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Characterization of intestinal absorption of mizoribine mediated by concentrative nucleoside transporters in rats

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Abstract

Mizoribine, an imidazole nucleoside, is an inhibitor of purine synthesis and has been used as an orally available immunosuppressive agent in human renal transplantation. In the present study, the intestinal absorption of mizoribine was characterized by examining the contribution of concentrative nucleoside transporters (CNT1, CNT2) in rats. When mizoribine was administered orally in conscious rats, the bioavailability of mizoribine estimated by urinary excretion percentage of unchanged mizoribine was a dose dependent: 53.1±6.0% at 5 mg/kg and 24.0±5.1% at 20 mg/kg. In in-situ loop studies, the disappearance rate, or absorption rate, of mizoribine from the intestinal lumen was comparable between 1 and 5 mg/kg, but significantly lower at 25 mg/kg. Coadministration of adenosine (a substrate of both CNT1 and CNT2), thymidine (a CNT1 substrate) and inosine (a CNT2 substrate) significantly suppressed the intestinal mizoribine absorption, depending on the nucleoside concentrations coadministered. Gemcitabine (a pyrimidine nucleoside analogue, a CNT1 substrate) and ribavirin (a purine nucleoside analog, a CNT2 substrate) also significantly suppressed the mizoribine intestinal absorption. Bile salts such as sodium cholate and sodium glycocholate (10 mM) also significantly suppressed the intestinal mizoribine absorption, but not ribavirin absorption. Mizoribine is an amphoteric compound, however, the suppression of intestinal absorption by bile salts was not ascribed to the electrostatic interaction or micellar formation between mizoribine and bile salts. In conclusion, the intestinal absorption of mizoribine is mediated by CNT1 and CNT2, and nucleoside-derived drugs such as gemcitabine and ribavirin can suppress the intestinal absorption of mizoribine. Bile salts such as sodium glycocholate were also found to cause interaction with mizoribine.

Keywords: Mizoribine; Concentrative nucleoside transporter; CNT1; CNT2; Intestine; Absorption; Bile

1. Introduction

A variety of nucleoside-derived drugs, such as acyclovir, doxifluridine (5′-DFUR), mercaptopurine, ribavirin and zidovudine, are used as antiviral or antitumoral agents (Baldwin et al., 1999; Pastor-Anglada and Baldwin, 2001; Casado et al., 2002). In clinical practice, these drugs are administered orally irrespective of their hydrophilic properties, because of the contribution of some specific influx transport systems for physiological nucleosides in intestinal membranes. Nucleoside transporters are divided into two categories: Na^+-independent equilibrative nucleoside transporter (ENT) that is known as a facilitated diffusion system and Na^+-dependent concentrative nucleoside transporter (CNT) that is known as an active transport system. ENT family has two members (ENT1, ENT2) having 11 transmembrane domains and a glycosylated extracellular loop, and these proteins are located in the basolateral membrane of absorptive epithelia. CNT family contains three members (CNT1, CNT2, CNT3) having 13 transmembrane domains and a glycosylated C-terminus, and these proteins are located in the apical membrane of absorptive epithelia. CNT1 and CNT2 are expressed in a proximal-to-distal gradient along the rat intestine, whereas the expression level of CNT3 is quite low in rats (Casado et al., 2002; Lu et al., 2004). CNT1 transports uridine, thymidine, cytidine (pyrimidine nucleosides)

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and adenosine (a purine nucleoside), and CNT2 transports guanosine, adenosine (both purine nucleosides) and uridine (a pyrimidine nucleoside). CNT3 transports both purine and pyrimidine nucleosides (Ritzel et al., 2001; Gray et al., 2004). For example, gemcitabine, a pyrimidine analogue, is transported by CNT1 and CNT3, in addition to ENT1 and ENT2, but not by CNT2 (Mackey et al., 1999; Rauchwerger et al., 2000; Casado et al., 2002). Clofarabine, a purine nucleoside analog, is transported by oocytes with recombinant transporters in the following order: CNT3 > ENT2 > ENT1 > CNT2, but not by CNT1 (King et al., 2006). Thus, there is a distinct difference in substrate specificities between CNT1 and CNT2.

In addition to antiviral and antitumoral effects, some nucleoside analogues exhibit immunosuppressive effect. For example, mizoribine (or bredinin®), an imidazole nucleoside, has long been used as an orally available immunosuppressive agent in human renal transplantation in Japan. Recently, mizoribine was also found to show a potent efficacy as an anti-hepatitis C virus (HCV) reagent in combination with interferon-α, as well as ribavirin (Naka et al., 2005). The high and relatively steady oral bioavailability of mizoribine in healthy subjects has been recognized, in which the excretion percentages of unchanged mizoribine were 65–100% in fasted human (Honda et al., 2006; Stypinski et al., 2006). However, researches on membrane transport mechanism of mizoribine are few. Recently, Okada et al. (2006) found that inosine and inosinic acid inhibited the absorption of mizoribine, and reported the possible contribution of CNT2 (or N1 transporter) in the intestinal absorption of mizoribine in rats.

In the present study, we characterized the intestinal absorption of mizoribine by examining the contribution of CNT1, in addition to CNT2, in rats. Some possible factors that may influence the intestinal absorption of mizoribine were also examined, since the oral bioavailability of mizoribine is known to be suppressed by the intake of food in humans and rats.

2. Materials and methods

2.1. Materials

Mizoribine was a gift from Asahi Kasei Pharma Corporation (Tokyo, Japan). Ribavirin (Rebetol®) was obtained from Schering-Plough K.K. (Osaka, Japan). Gemcitabine (Gemzar® Injection) was obtained from Eli Lilly Japan K.K. (Kobe, Japan). Adenosine, inosine, thymidine, sodium cholate, and indomethacin were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Glycocholic acid sodium salt was from Calbiochem-Novabiochem Co. (Darmstadt, Germany). Taurocholic acid sodium salt was from MP Biomedicals, LLC (OH, USA). Capric acid sodium salt (sodium decanoate) and sodium dodecylsulfate (SDS) were from Sigma-Aldrich Japan K. K. (Tokyo, Japan), and quinidine was from Kanto Chemical Co., Inc. (Tokyo, Japan). 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). The rats were fed a standard laboratory diet for rats (CE-2, Clea Japan, INC., Tokyo, Japan) and water for more than 1 week prior to experiments. Experiments with animals were performed in accordance with the "Guidelines for proper conduct of animal experiments" from Science Council of Japan, that is opened to the public on internet.

2.3. In-vivo oral administration of mizoribine (blood and urine sampling)

Rats were anaesthetized with pentobarbital (30 mg/kg, i.p. injection) and a jugular vein was exposed. A 18 cm-long polyurethane tubing (PUC-15, 0.5 mm in diameter, Eicom Corporation, Kyoto, Japan) was sterilized with ethanol, filled with 100-fold diluted heparin with saline (10 units/ml), and inserted into the jugular vein in the direction of chest (about 3 cm in long). The tubing was fixed at the inserted position by silk suture and a drop of surgical glue (Aron Alpha A "Sankyo", Sankyo Co., Ltd. Tokyo, Japan). The residual tubing (approximately 15 cm) was transferred to the neck in the back by tracing beneath the fur skin, and was connected to the Automated Blood Sampling System (DR-VS, Eicom Corporation, Kyoto, Japan, http://www.eicom.co.jp/). Mizoribine was dissolved in water at a concentration of 2.5 mg/ml or 10 mg/ml, and each rat received at a volume of 2 ml/kg (5 mg/kg or 20 mg/kg dose) by stomach intubation after one night-fast. Blood (0.25 ml each) was taken at designated time intervals (0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 24 h) by the auto sampling system. The blood 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.

In separate experiments, one night-fasted rats received mizoribine (5 mg/2 ml/kg or 20 mg/2 ml/kg) by stomach intubation, and were housed in metabolic cages to collect urine. 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.

2.4. In-situ intestinal loop study of mizoribine in rats

Rats were fasted overnight, anaesthetized with pentobarbital (30 mg/kg, *i.p.* injection) and affixed supine on a surface kept at 37 °C to maintain the body temperature at approximately 36 °C. After washing the intestinal lumen with a sufficient amount of saline prewarmed at 37 °C, a 10 cm-long intestinal loop was made at jejunum (a segment from 5 cm below the bile duct opening) or ileum (a segment above the ileocecum) to characterize the intestinal absorption of mizoribine in rats. Mizoribine dissolved in saline at a concentration of 0.5 mg/ml, 2.5 mg/ml or 12.5 mg/ml was administered to 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). In a separate experiment, the intestinal absorption of mizoribine from the loop without washing the intestinal lumen was also examined using jejunum and ileum loops. In inhibition studies, mizoribine (1 mg/2 ml/kg) or ribavirin (1 mg/2 ml/kg) was administered together with other

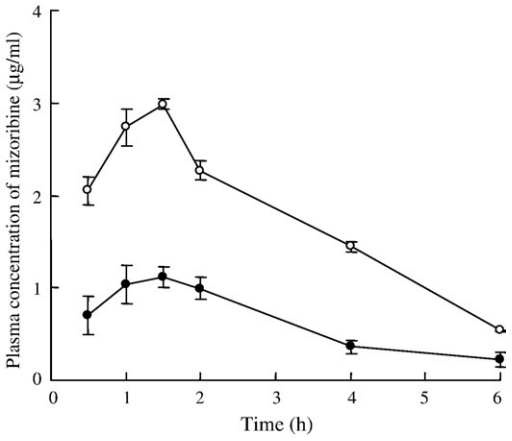

Fig. 1. Plasma concentration–time profiles of mizoribine after oral administration at a dose of 5 mg/kg (closed circle) or 20 mg/kg (opened circle).in male conscious rats. Each value represents the mean±S.D. (n=3).

compounds to examine their effects on intestinal absorption of mizoribine. Compounds examined were adenosine, thymidine, inosine, gemcitabine, ribavirin, capric acid sodium salt, indomethacine, quinidine, sodium dodecylsulfate, sodium cholate, glycocholic acid sodium salt, and taurocholic acid sodium salt for mizoribine. The effect of glycocholic acid sodium salt on intestinal absorption of ribavirin was also examined. Nucleoside compounds and nucleoside-derived drugs were added at different concentrations (3-fold, 10-fold or 30-fold molar excess of mizoribine) to the solution of mizoribine (0.5 mg/ml or 1.93 mM). Other compounds except nucleoside compounds were mixed with mizoribine or ribavirin at a concentration of 10 mM.

At 1 h after the administration of mizoribine or ribavirin, rats were killed by injecting a sufficient amount of saturated KCl solution to the heart. The intestinal loop containing mizoribine or 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.

2.5. Analysis

Concentrations of mizoribine in the supernatants of various biological samples (plasma, urine, intestinal homogenate) 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 same manner as reported by Homma et al. (1999).

Data were expressed as the mean±S.D. Differences among group mean values were assessed by the Kruskal-Wallis or ANOVA test followed by a post-hoc test (Tukey test) or Student's t-test. A difference of $P<0.05$ was considered statistically significant.

3. Results

3.1. Oral administration of mizoribine

Mizoribine was administered orally to conscious rats at a dose of 5 mg/kg or 20 mg/kg. Plasma concentrations of mizoribine (Fig. 1) and some pharmacokinetic parameters such as the peak plasma concentration (Cmax), time to reach Cmax (Tmax), area under the plasma concentration-time curve from 0 to 6 h (AUC_{0-6}), elimination rate constant from plasma (ke), and urinary excretion percentage for 24 h (% of dose) of mizoribine are summarized in Table 1. The difference in Cmax and AUC_{0-6} of mizoribine between 5 mg/kg and 20 mg/kg doses was not proportional with its doses. In particular, the urinary excretion percentage of mizoribine at a dose 20 mg/kg was almost the half of that at a dose of 5 mg/kg, suggesting the saturation of intestinal absorption of mizoribine in rats.

3.2. In-situ intestinal absorption of mizoribine

To confirm the dose dependency in the intestinal absorption of mizoribine, mizoribine was administered to the jejunum loop at a dose of 1, 5 or 25 mg/kg in rats. The absorption percentage of mizoribine, 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. 2).

3.3. Effect of nucleoside compounds and nucleoside-derived drugs on intestinal absorption of mizoribine

Effects of adenosine, thymidine and inosine on intestinal absorption of mizoribine were examined in in-situ intestinal loop method. The dose of mizoribine was 1 mg/2 ml/kg (corresponding to 1.93 mM of mizoribine in the dosing solution) and the concentrations of nucleoside compounds were varied to 3-fold, 10-fold, or 30-fold excess molar of mizoribine (Fig. 3). Adenosine (10-fold amount of mizoribine), tymidine (30-fold) and inosine (30-fold) significantly suppressed the intestinal absorption of mizoribine, and adenosine (a substrate of both CNT1 and CNT2) showed a greater inhibitory action as

Table 1
Pharmacokinetic parameters of mizoribine after oral administration in rats

Parameters	Dose of mizoribine	
	5 mg/kg	20 mg/kg
Cmax (μg/ml)	1.19±0.31	2.90±0.29
Tmax (h)	1.50±0.50	1.33±0.29
AUC_{0-6} (μg h/ml)	4.31±0.75	11.77±0.65
ke (h^{-1})	0.65±0.34	0.37±0.03
Urinary excretion (% of dose)	53.1±6.0	24.0±5.1 [a]

Values are expressed as the mean±S.D. (n=3). The value of AUC was calculated by a trapezoidal rule, and the urinary excretion of mizoribine was measured at 24 h after administration.

[a] Significantly different from the value at a dose of 5 mg/kg at a level of 0.05.

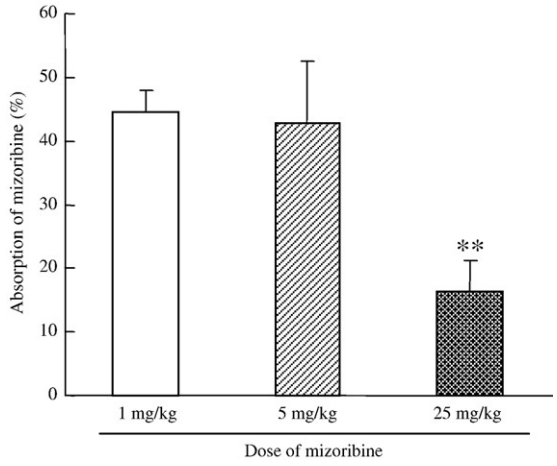

Fig. 2. Intestinal absorption of mizoribine in rats. Mizoribine was administered at a dose of 1, 5 or 25 mg/2 ml/kg to a 10 cm-long jejunum loop and the intestinal absorption (%) was estimated by measuring the remained amount of mizoribine in the loop 60 min after administration. Each value represents the mean±S.D. (n=3). ** P<0.01, significantly different from the value at doses of 1 and 5 mg/kg.

compared with those of thymidine (a CNT1 substrate) and inosine (a CNT2 substrate). These results suggested the contribution of CNT1 and CNT2 in intestinal absorption of mizoribine.

Effects of nucleoside-derived drugs such as gemcitabine (a CNT1 substrate) and ribavirin (a CNT2 substrate) on intestinal absorption of mizoribine were also examined (Fig. 4). Both gemcitabine and ribavirin significantly suppressed the intestinal absorption of mizoribine. In particular, ribavirin showed a greater inhibitory effect than gemcitabine when compared at a dose of 10-fold excess molar of mizoribine.

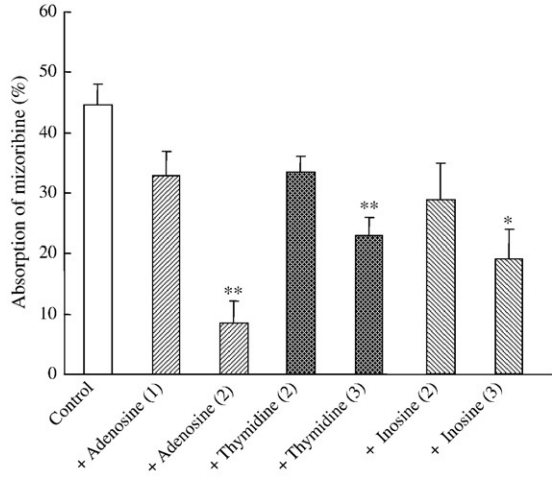

Fig. 3. Effects of various nucleoside compounds on intestinal absorption of mizoribine in rats. Mizoribine was administered to a 10 cm-long jejunum loop at a dose of 1 mg/2 mL/kg and concentrations of nucleoside compounds were (1) 3-fold, (2) 10-fold, and (3) 30-fold molar excess of mizoribine (1.93 mM). The intestinal absorption (%) was estimated by measuring the remained amount of mizoribine in the loop 60 min after administration. Each value represents the mean±S.D. (n=3). ** P<0.01, * P<0.05; significantly different from the value of control (mizoribine alone).

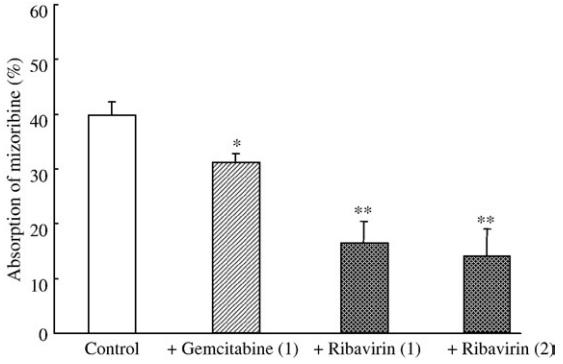

Fig. 4. Effects of gemcitabine and ribavirin on intestinal absorption of mizoribine in rats. Mizoribine was administered to a 10 cm-long jejunum loop at a dose of 1 mg/2 ml/kg, and the concentrations of nucleoside-derived drugs were (1) 10-fold or (2) 30-fold molar excess of mizoribine (1.93 mM). The intestinal absorption (%) was estimated by measuring the remained amount of mizoribine in the loop 60 min after administration. Each value represents the mean±S.D. (n=3). ** P<0.01, * P<0.05; significantly different from the value of control (mizoribine alone).

3.4. Effect of bile salts on intestinal absorption of mizoribine

In a preliminary study, mizoribine was administered into the intestinal loop without washing the intestinal lumen in some rats. In such cases, the absorption percentages of mizoribine from jejunum were lower than those in rats with lavaged intestinal lumen. On one hand, the effect of the washing of intestinal lumen prior to the intestinal absorption study of mizoribine was not observed in the ileum loop (Fig. 5). These results suggested the possible contribution of bile juice or bile salts in the intestinal absorption of mizoribine. As bile salts, sodium cholate, sodium taurocholate and sodium glycocholate were examined at a concentration of 10 mM. Sodium cholate and sodium glycocholate significantly suppressed the intestinal absorption of mizoribine (Fig. 6). On one hand, the intestinal absorption of ribavirin was not affected by sodium glycocholate. To clarify the

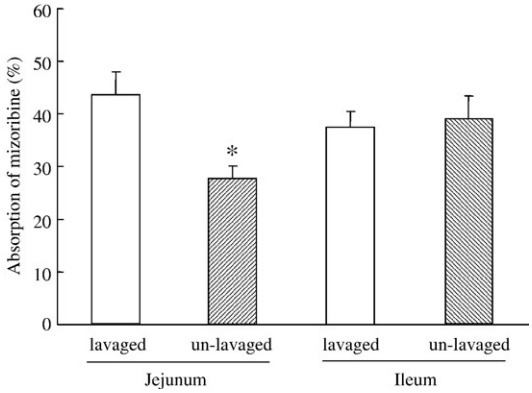

Fig. 5. Effect of the lavage of intestinal lumen on intestinal absorption of mizoribine in rats. Intestinal lumen was washed with a sufficient amount of saline prewarmed at 37 °C prior to the absorption study of mizoribine. Mizoribine was administered to a lavaged or un-lavaged 10 cm-long jejunum loop at a dose of 1 mg/kg, and the intestinal absorption (%) was estimated by measuring the remained amount of mizoribine in the loop 60 min after administration. Each value represents the mean±S.D. (n=3). * P<0.05; significantly different from the value of lavaged at jejunum.

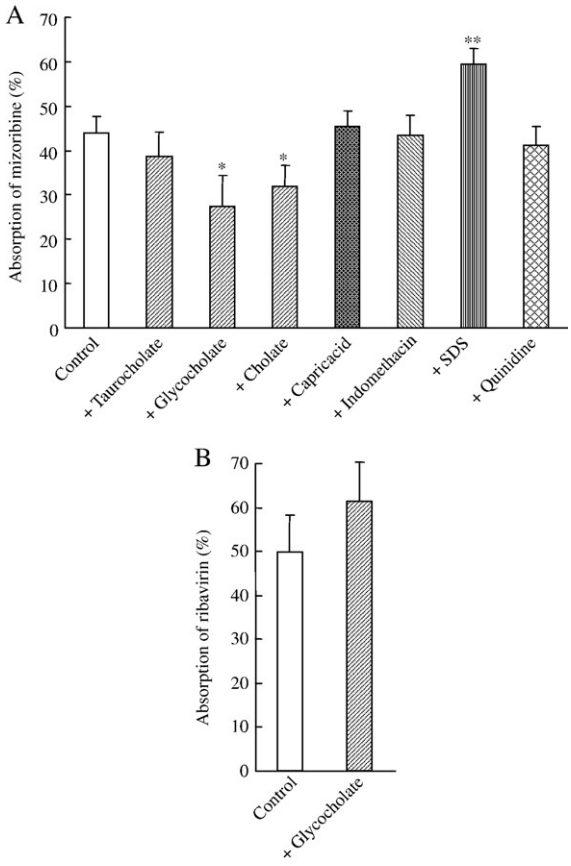

Fig. 6. Effects of various charged compounds on intestinal absorption of mizoribine (A) and effect of glycocholate of ribavirin (B) in rats. The concentration of various additives was 10 mM in the dosing solution. Mizoribine and ribavirin were administered to a 10 cm-long jejunum loop at a dose of 1 mg/2 ml/kg, and the intestinal absorption (%) was estimated by measuring the remained amount of the drug in the loop 60 min after administration. Each value represents the mean ± S.D. (n=3). ** P<0.01, * P<0.05; significantly different from the value of control (mizoribine alone).

suppression mechanism of bile salts, some charged compounds such as capric acid, indomethacin (negatively charged), quinidine (positively charged) and SDS (negatively charged surfactant) were examined by administering with mizoribine (Fig. 6). These charged compounds, except SDS, did not show any significant effects on intestinal absorption of mizoribine, whereas SDS significantly increased the intestinal absorption of mizoribine.

4. Discussion

Mizoribine, or bredinin®, was isolated from the culture filtrate of *Eupenicillium brefeldianum* as an immunosuppressive agent (Mizuno et al., 1974), and has been available on the market in inhibition of the rejection reaction in renal transportation and treating of nephritic syndrome, lupus nephritis and rheumatoid arthritis (Ishikawa 1999; Takei 2002; Tsuzuki 2002; Yokota 2002) since 1984 in Japan. Mizoribine is rapidly absorbed from the gastrointestinal tract and excreted into urine as an unchanged form at a percentage of 65–100% of dose in humans (Stypinski et al., 2006). Thus, the disposition of mizoribine from blood circulation is highly dependent on renal function (Takada et al., 1983; Abe et al., 2004; Honda et al., 2006). On one hand, the intestinal absorption mechanism, or membrane transport mechanism, of mizoribine is not yet fully understood. Recently, Okada et al. (2006) examined the effects of inosine and inosinic acid on intestinal absorption of mizoribine in rats, and found a significant suppression by them especially in proximal region of the intestine. The results suggested the possible contribution of CNT2 (or N1 transporter) in the intestinal absorption of mizoribine, since the intestinal absorption of inosine is known to be mediated by CNT2 (Patil et al., 1998). The greater absorption and suppression of mizoribine in proximal region would be due to the higher expression of CNT2 as compared with those in distal region. In the present study, we examined the contribution of CNT1, in addition to CNT2, and some possible factors that may influence the intestinal absorption of mizoribine in rats (Casado et al., 2002; Lu et al., 2004). The contribution of CNT3 in the intestinal absorption of mizoribine was not examined in the present study, since the expression level of CNT3 in rat intestine is reportedly very low (Casado et al., 2002; Lu et al., 2004).

When mizoribine was administered orally at two different doses (5 and 20 mg/kg), Cmax and AUC_{0-6} of mizoribine were not proportional with the dose (Fig. 1, Table 1). Also, the urinary excretion percentage, or oral bioavailability, of mizoribine at a dose of 20 mg/kg was the half of that at a dose of 5 mg/kg. This saturated intestinal absorption of mizoribine was also observed in in-situ loop studies, where the disappearance percentage from the loop 1 h after administration of mizoribine was significantly lower at a dose of 25 mg/kg than those at 1 and 5 mg/kg doses (Fig. 2). Mizoribine is a water soluble hydrophilic compound with a very low lipophilicity (log P is −2.87, that is calculated by Crippen's fragmentation method) (Ghose and Crippen, 1987). Thus, the participation of passive diffusion could be ruled out in the intestinal absorption mechanism of mizoribine. Among six possible nucleoside transporters (CNT1, CNT2, CNT3, ENT1, ENT2, ENT3), we examined the contribution of CNT1 and CNT2, because these transporters are abundantly expressed in the intestine of rats when their six transcripts were quantified using the branched DNA signal amplification assay (Lu et al., 2004). The substrate specificity of nucleoside transporters is complicated. For example, CNT1 transports uridine, thymidine, cytidine (pyrimidine nucleosides) and adenosine (a purine nucleoside) (Baldwin et al., 1999). CNT2 transports guanosine, adenosine (both purine nucleosides), uridine (a pyrimidine nucleoside), and inosine (Patil et al., 1998; Baldwin et al., 1999). Among these nucleoside compounds, adenosine, inosine, and thymidine were employed in the present study. Adenosine is a substrate of both CNT1 and CNT2, thymidine is a substrate of CNT1 and inosine is a substrate of CNT2. Adenosine and thymidine suppressed the intestinal absorption of mizoribine (Fig. 3), as well as inosine as reported by Okada et al. (2006). In particular, adenosine showed a greater inhibitory effect as compared with those of inosine and thymidine. The suppression by thymidine and the greater suppression by adenosine strongly suggested the participation of CNT1, in addition to CNT2, in the intestinal absorption of mizoribine.

Next, we examined the effects of gemcitabine and ribavirin on intestinal absorption of mizoribine. Gemicitabine, a pyrimidine analogue of deoxycytidine, is known to have high affinity to CNT1, CNT3, ENT1 and ENT2, but not to CNT2 (Mackey et al., 1999; Rauchwerger et al., 2000; Casado et al., 2002). Ribavirin is known to be transported by CNT2 (Okada et al., 2006). Both gemcitabine and ribavirin significantly suppressed the intestinal absorption of mizoribine, where ribavirin showed a greater inhibitory effect (Fig. 4). Thus, the contribution of CNT1 and CNT2 in the intestinal absorption of mizoribine was again confirmed, and these findings would suggest that mizoribine can cause interaction with many other nucleoside-derived drugs transported by CNT1 and CNT2 in the intestinal absorption of mizoribine.

In relation to the suppressive effects of nucleosides and nucleoside-derived drugs on the intestinal absorption of mizoribine, we also evaluated the effect of adenosine, which showed a greater inhibitory effect, on the intestinal membrane permeability by examining the intestinal absorption of phenolsulfonphthalein (PSP) and sulfanilamide in the presence of adenosine. PSP was used as a poorly absorbable hydrophilic model compound and sulfanilamide was used as a membrane permeable lipophilic compound. The absorption percentages of PSP (dose 1 mg/kg) from jejunum loop for 1 h were 10.1±6.6% and 15.1±13.5% in the absence and presence of adenosine (19.3 mM in the dosing solution), respectively. Those of sulfanilamide were 98.6±2.5% and 92.6±6.0%, respectively. Thus, no significant effect of adenosine on intestinal membrane permeability to PSP and sulfanilamide would suggest that the inhibitory effects of nucleoside compounds on intestinal mizoribine absorption would not be due to the reduction of membrane permeability, but due to the inhibition of the function of nucleoside transporters.

Then, we examined some possible factors that may influence the intestinal absorption of mizoribine in in-situ loop method. In preliminary experiments, we used both lavaged and un-lavaged intestine in estimating the intestinal absorption of mizoribine in jejunum and ileum. In that experiment, the washing of the intestinal lumen significantly increased the absorption of mizoribine in jejunum, but not in ileum (Fig. 5). Based on these results, we supposed that bile (or bile salts) affected the intestinal mizoribine absorption, and the greater suppression in jejunum than ileum was considered to be due to the higher concentrations of bile salts in the proximal intestine. Some bile salts such as sodium taurocholate, sodium glycocholate, and sodium cholate were examined whether they affect the intestinal absorption of mizoribine (Fig. 6). Both sodium glycocholate and sodium cholate significantly suppressed the intestinal absorption of mizoribine. The reason for the less effect of taurocholate is unclear at present, however, the small difference in the physicochemical properties of bile salts such as the solubilizing activity for Sudan III, the lipophilicity (Rm value) and affinity for calcium ion at pH 10.0 may contribute to the different inhibitory potencies (Murakami et al., 1984). In general, bile salts can increase the solubility of lipids, cholesterol and lipid-soluble vitamins as physiological surfactants and increase their absorption by delivering to the absorptive membrane through the unstirred water layer (Hofmann, 1999; Kullak-Ublick et al., 2004; Alrefai and Gill, 2007). However, for mizoribine, formation of mixed micells with bile salts would not be expected, since mizoribine is a hydrophilic compound. Thus, in the present study, the effects of bile salts were examined from the view points of charged interaction, because mizoribine is an amphoteric compound. Negatively charged compounds such as capric acid, indomethacine and sodium dodecylsulfate (SDS) and a positively charged compound quinidine did not show any significant effect on intestinal absorption of mizoribine. Instead, SDS increased the intestinal absorption of mizoribine, possibly due to the mucolytic activity (Takatsuka et al., 2006). Ribavirin is known to be absorbed by CNT2 in rats (Okada et al., 2006). For ribavirin, however, sodium glycocholate did not decrease the intestinal absorption, rather increased a little, though it did not reach to the significant difference (Fig. 6). Thus, it was speculated that the inhibitory effects of sodium glycocholate and sodium cholate on intestinal absorption of nucleoside-derived compounds may be specific to mizoribine, or specific to CNT1. Further study is necessary to clarify the inhibitory mechanism of some bile salts in the intestinal absorption of nucleosides and/or nucleoside-derived compounds.

In conclusion, it was found that the intestinal absorption of mizoribine is mediated by CNT1 and CNT2, and nucleoside-derived drugs such as gemcitabine and ribavirin can suppress the intestinal absorption of mizoribine. Bile salts such as sodium glycocholate were also found to cause interaction with mizoribine. These findings would be valuable in attaining safer and reliable oral bioavailability of mizoribine.

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