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Stereoselective pharmacokinetics and pharmacodynamics of disopyramide and its metabolite in rabbits

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Abstract

The extent to which interactions between enantiomers of disopyramide and between disopyramide and its metabolite, mono-N-dealkylated disopyramide (MND), contribute to stereoselectivity of the anti-arrhythmic effect has been investigated in rabbits by measuring the prolongation of the QUc interval. The plasma unbound fraction of disopyramide enantiomers was constant at a concentration range of 1.44–28.9 μM. An intravenous infusion study of the disopyramide enantiomer or racemate suggested that the *S*-enantiomer had a pharmacological effect, determined by linear regression analysis, approximately 3.3-times more potent than that of the *R*-enantiomer. Furthermore, the effect caused by racemic disopyramide was the sum of that elicited by both enantiomers individually. No significant difference was observed between the slope of linear regression analysis of intravenous infusion and that of intravenous bolus injection. Single intravenous bolus injection of MND did not affect the QUc intervals. In conclusion, the *S*-enantiomer of disopyramide was approximately 3.3-times more potent pharmacologically than the *R*-enantiomer. The relationship between plasma concentration of the disopyramide enantiomers and pharmacological effect was the sum of each enantiomer individually.

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Introduction

Disopyramide is a type Ia anti-arrhythmic agent used clinically for the treatment and prophylaxis of supraventricular and ventricular arrhythmias. Disopyramide has a relatively narrow therapeutic plasma concentration range (2–5 μg mL^{-1}) and shows concentration-dependent binding to plasma proteins in man (Hasselstrom et al 1991; Takahashi et al 1991b, 1993a).

Although disopyramide is available commercially as a racemic mixture, the pharmacokinetic properties of each enantiomer have been reported to be different in man and animals (Cook et al 1982; Lima et al 1985; Giacomini et al 1986; Takahashi et al 1993a; Masuhara et al 1995). Plasma protein binding of (*S*)-disopyramide is higher than that of (*R*)-disopyramide in man, and the total clearance of unbound (*S*)-disopyramide is also higher than that of (*R*)-disopyramide (Lima et al 1985; Takahashi et al 1991a). An interaction between enantiomers has been reported which affects the pharmacokinetics of disopyramide, including plasma protein binding and total clearance. Clearance of disopyramide shows stereoselectivity after administration of the racemate to man, whereas little difference in clearance of enantiomers is observed after enantiomer administration

(Giacomini et al 1986). Takahashi et al (1991a) demonstrated an interaction between plasma protein binding of disopyramide enantiomers and its major metabolite, mono-N-dealkylated disopyramide (MND), in man. A recent study demonstrated the enantioselective binding of disopyramide and other weak basic drugs to human α_1-acid glycoprotein, the main plasma binding protein for disopyramide (Hanada et al 2000).

A number of studies have reported a stereoselective pharmacological effect of disopyramide (Millo et al 1981; Pollick et al 1982; Kidwell et al 1987). However, these studies only compared the pharmacological effect of disopyramide enantiomers after administration of each enantiomer. Although the pharmacokinetic interaction between disopyramide enantiomers has been studied, the pharmacodynamic interaction between disopyramide enantiomers and between disopyramide and its metabolite is not well understood.

The purpose of this study was to clarify the pharmacodynamic interaction between disopyramide enantiomers and between disopyramide and MND. The relationship between stereochemistry and pharmacological effect was determined by measuring QUc intervals (QU interval corrected for heart rate, $QU/\sqrt{RR}$) as an index of the anti-arrhythmic effect in rabbits.

Materials and Methods

Chemicals and reagents

Racemic disopyramide, ^{3}H-labelled disopyramide (sp. act. 644 GBq mmol^{-1}) and clonidine hydrochloride were gifts from Nippon Roussel K. K. (Tokyo, Japan). Racemic disopyramide hydrochloride was obtained from Sigma Chemical Company (St Louis, MO). The free base of racemic disopyramide was obtained from Nippon Balk Co. (Osaka, Japan). The (+)- and (−)-enantiomers of disopyramide were separated by high-performance liquid chromatography (HPLC), and the chemical and stereochemical purities (both $> 98.0\%$) of each enantiomer were determined by optical rotation analysis, stereospecific HPLC resolution and elemental analysis. MND was synthesized as described by Karim et al (1972). All other chemicals used were of analytical grade.

Animals

The animals used in this study were handled in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, 1996). The animal ethics committee at Meiji Pharmaceutical University approved the study.

Japanese male White rabbits (2.32–3.45 kg; Tokyo Laboratory Animal Science Co., Ltd) were kept in individual cages under stable conditions of humidity ($60 \pm 5\%$) and temperature (22 ± 2°C). The rabbits were allowed free access to food and water for the duration of the experiment.

Binding of disopyramide enantiomers to plasma proteins

Binding of disopyramide enantiomers to plasma proteins was determined as described by Takahashi et al (1990) and Hanada et al (1998). Briefly, a tracer amount of ^{3}H-labelled disopyramide enantiomers or the corresponding unlabelled enantiomers were added to 0.5 mL freshly isolated plasma to yield an enantiomer plasma concentration ranging from 0.49–9.8 μM. After incubation at 37°C for 5 min, the plasma was filtered through a membrane filter (Ultrafree C3-LGC, Nihon Millipore, Tokyo, Japan) at 1700 *g* for 10 min at 37°C. The radioactivity of the filtrate and plasma was counted with a liquid scintillation counter (LSC-700; Aloka, Japan), and quenching was corrected by an external standardization procedure. No significant adsorption of disopyramide onto the membrane or ultrafiltration device was observed ($< 0.6\%$ for 0.33 nM (*S*)-disopyramide and 3.9% for 0.31 nM (*R*)-disopyramide) when 100-μL filtrate was ultrafiltered from 500 μL freshly drawn rabbit plasma.

Electrocardiogram

During the pharmacokinetic–pharmacodynamic studies, the rabbits were placed in the supine position. The electrocardiogram (ECG) was monitored with plate electrodes using I and II leads. The signals obtained were amplified with an ECG polygraph (Nippon Denki Sanei, Tokyo, Japan) at a chart speed of 100 mm s^{-1}. Each registration consisted of 30 complete heart cycles, and the mean value was calculated. The QT interval and/or QTc value, where the QT interval was corrected for heart rate using the Bazett equation, were usually used, but it was difficult to separate the QT segment and consecutive U wave from each other in rabbits. In this study, the QU interval was determined as a measure of the anti-arrhythmic effect on the ECG.

Experimental protocol

After 10-min stabilization, the control QU complex and RR intervals were recorded every 10 min for 30 min

before administration of the drugs, and at blood sampling times after administration of the drugs.

Intravenous constant infusion

Racemic disopyramide, (*S*)- or (*R*)-disopyramide was infused into the marginal ear vein using an infusion pump. The infusion rates were 2.5, 5.0 or 9.0 mg kg^{-1} h^{-1} for each enantiomer, and 3.75, 7.5 or 11.25 mg kg^{-1} h^{-1} for racemic disopyramide. For loading, the drugs were administered at twice the infusion rate described above for 15 min. As a control, saline was infused at a rate of 3.0 mL h^{-1}.

Intravenous bolus injection

(*S*)-disopyramide (4 mg kg^{-1}), (*R*)-disopyramide (6 mg kg^{-1}), racemic disopyramide (6 mg kg^{-1}), racemic MND (6 mg kg^{-1}), or a mixture of racemic disopyramide and MND (both 6 mg kg^{-1}) was injected as a bolus into the marginal ear vein.

Blood samples were taken from the opposite marginal ear vein before, and 5, 30, 60, 90 and 120 min after the start of intravenous infusion of disopyramide, and 3, 5, 7, 10, 15, 20 and 30 min after intravenous bolus injection of disopyramide. The blood was centrifuged at 2000 *g* for 15 min and the plasma was stored at −20°C until assay. The ECG was recorded simultaneously at the time of blood sampling.

Analysis of disopyramide and MND enantiomers in plasma

The concentrations of disopyramide and MND enantiomers in plasma were determined according to the method of Takahashi et al (1990). The HPLC system consisted of a Shimadzu HPLC apparatus (Kyoto, Japan), a LC-6A pump, and a CR-3A Chromatopac integrator. Samples were introduced into a 50-μL loop (Rheodyne, Cotati, CA). Disopyramide was detected with a SPD-6A spectrometric detector at a wavelength of 260 nm with a range of 0.02 aufs. Separation of disopyramide and MND enantiomers was performed with a Chiralcel OF column (50 × 4.6 mm i.d., Lot No. 50118; Daicel Chemical Industries, Tokyo, Japan). The mobile phase consisted of n-hexane, 2-propanol and diethylamine (82:18:0.1, v/v) at a flow rate of 0.6 mL min^{-1}.

Plasma (150 μL) was mixed with 100 μL internal standard (50 μg racemic clonidine) and 100 μL 2 M NaOH, and the mixture was shaken vigorously with 5 mL benzene for 10 min. After centrifugation (2000 *g* for 10 min), the organic phase was transferred to another tube and evaporated to dryness under a stream of dry nitrogen at 40°C. The residue was dissolved in the mobile phase (100 μL), and a sample injected onto the HPLC. Recoveries of (*S*)-disopyramide (0.72, 2.16 and 3.60 μg mL^{-1}), (*R*)-disopyramide (0.72, 2.16 and 3.60 μg mL^{-1}), (*S*)-MND (1.40, 4.19 and 6.98 μg mL^{-1}) and (*R*)-MND (1.40, 4.19 and 6.98 μg mL^{-1}) were 99.6 ± 4.08% (n = 5), 101 ± 4.64% (n = 5), 82.8 ± 4.66% (n = 5) and 81.9 ± 4.62% (n = 5), respectively. Respective coefficients of between-day variations were below 6.13%, 5.13%, 9.55% and 6.94%. The detection limits of disopyramide and MND enantiomers, determined as the signal-to-noise ratio of 3, were 80 and 200 ng mL^{-1}, respectively.

Data analysis

Pharmacokinetic analysis

The plasma concentration (Cp)–time (t) profiles of disopyramide and MND enantiomers after intravenous bolus injection were fitted to a two-compartment model ($Cp = Ae^{-\alpha t} + Be^{-\beta t}$) using the non-linear least squares fitting program MULTI, in which plasma concentration data were weighed by concentration^{-1} (Yamaoka et al 1981). Using the pharmacokinetic parameters obtained, total clearance (CL_{tot}) and volume of distribution at steady state (Vd_{ss}) were estimated using the following equations:

$$AUC = (A/\alpha) + (B/\beta) \quad (1)$$

$$AUMC = (A/\alpha^2) + (B/\beta^2) \quad (2)$$

$$CL_{tot} = Dose/AUC \quad (3)$$

$$Vd_{ss} = (Dose \cdot AUMC)/AUC^2 \quad (4)$$

Statistical analysis

The observed and estimated values were represented as the mean ± s.d. Linear regression analysis was used to assess the relation between drug plasma concentration and effect over time. The Mann–Whitney rank sum test was used to compare slopes and intercepts of the concentration–response curve for each parameter in the groups receiving the various treatments. The difference between the means of two groups was compared using the Student's paired *t*-test or unpaired *t*-test. $P < 0.05$ was considered to indicate a significant difference.

Results

Binding of disopyramide enantiomers to plasma proteins

The unbound fraction of each enantiomer was almost constant irrespective of concentration ((*S*)-disopyramide 84.2 ± 4.9 %, (*R*)-disopyramide 74.5 ± 5.3 %, n = 6), and no significant difference was observed between the enantiomers over the concentration range studied (data not shown). These values were larger than those observed in human plasma (Takahashi et al 1990). Therefore, total disopyramide concentration in plasma was used throughout the experiments.

Relationship between plasma concentration and pharmacological effect of each enantiomer

Figure 1 shows the time-course of plasma concentration and QUc interval during intravenous infusion of each enantiomer into rabbits. Plasma concentration and pharmacological effect reached a steady state within 15 min of starting the infusion. MND, a major metabolite with activity in man, was not detected in rabbit plasma. In the controls (administered saline), no significant difference was observed in the QUc intervals during the experiments. However, the QUc intervals were significantly prolonged after disopyramide administration, and the effect of (*S*)-disopyramide was higher than that of (*R*)-disopyramide.

A relationship between plasma concentration and pharmacological effect of disopyramide was observed under steady state conditions after intravenous infusion of disopyramide enantiomer (Figure 2). The effect observed for (*S*)-disopyramide was 3.3-times more potent than that seen with (*R*)-disopyramide. Assuming that each enantiomer produced a pharmacological effect independently, the pharmacological effect was estimated at each sampling time under steady state conditions after administration of disopyramide racemate. The slope observed after administration of disopyramide racemate appeared to be the mean value of both enantiomers. Furthermore, the predicted value for the pharmacological effect of racemic disopyramide from the data obtained for each enantiomer was similar to the observed value after racemic disopyramide administration (slope = 1.16, r = 0.751, $P < 0.01$; Figure 3). These results suggested that there was no interaction between the disopyramide enantiomers after administration of disopyramide racemate under steady state conditions.

Figure 4 shows the effect of MND on the QUc intervals after racemic MND administration. The time course was similar for each MND enantiomer. MND did not affect the QUc interval. To study any possible interaction between disopyramide enantiomers and MND enantiomers, a mixture of racemic disopyramide and MND was administered at a dose of 6 mg kg^{-1} each to rabbits. However, no significant difference between racemic disopyramide alone and coadministration with MND was observed (data not shown).

To determine the relationship between the dosing rate of disopyramide and pharmacological effect, as observed for propranolol (Takahashi et al 1993b), disopyramide was administered by intravenous bolus injection (Figure 5). Tables 1 and 2 show the pharmaco-

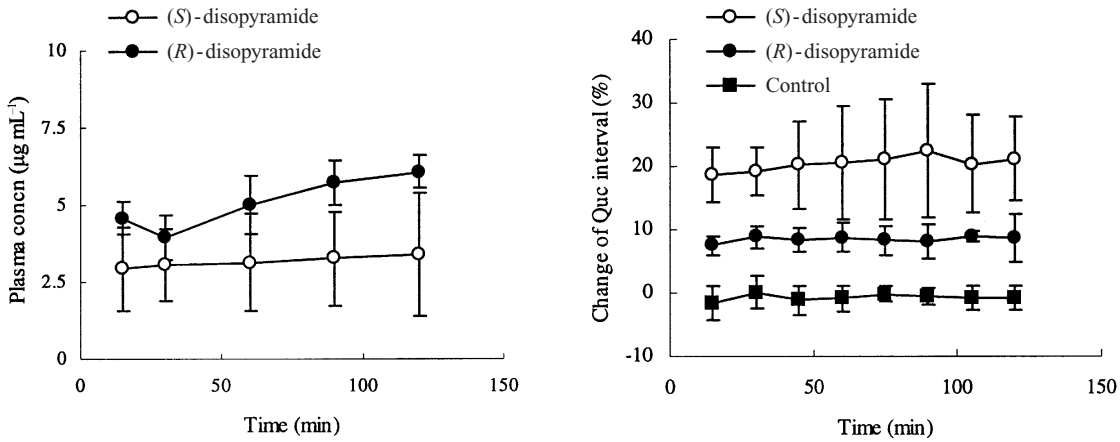

Figure 1 Time-courses of plasma disopyramide enantiomer concentration and pharmacological effect (% change in QUc interval) after intravenous infusion of (*S*)-disopyramide, (*R*)-disopyramide or saline to rabbits. (*S*)-disopyramide: 9.0 mg kg^{-1} h^{-1} (n = 4); (*R*)-disopyramide: 9.0 mg kg^{-1} h^{-1} (n = 4); saline: 3.0 mL h^{-1} (n = 8).

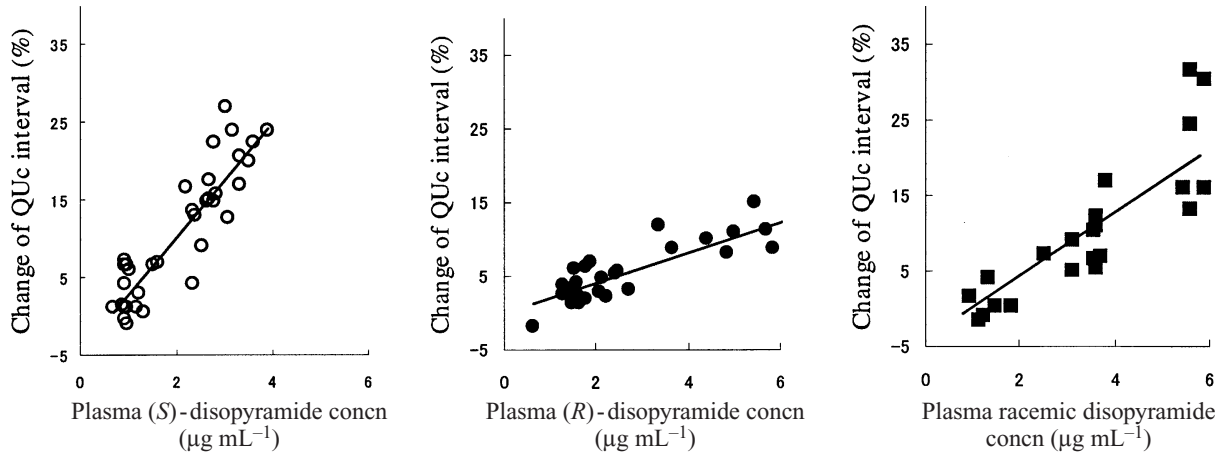

Figure 2 Relationship between disopyramide concentration at steady state and pharmacological effect (% change in QUc interval) after intravenous infusion of (*S*)-disopyramide, (*R*)-disopyramide or racemic disopyramide to rabbits. Solid line represents linear regression line. (*S*)-disopyramide: y = 8.17x − 5.16; (*R*)-disopyramide: y = 2.63x − 1.33; racemic disopyramide: y = 5.72x − 4.32.

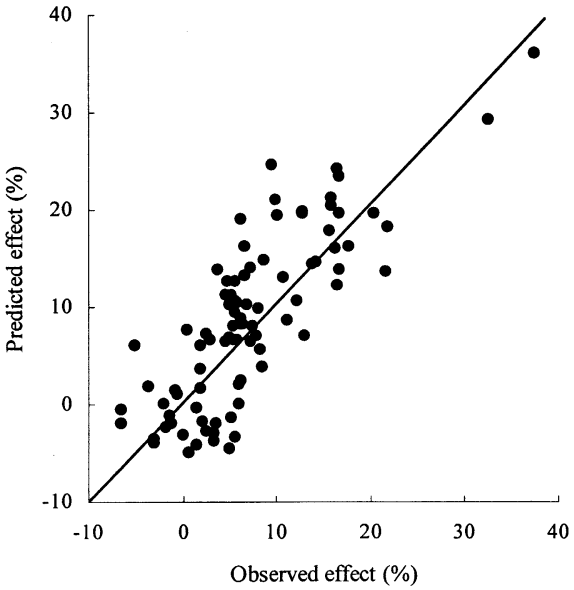

Figure 3 Relationship between predicted values and observed values of pharmacological effect (% change of QUc intervals) after intravenous infusion of racemic disopyramide to rabbits. Solid line represents linear regression line (y = 1.16x − 0.319) and dotted line represents y = x. The effect after racemic disopyramide administration was predicted as a sum of each enantiomers using a relationship between the effect and plasma concentration of each enantiomer at steady state.

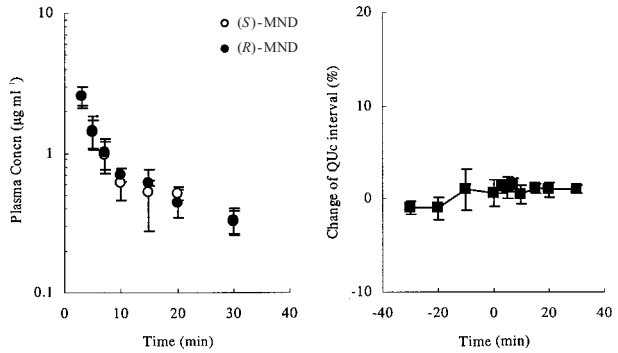

Figure 4 Time-courses of plasma mono-N-dealkylated disopyramide (MND) enantiomer concentration and pharmacological effect (% change in QUc interval) after intravenous bolus administration of racemic MND (6 mg kg^{-1}) to rabbits (effect of MND on the QUc intervals after racemic MND administration). Each point represents the mean ± s.d. (n = 4).

kinetic and pharmacodynamic parameters obtained after disopyramide enantiomer and racemate administration, respectively. There was no significant difference in total clearance of enantiomer between enantiomer administration and racemate administration, whereas the volume of distribution at steady state of disopyramide enantiomer after racemic administration was decreased significantly compared with that obtained after administration of each enantiomer. The pharmacodynamic parameters obtained after racemic disopyramide administration, M (slope) and I (intercept), were no different from those obtained after administration of the enantiomers. Furthermore, the pharmacodynamic parameters were not affected by the rate or method of administration (bolus injection or constant infusion).

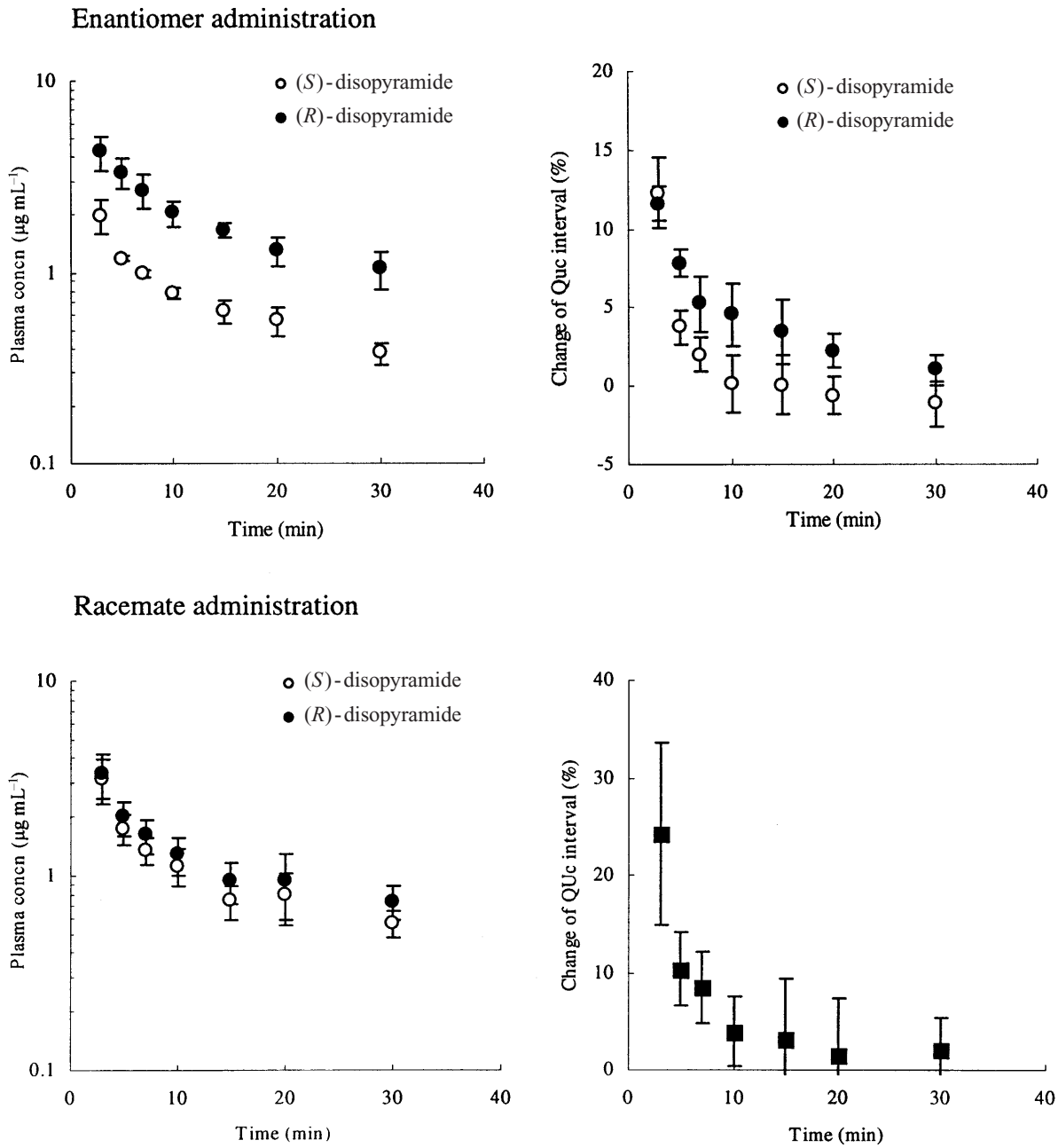

Figure 5 Time-courses of plasma disopyramide enantiomer concentration and pharmacological effect (% change in QUc interval) after intravenous bolus administration of disopyramide enantiomer ((*S*)-disopyramide 4 mg kg^{-1}; (*R*)-disopyramide 6 mg kg^{-1}) or racemic disopyramide (6 mg kg^{-1}) to rabbits. Each point represents the mean ± s.d. (n = 4).

Discussion

Although the unbound fraction of (*S*)-disopyramide was slightly greater than that of (*R*)-disopyramide when each enantiomer was added into plasma at a concentration range of 0.49–9.8 μM, there was no significant difference between the enantiomers. Linear binding of each disopyramide enantiomer in rabbit plasma has been reported by Huang & Oie (1983b). Disopyramide binds specifically to plasma proteins, especially α_1-acid glycoprotein, in man. However, there was no significant difference in the unbound fraction of each disopyramide

Table 1 Pharmacokinetic parameters of disopyramide enantiomer after intravenous bolus administration of disopyramide enantiomers and pharmacodynamic parameters of disopyramide enantiomers after intravenous bolus administration and constant infusion of disopyramide enantiomer to rabbits.

Pharmacokinetic parameter	(*S*)-disopyramide	(*R*)-disopyramide
CL_{tot} (mL min^{-1} kg^{-1})	81.9 ± 11.9	46.4 ± 26.8
Vd_{ss} (L kg^{-1})	2.30 ± 1.58	2.53 ± 0.75
M (effect %, mL ng^{-1})		
Intravenous bolus injection	8.52 ± 2.02*	3.32 ± 0.625
Intravenous constant infusion	8.17	2.63
I (effect %)		
Intravenous bolus injection	−5.80 ± 1.93	−2.66 ± 1.58
Intravenous constant infusion	−5.16	−1.33

Data represent the mean ± s.d. (n = 4). *$P < 0.05$ compared with (*R*)-disopyramide.

Table 2 Pharmacokinetic parameters of disopyramide enantiomer after intravenous bolus racemic disopyramide administration to rabbits.

Pharmacokinetic parameters	(*S*)-disopyramide	(*R*)-disopyramide
CL_{tot} (mL min^{-1} kg^{-1})	44.3 ± 6.81	37.5 ± 8.62
Vd_{ss} (L kg^{-1})	1.14 ± 0.71	1.27 ± 0.70

Data represent the mean ± s.d. (n = 4).

enantiomer and the binding of disopyramide to rabbit plasma was relatively low and linear, suggesting that disopyramide may bind non-specifically to rabbit plasma proteins.

The volume of distribution at steady state after administration of racemic disopyramide was decreased significantly compared with the distribution volume after enantiomer administration. This suggested that an interaction in tissue binding occurred between the enantiomers. This result was supported by the fact that binding of disopyramide enantiomers to phosphatidylserine, one of the substances in tissue binding to weak basic drugs, also decreased in the presence of another enantiomer (Hanada et al 1998). The partition coefficient between buffer (pH 4.0)–hexane containing phosphatidylserine of disopyramide enantiomer ((*S*)-disopyramide 0.28; (*R*)-disopyramide 0.38) was significantly decreased using racemic disopyramide ((*S*)-disopyramide 0.08; (*R*)-disopyramide 0.29). In this study, however, no stereoselectivity of volume of distribution was observed. Further study for quantitative analysis of phosphatidylserine binding of disopyramide enantiomer would be needed.

On the basis of electrophysiological studies in-vivo and in-vitro, a number of reports have shown a relationship between disopyramide concentration and electrophysiological effect of each enantiomer. Millo et al (1981) reported that the in-vitro action potential duration (APD) in canine Purkinje fibres was prolonged significantly after treatment with (*S*)-disopyramide, whereas it was shortened after treatment with (*R*)-disopyramide. In addition, other investigators have demonstrated that enantiomers of disopyramide prolonged the APD. Vanhoutte et al (1991) reported that the effect on APD was concentration-dependent, enantiomers of disopyramide prolonged APD, while enantiomers of MND were less effective using guinea-pig papillary muscles in-vitro. Furthermore, Kidwell et al (1989) suggested that (*S*)-disopyramide was more potent than (*R*)-disopyramide against QTc intervals, APD and V_{max} in a canine superfusion model in-vivo.

In this study, the relationship between plasma concentration of each enantiomer and pharmacological effect demonstrated that (*S*)-disopyramide was approximately three-times more potent than (*R*)-disopyramide using ECG analysis of the prolongation of QUc values in rabbits. Our finding was supported by the observations of several investigators who stressed that the pharmacological effect of (*S*)-disopyramide was higher than that of (*R*)-disopyramide in man and other animal species. However, it is impossible to predict the pharmacological effect in man after administration of racemic disopyramide from these findings because of problems such as non-linear plasma protein binding in man and interactions between the two enantiomers of disopyramide and MND. In rabbits, non-linear plasma protein binding of disopyramide and MND did not occur, and interactions between these compounds were not observed. Therefore, it was considered that the rabbit was a suitable animal species to estimate the extent to which racemic disopyramide contributed to the anti-arrhythmic effect. Our findings demonstrated that prolongation of the QUc interval caused by administration of racemic disopyramide was the sum of the prolongation induced by each disopyramide enantiomer individually. Moreover, MND racemate did not prolong the QUc interval, and did not affect the prolonged QUc intervals induced by disopyramide racemate admin-

istered intravenously. The results obtained after intravenous administration of MND seemed to be supported by previous data (Chiang et al 1985).

Conclusion

(*S*)-disopyramide was shown to be approximately three-times more potent pharmacologically than (*R*)-disopyramide in rabbits. The relationship between plasma concentration and pharmacological effect after intravenous administration of racemic disopyramide was the sum of the effect induced by each enantiomer administered individually. Moreover, MND, which is the main metabolite of disopyramide, did not prolong the QUc interval and did not affect the prolongation of the QUc interval induced by disopyramide.

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