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Uridine pharmacokinetics in cancer patients*

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Summary. The availability of uridine can alter the sensitivity of tumor cells to antimetabolites such as *N*-phosphonacetyl-L-aspartic acid (PALA) and acivicin by virtue of the cell's ability to salvage preformed metabolites from its environment. We investigated the pharmacokinetics of physiologically relevant amounts of uridine in cancer patients in a pilot study to further our understanding of uridine metabolism in the human body. Four cancer patients, two males and two females, were given an i.v. bolus of a trace amount of radiolabeled uridine. The nucleoside disappeared from the plasma in a triphasic manner, with initial half-lives of 0.57 ± 0.28 and 1.79 ± 0.62 min and a terminal half-life of 17.5 ± 7.3 min. The volume of distribution was 481 ± 70 ml/kg, and the plasma uridine clearance was calculated to be 1.70 ± 0.42 l/min. Simultaneous plasma and bone marrow uridine concentrations were measured in a separate group of seven healthy volunteers. The uridine concentration in plasma was 2.32 ± 0.58 μM, and that in the bone marrow plasma was 10.44 ± 5.06 μM. These results suggest a very rapid turnover of uridine in the plasma when the nucleoside is present at physiologic concentrations, and that there is a locally high concentration of uridine available for salvage in the bone marrow.

Introduction

Uridine is an endogenous nucleoside that can modulate the activity of a variety of antimetabolites affecting the de novo pyrimidine synthetic pathway. Micromolar concentrations of uridine that are routinely found in human plasma have been shown to affect the activity of *N*-phosphoracetyl-L-aspartic acid (PALA) [2, 5, 7, 8]. Our current knowledge of uridine metabolism is derived primarily from studies on rodents. Gasser et al. have reported that the circulating uridine in rat blood was synthesized by the liver de novo, and that over 90% of the newly synthesized uridine was subsequently metabolized by the same organ to carbon dioxide and water after a single pass [3]. As a first step toward characterizing uridine metabolism in the human body, we studied the clearance of trace amounts of radiolabeled uridine in the plasma of cancer patients. In addition, we studied the possible compartmentalization of uridine in the human body by measuring its concentrations concurrently in the plasma and bone marrow of healthy volunteers, and the results of our pilot study are reported here.

Material and methods

Subjects and study design. Two males and two females with histologically confirmed malignancy and no evidence of liver involvement participated in the investigation of plasma pharmacokinetics. All had ECOG performance statuses of 0–1. This study was approved by the institutional human subjects committee, and an IND for (5,6^{3}H)-uridine was obtained from the Food and Drug Administration. The use of this radiolabeled compound in humans was also approved by the UCSD Radiation Drug Research Committee. Written informed consent was obtained from all subjects. Two i.v. infusion lines were placed in the antecubital vein on the opposing arms of the patient; one was used for injection and one for blood sampling. A total of 0.25 mCi of (5,6^{3}H)-uridine (40–60 Ci/mmol, Amersham, Arlington Heights, Ill.) in 5 ml sterile saline was injected as an i.v. bolus over 10 s (total uridine doses of 1.55–2.29 mg/patient), followed by a 5-ml saline flush. The syringe and cannula were later extensively washed with methanol to determine exactly what fraction of the intended dose of radioactivity had actually been injected, and what fraction was left in the syringe and tubing. Blood samples were drawn from the catheter in the opposite arm into EDTA tubes at 30-s intervals for the first 5 min and every minute for the next 25 to 45 min. The sampling line was flushed with saline between successive samplings to minimize cross-contamination. Formed elements were removed rapidly from the plasma by centrifugation at 4° C, and the plasma samples were analyzed immediately by the high performance liquid chromatography procedure outlined below.

Bone marrow sampling. The bone marrow donation protocol was approved by the Institutional Review Board, and

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informed consent from each donor was obtained prior to the procedure. Bone marrow aspirates were obtained from seven normal, healthy volunteers (five males and two females). A 9-ml sample was drawn into 1 ml heparinized saline from the posterior iliac crest following local anesthesia. A concurrent blood sample was drawn from the antecubital vein. Marrow and blood samples were immediately centrifuged at 500 *g* for 5 min at 4° C. Both the bone marrow and blood plasma were separated from formed elements and frozen at −20°C until analysis. Uridine was stable in plasma under these conditions for at least 6 months, and the typical storage time was less than 2 weeks.

Uridine measurement. The total radioactivity in the plasma was measured by counting a 100-µl aliquot of plasma before further processing. The amount of injected uridine in the plasma samples was determined by counting the radioactivity associated with the uridine peak after separation from other metabolites by high performance liquid chromatography [2]. Briefly, the blood and marrow plasma samples were deproteinized by centrifugation through AMICON CF-25 membrane cones (Danvers, Mass.) at 1500 *g* for 30 min. The uridine concentration in the ultrafiltrates was measured as has previously been reported [2] using a high performance liquid chromatographic system consisting of the following Waters Associates equipment: model 6000A pump, model 440 UV absorbance detector, model U6K injector, Z-module radial compression unit, and a model 730 data module. The column was a Waters Associates µ-Bondapak C-18 cartridge with a proximal guard column of the same packing material. The isocratic buffer used was 50 m*M* KH_2PO_4, pH 3.75, maintained at the flow rate of 2.0 ml/min. Uridine identification was confirmed by 254/280 nm absorbance ratios and coelution of patient samples with purified standards. The uridine peak was baseline resolved with a typical retention time of 9.7 min. Post-column fractions (2.0-ml aliquots) were collected with a fraction collector (ISCO 1850, Lincoln, Neb.), and the amount of radioactivity in each fraction was quantified by liquid scintillation counting using a cocktail-to-sample ratio of 9:1 (Bio-HP, Fisher Scientific, NJ). Counting efficiency and quenching was corrected for by injecting control plasma samples spiked with known amounts of radiolabeled uridine into the HPLC and the peaks were eluted under the same conditions.

Pharmacokinetic modeling. Radiometric data for total plasma and uridine-specific activity were analyzed by computer-assisted modeling. A model-independent curve-fitting program, RSTRIP (Micromath Inc., Salt Lake City, Utah), was used in our pharmacokinetic analysis. This program uses a parameter known as the Model Selection Criterion (MSC) to determine the optimal number of exponential terms needed to describe a data set. The MSC is a modified Akaike Information Criterion (AIC) given by the formula:

$$AIC = N * \ln\left(\sum_{i=1}^{n} w_i (Yobs_i - \overline{Y}obs)^2\right) + 2p$$

The formula for the MSC used for our curve fitting is as follows:

$$MSC = \ln\left(\frac{\sum_{i=1}^{n} (Yobs_i - \overline{Y}obs)^2}{\sum_{i=1}^{n} (Yobs_i - Ycal_i)^2}\right) - \frac{2p}{N}.$$

MSC values usually range from 3 (acceptable fit) to 6 (excellent fit). A value of 5 is roughly equivalent to an r^2 value of 0.98. All values cited in the text and tables are mean ± SD except when noted.

Results

Plasma pharmacokinetics

We studied the disappearance of total radioactivity and uridine-specific radioactivity in the plasma of four patients with confirmed malignancies not involving the liver. Patients with known liver disease were excluded; if the human body handles uridine in the same manner as has been reported in rodents, then liver disease may unpredictably alter the observed pharmacokinetic parameters. The characteristics of these four patients are presented in Table 1. The disappearance of total plasma radioactivity, which includes both uridine and its metabolites, as well as of radioactivity specifically associated with uridine are depicted

Table 1. Patient characteristics

Patient	Sex	Age (years)	Weight (kg)	Tumor type	ECOG[a]
K. H.	F	40	56.7	ovarian	0
B. S.	M	26	77.1	Hodgkin's	0
K. B.	M	62	72.5	colorectal	0
A. S.	F	63	71.2	ovarian	1

[a] Eastern Cooperative Oncology Group patient performance status

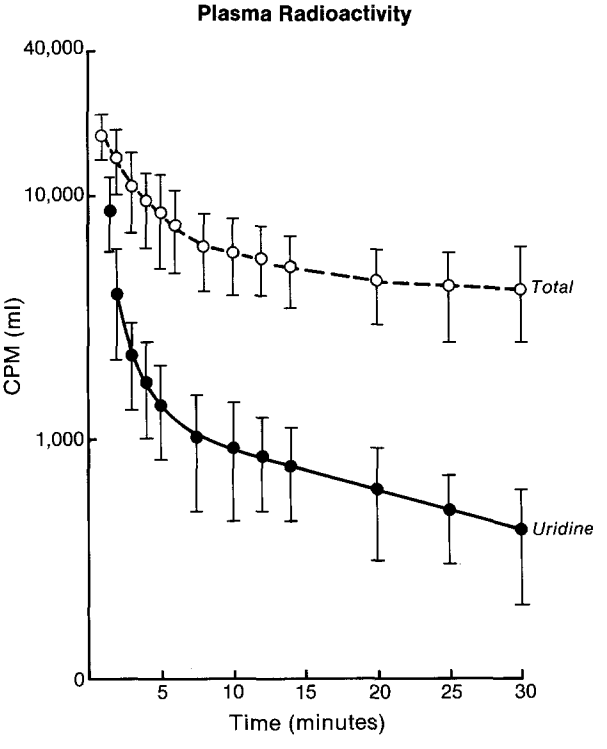

Fig. 1. Averaged total plasma radioactivity (○) and uridine-associated radioactivity (●) over time in the four cancer patients who received (5,6-^{3}H) uridine as a rapid i.v. bolus. Actual blood sampling intervals were more frequent and the sampling periods were extended to beyond 1 h. The bars represent one standard deviation from the mean value

Table 2. Uridine pharmacokinetic parameters*

Patient	Vd (ml/kg)	Half-life (min)			Clearance (TB)
		a	b	c	
K. H.	606.2	0.54	2.59	26.4	1.08 l/min
B. S.	457.6	0.18	1.13	12.8	1.90 l/min
K. B.	448.5	0.77	1.52	20.6	1.79 l/min
A. S.	413.8	0.79	1.95	10.2	2.04 l/min

* Values are calculated from fitted curves using the model-independent pharmacokinetic curve-fitting program RSTRIP

Table 3. Plasma and marrow uridine concentration in normal volunteers

Donor	Sex	Uridine concentration (μM)		
		Plasma	Marrow	Ratio[a]
J. G.	F	2.60	6.72	2.6
A. W.	F	1.60	9.60	6.0
C. M.	M	2.48	7.32	3.0
R. R.	M	2.00	9.00	4.5
C. F.	F	3.44	22.10	6.4
J. L.	F	1.68	11.80	7.0
J. J.	M	2.41	6.52	2.7
Mean ± SD		2.32 ± 0.58	10.44 ± 5.06[b]	4.6 ± 1.9

[a] Bone marrow uridine:plasma uridine

[b] Significantly different from plasma values; $P < 0.005$, Student's *t*-test

in Fig. 1. Total plasma radioactivity declined in a biphasic manner and was adequately defined by an equation with two exponential terms (MSC = 5 or better in all patients) with a mean initial half-life of 3.9 ± 2.1 min and a mean terminal half-life 77 ± 15 min. It is apparent that uridine was very rapidly converted to metabolites and that even within a few minutes it made up only a minor portion of the total radioactivity present in the plasma. The mean initial half-lives for uridine-associated radioactivity were 0.57 ± 0.28 and 1.79 ± 0.62 min, and the mean terminal half-life was 17.5 ± 7.3 min. The pharmacokinetic parameters for each patient are presented in Table 2. The calculated volume of distribution of uridine averaged to 481 ± 70 ml/kg, which translated to approximately 33 l in a 70-kg person. This value is consistent with the distribution of uridine into total body water. The average total body clearance (CL_{tb}) of uridine was 1.70 ± 0.42 l/min.

Uridine concentration in bone marrow

Table 3 presents the concentration of uridine present in simultaneously obtained marrow and plasma samples in seven healthy volunteers. The uridine concentration in the marrow averaged five times higher than that in the plasma. The mean marrow uridine concentration was 10.44 ± 5.06 μ*M*, whereas the mean plasma uridine concentration was 2.32 ± 0.58 μ*M*.

Discussion

One mechanism by which tumor cells can protect themselves against the cytotoxic affects of inhibitors of the de novo pyrimidine synthetic pathway is by salvaging preformed pyrimidines from their environment [1, 4]. The fact that modulation of cytotoxicity can take place even at very low concentrations of uridine [1, 8] suggests that regional differences in uridine concentration, and factors that alter plasma uridine, are important determinants of pyrimidine antimetabolite activity. In this pilot study we examined the rate of disappearance of radiolabeled uridine following i.v. injection and measured the steady-state concentration of uridine in the bone marrow relative to the plasma in human subjects.

When given in trace amounts, radiolabeled uridine disappears very quickly from the plasma in a triphasic fashion, with a mean terminal half-life of approximately 15 min. It was also apparent that within a few minutes the majority of the injected uridine had been converted to metabolites, indicating that the turnover of uridine at physiologic concentrations is extremely rapid. We did not characterize the radioactive metabolites of uridine in this study because of the need to use higher doses of radioactivity in the patients. Uracil is likely the first metabolite in the catabolism of plasma uridine, since previous studies using high doses of nonradiolabeled uridine have reported elevations in plasma uracil immediately following uridine administration [9, 12]. Although the number of patients studied here was small, as is customary for nontherapeutic investigations, the same pattern of plasma clearance is evident in all of them. It is possible that there are additional half-lives for plasma uridine not detected in our present study due to limited radiometric sensitivity. The concurrent observation that total plasma radioactivity was cleared at a much slower rate (half-life of 80 min) in these patients, however, suggests that we were seeing rapid catabolism of the nucleoside. Levya et al. [9] have reported a mean terminal half-life of 118 min in patients given 1–12 g/m^2 uridine i.v., doses that produced millimolar concentrations in the plasma. Some patients in the Levya study also received 5-fluorouracil concurrently. Although these authors have reported that the peak plasma concentrations and AUC varied linearly with dose, there was a trend in their data toward longer terminal half-lives in courses where larger amounts of uridine were infused. The different terminal half-life values between the study reported by Levya et al. and the present study may reflect the clearance of physiologic concentrations of uridine vs the clearance of high concentrations of the same nucleoside.

The very rapid turnover of plasma uridine observed in our patients is consistent with the results of studies done on the rat [10] and the dog [11]. Since the calculated clearance values for our cancer patients are greater than the known blood flow to any single organ, our data suggest multiple sites of metabolism or uptake of plasma uridine in humans, in contrast to the rat model, where the liver was identified as the main site of both catabolism and synthesis of uridine [3]. The bone marrow uridine concentrations averaged 4.6 times higher than plasma uridine concentrations (range, 2.6- to 7.0-fold) in our healthy volunteers. The reason for this difference is not immediately apparent, but it indicates that a concentration gradient exists between the marrow and the plasma, suggesting that either marrow elements are synthesizing uridine and exporting it to the plasma or preformed uridine is being locally released from blood cells that circulate to the marrow. In

any case, the local concentrations of uridine in the marrow are in the range that will antagonize the activity of PALA in tissue culture [2, 7], and this may provide an explanation for why PALA produces less myelosuppression than antimetabolites not antagonized by uridine. The published concentrations of plasma uridine in cancer patients are in the range of 3–8 μ*M* [2, 6, 9], and in a small number of patients we have found that plasma uridine concentrations are quite stable when measured on serial days [2]. This implies that the plasma uridine is so tightly regulated by some control mechanism that the plasma concentration remains within a relatively narrow range. The nature of such regulatory mechanism(s) and the physiologic significance of plasma uridine concentrations in human cancer remain to be elucidated through additional clinical investigations.

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