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Phase I trial of combination therapy of cancer with *N*-phosphonacetyl-L-aspartic acid and dipyridamole*

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Summary. While *N*-phosphonacetyl-L-aspartic acid (PALA), an inhibitor of de novo pyrimidine biosynthesis, demonstrated a unique spectrum of activity during preclinical drug evaluation, multiple clinical trials have shown it to possess minimal clinical activity. One explanation for the disappointing results is the possibility that tumor cells are able to utilize circulating uridine in the synthesis of pyrimidines (salvage pathway). Dipyridamole, an inhibitor of nucleoside transport, has been demonstrated experimentally to potentiate the cytotoxicity of PALA significantly. In addition, this agent has a long safety record when used clinically in man. A phase I trial of this two-drug combination was therefore conducted, with a fixed oral dose of dipyridamole (50 mg/m^2 every 6 h) and an escalating i. v. dose of PALA administered every 3 weeks. The dose-limiting toxicity with this schedule was diarrhea and abdominal cramping pain at a PALA dose of 3900–4200 mg/m^2. Among the 65 patients participating in this trial 4 objective responses (2 partial, 2 minimal) were observed. Because of the potential for unique clinical synergy between PALA and dipyridamole further investigation should be considered.

Introduction

N-Phosphonacetyl-L-aspartic acid (PALA) is a rationally designed analogue of the transition-state intermediate of aspartic transcarbamylase [4, 27, 30], the second enzyme of de novo pyrimidine biosynthesis [20]. In preclinical evaluation the drug was shown to possess unique activity, as it was cytotoxic to several slow-growing experimental tumors (Lewis lung carcinoma, B16 melanoma) but inactive against the more rapidly growing murine leukemias [12, 13]. Unfortunately, in spite of the potential this agent demonstrated in murine models, multiple clinical trials have failed to reveal significant activity of PALA against any tumor type in man [5–7, 17, 18, 22, 23, 28].

While a variety of mechanisms have been proposed to explain the discrepancy between the murine and human activity of PALA [10, 16], one possible explanation for the clinical failure of this drug is the ability of tumor (and normal) cells to salvage exogenous pyrimidine nucleosides and bypass the enzymatic block produced by PALA. It has been demonstrated experimentally that uridine can block both the toxicity and the antitumor effects of PALA [27], has been shown in vitro to cause marked potentition of the cytotoxicity of PALA against several tumor cell lines as well as against normal human bone marrow at concentrations clinically achievable in man, presumably by preventing the cells from utilizing the salvage pathway of pyrimidine biosynthesis [1, 2].

We report here the results of a phase I trial of a combination therapy program employing dipyridamole, administered p. o. at a fixed dose level, and PALA, delivered by i. v. infusion in escalating doses every 3 weeks. This trial was conducted to determine both the toxicity and the possible clinical efficacy of this unique drug combination.

Materials and methods

Patients. This phase I trial was conducted in 65 patients with a histologically confirmed diagnosis of advanced cancer. There were 44 men and 21 women, with a median age of 63 (range 29–82). The ECOG performance status was 1 or 2 in 50 patients, while 15 patients had a performance status of 3. Only 4 patients had not received any prior therapy; 55 patients had received prior chemotherapy, 17 of whom had received more than four previous chemotherapeutic agents. There were 17 patients with non-small cell lung cancer, while 14 had colorectal cancer and 10 had a sarcoma. The remaining patients had a mixture of other solid tumors, 1 patient having a hematologic malignancy (acute lymphoblastic leukemia). Written informed consent was obtained from patients prior to the institution of therapy. Diet was ad libitum.

Drug administration. Dipyridamole (Persantine) was initially administered to all patients at a dose of 50 mg/m^2 p. o. every 6 h. This was believed to be the maximum amount of drug which could be taken by patients p. o. on a long-term basis. Most patients received one 75-mg tablet

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every 6 h (300 mg/day). The drug was begun 1 week before the first PALA administration, in order to evaluate the toxicity of dipyridamole and to achieve quasi-steady-state levels before the second drug was given. Patients experiencing excessive toxicity with the initial dose level of dipyridamole had the dose decreased by 25% before PALA was administered. Patients continued to receive dipyridamole as long as they remained on the clinical trial.

PALA was provided by the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. The PALA was diluted in 50 ml 0.9% saline and administered i. v. over 30 min. Treatment with this agent was repeated every 3 weeks. The starting dose of PALA was 500 mg/m^2, one-tenth the dose previously demonstrated to result in acceptable toxicity in phase I trials where this agent was administered alone as a short infusion (30–60 min) [6, 17, 22]. The dose of PALA was escalated in 300- to 500-mg/m^2 increments when three courses in three patients were fully evaluable and found to be associated with non-dose-limiting side-effects.

Evaluation of toxicity and efficacy. All patients had a complete history taken and full physical examination performed prior to the initiation of therapy and at 3-weekly intervals. The protocal called for a complete blood count and platelet count before each PALA administration and weekly thereafter. Severe gastrointestinal toxicity was defined as that requiring hospital admission to treat diarrhea, mouth sores, or pain.

A partial remission was defined as a 50% or greater decrease in the sum of the products of the perpendicular diameters of measurable lesions with no progression in any other lesion and no evidence of new disease, all lasting at least 1 month. A minimal response was defined as a decrease of less than 50% in the size of a measurable lesion with no progression elsewhere.

Analysis of influence of treatment program on plasma uridine levels. Selected patients had blood samples taken before and during their treatment. The samples were collected in chilled vacutainer tubes containing EDTA. They were certrifuged (500 *g* for 5 min and the plasma fractions were deproteinized by centrifugation (2000 *g*) through Amicon Centriflo ultrafiltration membrane cones CF-25 (Amicon Corp., Danvers, Mass). Details of the HPLC assay for plasma uridine have been presented elsewhere [3]. Briefly a carefully measured volume of the ultrafiltrate was injected onto a Z-module radial compression C-18 μ-Bondapak column, and the absorbance at 254 nm was recorded for nucleoside quantitation. The isocratic buffer used was 50 m*M* KH_2PO_4, pH 3.75, maintained at a flow rate of 2.0 ml/min. Uridine identification was confirmed by 254/280 nm absorbance ratios and internally spiked samples. The typical retention time for uridine in this HPLC system was 10 min.

Results

Toxicity

In all, 65 patients with a histologically confirmed diagnosis of advanced cancer were entered on this phase I trial, but 1 declined treatment after entry and never received either drug. The orally administered dose of dipyridamole was generally well tolerated. Only 5 patients complained of headache at any point while taking dipyridamole, and 1 of these opted to withdraw from the study when a 25% reduction in dipyridamole dose failed to relieve headache. The 4 other patients had substantial relief of headache with a 25% dose reduction in dipyridamole, and remained on study. The only dipyridamole toxicity noted other than headache was mild nausea and upper abdominal discomfort, which occurred in 2 patients at the dosage of 75 mg every 6 h. This did not require dose modification. Eleven additional patients had no difficulty tolerating the dipyridamole but withdrew from the treatment program prior to receiving their first dose of PALA because of disease progression. There was no definable hematologic toxicity from dipyridamole by itself.

A total of 128 courses of PALA were administered to 52 patients who remained on study after receiving 7 days of dipyridamole pretreatment. Table 1 presents the toxicity of the PALA/dipyridamole combination. In general toxicity was mild until the 3000 mg/m^2 dose level was reached. Above this level, several patients developed mucositis,

Table 1. Toxicity of PALA in combination with dipyridamole

Dose (mg/m^2)	No. of patients	No. of courses	No. of courses associated with		
			Rash	Mucositis	Diarrhea/abdominal cramping
500	8	8	0	0	1 (grade I)
800	2	3	0	0	0
1100	4	4	0	1 (grade I)	0
1300	1	1	0	0	0
1500	6	6	0	0	0
2000	5	6	0	0	0
2500	5	9	0	0	0
3000	13	23	2	3	4 (2)
3300	14	21	2 (1)	1 (1)	1 (1)
3600	9	12	0	3	6
3900	4	10	2	1	4 (1)
4200	9	18	3 (1)	1	6 (3)
4500	4	7	0	1	4 (1)

Number in parentheses indicates the number of courses with severe toxicity (ECOG grade 3–4+) in each case. Other courses associated with toxic events were either ECOG 1 or 2

rash, diarrhea, and abdominal cramping. At the higher dose levels, diarrhea and abdominal pain were occasionally quite severe and were the dose-limiting toxicities. As is often the case when gastrointestinal toxicity is the dose-limiting toxicity, both the incidence and the severity of the diarrhea and abdominal cramping were somewhat erratic, making it difficult to define an exact maximum tolerated dose. However, at the 4200 mg/m^2 dose of PALA three episodes of severe diarrhea and cramping developed which required hospitalization. Our recommended phase II dose PALA when administered with dipyridamole is 3600–3900 mg/m^2.

Myelosuppression was very mild, and no definite pattern of white blood cell count or platelet count depression and recovery was definable. One patient experienced thrombocytopenia to a platelet nadir of 40 000/ml^3 with PALA administered at 500 mg/m^2. He had previously received extensive radiation therapy for multiple bone metastases, and was considered to have a limited bone marrow reserve. The second patient developed severe bone marrow suppression following treatment with PALA at the 3000 mg/m^2 dose level, but was found on bone marrow biopsy to have extensive replacement of the bone marrow with malignant melanoma. These were the only two courses associated with the white blood count nadir below 3000/m^3 or platelet count nadir below 100 000/m^3. There was no evidence of renal, hepatic, or neurologic toxicity. Nausea, when it occurred, was mild. Several patients noted tingling of their hands and lips, which occurred during the PALA infusion and persisted for 10–30 min thereafter.

Response to therapy

A total of 38 of the 52 patients were evaluable for response by virtue of having measurable disease and having received at least one course of treatment. There were 2 partial and 2 minimal responses. One partial response occurred in a previously untreated patient with adenocarcinoma of the lung; this lasted for 4 months. The other partial response occurred in a previously treated patient with soft tissue sarcoma metastatic to the lung and was of 2 months' duration.

Influence of therapy on plasma uridine levels

Plasma uridine levels were monitored in 10 patients during the 1 week treatment period with oral dipyridamole alone. The mean pretreatment plasma uridine concentration was 3.49 ± 1.28 (SD) μM. Dipyridamole treatment caused a reduction in mean plasma uridine concentration to 2.9 ± 0.70 μM 9 h after the first oral dose. A peak plasma dipyridamole concentration of 1.86 ± 0.99 μM ($P < 0.05$, paired t-test, compared with baseline values) was achieved approximately 2 h after oral dosing. Plasma uridine concentrations were also monitored in a group of 9 patients who had a reduced plasma uridine concentration of 2.46 ± 0.61 μM after 1 week of dipyridamole ($P < 0.05$ compared with baseline). In the same 9 patients administration of PALA caused a further reduction to 0.87 ± 0.23 μM 7 h after the first i. v. dose ($P < 0.01$ compared with baseline). Daily plasma uridine measurements in 2 patients after a single PALA dose of 4.2 g/m^2 showed that their plasma uridine continued to decline for 5 h after the PALA dose, then remaining depressed for 6 days in 1 patient and 11 days in the other.

Discussion

In experimental systems, uridine has been demonstrated to completely reverse the toxic and antineoplastic properties of PALA [11, 27]. Many types of human cancer cells are capable of utilizing endogenous nucleosides to bypass the block produced by PALA in de novo pyrimidine synthesis, as human plasma contains uridine in sufficient concentrations to serve as a substrate for the salvage pathway [14]. Dipyridamole, by interfering with transport of nucleosides across cell membranes [24–26], can inhibit the cell's ability to make use of this salvage pathway and result in dual blockage of pyrimidine biosynthesis when administered with PALA. The potentiation of PALA-induced cytotoxicity by clinically achievable concentrations of dipyridamole has been demonstrated for a variety of human cancer cell lines [1, 2]. While cytotoxic synergy was also demonstrated in vitro against normal human bone marrow cells, it is possible that the in vivo microenvironment (the levels of endogenous nucleosides) surrounding a tumor is different from that of marrow elements, and this dual blockage may, therefore, be partialy selective for malignant tissue.

In this phase I clinical trial we have demonstrated that a combination chemotherapy regimen of p. o.-administered dipyridamole and i. v.-delivered PALA can be safely given with a profile of toxicity that is similar to that observed when PALA is administered alone. The oral dose of dipyridamole used in this trial was probably close to a maximum tolerated dose. In 8% of the patients dose reduction was required for headache. The peak plasma concentration of 1.86 ± 0.97 (SD) micromolar is in the range demonstrated to be biochemically effective in modulating the toxicity of PALA in tissue culture [2]. In addition, this dose of dipyridamole had a statistically significant effect in reducing plasma uridine levels by 35%. Nevertheless, it remains an issue whether a larger dose of dipyridamole could be used to achieve a greater degree of biochemical modulation.

Because the dose-limiting toxicity in this study was gastrointestinal, and because the incidence of this toxicity was somewhat erratic, it was difficult to define the maximum tolerated dose with an acceptable degree of accuracy. It appears to us that doses in excess of 3900 mg/m^2 will not be well tolerated, and our recommended phase II dose is 3600–3900 mg/m^2. The maximum tolerated dose as defined by this study is approximately 70%–80% of the dose that is tolerated when PALA is given as a single agent. This suggests that the dose of dipyridamole used in this study enhanced PALA toxicity somewhat, which would be consistent with the effects of dipyridamole on plasma uridine levels and dipyridamole's ability to enhance toxicity to normal cells in tissue culture [1]. However, because of the difficulty in defining an exact maximum tolerated dose, no definitive statement can be made regarding synergy between these two agents with regard to a change in toxicity or antitumor efficacy.

It is of particular interest that dipyridamole has been demonstrated in this study to decrease plasma uridine levels. The liver is thought to be both the major source of plasma uridine and the major site of its degradation [8]. Dipyridamole blocks the uptake of uridine into cells, and thus it might have been anticipated that dipyridamole would increase plasma uridine levels by interfering with uridine uptake in the liver. However, dipyridamole can also block efflux of uridine from cells [9, 19, 29], and this

may be the overriding influence resulting in a net decrease in plasma uridine levels. When PALA was added in addition to dipyridamole, there was a mean further decrease of 64% in plasma uridine levels. While this is somewhat greater than has been observed in rodents [15, 21], we cannot conclude that this decrease is greater than would be anticipated from PALA given alone without dipyridamole. Additional details of the dipyridamole and PALA effect on plasma uridine and a discussion of these issues are presented elsewhere [3].

While several patients demonstrated responses to this program, the overall response rate (4 of 65 patients entered; 4 of 38 patients evaluable) was not clearly different from that observed in trials where PALA was administered alone. However, the dual blockade of pyrimidine synthesis resulting from treatment with the PALA/dipyridamole drug combination represents a unique approach to the therapy of malignant disease and deserves consideration for additional clinical investigative efforts.

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