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, the enzyme activity with increasing PALA dose was < 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 UDP 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 DNA. Depletion of UDP and CDP by PALA results in decreased dUMP pools and, hence, less competition with FdUMP for binding to thymidylate synthase. Finally, decreased dCTP pools cause further inhibition of DNA synthesis beyond that resulting from the FdUMP-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.

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2 The abbreviations used are: PALA, N-(phosphonacetyl) L-aspartate; ACTase, aspartate carbamoyltransferase; 5-FU, 5-fluorouracil; Urd, uridine; Cyd, cytidine; PRPP, phosphoribosylpyrophosphate; FdUMP, fluoro-5-uracil monophosphate; LCV, leucovorin; PBMC, peripheral blood mononuclear cell; HPLC, high-performance liquid chromatography; C₅₀₅, steady-state plasma concentration; CEA, carcinoembryonic antigen.
indirect assay measuring changes in plasma Urd. Because of interpa-
asst of ACTase activity in peripheral blood mononuclear cells and an
ined using two different assays using surrogate normal tissues: a direct
5-FU dose selected for this latter part of the trial would be one dose
given 24 h prior to the start of the 72-h infusion of 5-FU/LCV. The
capable of inhibiting ACTase in most clinical tumors. In the second
oversimplification to assume that a fixed, low dose of PALA would be
by PALA and the ensuing depletion of pyrimidine nucleotides in
level below that associated with unacceptable, dose-limiting toxicity
(27). Inhibition of ACTase was accompanied by changes in UTP
from 16 patients at baseline and again following PALA treatment
will enter the synthetic phase of the cell cycle during drug exposure
concentrations of PALA were required for biochemical effects to be
Less sensitive tumors tended to have higher ACTase levels, and higher
to correlate with the proliferative rate of the tumor and host tissue.
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
correlate with the proliferative rate of the tumor and host tissue.
Lymphoid leukemia 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 tu-
mors 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
intra- and extra-cellular distribution of pyrimidine nucleotides in cancer
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
able to inhibit 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 exam-
ined 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 interpa-
tient 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, orotidine, 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 30 patients. Patients with adenocarcinoma arising in
the gastrointestinal tract were required to have an Eastern Cooperative Oncol-
ogy Group performance status ≥2, a leukocyte count >4000/μl, a platelet
count >100,000/μl, serum bilirubin <2.0 mg/dl, serum glucosam-oxaloacetic
transaminase <4-fold normal, and serum creatinine <2.0 mg/dl. Patients must
have received no more than one prior chemotherapeutic 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 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 nonhemato-
logic 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 he-
matological toxicity an acceptable target degree of toxicity for our patients.
The 5-FU dose escalation scheme for individual patients was more conserva-
tive than 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 granu-
locyte 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 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.** Heparinated 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 hemacytometry, and the remaining cells were then centrifuged at 400 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 mM 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; dihydroorotate 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/orosidine, 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 Cpmax.

**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>
<thead>
<tr>
<th>Table 1 Patient characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. patients</td>
</tr>
<tr>
<td>Median age (y; range)</td>
</tr>
<tr>
<td>Performance status</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>Prior chemotherapy</td>
</tr>
<tr>
<td>Prior radiation therapy</td>
</tr>
<tr>
<td>Site of primary disease</td>
</tr>
<tr>
<td>Colorectal</td>
</tr>
<tr>
<td>Pancreas</td>
</tr>
<tr>
<td>Gastric</td>
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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>Muocositis</th>
<th>Diarrhea</th>
<th>Granulocyte nadir (μl)</th>
<th>Platelet nadir (X10³/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grade 2</td>
<td>Grade 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>Range</td>
<td>Median</td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td>5-FU (mg/m²/day)</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>
</tr>
<tr>
<td>1750</td>
<td>4</td>
<td>2 (50%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2000</td>
<td>4</td>
<td>3 (75%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2300</td>
<td>5</td>
<td>3 (60%)</td>
<td>2 (40%)</td>
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<tr>
<td>PALA 24 h prior to 72-h infusion of 5-FU (2000 mg/m²-day) with 500 mg/m²-day LCV</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PALA (mg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>375</td>
<td>3</td>
<td>1 (33%)</td>
<td>1 (33%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>562</td>
<td>3</td>
<td>1 (33%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>844</td>
<td>4</td>
<td>1 (25%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1266</td>
<td>6</td>
<td>6 (75%)</td>
<td>0</td>
<td>4 (50%)</td>
<td>0</td>
</tr>
<tr>
<td>1899</td>
<td>6</td>
<td>5 (63%)</td>
<td>0</td>
<td>2 (33%)</td>
<td>0</td>
</tr>
<tr>
<td>2848</td>
<td>3</td>
<td>1 (33%)</td>
<td>2 (66%)</td>
<td>0</td>
<td>0</td>
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</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 experienced moderately severe conjunctival toxicity at the higher 5-FU dose. The overall incidence of dose-limiting granulocyte toxicity occurring across all patient cycles did not appear to be related to PALA dose. Among 18 patients entered at PALA doses ranging from 375 to 1266 mg/m², grade 3–4 mucositis, diarrhea, and skin rash occurred in 22, 11, and 6%, respectively. With 1899 mg/m² PALA, however, dose-limiting mucositis and skin rash ultimately occurred in 4 of 6 patients. Ocular toxicity in the form of conjunctivitis and excessive lacrimation was seen in only 3 of 40 patients (7.5%) treated with ≤1266 mg/m² PALA but occurred in 4 of 6 patients treated with 1899 mg/m² PALA.

Table 3 Most severe degree of toxicity across all cycles of therapy

<table>
<thead>
<tr>
<th>Entry dose</th>
<th>Ps</th>
<th>Nausea/vomiting (%)</th>
<th>Muocositis (%)</th>
<th>Diarrhea (%)</th>
<th>Cutaneous (%)</th>
<th>Granulocyte (%)</th>
<th>Platelet (%)</th>
<th>Hemoglobin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU (mg/m²/day)</td>
<td></td>
<td>Grade 2</td>
<td>Grade 3</td>
<td>Grade 2</td>
<td>Grade 3</td>
<td>Grade 2</td>
<td>Grade 3</td>
<td>Grade 2</td>
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<tr>
<td>5-FU (mg/m²/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1150–1525</td>
<td>9</td>
<td>11</td>
<td>11</td>
<td>66</td>
<td>11</td>
<td>11</td>
<td>22</td>
<td>0</td>
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<tr>
<td>1750</td>
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<td>0</td>
<td>0</td>
<td>75</td>
<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td>2000</td>
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<td>0</td>
<td>100</td>
<td>0</td>
<td>50</td>
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<td>PALA 24 h prior to 72-h infusion of 5-FU (2000 mg/m²-day) with LCV starting cycle 1</td>
<td></td>
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<td></td>
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<td>PALA (mg/m²)</td>
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<td>25</td>
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<td>88</td>
<td>12</td>
<td>62</td>
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<td>25</td>
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<tr>
<td>1899</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>83</td>
<td>17</td>
<td>0</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>2848</td>
<td>3</td>
<td>0</td>
<td>33</td>
<td>33</td>
<td>66</td>
<td>33</td>
<td>0</td>
<td>33</td>
</tr>
</tbody>
</table>

Eleven patients entered in part one of the study at 5-FU doses of either 1750, 2000, or 2300 mg/m²/day received one or more cycles with 250 mg/m² PALA; 2 of these patients (18%) could not tolerate the combination of 250 mg/m² PALA with 2000 mg/m²/day 5-FU and LCV. Three of 10 patients (30%) treated with PALA doses of 375 to 844 mg/m² and 3 of 8 (38%) patients treated with 1266 mg/m² ultimately experienced dose-limiting toxicity. In contrast, 83% (5 of 6 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. 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.

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 nonmeasurable, 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 measurable (9 patients) or evaluable (12 patients) disease, 5 partial responses were noted (24%, exact 95% confidence interval 8–47%). Three of the responding patients had colorectal cancer (33%), and one each had gastric (33%) or pancreatic cancer (8%). Although CEA

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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 Cpss was examined in two ways. We first compared the Cpss 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 (P2 = 0.68 and 0.37 at 1750 and 2000 mg/m² 5-FU, respectively, Wilcoxon rank sum test). We also analyzed the Cpss 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 (P2 = 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 Cpss as a function of increasing 5-FU dose (P2 ≤ 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 apparent between 5-FU Cpss and either response or time to treatment failure.

We next examined the association of 5-FU Cpss 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 Cpss ≤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 Cpss ≥9.0: life-threatening gastrointestinal toxicity, 14% (P2 = 0.02); granulocytopenia, 41% (P2 = 0.01); thrombocytopenia, 14% (P2 = 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).

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). No significant trend was evident when the data were analyzed as to returned toward baseline by 96 h in most patients. Similarly, no the actual ACTase activity at 96 h compared to the matched baseline ACTase activity, \( P_2 = 0.02 \).

Dose level was considered (% baseline ACTase activity versus PALA dose, \( P_2 = 0.008 \); proportion of cycles with a >50% decrease in the 2848-mg/m² dose of PALA, each cycle had pronounced inhibition of ACTase. The overall trend for dose-dependent inhibition of ACTase was significant (\( P_2 = 0.005 \), Jonckheere’s test). Similarly, no data from 28 paired samples at 0 and 96 h. The overall trend for dose-dependent inhibition of ACTase was significant (\( P_2 = 0.015 \), Mantel test for trend). A similar the 2848-mg/m² dose of PALA, each cycle had pronounced inhibition of ACTase. The overall trend for dose-dependent inhibition of ACTase was significant (\( P_2 = 0.015 \), Mantel test for trend). A similar the 2848-mg/m² dose of PALA, each cycle had pronounced inhibition of ACTase. The overall trend for dose-dependent inhibition of ACTase was significant (\( P_2 = 0.015 \), Mantel test for trend). A similar the 2848-mg/m² dose of PALA, each cycle had pronounced inhibition of ACTase. The overall trend for dose-dependent inhibition of ACTase was significant (\( P_2 = 0.015 \), Mantel test for trend). A similar the 2848-mg/m² dose of PALA, each cycle had pronounced inhibition of ACTase. The overall trend for dose-dependent inhibition of ACTase was significant (\( P_2 = 0.015 \), Mantel test for trend). A similar the 2848-mg/m² dose of PALA, each cycle had pronounced inhibition of ACTase. The overall trend for dose-dependent inhibition of ACTase was significant (\( P_2 = 0.015 \), Mantel test for trend). A similar the 2848-mg/m² dose of PALA, each cycle had pronounced inhibition of ACTase. The overall trend for dose-dependent inhibition of ACTase was significant (\( P_2 = 0.015 \), Mantel test for trend). A similar the 2848-mg/m² dose of PALA, each cycle had pronounced inhibition of ACTase. The overall trend for dose-dependent inhibition of ACTase was significant (\( P_2 = 0.015 \), Mantel test for trend). A similar the 2848-mg/m² dose of PALA, each cycle had pronounced inhibition of ACTase. The overall trend for dose-dependent inhibition of ACTase was significant (\( P_2 = 0.015 \), Mantel test for trend). A similar

Because ACTase was not uniformly inhibited at 24 h with the intermediate doses of PALA, we then wished to determine whether inhibition was greater at an earlier time in 6 patients treated with 1266 mg/m² and 1 patient treated with 1899 mg/m² PALA. Since recovery of ACTase activity was apparent in most patients by 96 h, the extent of inhibition was also examined at 72 h in 6 of these patients. In each patient, maximal inhibition of ACTase activity occurred by 4 h (median, 23% of baseline; range, 7–65%). In 5 of the 7 patients, partial to complete recovery of ACTase activity had occurred by 24 h (median, 33% of baseline; range, 23–90%); at 72 h, the median ACTase activity was 51% of baseline (range, 37–92%). The proportion of cycles with ≥50% inhibition of ACTase activity was as follows: 4 h, 86%; 24 h, 71%; 72 h, 50%.

Effect of PALA on Plasma Urdidine Levels. PALA administration has been associated with a reduction in plasma Urd levels in mice and humans (37–39); decreased export of Urd from the liver to the plasma as a result of PALA-mediated inhibition of ACTase likely plays a major role, but increased salvage of Urd by other tissues may also contribute. In our patients, a modest decrease in plasma Urd levels was seen in most patient cycles at all PALA doses (Table 5);

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

<table>
<thead>
<tr>
<th>PALA dose (mg/m²)</th>
<th>250</th>
<th>375–844</th>
<th>1266</th>
<th>1899</th>
<th>2848</th>
<th>( P_2 (\text{test})^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>24 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. cycles (no. patients)</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 act:median</td>
<td>66</td>
<td>56</td>
<td>44</td>
<td>46</td>
<td>28</td>
<td>0.01 (F)</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><strong>96 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. cycles (no. patients)</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 act:median</td>
<td>120</td>
<td>80</td>
<td>71</td>
<td>63</td>
<td>61</td>
<td>0.33 (J)</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>

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**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>
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<tbody>
<tr>
<td><strong>24 h</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>No. cycles (no. patients)</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 Urd*: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><strong>96 h</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. cycles (no. patients)</td>
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<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>≥30% decrease (%)</td>
<td>100</td>
<td>66</td>
<td>71</td>
<td>80</td>
<td>100</td>
</tr>
</tbody>
</table>

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* 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
toxicity that did not improve despite 5-FU dose reduction. Even with
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/day 5-FU was not significantly different with PALA doses
intensity across all cycles of therapy for patients treated with >1750
curred during the initial cycle with 2300 mg/m²/day 5-FU and high-
^ 1266 mg/m², but it was significantly lower with PALA >1899
DISCUSSION
these changes did not closely parallel the degree of ACTase inhibi-
tion in PBMCs. Because no difference in plasma Urd levels at either
24 h (P₂ = 0.50), 72 h (P₂ = 0.45), or 96 h (P₂ = 0.45) was appar-
ent between various PALA doses, the data for all PALA doses was
combined. The average baseline plasma Urd level was 2.9 ± 0.2 μM
(range, 1.2–6.2 μM). A significant decrease in plasma Urd levels was
evident at all times up to 96 h after PALA treatment (Fig. 4). A
≥50% decline in Urd levels, however, was evident in only 9 of 43
cycles (21%) and 6 of 20 cycles (30%) at 24 and 96 h, respectively.

Fig. 4. Non-dose-dependent effect of PALA on plasma uridine levels. Plasma Urd
levels were determined pretreatment and at 24-h intervals following PALA treat-
ment. Samples within a matched set were drawn at the same time of day and were subjected to
HPLC analysis. There was no apparent trend for decreasing Urd levels posttreatment with
increasing PALA dose, therefore, data from all PALA doses are combined. The numbers of
paired patient samples at each time are as follows: 24 h, 43; 48 h, 8; 72 h, 13; 96 h, 20.
The baseline and posttreatment Urd levels were compared by the Wilcoxon signed rank
test; the differences from matched baseline samples were significant at all times (P₂ =
0.03, 48 h; P₂ ≤ 0.002 at 24, 72, and 96 h). Columns, means; bars, ±SEM.

Forty-nine assessable patients with adenocarcinoma of the gastro-
intestinal tract were treated in this trial. Dose-limiting toxicity oc-
curred 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 endpoints: target enzyme
depth in PBMCs and plasma Urd levels. The delivered 5-FU dose
intensity across all cycles of therapy for patients treated with ≥1750
mg/m²/day 5-FU was not significantly different with PALA doses
≤1266 mg/m², but it was significantly lower with PALA ≥1899
mg/m². Compared to each patient’s own baseline, PALA ≤844 mg/m²
failed to appreciably inhibit ACTase activity in PBMCs at 24 h in most
patients. More consistent inhibition was seen with ≥1266 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
ward 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 ad-
ministration. 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 docu-
menting the pivotal role of the liver in regulating the concentration of
circulating Urd (40, 41). Radiolabeled-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 nucleo-
tides 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 syn-
thesis, 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 syn-
thesis 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 end points 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 bio-
chemical 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 inhibi-
tion 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 ACT-
ase 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 resus-
pending the cell pellet in a small volume of buffer, 50 μl, such that
cytosol constituted 75% of the final assay volume (40 μl). 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 re-
spect to carbamoyl phosphate, we reasoned that the concentration of
carbamoyl phosphate should be low, to maximize the assay sensitiv-
ity to PALA inhibition, and yet be in the concentration range likely
to be found intracellularly. We selected 30 μg carbamoyl phosphate
for our assay, which approximates the Kₘ as determined in prelimi-
nary studies using our assay (32). Despite the theoretical limitations of
a cytosolic assay of ACTase, previous investigators have demon-
strated a correlation between the degree of pyrimidine nucleotide
pool depletion and the degree of enzyme inhibition in both in vitro
and in vivo models. Data from a separate clinical trial combining
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 orotate and orotidine 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 orotate, 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 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 C₀ ≥ 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 the 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

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