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A phase II trial of PALA + dipyridamole in patients with advanced soft-tissue sarcoma*

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Summary. A total of 21 patients with advanced soft tissue sarcoma enrolled in a phase II trial of 3.5 g/m^2 *N*-phosphonacetyl-L-aspartate (PALA) given intravenously every 3 weeks plus 50 mg/m^2 dipyridamole (Persantine) given orally every 6 h. Dipyridamole administration was initiated 1 week before the first dose of PALA. Peak and trough plasma concentrations of dipyridamole were measured before and after the first dose of PALA in 14 patients. In all, 19 patients were evaluable for therapeutic response. One subject experienced partial regression of a pulmonary metastasis; no other major response was observed. Diarrhea was the most prominent toxicity; in one patient it was life-threatening and was associated with a severe rash. On the day preceding PALA administration, the median peak plasma concentration of dipyridamole was 2,208 ng/ml and the median trough value was 904 ng/ml. Similar values were obtained on the day of PALA administration. Although the levels achieved were similar to those required to modulate the activity of PALA in preclinical systems, the therapeutic results obtained in the present study were not superior to those reported for PALA alone in previously treated patients with soft-tissue sarcoma.

Introduction

Systemic therapy for advanced soft-tissue sarcoma is inadequate. As a single agent, doxorubicin induces major responses in approximately 15%–30% of patients with advanced disease [4, 27]. Ifosfamide has demonstrated a similar degree of clinical activity [1, 5]. Higher response rates have been reported in patients treated with combination chemotherapy [3, 31], but prospective randomized trials have not demonstrated a survival advantage for such patients as compared with those given doxorubicin alone [2, 4, 26].

PALA (*N*-phosphonacetyl-L-aspartate), a transition-state inhibitor of aspartate transcarbamylase, blocks de novo pyrimidine biosynthesis [8]. In spite of promising preclinical activity [18], PALA has generally demonstrated little efficacy as a single agent in human cancer. However, among 17 patients with soft-tissue sarcoma who were given PALA during the drug's phase I evaluation, 1 experienced partial regression, 2 showed less than partial regression, and 4 displayed stable disease [11, 12, 14, 16, 29]. Nonetheless, in a formal phase II trial, no major responses were seen among 17 patients with doxorubicin-resistant advanced soft-tissue sarcoma [19].

One possible explanation for this disappointing lack of clinical activity is that human tumor cells may use circulating uridine that is taken up via a facilitated transport mechanism, bypassing the blockade of pyrimidine biosynthesis in tumor cells. In preclinical systems, uridine reverses the toxicity and therapeutic activity of PALA [17]. Interference with the uptake of uridine might therefore be an important strategy for enhancing the antineoplastic activity of PALA.

Dipyridamole, which is widely used as a vasodilator and anti-platelet agent, is a known inhibitor of nucleoside transport [23–25]. In vitro, dipyridamole markedly potentiates the cytotoxicity of PALA to experimental tumor cell lines and human bone marrow [6]. Furthermore, in a phase I study, the combination of PALA and dipyridamole induced two minor regressions and one partial response among eight patients with soft-tissue sarcoma [21]. The present trial was conducted to define further the activity of this combination in patients with soft-tissue sarcoma.

Patients and methods

In all patients, the diagnosis of soft-tissue sarcoma was confirmed by the Department of Pathology at Memorial Hospital. Only subjects with metastatic disease that had not previously been treated with chemothera-

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py or those who had undergone only one regimen were eligible. Patients must not have received chemotherapy or radiation therapy within 4 weeks of treatment and were required to have recovered from any toxicities attributable to prior therapy. Measurable disease was required, as was a performance status of $\geq 50\%$ (Karnofsky scale), a WBC count of $\geq 3{,}000/\text{ml}$, a platelet count of $\geq 100{,}000/\mu\text{l}$, a serum creatinine level of ≤ 1.5 mg/dl, and a total serum bilirubin value of ≤ 2 mg/dl.

The pretreatment evaluation included a complete history and physical examination, a complete blood count (CBC), a biochemical profile [blood urea nitrogen (BUN), uric acid, calcium, total bilirubin, total protein, albumin, alkaline phosphatase, phosphorus, serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH)], determination of serum creatinine values, and a chest radiograph. Signed informed consent was obtained from all patients. This study was approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center.

Dipyridamole (Persantine, Boehringer Ingelheim, Inc.) was given orally at a dose of 50 mg/m^2 (rounded off to the nearest 25 mg) every 6 h beginning 5–7 days prior to the first dose of PALA. Dipyridamole was continued as long as the patient remained on study; in the event of headache or epigastric distress, the dose was reduced by 25 mg/dose.

PALA (National Cancer Institute, Bethesda, Md.) was given intravenously over approximately 1 h every 3 weeks. The initial dose was 3,500 mg/m^2. Subsequent doses were increased to 4,000 mg/m^2 in the absence of gastrointestinal toxicity; in the presence of grade 2 or worse gastrointestinal toxicity, subsequent doses were reduced to 2,500 mg/m^2. Patients were scheduled to receive a minimum of two courses of PALA in the absence of definite disease progression or severe toxicity.

During the study, a CBC, screening profile, and determination of serum creatinine values were done on each day of planned PALA therapy. A physical examination including tumor measurements was performed every 3 weeks. When necessary for the assessment of measurable lesions, computer-assisted tomography (CT) scans were performed at 6-week intervals. Tumor measurements were recorded in centimeters using the longest diameter of each measurable lesion and its greatest perpendicular measurement. A complete response required the disappearance of all evidence of tumor and the resolution of all tumor-related symptoms for a minimum of 8 weeks. A partial regression was defined as a reduction of $\geq 50\%$ of the sum of the measurements of all measurable lesions for at least 1 month. Regression amounting to >25% of the tumor mass but less than that required for a partial regression was designated as minor regression. An increase in measurable disease of $\geq 25\%$ of the pretreatment value or the appearance of any new lesion represented progression, whereas patients whose tumors demonstrated a change amounting to <25% of the pretreatment value over a period of 90 days were considered to have stable disease. The WHO scale [22] was used to grade toxicity.

For assessment of the peak and trough levels of dipyridamole and for evaluation of any influence of PALA administration on these levels, blood was sampled 1 day before the first dose of PALA as well as on the day of the first PALA dose. Trough samples were taken immediately before the midday dose of dipyridamole, and peak levels were determined 75 min after the dose of dipyridamole [20]. Each dose of PALA immediately preceded the midday dose of dipyridamole. Total plasma dipyridamole concentrations were measured using a modified assay method of high-performance liquid chromatography with fluorescence detection [30] at Boehringer Ingelheim Pharmaceuticals, Inc. (Ridgefield, Conn.).

Results

A total of 21 patients were enrolled in this trial. One subject was ineligible because of extensive prior therapy. Another patient developed a severe headache following 1 day of treatment with dipyridamole and refused further therapy. Thus, 19 patients were evaluable for therapeutic response; their characteristics are presented in Table 1.

Table 1. Patient's characteristics

			Patients (*n*)
Evaluable for response			19
Age:			
	Median, 45 years		
	Range, 23–77 years		
Performance status:			
	Median, 90%		
	Range, 70%–90%		
Men/Women			8/11
Primary site/diagnosis:			
	Extremities		9
		Synovial cell sarcoma	3
		Liposarcoma	3
		Spindle-cell sarcoma	2
		Hemangiopericytoma	1
	Abdomen/retroperitoneum		8
		Leiomyosarcoma	4
		Spindle-cell sarcoma	2
		Synovial cell sarcoma	1
		Liposarcoma	1
	Undetermined		2
		Leiomyosarcoma	2
Prior therapy:			
	Adjuvant chemotherapy		1
	Therapy for metastatic disease		
		Chemotherapy alone	14
		Chemotherapy and radiation	4
	Therapy with Adriamycin		14

Table 2. Toxicity

	Number of patients with the toxicity	
	Any grade	Grade 3 or worse
Diarrhea	12	9
Myelosuppression	5	3
Nausea/vomiting	8	7
Skin rash	6	1
Headache	8	0
Mucositis	2	1
Fever	3	0

A 70-year-old woman with metastatic synovial sarcoma whose disease had progressed in spite of doxorubicin treatment experienced nearly complete resolution of a pulmonary nodule measuring 3×3.2 cm that had appeared near the site of a suture line from a previous pulmonary resection. This lesion, obvious on the baseline chest X-ray, could not be seen 3 months later, although a CT scan showed residual nodularity in this area. Therapy was discontinued after 1 year, and the nodule promptly reappeared. The lesion was then resected, and the diagnosis of metastatic sarcoma was confirmed pathologically. The patient subsequently developed a second pulmonary nodule that did not respond to treatment with dipyridamole + PALA. Three patients displayed stable disease for 3, 3, and

Table 3. Total plasma dipyridamole levels in 14 patients as measured prior to and on the day of PALA therapy[a]

Day before initiation of PALA:		
	Trough	903.9 (454.1–1,884) ng/ml
	Peak	2,208.2 (915.6–3,916.5) ng/ml
Day of PALA therapy:		
	Trough	937.1 (423–2,876.6) ng/ml
	Peak	2,123.8 (583.4–5,219.2) ng/ml

[a] Data represent the median values; ranges are indicated in parentheses

5 months, respectively. All other patients developed progressive disease.

Toxicity observed in the 19 evaluable patients is demonstrated in Table 2. The median number of PALA doses given per patient was 2 (range, 1–12). Four patients received only one dose of PALA as a result of unequivocal progression of disease within 4 weeks of the initiation of treatment. Myelosuppression was uncommon and generally mild. The median WBC nadir was 7.7×10^3 leukocytes/μl (range, $1.6–11.6 \times 10^3$/μl) and the median platelet nadir was 310×10^3/μl (range, $50–710 \times 10^3$/μl). Diarrhea was the major toxicity, with one patient developing life-threatening diarrhea associated with a severe skin rash. The dose of PALA was increased in nine cases and reduced in five. Eight patients complained of headaches, requiring a reduction of the dipyridamole dose in two cases.

Pharmacokinetic results are presented in Table 3. The dipyridamole concentrations achieved were similar to those required for modulation of PALA in vitro. No relationship was observed between peak or trough dipyridamole concentrations and toxicity, nor did the one responding patient show levels of dipyridamole that were extreme (trough, 423–495 ng/ml; peak, 1,782–2,058 ng/ml). Administration of PALA did not have an obvious effect on dipyridamole kinetics.

Discussion

The observed response rate in this trial was 5% (95% confidence interval, 0–23%). Although all patients had previously undergone chemotherapy, the median performance status was 90%. However, with the exception of the one responding patient, there was little, if any, evidence of antitumor activity. Thus, it is unlikely that the accrual of additional patients to this trial would have led to a different conclusion.

There are several possible explanations for the low response rate observed in this study. The cytotoxicity of PALA to normal and malignant cell lines can be increased by 1 μM dipyridamole [6]. The doses and schedule used in our trial were based on the results of the phase I study conducted by Markman et al. [21], in which plasma dipyridamole concentrations measured during treatment with 50 mg/m^2 oral dipyridamole q6h demonstrated that the peak concentration of free drug was 1.86 ± .99 μM. Thus, the levels achieved clinically were in a range that would be predicted to modulate the activity of PALA. In addition, this dose of dipyridamole results in reduced plasma uridine concentrations [7].

Wide variability in plasma dipyridamole concentrations following oral administration of the drug has been reported [20]. In the present study, peak total plasma dipyridamole concentrations lay in the range of 2–8 μM; however, we did not measure free plasma levels. Dipyridamole binds avidly to α_1-acid glycoprotein in blood [28], and it may be that concentrations of free dipyridamole higher than those achieved in our patients are necessary to inhibit nucleoside transport. It is unlikely that higher doses of dipyridamole can be given orally. Due to a severe headache one patient in this trial discontinued treatment before PALA administration could be initiated; in two other cases, dose reduction was required. Intravenous administration of dipyridamole might overcome this problem [13].

However, even if the concentrations of dipyridamole were adequate, other factors important in regulating the intracellular concentration of uridine may have affected the clinical results. The intracellular concentration of uridine represents a balance between facilitated diffusion (which is inhibited by dipyridamole), energy-dependent concentrative transport, and uridine metabolism [15]. Whether transport systems insensitive to dipyridamole exist in human sarcomas is not known. In murine systems, 5-benzylacyclouridine (BAU), an inhibitor of uridine phosphorylase, blocks the degradation of uridine, increases the plasma concentration of uridine, enhances its incorporation into normal tissues, and decreases its concentration in experimental tumors [10]. The simultaneous administration of BAU and dipyridamole might protect normal but not neoplastic tissues, enabling the effective use of high doses of PALA (or fluorouracil) without producing excessive host toxicity [9].

At the doses and on the schedule used, the activity of PALA + dipyridamole seen in this clinical study is insufficient to warrant further evaluation of this combination in patients with soft-tissue sarcoma. Evaluation of other PALA-based combinations for the treatment of patients with advanced sarcomas should await preclinical studies that demonstrate enhanced efficacy in the absence of increased toxicity.

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