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Study on intestinal absorption sites of mizoribine and ribavirin, substrates for concentrative nucleoside transporter(s), in rats

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ABSTRACT

The absorption sites of mizoribine (an imidazole nucleoside) and ribavirin (a purine nucleoside) in the small intestine were evaluated in rats. The intestinal absorption of mizoribine is known to be mediated by rat concentrative nucleoside transporter (CNT)1 and CNT2. In contrast, the absorption mechanism of ribavirin in rats is not yet fully understood. Thus, the intestinal absorption of ribavirin was characterized firstly. In in-situ jejunum loop studies, the absorption percentage of ribavirin at a dose of 25 mg/kg was significantly lower than those after 1 mg/kg and 5 mg/kg doses. Coadministration of adenosine, inosine and mizoribine, but not thymidine and gemcitabine, significantly suppressed the intestinal absorption of ribavirin, indicating that ribavirin absorption is mediated by CNT2 in rats. In in-situ loop studies, mizoribine and ribavirin were absorbed to the same extents both in the proximal and distal small intestine. In vivo study was carried out using mizoribine, in which the gastric emptying rates altered by a subcutaneous injection of metoclopramide or scopolamine butylbromide exerted no significant effects on the values of peak plasma level (Cmax), area under the plasma concentration–time profile from 0 to 6 h (AUC_{0-6}), and urinary excretion percentage of mizoribine given orally, though the time to reach Cmax (Tmax) of mizoribine was altered by each treatment. In conclusion, mizoribine and ribavirin were found to be absorbed efficiently to the same extents from the whole small intestine. Also, the altered gastric emptying rates exerted no significant effects on the oral bioavailabilities of mizoribine and ribavirin.

1. Introduction

A variety of nucleoside analogues are given orally in the treatments of various viral, tumoral or immunological diseases in clinical practice. The intestinal absorption of nucleoside analogues is mediated by Na^+-dependent concentrative nucleoside transporters (CNTs) located in the brush-border membrane and Na^+-independent equilibrative nucleoside transporters (ENTs) located in the basolateral membrane of absorptive epithelia (Baldwin et al., 1999; Pastor-Anglada and Baldwin, 2001; Casado et al., 2002; Pastor-Anglada et al., 2007; Molina-Arcas et al., 2008). The CNT family contains CNT1, CNT2 and CNT3. CNT1 transports pyrimidine nucleosides (uridine, thymidine, and cytidine) and adenosine (a purine nucleoside). CNT2 transports purine nucleosides (guanosine and adenosine) and uridine (a pyrimidine nucleoside). CNT3 transports both purine and pyrimidine nucleosides (Ritzel et al., 2001; Gray et al., 2004).

Mizoribine (or bredinin®), an imidazole nucleoside, has long been used as an orally available immunosuppressive agent in human renal transplantation in Japan. Recently, we characterized the intestinal absorption of mizoribine by examining the contribution of CNT1, in addition to CNT2, in rats, and found that the intestinal absorption of mizoribine is mediated by both CNT1 and CNT2 (Mori et al., 2008a). Ribavirin, a purine nucleoside, is a broad-spectrum antiviral drug against both RNA and DNA viruses, and used together with pegylated or non-pegylated interferon-α in the treatment of patients with hepatitis C virus (Palumbo, 2009; Hartwell and Shepherd, 2009). Ribavirin is a substrate of human CNT2, CNT3, ENT1 and ENT2, as evaluated in vitro by using membrane vesicles prepared from human intestine, human placental epithelial cells, Xenopus oocytes expressing human nucleoside transporters, sandwich-cultured human hepatocytes, and human erythrocytes (Patil et al., 1998; Owen et al., 2005; Yamamoto et al., 2007; Govindarajan et al., 2008). However, it is known that CNTs show species difference in its substrate specificity as follows; didanosine and cladribine are transported by rat CNT2, but not by human CNT2 (Li et al., 2001; Gerstin et al., 2002; Owen et al., 2006). It was recently reported that the intestinal absorption of ribavirin from rat intestinal loop and the uptake of ribavirin to human intestinal epithelial LS180 cells were significantly suppressed by coadministration of 10 mg/ml inosine, and Na^+-independent equilibrative nucleoside transport contributes significantly to intestinal absorption of ribavirin at relatively high concentrations (Takaai et al., 2008).

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In the present study, firstly, we characterized the intestinal absorption of ribavirin by examining the contribution of CNT1, in addition to CNT2, in rats. Then we evaluated the absorption sites of ribavirin and mizoribine after oral administration by using rats with altered gastric emptying rates. The study on the absorption site of transporter-mediated drugs will be expected to reveal the role of transporters functionally in vivo.

2. Materials and methods

2.1. Materials

Ribavirin was obtained as Rebetol® (capsule for oral administration, dry powder) from Schering-Plough K.K. (Osaka, Japan), and gemcitabine, or Gemzar® Injection (solution), was from Eli Lilly Japan K.K. (Kobe, Japan). These commercially available therapeutic drugs were used without further purification. Mizoribine (dry powder, purity: 99.8%) was a gift from Asahi Kasei Pharma Corporation (Tokyo, Japan). Adenosine, inosine, and thymidine were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Fluoresceine isothiocyanate-dextran with molecular weight of approximately 10,000 (FD-10S) was obtained from Sigma-Aldrich Japan K. K. (Tokyo, Japan). Metoclopramide (Primperan® Injection) and scopolamine butylbromide (Buscopan® Injection) were from Astellas Parma Inc. (Tokyo, Japan) and Nippon Boehringer Ingelheim Co., Ltd. (Tokyo, Japan), respectively. All other chemicals used were of the highest purity available.

2.2. Animals

Male Sprague–Dawley (SD) rats weighing about 250 to 350 g were purchased from Japan SLC, Inc. (Shizuoka, Japan). Rats were fed a standard laboratory diet (CE-2, Clea Japan, INC., Tokyo, Japan) and water for more than 1 week prior to the experiments. Rats were fasted overnight with free access of water prior to the oral administration and in-situ loop studies. Experiments with animals were performed in accordance with the "Guide for Animal Experimentation" from the Committee of Research Facilities for Laboratory Animal Sciences, Hiroshima International University, which is in accordance with the "Guidelines for proper conduct of animal experiments" from Science Council of Japan.

2.3. In-situ intestinal loop study in rats

Rats were anesthetized with pentobarbital (30 mg/kg, i.p. injection). The in-situ intestinal loop study was carried out in the same manner as reported previously (Mori et al., 2008a). Briefly, bile duct was ligated and the intestinal lumen was washed with a sufficient amount of saline prewarmed at 37 °C after cannulating polyethylene tubings at the upper duodenum and lower ileum of the small intestine. Then, a 10 cm-long intestinal loop was made by ligating both ends of the intestinal loop at proximal (a segment from 5 cm below the bile duct opening) or distal (a segment above the ileocecum) small intestine of anesthetized rats. Ribavirin was dissolved in saline at a concentration of 0.5 mg/ml, 2.5 mg/ml or 12.5 mg/ml, and the solution was administered to the loop via the polyethylene tubing (PE 10) inserted into the loop at a volume of 2 ml/kg (corresponding to a dose of 1 mg/kg, 5 mg/kg, or 25 mg/kg, respectively). Such in-situ intestinal loop study was also carried out for mizoribine to evaluate the absorption sites using proximal and distal small intestine. Mizoribine dissolved in saline at a concentration of 0.5 mg/ml was administered to the loop at a volume of 2 ml/kg (corresponding to a dose of 1 mg/kg).

In inhibition studies, ribavirin (1 mg/2 ml/kg) was administered together with adenosine, thymidine, inosine, gemcitabine, or mizoribine. These nucleoside compounds and nucleoside-derived drugs were added at different concentrations (10-fold (20.5 mM) or 30-fold (61.5 mM) molar excess of ribavirin) to the solution of ribavirin (0.5 mg/ml, corresponding to 2.05 mM). At 1 h after the administration of ribavirin, rats were killed by injecting a sufficient amount of saturated KCl solution to the heart. The intestinal loop containing ribavirin was isolated, and the isolated loop was weighed and homogenized with the tissue homogenizer (21,000 rpm, 2 min) after adding 9-fold volume of distilled water. To the 10% intestinal homogenate (0.5 mL), 0.5 ml of acetonitrile was added and the suspension was centrifuged at 1000 *g* for 5 min to obtain the supernatant.

2.4. Effect of altered gastric emptying rate on in vivo oral absorption of mizoribine (blood and urine sampling)

As a control, mizoribine dissolved in water at a concentration of 2.5 mg/ml was administered to rats at a volume of 2 ml/kg (5 mg/kg) by gastric tube. Blood (0.25 ml each) was taken at designated time intervals (0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 h) from jugular vein under light anesthesia with ethylether each time. In separate experiments, one night-fasted rats received mizoribine (5 mg/2 ml/kg) by gastric tube, and were housed in metabolic cages to collect urine. The gastric emptying rate was altered by injecting metoclopramide (15 mg/kg) or scopolamine butylbromide (10 mg/kg) subcutaneously (Jacoby and Brodie, 1967; Medicine interview form for Buscopan® Injection, Nippon Boehringer Ingelheim Co., Ltd., Tokyo, Japan). At 15 min after the injection, rats received mizoribine (5 mg/kg) orally and blood or urine samples were collected.

2.5. Evaluation of altered gastric emptying rate in rats

The gastric emptying rate was evaluated by measuring the residual amounts of FD-10S, a poorly absorbable compound, in the stomach after oral administration in the same manner as reported previously (Mori et al., 2008b). Briefly, rats received metoclopramide (15 mg/kg) or scopolamine butylbromide (10 mg/kg) subcutaneously, and 15 min later, rats received FD-10 S solution orally by gastric tube at a dose of 5 mg/kg (a dosing volume of 1 ml/kg). At 1 min prior to the designated time (15 min or 60 min after the administration of FD-10 S), rats were lightly anesthetized with ethyl ether, and sacrificed by injection of an excess amount of sodium pentobarbital into the heart. The abdomen was opened, and the cardia and pylorus were ligated. The isolated stomach was weighed, added with 9-fold volumes of distilled water, homogenized with a tissue homogenizer (21,000 rpm, 2 min), and the remained amount of FD-10S in the stomach homogenates was measured.

2.6. Analysis

The blood sampled was centrifuged at 1000 *g* for 5 min to collect plasma. To each 100 μl plasma sample, an equal volume of acetonitrile was added, and the suspension was centrifuged at 1000 *g* for 5 min to obtain supernatant. To the urine, an equal amount of acetonitrile was mixed for deproteinization, and the suspension was centrifuged at 1000 *g* for 5 min to collect supernatant. The isolated stomach containing FD-10S was weighed, added with 9-fold volumes of distilled water, and homogenized with a tissue homogenizer (21,000 rpm, 2 min). The homogenate was centrifuged at 4000 *g* for 5 min to obtain the supernatant.

Concentrations of mizoribine in the supernatants of biological samples (plasma and urine) were determined by HPLC according to the reported method (Hosotsubo et al., 1988). Briefly, the column used was a Shimpack CLC-NH_2 (6.0 mm I.D.×150 mm, Shimadzu Corporation, Kyoto, Japan) and mobile phase was a mixture of 1/15 M phosphate buffer (pH 2.5) and acetonitrile, in a ratio of 27.5: 72.5 (v/v). The flow rate of mobile phase was 1.3 ml/min, and detection was made at wavelength of 280 nm. The concentration of ribavirin was measured by HPLC in the similar manner as reported by Homma et al. (1999). Briefly, the column used was a Lichrospher 100 RP-18(e) column (Cica-Merk,