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Variability of Bioavailability and Intestinal Absorption Characteristics of Bisoprolol

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Summary: We previously reported that renal function is partly responsible for the interindividual variability of the pharmacokinetics of bisoprolol. The aim of the present study was to examine the variability of bioavailability (F) of bisoprolol in routinely treated Japanese patients and intestinal absorption characteristics of the drug. We first analyzed the plasma concentration data of bisoprolol in 52 Japanese patients using a nonlinear mixed effects model. We also investigated the cellular uptake of bisoprolol using human intestinal epithelial LS180 cells. The oral clearance (CL/F) of bisoprolol in Japanese patients was positively correlated with the apparent volume of distribution (V/F), implying variable F. The uptake of bisoprolol in LS180 cells was temperature-dependent and saturable, and was significantly decreased in the presence of quinidine and diphenhydramine. In addition, the cellular uptake of bisoprolol dissolved in an acidic buffer was markedly less than that dissolved in a neutral buffer. These findings suggest that the rate/extent of the intestinal absorption of bisoprolol is another cause of the interindividual variability of the pharmacokinetics, and that the uptake of bisoprolol in intestinal epithelial cells is highly pH-dependent and also variable.

Keywords: bisoprolol; intestinal absorption; bioavailability; nonlinear mixed effects model (NONMEM); LS180 cell

Introduction

Bisoprolol is a selective β1-blocker and has been used widely in patients with cardiovascular diseases such as hypertension, angina pectoris, cardiac arrhythmias, and chronic heart failure. After oral administration, 50% of the absorbed dose of bisoprolol is metabolized in the liver, while 50% of the absorbed dose is excreted into the urine as the unchanged form.1) We previously investigated the pharmacokinetics of bisoprolol in 40 middle-aged and elderly Japanese patients.2) The oral clearance (CL/F) of bisoprolol was positively correlated with the creatinine clearance (CLcr), indicating that renal function is one of the main causes of the interindividual variability of the pharmacokinetics of bisoprolol. However, despite taking the renal function into account, some interindividual variability remains in the pharmacokinetics of bisoprolol.2)

Recently, we have reported that the variability of bioavailability (F) is one of the causes of the interindividual pharmacokinetic variability of mizoribine, an immunosuppressive agent.3,4) That is, we analyzed the serum concentration data of mizoribine in adult and pediatric recipients of renal transplantation using a nonlinear mixed effect model (NONMEM) program. We found a significant positive correlation between CL/F and the apparent volume of distribution (V/F) in both studies.3,4) Since the plasma protein binding of mizoribine is negligible, the systemic clearance (CL) of the drug may not be correlated with the volume of distribution (V). In addition, mizoribine is not subjected to hepatic first-pass metabolism, and is exclusively excreted into the urine as the unchanged form. Therefore, interindividual variability in F of mizoribine is one of the causes of the interindividual pharmacokinetic variability of the drug, and the intestinal absorption step is plausibly responsible for the interindividual variability of the F of mizoribine.3,4) The primary aim of the present study was to evaluate the variability of the F of bisoprolol. That is, we analyzed the plasma concentration data of bisoprolol in 52 Japanese patients using a NONMEM program, and evaluated the correlation between CL/F and V/F of the drug. Because of the low plasma protein binding of bisoprolol (30%), CL of the drug may not be correlated with V.1) Therefore, the significant positive correlation between CL/F and V/F will suggest the interindividual variability of F of bisoprolol. In addition, bisoprolol is partly metabolized by cytochrome P450 (CYP) 2D6 and 3A4; however, the hepatic first-pass
extraction ratio of the drug is low (<10%). Therefore, if the F of bisoprolol is variable, the intestinal absorption step can be another cause for the interindividual variability of the pharmacokinetics of the drug.

The mechanism of the intestinal absorption of organic cations had been explained as passive diffusion of unionized forms according to the pH-partition theory. On the other hand, Katsura et al. investigated the transport characteristics of procainamide, a cationic anti-arrhythmic drug, using rabbit intestinal brush-border membrane vesicles. They reported that the uptake of procainamide in the vesicles was stimulated by an outward H+ gradient, and that the initial uptake of procainamide was inhibited by diphenhydramine. In addition, we previously investigated the transport mechanism of procainamide in human intestinal epithelial LS180 cells. The uptake of procainamide in LS180 cells was markedly decreased by acidification of the apical medium, and was significantly inhibited by hydrophobic cationic drugs such as quinidine, diphenhydramine, and pyrilamine. These results suggested that the pH-dependent transport system is at least partly involved in the uptake of procainamide into intestinal epithelial cells, and that the rate/extent of the intestinal absorption of procainamide varies considerably. The secondary aim of the present study was to examine the intestinal absorption characteristics of bisoprolol. That is, we investigated the mechanisms and characteristics of the cellular uptake of bisoprolol using human intestinal epithelial LS180 cells.

Materials and Methods

Materials: Bisoprolol hemifumarate was obtained from Mitsubishi Tanabe Pharma Co. (Osaka, Japan). [14C]Procainamide hydrochloride (2.04 GBq/nmol) and [3H]mannitol (740 GBq/nmol) were purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO). Procainamide hydrochloride and tetraethylammonium (TEA) chloride were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Quinidine hydrochloride monohydrate, metformin hydrochloride, and pyrilamine maleate salt were purchased from Sigma Aldrich (St. Louis, MO). Diphenhydramine hydrochloride was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). All other chemicals were of the highest purity available.

Clinical pharmacokinetic data of bisoprolol: We previously estimated the pharmacokinetic parameters of bisoprolol in 40 Japanese patients with cardiovascular diseases. In the present study, 12 patients were newly enrolled, and the pharmacokinetic parameters of bisoprolol in 52 Japanese patients were re-estimated. The subjects were Japanese patients consisting of 40 males and 12 females between 40 and 89 (mean: 64.0) years old, and their mean body weight was 66.0 kg. All patients gave written consent to participate in this study, which was approved by the ethics committee of University of Toyama. Seven patients had congestive heart failure characterized as New York Heart Association (NYHA) class II, but no patients had severe hepatic or renal failure. All patients had been routinely treated with oral administration of bisoprolol hemifumarate (Mainitate Tablets; Mitsubishi Tanabe Pharma Co., Osaka, Japan) at doses of 0.625–10 mg/day, and the drug was administered once a day to all patients. The total number of blood samples obtained at steady-state following repetitive administration was 118. That is, one or two blood samples for all 52 patients were obtained between 2.3 and 7.0 h after dosing. Additional blood samples were obtained just before dosing from 48 patients.

Cell culture: LS180 cells at passage 38 were obtained from the American Type Culture Collection (Manassas, VA). The cells were seeded at a density 5 × 10^4 cells/cm² on a 3.8 cm² plastic dish using a Falcon multiwell plate (BD Bioscience, Bedford, MA). LS180 cells were maintained with Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (Biowest, Nuaillé, France) in an atmosphere of 5% CO₂–95% air at 37°C for 7 days. All uptake experiments were carried out with LS180 cells between passages 60 and 68.

pH-dependent cellular uptake of procainamide and bisoprolol into LS180 cells: The pH-dependent cellular uptake of procainamide and bisoprolol was examined using LS180 cells. The composition of the incubation medium was as follows: 125 mM NaCl, 4.8 mM KCl, 5.6 mM d-glucose, 1.2 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄·7H₂O, and 25 mM 2-[4-(2-hydroxyethyl)-piperazinyl]-1-ethanesulfonic acid (HEPES) (pH 6.4 or 7.4). In order to evaluate the effect of the extracellular pH on the cellular uptake of procainamide and bisoprolol, 25 mM HEPES was replaced with Tris (pH 8.4). The cells were first pre-incubated for 55 min at 37°C with 2.0 mL incubation medium, followed by a 5-min incubation with 500 μL fresh incubation medium.

The incubation medium was replaced with 500 μL incubation medium containing 100 μM procainamide of which 3.64 μM (0.1 μCi/well) was [3H]procainamide. After the cells were incubated with 100 μM procainamide for another 10 min at 37°C, they were immediately washed with ice-cold phosphate buffer and collected. The amount of [3H]mannitol in the cells was also determined as the amount of extracellular trapping of procainamide. The amount of radio-labeled compounds in the cells was determined using a liquid scintillation counter.

The cellular uptake of 100 μM incubation medium containing 100 μM bisoprolol after a 60-min pre-incubation as described above. After the cells had been incubated with 100 μM bisoprolol for another 10 min at 37°C, they were immediately washed with ice-cold phosphate buffer and collected with distilled water. The cellular suspension was vortexed sufficiently, followed by sonication for 5 min, and stored at −30°C until the assay of bisoprolol.

Characteristics of cellular uptake of bisoprolol in LS180 cells: To evaluate the effect of temperature on the uptake of bisoprolol in LS180 cells, the uptake of 100 μM bisoprolol at 37 or 4°C was assessed. Briefly, the cells were first pre-incubated for 55 min at 37 or 4°C with 2.0 mL incubation medium (pH 7.4), followed by a 5-min incubation with 500 μL fresh incubation medium (pH 7.4) at 37 or 4°C. After the cells had been incubated with 100 μM bisoprolol for another 5 min at 37 or 4°C, they were immediately washed with ice-cold phosphate buffer and collected as described above.

The effect of intracellular pH on the cellular uptake of 100 μM bisoprolol in LS180 cells was evaluated at 37°C as reported by Mizuuchi et al. with a minor modification. Briefly, the cells were first pre-incubated for 30 min with 500 μL incubation medium (pH 7.4) in the absence (control and Acute-NH₄Cl treatment) or presence (Pre-NH₄Cl treatment) of 30 mM NH₄Cl. The incubation medium was replaced with 500 μL incubation medium (pH 7.4) containing 100 μM bisoprolol in the absence (control and Pre-NH₄Cl treatment) or presence (Acute-NH₄Cl treatment) of 30 mM NH₄Cl. After the cells had been incubated with 100 μM bisoprolol for another 5 min at 37°C, they were immediately washed with ice-cold phosphate buffer and collected as described above.
The effect of various organic compounds on the cellular uptake of 100 μM bisoprolol in LS180 cells was evaluated at 37°C. Briefly, the cells were first pre-incubated for 55 min with 2 mL incubation medium (pH 7.4), followed by a 5-min incubation with 500 μL fresh incubation medium (pH 7.4) supplemented with 5 mM organic cations. The incubation medium was replaced with 500 μL incubation medium (pH 7.4) containing 100 μM bisoprolol and 5 mM organic cations. After the cells had been incubated with 100 μM bisoprolol for another 10 min at 37°C, they were immediately washed with ice-cold phosphate buffer and collected as described above.

To estimate the pharmacokinetic parameters for bisoprolol uptake in LS180 cells, the concentration-dependent uptake of bisoprolol was evaluated. That is, the cells were first pre-incubated for 55 min at 37°C with 2 mL incubation medium (pH 7.4), followed by a 5-min incubation with 500 μL fresh incubation medium (pH 7.4). After the cells had been incubated with 0.01–10 mM bisoprolol for another 10 min at 37°C, they were immediately washed with ice-cold phosphate buffer and collected as described above.

**Assay of bisoprolol:** The amount of bisoprolol in the sample was determined by a reversed-phase HPLC method as described previously with a minor modification. Briefly, a 200-μL aliquot of the sample was alkalinized with 1.5 mL glycine buffer (0.1 M, saturated with NaCl, pH 10.6), and mildly extracted with 5 mL diethylether for 20 min. Bisoprolol was back-extracted from the organic phase with 0.2 mL of 0.05 N HCl for 20 min. A 50-μL aliquot of HCl solution was injected into an HPLC system. The column was COSMOSIL 5C18-AR-II (15 cm × 4.6 mm; i.d. 4.5 μm particle size; Nacalai Tesque). The mobile phase consisted of 10 mM KH2PO4 that contained 0.59% (w/v) triethylamine and 0.2 mL of 0.05 N HCl for 20 min. A 50-μL sample was alkalinized with 1.5 mL glycine buffer (0.1 M, pH 10.6), and mildly extracted with 5 mL 10 mM bisoprolol for another 10 min at 37°C, they were immediately washed with ice-cold phosphate buffer and collected as described above.

**Pharmacokinetic analysis:** The clinical pharmacokinetic parameters of bisoprolol were estimated as described previously with a minor modification. That is, the 1-compartment model with repetitive bolus dosing was parameterized in terms of CL/F and V/F. \( CL/F \) and \( V/F \) in the \( i \)th individual (\( CL/F_i \) and \( V/F_i \), respectively) were modeled using the following equations:

\[
CL/F_i = (\theta_1 \cdot WT + \theta_2 \cdot CL_{cr}) \cdot (1 + \eta_{CL/F_i})
\]

\[
V/F_i = \theta_3 \cdot WT \cdot (1 + \eta_{V/F_i})
\]

where \( \theta_1 \cdot WT + \theta_2 \cdot CL_{cr} \) and \( \theta_1 \cdot WT \) are the predicted population mean of oral clearance and apparent distribution volume, respectively, \( WT \) is the individual body weight (kg). The \( CL_{cr} \) value (L/h) was calculated using the Cockcroft-Gault equation:

\[
CL_{cr} = \frac{(140 - AGE) \cdot WT}{72 \cdot S_G} \cdot 0.85 \cdot \frac{60}{1,000}
\]

where \( AGE \) and \( S_G \) are age (year) and serum creatinine concentration (mg/dL), respectively. \( S_G \) is one for females and zero for males. In the present study, random variables \( \eta_{CL/F_i} \) and \( \eta_{V/F_i} \) were assumed to be distributed normally with means of zero and covariance of \( \omega_{CL/F_i}^2 \), \( \omega_{CL/F_i}^2 \), and \( \omega_{CL/F_i}^2 \). Finally, the \( j \)th observed plasma concentration in the \( i \)th patient \( (C_{p,j}^o) \) was assumed to be randomly and normally distributed from the \( j \)th predicted plasma concentration in the \( i \)th patient \( (C_{p,j}^i) \):

\[
C_{p,j} = C_{p,j}^i + \epsilon_{ij}
\]

where \( \epsilon_{ij} \) is a random variable that describes intrapatient variability with a mean of zero and variance of \( \sigma^2 \).

The kinetic parameters for the uptake of bisoprolol in LS180 cells were estimated using the following equation:

\[
V = \frac{V_{max} \cdot [S]}{K_m + [S]} + K_d \cdot [S]
\]

where \( V \) and \( V_{max} \) are the uptake rate (nmol/10 min/3.8 cm²) and the maximum uptake rate (nmol/10 min/3.8 cm²), respectively. \( [S], K_m \), and \( K_d \) are the initial concentration (mM), the Michaelis constant (mM), and the coefficient of diffusion (μL/10 min/3.8 cm²), respectively.

Pharmacokinetic analyses were performed with NONMEM software (double precision NONMEM Version VI Level 2.0, PREDP Version V Level 2.0, and NM-TRAN Version IV Level 2.0) running on a Windows 7 computer. In the present study, we used a first-order estimation method and the NONMEM-PREDPP library subroutines ADVAN1 and TRANS2 for the 1-compartment model with bolus dosing.

**Statistical analysis:** The statistical significance of the population pharmacokinetic parameters was evaluated with the likelihood ratio test using the minimum value of the objective function \(-2 \log (LLD)\) produced by NONMEM. The difference of \(-2 \log (LLD)\) is asymptotically distributed as \( \chi^2 \) with degrees of freedom equal to the number of parameters that were fixed to the hypothesis value. That is, when the LLD value between two models allowing a parameter of interest freely estimated versus a fixed hypothetical value was greater than 3.84, the parameter value was considered to be statistically significant (p < 0.05). In addition, NONMEM provides estimates of the standard error (SE) for all parameters, and SE can be used to define 95% confidence intervals (CI) for true parameter values: 95% CI = (the estimated parameter value) ± 1.96(SE).

Multiple comparisons were performed using Scheffé’s test following one-way ANOVA, provided that the variances of groups were similar. If this was not the case, a Scheffé-type test was applied following Kruskal-Wallis analysis. A p value less than 0.05 was considered to be statistically significant.

**Results**

**Correlation between CL/F and V/F of bisoprolol in Japanese patients with cardiovascular diseases:** Population pharmacokinetic parameters were estimated from the 118 serum concentration data of bisoprolol in 52 recipients using the NONMEM software. Figure 1 shows the plasma concentration of bisoprolol in 52 patients, and Table 1 shows the population pharmacokinetic parameters of bisoprolol and their 95% CI estimated with the basic model (assuming no correlation between CL/F and V/F) and the covariance model (assuming a correlation between CL/F and V/F). The difference in the LLD value between basic and covariance models was 21.145, indicating that the covariance model is more appropriate than the basic model. The mean values of \( \theta_1 \), \( \theta_2 \), and \( \theta_3 \) with the covariance model (0.0560 L/h/kg, 1.22, and 2.65 L/kg, respectively) were very similar to those with the basic model (0.0586 L/h/kg, 1.21, and 2.64 L/kg, respectively) (Table 1). The \( \omega_{CL/F}^2 \) value with the covariance model (0.0445) was also similar to that with the basic model (0.0427). However, the \( \omega_{CL/F}^2 \) value with the covariance model (0.0367) was larger than that with the basic model.
The low plasma protein binding of the drug (30%). Therefore, there is a close correlation between the basic and covariance models. There was a significant positive correlation between \( \eta_{CL/F} \) and \( \eta_{VF/F} \) in the covariance model as compared with that in the basic model (Fig. 2). There may not be a close correlation between \( CL/F \) and \( V/F \) of bisoprolol because of the low plasma protein binding of the drug (30%). Therefore, the present findings suggested that variability of \( F \) is another cause of the interindividual pharmacokinetic variability of bisoprolol.

**Table 1. Population pharmacokinetic parameters of bisoprolol with basic and covariance models**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Basic model</th>
<th>Estimates</th>
<th>95% CI</th>
<th>Covariance model</th>
<th>Estimates</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \theta_1 ) (L/h/kg)</td>
<td>0.0586</td>
<td>0.0257-0.0915</td>
<td>0.0560</td>
<td>0.0319-0.0801</td>
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</tr>
<tr>
<td>( \theta_2 ) (L/kg)</td>
<td>2.64</td>
<td>2.47-2.81</td>
<td>2.65</td>
<td>2.46-2.84</td>
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<td></td>
</tr>
<tr>
<td>( \omega_{CL/F}^2 )</td>
<td>0.0427</td>
<td>0.0147-0.0707</td>
<td>0.0445</td>
<td>0.0218-0.0672</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \omega_{VF/F} )</td>
<td>0 (FIX)</td>
<td>0.0368</td>
<td>0.0150-0.0586</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \omega_{VF/F}^2 )</td>
<td>0.00913</td>
<td>-0.0179-0.0362</td>
<td>0.0367</td>
<td>0.0044-0.0690</td>
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</tr>
<tr>
<td>( \sigma ) (ng/mL)</td>
<td>1.83</td>
<td>1.12-2.34</td>
<td>1.48</td>
<td>1.06-1.81</td>
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</tr>
<tr>
<td>(-2 \log \text{likelihood})</td>
<td>361.299</td>
<td>340.154</td>
<td></td>
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</tr>
</tbody>
</table>

**Fig. 1. Dose-corrected plasma bisoprolol concentrations at steady state in 52 patients**

**Fig. 2. The relationship between \( \eta_{CL/F} \) and \( \eta_{VF/F} \) with the basic (A) and covariance (B) models**

**Fig. 3. Effect of extracellular pH on the uptake of 100 µM procainamide (A) and bisoprolol (B) in LS180 cells**

The cells were incubated with \(^{14}C\)procainamide or bisoprolol for 10 min. Each column represents the mean ± SE of 4 experiments. *\( p < 0.05 \), significantly different from pH 7.4.

**Fig. 4. Effect of temperature and intracellular pH on the uptake of 100 µM bisoprolol in LS180 cells**

The cells were incubated with 100 µM bisoprolol for 5 min at 37 or 4°C. Each column represents the mean ± SE of 4-12 experiments. *\( p < 0.05 \), significantly different from the control.

With that of procainamide, a known substrate of the postulated pH-dependent transport system (Fig. 3). The uptake of 100 µM procainamide in LS180 cells was markedly increased by alkalization of the extracellular medium (Fig. 3A). Similarly, extra cellular alkalization significantly stimulated the uptake of 100 µM bisoprolol (Fig. 3B).

**Effect of temperature and intracellular pH on the uptake of bisoprolol in LS180 cells: Figure 4** shows the effects of temperature and intracellular pH on the uptake of bisoprolol in LS180 cells. The uptake of bisoprolol into LS180 cells at 4°C was much less than that at 37°C. Pre-NH4Cl treatment, which induces the acidification of intracellular pH, increased the uptake of bisoprolol in LS180 cells. On the other hand, the uptake of bisoprolol was decreased by Acute-NH4Cl treatment, which induces the alkalization of intracellular pH (Fig. 4). These findings indicated that the uptake of bisoprolol in LS180 cells is stimulated by an outward H+ gradient.

**Effect of various cationic compounds on the uptake of bisoprolol in LS180 cells:** The effect of 5 mM various organic cations on the 10-min uptake of 100 µM bisoprolol was also evaluated in LS180 cells (Fig. 5). The uptake of bisoprolol was not affected by the typical hydrophilic organic cations such as TEA, metformin, and cimetidine (Fig. 5). On the other hand, quinidine, diphenhydramine, and pyrilamine, which are substrates and/or
The mean and/or variability of \( F \) of some drugs in routinely-treated patients may be different from those in healthy subjects, although the precise mechanism is still unclear. For instance, we treated patients may be different from those in healthy subjects, and the F value of the drug in the recipients was more variable (range: 12–81%). In the case of bisoprolol, the \( F \) of the drug in healthy volunteers was reported to be high (90%). However, to our knowledge, there is no report about the \( F \) value of bisoprolol in routinely-treated patients. In the present study, \( CL/F \) of bisoprolol in 52 Japanese patients was positively correlated with \( V/F \), implying variable \( F \) (Table 1 and Fig. 2). Further clinical research may be needed to evaluate the mean and variability of \( F \) of drugs in routinely-treated patients.

In the present study, the carrier-mediated uptake of bisoprolol into LS180 cells was highly pH-dependent and variable (Figs. 3–6). In addition, the uptake of bisoprolol into the cells is plausibly mediated by the postulated \( H^+ / \text{tertiary amine} \) antiport system. To our knowledge, the intestinal \( H^+ / \text{tertiary amine} \) antiport system was firstly reported by Mizuuchi et al. in 1999.

That is, they reported that the cellular uptake of diphenhydramine at the apical membrane in human intestinal epithelial Caco-2 cells was pH- and temperature-dependent, and was inhibited by tertiary amine compounds such as chlorpheniramine, procainamide, and imipramine. It has been reported that the postulated \( H^+ / \text{tertiary amine} \) antiport system is expressed on not only Caco-2 cells but also rabbit intestine and LS180 cells. We previously reported that quinidine, another tertiary amine drug, is taken up into not only Caco-2 but also LS180 cells by the postulated \( H^+ / \text{tertiary amine} \) antiport system. In addition, our preliminary experiments have shown that the uptake of procainamide and bisoprolol in Caco-2 as well as LS180 cells was decreased with decreasing extracellular pH (unpublished data).

Furthermore, there are several reports indicating that the postulated \( H^+ / \text{tertiary amine} \) transport system seems to transport not only tertiary amine drugs, but also secondary and primary amine drugs, such as 3,4-methylenedioxyamphetamine and phenylethylamine. Further studies will be needed to identify the postulated \( H^+ / \text{tertiary amine} \) antiport system to clarify the contribution of the \( H^+ / \text{tertiary amine} \) antiport system to the uptake of organic cations in the human intestine, and to evaluate the functional variability of the transporter.

In conclusion, the findings in the present study suggest that variable \( F \) is another cause of the interindividual variability of the pharmacokinetics of bisoprolol, and that the rate/extent of the intestinal absorption of bisoprolol is highly pH-dependent and also variable.

**Discussion**

The mean and/or variability of \( F \) of some drugs in routinely-treated patients may be different from those in healthy subjects, although the precise mechanism is still unclear. For instance, we have previously reported that the mean \( F \) value of mizoribine in 30 healthy Japanese males was 86% (range: 60–99%). On the other hand, Ihara et al. evaluated the pharmacokinetics of mizoribine in 14 kidney transplant recipients. The mean \( F \) value of mizoribine in the recipients (41%) was lower than that in healthy volunteers, and the \( F \) value of the drug in the recipients was more variable (range: 12–81%). In the case of bisoprolol, the \( F \) of the drug in healthy volunteers was reported to be high (90%). However, to our knowledge, there is no report about the \( F \) value of bisoprolol in routinely-treated patients. In the present study, \( CL/F \) of bisoprolol in 52 Japanese patients was positively correlated with \( V/F \), implying variable \( F \) (Table 1 and Fig. 2). Further clinical research may be needed to evaluate the mean and variability of \( F \) of drugs in routinely-treated patients.

**Fig. 5. Effect of various compounds on the uptake of 100 \( \mu \text{M} \) bisoprolol in LS180 cells**

The cells were incubated with 100 \( \mu \text{M} \) bisoprolol for 10 min in the presence of 5 mM organic cations at pH 7.4. Each column represents the mean ± SE of 5–12 experiments. * \( p < 0.05 \), significantly different from the control.

**Fig. 6. Concentration dependence of the uptake of bisoprolol in LS180 cells**

The cells were incubated with bisoprolol for 10 min in the absence (open circles) or presence (closed squares) of 5 mM diphenhydramine at pH 7.4. Each point represents the mean ± SE of 4–7 experiments. The apparent \( K_m, V_{\text{max}}, \) and \( K_d \) values for bisoprolol uptake were 0.591 ± 0.124 mM, 26.9 ± 2.2 nmol/10 min/3.8 cm\(^2\), and 4.80 ± 0.16 \( \mu \text{L}/10 \text{min}/3.8 \text{cm}^2 \), respectively.

**References**


