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ORIGINAL ARTICLE

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Effects of PR-350, a newly developed radiosensitizer, on dihydropyrimidine dehydrogenase activity and 5-fluorouracil pharmacokinetics

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Abstract This study was designed to investigate the effects of PR-350, a newly developed radiosensitizer, on dihydropyrimidine dehydrogenase (DPD) activity and 5-fluorouracil (5-FU) pharmacokinetics in 8-week-old male Sprague-Dawley rats. In an in vitro study with hepatic cytosol, DPD activity was dose-dependently reduced by PR-350 at 0.5, 1.0, and 2.0 mmol/l to 75.5%, 64.9%, and 61.5%, respectively, of the control values. In an ex vivo study, DPD activities in hepatic cytosols obtained from animals which had received PR-350 over 4 days (200 mg/kg per day) were not significantly different from those in animals which had not. In an in vivo study, none of the pharmacokinetic parameters obtained from the plasma concentration-time profile of 5-FU were significantly altered by single i.v. injections of PR-350 (50, 100, or 200 mg/kg). However, (*E*)-5-(2)-(bromovinyl)uracil (BVU), a DPD inhibitor, significantly increased the half-life and area under the curve of 5-FU to 238.1% and 323.2%, respectively, of the control values. Administration of PR-350 over 4 days (200 mg/kg per day) did not affect either of these parameters. The administration of PR-350 significantly reduced the clearance (73.5% of control) and volume of distribution (71.0% of control) of 5-FU, but the alterations were much less than those caused by BVU. These results suggest that the effect of PR-350 on 5-FU pharmacokinetics is much less than that of BVU and that the enhancement of 5-FU toxicity by PR-350 is less than we initially anticipated.

Key words Radiosensitizer · PR-350 · 5-Fluorouracil · Dihydropyrimidine dehydrogenase · Pharmacokinetics · Interaction

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Introduction

Many hypoxic cell radiosensitizers have been developed because the radioresistance of hypoxic cells in solid tumors has been regarded as one of the limiting factors in the local control of cancer by radiotherapy [4, 6, 15]. Although misonidazole has shown marked radiosensitizing effects in hypoxic cells in solid tumors, its neurotoxicity limits the administration of adequate doses [19]. Etanidazole, pimonidazole, and several other sensitizers have been developed in an attempt to provide sensitizers with lower toxicity and/or higher radiosensitizing activity [1, 2, 14, 16].

PR-350, (±)-(2*RS*,3*SR*)-[3-(2-nitroimidazol-1-yl)-methoxy] butane-1,2,4-triol, was developed by POLA Chemical Industries (Yokohama, Japan) as a radiosensitizer. Previous studies have shown it to be as efficient as and less toxic than etanidazole [5, 8]. The pharmacokinetics of PR-350 in mice have been described by Hirokawa et al. [5]. Open trial phase I clinical studies of PR-350 have been undertaken in patients with pancreas cancer, and phase II studies began in 1999. Clinically, PR-350 is expected to be coadministered with anticancer drugs such as 5-fluorouracil (5-FU).

In 1993, 15 deaths associated with the coadministration of sorivudine (SRV), the antiviral drug for herpes zoster, and one of the oral 5-FU prodrugs were reported in Japanese patients [11]. These deaths seemed to be caused by an increase in the tissue 5-FU levels as a result of inactivation of hepatic dihydropyrimidine dehydrogenase (DPD) by (*E*)-5-(2-bromovinyl)uracil (BVU) formed from the antiviral SRV [3, 9, 10, 11]. 5-FU is a fluorinated pyrimidine analogue that is used widely in the treatment of a variety of solid tumors, including breast, colorectal, head and neck, gastric, and pancreatic cancers [13]. It is anticipated that the new treatment protocols involving the coadministration of PR-350 and 5-FU will be used for the treatment of many kinds of tumors and cancers. However, there has been no report on the interaction between PR-350 and 5-FU. Therefore,

we investigated the effects of PR-350 on the activity of DPD, a rate-limiting enzyme in 5-FU catabolism, and on 5-FU pharmacokinetics in rats.

Materials and methods

Chemicals

PR-350 was supplied by POLA Chemical Industries (Yokohama, Japan). [6-^{3}H]5-FU (>99% radiochemical purity, 555 Bq/mmol) was purchased from Amersham Corporation (Arlington Heights, Ill.). Unlabeled 5-FU was a generous gift from Kyowa Hakkou Kogyo Company (Tokyo, Japan). The radiolabeled drug was diluted with unlabeled 5-FU to give appropriate specific activities. [6-^{3}H]5-FU was incubated at a final drug concentration of 40 μmol/l and a final radioactivity concentration of 0.5 μCi/sample (1.0 ml). BVU was purchased from Sigma Chemical Company (St. Louis, Mo.). All other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan) and were of analytical grade.

Experimental procedures

In vitro study

Male Sprague-Dawley rats ($n=3$) at 8 weeks of age, weighing 284 to 289 g, were purchased from SEAS Company (Saitama, Japan) and were housed in air-conditioned quarters under a 12-h light/dark cycle and were given laboratory chow (CE-2; Nippon Clea, Tokyo, Japan) and water ad libitum. After the rats were killed by decapitation, the livers were quickly removed, diced, frozen in liquid nitrogen, and stored at –80 °C for cytosol preparation. Cytosol was prepared using a previously described method [17]. The protein concentration of the cytosolic fraction was determined by the method of Lowry et al. [7]. The incubation mixture consisted of 35 mmol/l potassium phosphate (pH 7.4), 2.5 mmol/l magnesium chloride, 10 mmol/l 2-mercaptoethanol, 200 μmol/l NADPH, and 250 μg cytosolic protein. Each sample was preincubated at 37 °C for 20 min in the presence of PR-350 at 0.5, 1.0 or 2.0 mmol/l or in the absence of the drug. The reaction was started by the addition of [6-^{3}H]5-FU (40 μmol/l). After 20 min, the reaction was stopped by the addition of 5% perchloric acid (0.5 ml). 5-FU and its metabolites were separated by reverse-phase high-performance liquid chromatography (HPLC) [17], and radioactivities were counted with an LSC-1000 scintillation counter (Aloka, Tokyo, Japan).

Ex vivo study

Male Sprague-Dawley rats at 8 weeks of age, weighing 294 to 312 g, were used. The animals were treated with PR-350 i.v. over 4 days (200 mg/kg per day) or the same volume of physiological saline (20 ml/kg) into the tail vein ($n=3$ in each group). All animals were killed by decapitation 1 h after the final administration of PR-350 or vehicle alone. Preparation of cytosol and measurement of DPD activity were done as described above.

In vivo study I

Male Sprague-Dawley rats at 8 weeks of age, weighing 277 to 310 g, received a single i.v. injection of PR-350 (50, 100, or 200 mg/kg) or the same volume of physiological saline (20 ml/kg) into the tail ($n=3$ in each group). Just after the injection, rats received a single i.v. injection of 40 mg/kg 5-FU containing 20 μCi/ml [6-^{3}H]5-FU (80 μCi/kg) into the tail vein at around 10 a.m. [18]. Blood (300 μl) was collected via another tail vein at 5, 15, 30, 45, 60, and 90 min after the 5-FU injection. Blood samples were mixed with a minimal amount of heparin and centrifuged, and the separated plasma was stored at –30°C until assay. Plasma (100 μl) was mixed with the same volume of 5% perchloric acid and the mixture centrifuged. The supernatant was removed, filtered (Ultrafree-MC, 0.45 μm; Millipore Corporation, Bedford, Mass.), and injected into the HPLC system used for the DPD activity assay [17]. The plasma 5-FU concentration was estimated as the disintegrations per minute count of the 5-FU fraction [18]. The area under the plasma concentration-time curve (AUC) of 5-FU was determined by the trapezoidal rule. The values were extrapolated to infinity by dividing the last measured plasma concentration by the slope of the terminal elimination phase (β) obtained with least-squares linear regression analysis. The elimination half-life ($t_{1/2}$) was calculated as $\ln 2/\beta$. Total clearance (CL) was calculated as dose/AUC and volume of distribution (V_d) was determined by the equation $V_d = CL/\beta$.

In vivo study II

Male Sprague-Dawley rats at 8 weeks of age, weighing 225 to 307 g, were assigned to groups of six rats each. A first group received a 4-day i.v. injection of physiological saline (20 ml/kg) and a single i.p. injection of BVU at 43.4 mg/kg suspended in Dulbecco's modified Eagle's medium (DMEM, 2 ml/kg) 1 h before the final injection of physiological saline (BVU group). A second group received a 4-day i.v. injection of PR-350 at 200 mg/kg dissolved in physiological saline (20 ml/kg) and a single i.p. injection of DMEM (2 ml/kg) 1 h before the final injection of PR-350 (PR-350 group). A third group received a 4-day i.v. injection of physiological saline (20 ml/kg) and a single i.p. injection of DMEM (2 ml/kg) 1 h before the final injection of physiological saline (control group). At about 10 a.m., just after the final i.v. injection, rats received a single i.v. injection of 5-FU at 40 mg/kg containing 20 μCi/ml [6-^{3}H]5-FU (80 μCi/kg) into the tail vein. The measurement of blood 5-FU concentrations was done as described above.

Statistical analysis

Data are presented as means ± SD. Mean values were compared by one-way analysis of variance followed by Scheffe's test. *P*-values less than 0.05 were considered statistically significant.

Results

The effects of various concentrations of PR-350 on the DPD activity in hepatic cytosol (in vitro study) are depicted in Fig. 1. When the reaction mixture was in-

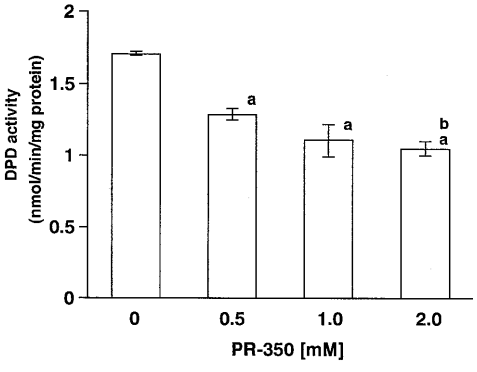

Fig. 1 Effects of various concentrations of PR-350 on DPD activity in hepatic cytosol ($^aP<0.001$ vs 0 mmol/l, $^bP<0.05$ vs 0.5 mmol/l)

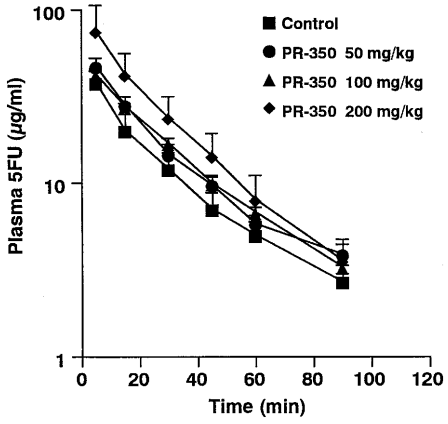

Fig. 2 Plasma concentrations (means ± SD) of 5-FU after a 40 mg/kg (80 μCi/kg) i.v. dose following a single injection of PR-350 (50, 100, or 200 mg/kg). Physiological saline was administered at the same time to control animals

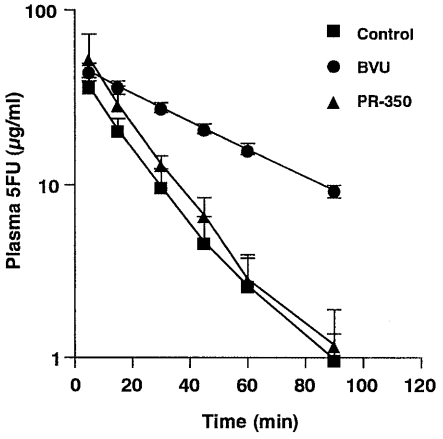

Fig. 3 Plasma concentrations (means ± SD) of 5-FU after a 40 mg/kg (80 μCi/kg) i.v. dose after a 4-day administration of PR-350 at 200 mg/kg per day or a single dose of BVU. The vehicles for PR-350 and BVU were administered to control animals

cubated with PR-350 0.5, 1.0, or 2.0 mmol/l, the DPD activities were dose-dependently reduced to 75.5%, 64.9%, or 61.5%, respectively, of the control values.

The 4-day-treatment with PR-350 at 200 mg/kg per day did not affect DPD activity in hepatic cytosol (ex vivo study). The activities after treatment with and without PR-350 were not significantly different (1.79 ± 0.24 and 1.90 ± 0.25 nmol/min per mg protein, respectively). Treatment with PR-350 also did not significantly affect liver weight (control 12.8 ± 0.3 g, PR-350 11.8 ± 0.7 g) or body weight (control 304.7 ± 7.0 g, PR-350 302.0 ± 8.0 g).

The effects of various doses of PR-350 on the plasma concentration-time profile of 5-FU (in vivo study I) are shown in Fig. 2. Single i.v. injections of PR-350 at doses of 50, 100, or 200 mg/kg did not affect 5-FU pharmacokinetics (Table 1) obtained from the plasma concentration-time profile of 5-FU.

The plasma concentration-time profile of 5-FU after the 4-day treatment with PR-350 at 200 mg/kg per day was compared with that after the single i.p. administration of BVU at 43.4 mg/kg (in vivo study II, Fig. 3). The pharmacokinetic parameters obtained from the profiles are summarized in Table 2. A single i.p. administration of BVU significantly increased the $t_{1/2}$ and AUC of 5-FU and significantly reduced the CL. However, after the 4-day administration of PR-350 the $t_{1/2}$ and AUC of 5-FU were not altered. The CL of 5-FU was significantly reduced by administration of PR-350 to 73.5% of the control value, but the reduction was much less than that found following administration of BVU. There was no significant difference in body weight between the three groups (control 272.2 ± 32.1 g, PR-350 270.0 ± 27.4 g, BVU 274.7 ± 25.0 g).

Discussion

We investigated the effects of PR-350, a new radiosensitizing agent, on DPD activity in hepatic cytosol in in vitro and ex vivo studies and on 5-FU pharmacokinetics in rats. When the reaction mixture was incubated with PR-350 at 0.5 to 2.0 mmol/l the DPD activity was dose-dependently reduced in the in vitro experiment. Although the exact mechanism of the inhibition of DPD activity by PR-350 remains to be elucidated, these findings suggest that treatment with PR-350 may affect DPD activity in the liver and the pharmacokinetics of 5-FU, a substrate of DPD.

We therefore examined the effect of a 4-day-treatment with PR-350 at 200 mg/kg per day on DPD activity and the pharmacokinetics of 5-FU in rats. Since Hirokawa et al. [5] and Oya et al. [12] have reported that a significant radiosensitizing effect can be expected even after a single i.v. dose of PR-350 at 100 mg/kg in mice, we also examined the effect of a single i.v. dose of PR-350 at 50, 100, or 200 mg/kg on 5-FU pharmacokinetics. Although the administration of PR-350 at 200 mg/kg per day for 4 days may be too much for obtaining the radiosensitizing effect, extreme doses of PR-350 in the ex vivo experiment did not affect DPD activity or the liver and body weights. Hirokawa et al. [5] reported

Table 1 Effects of single doses of PR-350 on the pharmacokinetic parameters of 5-FU. Values are means ± SD (differences are not significant)

	$t_{1/2}$ (min)	AUC (μg · min/ml)	CL (ml/min/kg)	V_d (ml/kg)
Control ($n = 3$)	26.7 ± 3.3	1012 ± 332	42.1 ± 11.9	1656 ± 639
PR-350 50 mg/kg ($n = 3$)	26.2 ± 4.5	1274 ± 248	32.2 ± 6.2	1213 ± 210
PR-350 100 mg/kg ($n = 3$)	25.1 ± 1.4	1291 ± 141	31.2 ± 3.5	1132 ± 138
PR-350 200 mg/kg ($n = 3$)	20.9 ± 3.3	1723 ± 1341	33.4 ± 20.8	1038 ± 769

Table 2 Effects of both 4-day administration of PR-350 and a single dose of BVU on the pharmacokinetic parameter of 5-FU

	$t_{1/2}$ (min)	AUC (μg · min/ml)	CL (ml/min/kg)	V_d (ml/kg)
Control ($n=6$)	16.0 ± 1.6	766 ± 160	53.9 ± 10.1	1229 ± 164
BVU ($n=6$)	$38.4 \pm 4.2^{aaa,bbb}$	$2476 \pm 157^{aaa,bb}$	$16.2 \pm 1.0^{aaa,b}$	893 ± 65^{aa}
PR-350 ($n=6$)	15.3 ± 2.7	1055 ± 243	39.6 ± 8.7^{a}	872 ± 220^{aa}

$^{aaa}P < 0.0001$, $^{aa}P < 0.001$, $^{a}P < 0.01$, vs control; $^{bbb}P < 0.0001$, $^{bb}P < 0.001$, $^{b}P < 0.01$, vs PR-350

much lower blood concentrations of PR-350 (0.54 mmol/l and 0.09 mmol/l) in mice 10 min and 60 min, respectively, after a single i.v. dose of PR-350 at 100 mg/kg than those found in our in vitro experiment. It is unlikely that the drug accumulates in the liver with daily administration because the elimination half-life is relatively short (1.6 h). These findings and the current study suggest that a high concentration of PR-350 might inhibit DPD activity in vitro. However, after a 4-day treatment with a therapeutic dose of PR-350, the concentrations in the liver never reached a value high enough to inhibit DPD activity.

In the pharmacokinetic experiment with single i.v. administrations of PR-350, none of the 5-FU pharmacokinetic parameters was affected at any of the three doses (50, 100, and 200 mg/kg). The effects of a 4-day treatment with PR-350 at 200 mg/kg per day on the pharmacokinetics of 5-FU were compared with those of a single treatment with BVU, a DPD inhibitor. The 4-day treatment with PR-350 at 200 mg/kg per day altered neither the $t_{1/2}$ nor the AUC of 5-FU, while the single treatment with BVU increased both of these parameters significantly. The CL and the V_d were significantly reduced following PR-350 treatment, but to a much lesser extent than following BVU treatment. The decreased CL associated with PR-350 treatment may have been related to a reduction in V_d since the $t_{1/2}$ was not affected. The mechanism involved in the reduction of V_d following treatment with PR-350 at 200 mg/kg is unknown. However, this reduction occurred only with the 4-day treatment, and not with the single administration of PR-350 at 200 mg/kg. In the clinical situation, a single i.v. injection of PR-350 just before patients receive radiotherapy is considered to be effective, and PR-350 at 100 mg/kg has been reported to be enough to obtain a significant radiosensitizing effect in mice [5, 12].

Although a simplistic extrapolation of our present results to humans is inappropriate, these findings suggest that coadministration of PR-350 and 5-FU at a therapeutic dose would be safe in patients. Naturally, further study is needed to investigate whether the administration of PR-350 affects the pharmacokinetics and the pharmacodynamics of 5-FU in humans. When PR-350 is administered repeatedly, precautions are required in patients with a relatively low intrinsic DPD activity because the DPD activity is highly variable between subjects.

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