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ORIGINAL ARTICLE

Population pharmacokinetics of mizoribine in adult recipients of renal transplantation

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Abstract
Background The aim of the present study was to estimate the population pharmacokinetic parameters of mizoribine in adult recipients of renal transplantation using a nonlinear mixed effects model (NONMEM) program.
Methods Pharmacokinetic data for population analysis were retrospectively collected from 114 recipients (66 males and 48 females) routinely treated with oral administration of mizoribine (25–450 mg/day). The range of creatinine clearance (CL_{cr}) was 7.6−136.1 mL/min (mean 49.2 mL/min).
Results The pharmacokinetics of mizoribine in adult recipients of renal transplantation was well described by a 1-compartment model with first-order absorption. The mean value of the absorption lag time (ALAG) and absorption rate constant (KA) was estimated to be 0.581 and 0.983 h^{-1}, respectively. Apparent volume of distribution (*V*/*F*) was modeled as a function of body weight (WT), and the mean value was estimated to be 0.858 × WT L. Oral clearance (CL/*F*) was modeled as a function of creatinine clearance (CL_{cr}), and the mean value was estimated to be 1.80 × CL_{cr} × 60/1000 L/h. In addition, there was a strong correlation between CL_{cr}-corrected CL/*F* and WT-corrected *V*/*F* in the adult recipients, indicating large interindividual variability in bioavailability (*F*) of mizoribine.
Conclusion The present findings suggested that not only the rate of renal excretion but also the extent of intestinal absorption of mizoribine is responsible for the large interindividual pharmacokinetic variability of the drug.

Keywords Mizoribine · Population pharmacokinetics · Bioavailability · Renal transplantation

Introduction

Mizoribine is an orally available immunosuppressive agent, which has been on the market since 1984 in Japan for the prevention of rejection in renal transplantation [1]. In contrast to other immunosuppressive agents (e.g., azathioprine), mizoribine has been shown to lack oncogenicity in animal experiments, and exhibits a clinically low incidence of severe adverse drug reactions (such as myelosuppression and hepatotoxicity), making it useful in long-term immunosuppression therapy [2]. Mizoribine is a highly hydrophilic compound, and plasma protein binding in human is negligible; therefore, it is conceivable that mizoribine is efficiently filtrated at the renal glomerulus. Indeed, mizoribine is absorbed from the gastrointestinal tract after oral administration, and the unchanged drug is predominantly excreted into the urine [3]. In addition, Takada et al. [4] evaluated the pharmacokinetics of mizoribine in renal transplant patients, and reported that the elimination rate of mizoribine from serum was dependent on kidney function.

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We previously performed a population pharmacokinetic analysis of mizoribine in healthy volunteers using a nonlinear mixed effects model (NONMEM) program [5, 6]. The serum mizoribine concentration data obtained from 36 healthy Caucasian male subjects were used in the population pharmacokinetic analysis. The mean absorption lag time (ALAG) and absorption rate constant (KA) were estimated to be 0.349 and 0.869 h^{-1}, respectively. Apparent volume of distribution (*V*/*F*) was modeled as a function of body weight (WT), and the mean value was estimated to be 0.834 × WT L. Oral clearance (CL/*F*) was modeled as a function of creatinine clearance (CL_{cr}), and the mean value was estimated to be 1.93 × CL_{cr} × 60/1000 L/h. In addition, the pharmacokinetic parameters in individual 36 subjects could be obtained from population estimates according to Bayes' theorem [5, 6]. The mean values of pharmacokinetic parameters (KA, *V*/*F*, and CL/*F*) in the subjects were almost constant within a dose range of 3–12 mg/kg. These results indicated that the pharmacokinetics of mizoribine in healthy volunteers are linear, and are well described by a 1-compartment model with first-order absorption [5]. However, it is still unknown whether the pharmacokinetic characteristics of mizoribine in adult recipients of renal transplantation are similar to those in healthy volunteers.

The aim of the present study was to evaluate the pharmacokinetic characteristics of mizoribine in adult recipients of renal transplantation. That is, we utilized routine therapeutic drug monitoring (TDM) data for mizoribine in order to estimate the population pharmacokinetic parameters of the drug. Pharmacokinetic analysis was performed using a NONMEM program, because population pharmacokinetics based on NONMEM analysis can simultaneously evaluate the mean pharmacokinetic parameters, the covariates affecting the pharmacokinetics of a drug, and also unknown inter- and intraindividual pharmacokinetic variability [5, 6]. We then compared the population pharmacokinetic parameters in the adult recipients with those in healthy volunteers.

Methods

Pharmacokinetic data

To estimate the population pharmacokinetic parameters of mizoribine, we collected 449 TDM data of mizoribine retrospectively. Routine TDM in the 114 adult recipients (66 males and 48 females) of renal transplantation was performed from 1987 to 2004 at 11 hospitals in Japan. Table 1 shows their demographic characteristics. The ranges of age, body weight, and creatinine clearance were 20–69 years old (mean 42.5 years), 33.4–92.0 kg (mean 55.6 kg), and 7.6–136.1 mL/min (mean 49.2 mL/min), respectively. These recipients were routinely treated with oral administration of mizoribine (Bredinin® Tablet, Asahi Kasei Pharma, Tokyo, Japan) at maintenance daily doses of 25–450 mg/day (mean 146.1 mg/day). Table 1 also shows the number of the recipients in each dosing interval. On the other hand, we could not collect information about the contents of meals and timing of food intake. In addition, data on the amount of sodium intake could not be collected, although mizoribine is a substrate of Na^+-coupled nucleoside transporter [7, 8]. Furthermore, the hepatic function of the adult recipients and the presence or absence of cholestasis were unclear. The serum concentration of mizoribine was measured using high performance liquid chromatography [9].

Table 1 Demographic characteristics of patient population

Total number of patients	114	
Male/female	66/48	
Age (years)	42.5 ± 11.1	(20–69)
Weight (kg)	55.6 ± 10.7	(33.4–92.0)
Creatinine clearance (mL/min)	49.2 ± 20.9	(7.6–136.1)
Maintenance daily dose (mg/day)	146.1 ± 79.5	(25–450)
Dosing interval (*n*)		
6 h	1	
8 h	4	
12 h	64	
24 h	44	
96 h	1	

Values are expressed as the mean ± SD (range)

Estimation of population pharmacokinetic parameters of mizoribine in adult recipients of renal transplantation

Population mean pharmacokinetic parameters and their interindividual variations were estimated with the NONMEM analysis, for which we used the first-order method in the present study [6]. The 1-compartment model with first-order absorption was parameterized in terms of ALAG, KA, *V*/*F*, and CL/*F* with NONMEM-PREDPP library subroutines ADVAN2 and TRANS2 [6]. ALAG in the *i*th recipient ($ALAG_i$) was modeled using the following equation:

$$ALAG_i = \theta_1 \quad (1)$$

where θ_1 is the predicted population mean of the absorption lag time (h). KA in the *i*th recipient (KA_i) was modeled using the following equation:

$$KA_i = \theta_2 \times \exp(\eta_{KA_i}) \quad (2)$$

where θ_2 is the predicted population mean of the absorption rate constant (h^{-1}), and η_{KA_i} is a random variable

distributed normally with a mean of zero and variance of ω^2_{KA}. *V/F* and CL/*F* in the *i*th recipient (V/F_i and CL/F_i, respectively) were modeled using the following equations:

$$V/F_i = \theta_3 \times WT \times \exp\left(\eta_{V/F_i}\right) \quad (3)$$

$$CL/F_i = \theta_4 \times CL_{cr} \times \frac{60}{1000} \times \exp\left(\eta_{CL/F_i}\right) \quad (4)$$

where WT is the body weight (kg), and $\theta_3 \times WT$ is the predicted population mean of the apparent volume of distribution (L), CL_{cr} is the creatinine clearance (mL/min), and $\theta_4 \times CL_{cr} \times 60/1000$ is the predicted population mean of oral clearance (in L/h). Random variables η_{V/F_i} and η_{CL/F_i} were assumed to be distributed normally with means of zero and covariance of $\omega^2_{V/F}$, $\omega_{V/F,CL/F}$, and $\omega^2_{CL/F}$. Finally, the *j*th observed serum concentration in the *i*th recipient (C_{ij}) was assumed to be randomly and normally distributed from the predicted value (C^*_{ij}):

$$C_{ij} = C^*_{ij} + \varepsilon_{ij} \quad (5)$$

where ε_{ij} is a random variable that describes intraindividual variability with a mean of zero and variance of σ^2.

Statistical analysis

Differences between the two groups were evaluated using Student's *t* test if the variances of the groups were similar. If this was not the case, Student's *t* test with Welch's correction was applied. $p < 0.05$ was considered to be statistically significant.

Results

Mizoribine concentration profiles in adult recipients of renal transplantation

The observed serum concentration of mizoribine, daily doses, and creatinine clearance in four typical recipients of renal transplantation are shown in Fig. 1. The serum concentration of mizoribine was variable among the recipients. In the recipient with higher CL_{cr} (ID = 80), the serum concentration of mizoribine was low, and its elimination half-life was short. On the other hand, the serum concentration of the drug was high in the recipient with lower CL_{cr} (ID = 85), and its elimination half-life was long. Furthermore, the change in creatinine clearance after surgery affected the serum concentration of mizoribine; that is, a gradual decrease of creatinine clearance corresponded to the increased serum concentration of mizoribine (ID = 47). On the other hand, the serum concentration of mizoribine decreased with the increase of creatinine clearance (ID = 76). These findings indicated that renal function was significantly responsible for the variability in pharmacokinetics of mizoribine in adult recipients of renal transplantation.

Population pharmacokinetic parameters of mizoribine in adult recipients of renal transplantation

Population pharmacokinetic parameters were estimated from the 449 TDM data of mizoribine in 114 recipients using the NONMEM software. NONMEM also provided estimates of the standard error (SE) for all parameters, and SE was used to define the 95% confidence interval (CI) for true parameter values: 95% CI = (estimated parameter value) ± 1.96 × SE [6]. Table 2 shows the population pharmacokinetic parameters of mizoribine and their 95% CI estimated by NONMEM analysis. The mean values of ALAG, KA, *V/F*, and CL/*F* were estimated to be 0.581 h, 0.983 h^{-1}, 0.858 × WT L, and 1.80 × CL_{cr} × 60/1000 L/h, respectively. These population mean pharmacokinetic parameters in adult recipients of renal transplantation were similar to those in adult healthy volunteers estimated previously (Table 2) [5]. In addition, the 95% CI of each parameter was small (Table 2), indicating that the pharmacokinetics of mizoribine in adult recipients of renal transplantation could be well described by a 1-compartment model with first-order absorption. On the other hand, the ω^2_{KA}, $\omega^2_{V/F}$, $\omega_{V/F,CL/F}$, and $\omega^2_{CL/F}$ values in the recipients were estimated to be 1.03, 0.259, 0.174, and 0.169, respectively, which indicated that there was larger interindividual variability in KA, *V/F*, and CL/*F*.

Pharmacokinetic parameters in individual adult recipients of renal transplantation

The pharmacokinetic parameters of mizoribine in individual recipients could be estimated according to Bayes' theorem using the NONMEM program and its post-hoc option [6]. Figure 2 shows the relationship between CL_{cr} and CL/*F* in recipients of renal transplantation. Despite large interindividual variability in CL/*F* among the recipients, CL/*F* seemed to be correlated with CL_{cr} (Fig. 2). We also evaluated the relationship between CL_{cr}-corrected CL/*F* and WT-corrected *V/F* in adult recipients of renal transplantation (Fig. 3). There was a strong correlation between (CL/*F*)/(CL_{cr} × 60/1000) and (*V/F*)/WT in the adult recipients, indicating that bioavailability (*F*) of mizoribine is another major factor responsible for the interindividual pharmacokinetic variability of the drug.

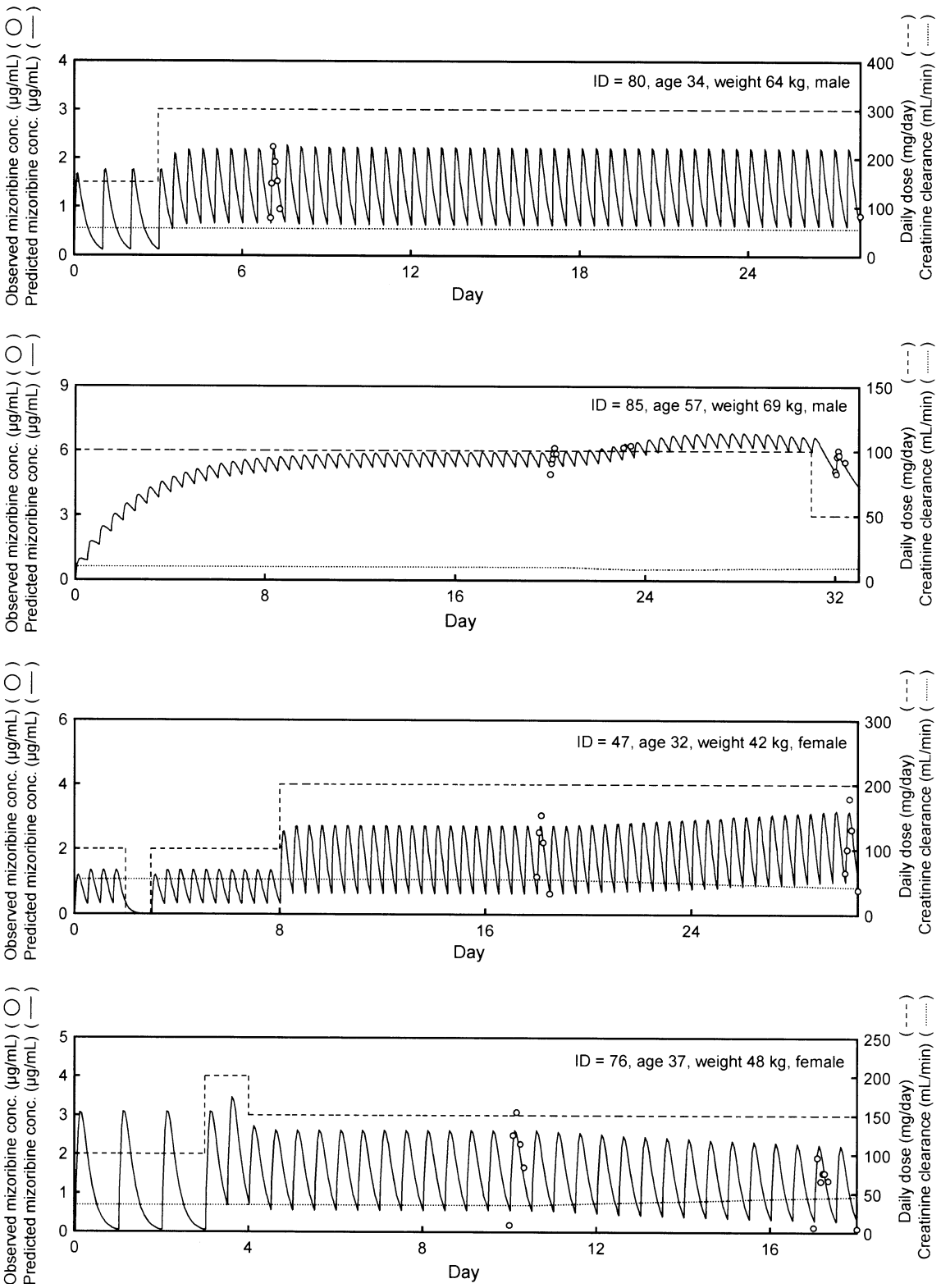

Fig. 1 Mizoribine concentration profiles, dose, and creatinine clearance in 4 typical recipients of renal transplantation. *Open circles*, *dashed lines*, and *dotted lines* indicate the observed mizoribine concentration, daily dose, and creatinine clearance, respectively. The pharmacokinetic parameters of mizoribine in individual recipients were estimated according to Bayes' theorem using the NONMEM program and its post hoc option [6]. *Solid lines* represent the predicted mizoribine concentration

Table 2 Population pharmacokinetic parameters of mizoribine in recipients of renal transplantation

	Recipients of renal transplantation		Healthy volunteers[a]
No. of subjects:	114		36
Parameters	Estimates	95% CI	Estimates
θ_1 (h)	0.581	0.516–0.646	0.349
θ_2 (h^{-1})	0.983	0.885–1.081	0.869
θ_3 (L/kg)	0.858	0.734–0.982	0.834
θ_4	1.80	1.60–2.00	1.93
ω^2_{KA}	1.03	0.44–1.62	0.0721
$\omega^2_{V/F}$	0.259	0.142–0.376	0.125
$\omega_{CL/F,V/F}$	0.174	0.100–0.248	n.d.[b]
$\omega^2_{CL/F}$	0.169	0.095–0.243	0.110
σ (μg/mL)	0.212	0.196–0.228	0.352

[a] Honda et al. [5]

[b] Not determined

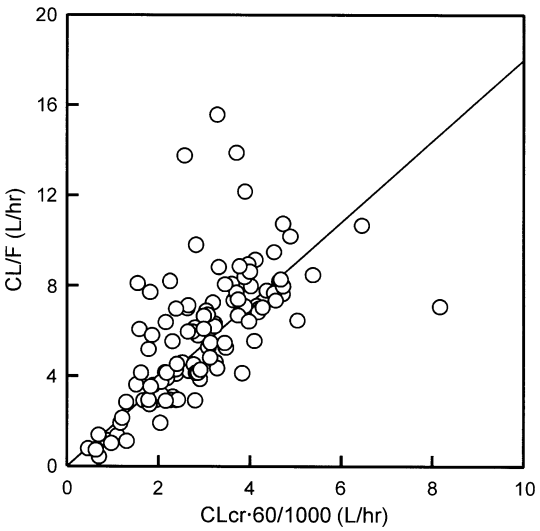

Fig. 2 Relationship between CL_{cr} and CL/*F* in recipients of renal transplantation. *Solid line* indicates CL/*F* = 1.80 × CL_{cr} × 60/1000

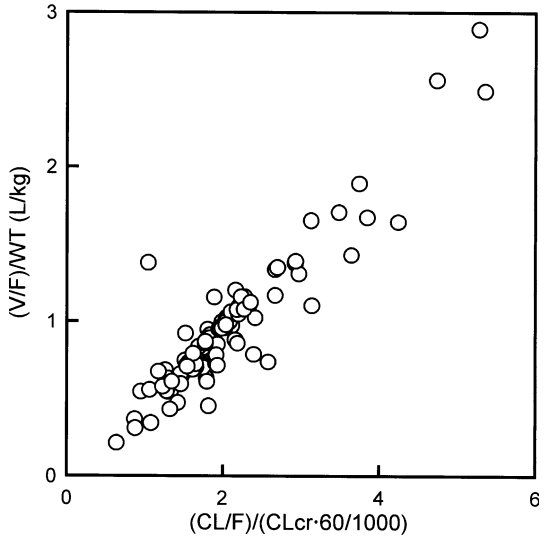

Fig. 3 Relationship between (CL/*F*)/(CL_{cr} × 60/1000) and (*V*/*F*)/WT of mizoribine in recipients of renal transplantation

Effect of the dosing interval on the pharmacokinetics of mizoribine in adult recipients of renal transplantation

To evaluate whether or not the dosing interval affects the pharmacokinetics of mizoribine in adult recipients of renal transplantation, the recipients were divided by dosing interval into the shorter-interval group (dosing interval ≤12 h) and the longer-interval group (dosing interval ≥24 h). Figure 4 shows the KA, WT-corrected *V*/*F*, and CL_{cr}-corrected CL/*F* values of mizoribine in 114 adult recipients of renal transplantation. The dosing interval did not affect the KA, (*V*/*F*)/WT, and (CL/*F*)/(CL_{cr} × 60/1000) values of mizoribine in the recipients (Fig. 4).

Discussion

In the present study, we utilized routine TDM data of mizoribine in adult recipients of renal transplantation in order to estimate the population pharmacokinetic parameters of the drug using a NONMEM program (Table 2). The population mean pharmacokinetic parameters in the adult recipients were similar to those in adult healthy volunteers, whereas interindividual pharmacokinetic variability seemed to be larger in the recipients than in healthy subjects (Table 2). In addition, we confirmed that renal function is a main factor responsible for the interindividual pharmacokinetic variability of mizoribine (Table 2; Figs. 1, 2). On the other hand, CL_{cr}-corrected CL/*F* in the recipients was highly correlated with WT-corrected *V*/*F*, suggesting that the bioavailability (*F*) of mizoribine was also responsible for the interindividual pharmacokinetic variability of the drug (Fig. 3).

Ihara et al. [3] evaluated the pharmacokinetics of mizoribine in 14 kidney transplant recipients, and reported that the cumulative urinary excretion (bioavailability) of mizoribine in the patients varied from 12 to 81%). Mizoribine is not metabolized in the body; therefore, the intestinal absorption step is plausibly responsible for the variability in bioavailability of mizoribine. It has been reported that two nucleoside transporter families are expressed in intestinal epithelial cells [10]. Concentrative nucleoside transporter (CNT) 1 and CNT2 are Na^+-dependent, and the movement of nucleoside regardless of its concentration

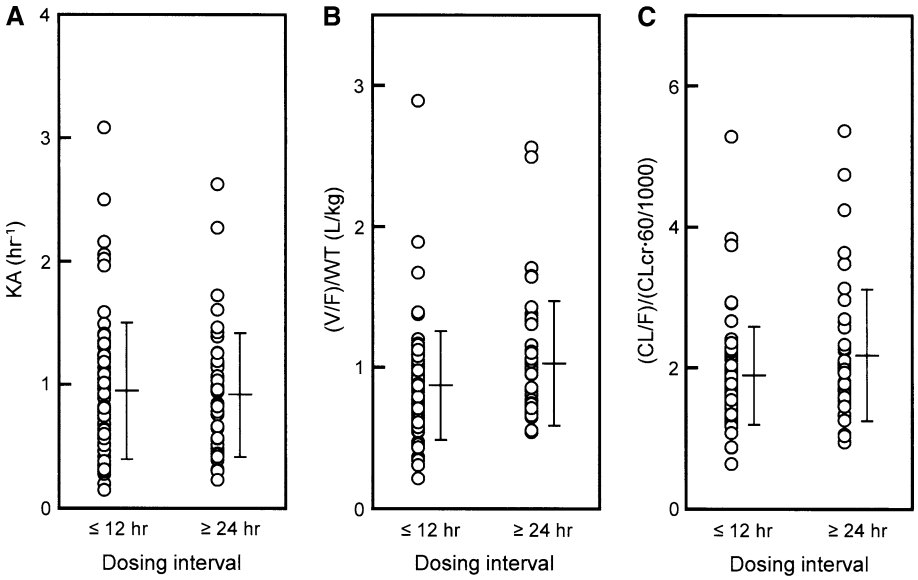

Fig. 4 Effect of the dosing interval of mizoribine on KA (**a**), (*V*/*F*)/WT (**b**), and (CL/*F*)/(CL_{cr} × 60/1000) (**c**) in adult recipients of renal transplantation. *Horizontal bars* represent the mean ± SD for each dosing interval

gradient is coupled to that of the sodium ion [11, 12]. On the other hand, equilibrative nucleoside transporter (ENT) 1 and ENT2, another nucleoside transporter family, are Na^+-independent, and mediate nucleoside transport in both directions depending on the nucleoside concentration gradient [12, 13]. We previously evaluated the membrane transport of mizoribine using human intestinal epithelial LS180 cells, and reported that the cellular uptake of mizoribine was mediated not only by CNT but also by ENT [7, 8]. In addition, Naito et al. [14] evaluated the effect of the genetic polymorphism of *CNT1 565G>A* on the bioavailability of mizoribine in Japanese kidney transplant recipients. The bioavailability of mizoribine in patients with *CNT1-G/A* and *-A/A* alleles was significantly lower than that with the *CNT1-G/G* allele. The authors therefore thought that *CNT1 565G>A* was one of the factors causing the variation in the bioavailability (intestinal absorption) of mizoribine [14]. However, the mechanisms responsible for interindividual variability in bioavailability of mizoribine have not yet been fully assessed. Recently, we have reported that mizoribine was actively exported from LS180 cells, probably by ATP-binding cassette (ABC) transporters, and that the activity of ABC transporter for mizoribine might be higher than that of nucleoside transporters (CNT and ENT) [13]. Further prospective clinical trials will be needed to clarify the contribution of genetic polymorphisms of both nucleoside transporters (CNT and/or ENT) and ABC transporters to the interindividual variability in bioavailability of mizoribine.

The clinical efficacy (adverse reactions) of mizoribine is considered to be correlated with the blood concentration of the drug; however, there are few reports about TDM-based dosage regimens of mizoribine in patients with renal transplantation [15, 16]. Sugitani et al. [15] evaluated the blood concentrations and clinical efficacy of mizoribine in 36 renal transplantation patients who were shifted from mycophenolate mofetil to mizoribine. The immunosuppressive effect of mizoribine was observed over a long period without severe adverse reactions in patients whose trough concentrations of mizoribine were to be kept higher than 1 μg/mL [15]. On the other hand, Sonda et al. [16] investigated the blood concentrations and adverse reactions of mizoribine in 46 renal transplantation patients. Stomatitis was observed in two patients whose trough concentrations of mizoribine were around 4 μg/mL. Thrombocytopenia was observed in one patient at the trough concentration of 6 μg/mL. In addition, one patient had liver dysfunction at the trough mizoribine concentration of 7 μg/mL. The authors thought that the warning range of the trough concentration of mizoribine was more than 4 μg/mL [16]. The findings in the present study will be useful for optimizing the dosage regimen of mizoribine in patients with renal transplantation.

In conclusion, the present findings suggested that not only the rate of renal excretion but also the extent of intestinal absorption of mizoribine are responsible for the large interindividual pharmacokinetic variability of the drug.

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