ABSTRACT

Serum uridine levels of N-(phosphonacetyl)-L-aspartate-treated patients from Phase I and continuing trials receiving 1000 to 2000 mg/sq m/day were measured by reverse-phase high-pressure liquid chromatography. For the five patients studied, the predose serum levels ranged from 2.6 to 6.4 μM, well within the normal range of 1.9 to 8.9 μM. All serum uridine levels decreased in the first 24 hr, but the drop was quite variable (7 to 85%). Uridine levels in all five patients remained below predose levels, with the largest drop for each patient being 37 to 85% at differing time points. The only patient to show a daily decrease was the only responder and the patient with the largest decrease. N-(Phosphonomethyl)-L-aspartate appears to have a limited reductive effect on human serum uridine levels.

INTRODUCTION

PALA² was synthesized by Collins and Stark (1) as an analog of the transition state intermediate of the reaction catalyzed by aspartate transcarbamylase, an early step in the de novo pyrimidine biosynthetic pathway. PALA is a potent inhibitor of aspartate transcarbamylase isolated from SV40-transformed baby hamster kidney cells (K = 1 × 10⁻⁸) (10) and from mouse spleen (K = 2.6 × 10⁻⁸) (4). PALA inhibits the de novo pathway in normal and tumor cells in culture (6, 7, 9, 11) and mouse spleen cells in vivo (12). The toxic effects of PALA against SV40-transformed baby hamster kidney cells in culture can be reversed by uridine, a nucleoside which can be utilized via the salvage pathway to maintain the intracellular pool of preformed pyrimidines, particularly uridine. Likewise, Johnson (5) has demonstrated a reversal of toxic and antitumor effects of PALA in mice by uridine.

Recent studies in our laboratory³ have shown that serum uridine levels in humans, rats, and mice are relatively constant for each species, suggesting that a regulatory mechanism exists in vivo for maintaining serum uridine levels. The level of circulating uridine is a reflection of both synthesis of uridine by donor organs and the utilization of uridine by tissues via the salvage pathway. In vivo studies of inhibitors of the de novo pyrimidine biosynthetic pathway have thus far neglected to take into consideration the effect of such inhibitors on circulating levels of preformed pyrimidines, particularly uridine. Because PALA is a potent inhibitor of the de novo pathway and because the serum is an available source of endogenous uridine that has the potential of reversing the cytotoxic effects of PALA, we initiated a study to determine the effects of PALA on human serum uridine levels. The results of this study are contained in this report.

MATERIALS AND METHODS

Serum samples were obtained from patients in the Phase I PALA trial (3) and continuing studies. The patients received 1000 to 2000 mg/sq m/day as a daily 15-min i.v. infusion for 5 consecutive days repeated every 3 weeks. Table 1 details the dose received and the diagnosis for the individual patients. The doses listed represent the highest tolerated dose for each patient.

To 250 to 500 μl of each serum sample, 10 nmol of the internal standard 5-methylcytidine and 2 ml of water were added. The samples were then centrifuged at 1000 × g for 20 min through an Amicon Centrifiq CF25 membrane cone (Amicon Corp., Cambridge, Mass.), and the filtrate containing the nucleosides was collected. To further purify the sample, a 10× 30-mm column of polyacrylamide-boronate gel (Affi-Gel 601; Bio-Rad Laboratories, Richmond, Calif.) was equilibrated with 10 ml of 0.25 M ammonium acetate solution. The nucleoside fraction was made about 0.25 M in ammonium acetate, placed on the column, and washed with 10 ml of 0.25 M ammonium acetate solution. The ribonucleosides were eluted with 15 ml of 0.1 M formic acid, collected, and lyophilized. The boronate column was washed with 20 ml of 0.1 M formic acid and stored in the refrigerator between uses (2).

The lyophilized samples were redissolved in 100 μl of water, and 5 μl of xanthine oxidase (Grade III; Sigma Chemical Co., St. Louis, Mo.) were added to oxidize any remaining xanthine and hypoxanthine (8). A 50- to 100-μl aliquot of sample was analyzed on an Altex Model 312 high-pressure liquid chromatograph equipped with a Partisil PXS 5/25 ODS column (Whatman, Inc., Clifton, N. J.). The samples were eluted with acetate buffer (0.01 M sodium acetate plus 0.01 M acetic acid, pH 4.5) at 1.5 ml/min. Between each run, the column was washed for 15 min with 95% methanol and 5% acetate buffer. Peak heights were determined at 254 nm using an Altex Model 153 UV detector. To ensure purity of the uridine and internal standard peaks, an Altex Model 155-30 variable wavelength UV detector set at 280 nm was used in series. The variation of individually analyzed replicate samples was less than 10%. A typical chromatogram is shown in Chart 1.

RESULTS AND DISCUSSION

An inhibitor of the de novo pyrimidine biosynthetic pathway might be expected to decrease levels of circulating uridine by inhibiting the synthesis of uridine by a donor organ(s) and/or 3 J. M. Kane, L. W. Anderson, D. D. Dietrick, and R. L. Cysyk. Serum uridine levels of N-(phosphonomethyl)-L-aspartate treated patients from Phase I and continuing trials receiving 1000 to 2000 mg/sq m/day were measured by reverse-phase high-pressure liquid chromatography. For the five patients studied, the predose serum levels ranged from 2.6 to 6.4 μM, well within the normal range of 1.9 to 8.9 μM. All serum uridine levels decreased in the first 24 hr, but the drop was quite variable (7 to 85%). Uridine levels in all five patients remained below predose levels, with the largest drop for each patient being 37 to 85% at differing time points. The only patient to show a daily decrease was the only responder and the patient with the largest decrease. N-(Phosphonomethyl)-L-aspartate appears to have a limited reductive effect on human serum uridine levels.

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RESULTS AND DISCUSSION

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The predose serum uridine levels for the 5 patients studied ranged from 2.6 to 6.4 μM (Chart 2). These values are within the normal range of 1.9 to 8.9 μM established for human volunteers. Chart 2 illustrates the time course studies for 5 patients receiving the indicated dose of PALA on 5 consecutive days. Samples from each patient were obtained every 24 hr, just prior to the next dose. All serum levels decreased in the first 24 hr; the decrease ranged from 7 to 65%. Serum uridine levels remained below predose levels throughout the observation period with the largest drop for each patient being 37 to 85% at differing time points. However, most of the levels remained in the normal range of 1.9 to 8.9 μM, with only a few time points for Patients 3, 4, and 5 dropping below 1.9 μM. Patient 5 had the largest decrease and was the only patient in which serum uridine levels decreased progressively every 24 hr. Patient 5 was the only responsive patient in the Phase I study at NIH, exhibiting a partial response to colon carcinoma metastatic to liver and lungs. In this patient, multiple lung metastases decreased by more than 50%, and a repeat liver scan indicated a decrease in the size of 3 major lesions following 3 cycles of therapy at 1500 mg/sq m/day. However, since the lowest uridine level of Patient 5 was not significantly different from that of Patients 3 and 4, the clinical responsiveness of Patient 5 cannot be attributed to the final absolute value for serum uridine achieved after treatment.

Several patients were studied in the 0- to 12-hr range after receiving PALA to determine if transient decreases in serum uridine occur. PALA has a plasma elimination t1/2 of 4.6 hr, and the serum uridine levels determined at the 24-hr time intervals in Chart 2 might have been a reflection of the recovery of the serum uridine pools after an initial depression. In 3 patients receiving 1500 mg/sq m, serum uridine levels were as follows: 6.1, 7.0, 4.2, 5.5, 3.3, and 5.7 μM at 0, 1, 2, 4, 8, and 24 hr, respectively; 2.7, 3.3, 1.1, and 2.2 μM at 0, 4, 12, and 24 hr, respectively; 5.1, 8.5, and 1.9 μM at 0, 8, and 24 hr, respectively. One patient receiving 1000 mg/sq m had serum uridine levels of 6.8, 4.7, and 2.5 μM at 0, 8, and 24 hr, respectively. Thus, although serum uridine levels fluctuated during the first 24 hr, they were not lower than the values determined at the 24-hr intervals (Chart 2).

In summary, PALA treatment results in a decrease in circulating uridine levels. However, this reductive effect is not extensive and appears not to be a major biological effect of this agent.
REFERENCES

Serum Uridine Levels in Patients Receiving N-(Phosphonacetyl)-l-aspartate


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