Plasma Uridine Changes in Cancer Patients Treated with the Combination of Dipyridamole and N-Phosphonacetyl-L-aspartate

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ABSTRACT

Dipyridamole (DP) and N-phosphonacetyl-L-aspartate (PALA) act synergistically in vitro against many cell lines and in vivo against human ovarian carcinoma xenographs. We have conducted a phase I clinical trial of DP p.o. (50 mg/m², every 6 h) in combination with PALA (starting at 500 mg/m² i.v. with 300-mg dose escalations). Sixty-five patients were entered into this study, and we have established the maximum tolerated dose of PALA to be 4.5 g/m² when combined with DP, which is approximately 80% of the previously reported maximum tolerated dose for PALA alone. The observed toxicities of DP plus PALA were mild and were similar to those reported for PALA alone. Bone marrow toxicities were not evident at any PALA dose. Ten patients with a mean pretreatment plasma uridine concentration of 3.49 ± 1.28 (SD) μM had their plasma uridine reduced to 2.29 ± 0.70 μM 9 h after DP p.o. A peak plasma DP concentration of 1.86 ± 0.59 μM was achieved approximately 2 h after p.o. dosing. Nine patients who had a reduced plasma uridine concentration of 2.46 ± 0.61 μM after 1 week of DP had their plasma uridine further reduced by PALA to 0.87 ± 0.23 μM 7 h post-PALA. Daily plasma uridine measurements in two patients during their DP treatment confirmed the previously described pattern for Day 1, but the data suggest a slight recovery (15%) in plasma uridine by Day 2. Daily sampling in two other patients after a single PALA dose of 4.2 g/m² showed that their plasma uridine declined 5 h after the PALA dose and remained depressed for 6 days in one patient and 11 days in the other. These results suggest that DP worked in synergy with PALA to lower circulating uridine in cancer patients. The mechanism for the ability of DP to reduce plasma uridine is not known, but there is evidence that DP can inhibit cellular uptake of uridine as well as its uptake. DP may reduce plasma nucleoside pools in addition to blocking nucleoside salvage and therefore have general applicability in other chemotherapy regimens.

INTRODUCTION

PALA is a potent inhibitor of ATCase, the enzyme responsible for the biosynthesis of carbamyl-L-aspartate in the pyrimidine metabolic pathway. Pyrimidine biosynthesis in mammalian cells was virtually completely blocked by PALA (3). In experiments with mice, PALA was found to markedly inhibit liver ATCase activity within 15 min of administration, and its effects persisted for at least 72 h after a single dose (4). Preclinical studies found PALA to have a unique antitumor spectrum with the rapidly proliferating murine leukemias being resistant and the more slowly growing Lewis lung carcinoma and B16 melanoma being sensitive to the effects of the drug (5, 6). In phase I pharmacological studies, PALA was found to have an initial phase τ₀ of 1 h and a terminal phase τᵣ of 5 h (7, 8).

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4The abbreviations used are: PALA, N-phosphonacetyl-L-aspartate; DP, dipyridamole; HPLC, high performance liquid chromatography; ATCase, L-aspartate transcarbamylase.

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analyses and radiograms were performed on Day 1. The patients were then given dipyridamole p.o. at a dose of 50 mg/m² (most patients received 15 mg) and were instructed to repeat every 6 h for as long as they remained on the study. On Day 7, the patients were brought back into the clinic for their PALA dose (starting at 500 mg/m² in the first few patients with 300-mg dose escalations as i.v. infusion over 20 min) after a standard workup as outlined for Day 1.

Blood Sampling. A pretreatment blood sample was drawn from each patient in the trial for measurement of plasma uridine and to serve as a dipyridamole blank. In 10 patients who had given their informed consent for multiple blood sampling, detailed time courses of plasma uridine levels were studied during their first dipyridamole doses. Anti-coagulated (EDTA) blood samples were obtained 2 and 1 h prior to DP dosing and at hourly intervals after the DP dose via an indwelling venous catheter. The samples were centrifuged (500 x g) immediately after their initial DP dose to beyond 15 h on Day 1 and subsequent sampling intervals was a modification of that reported by Wolfram and Bjornsson (28). Briefly, 1.0 ml of plasma was mixed with 20 μl of the internal standard solution (quinidine. HCl, 64 μg/ml) and 1.0 ml of 1 M sodium hydroxide. The sample was then extracted with 5.0 ml of diethyl ether by vortexing the mixture vigorously for 20 s. The organic phase was removed and evaporated to dryness under nitrogen. The samples were reconstituted in 100 μl of the mobile phase and a carefully measured volume was injected into the HPLC. The HPLC system consisted of the following Waters Associates equipment: Model 6000A pump; Model U6K injector; Z-module fitted with a C18-Bondapak cartridge and a proximal guard column of the same material; and a Model 420 fluorescence detector with an excitation wavelength set at 285 nm and an emission cutoff filter of 470 nm. DP and the internal standard were eluted with a mobile phase of methanol-water (60/40) containing 1-M heptanesulfonic acid, sodium salt (0.005 M) and acetic acid (0.1%), delivered at 2.5 ml/min through the column. Typical retention time for quinidine was 3.8 min, and that for DP was 5.8 min. The extraction procedure routinely recovers over 98% of quinidine and 95% of DP from the aqueous phase.

RESULTS

Toxicities of DP and PALA. DP p.o. at 50 mg/m² (every 6 h) was well tolerated by the patients in this study, with mild headache being the most common complaint. Five patients reported severe headaches with four of them requiring a dose reduction to 50 mg every 6 h. Two other patients reported nausea and upper abdominal pain. One patient withdrew from this study because of headache and twelve patients withdrew before receiving PALA due to disease progression. A total of 128 courses of PALA in combination with DP was administered to 52 patients. The toxicity of PALA plus DP was mild below the 3000-mg/m² dose level. The commonly observed toxicities above this dose were mucositis, rash, diarrhea, and abdominal cramps. We have established the maximum tolerated dose of PALA in combination with DP to be 4500 mg/m², with the dose limiting toxicities being severe diarrhea and abdominal pain (1). There was no evidence of bone marrow, renal, hepatic, or neurotoxicities. Nausea was mild and rarely observed, and several patients noticed tingling sensations in their hands and lips during PALA infusion which persisted for 10–30 min.

Effects of DP Treatment on Plasma Uridine. The acute effects of DP treatment on plasma uridine concentration were examined in a group of ten patients. Fig. 2 shows the averaged plasma uridine concentrations in these patients as a function of time before and during their DP treatment. Their mean pretreat-

<table>
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* One patient had primary breast tumor and sarcoma.

Fig. 1. Typical HPLC chromatogram of a patient’s plasma ultrafiltrate. The peak identifications and retention times were: C, cytidine, 5.07 min; UA, uric acid, 6.45 min; H, hypoxanthine, 7.70 min; U, uridine, 9.64 min. Peak identification was confirmed by 280/254 absorbance ratios and coelution with purified standards.
Effects of DP plus PALA Treatment on Plasma Uridine. In a group of nine patients who had a mean pretreatment plasma uridine concentration of 3.67 ± 1.22 μM which dropped to 2.46 ± 0.61 μM (P < 0.05, paired t test) after 1 week of DP, the acute effects of the further addition of PALA treatment on plasma uridine were studied. Their plasma uridine concentrations started falling 2 h after receiving PALA to a steady level of 0.87 ± 0.23 μM 7 h post-PALA. Fig. 3 shows the averaged plasma uridine concentration in these patients as a function of time. Plasma uridine was reduced markedly by the PALA treatment and remained depressed for more than 9 h posttreatment. In another two patients, the long term effects of a single PALA dose on plasma uridine concentration were studied. The blood sampling was extended to 15 h on Day 1 and the patients were asked to return for blood sampling daily for 3 weeks (before their next PALA dose). Fig. 4 shows that plasma uridine in these two patients remained depressed for 6 and 11 days, respectively, after a single PALA dose of 4200 mg/m². The uridine concentrations returned to pre-PALA levels 9 and 13 days after the PALA treatment.

Response to Therapy. There were 38 patients who were evaluable for response. One patient with adenocarcinoma of the lung who had not received prior chemotherapy experienced a partial clinical remission with the complete disappearance of a histologically confirmed malignant supraclavicular lymph node. His chest disease remained stabilized for 4 months while he was on DP-PALA. Another patient with metastatic soft tissue sarcoma resistant to previous chemotherapy experienced partial regression of his pulmonary nodules lasting for over 2 months. Two additional patients with endometrial carcinoma and leiomyosarcoma, respectively (both metastasized to the lung), demonstrated minimal responses (greater than 50% reduction of the sum of the products of all quantifiable tumor diameters in two planes) which lasted for 2 months. A fifth patient with a massive retroperitoneal leiomyosarcoma involving the liver, the inferior vena cava, and the right atrium experienced a minimal response and subjective improvement of the respiratory insufficiency which lasted for over 5 months.

DISCUSSION

We have demonstrated previously that of all the commonly encountered nucleosides, uridine was the only one capable of antagonizing the cytotoxic effects of DP and PALA in vitro. The mechanism of synergy between these two drugs seems to reside in the inhibition of de novo pyrimidine biosynthesis by PALA and the concurrent blockade of uridine salvage by DP (27). In addition to the usual objectives of a phase I clinical trial, we are also interested in the effects of DP and PALA on the circulating uridine levels in cancer patients. While PALA alone specifically decreases the level of pyrimidines in sensitive tumor cells compared to normal tissues (27, 29), the reported decrease in circulating uridine levels after PALA treatment in vivo was not dramatic. In mice, PALA treatment either as a single dose or as four daily doses reduced serum uridine level by 55% (30), while in the Sprague-Dawley rat uridine depression was found to be less than 40% 24 h after treatment (31). Similarly, in a phase I trial in patients receiving PALA (1000-
2000 mg/m²/day), serum uridine levels were noted to be maximally depressed by 37–85% (32). It is possible, in fact, that the observation of decreased circulating levels represents not only a decline secondary to the inhibition of pyrimidine de novo synthesis by PALA but also an increased uptake of uridine by the cells to be used in the salvage pathway. Several lines of evidence support this hypothesis: (a) enzyme activity of the salvage pathway has been demonstrated to be increased in tumors made resistant to PALA (33); and (b) while leukocyte ATCase activity is rapidly and markedly inhibited by PALA with 280 h being required for 50% recovery of ATCase activity (34), there was no evidence of myelotoxicity under these conditions suggesting the efficient use of salvage pathways by the bone marrow.

Our current findings provide some interesting information on the effects of DP and PALA in cancer patients. The observation that DP alone reduced the circulating level of uridine in our patients was unexpected. If this drug works via the blockade of cellular uptake of uridine, we might expect the plasma uridine to increase after treatment due to impaired hepatic extraction and blocked cellular uptake of uridine from the plasma. Such an increase has been observed in the case of plasma adenosine concentrations in patients and several species of laboratory animals being treated with DP (35). The mechanism by which DP decreases plasma uridine may be related to its ability to inhibit nucleoside efflux in addition to blocking influx, as has been shown recently in several mammalian cell lines (36–38). If the liver is indeed the major site of uridine biosynthesis as proposed by Gasser et al. (23), DP may inhibit the release of this nucleoside from the liver into the systemic circulation resulting in a drop in circulating uridine after DP treatment. More detailed pharmacokinetic studies on uridine metabolism in vivo are required before we can be certain of an explanation for this observation.

In combination with DP, PALA reduced the plasma uridine in these patients consistently to approximately 20% of the pretreatment level. The reduction is more reproducible and larger in magnitude than the previously reported 45% reduction in patients treated with PALA alone (32). We have also demonstrated that a single dose of PALA resulted in a prolonged reduction of plasma uridine which did not recover to pre-PALA levels until 9–13 days posttreatment. This finding is very valuable for the design of further trials in terms of the optimum frequency and dosage of chemotherapy to be used, if we can assume that plasma uridine is a biological indicator of the overall biochemical effects of the PALA-DP combination. Other nucleosides measured concurrently did not show any remarkable trend during DP and PALA treatment (data not shown).

Although the overall response rate in this phase I trial was not striking, we are particularly encouraged by the responses of the patients with soft tissue sarcomas to the DP-PALA regimen. We are unaware of any effective second line chemotherapeutic regimen to date for the treatment of this type of advanced malignancy. We plan to investigate further the efficacy of DP and PALA in the treatment of soft tissue sarcoma in a phase II trial. We do not know the mechanism by which DP lowers the plasma uridine concentration at the present time. The ability of this drug to effectively reduce the body’s plasma uridine pool (and possibly other metabolites) has important clincial implications. It is possible that DP can limit the availability of salvage metabolites in the body in addition to blocking their uptake. Other workers have already reported synergy between DP and antimitabolites such as methotrexate (39) and aciclovir (40, 41) against tumor cells in vitro. Reports on the results from clinical trials using DP in combination with other anticancer drugs will undoubtedly be forthcoming.

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