Phase I Trial of N-(Phosphonacetyl)-l-aspartate, Methotrexate, and 5-Fluorouracil with Leucovorin Rescue in Patients with Advanced Cancer

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ABSTRACT

Based on an animal model to improve the antitumor activity of 5-fluorouracil (FURA), a Phase I study of N-(phosphonacetyl)-l-aspartate, methotrexate, FURA, and leucovorin was conducted on 44 patients. Methotrexate was given in an intermediate dose (250 mg/m²) to overcome potential drug resistance, and N-(phosphonacetyl)-l-aspartate was given at a low dose (250 mg/m²) in order to allow escalation of FURA to toxicity. These two drugs were given 24 h before FURA to enhance maximal incorporation of FURA into RNA. Two schedules of administration were used: one every other week and one weekly for 2 weeks. The every other week schedule was well tolerated, with minimal gastrointestinal and hematological toxicity. However, the weekly for 2 weeks schedule was more toxic with increased mucositis, diarrhea requiring therapy, and decreased performance status of 20% in 4 of 6 patients. There were no responders in the every other week schedule. There was one partial response and three patients with stable disease in four evaluable patients on the weekly for 2 weeks schedule. At 24 h post-N-(phosphonacetyl)-l-aspartate-methotrexate treatment, PRPP levels were doubled in bone marrow biopsies, and increased 2.5- to 25-fold in tumor biopsies. We have currently added uridine rescue to this combination with the hope of further escalating the dose of FURA.

INTRODUCTION

5-Fluorouracil is the most active chemotherapeutic agent for the treatment of advanced colorectal cancer, although the response rate is only 15–20% (1). One strategy to improve its antitumor activity is to biochemically modulate FURA2 to make its antitumor activity more selective. FURA exerts its cytotoxic effect by at least two mechanisms: a) incorporation of FURA as FUTP into RNA, and b) inhibition of thymidylate synthetase (2–3). It is not intuitively obvious whether the DNA- or RNA-dependent mechanism of FURA activity correlates with its cytotoxicity in various carcinomas cell lines. Evans et al. (4–5) have shown that low levels of FURA cause growth inhibition of Sarcoma 180 cells (a mouse sarcoma line) by inhibiting thymidylate synthetase, while at high levels of FURA the incorporation of RNA seems to be dose limiting. In contrast, the growth inhibition of human Hep-2 cells by FURA is due to its incorporation into RNA.

PALA is an inhibitor of aspartate transcarbamylase, one of the initial enzymes in the de novo pathway for the biosynthesis of pyrimidines. Thus, when PALA is administered prior to FURA, the consequent decrease in UTP allows greater utilization of FUTP by RNA polymerase, resulting in increased incorporation of FURA into tumor RNA, and enhanced antitumor activity in several animal tumor systems (6). Aspartate transcarbamylase, the target enzyme for PALA, is in lower concentration in human and murine tumor tissues than in normal tissues (7); thus, a low, nontherapeutic, but still biochemically active dose of PALA may selectively and safely modulate the antitumor activity of a maximally tolerated dose of FURA in patients, as it does in tumor-bearing mice (8–9). Casper et al. (10) have demonstrated in patients that excellent inhibition of whole body pyrimidine synthesis can be achieved with a low weekly dose (250 mg/m²) of PALA, allowing the safe administration of FURA at its weekly maximally tolerated dose 24 h after PALA.

MTX is a folate antagonist that binds to and inhibits dihydrofolate reductase, resulting in depletion of tetrahydrofolates and the inhibition of both thymidylate production and de novo purine synthesis. The latter inhibition leads to an accumulation of PRPP, a substrate which can be rate limiting in the activation of FURA to its active nucleotides; thus, the MTX-induced increased PRPP levels result in greater intracellular conversion of FURA into its nucleotide and therefore greater antitumor activity (11). Synergism between these two drugs depends both on proper sequence; i.e., MTX and then FURA, and the interval (18–24 h) between the two agents (12–13). Given the above information, a Phase I study was conducted with PALA (at low dose) and MTX (at an intermediate dose) 24 h prior to FURA (dose escalated), followed by leucovorin rescue to determine the maximum tolerated dose and toxicity of FURA.

MATERIALS AND METHODS

Patients

Forty-four patients with advanced cancer were entered in this protocol. Criteria for entry in this study included histologically confirmed cancer (42 patients with colorectal adenocarcinoma and 2 with epidermoid cancer of the head and neck), disease not curable by radiation therapy or surgery, Karnofsky performance status >50, life expectancy of 8 weeks, WBC >3,500 × 10⁹/liter, platelet count >130,000 × 10⁹/liter, bilirubin <1.5 mg/dl, and creatinine <1 mg/dl, or creatinine clearance >65 ml min. Previously treated patients were eligible if they had not received myelosuppressive chemotherapy within the previous 4 weeks (6 weeks for nitrosoureas and mitomycin C). Patients were not put on study if they had received radiation therapy to major bone marrow-containing areas within 4 weeks.

Of the 44 patients entered, 38 received every other week therapy, and 6 received weekly therapy for 2 weeks. Throughout this paper, they will be referred to as group 1 and group 2, respectively. Table 1 describes base-line characteristics of our patients.

Base-line laboratory studies included complete blood count, platelet count, differential, biochemical screening profile, chest radiograph, carcinoembryonic antigen, creatinine, and 24-h creatinine clearance. Radionuclear liver scan, bone scan, and abdominal computed tomography were performed if clinically indicated.

PRPP Determination

To ascertain the biochemical effects of PALA and methotrexate as modulators of PRPP levels, biopsies of tumor tissue were taken prior...

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2 To whom requests for reprints should be addressed.

The abbreviations used are: FURA, 5-fluorouracil; FUTP, 5-fluorouridine triphosphate; PALA, N-(phosphonacetyl)-l-aspartate; MTX, methotrexate; PRPP, 5-phosphoribosyl-1-pyrophosphate; HPLC, high pressure liquid chromatography; FUMP, fluorouridine monophosphate.
Table 1  Base-line characteristics of Phase I patients

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (median)</th>
<th>Group 2 (median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>38</td>
<td>6</td>
</tr>
<tr>
<td>Age</td>
<td>58</td>
<td>61</td>
</tr>
<tr>
<td>Sex</td>
<td>25 M; 13 F</td>
<td>4 M; 2 F</td>
</tr>
<tr>
<td>WBC</td>
<td>7.2</td>
<td>6.6</td>
</tr>
<tr>
<td>Platelets</td>
<td>325</td>
<td>209</td>
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<tr>
<td>Albumin</td>
<td>4.15</td>
<td>4.25</td>
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<tr>
<td>Alkaline phosphatase</td>
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<td>108</td>
</tr>
<tr>
<td>Carcinoembryonic antigen</td>
<td>25.1</td>
<td>117</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>249</td>
<td>256</td>
</tr>
<tr>
<td>Phosphatidylserine</td>
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<td>80</td>
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<tr>
<td>Prior therapy with FUra</td>
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<td>5</td>
</tr>
<tr>
<td>Site of measurable disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Lung</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Abdomen</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Pelvic mass</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Chest wall mass</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Median no. of previous treatments with FUra-containing regimen</td>
<td>(range, 0–5)</td>
<td>(range, 0–4)</td>
</tr>
</tbody>
</table>
patients had diarrhea, causing dehydration and requiring therapy. Nausea and vomiting were minimal at this dose level, but 1 patient had mucositis, requiring a liquid diet. Although objective parameters of toxicity were not significant, symptomatic tolerance was severely impaired with 4 of 6 patients having a >20% decrease in performance status on treatment, with improvement of performance status when therapy was delayed or stopped. Four of the 6 patients continued therapy at 500 mg/m². This did not cause a decrease in performance status, but 2 of the 4 patients had grade 2+ hematological toxicity and 2 had grade 2+ diarrhea.

PRPP Levels. Bone marrow and cancer biopsies were analyzed for levels of PRPP in pretreatment and posttreatment (24 h after PALA and MTX) tissues from the same patient. PRPP levels were enhanced. Findings are in Tables 3 and 4, respectively.

RNA Containing Incorporated FUra Residues in Bone Marrow. Fig. 1 is a HPLC separation of an alkali digest of RNA prepared from nucleated bone marrow cells; Fig. 1A is bone marrow taken prior to FUra administration, and Fig. 1B is bone marrow 2 h after FUra administration (650 mg/m²) in the same patient. Note the appearance of a small peak in Fig. 1B that is absent in Fig. 1A. The identity of the peak indicated as 2',3'-FUMP was established by its absence in the chromatogram of pretreatment marrow, and by its cochromatographing with authentic 2',3'-FUMP. In this particular sample, there was 446 pmol of FUra incorporated into RNA/10⁶ cells.

Therapeutic Results. Although this study was a Phase I trial, we nevertheless evaluated 27 patients with measurable disease in the first group and 4 patients with measurable disease in the second group for antitumor response. There were no responses in the first group (every other week schedule), but 3 patients with previous progressive disease had stabilization of their disease for a median of 349 days (range, 95–586). In the second group (weekly for 2 weeks schedule), 1 of 4 evaluable patients had a partial response (>50% decrease in an abdominal wall mass), and 3 who had progressed on previous FUra-containing regimens had stabilization of disease for a median of 189 days (range, 92–236).

DISCUSSION

In an attempt to increase the efficiency of FUra, numerous trials have been designed to biochemically modulate FUra.

Aradan et al. (8) have shown in in vitro studies that the synergistic effect of the combination of PALA and FUra on human mammary carcinoma cell lines correlates with an increased proportion of FUTP in the pyrimidine nucleotide pool, and, consequently, with an enhanced incorporation of FUra into RNA. Similar results, including enhanced antitumor activity, were also demonstrated in vivo preclinically with low doses of PALA prior to FUra (15).

PALA, inactive clinically as a single agent, has been used in a number of clinical studies as a biochemical modulator of FUra. However, the appropriate ratios of agents and the sequence and time interval between administration of agents are critical determinants of success, and these parameters were frequently not incorporated into the design of the clinical protocols (16). Buroker et al. (17), using PALA at 625 mg/m² followed in 4 h by FUra at 300 mg/m² for 5 consecutive days, obtained only an 11% response rate. Muggia et al. (18), using a high dose of PALA (1.5 g/m²) combined with escalating doses of FUra to 800 mg/m² given every other week, obtained a partial response rate of 26%. It is possible that these high doses of PALA did not permit appropriate escalation of FUra or more frequent weekly administration of the PALA/FUra combination which may have led to a better response rate. In contrast, Aradan et al. (19) used a weekly schedule with a low dose of PALA (250 mg/m²), followed in 24 h with a continuous infusion of FUra at 2600 mg/m² over 24 h, and obtained a response rate of 48% in previously untreated patients.

The latter study (19) suggests that a low PALA dose is capable of selectively potentiating the effects of FUra in human tumor cells with relative sparing of host tissues, thus improving the therapeutic index. In mice, a low nontherapeutic dose of PALA can lower UTP pools in a spontaneous breast tumor model and can safely be administered 24 h prior to the maximum tolerated dose of FUra resulting in enhanced anticancer activity (9).

Cadman et al. (11) have shown that a MTX concentration of
10 μM was necessary to maximally increase intracellular PRPP. They administered MTX doses of 200 mg/m² in order to achieve the desired MTX concentration (20). We thus selected 250 mg/m² as our dose, since this dose was safely administered in most of the clinical investigations with sequential MTX and FUra (13), and would give us the desired micromolar concentration.

The PRPP data indicate that the levels of PRPP in human colorectal tumors (Table 4) can be markedly increased (25-fold to 100-fold) in one patient 24 h following PALA and MTX treatment, in contrast to a 5-fold increase in the CD8F1 tumor. The data in Table 3 suggest that PALA-MTX combination can have some modulating effect upon the content of PRPP in bone marrow cells. The magnitude of the change observed was 2-fold in our study, but has not been reproducibly measured in the murine model. It is our future hope to compare the amount of FUra incorporated into RNA after pretreatment with PALA/MTX relative to that incorporated into RNA after FUra alone in human tumors.

The recommended Phase II dose for the every other week schedule is FUra at 800 mg/m², and the dose for the weekly for 2 weeks schedule is FUra at 500 mg/m².

Although the actual dose given in the every other week schedule was greater than the weekly for 2 weeks schedule, slight activity was seen only with this latter regimen. Calculations of dose intensity gives equal importance to time delays and actual dose (21). It may be that giving the FUra 1 week earlier is more advantageous.

Our current interest is to decrease toxicity of FUra with the addition of uridine. In murine studies, the toxicity was reduced by the addition of uridine rescue permitting escalation of FUra and consequent marked antitumor effects (14, 22). New studies demonstrate that uridine rescue is effective in controlling FUra and consequent marked antitumor effects (14, 22). We thus selected 250 mg/m² as our dose, since this dose was safely administered in most of the clinical investigations with sequential MTX and FUra (13), and would give us the desired micromolar concentration.

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