High-Dose Trimetrexate and Minimal-Dose Leucovorin: A Case for Selective Protection? 1

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INTRODUCTION
TMTX, a 4-amino, quinazoline analogue of methotrexate, is a potent inhibitor of the enzyme DHFR (1). TMTX is more lipophilic than MTX, and cellular uptake is both noncompetitive with folate or MTX and appears to be nonsaturable in vitro (2). Thus, TMTX might be effective when cells are resistant to MTX by virtue of altered drug transport (3, 4, 5, 6). Furthermore, because folate and MTX transport are both mediated by the RFC, it is possible that altered MTX transport could also impair folate uptake, i.e., the tumor would also not accumulate folate normally. This implies that a tumor that has lost ability to transport MTX may be sensitive to TMTX and not be rescued by LV (7, 8). This phenomena is the basis for treating patients with Pneumocystis carinii with large doses of TMTX and LV because the parasite cannot transport reduced folates. Specifically, patients with P. carinii are given 45 mg/m2 TMTX and 80 mg/m2 LV daily for 21 days (9). In comparison, the maximally tolerated dose of TMTX as a single agent in patients with cancer is ~10 mg/m2 daily 5 days every 3 weeks (10). Thus, LV allows a massive increase in the amount of TMTX administered.

The goal of our feasibility study presented here was to find the smallest dose of LV to provide selective protection (rather than rescue) to normal cells.

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Materials. TMTX was supplied by US Bioscience (West Conshohocken, PA). Twenty-five mg were reconstituted in 2 ml of sterile water and then diluted with 50% dextrose water to concentrations of 5 or 10 mg/ml. The latter was used as the oral solution. LV is commercially available for i.v. administration as well as an oral tablet form.

Protocol. TMTX (90 mg/m²/day) was given as 45 mg/m² p.o. every 12 h for 14 consecutive days. A course was defined as 14 days of TMTX, followed by 14 days of no drug (i.e., one course is 4 weeks). The goal was to determine the minimally effective oral LV dose necessary to prevent grade 3 toxicity (i.e., significant enough to stop treatment or initiate increased LV rescue) and allow adherence to the 14 day on/14 day off schedule. LV was started at 5 mg/m² twice daily (taken with TMTX). Because we did not want to “over rescue” the tumor, i.e., miss efficacy, decreasing the LV in a patient seemed prudent. Thus “de-escalation” of LV was planned from the starting dose to 2.5 mg/m², 1.25 mg/m², and 0.5 mg/m² in cohorts of three patients. Moreover, if a patient had no toxicity at a given dose and safety at a lower dose of LV documented in another patient, a patient could receive a subsequent course of treatment at a lower dose. If two consecutive patients experienced more than grade 3 toxicity, the LV dose was increased to the previous level. Because mucositis with antifolates can be severe and take days to recover, we considered a grade 3 mucositis a dose-limiting toxicity, although not life threatening, because we judged it would delay the start of another course, which was our overall dose-limiting toxicity.

Monitoring. Pretreatment evaluation include a complete clinical history and physical examination with a Karnofsky score assignment. Baseline laboratory tests are noted above. Imaging studies to document tumor extent (localization, size, and volume) were done within a week of initiation of treatment. Ongoing monitoring during therapy included twice weekly complete blood count with differential and a chemistry panel every other week to assess for toxicities. Imaging studies were repeated every two courses or sooner if objective clinical deterioration prompted reevaluation. Plasma peak and trough levels of TMTX were obtained at the end of the first and second weeks with peaks drawn 1-2 h after a dose was given and the corresponding trough prior to the following dose to assess/confirr bioavailability (1, 11).

Supportive care such as antiemetics, analgesics, antibiotics for infections associated with neutropenia, platelet and red cell transfusions, and fluids were provided as needed. Drugs that were avoided included cytochrome P-450 inhibitors, antibiotics such as erythromycin and Biaxin, fluconazole, and H₂ blockers such as cimetidine and ranitidine. Emergency radiation was allowed to prevent or minimize acute loss of function so long as there was measurable disease outside the radiation field.

Toxicity. Toxicities were assessed according to the Common Toxicity Criteria developed by the Cancer Therapy Evaluation Program of the National Cancer Institute. As noted above, the objective was to give TMTX as described above in 28-day cycles.

Off-Study Criteria. A complete response was defined as total disappearance of tumor with no evidence of disease; a partial response was >50% decrease in size of all measurable lesions; no response or stable disease was no change or <25% change of lesions; and progressive disease as >25% increase in size of one or more lesions or the appearance of new lesions. Patients with complete or partial responses and who experienced less than or equal to grade 2 toxicity continued on treatment. Patients who had only stable disease upon reevaluation after two cycles were continued on therapy at the patient’s and investigator’s discretion. Patients with progressive disease at any time were taken off study. Patients were also removed from study if unacceptable toxicities developed (grade 4) that did not rapidly reverse upon discontinuation of TMTX and increased LV rescue.

Pharmacokinetics. Plasma TMTX (peak and trough) was determined by both high-performance liquid chromatography and radio-ligand binding assay as published previously (12, 13, 14). Trough samples were obtained just prior to a dose of drug and the peak 1–2-h post dose.

RESULTS
From March 1995 to April 1996, 11 patients were enrolled. One patient had poor compliance in the first cycle, was removed from the study, and is not presented here. The characteristics of the 10 patients in the study are provided in Table 1. There were 3 males and 7 females with an age range of 4 to 20 years. Six patients had an osteogenic sarcoma, and four had a primitive neuroectodermal tumor or medulloblastoma. All of the patients with osteogenic sarcoma had prior therapy that included high-dose MTX, Adriamycin, and platinum. Other therapies included

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Disease</th>
<th>Age (yr)</th>
<th>No. of courses</th>
<th>Time to disease progression (wk)</th>
<th>Lowest LV dose (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Osteogenic sarcoma</td>
<td>20</td>
<td>3</td>
<td>10</td>
<td>1.25</td>
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<tr>
<td>2</td>
<td>Osteogenic sarcoma</td>
<td>17</td>
<td>2</td>
<td>8</td>
<td>0.5</td>
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<td>3</td>
<td>Osteogenic sarcoma</td>
<td>18</td>
<td>3</td>
<td>12</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>Osteogenic sarcoma</td>
<td>18</td>
<td>2</td>
<td>9</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>Primitive neuroectodermal tumor</td>
<td>11</td>
<td>1</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Medulloblastoma</td>
<td>15</td>
<td>4</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>Primitive neuroectodermal tumor</td>
<td>4</td>
<td>4</td>
<td>15</td>
<td>2.5</td>
</tr>
<tr>
<td>8</td>
<td>Osteogenic sarcoma</td>
<td>8</td>
<td>3</td>
<td>9</td>
<td>2.5</td>
</tr>
<tr>
<td>9</td>
<td>Medulloblastoma</td>
<td>13</td>
<td>6</td>
<td>24</td>
<td>2.5</td>
</tr>
<tr>
<td>10</td>
<td>Osteogenic sarcoma</td>
<td>19</td>
<td>1</td>
<td>2</td>
<td>2.5</td>
</tr>
</tbody>
</table>
vincristine, ifosfamide, cyclophosphamide, etoposide, and radiation.

All 10 patients were treated with 45 mg/m² TMTX every 12 h for 14 days with concomitant LV. Both drugs were taken p.o. Twenty-nine courses were given. The median number of courses given per patient was three (range, 1–6). Nine courses in seven patients were at 5 mg/m² LV, 15 courses in seven patients at 2.5 mg/m², and 5 courses in four patients at <2.5 mg/m² (range, 0.5–1.25 mg/m²; Table 1).

**Toxicity.** Only one of the nine courses (11%) with 5 mg/m² LV resulted in any more than or equal to grade 3 toxicity, whereas 8 of 15 (53%) at 2.5 mg/m² LV resulted in more than or equal to grade 3 toxicity, but three of the seven patients (nos. 7, 8, and 9) at this dose received 11 of the 15 courses without any grade 3 toxicity. The most common toxicities observed were mucositis, thrombocytopenia, and neutropenia. A summary of the toxicities seen at a specific LV dose are summarized in Table 2.

**Mucositis.** A more than or equal to grade 3 occurred in 3 of 29 courses of TMTX: once at 5 mg/m² LV and twice at ≤2.5 mg/m². The start of a second course was delayed a week in these two cases, both of which were in a patient with osteogenic sarcoma who had been treated previously with high-dose MTX. The LV rescue was continued during the extra week.

**Hematological.** Thrombocytopenia was the most common myelotoxicity encountered (11 of 29 courses varying from grades 1 to 4 in severity). Platelet transfusions were given in two of the four courses with more than or equal to grade 3 thrombocytopenia. All four occurred at reduced doses of LV (≤2.5 mg/m²). There were only two episodes of grade 3 and one grade 4 neutropenia seen. One resulted in hospitalization for fever with an associated pneumonia. In the case of the grade 4 toxicity, TMTX was discontinued after 12 of the 14 days, and LV was continued for 5 days, until toxicity was ≤1. Only one red cell transfusion was given (one course with 2.5 mg/m² LV).

**Other Toxicities.** Mild, transient elevations in liver function tests during one course and two cases of hyperpigmentation of the skin of hands that did not require medical intervention or treatment modification were seen.

**Responses.** There were no complete responses. One patient with a metastatic medulloblastoma (patient number 6) had a minimal response in leptomeningeal disease by magnetic resonance imaging that was correlated with clinical improvement. He was on treatment for ~4 months. Several other patients had stabilization of their disease, as judged by number of courses and time to progression.

**Pharmacokinetics.** Peak and trough TMTX values were determined on either day 7 and/or day 14 of TMTX. For the 10 evaluable patients, 22 determinations were done. The mean peak was 6.6 µM ± 2.8, and the mean trough was 2.0 µM ± 1.5. These values are comparable with those reported previously for single-dose bioavailability studies done at a dose of 45 mg/m² as well as other pharmacokinetic and Phase I/II studies (1, 15, 16).

**DISCUSSION**

Mammalian cells cannot synthesize folate. Therefore, there is an absolute dietary requirement for this vitamin. Reduced folates function as cofactors in one-carbon transfer reactions required for thymidine, purine, and certain amino acid interconversions (1). Specific mechanisms for folate transport, metabolism, and accumulation exist to ensure the availability of intracellular reduced folates. There are two different transport systems by which folates gain entry into mammalian cells: the RFC and a hydrophobic membrane-associated folate receptor. Cellular retention of reduced folates is enhanced by polyglutamation catalyzed by the enzyme FPgs. Polyglutamation also increases the affinity of folate-dependent enzymes for folate. MTX (and other folate analogues) use and compete with folate for some or all of these same pathways. Mechanisms of resistance to MTX include alteration or amplification of DHFR, decreased polyglutamation, and impaired transport.

TMTX shares a mechanism of action with MTX, i.e., inhibition of DHFR. Like MTX, TMTX is cell cycle phase specific, having its greatest cytotoxic impact during S phase (17). There are differences, however, in drug uptake and cellular accumulation. TMTX, as a more lipophilic, quinazoline folate analogue, gains entry into cells via passive diffusion. TMTX does not require active transport mechanisms for entry; therefore, it does not compete with or inhibit uptake of reduced folates, and uptake appears to be nonsaturable in vitro (1, 2). It is also not a substrate for FGPs. TMTX has demonstrated antitumor activity in numerous cancers including breast cancer, head and neck cancers, esophageal cancer, prostate cancer, sarcomas, and non-small cell cancer of the lung (10). If resistance to MTX is based on impaired transport or reduced polyglutamation, TMTX could bypass these therapeutic obstacles (3–8). For sarcomas in particular, there is evidence that intrinsic resistance to MTX is based on transport (4, 5).

The goal of this protocol was to provide large doses of TMTX for an extended period of time using the minimally effective dose of LV to prevent excessive toxicities. LV was not

<table>
<thead>
<tr>
<th>LV dose (mg/m²)</th>
<th>No. of courses</th>
<th>Worst toxicity</th>
<th>Grade</th>
<th>No. of episodes</th>
<th>Treatment for toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>9</td>
<td>Mucositis</td>
<td>3</td>
<td>1</td>
<td>TMTX held 3 days</td>
</tr>
<tr>
<td>2.5</td>
<td>15</td>
<td>Mucositis/thrush</td>
<td>3</td>
<td>2</td>
<td>Melfl-benadryl and nystatin</td>
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<tr>
<td></td>
<td></td>
<td>Anemia</td>
<td>3</td>
<td>1</td>
<td>Transfusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neutropenia</td>
<td>3.4</td>
<td>1.1</td>
<td>Antibiotics</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thrombocytopenia</td>
<td>4</td>
<td>2</td>
<td>Transfusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AST increase</td>
<td>3</td>
<td>1</td>
<td>Doubled LV, TMTX stopped on day 8</td>
</tr>
<tr>
<td>0.5–1.25</td>
<td>5</td>
<td>Thrombocytopenia</td>
<td>3</td>
<td>1</td>
<td>Transfusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Neutropenia</td>
<td>3</td>
<td>1</td>
<td>Antibiotics</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AST increase</td>
<td>2</td>
<td>1</td>
<td>TMTX held last 2 days</td>
</tr>
</tbody>
</table>
used in the traditional sense of “rescue” as described for high-dose MTX but to provide “selective protection” of normal tissues in those patients who may have tumors with intrinsic or acquired resistance to MTX. In the therapy of P. carinii, LV (80 mg/m² i.v. daily) is given concurrently with TMTX (45 mg/m² i.v.) for 21 days. Here, for the treatment of patients with refractory malignancies, TMTX (45 mg/m²) was given p.o. every 12 h for 14 days. This dose was chosen on the basis of the bioavailability of oral TMTX demonstrated in the treatment of PCP in patients with AIDS and our own trial of patients with cancer that determined the bioavailability and pharmacokinetic parameters of TMTX given p.o. (1, 11).

On the basis of studies of folic acid homeostasis, LV was given concurrently starting at only 5 mg/m², with subsequent de-escalations as tolerated. As a vitamin, folate is highly conserved by the body. The minimum daily requirement of folate is estimated to be 4–5 µg/kg/day. Plasma folate is only 0.01–0.02 µM. Our starting dose of LV increases plasma folate to 0.1–0.2 µM, a value 10–20 times normal. The results here confirm that this is an adequate level to allow high-dose TMTX to be given repititively to patients. Because the bioavailability of TMTX for the dose here is ~66% and the LV:TMTX ratio (at initial dosing) was approximately 10:60, i.e., 0.166 and for patients with P. carinii, the ratio is 80:45 or 1.77; the dosing here altered the LV:TMTX ratio a minimum of 10-fold, even without regard to consideration of the twice daily dosing and a T1/2β of ~8 h.

Because we did not have the opportunity to test the tumors for alterations in transport and we did not want to “over rescue,” we closed the trial as a completed feasibility study when it was clear that the amount of LV needed was near the normal recommended daily allowance for folate, as compared with the dose used in the treatment of patients with P. carinii. We also treated patients for whom we had no higher priority protocol and/or whom might of been resistant to MTX because of prior exposure to the drug. With regards to the specifics of the protocol, since we found interpatient variability with regard to dose of LV required for heavily pretreated patients, we recommend initiating therapy at 5 mg/m² and if tolerated, decreasing the dose to 2.5 mg/m². Because three of seven patients (accounting for 11 of 15 courses) tolerated only 2.5 mg/m² LV very well and there was only one grade 4 hematological toxicity, in patients with good performance status, the starting dose of LV might be 2.5 mg/m². In addition, the possibility of increasing the time of treatment to 21 days followed by 7 days off is rational based upon the treatment of patients with P. carinii.

We suggest that the ability to detect the RFC and FPGS using molecular probes may now allow for identification of patients most likely to respond to high-dose TMTX and low-dose LV, a protocol to provide selective protection to normal tissue and “use” the tumor’s drug resistance to the patient’s advantage.

REFERENCES


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