Variability of Bioavailability and Intestinal Absorption Mechanisms of Metoprolol

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Summary: We previously reported that aging and/or cytochrome P450 2D6 polymorphism are responsible for the interindividual variability in the systemic clearance (CL) and bioavailability (F) of metoprolol. The aim of the present study was to evaluate the residual variability of F of metoprolol in routinely treated Japanese patients and to investigate the intestinal absorption mechanism of the drug using human intestinal epithelial LS180 cells. We first re-analyzed the blood concentration data for metoprolol in 34 Japanese patients using a nonlinear mixed effects model. The oral clearance (CL/F) of metoprolol was positively correlated with the apparent volume of distribution (V/F), suggesting the residual variability of F. The uptake of metoprolol into LS180 cells was significantly decreased by the acidification of extracellular medium pH, and was dependent on temperature and intracellular pH. Furthermore, the cellular uptake of metoprolol was saturable, and was significantly decreased in the presence of hydrophobic cationic drugs such as diphenhydramine, procainamide, bisoprolol, and quinidine. These findings indicate that residual variability of F is one of the causes of the interindividual pharmacokinetic variability of metoprolol, and that the interindividual variability of not only presystemic first-pass metabolism, but also intestinal absorption, may be responsible for the variable F of the drug.

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

Introduction

Metoprolol, a selective β₁-blocker, is widely used in patients with various cardiovascular diseases. It is almost exclusively eliminated by hepatic metabolism, and cytochrome P450 (CYP) 2D6 mainly metabolizes the drug. We previously investigated the pharmacokinetics of metoprolol in middle-aged and elderly Japanese patients, and reported that the CYP2D6 polymorphism and aging are the major factors contributing to the interindividual variability of the pharmacokinetics of metoprolol. That is, the oral clearance (CL/F) and the apparent volume of distribution (V/F) in patients with the CYP2D6*10 allele were significantly lower than those in patients with the CYP2D6*1/*1 or *1/*2 genotype. In addition, CL/F in older (>70 years old) patients was lower than that in younger (≤70 years old) patients. However, despite taking the CYP2D6 polymorphism and aging into account, considerable interindividual variability remains in the pharmacokinetics of metoprolol.

Recently, we have reported that the variability of bioavailability (F) is one of the causes of the interindividual pharmacokinetic variability of bisoprolol, another selective β₁-blocker. That is, we have analyzed the plasma concentration data of bisoprolol in routinely treated Japanese patients using a nonlinear mixed effects model (NONMEM) program. The pharmacokinetics of bisoprolol in the patients was variable, and CL/F of bisoprolol was positively correlated with V/F. Because of the low plasma protein binding of bisoprolol (30%), the systemic clearance (CL) of the drug may not be correlated with the volume of distribution (V). Therefore, variability in F of bisoprolol can be one of the causes of the interindividual pharmacokinetic variability of the drug. The primary aim of the present study was to evaluate the residual variability of F of metoprolol. That is, we re-analyzed the blood concentration data for metoprolol in 34 Japanese patients in the previous study using a NONMEM program, and evaluated the correlation between CL/F and V/F of the drug. If the residual variability of F is another cause of the interindividual variability

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of the pharmacokinetics of metoprolol, CL/F may be positively correlated with V/F of the drug.

F is determined by the fraction of the drug absorbed from the gastrointestinal tract and by the fraction saved from the presystemic first-pass metabolism. Although bisoprolol is metabolized by CYP2D6 and 3A4, the hepatic first-pass extraction ratio of the drug is low (<10%). Therefore, the intestinal absorption step, but not hepatic first-pass metabolism, can be mainly responsible for the variable F of bisoprolol. We recently investigated the cellular uptake of bisoprolol in human intestinal epithelial LS180 cells, which is a microvillus-expressing cell line that possesses characteristics of the small intestine, and has been used as an in vitro model of the intestine. The cellular uptake of bisoprolol dissolved in acidic buffer was markedly less than that dissolved in neutral buffer. In addition, the uptake of bisoprolol in LS180 cells was temperature-dependent and saturable, and was significantly decreased in the presence of hydrophobic cationic drugs such as quinidine, diphenhydramine, and pyrilamine. These results suggested that a transport system is at least partly involved in the uptake of bisoprolol into intestinal epithelial cells, and that the rate/extent of the intestinal absorption of bisoprolol varies considerably. The chemical structure and physicalchemical properties of metoprolol are similar to those of bisoprolol: the log p values of metoprolol and bisoprolol are 1.69 and 1.84, respectively, and the pKa values of the drugs are 9.18 and 9.16. If the intestinal absorption characteristics of metoprolol are also similar to those of bisoprolol, not only the hepatic first-pass metabolism, but also the intestinal absorption step may be responsible for the basically variable F of metoprolol. The secondary aim of the present study was to investigate the mechanisms of the cellular uptake of metoprolol using human intestinal epithelial LS180 cells.

Materials and Methods

Materials: Metoprolol tartrate salt, quinidine hydrochloride monohydrate, and naringin were purchased from Sigma Aldrich (St. Louis, MO). Diphenhydramine hydrochloride was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). Procainamide hydrochloride, tetraethylammonium (TEA) chloride, and rifampicin SV sodium salt were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Rifampicin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Fexofenadine hydrochloride was purchased from Toronto Research Chemicals Inc. (Toronto, Canada). All other chemicals were of the highest purity available.

Clinical pharmacokinetic data of metoprolol: To re-estimate the population pharmacokinetic parameters of metoprolol, we used 65 blood concentration data from 34 Japanese patients with cardiovascular disease, which were obtained in the previous study. In brief, the subjects were Japanese patients consisting of 21 males and 13 females between 56 and 83 years old, and their mean body weight (±S.D.) was 60.0 ± 11.0 kg. Five patients had symptomatic congestive heart failure (CHF): 4 patients were characterized as New York Heart Association (NYHA) class II, and 1 patient was NYHA class III. However, no patients had severe hepatic or renal failure, and none had received any potent inhibitor of CYP2D6 (e.g. amiodarone or quinidine). These patients had been treated routinely with oral administration of a rapid-release preparation of metoprolol tartrate (Seloken® Tablets, AstraZeneca, Tokyo, Japan) at doses between 40 and 120 mg/d, and the drug was administered twice a day to 8 patients and three times a day to 26 patients.

No patients had null alleles of CYP2D6 (*4, *5, *14), and they could be divided into three groups on the basis of CYP2D6 genotypes. Nine patients were homozygous for the CYP2D6*1 allele, and 5 were heterozygous for the CYP2D6*1/*10 alleles (Group 1). Seven patients were heterozygous for the CYP2D6*1/*10 alleles, and 6 were heterozygous for the CYP2D6*2/*10 alleles (Group 2). Seven patients were homozygous for the CYP2D6*10 allele (Group 3).

Cell culture: LS180 cells at passage 38 were obtained from the American Type Culture Collection (Manassas, VA). The cells were seeded at a density of 5 x 10⁵ 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 61 and 71.

Cellular uptake of metoprolol in LS180 cells: The pH-dependent cellular uptake of metoprolol 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-(2-hydroxyethyl)-1-piperaziny]ethanesulfonic acid (HEPES) (pH 6.4 or 7.4). In order to evaluate the effect of extracellular pH on the uptake of metoprolol, HEPES was replaced with 2-(N-morpholinono)-ethanesulfonic acid (pH 5.4). The cells were first pre-incubated for 25 min at 37°C with 2 mL incubation medium, followed by 5-min incubation with 500 µL fresh incubation medium in the presence or absence of 5 mM diphenhydramine. The incubation medium was replaced with 500 µL fresh incubation medium containing 100 µM metoprolol in the presence or absence of 5 mM diphenhydramine. After cells were incubated with 100 µM metoprolol for 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 metoprolol.

To evaluate the effect of temperature on the uptake of metoprolol to LS180 cells, the uptake of 100 µM metoprolol at 37 or 4°C was assessed. Briefly, the cells were first pre-incubated for 25 min at 37 or 4°C with 2 mL incubation medium (pH 7.4), followed by 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 metoprolol 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 metoprolol 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 2 mL 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 fresh incubation medium (pH 7.4) containing 100 µM metoprolol 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 metoprolol for another 5 min at 37°C, they were immediately washed with ice-cold phosphate buffer and collected as described above.

To estimate the pharmacokinetic parameters for metoprolol uptake in LS180 cells, the concentration-dependent uptake of metoprolol was evaluated. That is, the cells were first pre-incubated for 25 min at 37°C with 2 mL incubation medium (pH 7.4),
followed by 5-min incubation with 500 µL fresh incubation medium (pH 7.4). After the cells had been incubated with 0.01–4 mM metoprolol for another 10 min at 37°C, they were immediately washed with ice-cold phosphate buffer and collected as described above.

The effect of various compounds on the cellular uptake of 100 µM metoprolol in LS180 cells was evaluated at 37°C. Briefly, the cells were first pre-incubated for 25 min with 2 mM incubation medium (pH 7.4), followed by 5-min incubation with 500 µL fresh incubation medium (pH 7.4) supplemented with 0.003–5 mM organic cations or 1 mM organic anion transporting polypeptide (OATP) inhibitors. The incubation medium was replaced with 500 µL incubation medium (pH 7.4) containing 100 µM metoprolol plus 0.003–5 mM organic cations or 1 mM OATP inhibitors. After the cells had been incubated with 100 µM metoprolol for another 10 min at 37°C, they were immediately washed with ice-cold phosphate buffer and collected as described above.

**Assay of metoprolol:** The amount of metoprolol 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 mL glycine buffer (0.1 M, saturated with NaCl, pH 10.6), and mildly extracted with 5 mL diethylether for 20 min. Metoprolol was back-extracted from the organic phase with 0.6 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 0.6% (w/v) triethylamine adjusted to pH 3.3 with phosphate acid and acetonitrile (83/17, v/v). The peaks were monitored by a fluorescence detector (RF-10A; Shimadzu, Kyoto, Japan) at an excitation wavelength of 272 nm and an emission wavelength of 303 nm.

**Pharmacokinetic analysis:** The clinical pharmacokinetic parameters of metoprolol 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 ith individual (CL/Fi and V/Fi, respectively) were modeled using the following equations:

\[
\frac{CL}{Fi} = (\theta_1 + \theta_2 \cdot G1 - \theta_2 \cdot G3) \cdot \theta_3^{G1>70} \cdot WT_i \cdot (1 + \eta_{CL/Fi})
\]

\[
\frac{V}{Fi} = (\theta_4 + \theta_5 \cdot G1 - \theta_5 \cdot G3) \cdot WT_i \cdot (1 + \eta_{VI})
\]

where WT is the individual body weight (kg), \(\theta_1 + \theta_2 \cdot G1 - \theta_2 \cdot G3\), \(\theta_4^{G1>70} \cdot WT_i\) and \(\theta_4 + \theta_5 \cdot G1 - \theta_5 \cdot G3 \cdot WT_i\) are the predicted population mean of the oral clearance and the apparent distribution volume, respectively. G1 and G3 were fixed to be 1 and 0, respectively, for Group 1 (patients with CYP2D6*1/*1 and *1/*2). G1 and G3 were fixed to be 0 for Group 2 (patients with CYP2D6*1/*10 and *2/*10). G1 and G3 were fixed to be 0 and 1, respectively, for Group 3 (patients with CYP2D6*1/*10). In the present study, random variables \(\eta_{CL/F}\) and \(\eta_{VI}\) were assumed to be distributed normally with means of zero and covariance of \(\omega_{CL/F}^2\), \(\omega_{VI}^2\). Finally, the \(\eta_i\) observed blood concentration in the \(\eta_i\)th patient (\(C_{bi(i)}\)) was assumed to be randomly and normally distributed from the \(\eta_i\)th predicted blood concentration in the \(\eta_i\)th patient (\(C^{pbi(i)}\)):

\[
C_{bi(i)} = C^{pbi(i)} + \epsilon_{ij}
\]

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

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

The inhibitory constant (\(K_i\) in mM) of procainamide, bisoprolol, and quinidine for the uptake of metoprolol in LS180 cells was estimated using the following equations:

\[
IC_{50} = K_i \left(1 + \frac{[I]}{K_m}\right)
\]

\[
UT = (100 - NS) \cdot \frac{IC_{50}}{IC_{50} + [I] + NS}
\]

where \(IC_{50}\) and \([I]\) are the half maximal inhibitory concentration (mM) and the inhibitor concentration (mM), respectively. \(UT\) and \(NS\) are the uptake (% of control) and the nonsaturable component (% of control) of metoprolol, respectively.

**Statistical analysis:** The population pharmacokinetics models were evaluated by the minimum value of the objective function (~2 log likelihood) produced by NONMEM. In addition, NONMEM provides estimates of the standard error (S.E.) for all parameters, and S.E. can be used to define 95% confidence intervals (CI) for true parameter values: 95% CI = (the estimated parameter value) ± 1.96 S.E.

The statistical significance of differences between two groups was tested using Student’s t-test, provided that variances in the two groups were similar. Multiple comparisons were performed using Scheffé’s test following one-way ANOVA, provided that the variances in groups were similar. If this were 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 metoprolol in Japanese patients:** The 65 blood concentration data for metoprolol in 34 patients in the previous study were re-analyzed to estimate population pharmacokinetics parameters using the NONMEM software. Table 1 shows the population pharmacokinetic parameters of metoprolol 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 σ value could not be estimated precisely in the covariance model, probably because the number of data points for each subject was two or less. Then, the σ value was fixed at 4.54 ng/mL, and the other parameter values were estimated in the covariance model. The mean values of \(\theta_1, \theta_2, \theta_3, \theta_4, \text{ and } \theta_5\) with the covariance model (0.917, 0.441 L/h/kg, 0.709, 4.94 and 0.875 L/kg respectively) were similar to those with the basic model (0.938, 0.439 L/h/kg, 0.739, 4.52, and 0.637 L/kg respectively) (Table 1). The \(\omega_{CL/F}^2\)
value with the covariance model (0.0859) was also similar to that with the basic model (0.0885). However, the $\omega_{CL/F}^2$ value with the covariance model (0.0620) was larger than that with the basic model (0.0291) (Table 1). In addition, the coefficient of correlation ($\rho$) between $CL/F$ and $V/F$, which was calculated as follows: $\rho = \omega_{CL/F} \omega_{V/F}$, was found to be considerably high (0.851). The $-2 \log$ likelihood in the covariance model was less than that in the basic model, indicating that the covariance model is more appropriate than the basic model (Table 1). The individual $\eta_{CL/F}$ and $\eta_{V/F}$ values for metoprolol were obtained from population estimates for the covariance model according to Bayes’ theorem using the NONMEM post-hoc option. Figure 1 shows the relationship between $\eta_{CL/F}$ and $\eta_{V/F}$ with the covariance model. There was a significant positive correlation between $\eta_{CL/F}$ and $\eta_{V/F}$ in each genotype group (Fig. 1). There may not be a close correlation between $CL$ and $V$ of metoprolol because of the low plasma protein binding of the drug (12%). Therefore, the present findings suggested that the residual variability of $F$ is another cause of the interindividual pharmacokinetic variability of metoprolol.

The individual $CL/F$ and $V/F$ values were re-estimated with the covariance model according to Bayes’ theorem using the NONMEM post-hoc option (Supplemental Figs. 1 and 2). The $CL/F$ values with the covariance model (Supplemental Fig. 1) were similar to those with the basic model. On the other hand, the $V/F$ values with the covariance model (Supplemental Fig. 2) were more variable than those with the basic model. In addition,
The cells were incubated with 100 µM metoprolol for 5 min. Each column represents the mean ± S.E. of 6–14 experiments. *p < 0.05, significantly different from the control.

Fig. 5. Concentration dependence of the uptake of metoprolol in LS180 cells
The cells were incubated with 100 µM metoprolol for 10 min in the presence of organic cations at pH 7.4. Open diamond, TEA; open circles, procainamide; closed circles, bisoprolol; open triangles, quinidine. Each point represents the mean ± S.E. of 3–6 experiments.

findings indicated that the uptake of metoprolol in LS180 cells is stimulated by an outward H⁺ gradient. **Concentration-dependent uptake of metoprolol in LS180 cells:** Figure 5 shows the effects of 0.01–4 mM metoprolol in the presence or absence of 5 mM diphenhydramine (DPH) at pH 7.4. Each point represents the mean ± S.E. of 3–6 experiments. The apparent $K_{in}$, $V_{max}$, and $K_d$ values (±S.E.) for metoprolol uptake were estimated to be 0.129 ± 0.026 mM, 8.09 ± 0.58 nmol/10 min/3.8 cm², and 6.15 ± 0.15 µL/10 min/3.8 cm², respectively. Inset: Eadie-Hofstee plot of the uptake of metoprolol after correction for the nonsaturable component. $V$, the uptake in nmol/10 min/3.8 cm²; $S$, metoprolol concentration in mM.

**Effect of various compounds on the uptake of metoprolol in LS180 cells:** Figure 6 shows the effects of 0.003–5 mM organic cations on the uptake of 100 µM metoprolol in LS180 cells. A typical hydrophilic organic cation, TEA, did not inhibit the uptake of metoprolol. On the other hand, procainamide, bisoprolol, and quinidine, which were substrates and/or inhibitors of the postulated pH-dependent tertiary amine transport system, significantly decreased the uptake of metoprolol in a concentration-dependent manner (Fig. 6). The $K_i$ values (±S.E.) of procainamide, bisoprolol, and quinidine were estimated to be 1.09 ± 0.05, 0.191 ± 0.014, and 0.0431 ± 0.0023 mM, respectively. We also evaluated the effect of 1 mM OATP inhibitors, rifampicin, rifamycin SV, naringin, and fexofenadine, on the uptake of 100 µM metoprolol in LS180 cells (Supplemental Fig. 3). However, OATP inhibitors had no significant effect on the uptake of metoprolol (Supplemental Fig. 3).

**Discussion**

We have previously reported that there is a significant positive correlation between $CL/F$ and $V/F$ of not only bisoprolol, but also mizoribine and carvedilol. Mizoribine is not subjected to hepatic metabolism, and the unchanged drug is excreted almost exclusively into the urine. In addition, the plasma protein binding of mizoribine is negligible. Therefore, the variability of $F$ of mizoribine can be a cause of the positive correlation between $CL/F$ and $V/F$ of the drug, and the intestinal absorption step is mainly responsible for the variable $F$ of mizoribine. On the other hand, carvedilol is almost entirely eliminated by hepatic metabolism via aliphatic side-chain oxidation, aromatic ring oxidation, and the conjugation pathway. In addition, the hepatic extraction ratio of carvedilol is considerably high; therefore, the interindividual variability in the hepatic first-pass metabolism can be responsible for the variable $F$ and for the positive correlation between $CL/F$ and $V/F$ of the drug. Furthermore, the plasma protein binding of carvedilol is extremely high (95.4–98.2%), and there is considerable variability in the unbound fraction of the drug; therefore, $CL$ of carvedilol may also be correlated with $V$ to some extent.

In the present study, the covariance model was more appropriate for analyzing the clinical pharmacokinetic data on metoprolol, and the individual $\eta_{CL/F}$ values of metoprolol were positively correlated with the individual $\eta_{V/F}$ values (Table 1 and Fig. 1). There may not be a close correlation between $CL$ and $V$ of metoprolol because of the low plasma protein binding of the drug (12%). Therefore, the residual variability of $F$ of metoprolol can be a cause of the positive correlation between $CL/F$ and $V/F$ of the drug. In addition, the hepatic extraction ratio of metoprolol is considerably high; therefore, the interindividual variability in the hepatic first-pass metabolism can be at least partly responsible for the residual metabolism can be at least partly responsible for the residual variability of the drug; therefore, the interindividual variability in the hepatic first-pass metabolism can be at least partly responsible for the residual
variability of $F$ of the drug. In the present study, we further hypothesized that the intestinal absorption step could be also responsible for the residual variability of $F$ of metoprolol, and investigated the mechanism of the uptake of metoprolol into intestinal epithelial LS180 cells.

The uptake of metoprolol to LS180 cells was significantly decreased by the acidification of extracellular pH, and stimulated by an outward H$^+$ gradient (Figs. 3 and 4). In addition, the cellular uptake of metoprolol was temperature-dependent and saturable (Figs. 4 and 5). Furthermore, the uptake of metoprolol was significantly inhibited by hydrophobic organic cations, such as diphenhydramine, procainamide, bisoprolol, and quinidine, but was not affected by TEA or OATP substrates/inhibitors (Figs. 3 and 6 and Supplemental Fig. 3). These findings suggest that the pH-dependent transport system is at least partly involved in the uptake of metoprolol to intestinal epithelial cells. A plausible candidate for the metoprolol transporter is the postulated H$^+$/tertiary amine antiport system, which was first reported by Mizuuchi et al. in 1999.\textsuperscript{21} In addition, it has been reported that the postulated H$^+$/tertiary amine antiport system is expressed on human intestinal epithelial Caco-2 cells and LS180 cells, and is involved in the cellular uptake of tertiary amine compounds such as procainamide, quinidine, and diphenhydramine.\textsuperscript{9,11,21,22}

We previously evaluated the cellular uptake of procainamide and quinidine in LS180 cells.\textsuperscript{9} The cellular uptake of procainamide and quinidine was temperature-dependent and saturable, and was decreased by acidification of the apical medium. In addition, the uptake of procainamide and quinidine was significantly inhibited by hydrophobic organic cations such as pyrilamine, diphenhydramine, and imipramine, but was not affected by the typical hydrophilic cations TEA and choline.\textsuperscript{9} Recently, we have reported that the uptake of bisoprolol in LS180 cells is also mediated by the postulated H$^+$/tertiary amine antiport system.\textsuperscript{33} Further studies will be needed to identify the postulated H$^+$/tertiary amine antiport system, to clarify the contribution of the 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 indicate that the residual variability of $F$ is another cause of the interindividual pharmacokinetic variability of metoprolol, and that the interindividual variability of not only presystemic first-pass metabolism, but also intestinal absorption, may be responsible for the residual variability of $F$ of the drug.

References


