Bioavailability Assessment of Disopyramide Using Pharmacokinetic–Pharmacodynamic (PK–PD) Modeling in the Rat

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The relationship between the serum concentration and the pharmacological effect of disopyramide was investigated quantitatively to estimate the extent of its oral bioavailability (EBA\textsubscript{oral}) and to evaluate the drug interaction with miconazole, a CYP3A4 inhibitor. An integrated pharmacokinetic–pharmacodynamic (PK–PD) model was used to describe the relationship between the serum concentrations and changes in QT interval (pharmacological data) of disopyramide after intra-vascular infusion for 15 min (i.v. short-term infusion) to rats. A two-compartment model was applied to the pharmacokinetics of disopyramide. The pharmacological data after short-term infusion were well explained using a PK–PD link model. To validate the present PK–PD model, disopyramide was administered intra-vascularly in separate experiments, and the doses were predicted only from the pharmacological data. The model predicted doses were identical to the actual doses, regardless of the dosing rates. This result indicates that the PK–PD model used in the present study is appropriate, and that the relationship between the serum concentrations and changes in QT intervals is independent of the dosing (input) rate. When miconazole was co-administered orally 1 h before disopyramide infusion, the serum disopyramide concentrations were significantly higher than that following disopyramide alone. The raised serum concentrations under miconazole co-administration were well explained by nonlinear elimination clearance. The pharmacological effects of disopyramide under miconazole co-administration, were also greater than those following disopyramide alone. The results of the PK–PD analysis indicated that the enhanced pharmacological response under miconazole co-administration was simply caused by a pharmacokinetic change. The EBA\textsubscript{oral} values estimated from the pharmacological effects predicted the observed values reasonably well. In conclusion, we demonstrated following: (1) the pharmacological effect after intra-vascular administration of disopyramide is related quantitatively to the serum concentrations using a PK–PD model; (2) miconazole affects only the elimination clearance of disopyramide to enhance the pharmacological effect; (3) the EBA of disopyramide can estimated reasonably well from the pharmacological data using the PK–PD model; (4) there is no dosing-rate-dependent or dosing-route-dependent pharmacological effect of disopyramide.

Key words: bioavailability; pharmacological data; pharmacokinetics; pharmacodynamics; disopyramide; QT prolongation

Disopyramide is a class Ia antiarrhythmic agent and is mainly used for the treatment of ventricular and supraventricular tachyarrhythmias. Disopyramide depresses contractility and can precipitate heart failure, and it can often cause torsades de pointes associated with electrocardiographic QT prolongation. Disopyramide is partially metabolized by cytochrome P450 3A4 (CYP3A4)\textsuperscript{1} in the liver, and is excreted mainly in the urine. Therefore, it should be used with caution in association with other cardiac depressants including beta-blockers, other class Ia antiarrhythmic agents and inhibitors of the CYP3A subfamily, particularly CYP3A4,\textsuperscript{2} such as macroline antibiotics, azole antifungals, and cimetidine. Bioavailability monitoring as well as frequent cardiac monitoring is thus recommended when disopyramide is used therapeutically.

The bioavailability of a drug is defined as its rate and extent of absorption, and is one of the most important indexes for the evaluation of drug formulations. Most methods available for determining the extent of bioavailability (EBA), use plasma concentrations; however, there are several reasons for using pharmacological responses instead of plasma concentrations. For example, plasma drug levels may not be available due to lack of an appropriate analytical method, while high quality pharmacological response data are available. In that situation, the area under the pharmacological effect–time curve (AUE) is often used as an alternative to the area under the plasma concentration–time curve (AUC) for estimating EBA\textsuperscript{3,4}. However, the AUE is not always proportional to AUC\textsuperscript{5,6,7}. Recently, we reported a novel method for evaluating the EBA from pharmacological effects.\textsuperscript{8} This method is based on a one-to-one correspondence between the plasma concentrations (pharmacokinetics: PK) and pharmacological effects (pharmacodynamics: PD) that holds for different dosing rates and different dosing routes (oral administration, infusion and/or bolus injection).

The purpose of this study was to determine the applicability of the previous method to the antiarrhythmic agent, disopyramide. Bryson et al. and several other researchers have suggested that plasma disopyramide concentrations are directly related to the pharmacological effects (changes in QT interval), independent of the administration route.\textsuperscript{9–11} Therefore, estimation of EBA from QT intervals after oral administration of disopyramide is feasible. In this study, we first constructed a PK–PD model based on intra-vascular infusion data, and then the following questions were addressed. (1) Can the oral bioavailability (EBA\textsubscript{oral}) of disopyramide be estimated from pharmacological data accurately? (2) Does the pharmacological effect of disopyramide show any dosing rate-dependent characteristics? (3) Does miconazole, an azole antifungal agent, interact with disopyramide? (4) Is the disopyramide–miconazole interaction attributable to pharmacokinetic changes, pharmacodynamic changes, or both?

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MATERIALS AND METHODS

**Chemicals** Disopyramide phosphate, (±) miconazole nitrate and procaine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The lactated Ringer’s solution was from Otsuka Pharmaceuticals Ltd. (Tokyo, Japan). All other chemicals and solvents used were of reagent grade and were obtained commercially (Wako Pure Chemical Industries, Osaka, Japan).

**Animals and Surgery** Male Wistar rats (240—270 g) were purchased from Japan SLC, Inc. (Shizuoka, Japan). The rats were maintained in a constant environmental facility (temperature: 24±1°C, humidity: 55±10%) exposed to 12:12 h light–dark cycles and allowed free access to tap water and standard rat chow for at least 1 week. On the day of the experiment, rats were lightly anesthetized with pentobarbital sodium, and a combination of Silastic (Dow Corning Corp., Midland, MI, U.S.A.) and PE50 (Clay Adams, Parsippany, NJ, U.S.A.) catheters were implanted surgically in the femoral vein and right jugular vein for drug administration and blood sampling, respectively. Both catheters were externalized through the back in the neck region and secured. After closure, animals were housed individually and allowed to recover for at least 1 h. For the oral administration study, a cannula (PE50) was inserted temporarily into the stomach. All these animal experiments had previously been approved by the Animal Experimentation Committee of the Osaka University of Pharmaceutical Sciences.

**Determination of Serum Disopyramide Concentrations** Disopyramide was dissolved in lactated Ringer’s solution and injected into the jugular vein at doses of 10—50 mg/kg. Blood samples (0.3 ml) were withdrawn from the jugular vein at designated times up to 255 min after administration. After centrifugation (10000 rpm, 5 min, 4°C), serum was separated and stored at -20°C until analysis. Serum disopyramide concentrations were assayed by HPLC according to a slight modification of the methods of Masuhara et al.,12 and Witek et al.,13 Procaine hydrochloride was used as the internal standard. Detection was performed by monitoring UV absorption at 260 nm.

**Measurement of QT Interval** Signals from the standard limb lead II of the electrocardiograph (ECG) were recorded by means of a polygraph (PolyGraph 366 system, NEC San-ei Instruments, Co. Ltd., Tokyo, Japan) and a digital oscilloscope (System 400, Nicolet Japan Corporation, Tokyo, Japan), connected to a microcomputer (Dyna-Book J-3100SS, Toshiba Corporation, Tokyo, Japan). Disopyramide was infused intravenously for 15 min (short-term infusion study: 5—50 mg/kg) or 1—6 h (long-term infusion study: 25—200 mg/kg). For the oral administration study, 25—100 mg/kg disopyramide was given in a volume of 400 μl. The ECG was recorded continuously from predosing, up to 360 min (short-term infusion study) or 540 min (long-term infusion study or oral dosing study) postdosing. Since the QT interval has been reported to be independent of the heart rate in rats,14 we also used the raw QT interval for this study. The change in QT interval was defined as the difference from the mean interval over 25 min predosing.

**Measurement of in Vitro Protein-Binding** The in vitro protein binding of disopyramide in pooled rat serum was determined by ultrafiltration (concentrations range: 0.5—40 μg/ml) conducted at 6000 rpm, for 25 min (37°C) using an Ultrafree™ membrane filter (exclusion molecular size 5000; MC NMWL5000, Millipore Co., Bedford, MO, U.S.A.). HPLC was used to determine the free and total concentrations of disopyramide. The fraction unbound was calculated by the standard method.

**Miconazole Interaction Study** Miconazole was suspended in 10% Gum Arabic, and given orally at the dose of 300 mg/kg (dosing volume: 750 μl), 1 h before disopyramide administration. Unless otherwise specified, disopyramide was infused intravenously for 15 min (short-term infusion, dose range: 10—50 mg/kg). The serum disopyramide concentrations and the QT interval were measured as described above.

**Data Analysis** The concentration–time data were analyzed using a nonlinear regression program PKDM15 applying the algorithm by Berman et al.16 on a MicroVAX II computer (DEC, Maynard, MS, U.S.A.) or a PC/AT compatible personal computer. The differential equations were solved by the Runge–Kutta–Gill method and the AUC was calculated by the trapezoidal method. The EBA<sub>p,n</sub> was calculated as follows:

\[
EBA_{p,n} = \frac{AUC_{p,n}}{D_{p,n}} \times \frac{AUC_{n,n}}{D_{n,n}}
\]

where \(D_{p,n}\) and \(D_{n,n}\) are the oral and intravenous doses of disopyramide, respectively. \(AUC_{p,n}\) and \(AUC_{n,n}\) represent the area under the serum concentration–time curve after oral administration and i.v. administration, respectively.

**THEORETICAL**

Figure 1 represents the PK–PD model that describes the relationship between the serum disopyramide concentration and pharmacological effect (prolongation of QT interval) after intravenous administration of disopyramide. This model was constructed under the following assumptions: (1) the disposition of disopyramide is described by a conventional two compartment model; (2) the distribution and elimination of disopyramide are described by first-order kinetics; (3) the site of action of disopyramide is in the “effect” compartment; (4) the free fraction of disopyramide in the “effect” compartment is directly related to the free fraction of disopyramide in the central compartment; and (5) the relationship between the free fraction of disopyramide in the “effect” compartment and the pharmacological effect (prolongation of QT interval) is described by a sigmoid \(E_{\text{max}}\) model.

For the infusion study, the differential equations of the model are,

\[
\begin{align*}
V_1 \frac{dDP_1}{dt} &= K_e - (CL_{e1} + CL_{i1}) \cdot DP_1 + CL_{e1} \cdot DP_2 \\
V_2 \frac{dDP_2}{dt} &= CL_{e2} \cdot DP_1 - CL_{e2} \cdot DP_2 \tag{1} \\
DP_{i,n} &= F_{i,n} \cdot DP_1 \tag{2} \\
\frac{dC_c}{dt} &= k_{c,n} \left( DP_{i,n} - C_c \right) \tag{3}
\end{align*}
\]
Fig. 1. Diagrammatic Representation of the PK-PD Model after Intravenous Infusion and Oral Administration of Disopyramide to Rats

\[
\text{Change in QT interval} = \frac{E_{\text{max}} C_t}{EC_{50} + C_t} \quad (5)
\]

at \( t=0, DP_1=DP_2=C_t=0 \)

where \( K_0 \) (\( \mu g/min \)) is the infusion rate of disopyramide, \( DP_1 \) and \( DP_2 \) are the disopyramide concentrations (\( \mu g/ml \)) in the central (serum) and peripheral compartments, respectively, \( CL_{12} \), \( CL_{21} \) and \( CL_{10} \) are the clearances (ml/min/kg) of disopyramide. At steady-state, \( CL_{12} \) is equal to \( CL_{21} \), \( V_1 \) and \( V_2 \) are the distribution volumes (ml/kg) of each compartment, respectively. \( DP_{tu} \) is the free fraction of disopyramide (\( \mu g/ml \)) in the central compartment and \( F \) is the ratio of unbound and total concentrations of disopyramide in serum. \( C_t \) is the unbound concentration of disopyramide (\( \mu g/ml \)) in the effect compartment. \( k_{12} \) and \( k_{02} \) are the first-order rate constants (min\(^{-1}\)) of the effect compartment. \( E_{\text{max}} \) and \( EC_{50} \) are constants for a sigmoid \( E_{\text{max}} \) model, and \( \gamma \) is the Hill constant.

For the oral administration study, the differential equation for the concentration of disopyramide in the central compartment (Eq. 1) is replaced by Eqs. 6 and 7.

\[
\frac{dA}{dt} = -k_e A_t \quad (6)
\]

\[
V_1 \frac{dDP}{dt} = k_e A_t = (CL_{12} + CL_{10}) \cdot DP + CL_{21} \cdot DP_2 \quad (7)
\]

at \( t=0, A_t = D_{pu} F, DP_1 = DP_2 = 0 \)

where \( D_{pu} \) (mg/kg) is the oral dose of disopyramide, \( A_t \) is the amount of disopyramide in the gastro-intestinal tract remaining to be absorbed, \( k_e \) (min\(^{-1}\)) is the first-order absorption rate constant, and \( F \) is the bioavailability.

RESULTS

PK-PD Modeling The time-courses of the serum disopyramide concentrations after short-term (15 min) infusion are shown in Fig. 2A, as plotted points. The disopyramide concentrations declined biexponentially for each dose.

Since there was no difference in total body clearance among the doses (data not shown), the disposition of disopyramide in the rat was considered to be linear. These serum concentration data were fitted to the model shown in Fig. 1, and the PK parameters were estimated. The solid lines shown in Fig. 2A were obtained by the nonlinear least squares method, and the estimated PK parameters are listed in Table 1. As shown in Table 2, the ratio of unbound and total concentrations of disopyramide in serum was almost constant (0.822 ± 0.0017) over a wide range of total concentrations (0.5—40 \( \mu g/ml \)). This fact indicates that the serum protein binding of disopyramide is constant over the dose range in the in vivo study.

The time-courses of the pharmacological effect (change in QT interval) during and after short-term infusion of disopyramide (dose: 5—50 mg/kg) are shown in Fig. 2B, as plotted
points. The change in QT interval reached a peak \((\text{QT}_{\text{max}})\) up to 60 min after dosing and then disappeared rapidly. Fig. 3A and Fig. 3B represent the dose-dependence of the \(\text{QT}_{\text{max}}\) and the area under the QT prolongation–time curve \((\text{AUE})\) following short-term infusion. Since there was typical anti-clockwise hysteresis between the free disopyramide concentration in serum and the change in QT interval (Fig. 3C), a conventional link model\(^{15}\) shown in Fig. 1, was introduced. The solid lines shown in Fig. 2B were obtained by least squares fitting of the observed data to Eqs. 1—5, and the estimated PD parameters are listed in Table 1. The fitted lines shown in Fig. 2B describe the pharmacological effects adequately, regardless of dose.

**Effect of Dosing Rate on PK–PD Relationship** The effect of dosing rates on the PK–PD relationship of disopyramide was investigated in separate experiments. During and after administration of disopyramide at various infusion rates \((0.28–1.67 \text{ mg/kg/min})\) and infusion periods \((1 \text{ to } 6 \text{ h: long-term infusion study})\), changes in the QT interval were continuously recorded. Then, these pharmacological effects were fitted to the overall PK–PD model (Eqs. 1—5), and only the input rate \((\text{Ki})\) was estimated. All other PK and PD parameter values were fixed at the previous values (Table 1). The estimated doses (products of \(\text{Ki}\) and infusion period) were plotted against the actual doses, as shown in Fig. 4. The plotted points showed a straight line (slope = 1.01, intercept = 0, \(r^2=0.991\)), indicating that there is no input-rate-dependent-pharmacological effect and that the present PK–PD model is appropriate for describing the time-course of the pharmacological effect of disopyramide over a wide range of dosing rates.

**Interaction with Miconazole** The plotted points shown in Fig. 5A represent the time-course of the serum disopyramide concentrations following short-term infusion of disopyramide \((10, 25, 50 \text{ mg/kg})\) under co-administration of miconazole \((300 \text{ mg/kg, p.o.)}\). The elimination of disopyramide was significantly reduced by miconazole co-administration over the dose range examined. Since the total body clearances of disopyramide fell as the doses increased \((23.7, 16.3, \text{ and } 17.4 \text{ ml/min/kg} \text{ at a dose of } 10, 25, \text{ and } 50 \text{ mg/kg, respectively})\), typical nonlinear elimination kinetics of disopyramide were displayed under co-administration of miconazole. This nonlinearity may be related to enzymatic inhibition by miconazole of the metabolism of disopyramide. Then, we introduced Michaelis–Menten type kinetics to the elimination process of disopyramide. The differential equation for the amount of disopyramide in serum under miconazole co-administration is as follows.

\[
\frac{d\text{DP}_i}{dt} = \text{Ki} - \left( \frac{V_{\text{max}}}{\text{Ki} + \text{DP}_i} \right) \text{CL}_2 + \text{CL}_1 \cdot \text{DP}_i
\]

where \(V_{\text{max}}\) and \(\text{Ki}\) are the maximum rate and the Michaelis constant, respectively. The serum disopyramide concentrations under co-administration of miconazole were fitted to Eq. 8 and Eq. 2, and the PK parameters were estimated. The solid lines shown in Fig. 5A represent the results of the model fitting and the estimated parameters are listed in Table 3.

The plotted points shown in Fig. 5B represent the time-course of the QT interval after administration of disopyramide under co-administration of miconazole. The extent of QT prolongation increased compared with disopyramide alone (dashed lines), especially at high doses of disopyramide. There was no effect on the heart rate and on the QT interval after administration of miconazole alone (data not shown). If miconazole only affects the pharmacokinetic process (metabolic process) of disopyramide but not the pharmacological process, the present PK–PD model will be applicable to the change in QT interval under miconazole co-administration, without any parametric modification. Then, the QT prolongation–time profiles following miconazole co-

**Table 2. In Vitro Protein Binding of Disopyramide at Various Concentrations in Rat Serum**

<table>
<thead>
<tr>
<th>Total disopyramide concentration ((\mu\text{g/ml}))</th>
<th>Fraction of free disopyramide</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.797±0.00632</td>
</tr>
<tr>
<td>1</td>
<td>0.794±0.0237</td>
</tr>
<tr>
<td>2.5</td>
<td>0.834±0.0125</td>
</tr>
<tr>
<td>5</td>
<td>0.807±0.0407</td>
</tr>
<tr>
<td>10</td>
<td>0.822±0.0209</td>
</tr>
<tr>
<td>20</td>
<td>0.832±0.0130</td>
</tr>
<tr>
<td>40</td>
<td>0.883±0.0119</td>
</tr>
</tbody>
</table>

Data represent the mean±S.E. of 4 experiments.

---

**Fig. 3. Dose-Dependence of** (A) \(\text{QT}_{\text{max}}\) (B) \(\text{AUE}\), and (C) Relationship between the Serum Disopyramide Concentration and the Change in QT Interval after i.v. Short-Term Infusion of Disopyramide to Rats**

\(\text{QT}_{\text{max}}\) represents the maximum change in QT interval. \(\text{AUE}\) shows the area under the QT prolongation–time curve from 0 to 360 min. Doses in panel C are 10 mg/kg (□), 25 mg/kg (△), and 50 mg/kg (○). Plotted points represent the mean and S.E. of 3–4 experiments.
administration were simulated. The simulation curves shown in Fig. 5B (in solid lines) followed the observed data closely.

**Prediction of Serum Disopyramide Concentrations and Bioavailability after Oral Administration**

Figure 6A shows the time-course of QT prolongation after oral administration of disopyramide (25, 50 and 100 mg/kg). The change in QT interval reached a peak 1—2 h after administration and then disappeared. To estimate the absorption parameters, such as the kₐ and F of the model, the pharmacological data were fitted to Eqs. 2—7. The fitted lines described the actual data well (Fig. 6A) and the estimated absorption parameters are listed in Table 4. The solid lines shown in Fig. 6B represent simulated curves of the serum disopyramide concentrations following oral administration (25, 50, 100 mg/kg). From these simulated curves, the oral bioavailability (EBAₜ₀) was calculated. The predicted EBAₜ₀ value was 96.6%, as listed in Table 5. In order to assess the accuracy of this predicted EBAₜ₀ value, disopyramide was administrated orally to rats in separate experiments, and the time-course of the serum concentrations of disopyramide was determined. The plotted points shown in Fig. 6B are the actual serum
Table 4. Absorption Parameters after Oral Administration of Disopyramide to Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_t )</td>
<td>0.0028±0.0005</td>
</tr>
<tr>
<td>( F )</td>
<td>0.97±0.15</td>
</tr>
</tbody>
</table>

Data represent the computer-fitted value±S.D.

Table 5. Comparison of Model-Predicted AUC and EBA\(_{p,a}\) with the Observed Values after Oral Administration of Disopyramide to Rats

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>AUC (( \mu )g/ml min)</th>
<th>EBA(_{p,a}) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Predicted</td>
<td>Observed</td>
</tr>
<tr>
<td>25</td>
<td>734</td>
<td>453±47</td>
</tr>
<tr>
<td>50</td>
<td>1469</td>
<td>1306±151</td>
</tr>
<tr>
<td>100</td>
<td>2937</td>
<td>3124±259</td>
</tr>
</tbody>
</table>

The observed data represent the mean±S.E. of 6 experiments.

concentration data. Using these actual data, the EBA\(_{p,a}\) value for each dose was calculated. The actual values of EBA\(_{p,a}\) were 57.6% to 99.4% (Table 5), and these values were almost identical with those obtained in the simulation study. These findings indicate that the EBA\(_{p,a}\) estimated from the pharmacological effects predicted the actual values, accurately.

DISCUSSION

Pharmacokinetics and Pharmacodynamics of Disopyramide To describe the time-course of the serum concentrations of disopyramide, a conventional linear two-compartment model was used in this study. The results indicated that this model adequately describes the pharmacokinetics of disopyramide after short-term infusion in rats. The biological half-lives in serum were about 2.9 and 79.7 min for the distribution and elimination phase, respectively, and these values were comparable with those reported by Ranney et al.\(^{11}\) As in other reports,\(^{13}\) the free fraction of disopyramide in serum was almost constant (\( F_f = 0.822±0.0017 \)) over the dose range investigated. Therefore, the total serum concentrations of disopyramide, instead of free concentrations, were determined in the in vivo study, and the free concentrations of disopyramide in serum were calculated from Eq. 3.

Although QT prolongation has been reported to be directly proportional to the blood disopyramide concentration,\(^{8,10,18}\) a significant delay (a typical hysteresis) was observed in this study. Disopyramide was administered at a dosing rate of 0.33—3.33 mg/kg/min in our study, while rates of 0.1—1 mg/kg/min were used in those reports. Therefore, the low dosing rate might conceal the delay in the pharmacological effect of disopyramide; however, further investigations are required to resolve this.

To validate the present PK–PD model, the administered doses were predicted from the pharmacological data obtained in separate experiments at various infusion rates (Fig. 4). The results revealed that the predicted doses were in accord with the actual doses, independently of the doing rate. This fact indicates that the present model is appropriate for describing the PK–PD relationship of disopyramide, and that this PK–PD relationship is maintained over the dosing range investigated. These results are fundamentally consistent with the report of Whiting et al.\(^{10}\) which suggested that the sensitivity of the QT interval to disopyramide was preserved, regardless of the route of administration. Recently, we reported in a study of arginine-vasopressin (AVP) that the one-to-one correspondence between PK and PD does not hold for different dosing rates, and that this phenomenon was explained by the receptor-desensitization of AVP-receptor binding.\(^{8,11}\) In that situation, the accuracy of EBA obtained from pharmacological data was not satisfactory, unless an adequate dosing rate of the reference (intra vascular) formulation was selected. However, in the case of disopyramide, such receptor-desensitization might not occur. The QT prolongation of disopyramide (and, presumably, quinidine) is attributed to blocking some types of cardiac potassium channels.\(^{19,21}\) These drugs bind to the open-state of the potassium channel;\(^{22}\) however, the channels repeatedly open and close depending on the membrane potential and not on the binding of the ligands. Therefore, these potassium channels may not cause ligand-mediated desensitization. This might be the reason that the EBA of disopyramide could be estimated accurately from pharmacological data, regardless of the dosing rates.

PK–PD Relationship under Miconazole Co-administration The main metabolic pathway for disopyramide in mammals, including rats, is oxidative N-dealkylation in hepatic microsomes, and this pathway is catalyzed by CYP3A4.\(^{23}\) It is well known that azole antifungal agents, such as miconazole, and macrolide antibiotics, such as erythromycin, significantly inhibit the enzymatic activity of CYP3A, especially CYP3A4 in hepatic microsomes.\(^{20}\) In the present study, the serum concentrations of disopyramide under co-administration of miconazole were significantly higher than those without miconazole co-administration. These serum concentration data were reasonably described by a PK model with nonlinear elimination kinetics (Fig. 1 and Eq. 8). The results of the model analysis showed that the estimated values for the distribution volumes (\( V_f \) and \( V_f \)) and the distribution clearance (\( CL_{dv} \)) are almost identical with those values obtained in the disopyramide alone study. This fact suggests that miconazole affects only the elimination of disopyramide, but not its distribution, including protein binding in serum, by reducing the apparent amount of CYP3A4 in hepatic microsomes in rats. If the serum disopyramide concentration is sufficiently low compared with the \( K_m \) value (\( K_m \gg DP \)), the elimination clearance will be 3.18 ml/min/ kg, and this value is almost identical with the \( CL_{dv} \) of 32.6 ml/min/kg which was obtained in the disopyramide alone study. Although the co-administration of miconazole induced larger changes in the QT interval than those in the disopyramide alone study, the present PK–PD analysis suggests clearly that this enhancement of pharmacological response is simply due to a pharmacokinetic change. Therefore, we concluded that miconazole affects the free fraction of disopyramide at the site of action via the serum concentration by inhibiting metabolism; however, it does not affect the pharmacological action at all. Hanada et al. reported that co-administration of erythromycin, which is known to be another potent inhibitor of CYP3A4, with disopyramide produced a severe QT prolongation in rats, without any change in the plasma concentrations of disopyramide. From these re-
sults, they concluded that the disopyramide–erythromycin interaction was caused only by a pharmacological interaction, not a pharmacokinetic one. This is very different from our results. The most probable explanation for this discrepancy is the difference between the two inhibitors in their effect on the pharmacodynamics of QT prolongation, or their inhibitory effect on CYP3A4. More definitive experiments are needed to clarify this.

Although renal excretion is the major route of elimination from serum, we did not investigate the renal transport of disopyramide in the present study. Since the renal transport of disopyramide is known to be an active process, dose-dependent renal excretion would be expected. In the model analysis, only linear pharmacokinetics was suggested at dose levels up to 50 mg/kg of disopyramide (Fig. 2). Therefore, there was little possibility that the renal excretion would affect the pharmacokinetics of disopyramide, even under co-administration of miconazole.

**Assessment of \( \text{EBA}_{p, \alpha} \), Estimated by the Change in QT Interval** After oral administration of disopyramide, a linear absorption model was assumed in the present study. As reported by Cook et al. and Lee et al., disopyramide is rapidly and almost completely absorbed from the gastro-intestinal tract. Since there is no report of the degradation or metabolism of disopyramide in the gastro-intestinal tract, we did not built these processes into the model. As shown in the result section, the \( \text{EBA}_{p, \alpha} \) was estimated merely from pharmacological data, using the PK–PD model of the short-term infusion study. The estimated \( \text{EBA}_{p, \alpha} \) value at the dose of 50 mg/kg was identical to the actual \( \text{EBA}_{p, \alpha} \) value. This dose level is within the therapeutic dose range of disopyramide in humans (therapeutic serum concentration: 2–6 µg/ml). However, there was a tendency to overestimation at 25 mg/kg and lower doses. One of the reasons for this may be that we were obliged to use very small changes in QT interval (less than 5 ms), near the detection limit of the pharmacological effect. Nevertheless, these results demonstrated that the \( \text{EBA}_{p, \alpha} \) of disopyramide could be estimated adequately from the pharmacological effect (change in QT interval), independently of the dosing route.

In conclusion, we demonstrated the following points: (1) the pharmacological effect after intra-vascular administration of disopyramide was related quantitatively to the serum concentrations using a PK–PD model; (2) miconazole affected only the elimination clearance of disopyramide to enhance its pharmacological effect; (3) the \( \text{EBA} \) of disopyramide could be reasonably estimated from only the pharmacological data using the PK–PD model; (4) there was no dosing-rate-dependent or dosing-route-dependent pharmacological effect of disopyramide. Using present method, for example, the elimination clearance of disopyramide under miconazole co-administration can be predicted from the QT prolongation data alone and, thus, dosage reduction can easily be performed without time-consuming determination of serum drug concentrations. If this method is applicable to human data, such as Holter-type ECG monitoring, the results will be very useful in therapeutic situations.

**REFERENCES**