolites by infusion allows the establishment of constant, steady-state plasma levels similar to cell culture conditions.

MATERIALS AND METHODS

Female DBA/2J and C57BL/6 mice (20 to 22 g) were purchased from The Jackson Laboratory, Bar Harbor, Maine. The leukemia L1210 was used as described previously (23, 32). In all experiments 10⁶ L1210 cells were inoculated i.p. into recipient mice, and drug infusions were

1 This investigation was supported in part by USPHS Project Grants CA-17156 and CA-16056 for statistical analysis from NIH. A preliminary report of this investigation has appeared (33).

2 The abbreviations used are: MTX, methotrexate; dThd, thymidine; HPLC, high-pressure liquid chromatography.

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begun 24 hr later. The mean survival was calculated from the day of tumor inoculation, which was considered to be Day 0.

The drugs were obtained from Sigma Chemical Co., St. Louis, Mo., or Lederle Laboratories, Pearl River, N. Y. Drugs were administered via continuous i.v. infusion in unrestrained mice at a rate of 0.37 ml/hr. The technique was modified from that of Paul and Dave (27) to allow simultaneous infusion of 40 mice for up to 6 days with 5 Harvard Model 940 infusion pumps (Harvard Apparatus Co., Inc., Millis, Mass.) equipped with adapters for holding 8 glass syringes (5 ml). Disposable plastic syringes (12 ml; Sherwood, Deland, Fla.) fit these adapters and do not freeze during the long-term infusions. The shields and splints were constructed as described by Paul and Dave (27).

After the mouse is restrained, a lateral tail vein is punctured about 2.5 cm from the base of the tail with an 18-gauge needle and then cannulated with Intramedic P. E. 10 polyethylene tubing. The tubing is inserted into the vein to about 1 cm from the base of the tail. After spraying the tail lightly with surgical dressing, it is wrapped with 0.5-inch gauze and sprayed again with dressing to form a protective cocoon. The tail is taped to the splint, the shield is positioned, and the mouse is put into a 6- × 10-inch cage. The splint is attached by wire to an overhead support to keep the tail roughly perpendicular to the cage floor. The other end of the cannula is attached to a syringe with either a 27.5- or 30-gauge needle. Drugs were dissolved in either 0.9% NaCl solution or reduced salt to maintain isotonicity and adjusted to pH 7 to 7.4. Prior to administration all solutions were sterilized via passage through 0.22-μm Millipore filters.

The procedure for identification of plasma salvage metabolites by HPLC (Model 830: DuPont Instruments, Des Plaines, Ill.) is an early modification of that described by Rustum (31). Separation is achieved on a Zorbax ODS column eluted with 2.5 mM KH2PO4, pH 6.9. After centrifugation to remove RBC, the plasma was extracted with perchloric acid (final concentration, 4.4%), and the extract was neutralized with 1 N KOH and analyzed with HPLC by injection of 10 μl of the extracted sample.

RESULTS

Nucleoside Reversal of MTX Toxicity. A comparison of MTX toxicity in normal female DBA/2J and C57BL/6 mice indicated that the 2 strains exhibited a slight difference in sensitivity to 48 hr of continuous infusion of MTX alone (Chart 1). Whereas the 50% lethal dose of 6 and 11 mg/kg/day for the 2 strains was not statistically different, the difference in the 100% lethal dose between the 2 strains was reproducible in subsequent experiments. The lack of statistical significance for the 50% lethal dose values is related to the more shallow dose response curve obtained with the C57BL/6 mice. Simultaneous infusion of dThd (5 g/kg/day) and MTX for 48 hr, followed by an additional 48 hr of dThd alone, produced about 8- and 5-fold shifts in the MTX survival curves in the DBA/2J and C57BL/6 mice, respectively. This regimen provided significant protection (p < 0.05) from MTX toxicity in both strains of mice. dThd was infused for an additional 2 days after termination of the MTX to provide thymidylate during the resynthesis of dihydrofolate reductase in tissues (12). The infusion of a lower concentration of dThd (1 g/kg/day) was relatively ineffective in reducing MTX toxicity (p > 0.05). High doses of concurrent dThd (18 g/kg/day) for a total of 96 hr afforded almost complete protection against mortality in both strains with MTX up to 140 mg/kg/day, a 35-fold decrease in toxicity. This regimen was significantly (p < 0.05) more effective than the lower concentration of dThd in reducing MTX toxicity. Infusion of this concentration of dThd alone for 96 hr produced some toxicity in normal mice as evidenced by a 3.2-g weight loss at the end of infusion and a 20% mortality. Slightly lower doses of dThd (15 g/kg/day) alone yielded no gross toxicity.

Further studies were carried out on the modulation of MTX toxicity by salvageable nucleosides in normal female DBA/2J mice. As shown in Chart 1 and listed in Table 1, a 48-hr infusion of MTX at 32 mg/kg/day resulted in 0% survival. Coadministration of dThd for 96 hr offered partial although significant (p < 0.05) protection with 80% of the mice surviving and an average weight loss of 4.2 g/mouse. Infusion of both end products of folate metabolism, dThd and a source of purine, inosine, at optimal doses significantly (p < 0.05) prevented lethal toxicity and minimized weight loss. Lowering the concentration of dThd produced increased weight loss (Table 1). The infusion of inosine (4 g/kg/day) alone for 96 hr concurrently with MTX was also capable of significantly (p < 0.05) reducing mortality; however, a large weight loss was evident and the animal's appearance indicated that full reversal of MTX toxicity had not occurred. By using the same scheduling as in Table 1, the toxicity of MTX at 64 mg/kg/day could be significantly (p < 0.05) reversed with inosine alone or dThd plus inosine; however, dThd (5 g/kg/day) alone could not prevent mortality.

Effects of Normal Metabolites on the Antileukemic Activity of MTX. Chart 2 demonstrates the effects of concurrent infusion of various doses of dThd on the antitumor activity of a single concentration of MTX against leukemia L1210 in female DBA/2J mice. MTX (4 mg/kg/day) infused alone for 48 hr, although a nonlethal dose in normal mice, is stressful to the tumor-bearing animals, which die ahead of the day of tumor inoculation, which was considered to be Day 0.
of controls (Chart 2). Doses of dThd below 1 g/kg/day were not sufficient to reverse MTX toxicity substantially. At this concentration of MTX, a significant (p < 0.05) therapeutic effect was achieved with dThd at 2.5 g/kg/day, allowing an average survival of 12.8 ± 1.8 days (S.D.). As the dose of concurrent dThd was increased to 18 g/kg/day, significant (p > 0.05) antitumor activity was abolished. Infusion of similar high levels of dThd alone had no significant (p > 0.05) antitumor activity. Although maximum survival was obtained with dThd at 2.5 g/kg/day, the higher dose of dThd (5 g/kg/day) was used in subsequent antitumor experiments, for it provided better toxicity reversal and minimal weight loss.

The effects of concurrent infusions of normal metabolites on the antileukemic activity of MTX are summarized in Table 2 and Chart 3. The infusion of MTX alone for 48 hr is rather ineffective against L1210, yielding only a 33% increase in life span over controls (Chart 3). An optimum schedule of MTX (i.p., 3 doses on alternate days) previously yielded a 73% increase in life span over controls with this tumor in DBA/2J mice (6). Co-administration of dThd and MTX for 48 hr potentiates MTX activity, allowing a 14-day average survival time with MTX at 4 mg/kg/day. At higher concentrations of MTX, the 2 extra days of dThd infusion are necessary for prevention of toxicity while maintaining MTX antitumor activity. Maximum therapeutic selectivity and an increase in life span of 125% were obtained with MTX at 16 mg/kg/day infused 48 hr concurrently with dThd (5 g/kg/day) for 96 hr. As the MTX concentration was further increased, the mice died of toxicity. In contrast, concurrent infusion of dThd at 15 g/kg/day prevented toxicity and weight loss at high doses of MTX; however, it reversed tumor kill also and no increase in survival was achieved. As shown in Chart 4, survival curves for the various regimens were constructed at the optimum concentration of MTX for each regimen to facilitate statistical analysis as described by Breslow (4). The infusion of

### Table 1

<table>
<thead>
<tr>
<th>MTX alone</th>
<th>MTX + dThd (5 g/kg/day)</th>
<th>MTX + inosine</th>
<th>MTX + inosine + dThd</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>30 ± 0.7</td>
<td>4.2 ± 1.0</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>80</td>
<td>100</td>
<td>2.5 ± 1.3</td>
<td>4.7 ± 0.6</td>
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</table>

<table>
<thead>
<tr>
<th>MTX (mg/kg/day)</th>
<th>MTX + inosine</th>
<th>MTX + inosine + dThd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.6 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.4 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9.0 ± 0.9</td>
<td>8.8 ± 0.8</td>
</tr>
<tr>
<td>8</td>
<td>4.0 ± 1.5</td>
<td>9.8 ± 0.8</td>
</tr>
<tr>
<td>16</td>
<td>6.6 ± 0.5</td>
<td>8.6 ± 0.5</td>
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<td>32</td>
<td>6.8 ± 0.4</td>
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<table>
<thead>
<tr>
<th>TdR (gm kg⁻¹ DAY⁻¹)</th>
<th>CONTROLS</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
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<tr>
<td>5</td>
<td>12</td>
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<td>10</td>
<td>16</td>
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Chart 2. Effects of varying concentrations of dThd (TdR) on the antitumor activity of 1 dose of MTX against L1210 in DBA/2J female mice. The drugs were administered on Day 1, 24 hr after tumor inoculation. The average survival time of the mice treated with MTX (4 mg/kg/day) alone is 6.6 ± 0.5. MTX and dThd were infused for 48 hr, and then the dThd was continued alone for an additional 2 days. Each group contained 5 mice. Controls, average survival time of untreated leukemic mice of 8.4 ± 0.5. The average survival (12.8 ± 1.8) of the optimum combination was significantly (p < 0.05) better than controls or MTX alone as determined by the analysis of Breslow (4).

**Modulation of MTX Activity by Infusion of dThd**

Each group contained at least 5 DBA/2J mice (20 to 21 g) inoculated with 10⁶ L1210 cells (i.p.) 24 hr prior to drug treatment. Average survival of controls without drug treatment was 8.2 ± 0.8 days.

<table>
<thead>
<tr>
<th>MTX (mg/kg/day)</th>
<th>MTX + inosine</th>
<th>MTX + inosine + dThd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.6 ± 0.8</td>
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<td>2</td>
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<td>8</td>
<td>4.0 ± 1.5</td>
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<tr>
<td>16</td>
<td>6.6 ± 0.5</td>
<td>8.6 ± 0.5</td>
</tr>
<tr>
<td>32</td>
<td>6.8 ± 0.4</td>
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</table>

| Mice were infused for 48 hr with MTX and for 96 hr with inosine (4 g/kg/day). |
| Mice were infused for 48 hr with MTX and for 96 hr with dThd (5 g/kg/day) and inosine (4 g/kg/day). |
| Mean ± S.D. |
MTX alone at 1 mg/kg/day for 48 hr produced significant (p < 0.05) antitumor effects with an average survival of 11.0 ± 1.1 days as compared to 8.7 ± 0.6 for untreated controls. The coinfusion of dThd (5 g/kg/day) with the MTX (4 mg/kg/day) for 48 hr is significantly (p < 0.05) more effective than MTX alone against L1210, resulting in an average survival time of 14.2 ± 2.8 days. When the infusion of dThd (5 g/kg/day) was continued for 48 hr beyond the MTX (16 mg/kg/day), the antitumor effects were significantly (p < 0.05) increased beyond the previous combination, with an average survival time of 19.6 ± 2.6 days in this experiment. No significant (p > 0.05) antitumor effects were observed at this concentration of MTX when the concentration of dThd was increased to 15 g/kg/day on the same schedule.

Table 2 demonstrates the reversal of antileukemic activity by coadministration of inosine with the MTX-dThd combination. The infusion of inosine alone with the antifolate blocked most antitumor activity with MTX at 1 and 2 mg/kg/day, consistent with the previous results obtained with allopurinol (7). At high doses of MTX in the leukemic mice, the inosine alone was not capable of preventing toxic deaths and the mice died ahead of controls (Table 2). This is consistent with the severe weight loss observed in normal mice infused with MTX at 32 mg/kg/day plus purine alone (Table 1).

Concentration of Salvage Metabolites in Plasma during Infusions. In normal DBA/2J plasma, the concentration of hypoxanthine as determined by HPLC is approximately 1 to 2 μM, whereas that of inosine is 20 μM. Although the thymine concentration is below detection (<1 μM), the concentration of dThd is about 0.5 to 1 μM, consistent with the previous report by Hughes et al. (14) using a radioimmune assay to measure serum dThd. As shown in Chart 5, the infusion of dThd at 5 g/kg/day dramatically increased the circulating level of thymine as well as dThd and achieved a plasma concentration of dThd of about 100 μM. Infusion of this dose of dThd did not substantially alter the concentration of other salvage metabolites in plasma. Similar results were observed in replicate experiments and when this dose of dThd was infused with MTX (32 mg/kg/day). However, the infusion of dThd at 15 g/kg/day increased the concentration of hypoxanthine and other metabolites in plasma in addition to that of dThd and thymine (Chart 5). The approximate plasma concentrations of metabolites in this and replicate experiments were: dThd, 800 μM; thymine, 400 μM, inosine, 20 μM; and hypoxanthine, 13 μM. Increased levels of purines were also observed in plasma when this concentration of dThd was infused with MTX (140 mg/kg/day).

DISCUSSION

Effects of End Products on MTX Activity. Whereas, in cell culture, extensive data have been generated on the biological effects of salvage metabolites on MTX activity, the in vivo evaluation of these observations has been complicated by the short plasma half-life of the metabolites (5, 30). Their administration by continuous i.v. infusion results in the establishment of constant, steady-state plasma levels similar to cell culture conditions and allows a direct translation of cell culture results to the in vivo situation. As shown in Tables 1 and 2, concurrent infusion of dThd and a source of salvageable purine, inosine, with MTX completely reversed both toxicity and antitumor activity in vivo comparable to what was previously observed in cell culture (3, 8–10, 36). Concurrent infusion of inosine alone also blocked MTX-induced lethality (Table 1). This somewhat surprising observation is probably a consequence of the 0.5 to 1 μM plasma dThd in the mouse (see “Results” and Ref. 14). In the presence of adequate purge, this concentration of dThd is able to partially reverse the growth inhibitory effects of MTX on mammalian cells in culture, with optimum reversal occurring at 6 to 10 μM (7, 28). The substantial weight loss (4.7 g) observed following the infusion of inosine and MTX indicated that only a partial reversal of toxicity was achieved, as might be expected in the presence of this low concentration of dThd. The minimal weight loss observed following the infusion of additional dThd with this combination (Table 1) and the lack of significant antitumor activity in the presence of inosine.

Chart 5. Evaluation of salvage metabolites in plasma by HPLC during the infusion of dThd (TdR) at 5 g/kg/day. Blood was collected and pooled from 3 mice after 48 hr of infusion and evaluated as described in “Materials and Methods.” Peaks were identified and quantitated by elution position and peak area of marker compounds. Full scale absorbance at 254 nm as indicated by the height of the ordinate is 0.02 absorbance unit. T, thymine; Hx, hypoxanthine.
(Table 2) are also consistent with this proposal.

As reported by Borsa and Whitmore (3) and Tattersall et al. (36), the addition of dThd alone partially reversed the growth inhibitory effects of MTX in certain cell lines in culture, whereas other tumor cell lines required both dThd and purine for any substantial reversal of toxicity (8, 10, 36). Logically, MTX-induced depletion of reduced folates via inhibition of dihydrofolate reductase (24) could affect both thymidylate and purine nucleotide biosynthesis since both synthetic pathways require reduced folate cofactors. Thus, the critical site(s) of MTX inhibition producing cytotoxicity in a particular cell or tissue may depend upon the contribution of de novo thymidylate and/or purine pathways versus salvage reactions for the maintenance of nucleotides in that tissue. MacKinnon and Deller (21) and Lajtha and Vane (17) reported that the intestine and bone marrow, respectively, have very low capabilities for de novo purine nucleotide synthesis and may therefore depend primarily upon salvageable purines to meet cellular needs.

The liver has been implicated as a source of and site for interconversion and release of nucleotide precursors into the circulation (17, 20) for use by tissues such as the intestine and marrow and especially RBC, which lose the capability of de novo synthesis upon maturation (6, 19). In addition to hepatic output and diet, the intestinal mucosa may be in an advantageous position to make use of purines and pyrimidines available from the digestion of large numbers of leukocytes, which are removed from the circulation daily via passage into the intestinal lumen (18). With a diminished capability for de novo purine nucleotide synthesis, some normal tissues may sustain adequate purine pools via salvage; under these conditions MTX would have negligible effects on the level of intracellular purine nucleotides. Thus, the toxicity of MTX to dividing host tissues, such as intestine and bone marrow, may be chiefly a limitation of thymidylate biosynthesis. However, in normal mice the dThd (5 g/kg/day) protection was incomplete, similar to that observed in the cultured cell lines, and survival decreased with increasing dose of MTX. With MTX at 64 mg/kg/day, the lower dose of concurrent dThd no longer afforded any reversal of mortality (Chart 1). This MTX dose dependence for survival is inconsistent with the postulate of end product reversal of its effects produced by infusion of dThd and salvage of naturally occurring purines by the normal tissues. Via this mechanism even the highest doses of MTX should have been nontoxic (3, 8-10, 36).

The infusion of larger doses of dThd (15 g/kg/day) was more effective in reducing the toxicity of high doses of MTX, although antitumor activity was blocked also. As shown in Chart 6, however, the infusion of this high concentration of dThd altered the level of other salvage metabolites such as hypoxanthine in addition to increasing the plasma concentration of dThd and thymine. The mechanism of this induced increase in circulating purines is not understood. The concentrations of dThd and hypoxanthine observed under these conditions should be sufficient to achieve a total end product reversal of MTX toxicity. This would be consistent with the total loss of MTX antitumor activity and toxicity observed in Charts 1 to 3 following simultaneous infusion of high doses of dThd.

**Metabolic Modulation of MTX Activity.** Another mechanism that could account for the ability of dThd to partially reverse MTX toxicity with retention of antitumor activity involves the indirect inhibition of thymidylate synthetase. The synthesis of TMP catalyzed by thymidylate synthetase involves the transfer of a 1-carbon unit from 5, 10-methyltetrahydrofolate acid and the loss of the C-6 hydrogen from the cofactor to the methyl group of TMP (2). The dihydrofolate acid thus formed must be reduced by dihydrofolate reductase to tetrahydrofolate acid, for it is only at this level of reduction that the folate can accept 1-carbon units to form the cofactors for intracellular reactions (Chart 7).

Interruption of the dihydrofolate reductase reaction, as by MTX, with the continuing synthesis of TMP results in depletion of reduced folate cofactor pools and growth inhibition (24). However, this depletion of reduced folate cofactor pools would not occur in cells exposed to MTX, which contained no TMP synthetase activity, except via growth dilution. Thus, bacterial or mammalian cell mutants
with substantially decreased TMP synthetase activity can be readily isolated by cloning the cells in medium containing MTX and thymine or dThd (25). Under these conditions no purine toxicity is expressed. Limitation of the thymidylate synthetase reaction concurrently with inhibition of dihydrofolate reductase might provide a balanced slowdown in the rate of the folate oxidation-reduction cycle. Such a slowdown would spare a cell from rapid depletion of its reduced folates, albeit at the expense of thymidylate synthesis. When added to the medium of cells in culture, the anabolism of dThd to the triphosphate produces an expanded intracellular dTTP pool (18, 36). The deoxytriphosphate behaves as a feedback effector of ribonucleotide reductase and thereby alters or depletes the concentrations of the other deoxyribonucleotides. Thus, the generation of excess dTTP in tissues in vivo by continual provision of dThd could inhibit the synthesis of dUMP via allosteric regulation. Indeed, Jackson (15) recently reported a dramatic reduction in dUMP pools in Novikoff hepatoma cells in culture in the presence of 20 μM dThd. A decrease in the dUMP pool would then impede the thymidylate synthetase reaction and thus the oxidation of the cofactor, 5,10-methylenetetrahydrofolate acid, via substrate limitation (Chart 7). The end result of the metabolic modulation is provision of a tissue with thymidylate and at the same time a sparing of its pool of reduced folate cofactors, allowing other folate-requiring reactions such as purine de novo synthesis.

An integral part of this mechanism is that a partial reversal of MTX toxicity may well be achieved dependent on the balance of the 2 key enzymes in the folate oxidation-reduction cycle (Chart 7). Such a partial reversal has been observed both in cell culture (3, 11, 36) and in vivo (Chart 1). Antitumor selectivity would be achieved through the differential ability of dThd to induce an inhibition of thymidylate synthetase in various cells. Little or no protection from MTX toxicity with the addition of dThd has been observed for most mammalian cell lines in culture (8, 9, 36). Thus, the mechanism of MTX tumor cell kill in the presence of dThd would be a limitation of purine nucleotide biosynthesis, with L1210 undergoing a "purineless" death similar to that observed in L5178Y cells in culture by Hryniuk (13). Additional support for this concept is derived from the observations that the antitumor activity obtained with the MTX-dThd combination is reversed by the administration of inosine (Table 2) and the high plasma concentration of dThd (100 μM or greater) required to achieve the modulation. A lower concentration of dThd, which was effective in blocking toxicity in the presence of inosine (Table 1), was ineffective alone in preventing MTX toxicity. Similar results were obtained by Pinedo et al. (28) who evaluated the ability of nucleosides to prevent the cytotoxicity of MTX to mouse bone marrow cells in vitro.

Although these results indicate that the therapeutically active concentration of MTX in the mouse is clearly improved by coadministration of dThd, the implications of extrapolating these data to the clinic are complicated by the salvage metabolite effects themselves. In contrast to the mouse serum, human sera does not contain xanthine oxidase (1), and thus the report of 40 μM hypoxanthine in human sera is not surprising (26). Variation in endogenous purines and other normal metabolites may play a significant role in modulating MTX activity against human tumors. Although MTX and concurrent dThd has been initiated into clinical trial with some therapeutic success (5), clearly, further studies are necessary to evaluate the role of salvage metabolites in clinical MTX as well as other antimetabolite chemotherapies.

ACKNOWLEDGMENTS

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