Note

Effect of Genetic Polymorphisms of SLC28A1, ABCG2, and ABCC4 on Bioavailability of Mizoribine in Healthy Japanese Males

Miki Fukao 1, Kazuya Ishida 1, Takuya Sakamoto 1, Masato Taguchi 1, Hiroyoshi Matsukura 2, Toshio Miyawaki 1 and Yukiya Hashimoto 1,*

1Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama, Japan
2Department of Pediatrics, Saiseikai Toyama Hospital, Toyama, Japan

Summary: The aim of the present study was to investigate the genetic factors responsible for the interindividual variability in the bioavailability of mizoribine. Thirty healthy Japanese men aged 20–49 years and weighing 53–75 kg participated in the present study and took 150 mg of mizoribine. Urine samples were collected periodically for 12 h after the dose, and the bioavailability of mizoribine was calculated from the estimated total urinary excretion from time zero to infinity. The bioavailability of mizoribine in the 30 subjects ranged from 60.3% to 99.4%. The mean bioavailability of mizoribine in subjects with the concentrative nucleoside transporter 1 (SLC28A1) 565-A/A allele (75.4%) was significantly lower than that in subjects with the SLC28A1 565-G/G allele (90.1%). On the other hand, the bioavailability of mizoribine was not affected by polymorphisms of breast cancer resistance protein (ABCG2) C421A and multidrug resistance-associated protein 4 (ABCC4) G2269A. The findings in the present prospective study suggested that the genetic test for the SLC28A1 G565A polymorphism is promising for predicting the Japanese subjects with lower bioavailability of mizoribine.

Keywords: mizoribine; bioavailability; SLC28A1; ABCG2; ABCC4

Introduction

Mizoribine is an orally available immunosuppressive agent that has been on the market since 1984 in Japan for the prevention of rejection in renal transplantation.1 Additional indications for which mizoribine has subsequently been approved are lupus nephritis, rheumatoid arthritis, and nephrotic syndrome.2 Mizoribine is a highly hydrophilic compound and is not metabolized in the body. The unchanged drug binds only slightly to plasma proteins and is excreted predominantly in the urine; therefore, renal function has been considered to be one of the main causes of the pharmacokinetic variability of mizoribine. On the other hand, Ihara et al. evaluated the pharmacokinetics of mizoribine in 14 kidney transplant recipients. The cumulative urinary excretion of mizoribine (bioavailability) in the patients was variable, and ranged from 12% to 81% of the dose (mean: 41%).3 These findings suggested that the extent of intestinal absorption is another factor affecting the pharmacokinetic variability of mizoribine.

Concentrative nucleoside transporter 1 (CNT1/SLC28A1) and 2 (CNT2/SLC28A2) are Na+–dependent, and the movement of nucleoside regardless of its concentration gradient is coupled to that of sodium ions.4,5 SLC28A1 and SLC28A2 are localized on the apical side of enterocytes, and these transporters differ in their substrate specificities: SLC28A1 transports pyrimidine-nucleoside preferentially, whereas SLC28A2 transports purine-nucleoside preferentially.4-6 Recently, Naito et al. evaluated the effect of polymorphisms of SLC28A1 and SLC28A2 on the bioavailability of mizoribine in Japanese kidney transplant recipients.7 SLC28A2 C65T and C225A polymorphisms did not affect the bioavailability of mizoribine. On the other hand, the bioavailability of mizoribine in patients with SLC28A1 565-G/G and -A/A alleles was significantly lower than that with the wild-type SLC28A1 565-G/G allele.7

Not only influx but also efflux transporters expressed in the apical membrane of intestinal epithelial cells may affect the bioavailability of substrate drugs.8 Breast cancer resistance protein (BCRP/ABCG2) transports hydrophilic...
compounds such as human immunodeficiency virus type 1 nucleoside reverse transcriptase inhibitors and anticancer nucleoside.\textsuperscript{9,10} It has been reported that there are several single nucleotide polymorphisms in \textit{ABCG2}, and that \textit{ABCG2 C421A} is the most frequent polymorphism at an allelic frequency of 26.6–35.0\% in the Japanese population.\textsuperscript{11} Yamasaki \textit{et al.} evaluated the effect of the genetic polymorphism of \textit{ABCG2 C421A} on the pharmacokinetics of sulfasalazine in healthy male volunteers.\textsuperscript{12} The area under the plasma concentration–time curve (AUC) of sulfasalazine in subjects with the \textit{ABCG2 421-C/A} and -A/A alleles was significantly higher than in those with the \textit{ABCG2 421-C/C} allele.\textsuperscript{13} On the other hand, multidrug resistance-associated protein 4 (MRP4/ABCC4) is expressed in the intestinal epithelial cells\textsuperscript{15,14} and mediates transport of nucleobase, nucleoside, and nucleotide analogs including cyclic nucleotides (cAMP and cGMP).\textsuperscript{15,16} Ban \textit{et al.} evaluated the effect of \textit{ABCC4} polymorphism on the pharmacodynamics of azathioprine/6-mercaptopurine.\textsuperscript{17} They reported that the \textit{ABCC4 G2269A} polymorphism was common in the Japanese population (allele frequency: 14.7\%), and that the white blood cell count in Japanese patients with the \textit{ABCC4} variant allele was significantly lower than in those with the \textit{ABCC4} wild type.\textsuperscript{17}

In the present study, we estimated the bioavailability of mizoribine in 30 healthy Japanese males by calculating cumulative urinary excretion following 150-mg oral dosing. We then evaluated the effect of the common genetic polymorphisms of \textit{SLC28A1}, \textit{ABCG2}, and \textit{ABCC4} on the bioavailability of mizoribine.

\textbf{Methods}

\textbf{Subjects and study protocols:} Thirty healthy Japanese males aged 20–49 years (mean: 24.5) and weighing 53–75 kg (mean: 62.9) participated in the present study. All subjects gave written informed consent to participate in the study, which was approved by the ethics committee of the University of Toyama. The subjects took 500 mL of water on awakening and were given 150 mg (three 50-mg tablets) of mizoribine (Bredinin tablet; Asahi Kasei Pharma, Tokyo, Japan) with a glass of water under fasting conditions at least 1 h after awakening. Urine samples were collected at 1, 2, 3, 4, 6, 8, 10, and 12 h after the dose, and all subjects drank a glass of water at the time of each urine collection. In addition, all subjects had a light breakfast at least 2 h after the dose. The amount of mizoribine in each urine sample was measured using high-performance liquid chromatography (HPLC), as described previously.\textsuperscript{18}

\textbf{Genotyping of \textit{SLC28A1}, \textit{ABCG2}, and \textit{ABCC4}:} Genomic DNA was isolated from the peripheral blood using QIAamp DNA blood Mini Kits (Qiagen, Hilden, Germany) according to the manufacturer’s instructions and was stored at −80°C. The \textit{SLC28A1 G565A} polymorphism was determined using the polymerase chain reaction–restriction fragment length polymorphism method.\textsuperscript{17} The \textit{ABCG2 C421A} polymorphism was determined using the TaqMan Drug Metabolism Genotyping Assay (Applied Biosystems, Foster, CA, USA).\textsuperscript{12} The \textit{ABCC4 G2269A} polymorphism was identified by direct sequencing.\textsuperscript{17}

\textbf{Estimation of pharmacokinetic parameters of mizoribine in individual subjects:} To estimate the pharmacokinetic parameters of mizoribine in the subjects, we assumed a one-compartment model with first-order absorption.\textsuperscript{19} The urinary excretion rate of mizoribine \((X_u)\) may be described by the following equation:

\[
X_u = k_e \cdot A = k_e \cdot \frac{D \cdot F \cdot k_a}{k_a - k_e} \left( e^{-k_a t} - e^{-k_e t} \right) \tag{1}
\]

where \(k_e\) and \(k_a\) are the elimination and absorption rate constants, respectively, \(A\) is the amount of mizoribine in the body at time \(t\), \(D\) is the dose (150 mg), and \(F\) is the bioavailability of mizoribine. The urinary excretion rate \((X'_u)\) interpolated and extrapolated from the data in the terminal elimination phase (at 6–12 h after the dose) can be described by the following equation:

\[
X'_u = k_e \cdot D \cdot F \cdot k_a \frac{D \cdot F \cdot k_a}{k_a - k_e} \cdot e^{-k_e t} \tag{2}
\]

Therefore, the \(k_e\) value was estimated using the following equation (Fig. 1):

\[
\ln X'_u = \ln \left( k_e \cdot D \cdot F \cdot k_a \frac{D \cdot F \cdot k_a}{k_a - k_e} \right) - k_e \cdot t \tag{3}
\]

In addition, the elimination half-life \((t_{1/2})\) was estimated using the following equation:

\[
t_{1/2} = \frac{\ln 2}{k_e} \tag{4}
\]

The amount of mizoribine in the urine after the final collection of urine \((A_{12 \rightarrow \infty})\) is the cumulative urinary excretion \((A_{0 \rightarrow 12} + A_{12 \rightarrow \infty})\), and the \(F\) value of mizoribine were estimated using the following equations:

\[
A_{12 \rightarrow \infty} = \frac{X_{u_{12}}}{k_e} \tag{5}
\]

\[
F = \frac{A_{0 \rightarrow 12} + A_{12 \rightarrow \infty}}{D} \tag{6}
\]

where \(X_{u_{12}}\) is the estimated urinary excretion rate at 12 h after the dose, and \(A_{0 \rightarrow 12}\) is the total amount of mizoribine in the urine collected between 0 and 12 h after the dose. Furthermore, we estimated the \(k_e\) value using the urinary excretion rate in the early absorption phase (at 0–3 h after the dose), \(t.e.,\) from Eqs. (1) and (2), the difference between \(X'_u\) and \(X_u\) is described as:

\[
X'_u - X_u = k_e \cdot D \cdot F \cdot k_a \frac{D \cdot F \cdot k_a}{k_a - k_e} \cdot e^{-k_e t} \tag{7}
\]

Therefore, the \(k_e\) value can be estimated using the following equation (Fig. 1):
\[
\ln(X_0' - X_a) = \ln\left(\frac{k_e \cdot D \cdot F \cdot k_d}{k_e - k_d}\right) - k_a \cdot t
\]  
(8)

In addition, the absorption lag time (ALAG) was estimated by the intersection of Eqs. (3) and (8).

**Statistical analysis:** Differences between two groups were evaluated using Student's t test, provided that the variances of the groups were similar. If this was not the case, the Mann-Whitney U test was applied. Multiple comparisons were performed using Scheffé's test following one-way ANOVA, provided that the variances of groups were similar. \(P\) values of less than 0.05 were considered to be statistically significant.

Equation (9) was used to evaluate the effect of the genetic polymorphisms of SLC28A1, ABCG2, and ABCC4 on the bioavailability of mizoribine, and multiple regression analysis was performed using the nonlinear mixed effect model (NONMEM) software:

\[
F(\%) = \theta_1 + \theta_2 \cdot SLC28A1 + \theta_3 \cdot ABCG2 + \theta_4 \cdot ABCC4
\]  
(9)

where \(SLC28A1 = 0\) for subjects with the 565-G/G allele, 1 for those with the 565-G/A allele, and 2 for those with the 565-A/A allele; \(ABCG2 = 0\) for subjects with the 421-C/C allele, 1 for those with the 421-C/A allele, and 2 for those with the 421-A/A allele; \(ABCC4 = 0\) for subjects with the 2269-G/G allele, 1 for those with the 2269-G/A allele, and 2 for those with the 2269-A/A allele. NONMEM provides estimates of the standard error (S.E.) for the \(\theta\) values, and S.E. can be used to define 95% confidence intervals (CI) for true parameter values: 95% CI = (the estimated parameter value) \pm 1.96 \cdot \text{S.E.}^{20} \text{If the null value was outside the 95% CI, the } \theta \text{ value was considered to be statistically significant.}

**Results**

**Polymorphisms of SLC28A1, ABCG2, and ABCC4 in 30 healthy Japanese males:** We first evaluated the genetic polymorphism of SLC28A1, ABCG2, and ABCC4 in the subjects. Seven subjects had the SLC28A1 565-G/G allele, eighteen subjects had the SLC28A1 565-G/A allele, and five subjects had the SLC28A1 565-A/A allele. Nineteen subjects had the ABCG2 421-C/C allele, ten subjects had the ABCG2 421-C/A allele, and one subject had the ABCG2 421-A/A allele. Twenty-five subjects had the ABCC4 2269-G/G allele, four subjects had the ABCC4 2269-G/A allele, and one subject had the ABCC4 2269-A/A allele.

**Pharmacokinetic parameters of mizoribine in healthy Japanese males:** We next estimated the pharmacokinetic parameters of mizoribine in 30 healthy male Japanese volunteers. Figure 1 shows the urinary
excretion rate of mizoribine in four typical volunteers. The ranges of $k_v$, $t_{1/2}$, $F$, $k_a$, and ALAG of mizoribine were 0.191–0.454 h$^{-1}$ (mean: 0.270 h$^{-1}$, median: 0.261 h$^{-1}$), 1.53–3.63 h (mean: 2.62 h, median: 2.65 h), 60.3%–99.4% (mean: 85.6%, median: 88.0%), 0.670–1.262 h$^{-1}$ (mean: 0.893 h$^{-1}$, median: 0.872 h$^{-1}$), and 0.226–0.634 h (mean: 0.400 h, median: 0.382 h), respectively. These findings indicated that the $t_{1/2}$, $k_v$, and ALAG values and their variability estimated in the present study are comparable to those estimated by a previous analysis of serum concentration data, and that there is considerable interindividual variability not only in elimination ($k_v$ and $t_{1/2}$), but also in bioavailability ($F$) and intestinal absorption ($k_a$ and ALAG) of mizoribine in healthy Japanese subjects.

**Effect of SLC28A1, ABCG2, and ABCC4 polymorphisms on bioavailability of mizoribine in healthy Japanese males:** Figure 2A shows the effect of SLC28A1 G565A on the bioavailability of mizoribine. The mean $F$ value in subjects with the SLC28A1 565-G/A allele (86.7%) was only slightly lower than that in subjects with the SLC28A1 565-G/G allele (90.1%) (Fig. 2A). On the other hand, the mean $F$ value in subjects with the SLC28A1 565-A/A allele (75.4%) was significantly lower than that in subjects with the SLC28A1 565-G/G allele (Fig. 2A). The findings confirmed that the SLC28A1 G565A polymorphism significantly affected the bioavailability of mizoribine in the Japanese subjects tested.

We further evaluated the effect of ABCG2 C421A and ABCC4 G2269A polymorphisms on the bioavailability of mizoribine in healthy Japanese subjects. We could not detect a systematic effect of ABCG2 C421A and ABCC4 G2269A polymorphisms on the $F$ value of mizoribine (Figs. 2B and 2C); therefore, we performed multiple regression analysis of the $F$ values in the tested population (Eq. 9). Values for $\theta_1$, $\theta_2$, $\theta_3$, and $\theta_4$ were estimated to be 93.5%, −7.6%, −2.3%, and 0.7%, respectively. The null value was expectedly outside the 95% CI of $\theta_2$; however, the null value was inside the 95% CIs of $\theta_1$ and $\theta_4$ (Table 1), i.e., the ABCG2 C421A and ABCC4 G2269A polymorphisms did not significantly affect the interindividual variability in bioavailability of mizoribine in healthy Japanese males.

**Discussion**

Naito et al. evaluated the effect of the SLC28A1 G565A polymorphism on the bioavailability of mizoribine in kidney transplant recipients and reported that the median bioavailabilities of mizoribine in recipients with the SLC28A1 565-G/G, -G/A, and -A/A alleles were 62.4%, 42.0%, and 41.4%, respectively. In the present study, we observed a significant effect of the SLC28A1 G565A polymorphism on the bioavailability of mizoribine in healthy Japanese males. However, the bioavailability of mizoribine in healthy subjects was higher than that in kidney transplant recipients, i.e., the median (mean) bioavailabilities of mizoribine in healthy Japanese subjects with the SLC28A1 565-G/G,
-G/A, and -A/A alleles were 90.3% (90.1%), 88.8% (86.7%), and 73.3% (75.4%), respectively. To our knowledge, it is unclear whether the kidney transplantation itself and/or primary diseases of the recipients affect the SLC28A1 expression and/or function in the intestine.\(^7\) On the other hand, we designed the present study to analyze the urinary excretion rate of mizoribine quantitatively and to collect an adequate volume of urine periodically from each subject; all subjects drank approximately 2 L of water in total during the trial. One possible explanation for the higher bioavailability of mizoribine in our study population may lie in the hypothesis that the drinking of water increases the dispersion of the hydrophilic drug from the tablet in the intestinal tract and the extent of absorption by the intestinal epithelial cells.

We previously evaluated the cellular uptake of mizoribine in human intestinal epithelial LS180 cells.\(^21\) The steady-state cell/medium \((C/M)\) ratio of mizoribine in the low (1 mM) extracellular Na\(^+\) condition was much lower than 1.0 (approximately 0.1). In addition, the steady-state \(C/M\) ratio of mizoribine was approximately 0.2, even in the high (126 mM) extracellular Na\(^+\) condition. On the other hand, digitonin, which is a mild nonionic detergent that permeabilizes the plasma membrane, increased the \(C/M\) ratio of mizoribine toward 1.0 with increasing concentration of the detergent. These results suggested that mizoribine is actively exported from the intestinal epithelial cells by some efflux transporter(s).\(^{21}\)

In the present study, we evaluated the effects of common genetic polymorphisms of \(ABCG2\) and \(ABCC4\) on the bioavailability of mizoribine; however, \(ABCG2\) \(C421A\) and \(ABCC4\) \(G2269A\) polymorphisms did not significantly affect the bioavailability of mizoribine (Figs. 2B and 2C, Table 1). It has been reported that \(ABCC5\) and \(ABCC11\) also confer resistance to nucleoside-based agents such as 5-fluorouracil and 9-(2-phosphonomethoxyethyl)adenine.\(^{23}\) However, it is still unclear whether the genetic polymorphisms of \(ABCC5\) and/or \(ABCC11\) are associated with functional changes in efflux transporters.\(^{23}\) Further study will be needed to clarify the factor(s) responsible for the residual variability in the bioavailability of mizoribine.

In conclusion, the SLC28A1 \(G565A\) polymorphism was a significant factor in the interindividual variability in the bioavailability of mizoribine, even in a healthy Japanese male population with fewer miscellaneous confounding factors. The present findings, in conjunction with the consistent findings by Naito et al.,\(^7\) suggest that the genetic test for the SLC28A1 \(G565A\) polymorphism is promising for predicting the Japanese subjects with lower bioavailability of mizoribine.

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References


