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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.

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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,

Tokyo, Japan), and mobile phase was 30 mM phosphate buffer (pH 6.5) containing 0.1% acetonitrile at a flow rate of 0.7 ml/min. Detection was made at wavelength of 235 nm. The concentration of FD-10S in the supernatant of stomach homogenate was measured by fluorometry at wavelengths of 496 nm for excitation and 516 nm for emission.

Data were expressed as the mean ± S.E. Differences among group mean values were assessed by the Kruskal–Wallis or ANOVA test followed by a post-hoc test (Tukey test). A difference of $P<0.05$ was considered statistically significant.

3. Results

3.1. Dose dependency in in-situ intestinal absorption of ribavirin

The dose dependency in the intestinal absorption of ribavirin was examined by administering ribavirin at a dose of 1, 5 or 25 mg/kg to the jejunum loop in rats. The absorption percentage of ribavirin, estimated by the disappearance percentage from the loop for 1 h, at a dose of 25 mg/kg was significantly lower than those after 1 mg/kg and 5 mg/kg doses (Fig. 1).

3.2. Effect of nucleosides and nucleoside analogues on intestinal absorption of ribavirin

Effects of adenosine, thymidine and inosine on intestinal absorption of ribavirin were examined in in-situ intestinal loop method. The dose of ribavirin to the loop prepared at the proximal region was 1 mg/2 ml/kg (corresponding to 2.05 mM of ribavirin in the dosing solution) and the concentrations of nucleosides coadministered were varied to 10-fold (20.5 mM) or 30-fold (61.5 mM) excess molar of ribavirin (Fig. 2). Adenosine (20.5 mM) and inosine (20.5 mM and 61.5 mM), but not tymidine (61.5 mM), significantly suppressed the intestinal absorption of ribavirin. These results indicated the contribution of CNT2, but not CNT1, in the intestinal absorption of ribavirin.

Effects of nucleoside-derived drugs such as gemcitabine, a CNT1 substrate, and mizoribine, a substrate of both CNT1 and CNT2, on the intestinal absorption of ribavirin were also examined, and mizoribine, but not gemcitabine, significantly suppressed the intestinal absorption of ribavirin (Fig. 3).

3.3. Effect of administrational site on intestinal absorption of mizoribine and ribavirin

Mizoribine, a substrate for both CNT1 and CNT2, was administered into loops prepared at the proximal and distal small intestine to evaluate the main absorption site in the intestine. The absorption percentage of mizoribine was comparable between the two different regions (Fig. 4). Rivabirin, a substrate for CNT2, also showed comparable absorption percentages between the proximal and distal small intestine (Fig. 4).

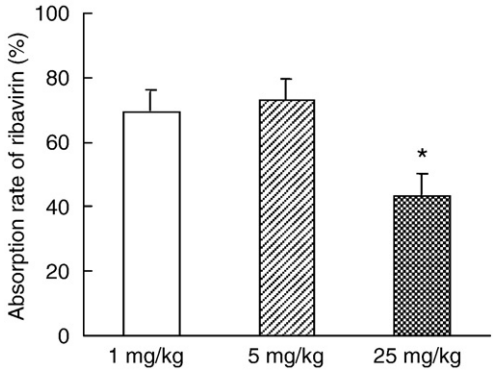

Fig. 1. Intestinal absorption of ribavirin in rats. Ribavirin was administered at a dose of 1, 5 or 25 mg/2 ml/kg to a 10 cm-long jejunum loop and the intestinal absorption rate (%) was estimated by measuring the remained amount of ribavirin in the loop 60 min after administration. Each value represents the mean ± S.E.M. ($n=4$). *$P<0.05$, significantly different from the value at doses of 1 and 5 mg/kg.

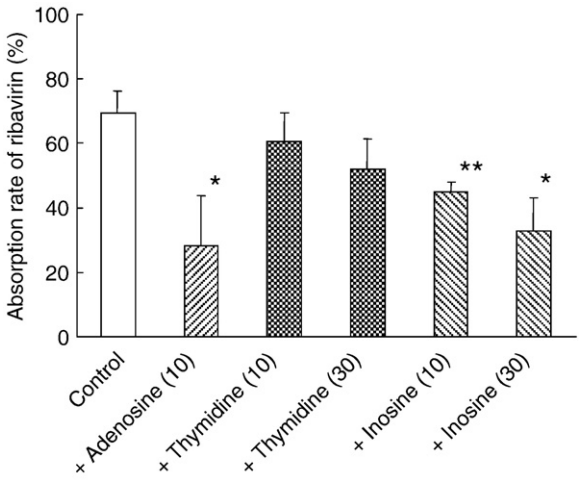

Fig. 2. Effects of various nucleoside compounds on intestinal absorption of ribavirin in rats. Ribavirin was administered at a dose of 1 mg/2 mL/kg to a 10 cm-long jejunum loop, and concentrations of nucleoside compounds coadministered were 10-fold (20.5 mM) or 30-fold (61.5 mM) molar excess of ribavirin (2.05 mM). The intestinal absorption rate (%) was estimated by measuring the remained amount of ribavirin in the loop 60 min after administration. Each value represents the mean ± S.E.M. ($n=4$). **$P<0.01$, *$P<0.05$; significantly different from the value of control (ribavirin alone).

3.4. Effect of altered gastric emptying rates on intestinal absorption of mizoribine

Gastric emptying rate was altered by subcutaneous injection of either metoclopramide or scopolamine butylbromide. In untreated control rats, the remained amounts of FD-10S in the stomach after oral administration were 40% of dosed amount at 15 min and 12% at 60 min after administration. The treatment with metoclopramide markedly increased the gastric emptying rate, where the remained amount of FD-10S in the stomach 15 min after oral administration decreased from 40% to 10% of the dosed amount in metoclopramide-treated rats. In contrast, the treatment with scopolamine butylbromide markedly decreased the gastric emptying rate, where the remained amount of FD-10S in the stomach 60 min after oral

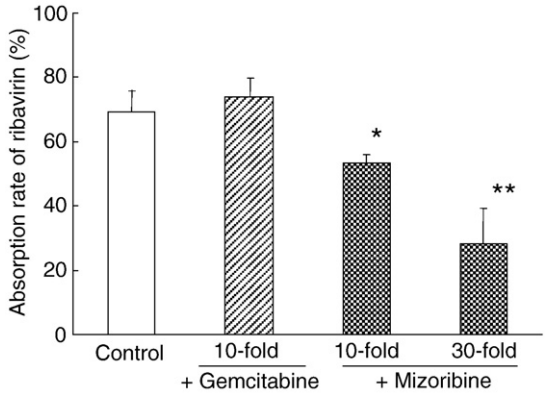

Fig. 3. Effects of gemcitabine and mizoribine on intestinal absorption of ribavirin in rats. Ribavirin was administered at a dose of 1 mg/2 ml/kg to a 10 cm-long jejunum loop, and the concentrations of nucleoside-derived drugs coadministered were10-fold (20.5 mM) or 30-fold (61.5 mM) molar excess of ribavirin (2.05 mM). The intestinal absorption rate (%) was estimated by measuring the remained amount of ribavirin in the loop 60 min after administration. Each value represents the mean ± S.E.M. ($n=4$). **$P<0.01$, *$P<0.05$; significantly different from the value of control (ribavirin alone).

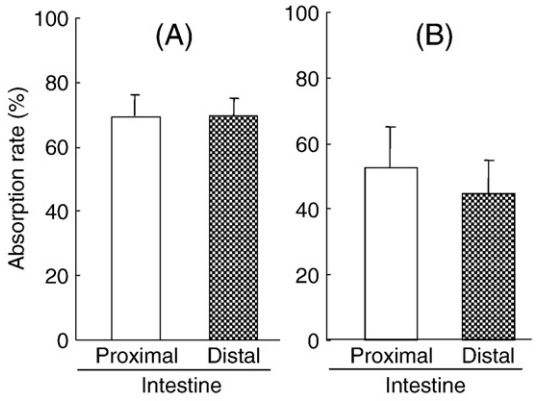

Fig. 4. Effect of absorption sites on intestinal absorption of ribavirin (A) and mizoribine (B) in rats. Ribavirin and mizoribine were administered at a dose of 1 mg/2 ml/kg to a 10 cm-long intestinal loop prepared at two different intestinal sites. The intestinal absorption rate (%) was estimated by measuring the remained amount of mizoribine or ribavirin in the loop 60 min after administration. Each value represents the mean ± S.E.M. ($n = 4$).

administration increased 62% from 12% of the dosed amount in treated rats.

The effect of altered gastric emptying rate on intestinal absorption of nucleoside analogues was evaluated by using mizoribine. Mizoribine (5 mg/kg) was administered orally to rats with normal or altered gastric emptying rates, and the time profile of plasma concentrations and urinary excretion percentage of mizoribine during 24 h after administration were determined (Fig. 5 and Table 1). Treatment with metoclopramide exerted no significant effects on the extent of oral bioavailability of mizoribine, as evaluated by peak plasma concentration (Cmax), the time to reach Cmax (Tmax), area under the concentration–time curve from 0 to 6 h (AUC_{0-6}), and plasma elimination rate constant (ke) in the late phase of mizoribine disposition. Treatment with scopolamine butylbromide significantly delayed the Tmax, but did not affect the values of Cmax, AUC_{0-6}, and ke of mizoribine, as compared with those in untreated control rats. The urinary excretion percentage of mizoribine in rats with altered gastric emptying rates was also comparable with that in untreated control rats (Table 1).

4. Discussion

In the present study, we evaluated the absorption sites of mizoribine and ribavirin by administering into the intestinal loop prepared at different sites along the small intestine. The effect of

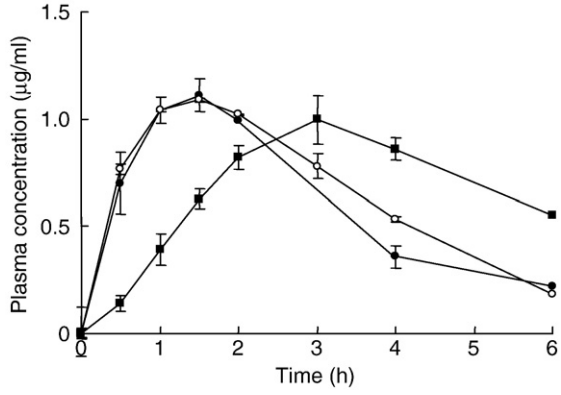

Fig. 5. Plasma concentration–time profiles of mizoribine after oral administration at a dose of 5 mg/kg in male conscious control rats (solid circle) and rats pretreated with metoclopramide (opened circle) or scopolamine butylbromide (solid square). Metoclopramide (15 mg/kg) and scopolamine butylbromide (10 mg/kg) were injected subcutaneously 15 min prior to the oral administration of mizoribine. Each value represents the mean ± S.E.M. ($n = 3$).

Table 1
Pharmacokinetic parameters of mizoribine after oral administration in rats untreated, and pretreated with metoclopramide or scopolamine butylbromide.

Parameters of mizoribine	Untreated control rats	Metoclopramide-treated rats	Scopolamine butylbromide-treated rats
Cmax (μg/ml)	1.19 ± 0.18	1.10 ± 0.01	1.12 ± 0.08
Tmax (h)	1.50 ± 0.29	1.33 ± 0.17	3.33 ± 0.33[a]
AUC_{0-6} (μg h/ml)	4.31 ± 0.43	4.35 ± 0.23	7.37 ± 1.97
Elimination rate constant (h^{-1})	0.65 ± 0.20	0.56 ± 0.08	0.31 ± 0.06
Urinary excretion (% of dose)	40.1 ± 3.70	41.7 ± 6.00	42.9 ± 8.49

Rats received metoclopramide (15 mg/kg) or scopolamine butylbromide (10 mg/kg) subcutaneously, and 15 min later, rats received mizoribin orally by gastric tube at a dose of 5 mg/kg. Values are expressed as the mean ± S.E.M. ($n = 3$ for plasma pharmacokinetic data, $n = 4$ for urinary excretion data). The value of AUC_{0-6} (μg h/ml) was calculated by a trapezoidal rule, and the urinary excretion of ribavirin was measured during 24 h after administration.

[a] $P < 0.05$; significantly different from the values of untreated control and metoclopramide treatment.

altered gastric emptying rates on the intestinal absorption of mizoribine was also evaluated in rats in vivo. Such study on the intestinal absorption sites will be expected to reveal the functional role of various transporters in intestinal drug absorption. For example, it is known that riboflavin has a narrow absorption window in the upper part of the small intestine, and the gastric retention, or gastric emptying rate, of dosage formulation given by the oral route is the important factor in determining the oral bioavailability of riboflavin. Floating pellet and gastroretentive Accordion Pill are known to increase the oral absorption of riboflavin (Hamdani et al., 2006; Kagan et al., 2006; Ahmed and Ayres, 2007). As well, some hydrophilic β-lactam antibiotics are transported by a proton-coupled oligopeptide transporter (PepT1), and an excellent correlation has been observed between the expression levels of PepT1 mRNA and the permeability coefficient of cefadroxil in the rat small intestine (Tsuji and Tamai, 1996; Tamai et al., 1997; Naruhashi et al., 2002). Like this, the transport ability of transporter-mediated drugs is closely related with the regional-specific expression levels of the transporter. In addition, in clinical practice, other factors such as the supply of driving force, the concentration of the substrates in the luminal fluid, luminal pH, luminal fluid volume, and intestinal transit of the drug also should be taken into consideration. For example, it is reported that coadministration of Eudragit L100-55, a proton-releasing polymer that supplies H^+, a driving force for PepT1, increased the plasma concentration of cefadoxil given orally, whereas Eudragit RSPO, a proton-nonreleasing analogous polymer, did not show any effect (Nozawa et al., 2003).

In the present study, at first, we evaluated the contribution of CNT1 and CNT2 in the intestinal absorption of ribavirin. CNT1 and CNT2 are expressed in a proximal-to-distal gradient along the rat intestine, whereas the expression level of CNT3 is quite low (Pastor-Anglada and Baldwin, 2001; Casado et al., 2002; Lu et al., 2004). Also, ribavirin is known to be a substrate of CNT2, CNT3, ENT1 and ENT2 in human (Patil et al., 1998; Owen et al., 2005; Yamamoto et al., 2007; Govindarajan et al., 2008). In contrast, the information regarding the substrate specificity of ribavirin in rats is not enough. In the present study, the intestinal absorption of ribavirin was dose-dependent (Fig. 1), as well as that of mizoribine, in rats (Mori et al., 2008a). As shown in Fig. 2, adenosine (a substrate for CNT1 and CNT2) and inosine (a substrate for CNT2), but not tymidine (a substrate for CNT1), significantly suppressed the intestinal absorption of ribavirin. Also, mizoribine (a substrate for CNT1 and CNT2), but not gemcitabine (a CNT1 substrate), suppressed the intestinal absorption of ribavirin. These results indicated that ribavirin is a substrate of CNT2, but not of CNT1, in rats, as well as in human. Ribavirin was effectively absorbed from both proximal (lower part of the opening of the bile duct) and distal (upper part of the

ileocecum) small intestine, and the extents of intestinal absorption were comparable between proximal and distal small intestine (Fig. 4). Mizoribine, a substrate for CNT1 and CNT2 (Mori et al., 2008a), was also absorbed to the same extents at two different regions along the small intestine (Fig. 4). These results indicated that nucleoside analogues being a substrate of CNT1 or CNT2 or both of CNT1 and CNT2 can be absorbed to the same extents from the whole small intestine.

The effect of altered gastric emptying rate on the intestinal absorption of nucleoside analogues was evaluated by using mizoribine. Mizoribine is known to be excreted into urine as an intact form, and the oral bioavailability of mizoribine in human is 65–100% (Stypinski et al., 2006). This means that the extent of oral bioavailability of mizoribine can be estimated by measuring the urinary excretion percentage of mizoribine. In contrast, ribavirin is excreted into urine by 1/3 of absorbed dose and the remainder is cleared by metabolism (Paroni et al., 1989; Preston et al., 1999).Also, ribavirin is taken up by erythrocytes in blood and accumulated as ribavirin triphosphate. The blood ribavirin concentrations gradually increase to steady-state level several weeks after the initiation of ribavirin treatment (Jarvis et al., 1998; Maeda et al., 2004; Homma et al., 2004). In such case, it will be not easy to estimate oral bioavailability of ribavirin correctly from their plasma levels. Thus, in the present study, the effect of altered gastric emptying rates on intestinal absorption of nucleoside compounds was examined by using mizoribine. Rats received metoclopramide subcutaneously to increase gastric emptying rate (Jacoby and Brodie, 1967) or scopolamine butylbromide, an antiperistaltic agent, to decrease gastric emptying rate (Lin et al., 1997; Katoh et al., 2003). The treatment with metoclopramide markedly increased gastric emptying rate and the treatment with scopolamine butylbromide markedly decreased the gastric emptying rate, as evaluated by the remained amounts of FD-10S in the stomach after oral administration. The increased gastric emptying rate showed no significant effect on the extent and rate of oral bioavailability of mizoribine. Thus, it was considered that mizoribine discharged from the stomach was absorbed rapidly and efficiently by CNT1- and CNT2-mediated transport system from any regions of the small intestine, since CNT1 and CNT2 are expressed efficiently along the small intestine. In contrast, the decreased gastric emptying rate markedly decreased the initial absorption rate of mizoribine, possibly due to the slow delivery of mizoribine to the absorption site (proximal region of the intestine). It will be generally considered that the decreased gastric emptying rate, or slow delivery of the compound to the absorption site, can increase the oral bioavailability of the compounds with a narrow absorption window in the upper part of the small intestine, like riboflavin, because, in such case, the transporter can work at a full function without saturation. In contrast, the extents of oral bioavailability of mizoribine were comparable among three different gastric emptying rates (control, increased, and decreased gastric emptying rates) (Fig. 5, Table 1), indicating that there is no specific absorption window for mizoribine and mizoribine is absorbed to the same extents from whole small intestine in rats.

In conclusion, it was found that the intestinal absorption of ribavirin is mediated by CNT2, but not by CNT1, in rats, as well as in human. Substrate compounds for CNT2 alone or both for CNT1 and CNT2 can cause drug interaction with ribavirin, more or less, depending on the concentration of the compounds. Without competitive substrate compounds, both ribavirin and mizoribine were absorbed efficiently from the whole small intestine, and the modulation of gastric emptying rates may not exert significant effect on the extent of oral bioavailability of both drugs. These findings may give a clue in considering the modulated oral bioavailability of various nucleoside analogues.

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