A Phase I Study of Continuous Infusion 5-Fluorouracil plus Calcium Leucovorin in Combination with N-(Phosphonacetyl)-L-aspartate in Metastatic Gastrointestinal Adenocarcinoma


ABSTRACT

Preclinical studies suggest that the biochemical effects of N-(phosphonacetyl)-L-aspartate (PALA), an inhibitor of aspartate carbamoyltransferase (ACTase), may increase the metabolic activation of 5-fluorouracil (5-FU) and enhance its cytotoxicity through both RNA- and DNA-directed mechanisms. In this Phase I trial, 22 evaluable patients with adenocarcinoma of the gastrointestinal tract were entered at escalating doses of 5-FU starting at 1150 mg/m²/day given as a concurrent 72-h i.v. infusion with a fixed dose of leucovorin (LCV), 500 mg/m²/day. The dose of 5-FU was escalated within patients according to individual tolerance, and then PALA at 250 mg/m² was added 24 h prior to the initiation of the 5-FU/LCV infusion of the subsequent cycle. Dose-limiting mucositis and myelosuppression occurred during the initial cycle in 3 of 5 patients treated with 2300 mg/m²/day 5-FU; therefore, the recommended dose of 5-FU with concurrent LCV is 2000 mg/m²/day. Twenty-seven additional patients were then treated with escalating doses of PALA ranging from 375 to 2848 mg/m², i.v., followed 24 h later by 2000 mg/m²/day 5-FU with high-dose LCV. Dose-limiting mucositis and myelosuppression occurred during the initial cycle in 2 of 3 patients entered at 2848 mg/m² PALA. Dose-limiting mucositis and skin rash ultimately required both PALA and 5-FU dose reductions in 4 of 6 patients treated with 1899 mg/m² PALA. Toxicity was similar, however, in patients receiving PALA at doses ranging from 375 to 1266 mg/m². The mean steady-state plasma concentration of 5-FU at 2000 mg/m²/day was 6.5 ± 0.9 μM; patients with 5-FU levels >9 μM had a significantly higher incidence of serious gastrointestinal and hematological toxicity. Compared to each patient’s own baseline, a significant trend for decreasing ACTase activity with increasing PALA dose was evident using cytosol isolated from peripheral blood mononuclear cells 24 h after PALA treatment (P < 0.01). PALA ≤844 mg/m² failed to appreciably inhibit ACTase activity at 24 h in most patients; furthermore, a decrease in ACTase activity by >50% from baseline was seen in only 29% of cycles. More consistent inhibition of ACTase activity was seen with PALA ≥1266 mg/m². Even with the highest PALA doses, however, ACTase activity returned to baseline by 96 h in most patients. In contrast, a modest decrease in plasma uridine levels was noted at all PALA doses, but the decrease was ≤50% in only 21% of cycles at 24 h. PALA ≥1266 mg/m² could be safely combined with a 72-h i.v. infusion of 5-FU 2000 mg/m²/day with LCV 500 mg/m²/day starting 24 h after PALA. Because the delivered 5-FU dose intensity for patients entered at or above 1750 mg/m²/day in this trial was similar at PALA doses ≤1266 mg/m², we have selected 1266 mg/m² for future studies.

INTRODUCTION

PALA² is a potent transition state analog inhibitor of ACTase (EC 2.1.3.2), the enzyme involved in the second step of de novo pyrimidine biosynthesis (1, 2). PALA was selected for clinical development based on its impressive preclinical activity as a single agent in murine solid tumors. Unfortunately, PALA given alone was clinically inactive in both leukemias and solid tumors (2). Interest then shifted to clinical application of PALA as a biochemical modulator of other antimetabolites. In several preclinical models, pretreatment with PALA enhanced the cytotoxicity of 5-FU by several mechanisms (2–8). PALA-mediated depletion of UTP and CTP pools results in increased Urd/Cyd kinase activity through decreased feedback inhibition and, hence, may lead to increased formation of fluorouridine monophosphate. Blockade by PALA of the de novo pyrimidine pathway leads to decreased orotate formation; the resulting increased PRPP levels favor the direct synthesis of fluorouridine monophosphate from 5-FU by orotate phosphoribosyltransferase. Increased formation of 5-FU ribonucleotides coupled with decreased competing substrates increases fluorouridine triphosphate incorporation into RNA. Depletion of UDP and CDP by PALA results in decreased dUMP pools and, hence, less competition with dUDP for binding to thymidylate synthase. Finally, decreased dCTP pools cause further inhibition of DNA synthesis beyond that resulting from the dUMP-mediated blockade of thymidylate synthase and subsequent dTTP pool depletion. Thus, PALA may potentiate both the RNA-directed and DNA-directed toxicities of 5-FU.

Casper et al. (9) conducted a Phase I study of PALA given 24 h prior to i.v. bolus 5-FU. The biochemical effects of PALA were monitored using a surrogate normal tissue end point: the effect on pyrazofurin-mediated urinary excretion of orotic acid and orotidine. Because 250 mg/m² PALA was associated with a biochemical effect and allowed administration of full-dose bolus 5-FU, it was selected for subsequent clinical studies. Ardalan et al. (10) combined 250 mg/m² PALA with a 24-h infusion of 5-FU starting 24 h after the PALA dose; treatment was repeated weekly (10). The recommended dose of 5-FU with or without 250 mg/m² PALA was 2600 mg/m²/week. A 40% response rate was noted in 28 previously untreated patients with advanced gastrointestinal adenocarcinoma (10). Phase II studies confirmed the activity of this regimen in colorectal cancer (43% response rate in 37 patients), but the results have been less encouraging in patients with advanced pancreatic cancer (14% response rate in 35 patients) (11, 12). The weekly schedule of low-dose PALA followed 24 h later by 24 h infusional 5-FU is currently being compared to the same schedule of 5-FU alone and to other 5-FU-modulated regimens in two Phase III trials by the Southwest Oncology Group and the Eastern Cooperative Oncology Group.

Preclinical and clinical studies have shown improved anticancer activity with the addition of LCV to 5-FU (16–18). The potential for PALA to reduce competing dUMP pools and thereby potentiate inhibition of thymidylate synthase by dFdUMP suggests that the combination...
tion of PALA, 5-FU, and LCV may be complementary. Since 5-FU has a very short plasma half-life, infusional schedules provide a means of maintaining 5-FU plasma exposure for prolonged periods. As the duration of infusion increases, the probability that a given tumor cell will enter the synthetic phase of the cell cycle during drug exposure also increases. Preclinical studies confirm a schedule-dependent cytotoxicity of 5-FU, with better antitumor effects when the duration of exposure exceeds 24 h (13–16). Preclinical studies also suggest that continuous exposure to LCV for ≥24 h favors the formation of intra-cellular reduced folate polyglutamates, which have prolonged intra-cellular retention and a greater capacity to participate in ternary complex formation compared with monoglutamate counterparts (19–21). Therefore, we wished to test the feasibility of combining these three strategies to potentiate 5-FU cytotoxicity. Our approach in the present study was to first determine the highest tolerated dose of 5-FU and LCV given as a concurrent 72-h i.v. infusion.

Biochemical effects of PALA are first evident within several hours; pyrimidine nucleotide pool depletion and PRPP accumulation may become more pronounced during a 12- to 24-h period. The duration of these biochemical effects varies according to the type of host and tumor tissue studied; in general, sensitive tissues show persistent enzyme inhibition for many days following PALA administration, whereas more rapid recovery is noted in less sensitive tissues. Some preclinical studies in mice bearing PALA-sensitive murine solid tumors such as Lewis lung carcinoma and spontaneous breast tumor suggested that subtherapeutic doses of PALA may selectively produce depletion of Urd and Cyd nucleotide pools in tumor tissue (7, 22). Other preclinical studies, however, documented a heterogeneity in the in vitro and in vivo sensitivity of various cancer cell lines to PALA-mediated inhibition of ACTase (2, 23–25). ACTase activity appeared to correlate with the proliferative rate of the tumor and host tissue. Less sensitive tumors tended to have higher ACTase levels, and higher concentrations of PALA were required for biochemical effects to be evident. Furthermore, ACTase activity in many tumor tissues was much higher compared to normal tissues (26). Moore et al. (27) reported the change in ACTase activity in serial tumor biopsies taken from 16 patients at baseline and again following PALA treatment (administered on one of several schedules). Baseline ACTase activity varied by 4-fold among tumor specimens. Sixteen to 24 h after the initial dose of 1000, 3600, or 6000 mg/m² PALA, ACTase was inhibited by 17% (n = 1), 47% (n = 1), and 75% (n = 2), respectively (27). Inhibition of ACTase was accompanied by changes in UTP pools: after 1000 mg/m² and 5000–6000 mg/m² PALA, UTP pools were decreased by 16% (n = 1) and 43% (n = 3), respectively (40). The only specimens that showed a >60% decrease were from patients having low initial ACTase activities who were treated with ≥3600 mg/m² PALA doses. The authors concluded that inhibition of ACTase by PALA and the ensuing depletion of pyrimidine nucleotides in human tumors occurred in a dose-dependent manner.

These preclinical and clinical observations argue that it may be an oversimplification to assume that a fixed, low dose of PALA would be capable of inhibiting ACTase in most clinical tumors. In the second part of the study, we, therefore, planned to escalate the dose of PALA given 24 h prior to the start of the 72-h infusion of 5-FU/LCV. The 5-FU dose selected for this latter part of the trial would be one dose level below that associated with unacceptable, dose-limiting toxicity during cycle 1 in more than one-third of patients. We measured 5-FU pharmacokinetics, and the biochemical effects of PALA were examined using two different assays using surrogate normal tissues: a direct assay of ACTase activity in peripheral blood mononuclear cells and an indirect assay measuring changes in plasma Urd. Because of interpatient variability, each patient served as his or her own control; within a matched patient cycle, the pre- and posttreatment samples were drawn at the same time of day. The results of this study are contained in this report.

MATERIALS AND METHODS

Materials. [14C]Carbamoyl phosphate, dilithium salt (17.6 mCi/mmol), was obtained as a crystalline solid, 98% pure, from NEN Research Products (DuPont Co., Wilmington, DE) and was stored at −70°C. Immediately prior to daily use of [14C]carbamoyl phosphate, an aliquot of the crystalline solid was diluted in buffer (50 mM Tris–HCl, pH 8.0) and placed on ice; the radioactivity was determined, and the aqueous solution was frozen on dry ice until it was added to the reaction mixture. Carbamoyl phosphate, carbamoyl aspartate, dihydroorotate, orotic acid, oroticine, and all nucleotides were obtained from Sigma Chemical Co. (St. Louis, MO). Phosphate-buffered saline (1X, pH 7.4) was obtained from Biofluids, Inc. (Rockville, MD). Gelman Acrodisc® filters (LC13 polyvinylidifluoride, 0.45 μm) were obtained from PGC Sciences (Gaithersburg, MD). PALA was provided by the Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute. 5-FU was obtained from commercial sources.

Eligibility. This study was activated in September 1989 and closed in March 1992 after accruing 50 patients. Patients with adenocarcinoma arising in the gastrointestinal tract were required to have an Eastern Cooperative Oncology Group performance status ≥2, a leukocyte count >4000/μl, a platelet count >100,000/μl, serum bilirubin <2.0 mg/dl, serum glumatic-oxaloacetic transaminase <4-fold normal, and serum creatinine <2.0 mg/dl. Patients must have received no more than one prior chemotherapy regimen for metastatic disease. Prior 5-FU treatment, but not 5-FU/LCV, was permitted. Patients must have recovered from prior surgery, chemotherapy, and radiotherapy. The primary end point of the study was documentation of the tolerability of the regimen; thus, measurable or evaluable disease was not required. This study had the approval of the Institutional Review Board, and all patients gave written informed consent.

Treatment Plan. In the initial part of the study, cohorts of three patients were entered at each 5-FU dose level. All patients received 500 mg/m² LCV, i.v., over 30 min as a a loading dose which was then immediately followed by a 72-h concurrent continuous i.v. infusion of 500 mg/m²/day 5-FU/LCV mixed with 5-FU. Heparin (10 units/ml) was added to the infusion solution. Patients were instructed to flush their central access lines every 8 h during the 5-FU/LCV infusion to prevent possible catheter blockage caused by drug precipitation. The starting dose of 5-FU was 1150 mg/m²/day, 50% of the recommended dose of 5-FU given as a 72-h infusion without LCV (28). Because of the possibility for increased toxicity with the addition of LCV, we chose a conservative dose escalation scheme for 5-FU, and proceeded in 15% increments (mg/m²/day): 1150, 1325, 1525, 1750, 2000, 2300, 2650, 3050, 3500. Cycles were repeated at 21-day intervals provided that the absolute granulocyte count had recovered to ≥1500/μl, the platelet count was ≥80,000/μl, and all nonhematological toxicity had resolved.

The daily 5-FU dose was reduced 15% for grade 3 or worse nonhematological toxicity, a granulocyte nadir <500/μl, or a platelet nadir <25,000/μl. The dose of LCV was not modified. 5-FU dose escalations would cease when an absolute granulocyte nadir <500/μl or platelet nadir <25,000/μl and/or grade 3 or 4 nonhematological toxicity occurred during the initial cycle in more than one-third of patients treated at that dose level.

We allowed patients to escalate their dose of 5-FU according to individual tolerance and considered grade 2 nonhematological toxicity and grade 3 hematological toxicity an acceptable target degree of toxicity for our patients. The 5-FU dose escalation scheme for individual patients was more conservative that that used for dose-escalation between patient cohorts. Individual patients treated at a given 5-FU dose level were allowed to receive a 15% escalation of 5-FU in the subsequent cycle provided that the absolute granulocyte count nadir was ≥1200/μl, the platelet nadir was ≥60,000/μl, and nonhematological toxicity was equal to or less than grade 1 in severity. When patients reached an acceptable dose of 5-FU with LCV, they received PALA at a fixed dose of 250 mg/m²/day i.v. over 30 min 24 h prior to the 5-FU/LCV infusion in subsequent cycles.

In the second part of the study, escalating PALA doses were given 24 h prior to starting the loading dose of 5-FU/LCV followed by the concurrent 72 h infusion of 500 mg/m²/day LCV plus 2000 mg/m²/day 5-FU (defined in the first part of...
the study to be one dose level below that associated with dose-limiting toxicity during cycle one in more than one-third of patients). The PALA dose was increased by 50% increments in patient cohorts as follows (mg/m²): 250, 375, 562, 844, 1266, 1899, 2848. Dose escalation of PALA was to be stopped either if dose-limiting toxicity occurred during the initial cycle or if a treatment delay of >2 weeks was necessary in more than one-third of patients treated at that level. Each patient received a fixed dose of PALA but could receive a 5-FU dose escalation if clinical toxicity was minimal.

Patients continued to receive therapy until disease progression. Standard response criteria were used as previously described (29). Time to treatment failure was computed actuarially using the Kaplan-Meier method beginning with the on-study date (30).

After assessment of the biochemical data, the protocol was amended to administer 1266 mg/m² PALA i.v. immediately prior to the loading dose of LCV and the 72-h infusion of 5-FU (1750 mg/m²/day) and LCV. Biochemical information concerning the effect of 1266 mg/m² on ACTase activity from three patients treated with this amended regimen are included in the results, but they are not included in the analysis of toxicity or response.

Effects of PALA on ACTase Activity in Peripheral Blood Mononuclear Cells and Tumor Tissue. Heparinized peripheral blood samples were obtained prior to and 24 and 96 h after PALA. Additional samples were collected in some patients at 4, 48, and 72 h after PALA. PBMCs were isolated using differential centrifugation in lymphocyte separation medium according to a modification of a published method as recommended by the manufacturer (Organon Teknika, Durham, NC) (31). The PBMCs were then suspended in 1 ml of phosphate-buffered saline; an aliquot was counted by hemacytometer, and the remaining cells were then centrifuged at 400 x g at 4°C. The supernatant was then gently aspirated, and the cell pellet was stored at -70°C until analysis. The median number of PBMCs isolated from 10 ml of blood was 10 million (range, 1-34 million).

To measure ACTase activity, we developed a direct HPLC assay using [14C]carbamoyl phosphate and excess cold aspartate which allowed the product of the reaction, [14C]carbamoyl aspartate, to be readily separated from the substrate as well as distal metabolites (32). In preliminary experiments, we optimized the conditions for this cytosolic assay to improve its sensitivity to detecting inhibition of ACTase by PALA (32). The Km of aspartate (in the presence of excess carbamoyl phosphate) was 1.9 mM. Because PALA is a competitive inhibitor with respect to carbamoyl phosphate, we wished to use a relatively low concentration of carbamoyl phosphate in order to maximize the assay sensitivity to PALA inhibition. Selecting a concentration of carbamoyl phosphate which approximated its Km as determined by this assay seemed reasonable, because it should be in the range likely to be found intracellularly. The Km of carbamoyl phosphate in the presence of 5 mM aspartate was determined in five separate experiments; the mean value was 59 µM, and we selected 50 µM carbamoyl phosphate for the remainder of the study.

The cell pellet was brought up in 50 µL of 50 mM Tris-HCl (pH 8.0) (about 5-fold the volume of the cell pellet); after sonication, the volume of cytosol recovered was recorded. Thirty µL of supernatant was mixed in a total assay volume of 40 µL containing 2 mM aspartate. The proportion of cytosol used in the final assay mixture was similar for baseline and post-PALA samples, 50 ± 9 (mean ± SD) and 50 ± 8% for 0 and 24 h, respectively. The reaction was started by the addition of approximately 77,500 dpm [14C]carbamoyl phosphate (final concentration, 50 µM), and the reactants were incubated in a 37°C water bath. Aliquots were taken at intervals from 2 to 10 min, and the reaction was quenched by adding 500 µL 1 M ammonium phosphate, pH 3.0 (the initial mobile phase), followed by immediate placement of the tubes on dry ice.

The samples were then stored at -70°C until analysis.

HPLC Assay. A Waters analytical HPLC system (Millipore Corp., Marlborough, MA) with on-line liquid scintillation flow detector (Flo-One Beta, Radiomatic, Tampa, FL) was used (33). An SAX Radial-Pak column (Waters) was developed using two buffers as the mobile phase: buffer A was 0.001 M ammonium phosphate, pH 3.0, and buffer B was 0.375 M ammonium phosphate, pH 4.5. An isotropic gradient of 100% buffer A was run at 2 ml/min for 3 min; the following linear gradients were then run: to 90% A/10% B over 17 min, to 80% A/20% buffer B over 10 min, to 70% A/30% B over 8 min, and then to 100% B over 4 min. The column was then allowed to equilibrate for 10 min with initial conditions before the next run. An aliquot of sample was filtered through a Gelman Acrodisc and was injected along with cold standards into the HPLC system. UV absorbance was monitored between 200 nm and 280 nm; dihydropyrimidines and carbamoyl aspartate standards were detected at 200–210 nm; the remaining pyrimidine nucleotides and nucleotides were monitored at 260 nm. The retention times were as follows: dihydroorotate/ orotate/oroside, 14 min; carbamoyl aspartate, 27 min; carbamoyl phosphate, 36 min.

Enzyme activity was determined from the linear portion of the curve, and was expressed as pmol metabolites formed/min/million nucleated cells. In some cases in which a cell count was not available, the activities in the baseline and post-PALA samples were expressed as pmol/min/mg protein. Protein was determined by the method of Bradford (34). The Jonckheere test was used to compare the trends in distribution of ACTase activity values following PALA therapy as a function of increasing PALA dose (35). The Mantel test was used to identify whether there was a trend in the proportion of cycles in which ACTase activity was inhibited by at least 50% as a function of PALA dose (36).

Effect of PALA on Plasma Urd Levels. Five ml of peripheral venous blood were collected in a heparinized tube prior to and 24, 48, 72, and 96 h after PALA treatment. Plasma Urd levels were measured by a previously published HPLC assay (37, 38).

Pharmacokinetics of 5-FU. Venous samples were collected in heparinized tubes pretreatment and again at either 24, 48, or 72 h after 5-FU/LCV infusion was started but prior to termination of the 5-FU/LCV infusion. The samples were obtained between 10:00 am and 4:00 pm. Samples were placed on ice immediately and spun at 800 x g at 4°C for 10 min; the plasma was then separated and frozen at -70°C until analysis. The samples were extracted and analyzed by reversed-phase HPLC as previously described (29). Clearance was calculated by dividing the 5-FU dose rate by the Cpm.

RESULTS

Fifty patients were entered into study (Table 1). One patient withdrew after a single dose of 250 mg/m² PALA (for geographical reasons); he is not considered evaluable for either toxicity or response.

Toxicity. In the initial part of the study, cohorts of 3–5 patients were entered at escalating dose levels of 5-FU starting at 1150 mg/m²/day with concurrent high-dose infusional LCV. The dose of 5-FU was escalated in each individual according to tolerance (grade 2 nonhematological toxicity and/or grade 3 hematological toxicity was considered acceptable), and then 250 mg/m² PALA was added the next cycle 24 h prior to the start of 5-FU/LCV. Dose-limiting mucositis and myelosuppression occurred in 3 of 5 patients entered at 2300 mg/m²/day 5-FU during their initial cycle (Table 2). Five patients entered at 5-FU dose levels ≤2000 mg/m²/day escalated to ≥2300 mg/m²/day before reaching dose-limiting toxicity; 4 of these tolerated 2300 mg/m²/day 5-FU. Therefore, 4 of 10 patients ultimately experienced dose-limiting toxicity while receiving 2300 mg/m²/day 5-FU with high-dose LCV. The worst degree of toxicity across all cycles is shown in Table 3 according to the entry dose of 5-FU.

Four patients entered during the initial phase of the trial had disease progression and were taken off study before receiving PALA. Fifteen matched cycles are available in which the same dose of 5-FU/LCV was administered and 250 mg/m² PALA was given during the subse-

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Total no. patients</th>
<th>Median age (yr; range)</th>
<th>Performance status</th>
<th>Prior chemotherapy</th>
<th>Prior radiotherapy</th>
<th>M/F</th>
<th>Prior chemotherapy</th>
<th>Prior radiotherapy</th>
<th>Median no. cycles</th>
<th>Median duration prior chemotherapy</th>
<th>Median duration prior radiotherapy</th>
<th>Site of primary disease</th>
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<tr>
<td>50</td>
<td>59.5 (34–78)</td>
<td>15 (30%)</td>
<td>24 (48%)</td>
<td>11 (22%)</td>
<td>31/19 (62/38%)</td>
<td>26 (52%)</td>
<td>2 (4%)</td>
<td>24 (48%)</td>
<td>13 (26%)</td>
<td></td>
<td>30 (60%)</td>
</tr>
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</table>
PHASE I TRIAL OF PALA WITH HIGH-DOSE I.V. 5-FU/LCV

Table 2 Toxicity during the initial cycle

<table>
<thead>
<tr>
<th>Dose</th>
<th>No. of patients</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Platelet nadir (x10^9/μl)</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Median Range</th>
<th>Median Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU (mg/m²/day)</td>
<td>72-h infusion of 5-FU with 500 mg/m²/day LCV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1150–1525</td>
<td>9</td>
<td>2 (22%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4028</td>
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<td>1750</td>
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<td>3 (75%)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2405</td>
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</tr>
<tr>
<td>2300</td>
<td>5</td>
<td>3 (60%)</td>
<td>2 (40%)</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>404</td>
<td>22–3619</td>
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<td>PALA 24 h prior to 72-h infusion of 5-FU (2000 mg/m²-day) with 500 mg/m²/day LCV</td>
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<tr>
<td>PALA (mg/m²)</td>
<td>72-h infusion of 5-FU-LCV</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1325</td>
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<td>0</td>
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<td>1266</td>
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<td>6 (75%)</td>
<td>0</td>
<td>4 (50%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>2 (33%)</td>
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<td>0</td>
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<td>3</td>
<td>1 (33%)</td>
<td>2 (66%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>836</td>
<td>32–2009</td>
</tr>
</tbody>
</table>

The incidence and severity of mucositis, diarrhea, and neutropenia were comparable in these matched cycles, indicating that the 250-mg/m² dose of PALA did not enhance the toxicity of 5-FU (data not shown).

Because the degree of toxicity was considered to be unacceptable with 2300 mg/m²/day 5-FU, the 2000-mg/m²/day 5-FU dose level was selected for the second part of the study. Separate patient cohorts were entered at escalating doses of PALA beginning the first treatment cycle. Dose-limiting toxicity was not observed during the initial cycle until the 2848-mg/m² PALA dose level (Table 2). Severe and life-threatening mucositis occurred in 2 of 3 patients; one of these patients also developed grade 4 pancytopenia and died on day 19 with fever, probable pulmonary embolus, and hypotension.

Only 2 of 10 patients entered at PALA doses of 375–844 mg/m² with 2000 mg/m² 5-FU were able to tolerate a higher 5-FU dose. Of 14 patients entered at 1266 and 1899 mg/m² PALA, only one patient received a 5-FU dose escalation to 2300 mg/m²/day. This patient entered at escalating doses of PALA beginning the first treatment cycle and thus takes into account cumulative dose reductions and treatment delays. For patients entered at or above 1750 mg/m²/day 5-FU, the delivered 5-FU dose intensity was similar with PALA doses ranging from 250 to 1266 mg/m². Patients treated with 1899 mg/m² PALA ultimately required a 5-FU dose reduction. The 5-FU dose intensity indicates the total amount of 5-FU delivered across all cycles of therapy and thus takes into account cumulative dose reductions and treatment delays.

**Response Rate.** Assessment of response was not the primary end point of this study, and measurable disease was not required. Twenty-five assessable patients had not received prior chemotherapy (colorectal, 9; gastric, 3; pancreas, 13). Four of these patients had nonevaluable, nonevaluable disease. Three of these nonevaluable patients had pancreatic cancer and remained stable while receiving therapy for 9.5, 13.5, and 15.5 months; one patient with gastric cancer withdrew after three cycles because of toxicity. Among 21 patients with either evaluable, nonevaluable disease. Three of these nonevaluable patients had colorectal cancer (33%), and one each had gastric (33%) or pancreatic cancer (8%). Although CEA was similar with PALA doses ranging from 250 to 1266 mg/m². Patients treated with 1899 and 2848 mg/m² PALA, however, received a significantly lower 5-FU dose intensity (Fig. 1).
levels were not used as a response criterion, the following values were noted. The median CEA value pretreatment in 23 patients was 15.9 units/ml (range, 0.7–150 units/ml, n = 24), and the lowest CEA value during therapy was a median of 4.4 units/ml (range, 0.6–663 units/ml); 8 patients (35%) had a decrease in their CEA value by >50%. With a median potential follow-up of 19 months, the median time to treatment failure in 25 patients with no prior chemotherapy was 6.4 months. The median time to treatment failure was 9.4 months and 5.3 months in patients with colorectal and pancreatic cancer, respectively.

One partial response was observed among 24 patients who had received prior chemotherapy (4%). The median CEA value pretreatment in 14 patients was 20 units/ml (range, 2.3–1185 units/ml), and the lowest CEA value during therapy was a median of 14.8 units/ml (range, 3.0–393 units/ml). The median time to treatment failure was 2.2 months.

5-FU Steady-State Plasma Concentrations. The effect of PALA on 5-FU C_{ps} was examined in two ways. We first compared the C_{ps} of 5-FU at each dose level in cycles without and with PALA (combining all PALA doses). PALA did not significantly affect 5-FU plasma levels (P = 0.68 and 0.37 at 1750 and 2000 mg/m² 5-FU, respectively, Wilcoxon rank sum test). We also analyzed the C_{ps} of 5-FU in patients treated with 2000 mg/m² according to PALA doses of 0 to 2848 mg/m². There was no significant trend in 5-FU plasma levels as a function of PALA dose (P = 0.19, Jonckheere’s test for trend). Therefore, all data for each PALA level was combined; the trend was highly significant for a linear increase in mean 5-FU C_{ps} as a function of increasing 5-FU dose (P = 0.0001, Fig. 2). Conversely, the clearance of 5-FU decreased with increasing 5-FU dose. At 2000 mg/m²/day, the mean 5-FU plasma level was 6.5 ± 0.9 μM, and the average clearance was 2651 ± 324 ml/min/m². The one patient with fatal toxicity treated at the 2000-mg/m² 5-FU dose level with 2848 mg/m² PALA had a 5-FU plasma level of 48 μM; the patient’s calculated clearance was 223 ml/min/m², indicating profound impairment. The average clearance for 19 patient cycles at 1150–1525 mg/m² 5-FU was 3011 ± 356 ml/min/m² but was 2247 ± 443 ml/min/m² in 9 cycles at 2645–3041 mg/m². No correlation was present between 5-FU C_{ps} and either response or time to treatment failure.

We next examined the association of 5-FU C_{ps} with the severity of toxicity in patients treated with PALA doses ≤1266 mg/m², which did not appear to modify 5-FU toxicity. Although there was variability in individual patient tolerance, 9 μM appeared to be a threshold above which the incidence of serious toxicity increased substantially. In 91 cycles with a 5-FU C_{ps} ≤8.9 μM, the incidence of severe or life-threatening gastrointestinal toxicity, granulocytopenia (<1000/μL) and thrombocytopenia (<50,000/μL) was 1, 14, and 0%, respectively. The incidence of serious toxicities was significantly higher in 22 cycles with a 5-FU C_{ps} ≥9.0: life-threatening gastrointestinal toxicity, 14% (P = 0.02); granulocytopenia, 41% (P = 0.01); thrombocytopenia, 14% (P = 0.007, Fisher test).

Effect of PALA on ACTase Activity. PBMCs were isolated from 28 patients pretreatment and 24 and 96 h after PALA. While the time of sampling varied from cycle to cycle, all samples within a matched patient set were obtained at the same time of day; furthermore, determination of ACTase activity for each matched set of samples was performed at the same time. Our HPLC assay allowed measurement of ACTase activity in 100% of pretreatment patient samples. The average baseline ACTase activity in PBMCs isolated on 65 occasions was 31.7 ± 2.1 pmol/min/10⁶ cells (range, 7.6–88).

Fig. 3a shows the absolute ACTase activity in cytosol from PBMCs obtained at baseline and 24 h following PALA treatment in 58 matched patient cycles. The variation in the baseline ACTase activity between the PALA doses was not statistically significant (P = 0.18) but emphasizes the importance of comparing posttreatment enzyme activity to each patient’s own baseline obtained at the same time of day. ACTase activity was similar at baseline and 24 h in patients treated with 250 mg/m². A dose-dependent decrease in ACTase activity at 24 h was apparent (P = 0.011, Jonckheere’s test for trend); an appreciable decrease from baseline was seen with a ≥1266-mg/m² dose of PALA.

Table 4 presents the median ACTase activity at 24 and 96 h as a percentage of the baseline value using data from all available cycles. With PALA doses ≤844 mg/m², ACTase was inhibited by at least 50% in only one-third of the cycles. With 1266 and 1899 mg/m² PALA, ACTase was inhibited by ≥50% in more than half of the cycles. With
whether a >50% decrease compared to baseline was seen (Table 4). Similarly, no increase in plasma Urd levels after treatment across PALA levels at 24, 72, and 96 h (P = 0.45-0.50). ACTase activity was expressed in the baseline and posttreatment sample as pmol/min/mg protein, and the % baseline was then calculated. Within a matched set, all samples were obtained at the same time of day. In 6 cases in which cell counts were not available, ACTase activity was expressed in the baseline and posttreatment sample as pmol/min/mg protein, and the % baseline was then calculated.

Fig. 3. Dose-dependent effect of PALA on ACTase activity in PBMCs. ACTase activity was determined by analyzing the conversion of [14C]carbamoyl phosphate to [14C]-carbamoyl aspartate by union-exchange HPLC as described in “Materials and Methods.” Within a matched set, the baseline and posttreatment samples were obtained at the same time of day. a, data from paired samples from 58 patient cycles at 0 and 24 h. The numbers of cycles at each PALA dose level (mg/m²) are as follows: 250, 6; 375-562, 6; 844, 4; 1266, 23; 1899, 8; 2848, 5. ACTase activity at 96 h was not significantly different from baseline values, and there was no trend for greater inhibition of ACTase activity with increasing PALA dose. Columns, means; bars, ±SEM.

Table 4 Dose-dependent inhibition of ACTase activity in PBMCs by PALA

<table>
<thead>
<tr>
<th>PALA (mg/m²)</th>
<th>250</th>
<th>375-844</th>
<th>1266</th>
<th>1899</th>
<th>2848</th>
<th>P₂(test)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. cycles</td>
<td>7 (3)</td>
<td>10 (6)</td>
<td>26 (13)</td>
<td>15 (6)</td>
<td>5 (3)</td>
<td></td>
</tr>
<tr>
<td>% 0 h ACTase</td>
<td>66</td>
<td>56</td>
<td>44</td>
<td>46</td>
<td>28</td>
<td>0.01 (F²)</td>
</tr>
<tr>
<td>Range (%)</td>
<td>35-189</td>
<td>34-97</td>
<td>13-366</td>
<td>19-142</td>
<td>20-35</td>
<td></td>
</tr>
<tr>
<td>≥50% decrease (%)</td>
<td>29</td>
<td>30</td>
<td>62</td>
<td>53</td>
<td>100</td>
<td>0.01 (M²)</td>
</tr>
<tr>
<td>96 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. cycles</td>
<td>2 (2)</td>
<td>6 (4)</td>
<td>8 (5)</td>
<td>9 (5)</td>
<td>5 (3)</td>
<td></td>
</tr>
<tr>
<td>% 0 h ACTase</td>
<td>120</td>
<td>80</td>
<td>71</td>
<td>63</td>
<td>61</td>
<td>0.33 (J)</td>
</tr>
<tr>
<td>act:median</td>
<td>67-177</td>
<td>45-128</td>
<td>37-240</td>
<td>28-101</td>
<td>38-182</td>
<td></td>
</tr>
<tr>
<td>≥50% decrease (%)</td>
<td>0</td>
<td>20</td>
<td>25</td>
<td>22</td>
<td>40</td>
<td>0.34 (M)</td>
</tr>
</tbody>
</table>

ACTase activity was determined in cytosol isolated from PBMCs obtained at 0, 24, and 96 h by HPLC analysis as described in “Materials and Methods.” Within a matched set, all samples were obtained at the same time of day. In 6 cases in which cell counts were not available, ACTase activity was expressed in the baseline and posttreatment sample as pmol/min/mg protein, and the % baseline was then calculated.

Statistical analysis was by either the Jonckheere (J) or Mantel (M) test for trend.

Table 5 Non-dose-dependent decrease in plasma Urd levels during PALA treatment

<table>
<thead>
<tr>
<th>PALA (mg/m²)</th>
<th>250</th>
<th>375-844</th>
<th>1266</th>
<th>1899</th>
<th>2848</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. cycles</td>
<td>7 (4)</td>
<td>6 (4)</td>
<td>15 (10)</td>
<td>11 (6)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>% 0 h Urd²:median</td>
<td>60</td>
<td>66</td>
<td>71</td>
<td>67</td>
<td>56</td>
</tr>
<tr>
<td>Range (%)</td>
<td>38-111</td>
<td>57-130</td>
<td>38-247</td>
<td>27-204</td>
<td>27-74</td>
</tr>
<tr>
<td>≥30% decrease (%)</td>
<td>71</td>
<td>67</td>
<td>47</td>
<td>82</td>
<td>80</td>
</tr>
<tr>
<td>96 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. cycles</td>
<td>1 (1)</td>
<td>3 (3)</td>
<td>7 (6)</td>
<td>5 (4)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>% 0 h Urd:median</td>
<td>38</td>
<td>53</td>
<td>61</td>
<td>54</td>
<td>65</td>
</tr>
<tr>
<td>Range (%)</td>
<td>46-74</td>
<td>12-132</td>
<td>29-72</td>
<td>21-67</td>
<td></td>
</tr>
<tr>
<td>≥30% decrease (%)</td>
<td>100</td>
<td>66</td>
<td>71</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

Plasma Urd levels were determined by HPLC analysis in samples obtained pretreatment and at 24-h intervals after PALA treatment. Samples within a matched patient set were obtained at the same time of day.
PALA dose levels and persisted for at least 96 h. Karle et al. (38) activity, a modest decrease in plasma Urd levels was evident at all ward baseline by 96 h in most patients. Ultimately required a PALA dose reduction because of mucocutaneous unacceptable. Similarly, most patients treated with 1899 mg/m2 PALA PBMCs was observed with 2848 mg/m2 PALA, the toxicity was failed to appreciably inhibit ACTase activity in PBMCs at 24 h in most mg/m2. Compared to each patient's own baseline, PALA >1899 mg/m2 ranging from 250 to 2848 mg/m2, and biochemical assessment was dose leucovorin. Therefore, we selected 2000 mg/m2/day 5-FU for the incurred during the initial cycle with 2300 mg/m2/day 5-FU and high- ^1266 mg/m2, but it was significantly lower with PALA >1899 cycles (21%) and 6 of 20 cycles (30%) at 24 and 96 h, respectively.

**DISCUSSION**

Forty-nine assessable patients with adenocarcinoma of the gastrointestinal tract were treated in this trial. Dose-limiting toxicity occurred during the initial cycle with 2300 mg/m2/day 5-FU and high-dose leucovorin. Therefore, we selected 2000 mg/m2/day 5-FU for the second part of the trial. Patients were treated at seven PALA doses ranging from 250 to 2848 mg/m2, and biochemical assessment was performed at each dose using two surrogate end points: target enzyme activity in PBMCs and plasma Urd levels. The delivered 5-FU dose intensity across all cycles of therapy for patients treated with ñ1750 mg/m2/day 5-FU was not significantly different with PALA doses ñ1266 mg/m2, but it was significantly lower with PALA ñ1899 mg/m2. Compared to each patient's own baseline, PALA ñ844 mg/m2 failed to appreciably inhibit ACTase activity in PBMCs at 24 h in most patients. More consistent inhibition was seen with ñ1266 mg/m2 PALA. Although more profound inhibition of ACTase activity in PBMCs was observed with 2848 mg/m2 PALA, the toxicity was unacceptable. Similarly, most patients treated with 1899 mg/m2 PALA ultimately required a PALA dose reduction because of mucocutaneous toxicity that did not improve despite 5-FU dose reduction. Even with the highest PALA doses, however, ACTase activity had returned toward baseline by 96 h in most patients.

In contrast to the dose-dependent effects of PALA on ACTase activity, a modest decrease in plasma Urd levels was evident at all PALA dose levels and persisted for at least 96 h. Karle et al. (38) reported a decrease in plasma Urd of 7–65% 24 h after the initial dose of PALA (1000 to 2000 mg/m2/day daily for 5 days); maximum decreases by day 5 were 37–85%. Chan et al. (39) found that decreases in plasma Urd levels were evident within 4 h of PALA administration. Previous studies using the isolated rat liver perfusion model have shown that the net export of Urd from the liver increased as the concentration of Urd in the perfusate decreased, thus documenting the pivotal role of the liver in regulating the concentration of circulating Urd (40, 41). Radiolabeled Urd entering the liver was extensively catabolized, with a half-life of 5–7 min; only a small portion of the radioactivity appeared as uracil nucleosides or nucleotides in liver extracts (40, 41). These results suggested that the liver appeared to degrade essentially all incoming Urd and that the hepatic pools of uracil nucleotides, formed predominantly by *de novo* synthesis, were responsible for the plasma Urd concentrations leaving the liver. The pharmacological basis of the decrease in plasma Urd levels during PALA treatment, therefore, may largely reflect decreased synthesis and export of Urd by the liver. Increased salvage of preformed Urd by various tissues may also play a role, but its contribution has not yet been established.

Under ideal circumstances it would be best to obtain paired tumor samples in patients prior to and at various intervals following PALA treatment. Since the population for this trial consisted of patients with gastrointestinal malignancies, tumor tissue was not easily accessible. Serial tumor biopsies would require repetitive invasive procedures. In an initial Phase I study, these invasive biopsies would be of no direct benefit to the patient. Therefore, we elected to examine two different surrogate endpoints because it was possible to obtain serial blood samples at various intervals following PALA therapy with minimal discomfort and risk to the patient. A complete picture of the biochemical effects of PALA would entail simultaneous measurement of target enzyme inhibition, the biochemical consequences, and evidence of modulation of 5-FU incorporation into RNA or greater inhibition of thymidylate synthase in both tumor tissue and normal host tissues. Unfortunately, it is not practical to perform multiple biochemical studies on limited clinical material.

We wished to include a direct assay of ACTase activity because the depletion of Urd nucleotide pools are a consequence of enzyme inhibition. There are limitations to the cytosolic assay of ACTase activity we used, and it may underestimate the extent of enzyme inhibition in the patient. The assay requires removal of the cells from the patient, followed by several steps to isolate the cells. Preparation of the cytosolic extract necessitates further dilution. Inhibition of ACTase by PALA is dependent on both the concentration of PALA and the concentration of the competing normal substrate, carbamoyl phosphate. We attempted to minimize the dilutional effects by resuspending the cell pellet in a small volume of buffer, 50 μl, such that cytosol constituted 75% of the final assay volume (40, 41). Previ- ously reported methodologies for measurement of cytosolic ACTase activity indicated that the proportion of cytosol used in the assay mixture represented only 17–25% of the assay volume. Saturating concentrations of carbamoyl phosphate have been used in previously reported assays. Because PALA is a competitive inhibitor with respect to carbamoyl phosphate, we reasoned that the concentration of carbamoyl phosphate should be low, to maximize the assay sensitivity to PALA inhibition, and yet be in the concentration range likely to be found intracellularly. We selected 50 μM carbamoyl phosphate for our assay, which approximates the K_m as determined in preliminary studies using our assay (32).
weekly PALA with prolonged infusional 5-FU also suggest that the 250-mg/m² dose of PALA did not produce consistent inhibition of ACTase activity in peripheral blood leukocytes (42).

The majority of current trials use the 250-mg/m² dose of PALA. Casper et al. (9) concluded that the 250-mg/m² dose of PALA was most reasonable because it was associated with biochemical effects but did not increase the toxicity of bolus 5-FU; in contrast, clinical toxicity was greater with higher PALA doses. The biochemical methodology used to select this low PALA dose (9), however, has several limitations. The assessment of inhibition of ACTase by PALA was indirect and required the administration of a second drug, pyrazofurin. Pyrazofurin produces increased serum levels and urinary excretion of orotidine and orotidyine by blocking a later enzymatic step in de novo pyrimidine synthesis (43). The study design called for a separate control group rather than having each patient serve as his/her own control, and information concerning the degree of interpatient variability was not provided. Incomplete inhibition of ACTase by PALA may not result in critical depletion of Urd nucleotide pools yet still be accompanied by an appreciable decrease in pyrazofurin-mediated urinary excretion of orotidine, thus leading to an overestimate of the biochemical activity of PALA (44). This assay also reflects a PALA effect on normal tissues and may largely reflect inhibition of de novo pyrimidine synthesis in the liver, an organ that is highly sensitive to the effects of PALA (discussed below). Despite these theoretical limitations, our data demonstrating the lack of a dose-dependent effect of PALA on plasma Urd levels, which may also reflect hepatic effects of PALA, are in agreement with the data of Casper et al.

One might then question whether the liver is a more accurate surrogate tissue than PBMCs. Preclinical data suggest that the liver may be an especially sensitive target organ to PALA-mediated inhibition of ACTase. Johnson et al. (23) reported dose-dependent hepatic toxicity with sparing of the intestinal epithelium and bone marrow following the administration of PALA by the i.p. route. Jayaram et al. (45) compared the duration of ACTase inhibition in normal organs and tumor tissue in mice treated with a single i.p. injection of 1200 mg/m² PALA. Profound and prolonged inhibition of ACTase activity was evident in hepatic tissue: 88% inhibition was noted at 12 days, whereas partial recovery to 50% of control was noted by 8 days in spleen tissue and in a PALA-sensitive tumor. Chisena et al. (46) compared inhibition of de novo pyrimidine biosynthesis in liver, intestinal, and tumor tissue using a sensitive gas chromatography/mass spectroscopy assay which can accurately quantitate incorporation of stable-label [15N]alanine into uracil nucleotides (46). Hepatic synthesis was exquisitely sensitive to 150 mg/m² PALA and was completely inhibited for up to 48 h after dosing, whereas Nettsehsen carcinoma was unaffected (45). Miller et al. (47) measured ACTase activity in several normal tissues 19–25 h following the i.v. administration of 1000 mg/m² PALA in dogs. ACTase activity was reduced to 37, 7, and 21% of baseline in intestinal mucosa, liver, and bone marrow, respectively (47). The occurrence of dose-limiting hepatic toxicity with 250 mg/m² PALA given in conjunction with weekly bolus 5-FU suggests that low-dose PALA apparently modulates the toxicity of 5-FU in hepatic tissue on this particular schedule (48).

As yet we have only limited information concerning ACTase activity in tumor tissue using our assay. ACTase activity in three patients (with pancreatic, colorectal, and adenocarcinoma of unknown primary) with malignant ascites was 17.2, 104, and 162 pmol/min/10⁶ cells. For reference, the average activity in PBMCs in our study was 32 pmol/min/10⁶ cells. Baseline ACTase activity in three patients with acute leukemia (myelogenous, one patient; prolymphocytic, 2 patients) was 144–162 pmol/min/10⁶ cells, whereas it was lower, 44 pmol/min/10⁶ cells, in a patient with chronic lymphocytic leukemia. When baseline ACTase activity in PBMCs in this study was expressed on a per mg protein basis, it was 874 ± 114 pmol/min/mg protein (range, 173–2001 pmol/min/mg protein, n = 22). The baseline ACTase activity in 10 human tumor specimens in the study by Moore et al. (27) was 882 ± 139 pmol/min/mg protein (range, 435–1750 pmol/min/mg protein). Since the methodologies for the assays differed, the ACTase activities are not strictly comparable. Nevertheless, it suggests that ACTase activity in PBMCs may be in a similar range to that reported for human tumors.

We found that patients with a daytime Cₚ₉₀ ≥9 μM had a higher incidence of serious hematological and gastrointestinal toxicity. This observation raises the possibility that pharmacokinetic monitoring after the initial 24 h of 5-FU/LCV infusion might allow dose adjustment for the remainder of the 72-h infusion in an effort to avoid prohibitive toxicity. Several investigators have previously identified a correlation between 5-FU plasma levels and clinical toxicity on infusional schedules (28, 49). A similar correlation with clinical response has not been noted in either this or previous trials (28, 49).

Because of the Phase I nature of this trial, an accurate estimate of the activity of the regimen cannot be made. We observed a 24% response rate in a mixed population of untreated patients with adenocarcinoma of the gastrointestinal tract. The overall median time to treatment failure, however, compares favorably with recently published series (18, 50, 51). The potential utility of this regimen will need to be determined in future studies.

For future studies involving a 72-h i.v. infusion of 5-FU with high-dose LCV, we recommend 2000 mg/m²/day. PALA doses ≤1266 mg/m² may safely be given 24 h prior to the start of a 72-h infusion of 2000 mg/m²/day 5-FU with high-dose LCV. We selected 1266 mg/m² PALA for future studies not only on the basis of biochemical information but also on clinical grounds because it was the highest PALA dose that did not significantly compromise the actual delivered 5-FU dose intensity in this trial. We found that inhibition of ACTase activity in PBMCs was maximal by 4 h after PALA and that ACTase activity had largely recovered by 96 h (the end of the 72-h 5-FU/LCV infusion). Preclinical studies indicated that the initial biochemical effects of PALA were evident within a few hours; with persistent enzyme inhibition, the magnitude of pyrimidine nucleotide depletion could become more profound by 24 h (2, 4, 6, 22, 23, 25). Liang et al. (5) found that a 12-h preexposure to PALA enhancedFdUMP formation, decreased competing dUMP pools, and enhanced inhibition of thymidylate synthase. Major et al. (6) reported that the elevation of PRPP pools following PALA treatment reached a maximum by 6–9 h. Both 3- and 24-h preexposures to PALA enhanced 5-FU incorporation into RNA in a human breast cancer cell line (4). With a 72-h infusion schedule, therefore, the 24-h interval between PALA and the start of the 5-FU/LCV infusion may not be necessary. We are currently exploring the feasibility of giving PALA immediately prior to the start of the 5-FU/LCV infusion.

Because of interpatient variability in the degree of ACTase inhibition following PALA, biochemical monitoring of target enzyme activity may permit more rational adjustment of the PALA dose in individual patients. An area of crucial importance is the effect of PALA on ACTase activity in malignant tissue. The development of this sensitive, direct HPLC assay of ACTase activity may facilitate such biochemical monitoring in tumor specimens from patients enrolled in future clinical trials of PALA/5-FU. The development of more sensitive laboratory methodologies will be needed to permit assessment of the ultimate target in tumor samples: modulation of 5-FU metabolism and its incorporation into RNA and inhibition of thymidylate synthase activity.
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A Phase I Study of Continuous Infusion 5-Fluorouracil plus Calcium Leucovorin in Combination with $N$-(Phosphonacetyl)-l-aspartate in Metastatic Gastrointestinal Adenocarcinoma

J. L. Grem, N. McAtee, S. M. Steinberg, et al.


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