Preclinical Evaluation of 5-Iodo-2-pyrimidinone-2'-deoxyribose as a Prodrug for 5-Iodo-2'-deoxyuridine-mediated Radiosensitization in Mouse and Human Tissues

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ABSTRACT

We reported previously that p.o. administered 5-iodo-2-pyrimidinone-2'-deoxyribose (IPdR) was efficiently converted to 5-iodo-2'-deoxyuridine (IUdR) in athymic mice (T. J. Kinsella et al., Cancer Res., 54: 2695-2700, 1994). Here, we further evaluate IPdR metabolism, systemic toxicity, and percentage DNA incorporation in athymic mouse normal tissues and a human colon cancer xenograft (HT29) using higher p.o. doses of IPdR. These data are compared to results using a continuous infusion of IUdR at the maximum tolerable dose. We also evaluate IPdR metabolism in cytotoxic extracts from normal human liver, normal human intestine, and human colorectal cancer specimens.

Aymic mice tolerated a daily p.o. bolus of up to 2 g/kg IPdR for 6 days with minimal host toxicity (<10% body weight loss). There was rapid conversion of IPdR to IUdR, with peak plasma levels of IUdR of 40-75 μM at 10 min following a p.o. IPdR bolus of 250-1500 mg/kg. The percentage IUdR-DNA in the HT29 s.c. human xenografts increased 1.5 times (2.3-3.6%) with IPdR doses above 1 g/kg/day for 6 days, whereas the percentage IUdR-DNA incorporation in two proliferating normal tissues (4-4.5% in intestine; 1.6-2.2% in bone marrow) and a quiescent normal tissue (≤1% in liver) showed <1.5-fold increases with the IPdR dose escalation between 1-2 g/kg/day for 6 days. In contrast, using a continuous infusion of IUdR at 100 mg/kg/day, significant systemic toxicity (>20% body weight loss) was found by day 6 of the infusion. Steady-state plasma IUdR levels were 1.0-1.2 μM during the 6-day infusion, and percentage IUdR-DNA incorporations of 2.3, 8, 6, and 1% were measured in s.c. tumors, normal intestine, normal bone marrow, and normal liver, respectively, following the 6-day infusion. Thus, the p.o. IPdR schedule has an improved therapeutic index, based on percentage IUdR-DNA incorporation in normal and tumor tissues, compared to continuous infusion IUdR at the maximum tolerable dose in athymic mice with this human tumor xenograft. Additionally, a tumor regrowth assay to assess the radiation response of HT29 s.c. xenografts showed a 1.5-fold enhancement (time to regrowth to 300% initial tumor volume) with IPdR (1000 mg/kg/day for 6 days) plus fractionated irradiation (XRT; 2 Gy/day for 4 days), compared to XRT (2 Gy/day for 4 days) alone. No enhancement in the radiation response of HT29 s.c. xenografts was found with continuous infusion IUdR (100 mg/kg/day for 6 days) plus XRT (2 Gy/day for 4 days), compared to XRT alone.

Using cytosolic extracts from normal human liver specimens, we found a rapid (15-min) conversion of IPdR to IUdR. Coincubation of liver cytosol with IPdR and allopurinol, an inhibitor of xanthine oxidase, had no inhibitory effect on IPdR metabolism, whereas coincubation with IPdR and isovannilin or menadione, analogue substrates for aldehyde oxidase, selectively reduced the amount of IPdR oxidized to IUdR. Significantly less metabolism of IPdR to IUdR was seen in cytosolic extracts from normal human intestine specimens, and no metabolism of IPdR was found in cytosolic extracts from colorectal liver metastases in two patients and from the HT29 human colon cancer xenografts in athymic mice.

These additional data indicate that IPdR has the potential for clinical use as a p.o. prodrug for IUdR-mediated radiosensitization of resistant human cancers.

INTRODUCTION

The pyrimidinone nucleosides were initially developed as antiviral agents, based on the hypothesis that nucleosides without an amino group or oxygen at position 4 would be substrates for viral but not mammalian TdR kinase (1). IPdR was found to have significant activity in herpes simplex virus-infected HeLa cells in vitro and in vivo following p.o. administration without toxicity to uninfected cells or mice (2, 3). Although initial studies suggested that p.o. IPdR did not require metabo-
lism to IUdR for antiviral activity (2, 3), more recent studies by the same investigators demonstrated an aldehyde oxidase, which is present in both rat and mouse liver, that efficiently converts IPdR to IUdR (4). Other normal tissues in the rat and athymic mouse including intestine, bone marrow, lung and kidney show 1 to 2 log less activity of IPdR aldehyde oxidase (4, 5). These findings led Cheng and collaborators (4) to postulate that IPdR might increase the percentage IUdR-DNA incorporation and subsequent radiosensitization of actively proliferating primary or metastatic tumors in liver, while minimizing drug toxicity and/or radiosensitization to the adjacent quiescent normal liver parenchyma and possibly other rapidly proliferating normal tissues, including bone marrow and intestine.

In a recent publication, we tested Cheng’s hypothesis using an athymic mouse model where a human colon cancer cell line was established as a xenograft in s.c. flank tissue and/or as liver metas-
tases. The colon cancer cell line was HCT29. We also evaluated the metabolism of IPdR in cytosolic extracts from normal human intestine, from normal human liver, and from two patient specimens of colorectal cancer metastatic to liver. Previous investigations reported that cytosolic extracts from normal liver in rats catalyzed the oxidation of IPdR to IUdR (2-4). Additionally, it was found, based on a series of enzyme inhibition and substrate competition studies, that the IPdR oxidation product in rat liver was attributable to aldehyde oxidase but not xanthine oxidase (2-4). A similar analysis of IPdR oxidation was carried out in this study using human normal and tumor tissues and using coinubation of IPdR with menadione or isovanillin as selective inhibitors of aldehyde oxidase or coinubcation with allopurinol as a selective inhibitor for xanthine oxidase (14-17).

We also evaluate the metabolism of IPdR in cytosolic extracts from normal human intestine, normal human liver, and from two patient specimens of colorectal cancer metastatic to liver. Previous investigations reported that cytosolic extracts from normal liver in rats catalyzed the oxidation of IPdR to IUdR (2-4). Additionally, it was found, based on a series of enzyme inhibition and substrate competition studies, that the IPdR oxidation activity in rat liver was attributable to aldehyde oxidase but not xanthine oxidase (2-4). A similar analysis of IPdR oxidation was carried out in this study using human normal and tumor tissues and using coinubation of IPdR with menadione or isovanillin as selective inhibitors of aldehyde oxidase or coinubcation with allopurinol as a selective inhibitor for xanthine oxidase (14-17).

Our group and others have shown the potential of selective IUdR (or the related halopyrimidines, BUDR) in the radiosensitization of resistant human tumors in clinical trials of patients with unresectable liver metastases from colorectal primaries (10, 18, 19). This patient group, along with patients with high-grade brain tumors (12) and sarcomas (13), would seem appropriate for future clinical trials of p.o. IPdR radiosensitization.

**MATERIALS AND METHODS**

**Drugs and Chemicals.** IPdR was synthesized and provided by Sparta Pharmaceuticals, Inc. (Research Triangle Park, NC). IPdR was dissolved in water and sterile-filtered. IUdR (Sigma Chemical Co., St. Louis, MO) was dissolved in 1 N NaOH and diluted to 0.5 N NaOH at the appropriate concentrations for continuous infusion. Nucleoside standards (IUdR, chlorodeoxyuridine, and TDdR) and enzymes for DNA digestions were obtained from Sigma. Allopurinol, isovanillin, menadione, DTT, pepstatin A, phenylmethylsulfonfluoride, and (E)-5-(2-bromovinyl)uracil for the IPdR oxidase assay were also purchased from Sigma. To prepare the cytosol from human tissues, homogenization buffer (0.154 M KCl, 0.1 M Na+ and K+ phosphates, and 1 mM EDTA, pH 7.4), reaction buffer (0.1 M Na+ and K+ phosphates, 1 mM EDTA, and 5.0 mM MgCl2, pH 7.4), and mucosal buffer (0.154 M KCl, 0.1 M Na+ and K+ phosphates, 10 mM DTT, 0.1 mM pepstatin A, 5 mM EDTA, and 0.25 mM phenylmethylsulfonyl fluoride, pH 7.4) were purchased from Quality Biological, Inc. (Gaithersburg, MD). All cell culture media, supplements, and related chemicals were obtained from Life Technologies, Inc. (Grand Island, NY).

**Cell Culture.** The HT29 human colon carcinoma cell line was obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained in α-MEM, supplemented with 10% FBS, nonessential amino acids, glutamine, and gentamicin (α-MEM+), at 37°C in a humidified 5% CO2 atmosphere. Cells were passaged twice a week to maintain exponential growth. The in vitro population doubling time was 22 h for HT29 cells. Cultures were routinely tested and found to be free of mycoplasma contamination by DNA staining.

**In Vivo Systemic Toxicity Studies and Mouse Tissue Sampling.** Six- to 10-week-old female athymic nude mice (20-25 g; Harlan Sprague-Dawley, Madison, WI), with and without HT29 tumor xenografts transplanted to the s.c. tissue of the caudal dorsal flank, were housed under laminar flow ventilation with food and water provided ad libitum. IPdR was...
administered p.o. using a gastric tube, placed once daily, at doses of 500, 1000, 1500, and 2000 mg/kg/day for 6 days. In animal groups receiving IPdR at 1500 and 2000 mg/kg/day, the p.o. dose was delivered slower over 15–20 min to reduce the acute morbidity and mortality of pulmonary aspiration with the larger administered volume. As a control, IUdR was administered as a continuous infusion at 50 and 100 mg/kg/day for 6 days using miniosmotic pumps (Alzet model 2001; Alza Corporation, Palo Alto, CA) implanted s.c. under sterile conditions and general anesthesia (pentobarbital, 55 mg/kg given i.p.) as previously published (8). The miniosmotic pumps have a capacity of 221 μl and a pumping rate of 1 μl/h. In animals receiving p.o. IPdR or continuous-infusion IUdR, weights were monitored daily, along with visual inspection of the mice, to assess morbidity for 4 days before and during the 6-day treatment. Groups of six mice with HT29 xenografts received the various doses of p.o. IPdR or continuous-infusion IUdR for 6 days, as described above. An additional group of six control mice for the IPdR systemic toxicity study received a similar volume of sterile water by gastric tube. A similar group of six control mice for analysis of systemic toxicity of continuous-infusion IUdR received 0.5 N NaOH for 6 days via miniosmotic pumps, as described above. Following the 6-day treatment with p.o. IPdR or the 6-day treatment with continuous-infusion IUdR, mice for the systemic toxicity analyses were euthanized by cervical dislocation. Tissue samples of normal mouse liver, normal mouse intestine, and s.c. tumor xenografts were harvested and frozen in liquid nitrogen. Normal mouse bone marrow was harvested from the femurs of mice by aspirating with α-MEM+, liquid nitrogen. Normal mouse bone marrow was harvested from the femurs of mice by aspirating with α-MEM+, washing twice with PBS and pelleting by centrifugation at 1000 × g for 10 min.

**s.c. Tumor Implantation.** Exponentially growing HT29 tumor cells were detached from tissue culture plates using 0.1% trypsin in PBS-EDTA, suspended in α-MEM+, resuspended in ice-cold PBS, and then counted with an Elzone Particle Counter (Particle Data, Inc., Elmhurst, IL). Two × 10^6 tumor cells in 50 μl of PBS were injected s.c. into the caudal dorsal flank of 6–10-week-old female athymic nude mice. All mice were examined daily, and s.c. tumor dimensions in implanted mice were measured three times weekly. Over 90% of the implanted mice developed palpable HT29 tumors within 7–9 days of s.c. injection. Once the tumors reached a cross-sectional area of 0.25–0.30 cm², the daily p.o. doses of IPdR or the two dose levels of continuous infusion of IUdR were started. At least, two groups of six mice with s.c. HT29 tumors were used for assessment of systemic toxicity and measurement of the percentage IUdR-DNA incorporation into normal and tumor tissues at each IPdR and IUdR dose level.

**Tumor Regrowth Assay.** To assess the extent of in vivo p.o. IPdR-related or continuous-infusion IUdR-related radiosensitization in HT29 s.c. xenografts, a standard regrowth delay assay was used. In the first experiment, mice with s.c. xenografts measuring 0.25–0.30 cm² were randomized to receive either XRT alone (2 Gy/day for 4 days or 4 Gy/day for 4 days on days 3–6 of treatment) or p.o. IPdR (1000 mg/kg/day for 6 days on days 1–6 of treatment) and XRT (2 Gy/day for 4 days on days 3–6 of treatment). Tumor-bearing control mice received either sham irradiation alone (for 4 days) or sham irradiation and p.o. IPdR (1000 mg/kg/day for 6 days). Groups of six mice were treated according to the three treatment schedules or two control schedules. Following the treatment or control schedules, the cross-sectional area of the s.c. tumor was measured three times per week. The time to tumor growth to 300% of the initial tumor volume was used as an end point to measure the effect of treatment.

In a second experiment, groups of six mice with s.c. HT29 xenografts measuring 0.25–0.30 cm² were randomized to receive either XRT alone according to the same schedule as above or to receive continuous-infusion IUdR at the MTD (100 mg/kg/day for 6 days on days 1–6 of treatment) and XRT (2 Gy/day for 4 days on days 3–6 of treatment). Additionally, groups of six tumor-bearing control mice for this experiment received either sham irradiation alone (for 4 days) or sham irradiation and continuous-infusion IUdR at the MTD. The time to tumor growth to 300% of the initial tumor volume was used as the end point to measure the effect of treatment, as above.

For tumor irradiation, mice were restrained without sedation in lead jigs using a cutout to expose the s.c. tumor in the posterior third of the dorsal trunk. Irradiation was performed using a Philips RT 250 unit (Philips Medical Systems Inc., Shelton, CT), operating at 250-kV potential and 15 mA using a 0.2-mm copper filter with a dose rate of 2.27 Gy/min. To improve dose homogeneity, s.c. tumors were irradiated using opposed fields with equal weighting. Sham-irradiated animals were handled in a similar fashion.

**Statistical Analysis of Tumor Regrowth.** To estimate the time at which 300% growth was achieved, we used linear interpolation of the tumor growth data. Using that line, the day at which 300% growth was attained was computed. Our results were not sensitive to the number of points chosen for the linear regression.

The time to 300% growth was compared among the five treatment schedules from either the p.o. IPdR experiment or the continuous-infusion IUdR experiments using an ANOVA model. The model included a term to account for the particular day on which the experiment was performed. Pairwise comparisons were conducted using least-squares means, which account for the day on which the experiment was performed. In particular, we used Fisher’s least significant difference method for pairwise tests of the means (20). Fisher’s procedure requires a statistically significant overall ANOVA F test prior to pairwise comparison. Plots of the residuals were examined to assess the validity of standard ANOVA assumptions.

A SER was calculated as a simple ratio of the mean growth delay (time to 300% initial tumor volume) for IPdR (or IUdR) plus XRT (2 Gy/day for 4 days) to the mean growth delay for XRT alone (2 Gy/day for 4 days). To compute the SER, we used a natural logarithmic transformation on time to 300% growth. We then performed a two-sample t test on these logarithmic values. An advantage of using the logarithmic values is that we can derive confidence intervals directly for the SER. More precisely, the point estimate and confidence interval for the logarithmic-scale difference in means between the two treatments can simply be exponentiated to obtain the point estimate and confidence interval for the SER. Note that, with this method, the point estimate of the SER is the ratio of the geometric mean time to 300% growth in each group.

All analyses were performed in SAS using the GLM procedure (21).
Digestion of DNA from Mouse Tissues. Approximately 500 mg of s.c. tumor, normal liver, and normal intestinal tissues were minced separately in Petri dishes containing 2.0 ml of ice-cold PBS. Bone marrow aspirates were similarly processed. Released cells were pipetted into a culture tube on ice. The Petri dish was washed with 1 ml of PBS, and the cell suspensions of a particular tissue were combined. Cells were pelleted by centrifugation at 1000 x g at 4°C for 10 min. DNA was extracted and digested by the method of Belanger et al. (22). The digestion mixture was incubated overnight at 37°C. Four hundred-μl samples were ultrafiltered using Millipore Ultrafree-MC units (Millipore, Bedford, MA).

HPLC Nucleoside Analysis of IUdR-DNA Incorporation in Xenograft Tumor and Normal Mouse Tissues. HPLC analyses of IUdR replacement of TdR in DNA and plasma levels of IPdR, IUdR, and their metabolites, IP and IU, were performed with the use of a Waters 600E solvent delivery system on a 3.9 x 300 mm μBondapak reverse-phase column (Waters Corp., Milford, MA). Peak elution was monitored using a Waters 490E UV detector. The system was controlled and data were analyzed using Millenium Chromatography Manager, version 2.10.

Analysis of IUdR replacement of TdR in DNA was performed using the method of Belanger et al. (22). The mobile phase consisted of 100 mM sodium acetate buffer (pH 5.45) plus 7% (v/v) ACN. TdR and IUdR were detected at 290 nm. Peak identification and quantitation were performed against authentic nucleoside standards. The percentage IUdR-DNA incorporation was calculated as: [fr][IUdR][fd][TdR] + [IUdR][fr] x 100.

Plasma Levels of IPdR, IUdR, and Their Metabolites. Female athymic mice (20–25 g) were given IPdR as a single p.o. bolus at doses of 250, 500, 1000, and 1500 mg/kg. Groups of six mice were anesthetized with diethyl ether at 5, 10, 20, 45, 90, and 120 min posttreatment. The mice were then immediately euthanized by exsanguination through cardiac puncture using a 23-gauge needle into a heparinized 1-ml syringe. Typically, 0.6–0.8 ml of blood was recovered using this technique. Plasma was separated by centrifugation at 650 x g for 5 min in 1.5-ml microcentrifuge tubes. Extraction of the nucleoside analogues from the plasma was performed as published previously (5). Plasma analyses of IPdR, IUdR, IU, and IP were conducted as follows. The mobile phase for IPdR and IP analysis consisted of 100 mM sodium acetate buffer (pH 5.45), running a linear 1–8% ACN gradient over 15 min, at a flow rate of 1.0 ml/min. The mobile phase for IUdR and IU analysis consisted of 20 mM sodium acetate buffer (pH 4.0), running a linear 15-min 2–8% ACN gradient, at a flow rate of 1.0 ml/min. Peaks were detected at 290 nm (IUdR and IU) and 335 nm (IPdR and IP), using authentic standards. Typical retention times of IPdR, IUdR, IP, and IU were 21.9, 14.8, 14.0, and 8.3 min, respectively. Seventy % recovery of the nucleoside analogues was achieved using this method.

Human Tissue Procurement and Preparation. Human livers and small intestines that were medically unsuitable for organ transplantation were obtained under the auspices of the Washington Regional Transplantation Consortium (Washington, DC). Livers were placed in ice-cold Eurocollins transplantation buffer (23) and transported to the laboratory on ice. Livers were cut into cubes in a bath of ice-cold Eurocollins buffer, the cubes were placed into 50-ml polypropylene centrifuge tubes, and the tubes were stored at −80°C. Liver tissue was prepared and frozen within 3 h of the cross-clamp time. Small intestines were placed on ice, the lumens of the small intestines were perfused with 5 liters of ice-cold mucosal buffer, and the small intestines were transported to the laboratory on ice. The protocol for preparing small intestine tissue was as follows: the lumens of the small intestines were perfused with an additional 15 liters of ice-cold mucosal buffer, the small intestines were cut into 30-cm sections, the sections were cut longitudinally and placed flat, the mucosal layer was isolated by scraping, and the mucosal tissue was stored in 250-ml polypropylene centrifuge tubes at −80°C. Mucosal tissue was isolated and frozen within 3 h of the cross-clamp time.

Liver cytosols were prepared from frozen human liver samples by the following procedure; all steps were conducted at 4°C. Liver tissue (100 g) was thawed in homogenization buffer, homogenized first in a blender and then with a Dounce homogenizer, and the homogenate was centrifuged at 13,500 x g for 20 min. The resulting supernatant was filtered through gauze and centrifuged again at 13,500 x g for 20 min. The supernatant was centrifuged at 105,000 x g for 60 min, the resulting pellet was suspended in 150 ml of reaction buffer, and the suspension was centrifuged at 105,000 x g for 60 min. The supernatants were combined, and the protein content was determined with a Bio-Rad protein assay kit (Hercules, CA). Cytosol from two human hepatic metastatic tumor samples and the HT29 xenograft tumors were prepared by an identical procedure, except tissue was homogenized with a Dounce homogenizer only.

Small intestine cytosols were prepared from frozen human mucosal tissues by the following procedure; all steps were performed at 4°C. Human mucosal tissue (500 ml) was homogenized with a Dounce homogenizer, the homogenate was placed into 250-ml centrifuge bottles and centrifuged at 13,500 x g for 20 min, and the supernatant was decanted and saved. The pellet was homogenized with a Dounce homogenizer, and the homogenate was centrifuged again at 13,500 x g for 20 min. The supernatants from the 13,500 x g centrifugations were combined and centrifuged again at 13,500 x g for 20 min. The resulting pellet was discarded, and the supernatant was centrifuged at 105,000 x g for 60 min. The protein content of the resulting supernatant was determined with a Bio-Rad protein assay kit.

Cytosolic Incubations and IPdR Oxidase Assay. All cytosolic incubations were conducted in 50 mM Tris buffer (pH 7.5) and 1 mM EDTA and were incubated at 37°C for 15 min. Human liver, small intestine, or human tumor cytosolic protein samples (0.2–1.0 mg/ml) were incubated with IPdR (100 μM), in the presence or absence of TdR (1 mM), with menadione (10 μM), isovanillin (100 μM), or allopurinol (100 μM). Isovanillin (10 mM) was dissolved in reagent-grade ethanol, and 10 μl of this solution was added to 990 μl of reaction mixture to give a final ethanol concentration of 1% (v/v); control incubations contained 1% ethanol (v/v), which did not affect IPdR oxidase activity, when compared with control incubations containing no ethanol. Incubations were stopped by the addition of 5 ml of internal standard [0.2 μM 5-chloro-2'-deoxyuridine and 0.2 μM (E)-5-(2-bromovinyl)uracil in ACN], protein was removed by centrifugation, and the samples were dried with a Savant Speed-Vac. Samples were dissolved in 100 μl of water, and 10 μl of these solutions were analyzed by HPLC for IPdR oxidase activity. HPLC analyses of incubation samples were conducted...
with a Hewlett Packard 1050 system (Palo Alto, CA) coupled to a Keystone BDS-Hypersil-C18 column (2.0 × 150 mm; 5-μm particle size), fitted with a Keystone BDS-Hypersil-C18 Javelin precolumn (Bellafonte, PA). Samples were eluted with 25 mM potassium phosphate buffer (pH 6.5) and ACN as follows: the initial eluent profile was held at 100% 25 mM potassium phosphate buffer (pH 6.5) for 5 min; the ACN content was increased linearly to 20% over 20 min and then increased linearly to 40% over 10 min; the eluant was held at 40% ACN-60% 25 mM potassium phosphate buffer (pH 6.5) for 5 min; and the ACN content was then decreased linearly to 0% in 5 min, and the column was equilibrated with 100% 25 mM potassium phosphate buffer (pH 6.5) for 10 min. The absorbance of the eluate was monitored at 290 nm, and UV absorption spectra were recorded at 210–450 nm. Samples were quantified with standard curves for IUdR and IU; standards were prepared by an identical procedure as the experimental samples. All solutions of IPdR were made fresh to minimize degradation to its cyclized form (1).

RESULTS

The systemic toxicity of IPdR given as a daily p.o. bolus for 6 days over the range of 0.5–2 g/kg/day is presented in Fig. 1A. Using a change in the percentage body weight during treatment as an index of systemic toxicity, female athymic nude mice tolerated the higher doses of IPdR (>1000 mg/kg/day) with ≤10% body weight change. In comparison (Fig. 1B), mice tolerated a continuous infusion of IUdR at 50 mg/kg/day for 6 days with 10–15% weight loss but experienced ≥20% weight loss by day 6 using 100 mg/kg/day. By definition, the MTD of IUdR given by continuous infusion for 6 days was established at 100 mg/kg/day. In a previous study, we determined the MTD of IUdR given as a daily p.o. bolus for 6 days to be 250 mg/kg/day (5). We did not reach the MTD for a single, daily p.o. bolus of IPdR with this present dose escalation and 6-day schedule and elected no further escalation (>2000 mg/kg/day) because of increased risk of pulmonary aspiration with larger volumes and our interpretation of the percentage IUdR-DNA incorporation into normal and tumor tissues, as detailed below.

Fig. 2 shows the percentage IUdR-DNA incorporation (mean ± SD) into two normal proliferating mouse tissues [intestine (Fig. 2A) and bone marrow (Fig. 2B)] and a normal quiescent mouse tissue [liver (Fig. 2C)] at day 6 following the 4 dose levels of p.o. IPdR and the MTD dose level (100 mg/kg/day for 6 days) of continuous-infusion IUdR. In normal mouse intestine (Fig. 2A), the percentage IUdR-DNA incorporation increased from 2.5 ± 0.27% to 4.0 ± 0.33% with dose escalation of IPdR of 500–1000 mg/kg/day. However, there was little increase at 1500 (4.0 ± 0.34%) and 2000 mg/kg/day (4.5 ± 0.32%). For comparison, continuous-infusion IUdR using the MTD schedule resulted in an approximate 2-fold higher IUdR-DNA incorporation (7.9 ± 0.68%) in normal mouse intestine. In normal mouse bone marrow (Fig. 2B), IPdR doses of 500–2000 mg/kg/day resulted in 1.3 ± 0.15% to 2.4 ± 0.20% incorporation, whereas the percentage IUdR-DNA incorporation was more than 2-fold higher (6.1 ± 0.68%) using continuous-infusion IUdR. The percentage incorporation in normal mouse liver (Fig. 2C) remained at ≤1% at all IPdR and IUdR doses, reflecting the low proliferative activity in normal liver. The higher percentage DNA incorporation in one or both of these normal proliferating tissues (intestine and bone marrow) may explain, at least in part, the observed weight loss in mice receiving a continuous infusion of IUdR at 100 mg/kg/day for 6 days (Fig. 1B).

In Fig. 2, the percentage IUdR-DNA incorporation in the HT29 s.c. xenografts following p.o. IPdR is also compared with that following continuous-infusion IUdR (Fig. 2D). There appears to be up to a 1.5-fold increase in tumor cell incorporation with high dose (≥1500 mg/kg/day) IPdR for 6 days, compared to continuous-infusion IUdR at the MTD (100 mg/kg/day for 6 days). Using high-dose p.o. IPdR, the percentage IUdR-DNA incorporation ranged from 2.3 ± 0.16% to 3.6 ± 0.21% for
HT29 xenografts. Continuous-infusion IUdR at the MTD resulted in 2.3 ± 0.21% incorporation in the HT29 xenografts. The (steady-state) plasma concentration of IUdR measured during days 1, 3, and 6 of a continuous infusion of IUdR at the MTD ranged from 1.0 ± 0.3 μM to 1.2 ± 0.2 μM.

In Fig. 3A, we compare the effect on tumor growth of HT29 s.c. xenografts using p.o. IPdR, with and without irradiation. The results of four different treatment schedules are compared to a control (no treatment), including: p.o. IPdR alone (1000 mg/kg/day for 6 days); two schedules of XRT alone (2 Gy/day for 4 days or 4 Gy/day for 4 days); and the combination of IPdR, given on days 1–6, and XRT (2 Gy/day for 4 days), given on days 3–6. The time in days (mean ± SE) to reach 3 times (300%) the pretreatment cross-sectional area (day 0) was used to measure the effect of the different treatments. Significant effects on tumor growth delay were found in HT29 s.c. xenografts with the different XRT, with and without IPdR, treatment schedules but not with IPdR alone (P = 0.06). A significant effect on growth delay was found with XRT alone in both schedules (18.74 ± 1.17 days for 2 Gy/day for 4 days and 31.89 ± 1.34 days for 4 Gy/day for 4 days; P < 0.001) compared to untreated controls (9.67 ± 0.72 days). The combination of p.o. IPdR plus XRT (2 Gy/day for 4 days) to XRT (2 Gy/day for 4 days) alone is 1.53 for HT29 xenografts, with a 95% confidence interval of 1.27–1.85.

In Fig. 3B, we compare the effect on tumor growth of HT29 s.c. xenografts using continuous-infusion IUdR at the MTD (100 mg/kg/day for 6 days), with and without irradiation. The results of four different treatment schedules are compared to a control (no treatment) including: continuous-infusion IUdR alone; two schedules of XRT alone (2 Gy/day for 4 days or 4 Gy/day for 4 days); and the combination of continuous-infusion IUdR, given on days 1–6, and XRT (2 Gy/day for 4 days), given on days 3–6. Similar to the analysis for p.o. IPdR, with and without irradiation, the time in days (mean ± SE) to reach 3 times (300%) the pretreatment cross-sectional area (day 0) was used to measure the effect of the different treatments. Significant effects on tumor growth delay were found in these s.c. xenografts with the different XRT, with and without IUdR, treatment schedules but not with IUdR alone (P = 0.06). A significant effect on growth delay was again found with XRT alone in both schedules (22.43 ± 1.35 days for 2 Gy/day for 4 days and 36.41 ± 1.85 for 4 Gy/day for 4 days; P < 0.001), compared to untreated controls (9.72 ± 0.65 days). However, unlike the combination of p.o. IPdR and XRT, as seen in Fig. 3A, no significant effect on growth delay was found for the combination of continuous-infusion IUdR and XRT (23.25 ± 1.65 days for the combination, compared to 22.43 ± 1.35 days for 2 Gy/day for 4 days; P = 0.65). The radiosensitization...
Time (Days)

Fig. 3  A, tumor growth of HT29 s.c. xenografts following treatment with IPdR alone (1000 mg/kg/day for 6 days; ▲) or (4 Gy/day for 4 days; ▲) and the combination of IPdR (days 1–6) and XRT (2 Gy/day for 4 days, days 3–6; ▼). Control s.c. xenografts received no treatment (sham XRT; ●). B, tumor growth of HT29 s.c. xenografts following treatment with IUdR alone (100 mg/kg/day for 6 days; ▲) or (4 Gy/day for 4 days; ▲); (2 Gy/day for 4 days, days 3–6; ▼). Control s.c. xenografts received no treatment (sham XRT; ●). The time to reach 300% tumor volume was determined to be the end point for these tumor regrowth assays. Groups of six animals were included in each treatment or control for both the IPdR and IUdR experiments.

factor, defined above, was 1.02, with a 95% confidence interval of 0.81–1.15.

In Fig. 4, the plasma levels of IPdR, IUdR, and the two principal metabolites, IP and IU, are detailed for up to 2 h following a p.o. bolus of IPdR of 250, 500, 1000, or 1500 mg/kg in athymic mice. Plasma levels of IPdR and IUdR appeared to peak within 10–20 min. The peak plasma levels of IPdR at 20–45 min following p.o. boluses of IPdR of 250, 500, 1000, and 1500 mg/kg were 29.3 ± 7.6, 79.3 ± 20.1, 209.1 ± 70.4, and 251.6 ± 62.5 μM, respectively. The peak plasma levels of IUdR at 10–20 min following a p.o. bolus of IPdR using the four doses, as above, were 40.8 ± 5.4, 67.7 ± 7.3, 69.2 ± 3.6, and 75.6 ± 9.2 μM, respectively. Plasma levels of IUdR decreased more rapidly than did IPdR over the first 90 min. Plasma levels of IP and IU reached a peak within 45–90 min following a p.o. bolus of IPdR (Fig. 4).

Cytosolic extracts from five separate specimens of normal human liver showed high IPdR oxidase activity following a 15-min incubation (Table 1), whereas IPdR oxidase activity was ≥1–2 logarithms less after a 15-min incubation in cytosol extracts from two specimens of normal human small intestine. In addition, IPdR oxidase activity in the normal human liver and small intestine specimens was limited to cytosolic subcellular fractions, protein dependent, and microsomal and cofactor independent (data not shown). In contrast, cytosol extracts from two human colorectal tumors that were metastatic to the liver and from HT29 xenografts in athymic mice showed essentially no measurable oxidation of IPdR to IUdR.

To assess and differentiate between the involvement of aldehyde oxidase and xanthine oxidase in the oxidation of IPdR to IUdR in human tissues, human cytosolic protein from either normal human liver or normal human small intestine was incubated with IPdR in the presence of menadione (10 μM) or isovanillin (100 μM), a selective inhibitor or an analogue substrate, respectively, for aldehyde oxidase, or with albopunnol (100 μM), a selective mechanism-based inactivator of xanthine oxidase (14–17). The aldehyde oxidase inhibitors, menadione and isovanillin, significantly decreased IPdR oxidase activity in
Table 1  Human liver, human small intestine, and human tumor cytosolic IPdR oxidase

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tissue no.</th>
<th>IUdR (nmol/15 min/mg cytosolic protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>10</td>
<td>0.86 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>4.47 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6.52 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>8.31 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>8.93 ± 0.47</td>
</tr>
<tr>
<td>Small intestine</td>
<td>19</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Hepatic metastatic tumor</td>
<td>1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Human tumor xenografts</td>
<td>HT29</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* All incubations were conducted in triplicate and are shown as mean ± SD.

normal human liver cytosol by approximately 75%, whereas the xanthine oxidase inhibitor allopurinol had no inhibitory effect (Table 2). Coincubation of IPdR and TdR (1 mM) with normal human liver cytosolic protein resulted in higher concentrations of IUdR, presumably by inhibiting TdR phosphorylase-mediated IUdR catabolism. In contrast to the inhibitory profiles of menadione, isovanillin, and allopurinol observed in normal human liver, small intestine cytosolic IPdR oxidase activity was increased by menadione and was not significantly effected by isovanillin or allopurinol (Table 3). Additionally, incubation of small intestinal cytosol with both IPdR and TdR had no stimulatory effect on IUdR concentrations (data not shown).

**DISCUSSION**

The TdR analogue, IUdR, has been found to be an effective *in vitro* and *in vivo* radiosensitizing drug, with the extent of radiosensitization (up to 3 times) being directly related to the percentage IUdR-DNA incorporation (TdR replacement) in human tumor cells (6, 24). Although the exact mechanisms of interaction of IUdR with ionizing radiation are not completely understood, experimental data suggest both an enhancement of initial radiation-related DNA damage (principally double-strand breaks), as well as an inhibition of potentially lethal radiation damage repair (6, 24–27). Additionally, radiosensitization in human tumor cells is evident with only unifilar DNA substitution by IUdR. IUdR has been administered as a prolonged continuous i.v. infusion prior to and during X-irradiation, with recent Phase I/II studies suggesting clinically relevant radiosensitization of different types of poorly radioresponsive human tumors, including high-grade gliomas, unresectable and marginally resectable sarcomas, and liver metastases from colorectal carcinomas (10, 12, 13, 18, 19, 28).
corporate IUdR during DNA synthesis (S-phase). As such, one can ±

and in patients with liver metastases from colorectal adenocarcinoma, measured in patients with head and neck squamous cell cancers. Percentage IUdR-DNA incorporation in tumor cells have been also supported by the results of recent clinical trials of IUdR (or BUdR) as a tumor radiosensitizer seems established by the results of these mouse and human studies. A Phase I study of a continuous i.v. infusion of IUdR for 4–6 weeks during an accelerated, hyperfractionated course of radiation therapy in patients with high-grade gliomas is ongoing at the University of Wisconsin (Protocol CO-9392).

IUdR given by bolus infusion is rapidly metabolized, with a plasma half-life of ≤5 min in humans, but adequate steady-state plasma levels (≥1 μM) for radiosensitization can be maintained by prolonged continuous i.v. or intra-arterial infusion (10, 18, 28). A similar steady-state plasma level was maintained in athymic mice during a 6-day continuous infusion of IUdR at 100 mg/kg/day in this study. A prolonged infusion may also be necessary to maximize the proportion of tumor cells that incorporate IUdR during DNA synthesis (S-phase). As such, one can argue that the proportion of tumor clonogens that remain “unlabeled” will dictate the radiocurability of a tumor (29). Using a human tumor xenograft model similar to that used here, we recently reported that ≥95–99% of human colon cancer cells incorporated IUdR in DNA following a continuous exposure for approximately 5 times the tumor potential doubling time (8). Because the tumor potential doubling times of many solid tumors in humans have been measured to be in the range of 2.3% (Fig. 2, A and B). As a probable consequence, athymic mice lost ≥20% body weight at the completion of the infusion (Fig. 1B). We and others have found that further IUdR dose escalation to 200–250 mg/kg/day results in animal deaths with a 4–6-day continuous infusion (8, 32). Thus, the MTD of IUdR is 100 mg/kg/day for 6 days of continuous infusion in this athymic mouse model and results in percentage IUdR-DNA incorporation in the HT29 human tumor xenografts in the range of 2.3% (Fig. 2D). Additionally, we found no significant evidence of radiosensitization using continuous infusion IUdR at the MTD using a growth delay assay (Fig. 3B). The lack of radiosensitization presumably reflects the low percentage IUdR-DNA incorporation. For comparison to humans, a 2-week continuous i.v. infusion of IUdR at 1000 mg/m²/day resulted in significant neutropenia and thrombocytopenia and was associated with a median of 11% IUdR-DNA incorporation in circulating granulocytes (28). The available human data on percentage IUdR-DNA incorporation in.

**Table 2** The effect of selective aldehyde oxidase and xanthine oxidase inhibitors on human liver IPdR oxidase activity (mmol/15 min/mg cytosolic protein)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Specimen 1</th>
<th>Specimen 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPdR</td>
<td>0.05 ± 0.02</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>IPdR + menadione</td>
<td>0.13 ± 0.03</td>
<td>0.11 ± 0.02</td>
</tr>
<tr>
<td>IPdR + isovanillin</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.02</td>
</tr>
<tr>
<td>IPdR + allopurinol</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

*All incubations were conducted in triplicate and are shown as mean ± SD.

Statistically significant (P < 0.05) when compared with control incubations.

IUdR given by bolus infusion is rapidly metabolized, with a plasma half-life of ≤5 min in humans, but adequate steady-state plasma levels (≥1 μM) for radiosensitization can be maintained by prolonged continuous i.v. or intra-arterial infusion (10, 18, 28). A similar steady-state plasma level was maintained in athymic mice during a 6-day continuous infusion of IUdR at 100 mg/kg/day in this study. A prolonged infusion may also be necessary to maximize the proportion of tumor cells that incorporate IUdR during DNA synthesis (S-phase). As such, one can argue that the proportion of tumor clonogens that remain “unlabeled” will dictate the radiocurability of a tumor (29). Using a human tumor xenograft model similar to that used here, we recently reported that ≥95–99% of human colon cancer cells incorporated IUdR in DNA following a continuous exposure for approximately 5 times the tumor potential doubling time (8). Because the tumor potential doubling times of many solid tumors in humans have been measured to be in the range of 3–7 days (30), a continuous infusion of IUdR of at least 2–5 weeks would be required to maximize clinical radiosensitization in these human tumors. Our calculations of the duration of IUdR exposure, based on the athymic mouse-xenograft model (8), are also supported by the results of recent clinical trials of IUdR (or the related halopyrindine, BUdR), given by continuous infusion, in which both the percentage tumor cell labeling and percentage IUdR-DNA incorporation in tumor cells have been measured in patients with head and neck squamous cell cancers and in patients with liver metastases from colorectal adenocarcinomas (18, 19, 31). We reported that a 3-day continuous i.v. infusion of IUdR in patients with liver metastases resulted in 3% IUdR-DNA replacement and 35% labeling of tumor cells, whereas a 7-day continuous i.v. infusion of IUdR in head and neck cancer patients resulted in 7–8% IUdR-DNA replacement and >90% labeling (18, 31). Using a direct hepatic artery infusion of BUdR, a recent study from the University of Michigan reported 10–12% DNA replacement and up to 90% labeling of colorectal liver metastases after 7 days (19). Thus, the clinical rationale for the use of prolonged continuous infusion of IUdR (or BUdR) as a tumor radiosensitizer seems established by the results of these mouse and human studies. A Phase I study of a continuous i.v. infusion of IUdR for 4–6 weeks during an accelerated, hyperfractionated course of radiation therapy in patients with high-grade gliomas is ongoing at the University of Wisconsin (Protocol CO-9392).

Although the clinical responses in these poorly radioreponsive tumors are encouraging, systemic toxicity to rapidly proliferating normal tissues (principally bone marrow and intestine) limits the duration and dose rate of the IUdR infusion (10–13, 28). As a consequence, these normal tissues limit the potential therapeutic gain of IUdR-related tumor radiosensitization, particularly if these normal tissues (e.g., intestine) need to be included within the radiation treatment volume. Here, a 6-day continuous infusion of IUdR at 100 mg/kg/day resulted in a 6% IUdR-DNA incorporation in normal bone marrow and 8% IUdR-DNA incorporation in normal intestine (Fig. 2, A and B). As a probable consequence, athymic mice lost ≥20% body weight at the completion of the infusion (Fig. 1B). We and others have found that further IUdR dose escalation to 200–250 mg/kg/day results in animal deaths with a 4–6-day continuous infusion (8, 32). Thus, the MTD of IUdR is 100 mg/kg/day for 6 days of continuous infusion in this athymic mouse model and results in percentage IUdR-DNA incorporation in the HT29 human tumor xenografts in the range of 2.3% (Fig. 2D). Additionally, we found no significant evidence of radiosensitization using continuous infusion IUdR at the MTD using a growth delay assay (Fig. 3B). The lack of radiosensitization presumably reflects the low percentage IUdR-DNA incorporation. For comparison to humans, a 2-week continuous i.v. infusion of IUdR at 1000 mg/m²/day resulted in significant neutropenia and thrombocytopenia and was associated with a median of 11% IUdR-DNA incorporation in circulating granulocytes (28). The available human data on percentage IUdR-DNA incorporation in
tumors are summarized above. These differences in percentage IUdR-DNA incorporation in normal tissues and tumors in mice compared to humans may result from the higher endogenous plasma TdR levels in rodents \((\sim 10^{-9} \text{ M})\) compared to man \((\sim 10^{-7} \text{ M}; \text{Ref. 33})\).

In this in vivo study using athymic mice, we extend our preclinical evaluation of IPdR as an p.o. administered prodrug for IUdR in an attempt to further improve the therapeutic gain, compared to a continuous infusion of IUdR, which is typically used in clinical trials. We report that IPdR can be administered daily as a p.o. bolus at up to 2 g/kg/day for 6 days with minimal systemic toxicity in athymic mice, as measured by \(\leq 10\%\) body weight loss (Fig. 1A). Additionally, using the percentage IUdR-DNA incorporation in normal mouse intestine and mouse bone marrow as surrogate indices to predict systemic normal tissue toxicities, we found a 2-fold reduction in normal intestine and a 2–3-fold reduction in normal bone marrow with IPdR in the dose range of 1–2 g/kg/day for 6 days compared to the MTD dose of continuous infusion of IUdR (Fig. 2, A and B). As determined by its low proliferative rate, the percentage IUdR-DNA incorporation in normal liver is \(\leq 1\%\) with either drug, and this result is comparable to clinical data in normal human liver with continuous infusions of IUdR (18, 19). We also report an improvement in the therapeutic gain with high doses (\(\geq 1000\) mg/kg/day) of IPdR in an analysis of the percentage IUdR-DNA incorporation in the HT29 xenografts. A continuous infusion of IUdR at the MTD resulted in 2.3% incorporation in HT29 xenografts, compared to up to 3.6% using p.o. IPdR. Thus, from both our analyses of normal proliferating tissues and the HT29 human colon cancer xenograft, the p.o. administered prodrug has an improved therapeutic profile. Furthermore, significant tumor radiosensitization, as defined in our tumor regrowth assay, is possible with coadministration of p.o. IPdR during XRT (Fig. 3). Our calculated radiosensitizer enhancement ratio of \(>1.5\) for HT29 xenografts would be predicted to result in clinically relevant radiosensitization of resistant human tumors (10, 12, 13, 18, 19, 28).

To better explain this improved therapeutic index for IPdR, we further investigated the pharmacokinetics of bolus administration of IPdR (Fig. 4). As demonstrated in our first study (5), there is rapid and efficient conversion of IPdR to IUdR by hepatic aldehyde oxidase in athymic mice. The peak IUdR levels appear within 10–20 min and are markedly reduced within 45–90 min. Plasma levels of IPdR remain elevated at 45 and 90 min following bolus administration, particularly at the highest dose (1500 mg/kg). Although the persistent plasma levels of IPdR following 1500 mg/kg suggest a saturation of hepatic aldehyde oxidase, the levels may also result from the fact that this highest dose required a slower bolus administration over 15–20 min to reduce the risk of aspiration. Additionally, the higher levels of IPdR, as well as the principal metabolites IP and IU following IPdR doses of 1000 mg/kg and 1500 mg/kg, may also result from competition of both IUdR and IPdR for TdR phosphorylase, the first step in catabolism of pyrimidines. Such an effect was recently noted by our group in a similar in vivo athymic mouse study involving concomitant continuous infusions of IUdR and 5′-amino-5′-deoxythymidine (9).

Previous studies indicated that cytosolic extracts from mouse and rat hepatocytes and, to a lesser degree, rat kidney, small intestine, and spleen catalyzed the oxidation of IPdR to IUdR (4, 5). Moreover, it was concluded, based on a series of enzyme inhibitor and substrate competition studies, that IPdR oxidase activity was attributable to aldehyde oxidase and not xanthine oxidase (2–4). The results of the present investigation indicate that normal human liver has significant IPdR oxidase activity (Table 1). The human liver IPdR oxidase activity was cytosolic, protein dependent, cofactor independent, and inhibited by low concentrations of menadione and isovanillin but not allopurinol (Table 2). Menadione and isovanillin are selective inhibitors for aldehyde oxidase, and allopurinol is a selective inhibitor for xanthine oxidase (14–17). These results indicate that aldehyde oxidase but not xanthine oxidase is involved in the conversion of IPdR to IUdR in human liver. Moreover, the cytosolic localization and the lack of cofactors required for IPdR oxidase activity is consistent with aldehyde oxidase, which is a flavin adenine dinucleotide-containing enzyme that is oxidized by molecular oxygen but not by \(\beta\)-NAD\(^+\) or other electron-transferring enzymes (34, 35). The inability of menadione or isovanillin to inhibit IUdR formation completely suggests that an oxidase system other than aldehyde oxidase or xanthine oxidase may contribute to the overall IPdR oxidase activity. A second possibility is that the concentrations of menadione and isovanillin were insufficient to completely block aldehyde oxidase activity.

In contrast to human liver, human small intestine had significantly lower IPdR oxidase activity that was not inhibited by isovanillin or allopurinol but was stimulated by menadione (Table 3). These results indicate that human intestine cytosol has some IPdR oxidase activity; however, this activity cannot be assigned to aldehyde oxidase or xanthine oxidase, and the importance of this activity and the enzyme system responsible for this activity remains to be determined. In addition, the activity of aldehyde oxidase in small intestine is considerably lower than that found in liver when assayed with 6-methylpurine (36) and would be expected to have less contribution to IPdR oxidase activity. Here, we also confirm that IPdR oxidase activity is not detectable in the HT29 s.c. human tumor xenografts and in liver metastases obtained from two patients with colorectal cancer (Table 1).

In summary, we report that significant radiosensitization (as assessed by tumor growth delay; Fig. 3) of s.c. human tumor xenografts is possible, even with relatively low percentage IUdR-DNA incorporation (Fig. 2D) in athymic mice. Additionally, based on a 2–3-fold decrease in percentage IUdR-DNA incorporation in two dose-limiting normal tissues (Fig. 2, A and B) and a favorable pharmacokinetic profile for p.o. IPdR (Fig. 4) in athymic mice, we conclude that p.o. IPdR should be considered for testing in Phase I/II clinical trials in humans as a p.o. prodrug for IUdR radiosensitization of poorly radioresponsive tumors. Additionally, we demonstrate the presence of high levels of IPdR oxidase in normal human liver, which is an aldehyde oxidase and not a xanthine oxidase.

REFERENCES

Preclinical evaluation of 5-iodo-2-pyrimidinone-2'-deoxyribose as a prodrug for 5-iodo-2'-deoxyuridine-mediated radiosensitization in mouse and human tissues.
