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Comparison of the bioavailability of uridine in mice after either oral or parenteral administration

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Summary. We compared the bioavailability of uridine (Urd) (350 and 3500 mg/kg) administered either as a single SC injection or by gavage, in male $CD8F_1$ mice. Plasma samples were analyzed for Urd and uracil (Ura) using high-pressure liquid chromatography. After Urd (3500 mg/kg, SC), plasma Urd levels peaked at 4900 μM and then declined to pretreatment levels (< 10 μM) within 6 h. Plasma Ura concentrations peaked at 1400 μM and then declined initially more slowly than Urd. After Urd (3500 mg/kg, PO) plasma levels of Urd were fairly constant (range 33–82 μM) for up to 8 h and had returned to pretreatment levels at 16 h. Plasma Ura concentrations paralleled Urd, but were approximately ten-fold higher. Areas under the concentration-time curve for Urd showed that the bioavailability of Urd after PO administration was 7% of that after SC administration. After Urd (350 mg/kg, SC) Urd levels peaked at 210 μM returning to pretreatment levels within 2 h. Plasma Ura levels reached a peak with 300 μM and then declined initially more slowly than those of Urd. After Urd (350 mg/kg, PO) plasma Urd levels were not perturbed, although Ura levels peaked at 50 μM after which they declined and could no longer be detected at 4 h. These data indicate that (a) the bioavailability of Urd (350 or 3500 mg/kg) was lower when given PO than when it was administered by SC injection; and (b) Urd (3500 mg/kg) PO resulted in prolonged and relatively constant plasma Urd levels compared with Urd (3500 mg/kg) SC. These results suggest that Urd PO should be compared with parenterally administered Urd in attempts to increase the therapeutic index of 5-fluorouracil and of antimetabolite inhibitors of de novo pyrimidine biosynthesis.

Introduction

Marked disruptions in RNA and DNA synthesis following treatment with 5-fluorouracil (FUra) are due to the formation of two anabolites, 5-fluorouridine 5'-triphosphate and 5-fluorodeoxyuridine 5'-monophosphate [8]. In different tumor cell lines either the RNA- or the DNA-directed effects of FUra may be the principal determinants of its cytotoxicity [4, 16, 18, 30]. In normal mouse tissues, the dose-limiting gastroinetstinal toxicity of FUra, 5-fluorouridine, or 5-fluoro-2'-deoxyuridine correlates best with the incorporation of 5-fluorouridine 5'-triphosphate, derived from these fluorinated pyrimidines, into intestinal RNA rather than with levels of 5-fluorodeoxyuridine 5'-monophosphate [9].

The therapeutic index of FUra against mouse solid tumors can be increased by delayed administration of uridine (Urd) [13, 17]. In mice bearing the advanced colon tumor 26, Urd was administered by repeated IP injection, and two Urd schedules, each beginning 2 h after FUra, were successful. Urd was given either for two doses at 3500 mg/kg separated by an 18-h interval or at 800 mg/kg every 2 h for four doses [17]. In studies with mice bearing a day-1 B16 melanoma Urd was administered, beginning 24 h after FUra, according to a 5-day continuous SC infusion at a daily dose of 5 mg/kg [13].

Urd administered to mice by SC injection reverses the toxicity of antimetabolite inhibitors of de novo pyrimidine biosynthesis, including N-phosphonacetyl)-L-aspartate (PALA) and pyrazofurin [1, 10]. In mice bearing a day-1 SC-implanted Lewis lung carcinoma Urd is more effective than carbamyl-DL-aspartate in reversing the toxicity of PALA without completely reversing its antitumor action. In this schedule PALA was given by a single IP injection on days 1, 2, and 3 and Urd was given by SC injection at 350 mg/kg, twice daily, on days 1–18 [10]. In normal, nontumored mice the letahl toxicity of pyrazofurin, given as a single IP injection on days 1–3, was reversed by a single SC injection of Urd at 350 mg/kg on days 1–4 [1].

Clinical studies of the pharmacokinetics of Urd IV infusion and Urd rescue from FUra toxicity have recently been reported. In these studies Urd was administered by a 1-h IV infusion at doses of 1–12 g/m^2 [15].

Whereas parenteral Urd increases the therapeutic index of either FUra [13, 17] or PALA [10] against mouse tumors, there is no information as to the use of Urd given by the oral route in chemotherapy. It is of interest that Urd in the drinking water (20 mg/ml) of pregnant mice decreased PALA-induced embryotoxicity [26]. Oral Urd (1.9–5.6 g/m^2 per day) is also effective as replacement therapy in hereditary orotic aciduria, which is caused by a deficiency of orotate phosphoribosyl-transferase and/or orotidine 5'-phosphate decarboxylase [7].

The pharmacokinetics and physiological disposition of pharmacological doses of Urd, administered either parenterally or orally, remains to be fully defined. Therefore, we compared the bioavailability of Urd given by the oral and parenteral routes in mice at doses which, when administered parenterally, had been shown to increase the thera-

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peutic index of FUra or PALA and protect against the lethal toxicity of pyrazofurin. Such information might then allow for the development of rational dosage schedules, which could be used to test the effectiveness of oral Urd as a rescue agent to increase the therapeutic index of FUra, PALA, or pyrazofurin.

Materials and methods

Drugs and chemicals. Urd, Ura, 5-methyl cytidine, and tri-*n*-octylamine were purchased from Sigma Chemical Co. (St. Louis, Mo). Ammonium acetate (certified A.C.S.), sodium acetate (certified A. C. S.), 1,1,2-trichlorotrifluoroethane (reagent grade), and trichloroacetic acid (reagent grade) were obtained from Fisher Scientific Co. (Fairlawn, NJ).

Animals. Male BALB/c × DBA/2 ($CD8F_1$) mice weighing 18–24 g were obtained from the Animal Genetics and Production Branch, National Cancer Institute. Animals were caged in an air-conditioned room lighted from 6 a. m. to 6 p. m. and had free access to standard Purina chow and tap water. Mice were given free access to water but were fasted overnight prior to the experiment.

Plasma Urd levels. Urd was dissolved in 0.9% NaCl solution just prior to use at a concentration which permitted its administration at 0.01 ml/g mouse body weight. Mice each received Urd (350 or 3500 mg/kg) as either a single SC injection or a single oral dose by gavage. Fasted, untreated mice were taken as zero-time controls. At each time point, as indicated, five mice per group were sacrificed by decapitation, and individual blood samples were each collected in a Microfuge tube containing 50 µl heparin (0.1 mg/ml) in phosphate-buffered saline, pH 7.4. The samples were immediately centrifuged and individual plasma samples (50–250 µl) obtained. Each plasma sample was then made up to 0.5 ml with water to which was added 0.5 ml 10% TCA containing 5 nmol internal standard (5-methyl cytidine). The resulting suspension was centrifuged at 23 000 *g* for 5 min. The supernatant was transferred to another tube and vortexed with an equivalent volume of a 2:1 solution of 1,1,2-trichlotrifluoroethane and tri-*n*-octylamine. The tubes were centrifuged at 1000 *g* for 5 min, and the top layer containing the nucleosides was collected. The samples were then stored at −70 °C for subsequent analysis.

High-pressure liquid chromatography. A 100-µl aliquot of each sample was injected by a WISP 710B automatic injector (Waters Associates, Milford, Mass) onto an Altex model 312 high-pressure liquid chromatograph equipped with a Radial-PAK (C-18, reverse-phase) column (Waters Associates) in a radial compression module (Waters Associates). The samples were eluted with acetate buffer (10 m*M* sodium acetate plus 10 m*M* acetic acid) containing 0.05% triethylamine, pH 4.5, at 1.5 ml/min. Under these conditions the Ura, Urd, and 5-methyl cytidine peaks were eluted at 5, 11 and 17 min, respectively. Between each run the column was washed for 15 min with 50% methanol. Peak heights were recorded at both 254 nm (Altex model 153 UV detector) and 280 nm (Altex model 155-30 variable wavelength UV detector). The ratio of corresponding peak heights at each wavelength, when constant, indicated the absence of contaminating substances. The concentrations of Urd and Ura in each sample were calculated by comparing their peak heights against those of Urd and Ura standards which were injected in a 100-µl aliquot. Plasma samples were diluted so that the amounts of Urd and Ura injected onto the column were in the 0–25 nmol range. Standard curves for both Urd and Ura were linear from 0 to 25 nmol injected. The 5-methyl cytidine used as an internal standard consistently verified a greater than 95% recovery. Additional details of the chromatography procedure have been published elsewhere [11].

Results

Figure 1 shows the levels of Ura and Ura in plasma of mice given a single dose of Urd (3500 mg/kg) either by SC injection or PO. When Urd was administered SC, Urd levels peaked at 4900 µ*M* within 1 h and then declined and had returned to pretreatment levels by 6 h. In contrast, when Urd was administered PO the levels of Urd, which were 65 µ*M* by 0.5 h remained fairly constant (range 33–82 µ*M*) for up to 8 h, and then had returned to pretreatment levels by 16 h. AUC calculations for Urd from 0 to 8 h, by the trapezoidal rule, gave a value of 6640 and 490 µmol l^{-1} h^{-1} for SC and PO Urd, respectively. This indicated that the bioavailability of Urd (3500 mg/kg) after PO administration was 7% of that after SC administration.

When Urd was given SC, peak Ura levels of 1400 µ*M* were seen within 1 h. Between 1400 and 300 µ*M* Ura levels declined more slowly than Urd levels. Ura, which was 30 µ*M* at 6 h, could not be detected at 16 h. When Urd was

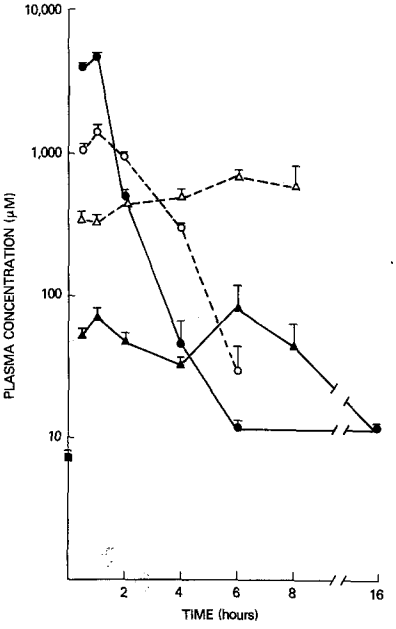

Fig. 1. Effect of Urd (3500 mg/kg, SC or PO) on Urd and Ura plasma levels in mice. Groups of five mice each were sacrificed at each time point indicated, and individual blood samples were collected. Urd and Ura were determined in each sample by HPLC. Untreated mice were taken as zero-time controls. The values obtained at each time point were averaged and the results expressed as the mean ±SE of five mice per group. ●———●, Urd and ○———○, Ura, after Urd, administered SC. ▲———▲, Urd and △———△, Ura, after Urd, administered PO. ■, Urd in zero-time controls

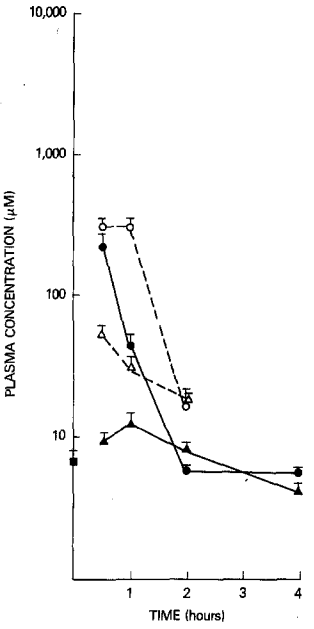

Fig. 2. Effect of Urd (350 mg/kg, SC or PO) on Urd and Ura plasma levels in mice. Groups of five mice each were sacrificed at each time point indicated, and individual blood samples were collected. Urd and Ura were determined in each sample by HPLC. Untreated mice were taken as zero-time controls. The values obtained at each time point were averaged and the results expressed as the mean ±SE of five mice per group. ●———●, Urd and ○———○, Ura, after Urd, administered SC. △———△, Urd and ▲———▲, Ura, after Urd, administered PO. ■, Urd in zero time controls

given PO the levels of Ura paralleled those of Urd but were approximately ten-fold higher (range 330–770 μ*M*) than those of Urd for up to 8 h and then could no longer be detected at 16 h.

Figure 2 shows the levels of Urd and Ura in plasma of mice given a single dose of Urd (350 mg/kg) either by SC injection or PO. When Urd was administered SC, peak Urd levels of 210 μ*M* were seen within 0.5 h, then declined and had returned to pretreatment levels within 2 h. After Urd was given PO, the plasma levels of Urd were essentially unchanged, which indicated a decreased bioavailability of Urd following PO compared with SC administration.

When Urd (350 mg/kg) was given SC, levels of Ura plateaued at 300 μ*M* at 0.5 and 1 h, then declined rapidly by 2 h and could no longer be detected at 4 h. When Urd was given PO, peak Ura levels of 50 μ*M* were seen at 0.5 h; the levels then declined and could no longer be detected by 4 h. At 0.5, 1, and 2 h the levels of Ura were higher than those of Urd, which never exceeded the physiological range.

AUC calculation for Urd from 0 to 2 h after Urd was given SC at either 3500 mg/kg (Fig. 1) or 350 mg/kg (Fig. 2) gave values of 5990 and 144 μmol/l^{-1} h^{-1}, respectively. In addition, following Urd 3500 mg/kg, SC the peak levels of Urd were higher than those of Ura (Fig. 1), whereas after Urd 350 mg/kg, SC peak levels of Ura were higher than those of Urd (Fig. 2).

Discussion

The uptake of Urd and other naturally occurring nucleosides is a carrier-mediated process [24]. Measurement of the transport of Urd across the rat jejunum shows that Urd does not undergo any metabolic alteration in crossing from the mucosal to serosal side [5, 22]. We found that the bioavailability of Urd (350 or 3500 mg/kg) was markedly lower after administration PO than after SC injection of the same doses of Urd. However, Urd (3500 mg/kg) PO resulted in more sustained and relatively constant elevation of plasma Urd levels than did Urd (3500 mg/kg) SC. The prolonged elevation in plasma Urd levels after Urd PO qualitatively resembles the results obtained during IV infusion of Urd to dogs [23]. This suggests that if high doses of Urd PO were to be tested as a rescue agent, e.g., following FUra, PALA, or pyrazofurin, then Urd could be administered at relatively long dosage intervals.

Urd, administered parenterally in tracer doses to rats or mice has a plasma half-life of less than 10 min and is extensively catabolized by the liver [6, 20, 21, 31]. Following IP injection of Urd (3500 mg/kg), plasma Urd levels peaked at approximately 6000 μ*M* and declined to less than 1000 μ*M* by 4 h [17]. Our results were similar in that when Urd at 3500 mg/kg was given by SC injection peak plasma Urd levels of 4800 μ*M* Urd were observed, and levels declined to less then 100 μ*M* by 4 h. It is likely that Urd (3500 mg/kg) SC could be as effective as Urd by IP injection for rescue from FUra toxicity.

The first product in the catabolism of Urd is the formation of Ura. This reaction, which occurs primarily in the liver [6, 19], is mediated by Urd phosphorlyase [14]. The next step, which is rate-limiting, is the reduction of Ura by dihydrothymine dehydrogenase [25]. The approximately ten-fold higher levels of Ura than of Urd which we observed in mouse plasma after Urd (3500 mg/kg) PO were presumably due to the action of Urd phosphorylase, which rapidly cleaves Urd entering the liver via the portal circulation [6, 19]. The sustained and relatively constant plasma levels of both Urd and Ura which were seen after Urd (3500 mg/kg) PO may be the result of two effects: (a) a prolonged systemic absorption of Urd resulting from saturation of Urd transport across the gastrointestinal mucosa; and (b) a continuous presystemic metabolism of Urd to Ura. It is also possible that Ura catabolism by dihydrothymine dehydrogenase was saturated [25]. For example, in patients given a 1-h infusion of Urd (5–12 g/m^2), plasma Ura concentrations increased during the infusion and either plateaued or continued to increase slightly (in the range 100–300 μ*M*) for up to 4 h after the end of the infusion, indicating saturation of Ura catabolism [15]. It is of interest that the catabolism of thymine, which uses the same pathways as Ura, approaches saturation near millimolar plasma levels [3].

AUC calculations of Urd from 0 to 2 h after Urd was given SC at 3500 or 350 mg/kg gave a ratio (42 : 1) which was substantially greater than the ratio of the doses of Urd which were administered. This indicated that after Urd (3500 mg/kg) was administered SC, at least initially its catabolism by Urd phosphorylase was saturated. This possibility was further strengthened by our observation that after Urd (3500 mg/kg) SC peak levels of Urd exceeded those of Ura whereas the reverse was true after Urd (350 mg/kg) SC.

We showed that physiological levels of plasma Urd were not affected after Urd (350 mg/kg) PO. However, Urd (350 mg/kg) SC increased plasma levels of Urd approximately 20-fold and they then returned to pretreat-

ment levels within 2 h. Urd (350 mg/kg, SC) given twice daily for 18 days increased the therapeutic index of PALA in mice bearing an SC-implanted Lewis lung carcinoma [10]. Urd (350 mg/kg) SC given once daily for 4 days protected normal, non-tumor-bearing mice against the lethal toxicity of pyrazofurin [1]. As Urd (350 mg/kg) PO did not elevate plasma Urd levels it appears that higher oral doses of Urd, e.g., 3500 mg/kg or greater, would be indicated in any attempts to increase the therapeutic index of FUra, PALA, or pyrazofurin.

The mechanism by which Urd reverses the toxicity of PALA is presumably due to the utilization of Urd by the salvage pathway, which replenishes the pyrimidine nucleotide pools and bypasses the drug-induced inhibition of de novo pyrimidine biosynthesis [1, 10]. In contrast, the mechanism by which Urd rescue increases the therapeutic index of FUra has not been defined [13, 17]. It is known that FUra nucleotides persist in cells and tissues for up to 3 days after treatment with FUra [2, 29]. Administration of exogenous Urd to mice should expand the intracellular Urd nucleotide pool [12, 17], which could prevent further utilization of persisting FUra nucleotides as well as enhancing recovery of RNA and DNA synthesis in host-sensitive tissue. It is also possible that exogenous Urd could exploit any differences which might exist in the RNA- and DNA-directed actions of FUra between tumor and host sensitive tissues. In mice bearing the colon tumor 26, examination of the effect of Urd rescue on the incorporation of FUra into RNA and the subsequent recovery from inhibition of DNA synthesis in bone marrow versus tumor indicates a relatively faster clearance of FUra-RNA from both tissues, but the rate of recovery DNA synthesis is markedly enhanced only in bone marrow [17].

At this time it is not clear what plasma levels of Urd are necessary to reverse the toxicity of FUra, PALA, or pyrazofurin to normal tissues. Reversal of the growth-inhibitory effects of PALA [12, 27, 28] and pyrazofurin [1] against tumor cells in culture can be achieved with Urd at 100 and 32 μM, respectively. Urd (350 mg/kg) SC is effective in reversing the lethal toxicity of either PALA [10] or pyrazofurin [1] in mice. We found that Urd (350 mg/kg) SC produced peak Urd plasma levels of 200 μM. With L1210 cells in culture, Urd at 20, 100, or 400 μM expands the Urd nucleotide pool approximately 50%, 100%, and 130%, respectively [12]. We found that Urd (3500 mg/kg) PO resulted in prolonged Urd plasma levels of 33–82 μM, concentrations which are likely to cause expansion of tissue Urd nucleotide pools. Whether Urd PO at a dose of 3500 mg/kg or higher would be effective in improving the therapeutic index of FUra, PALA, or pyrazofurin remains to be determined.

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