Pharmacodynamic Analysis of the Electrocardiographic Interaction between Disopyramide and Erythromycin in Rats

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Abstract □ Disopyramide (DP) is known to induce QT prolongation and Torsades de Pointes (TdP) when administered concomitantly with erythromycin (EM). To define and evaluate quantitatively the arrhythmogenic risk of the concomitant administration of DP and EM, we investigated the influence of EM on the pharmacokinetics and pharmacodynamics of DP in rats. The time profiles of change in QT interval and plasma concentration of each drug were evaluated during and after constant intravenous infusion of DP (6.0 or 15.0 mg/kg/h), EM (4.0 or 8.0 mg/kg/h), and coadministration of DP and EM (DP 6.0 mg/kg/h plus EM 4.0 mg/kg/h). Each agent induced QT prolongation at plasma concentrations within the therapeutic range in humans. DP-induced QT prolongation was proportional to its plasma concentration. In the case of EM, the E_{max} model with an "effect compartment" could explain the relationship between plasma EM concentrations and changes in QT interval. Although coadministration of EM with DP gave enhanced QT prolongation compared to dosing with DP alone, EM did not affect the pharmacokinetics of DP. In conclusion, it was shown that a pharmacodynamic interaction contributes to the electrocardiographic adverse reaction (i.e., QT prolongation) induced by coadministration of DP and EM in rats.

Introduction

Disopyramide (DP), a class Ia antiarrhythmic agent, is used for tachyarrhythmia and prolongs action potential duration (APD) in cardiac myocytes. In recent years, it has been reported that concomitant administration of DP and erythromycin (EM), a macrolide antibiotic, induced Torsades de Pointes (TdP) associated with electrocardiographic QT prolongation.[1,2] In humans, DP is metabolized mainly by the liver into the major metabolite, mono-N-dealkylated disopyramide (MND).[3] Since EM, which is known to inhibit CYP3A4,[4] is reported to inhibit the metabolism of DP,[5] this oxdation enzyme is thought to be from the CYP 3A subfamily, paticularly CYP3A4. This inhibition may lead to elevated plasma DP concentration, and resultant toxicity such as QT prolongation. However, one case of TdP associated with QT prolongation resulting from the coadministration of DP and EM without an elevation of plasma DP concentration was also reported.[1] Therefore, the elevation of plasma DP concentration may not completly explain this adverse reaction. In fact, EM itself is known to induce QT prolongation or TdP.[6–8] Lin et al.[9] reported a case of TdP associated with QT prolongation induced by the concomitant use of quinidine and EM without an increase of plasma quinidine concentrations; they concluded that the adverse reaction caused by quinidine and EM coadministration might be based on their pharmacodynamic interaction. However, the significance of the pharmacodynamic interaction between DP and EM remains to be evaluated quantitatively.

Since EM is well-known as a metabolic inhibitor, the interaction between DP and EM might be initially considered based on metabolic inhibition. However, as far as a pharmacodynamic interaction is involved, monitoring of plasma DP concentrations only should underestimate the risk of adverse reactions. In this case, it is necessary to investigate this interaction not only from the pharmacokinetic aspect but also from the pharmacodynamic one. The aim of this study, therefore, was to define and evaluate quantitatively the risk of QT prolongation induced by DP and EM in an animal model, where rats were employed to estimate the ECG abnormality, including QT prolongation.[9]

Materials and Methods

1. Materials and Animals—DP was purchased from Wako Pure Chemical Industries (Osaka, Japan). MND and *p*-chlorodisopyramide were kindly provided from Russel Uclaf (Paris, France). Erythromycin base (EM) was obtained from Dai Nippon Pharmaceutical Co. (Osaka, Japan). Acetonitrile, methanol, and dichloromethane were of HPLC grade from Wako Pure Chemical Industries. Other reagents of analytical grade were also purchased from Wako Pure Chemical Industries. Male Sprague−Dawley rats weighing 250−350 g were purchased from Nippon Bio-Supp. Center (Tokyo, Japan) and used for the experiments.

2. Pharmacodynamic Studies—Drug solutions used for pharmacodynamic studies were prepared as follows: DP was solubilized by physiological salt solution (PSS; NaCl: 135 mM, $NaHCO_3$: 11.9 mM, KCl: 5.4 mM, $CaCl_2$: 1.8 mM, $MgCl_2$: 1.0 mM) with 1 mol equiv of H_3PO_4. EM was easily dissolved in PSS. For coadministration experiments, EM were dissolved in DP phosphate solution described above (nearly pH 7.0). EM was comfirmed to be stable in all the solutions for intravenous administration. Each solution was infused into the jugular vein of rats as below by means of a syringe pump (Model 975, Harvard Apparatus, MA) at an infusion rate of 1.05 mL/h.

Rats were anesthetized with a mixture of urethane and α-chlorarose (1.1 mg/kg and 27.5 mg/kg i.p., respectively). The precordial and limb hair were removed using a hair-removing cream (Hair Remover, Kanebo, Tokyo). The trachea, jugular vein, and carotid artery were cannulated with polyethylene tubing. After cannulation, the animals were restrained in a supine position on a heatpad, and the electrodes were noninvasively fastened. The body temperature was maintained at 37.5 ± 0.5 °C throughout the experiment.

After stabilization of the ECG and body temperature, PSS was infused into the jugular vein for 10 min. Subsequently, DP (6 or

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15 mg/kg/h), EM (4 or 8 mg/kg/h), and the mixture of these agents (DP 6 mg/kg/h + EM 8 mg/kg/h) or PSS only (for control) was infused for 90 min. ECG was recorded 10 min before the start of infusion (−10 min), and 0, 1−7, 10, 15, 20, 30, 45, 60, 75, 90−97, 100, 105, 110, 120, 135, 150, 165, and 180 min after the start of infusion. The ECG was examined by a procedure previously reported.[10] Briefly, signals from the bipolar lead II were amplified by means of an electric amplifier (AB-621G, Nihon-Koden, Tokyo), stored in a personal computer (PC-9801VX, NEC, Tokyo) via A/D converter (Analog-Pro Jr., Canopus Electric, Kobe) at a sampling rate of 500 Hz and analyzed with Wave Master II (Canopus Electric, Kobe). Signals from the pressure transducer joined to the cannula of carotid artery were amplified by means of a pressure amplifier (AP-621G, Nihon-Kohden, Tokyo) and recorded by a pen recorder (WI-681G, Nihon-Kohden, Tokyo). ECG parameters such as heart rate and QT interval were derived manually from the average wave shape of recordings for 10 s. Blood was collected after the last recording of ECG to verify that the plasma drug concentration was equivalent to that in pharmacokinetic studies.

3. Pharmacokinetic Studies—*Pharmacokinetics during and after infusion of DP or EM*—Pharmacokinetic studies were performed in animals different from those used in the pharmacodynamic experiments in order to avoid any influence of blood sampling on ECG. Each drug solution was infused in the same manner as the pharmacodynamic experiments. Blood samples were collected from the carotid artery at 2, 5, 15, 45, 90, 92, 95, 105, 135, and 180 min after the start of drug administration. Blood samples were centrifuged to obtain plasma at 1500*g* for 10 min and determined by a procedure described below.

Pharmacokinetics after Portal Vein Administration of DP—Rats were fasted overnight before the experiment. Polyethylene cannula were inserted and fixed into the pyloric vein, the femoral vein and artery under ether anesthesia. The body temperature was maintained at 37.5 ± 0.5 °C throughout the experiment. After recovery from anesthesia, physiological saline or 50 mg/kg of EM was injected into the femoral vein at 5 min before the administration of DP. Then, 20 mg/kg of DP was injected into the portal vein. Each drug solution was prepared as follows: EM was solubilized in phosphate buffer solution (pH 4.0) (0.04 M KH_2PO_4, 0.04 M H_3PO_4), and DP was easily dissolved in physiological saline.

Blood samples (150 μL) were collected at 1, 3, 5, 15, 30, 60, 120, and 180 min after the DP administration and immediately centrifuged at 1500*g* for 10 min. Urine samples were collected for the intervals of 0−3, 3−24, and 24−48 h after the drug administration. The plasma and urine samples were stored at −20 °C until assay.

4. Effects of EM on Plasma Free Fraction (f_p) of DP—The plasma free fraction of DP (f_p) was determined in the presence or absence of EM by an ultrafiltration method. Drug-free blood was collected through the portal vein, heparinized, and centrifuged (1500g, 10 min) to separate plasma. DP was dissolved in plasma to make the concentrations of 0.1, 3.0, 6.0, and 15.0 μg/mL. To examine the influence of EM on f_p of DP, EM was added to the plasma samples containing EM to give a final concentration of 63 μg/mL. After preincubation at 37 °C for 5 min, an aliquot (500 μL) of the sample was put into the reservoir of an ultrafiltration device (Centrifree, Amicon, MA). Thereafter, it was centrifuged at 1000*g* for 2 min at 37 °C to obtain ultrafiltrate. The volume of the ultrafiltrate was measured as less than 15% of the original sample (500 μL). The concentration of DP in each fraction was determined as described below. The f_p value was calculated by dividing the DP concentration in the ultrafiltrate by that in the retained sample.

5. Determination of DP, MND, and EM Concentration—DP and MND concentrations in plasma or urine were determined using an HPLC method reported previously by Mayer et al.[11] with a minor modification. Briefly, an aliquot (50 μL) of plasma or urine sample was pipetted into a 10-mL glass tube. After addition of 50 μL of the internal solution (0.6 μg/mL *p*-chlorodisopyramide solution in 0.01 M HCl) and 2 mL of dichloromethane, the mixture was made alkaline with 5 μL of 1 M NaOH and then shaken for 10 min by a mechanical shaker. After centrifugation (10 min, 1500*g*), the lower organic layer was transferred into a 10-mL glass tube and evaporated to dryness under a stream of dry nitrogen at ambient temperature. The dry residue was dissolved into 50 μL of mobile phase, and 20 μL was applied onto the HPLC system.

HPLC was performed with a UV detector (UV−vis DETECTOR SPD-10A, Shimazu, Kyoto, Japan) and a C_{18} reversed-phase column (Cosmosil-packed column 5-C_{18}, 150 mm × 4.6 mm i.d., particle size 5 μm, Nacalai Tesque, Kyoto, Japan). Assay was carried out at ambient temperature. The mobile phase consisted of aqueous solution (0.125 M HCl, 0.125 M sodium 1-octanesulfonate, 0.072 M triethylamine) and acetonitrile (70:30, v/v). The flow rate was 1.0 mL/min, and the detection wavelength was set at 263 nm. The lower limit of reliable detection of this method was 0.1 μg/mL for DP and 0.05 μg/mL for MND. The plasma EM concentrations were determined by an HPLC-ECD method described previously.[12]

6. Pharmacokinetic−Pharmacodynamic Analyses of QT prolongation—The effects of DP and EM on QT interval were analyzed from both pharmacokinetic and pharmacodynamic aspects. The QT interval was defined as the time from the start of the QRS complex to the end of the T wave, at which the amplitude of the T wave declined to 10% of its maximum. The effect of a drug at each time was expressed as the deviation from the value before the drug administration (ΔQT). Mean QT interval just before the start of drug administration was 61.6 ± 0.98 ms (n = 24). The PR interval was defined as the time from the start of the P wave to the apex of the R wave.

The time profiles of plasma DP concentration (C_p) were analyzed by a conventional two-compartment open with a first-order elimination model described previously.[13] Pharmacokinetic parameters were estimated by simultaneous fitting of the data at each infusion rate.

Then, the relationship between the plasma concentration of DP and the effect of DP on QT prolongation (E_{DP}) was analyzed, assuming a linear relation as follows:

$$E_{DP} = KC_p \quad (1)$$

where C_p is the plasma DP concentration and K is the slope.

The time profiles of plasma EM concentration were also analyzed by a conventional two-compartment model described previously.[13] Pharmacokinetic parameters of α, β, k_{10}, k_{21}, and V_1 were estimated by simultaneous computer-fitting of the data at each infusion rate.

For the pharmacokinetic−pharmacodynamic analysis of EM, the "effect compartment model"[14] was applied because a delay of QT prolongation was observed. The drug concentration (C_e) in the effect compartment is described by equations reported by Holford and Sheiner.[14]

Then the relationship between C_e and the effect of EM on QT prolongation (E_{EM}) was analyzed. The E_{max} model was employed to relate C_e to E_{EM} as follows:

$$E_{EM} = \frac{E_{max}C_e}{EC_{50} + C_e} \quad (2)$$

where E_{max} is the maximum effect, and EC_{50} represents the concentration which gives half-maximal effect.

For coadministration of EM and DP, we assumed that the effect of the coadministration on QT prolongation (E_{DP+EM}) was estimated from the additive model described below.

$$E_{DP+EM} = E_{DP} + E_{EM} \quad (3)$$

Results

ECG Effects and Pharmacokinetics of DP—Figure 1 represents the time profiles of the change in QT interval (ΔQT) and the plasma DP concentration during and after the constant intravenous infusion of DP. The QT interval was prolonged in response to the increase in plasma DP concentration without a lag-time. After the end of the infusion, the QT interval was shortened as the plasma DP concentration declined. DP induced a dose-dependent prolongation of QT interval within the dose range tested; changes in QT prolongation at 90 min after the start of the infusion were 17.50 ± 1.94 ms and 38.50 ± 5.00 ms at the dosing rates of 6 mg/kg/h and 15 mg/kg/h, respectively. In addition, DP induced a significant prolongation of PR

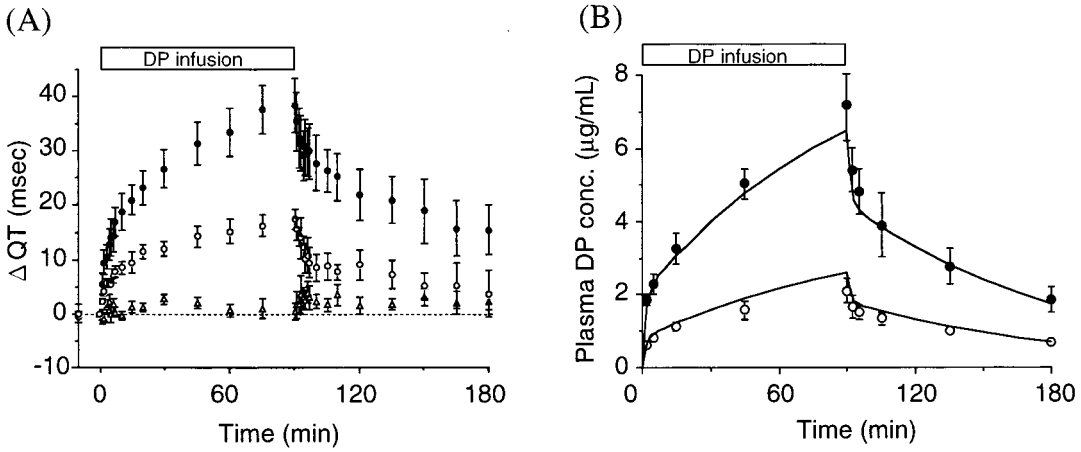

Figure 1—Time profiles of the change in QT interval (A) and the plasma concentration of DP (B) during and after i.v. infusion of DP. The solid lines in panel (B) are the fitting lines ●: DP 15 mg/kg/h, ○: DP 6 mg/kg/h, △: vehicle (mean ± SEM, $n = 3$–4).

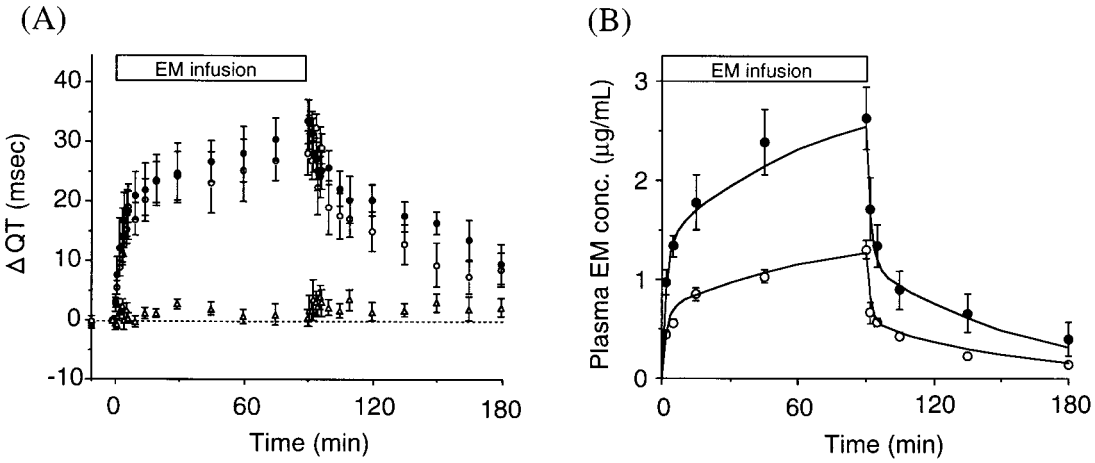

Figure 2—Time profiles of the change in QT interval (A) and the plasma concentration of EM (B) during and after i.v. infusion of EM. The solid lines in panel (B) are the fitting lines ●: EM 8 mg/kg/h, ○: EM 4 mg/kg/h, △: vehicle (mean ± SEM, $n = 3$–4).

interval and a slight prolongation of QRS interval (data not shown).

ECG Effects and Pharmacokinetics of EM—The time profiles of the change in QT interval (ΔQT) and the plasma EM concentration during and after the constant intravenous infusion of EM are shown in Figure 2. Although EM also evoked an obvious QT prolongation as observed in the case of DP, ceasing the infusion did not give an immediate shortening of the QT interval despite the prompt decrease in the plasma EM concentration. This observation indicates the presence of a delay in QT prolongation against plasma EM concentration. Within the EM concentration range under the present experimental conditions, saturation of the effect on QT interval was observed; changes in QT prolongation at 90 min after the start of the infusion were 28.20 ± 3.65 ms and 33.50 ± 3.75 ms at the dosing rates of 4 mg/kg/h and 8 mg/kg/h, respectively. Other ECG parameters, PR and QRS intervals, were not significantly altered (data not shown).

Pharmacokinetic–Pharmacodynamic Analyses of QT Prolongation—Figure 3 represents the relationship between plasma DP concentration and change in QT interval, and Figure 4 shows the relationship between EM concentration in plasma and change in QT interval. With regard to DP, as neither a delay nor saturation of QT prolongation was observed, the linear model (eq 1) was applied to the relationship between plasma DP concentration and E_{DP} (Figure 3). As judged from the anticlockwise hysteresis and saturation of the relationship in Figure 4, the E_{max} model with the effect compartment (eq 2) was

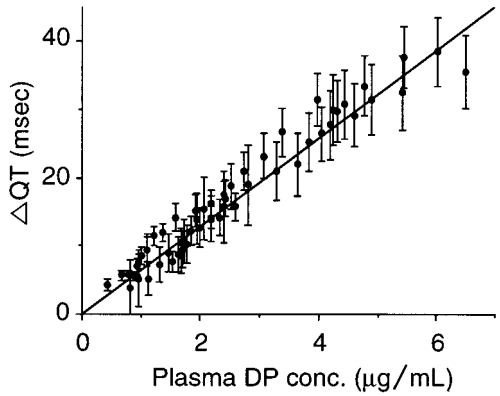

Figure 3—Relationship between the plasma concentration of DP and the change in QT interval. The solid line shows fitting curve calculated from eq 1 (mean ± SEM, $n = 4$).

successfully employed. The pharmacodynamic parameters for DP and EM are listed in Table 1.

Electrocardiographic Interaction on ECG Induced by DP and EM—Figure 5 represents the time profiles of the change in QT interval during and after the infusion of DP, EM, and their combination. The coadministration of DP and EM induced a larger QT prolongation than the administration of each drug alone. The simulation curves line calculated from eq 3 were in good agreement with the observed data. Although an electrocardiographic (ECG) interaction was seen between these drugs (right panel of

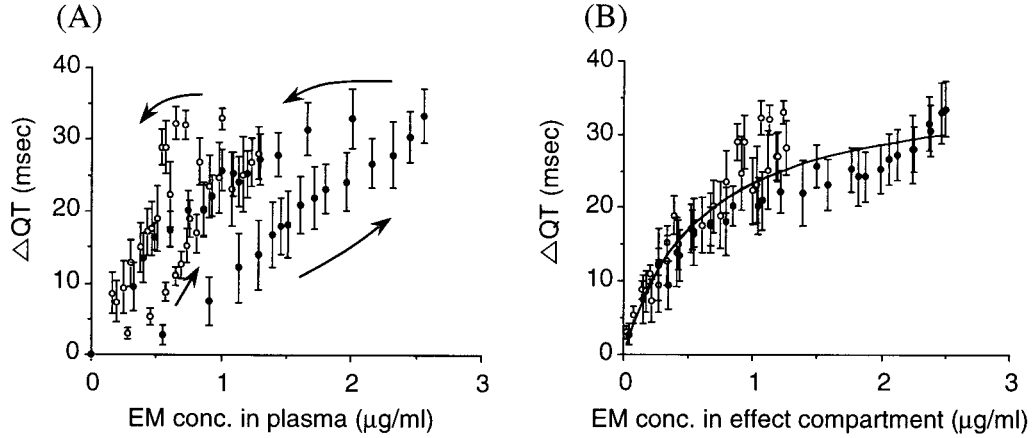

Figure 4—Relationship between the drug concentration of EM in plasma (A) or in effect compartment (B) and the change in QT interval. The solid line in panel (B) shows the fitting curve calculated from eq 2 (mean ± SEM, $n = 4$). ●: EM 8 mg/kg/h, ○: EM 4 mg/kg/h.

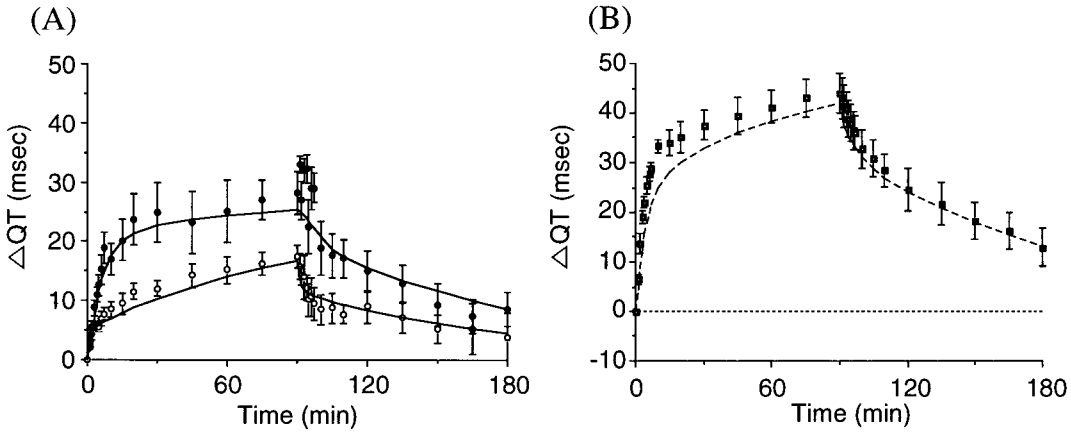

Figure 5—Changes in QT interval after the administration of DP or EM alone (A) and their concomitant administration (B). The solid lines in panel (A) are the fitting curves. The dotted line in panel (B) is the predicted curve calculated from eq 3 (mean ± SEM, $n = 4$). ●: DP 6 mg/kg/h, ○: EM 4 mg/kg/h, □: DP 6 mg/kg/h + EM 4 mg/kg/h.

Table 1—Pharmacodynamic Parameters for DP- or EM-Induced QT Prolongation (mean ± SD)

Parameter	DP	EM
K (ms·mL/μg)	6.45 ± 0.06	-
k_{e0} (min^{-1})	-	0.158 ± 0.023
E_{max} (ms)	-	37.10 ± 2.04
EC_{50} (mg/mL)	-	0.587 ± 0.091

Figure 5), EM did not affect the plasma concentration of DP and vice versa. This result led us to further investigate the pharmacokinetics of DP in the coadministration of EM.

Pharmacokinetic Interactions of DP and EM—Figure 6 represents the time profiles of plasma concentrations of DP and its metabolite, MND, after the portal vein administration of DP in the presence or absence of EM. The time profile of DP was described by a conventional two-compartment open model. Although the plasma concentration of MND reached maximum at 5 min after the administration of DP, the concentration of the metabolite was less than one tenth of that of the parent drug. After the administration of DP, 26.2 ± 2.1% of the dose was excreted as unchanged drug, while 4.1 ± 0.9% was excreted as MND in the urine within 24 h. Table 2 lists the pharmacokinetic parameters, which are in agreement with the published data.[15,16]

EM did not significantly change the pharmacokinetic parameters of DP, and both the plasma concentrations and the urinary excretion of the metabolite, MND, were not also significantly changed. Moreover, the plasma free fraction

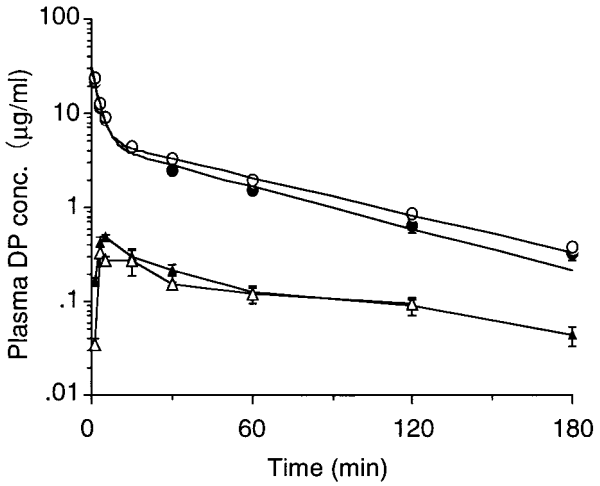

Figure 6—Time profiles of DP (circles) or MND (triangles) after portal vein administration of DP 20 mg/kg without (open symbols) or with (filled symbols) EM pretreatment (50 mg/kg i.v.) (mean ± SEM, $n = 4$). The solid lines of DP are the fitting curves calculated from two-exponential equation.

of DP (f_p), 0.800−0.875, was almost constant over the concentration range of 0.05−15.0 μg/mL and was not significantly changed in the presence of EM.

Discussion

In recent years, QT prolongation induced by noncardiovascular drugs has received much attention. Such drug-

Table 2—Pharmacokinetic Parameters of DP after Portal Vein Administration of DP (20 mg/kg) with or without EM Coadministration (50 mg/kg i.v.) to Rats

parameter	DP alone	DP + EM
V_{dss} (mL/kg)	2770 ± 100	2693 ± 155
AUC (μg/min/mL)	349 ± 28	413 ± 19
$t_{1/2}$ (β) (min)[a]	41.2 ± 3.3	45.5 ± 2.2
CL_{tot} (mL/min/kg)	57.9 ± 4.0	48.4 ± 2.31
CL_R (mL/min/kg)[b]	13.2 ± 1.7	12.2 ± 1.8

[a] $t_{1/2}$ (b): plasma eliminatiom half-life time for DP (mean ± SEM, $n = 5$). [b] CL_R: renal clearance of DP.

induced QT prolongation may be accentuated by coadministration of an antiarrhythmic agent, which itself prolongs QT interval. For instance, some cases have been reported that DP, coadministered with EM, caused TdP associated with QT prolongation,[1,2] a life-threatening adverse reaction. It is therefore important to investigate the interactions between antiarrhythmics and noncardiovascular agents which have the possibility of prolonging the QT interval.

Correction of QT Interval by Heart Rate—Since heart rate is known to affect the QT interval in humans, QTc, derived from "Bazett's formula", is widely applied in evaluation of QT prolongation. In this study, EM did not change the heart rate, whereas DP induced bradycardia, probably resulting from the blockade of sodium channels. However, Hayes et al. demonstrated that the QT interval is independent of heart rate in rats, in contrast to humans, rabbits, and guinea pigs.[17] In addition, it has been reported that the actual QT interval, without any correction for heart rate, may be preferable the QTc in heralding TdP.[18] Therefore, in this study, we employed the actual QT interval for evaluation of arrhythmogenic potency.

QT Prolongation Induced by DP or EM—In the present study, the administration of DP or EM alone induced a significant QT prolongation. DP is known to block sodium channels and potassium channels of cardiac myocytes and delay repolarization, which leads to prolongation of action potential duration (APD) and QT interval.[19] In our study in rats, DP induced QT prolongation within its therapeutic range (2.0−5.0 μg/mL). This range coincides with the concentrations where APD prolongation was reported on cardiac muscle of guinea pigs or rats by whole-cell voltage clamp technique.[20−23] Although MND, a major metabolite of DP, is documented to possess one-fourth the anticholinergic potency of DP,[24,25] it is virtually inactive in QT prolongation.[23,26] Therefore, MND was excluded from the further kinetic analysis.

EM is also known to inhibit potassium channels in ventricular myocytes.[27−29] This blockade may lead to delayed repolarization and prolongation of QT interval. However, the detailed mechanism has not been thoroughly elucidated.

An anticlockwise hysteresis was observed between plasma EM concentration and effect on QT prolongation, which indicates a delay of QT prolongation. The E_{max} model with "effect compartment" may adequately explain the relationship (Figure 3B). This hysteresis could be due to several factors, including delayed distribution of EM to the effect site, which is assumed to be potassium channels of ventricular myocytes. The binding site for EM in the potassium channel has not been identified yet. However, there is a possibility that the binding site is located in the intracellular or transmembrane domain of the channel and that it may take several minutes for the drug to reach the effect site, as speculated from the delay of QT prolongation. A possible explanation for this anticlockwise hysteresisis is the generation of an active metabolite of EM. While EM is reported to be active in QT prolongation, it is not reported whether the metabolite of EM is active or not. No major metabolites of EM were detected by ECD, so that we could not investigate metabolite pharmacokinetics. However, when we examined the time course of EM concentrations in the ventricle, it showed a delayed distribution of EM into the ventricle at the distribution rate similar with k_{eo} (data not shown).

In this study, EM induced a significant QT prolongation within the therapeutic range of plasma EM concentrations (0.5−3.0 μg/mL). Using the in vitro patch clamp technique, it has been reported that a higher concentration (up to 100 μM; 73.4 μg/mL) is necessary for EM to prolong the action potential duration (APD) or reduce potassium current (delayed rectifier potassium current).[27−29] Rubart et al.[27] discussed that the concentrations of EM required to induce early after-depolarizations (EADs) in vitro are higher than those in vivo. Antzelevitch et al.[29] demonstrated that APD prolongation was evoked at a relatively low concentration of EM (10 μM) in canine M cells, but the concentration was still above the therapeutic range. In the present study, even if the plasma protein binding of EM (16.9% for rats) was taken into consideration, the concentration which evoked QT prolongation in vivo was still lower than that reported in vitro,[27−29] indicating that the potency of EM for APD prolongation or potassium current blockade in ventricular myocytes in vitro may be weaker than that for QT prolongation in vivo. Further investigation is required to clarify the mechanism and sources of this discrepancy. A possible explanation is that the EM concentration near the potassium channels is different between in vivo and in vitro conditions, due to different cellular uptake and/or intracellular distribution under these conditions.

As no saturation was observed for DP-induced QT prolongation, the relationship between plasma DP concentration and change in QT interval was analyzed by a linear pharmacodynamic model (eq 1). Although we also employed the E_{max} model, the linear model showed a better goodness of fit than the E_{max} model. Doses of DP beyond the therapeutic range were not experimentally feasible due to adverse effects on rats. It is of interest that different pharmacodynamic models were applied to DP and EM: the linear model for DP and the E_{max} model for EM. Similar cases have been reported for other agents which induce QT prolongation, i.e., the relationship between drug concentration and change in QT interval was explained by the linear model for quinidine and an E_{max} model for terfenadine.[10] Whiting et al.[30] have also reported that the change in QT interval is linearly correlated with plasma DP concentration. Both DP and quinidine were reported to block several types of potassium channels on ventricular myocytes,[22,31] leading to a complex, stepwise inhibition, which may result in a linear relation between drug concentration and change in QT interval.

Pharmacodynamic Interaction between DP and EM—Enhanced QT prolongation was observed after coadministration of EM and DP compared to that after each administration of these drugs (Figure 5). However, the plasma concentrations of each agent were not changed by each other. This indicates that the interaction on ECG between these drugs can be attributed to their pharmacodynamic interaction, not a pharmacokinetic one, at least in the present experimental setting. Thus, a clinically important pharmacodynamic interaction between EM and DP can be observed without affecting pharmacokinetics.

The observed data after coadministration of EM and DP were simulated by simple addition of QT prolongations after separate EM and DP administrations. The simulated line is below the observed data during the administration. Then, at each point, we compared the ΔQT (ΔQT_{DP+EM}) in concomitant administration with the additional ΔQT (ΔQT_{DP}

$+ \Delta QT_{EM}$) using the Student *t*-test. As a result, significant differences were observed only at 4 and 5 min. Therefore, we cannot completely define this interaction as additive. However, we are not sure that the interaction between DP and EM is antagonistic. The clinical significance of the present study is that enhanced QT prolongation was shown in concomitant administration compared to individual administrations.

Effects of EM on Pharmacokinetics of DP—The pharmacokinetic parameters of DP obtained here were is close agreement with those noted in previous studies.[15,16] Since the total plasma clearance of DP was large and thought to be blood flow-limited, we evaluated the effects of EM on the pharmacokinetics of DP following portal vein administration of DP.

In the portal vein dosing of DP, EM pretreatment did not significantly change the pharmacokinetic parameters of DP (Table 2). In addition, no effect of EM on the plasma protein binding of DP was observed. In addition, the blood-to-plasma concentration ratio of DP (R_B) was also evaluated and it was not significantly changed by EM. While the plasma binding of DP is known to saturate within the therapeutic range in humans,[32] it was almost constant in rats under the current experimental range (0.05−15.0 μg/mL), suggesting interspecies difference in the plasma protein binding of DP, in accordance with a previous reports.[16]

Although it is expected that EM might cause the elevation of DP plasma concentrations in humans by metabolic inhibition, no pharmacokinetic interaction was seen in rats. The most likely explanation for these results may be interspecies differences in the metabolism of DP. In fact, the urinary excretion of MND, a major metabolite yielded by CYP3A, proved to be lower (up to 4% of the dose) in rats than in humans. It has also been reported[16,33] that in rats about 20% of the dose was excreted into urine as phenol and methoxyphenol metabolites, while these metabolites are not detected in humans. Therefore, the contribution of the metabolic pathway of DP, which EM could inhibit, to the total clearance may be lower in humans than in rats. The EM concentration in liver was enough compared to its inhibition constant (IC_{50} = 70 μg/mL) in vitro.[5] Another potential explanation for the lack of pharmacokinetic interaction between EM and DP is a setereoselective metabolism. DP is a racemic mixture of *R*(−) and *S*(+) enantiomers, and those clearances are reported to be different.[34] If EM inhibits one of the enantiomers, increase in AUC of DP as a racemic mixture may not reach significance. Echizen et al.[35] showed that the enantiomers share an enzyme in humans and mice, and that EM inhibited their metabolism equally in mice but not equally in human microsomes. However, no data are available for rats in vivo or in vitro. Since DP is used as a racemic mixture in clinical settings, the clinical significance of our study may not be diminished.

Other explanation is the administration route-dependency. EM was intravenously administered in this study. However, the repeated oral administration of EM might be required to induce the metabolic inhibition of CYP3A.[36] In addition, when orally administered, the intestinal metabolisms by CYP3A may be inhibited, which may lead to further increase in the DP concentrations if DP is metabolized by intestinal CYP3A.

Prediction of QT Prolongation in Humans—It has been reported[26,30,37−40] that DP prolongs the QT interval within the therapeutic range of plasma concentration (2.0−5.0 μg/mL) in humans. The findings obtained in the present study were consistent with the above reports. On the other hand, the plasma concentrations of EM have not been reported where QT prolongation or TdP occurred, possibly because of difficulty in the determination of EM concentration in plasma. Therefore, the quantitative relationship between concentration of EM and its effect on QT interval in clinical settings remains unknown. An EM-induced QT prolongation is reported[5] to occur not only after i.v. administration of a high dose (up to 1 g/body) in many cases, but also after oral administration of EM.[41] In the case of oral administration, plasma EM concentration is considered to be lower than in the case of i.v. injection, suggesting a possibility that EM may also induce QT prolongation within or near therapeutic plasma concentrations (0.5−3.0 μg/mL), which correlates well with the range examined in our present study. In addition, orally administered EM is also known to induce TdP associated with QT prolongation after concomitant treatment with quinidine.[9] Thus, our method presented here may be useful for predicting drug-induced QT prolongation in clinical settings, at least in a qualitative manner.

In conclusion, the present study suggests that pharmacodynamic interaction may increase the risk of QT prolongation after coadministration of DP and EM in humans. In concomitant treatment of DP and EM, therefore, it is our recommendation for patients' safety that ECG should be monitored periodically as necessary.

References and Notes

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