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A Phase I Trial of Combination Therapy with Continuous-Infusion PALA and Continuous-Infusion 5-FU

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Summary. *Thirty-four patients were treated with N-(phosphonacetyl)-L-aspartate (PALA) at a dose of 850 mg/m²/day × 5 by continuous intravenous infusion (days 1–5) and 5-fluorouracil (5-FU) on an escalating dose schedule of 300–630 mg/m²/day × 5 by continuous intravenous infusion (days 2–6). Dose-limiting oral mucositis occurred at a 5-FU dose of 560 mg/m²/day; other toxicities included nausea, vomiting, diarrhea, skin rash, and superficial venous phlebitis. Myelosuppression was rare. One partial response was observed in a patient with metastatic colorectal carcinoma. Plasma PALA levels were monitored in seven patients. Steady-state levels were achieved by the 2nd day of drug infusion and ranged between 10 and 20 μg/ml.*

Introduction

Clinical trials with 5-fluorouracil (5-FU) have been directed at enhancing the activity of this agent in combinations with *N*-(phosphonacetyl)-L-aspartate (PALA) [1, 10]. Phase I and phase II studies of 5-day continuous-infusion PALA with 5-day bolus infusion 5-FU have been performed at this institution. The dose-limiting toxicity associated with this regimen was mucositis, while diarrhea and an erythematous macular skin rash were also observed. Objective responses were observed in colorectal carcinoma, breast carcinoma, pancreatic carcinoma, large cell carcinoma of the lung, and malignant fibrous histiocytoma [8, 13].

The biochemical rationale for the combination of PALA/5-FU is based upon in vitro evidence that PALA enhances incorporation of 5-FU into tumor cell RNA and results in increased antitumor activity [6, 12]. As RNA synthesis continues throughout the cell cycle, continuous cellular exposure to 5-FU might be expected to maximize this antitumor effect. Thus, the administration of 5-FU by continuous infusion could be employed in the clinical setting to prolong cellular exposure to this drug. Further, a comparison of 5-FU alone administered either as a continuous infusion or by bolus injection for 5 days has demonstrated an equivalent or improved response rate and less toxicity with the prolonged IV infusion [11]. Consequently, it was of interest to determine whether PALA would enhance the activity of 5-FU when given continuously rather than by bolus infusion.

The present study describes the results obtained in a phase I trial of PALA and 5-FU given by continuous infusion. Plasma PALA levels were also monitored to ensure the achievement of concentrations that, according to in vitro data, would be sufficient to inhibit intracellular aspartate transcarbamylase (ATCase).

Materials and Methods

1. Clinical Studies. Eligible patients had histologically confirmed malignancy refractory to standard therapy, had a performance status of two or less (ECOG scale) and had fully recovered from toxicities associated with prior treatment (at least 3 weeks since prior therapy). Written informed consent was obtained prior to therapy.

PALA was provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The drug was prepared and stored as previously described [8]. 5-FU was obtained from the Adria Laboratories, Columbus, OH.

PALA was administered at a dose of 850 mg/m²/day × 5 by continuous IV infusion, based upon results of our previous phase I and phase II studies. The dose of 5-FU was derived from a modified Fibonacci escalating dose scheme with a starting dose (300 mg/m²/day) based on one-fifth the maximum tolerated dose (MTD) by continuous infusion for 5 days (1,500 mg/m²/day). Subsequently the dosage was increased to 450, 500, 560, and 630 mg/m²/day. The 5-FU infusion began 24 h following initiation

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of the PALA infusion at a separate venous site and continued for the succeeding 5 days. If venous access prevented infusion at separate sites, the two drugs were occasionally infused through the same IV line without evident incompatibility. The treatment cycle was 28 days. On subsequent courses of treatment, if patients experienced moderately severe oral mucositis (unable to tolerate semi-solid food) for 7 days or longer the dose of 5-FU was reduced to the next lower level on the dose escalation scale.

Prior to initiation of treatment, all patients had a complete history and physical examination. Laboratory evaluation included a 24-h creatinine clearance, CBC with differential, platelet count, BUN, creatinine and liver function tests; values had to be compatible with adequate drug metabolism and excretion before treatment commenced. In appropriate patients, measurable disease was documented prior to the first course of therapy. A partial response was defined by 50% or greater decrease in the sum of the products of the perpendicular diameters of measurable lesions. CBC, differential, and platelet counts were repeated weekly. Liver function tests, BUN, creatinine, CBC, differential and platelet counts were repeated prior to each course of therapy.

2. Plasma PALA Levels. Among selected patients, venous plasma specimens (5 ml) for PALA levels were drawn in evacuated tubes with EDTA at the end of each 24-h infusion of PALA. All plasma samples were stored at −20° C. PALA concentrations in human plasma samples were measured by gas chromatography/mass spectrometry/selected ion monitoring (GC/MS/SIM) using established procedures [2].

Results

1. Clinical Study

Forty-two patients were eligible for the study. Eight patients were inevaluable for toxicity or response: six patients were treated at inappropriately low doses of PALA and/or 5-FU, and two patients died of early non-toxic causes. The remaining 34 evaluable patients received 105 courses of therapy. A mean of three courses of therapy per patient was administered (median: 2 courses; range: 1−12 courses). The characteristics of the evaluable patients are listed in Tables 1 and 2.

Toxicity was primarily confined to the gastrointestinal tract (Table 3). Oral mucositis was the dose-limiting toxicity, and was observed in 46% of treatment courses. Severe mucositis was observed in patients treated at a dose of 560 mg/m^2/day, although eight courses at a dose of 630 mg/m^2/day induced only mild mucosal toxicity. Mucositis usually began on the 5th to 8th days of the treatment cycle and persisted for 5−14 days. Diarrhea was observed at all dose levels of 5-FU, always associated with oral mucositis, but remained only mild to moderate in severity. Three patients required reduction of their daily treatment to the next-lower dose of 5-FU in five subsequent courses of therapy as a result of severe and intolerable oral mucositis. The diarrhea that occurred in 30 courses of treatment (28%) usually

Table 1. Patients characteristics

Number of patients (male/female)	42 (22/20)
Number of patients evaluable for toxicity (male/female)	34 (18/16)
Total courses evaluable for toxicity	105
Ages of evaluable patients	Range: 21−73 Mean: 54 Median: 55
Previous therapy	
Chemotherapy	25
Radiation	2
Chemotherapy/radiation	7
Total patients evaluable for response	21
Previous 5-FU/5-FUdR in response-evaluable patients	13 (all colorectal)

Table 2. Diseases among patients evaluable for toxicity

Colorectal	19
Breast	2
Ovary	2
Angiosarcoma/breast	1
Adenocarcinoma/lung	1
Transitional cell/bladder	1
Adenocarcinoma/urachus	1
Melanoma	1
Adenocarcinoma/stomach	1
Pleural mesothelioma	1
Malignant fibrous histiocytoma	1
Adenocystic/salivary gland	1
Pseudomyxoma peritonei	1
Adenocarcinoma − unknown primary	1

Table 3. Toxicity[a]

Dose 5-FU (mg/m^2/day)	Day				
	0	1+	2+	3+	4+
Mucositis (courses of treatment)					
300	32	9	5		
450	15	5		3	
500	3	4	2		
560	7	2	6	3	1
630	2	6			
Diarrhea (courses of treatment)					
300	33	12	1		
450	20	3			
500	6	3			
560	9	8	2		
630	7		1		
Rash (courses of treatment)					
300	40	6			
450	15	8			
500	5	3			1
560	9	8	1	1	
630	4	4			

[a] Eastern Cooperative Oncology Group scale

developed and resolved during the course of oral mucositis. The PALA-associated skin rash occurred in 32 courses of treatment (30%) and was mild in all but two cases. In one cases it persisted for approximately 28 days and in the other it was diffuse and pruritic for 14 days.

Myelosuppression occurred in only two courses of treatment. A 5-FU dose of 300 mg/m^2/day × 5 administered to one patient resulted in a platelet nadir of 51,000/mm^3 on day 26. A second patient given 5-FU at 560 mg/m^2/day × 5 experienced a platelet nadir of 80,000/mm^3 on day 17. Myelosuppression was defined as a platelet count less than 100,000/mm^3 or WBC less than 3,000/mm^3.

Other toxicities included nausea and vomiting in 12 courses of therapy and superficial venous phlebitis near the infusion sites in 24 courses of treatment. The venous phlebitis was not dose-related, occurred only after initiation of 5-FU infusion, and appeared whether PALA and 5-FU were infused at the same venous site or at distant sites. The phlebitis occurred during the 6-day infusion course. Occasionally the overlying skin became erythematous, blistered, or superficially ulcerated. Healing occurred in all cases by the time of the next treatment cycle, often leaving a sclerotic venous cord. The phlebitis could be alleviated by the addition of hydrocortisone succinate (100 mg) to each daily dose of 5-FU. Balanitis was an unexpected toxicity which developed in two courses of therapy given to two patients; spontaneous healing occurred.

Twenty-one patients were evaluable for response to therapy. A partial response was observed in one patient with metastatic colorectal carcinoma, as evidenced by a $> 50\%$ reduction in the size of pulmonary nodules lasting 5 months. This patient had not been previously treated with 5-FU. Among 14 other patients with colorectal carcinoma, 13 had received 5-FU previously and had failed to respond to the PALA/5-FU combination. Responses were not observed in six patients with transitional carcinoma of the bladder, adenocarcinoma of the urachus, melanoma, adenoid cystic carcinoma of the salivary gland, malignant fibrous histiocytoma and pleural malignant mesothelioma.

2. *Plasma PALA Levels*

The plasma PALA levels obtained from seven patients are listed in Table 4. Steady-state plasma levels of PALA were obtained by the 2nd day of drug infusion. The data are reported as the mean of the plasma levels obtained on each of the last 4 days of PALA infusion.

Table 4. Plasma PALA levels

Patient	PALA dose (mg/m^2/day)	Mean concentration (μg/ml) ± SD
1	850	10.13 ± 2.17
2	850	15.78 ± 9.47
3	850	12.68 ± 3.33
4	850	13.65 ± 2.95
5	850	19.77 ± 7.31
6	850	13.43 ± 5.90
7	850	13.47 ± 3.67

Discussion

The biochemical rationale for the combination of PALA and 5-FU is based upon enhancing 5-FU incorporation into tumor cell RNA and thereby increasing antitumor response. Since RNA synthesis continues in other cell cycle phases not devoted to DNA replication, the prolongation of cellular exposure to 5-FU by continuous infusion could conceivably result in an improvement in drug effect. This phase I study has demonstrated that the continuous infusion of PALA and 5-FU in combination can be accomplished with acceptable toxicity. Indeed, compared with our phase I trial of continuous-infusion PALA and bolus-infusion 5-FU [13], higher daily doses of 5-FU can be infused by continuous infusion than by bolus infusion with equivalent gastrointestinal toxicity but lower hematologic toxicity. These findings are consistent with studies by Moertel et al. [9], Seifert et al. [11], and Lokich et al. [7], comparing rapid bolus 5-FU infusion with administration by prolonged or continuous infusion. In addition, Fraile et al. have provided evidence that continuous-infusion 5-FU results in lower levels of drug in the bone marrow than are observed with other administration methods, and consequently induces less myelotoxicity [3]. The dose-limiting toxicity in the present study was oral mucositis, while other toxicities included diarrhea, skin rash, superficial venous phlebitis, balanitis, and rarely myelosuppression.

The antitumor activity was limited with the dose-schedule employed in this study. A single response was observed in a patient with metastatic colorectal carcinoma not previously treated with fluorinated pyrimidines. No responses were observed in patients with metastatic colorectal carcinoma who had previously received fluorinated pyrimidines. Further, an additional 16 patients with other malignancies failed to respond to this regimen. These findings are consistent with the results of other PALA/5-FU studies [1, 8, 10, 13], which have demonstrated activity similar to that achieved with 5-FU alone.

The similar antitumor activity of the PALA/5-FU combination and 5-FU alone may result from several factors. The dose of PALA may be insufficient to inhibit tumor ATCase, although the plasma levels achieved in this study are known to inhibit ATCase in human leukocytes in vitro [5]. The direct measurement of ATCase inhibition in tumor cells, however, would be more revealing. Ideally, the lowest possible dose of PALA should be employed to inhibit tumor cell ATCase and thus enable the administration of the highest possible dose of 5-FU. In spite of optimal modulation of the de novo pyrimidine pathway, the activity of the PALA/5-FU combination would be similar to that of 5-FU alone if the tumor cells are sufficiently capable of salvaging pyrimidine nucleosides [4].

In conclusion, our dose scheduling of PALA and 5-FU by continuous infusion is ineffective in the treatment of colorectal carcinoma in patients previously treated with fluorinated pyrimidines. A direct comparison of PALA/5-FU and 5-FU alone in previously untreated patients with colorectal carcinomas would have to be instituted to determine whether PALA enhances the activity of 5-FU against this disease. The design of an optimal dose schedule would be defined by monitoring the appropriate biochemical and drug pharmacokinetic parameters.

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