Mechanisms of Pharmacokinetic Interaction between Propranolol and Quinidine in Rats

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In order to study the mechanism of propranolol–quinidine interaction, the effects of quinidine on propranolol pharmacokinetics were examined in male Wistar rats. The concurrent oral administration of quinidine (10 mg/kg) markedly increased the plasma concentration of propranolol (2.5 mg/kg), and the area under the propranolol concentration–time curve increased about 3.6-fold. These results are consistent with previous observations in man and indicate the possible usefulness of the male Wistar rat as an animal model for investigating the mechanisms of the drug interaction. When propranolol was given intravenously, a concurrent administration of quinidine increased the apparent distribution volume of propranolol, mainly by decreasing its plasma protein binding. However, the systemic clearance of propranolol was not significantly altered by quinidine. Thus, quinidine increased the availability of oral propranolol from 13.8±2.2 to 44.2±4.6% ($p<0.01$). Furthermore, quinidine delayed the elimination of propranolol from the isolated perfused rat liver.

These results indicate that quinidine reduces the presystemic elimination of propranolol in the liver, thereby increasing its systemic availability after oral administration.

Keywords propranolol; quinidine; drug interaction; pharmacokinetics; presystemic elimination; availability; combination therapy; rat

Combinations of antiarrhythmic agents are used when single agents would be ineffective or would have intolerable side-effects at the necessary doses. Propranolol and quinidine are given together to treat malignant arrhythmias, because in combination, their antiarrhythmic effect is greater.[2-6)] Results of *in vivo*[7-10)] and *in vitro*[11,12)] animal experiments support the combined use of propranolol and quinidine. Recently, it was found that when these drugs are given together in man not only does propranolol potentiate the action of quinidine, but quinidine potentiates the action of propranolol by increasing the plasma concentration of propranolol.[13,14)] However, the mechanism by which quinidine alters propranolol pharmacokinetics is still unclear.

In the present study, the effects of quinidine on propranolol pharmacokinetics were investigated in male Wistar rats.

Materials and Methods

Material DL-Propranolol hydrochloride and quinidine (free base) were obtained from Nacarai Tesque, Inc. (Kyoto, Japan). All other chemicals were of the highest grade available.

Pharmacokinetic Studies Male Wistar rats, weighing 200—270 g, were deprived of food but given free access to water for about 18 h prior to the experiments. Under light anesthesia with ether, the left carotid artery was cannulated with PE 10 polyethylene tubing and the exterior end of the catheter was passed under the skin to emerge at the nape of the neck for blood collection. The incisions were closed with sutures. Then heparin (1000 units/kg) was given intravenously. After recovery from ether anesthesia, drugs were given to the conscious rats. In the oral administration study, the rats received either a single dose of propranolol (2.5 mg/kg, as a free base) alone or in combination with quinidine (10 mg/kg, as a free base) *via* a gastric tube. In a separate experiment, propranolol alone, or in combination with quinidine, was injected into a femoral vein at the same dose used for the oral studies. Each rat was kept in an individual cage without restraint. Blood samples were withdrawn through the carotid artery cannula after 15, 30, 60, 90, 120, 180 and 240 min for oral studies and after 3, 5, 10, 20, 30, 60, 90 and 120 min for intravenous studies. Plasma was separated immediately by centrifugation and stored at −20 °C until assayed.

Plasma Protein Binding Binding of propranolol to rat plasma in the presence or absence of quinidine was measured by ultrafiltration using the Micropartition System (MPS-1, Amicon Corporation, Cambridge, MA).[15)] Heparinized plasma was obtained and pooled from three rats. Propranolol was dissolved in saline solution and added to 1 ml of plasma to yield plasma drug concentrations from 0.2 to 2.0 μg/ml. In the case of the quinidine combination, quinidine was also added to the plasma to yield a plasma drug concentration of 1.0 μg/ml. This was placed in the ultrafiltration device equipped with a YMT ultrafiltration membrane (Amicon Corporation) and centrifuged at 2000 × *g* for 15 min at 20 °C. The drug concentration in the plasma filtrate was analyzed by the same method as used for the plasma.

Liver Perfusion Studies Closed perfusion of the rat liver was performed according to the method of Mortimore *et al.*[16)] as described previously.[17)] Rats weighing 236—277 g were used. The liver was isolated under pentobarbital anesthesia and perfused *via* the hepatic portal vein with 20% (v/v) bovine blood cells and 5% (w/v) bovine serum albumin in Krebs–Henseleit buffer solution, equilibrated with 95% O_2 and 5% CO_2 to maintain a pH of 7.4 at 37 °C. The flow rate of the circulating perfusate was kept at 15 ml/min. The liver was allowed to equilibrate with the perfusate for about 10 min before drug administration. A drug solution (0.5 ml) was added to the 30 ml perfusate reservoir at 2.5 mg/kg propranolol with or without 10 mg/kg quinidine, and 0.2 ml samples of reservoir solution were obtained after 3, 5, 10, 15, 20, 25 and 30 min. After separation of blood cells, perfusate plasma was assayed in the same manner as the plasma.

Analysis of Propranolol Propranolol concentration was measured with a slight modification of the method previously reported.[13)] Plasma samples (0.1—0.5 ml) were alkalinized by the addition of 1 ml of 10% K_2CO_3. Then, 6 ml of ether containing an internal standard (verapamil) was added. The mixture was mixed with a vortex mixer for 2 min. After centrifugation at 2000 × *g* (4 °C) for 10 min, 5.5 ml of the organic layer was transferred into tapered centrifuge tubes. The ether was evaporated to dryness under reduced pressure. The residue was dissolved in 0.1—0.5 ml of the mobile phase. A 50 μl aliquot of the solution was injected onto a column of a high-performance liquid chromatography system (a Shimadzu Model LC-3A with a Shimadzu Model RF-500LC). After separation with a Zorbax-CN column (25 cm × 4.6 mm i.d.; 5 μm particle size; Shimadzu-Dupont, Japan) using a mobile phase of acetonitrile–0.0871 M phosphoric acid–H_2O (5 : 1 : 3, v/v) at a flow rate of 2 ml/min, propranolol was detected by fluorescence using an excitation wavelength of 296 nm and an emission wavelength of 353 nm. Under these analytical conditions, quinidine did not interfere with the determination of propranolol.

Data Analysis The area under the curve of orally administered propranolol (AUC_{oral}) was calculated by the trapezoidal rule and extrapolated to infinity. The terminal rate constant was determined by least-squares regression of the log-linear portion of the curve. Plasma concentration time course data of individual animals from the intravenous studies were fitted to the equation $C_t = Ae^{-\alpha t} + Be^{-\beta t}$ for the plasma concentration C_t at time t[18)] with the aid of a non-linear least-squares

regression computer program.[19] The AUC of intravenously administered propranolol (AUC_{iv}) was estimated from the equation, $AUC_{iv}=A/\alpha+B/\beta$. The apparent volume of the central compartment (V_c) was calculated as $V_c=\text{dose}/(A+B)$. The steady-state apparent volume of distribution (V_{ss}) was obtained from the equation $V_{ss}=\text{dose}\ (A/\alpha^2+B/\beta^2)/(A/\alpha+B/\beta)^2$. The systemic clearance (CL_s) was calculated from the equation $CL_s=\text{dose}/(A/\alpha+B/\beta)$. The V_c, V_{ss} and CL_s were all corrected for body weight. The elimination rate constant in the liver perfusion studies was determined by least-squares regression of the curve. Mean values are reported with standard errors. Statistical analysis was performed with Student's t-test (two-tailed) with $p<0.05$ as the minimal level of significance.

Results

Effect of Quinidine on the Pharmacokinetics of Propranolol

Figure 1 shows the plasma concentration of propranolol after oral administration at 2.5 mg/kg with or without 10 mg/kg of quinidine. Propranolol was rapidly absorbed from the gastrointestinal tract and the peak plasma concentration was reached within 30 min after administration. The time required to reach the peak was not significantly different between propranolol alone and propranolol with quinidine. When propranolol was given alone, its peak concentration in plasma was less than 30 ng/ml. When given with quinidine, it was about 5.2 times higher (22.2 ± 2.3 *vs.* 116.2 ± 7.8 ng/ml, $p<0.01$). The AUC of propranolol was about 3.6 higher when the two drugs were given together than when propranolol was given alone ($p<0.01$). These results are consistent with our previous observations in man.[13]

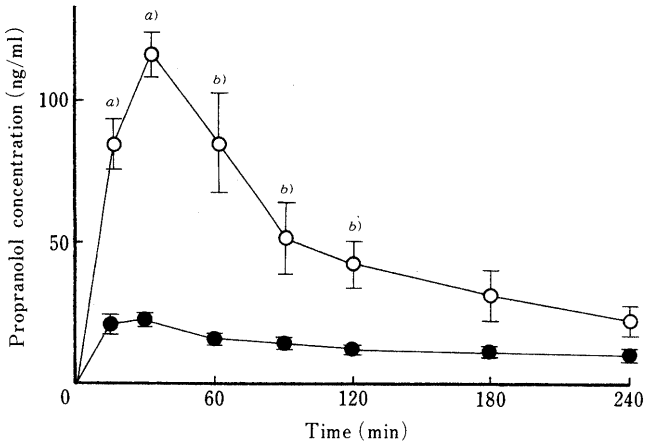

Fig. 1. Effect of Quinidine (10 mg/kg) on the Plasma Concentration of Propranolol (2.5 mg/kg) after Oral Administration in Conscious Rats

Each point and vertical bar represents a mean±S.E. of seven (●; propranolol alone) or six (○; propranolol with quinidine) rats. *a*) $p<0.01$, *b*) $p<0.05$.

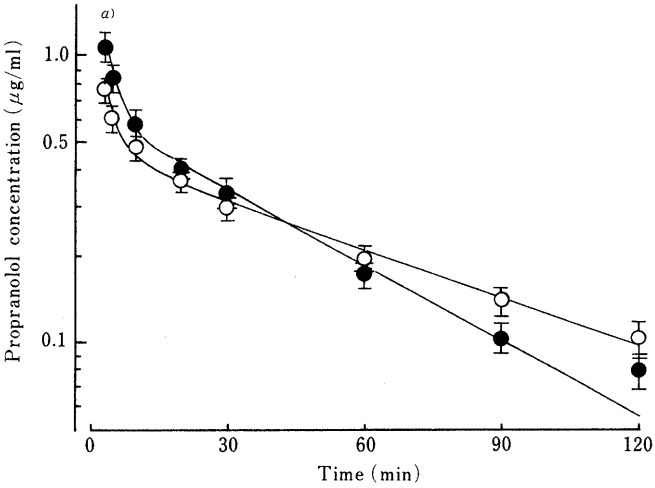

Fig. 2. Effect of Quinidine (10 mg/kg) on the Plasma Concentration of Propranolol (2.5 mg/kg) after Intravenous Administration in Conscious Rats

Each point and vertical bar represents a mean±S.E. of twelve (●; propranolol alone) or nine (○; propranolol with quinidine) rats. *a*) $p<0.05$.

Figure 2 shows the plasma concentrations of propranolol after rapid intravenous administration at the same dose used for the oral studies. The plasma concentration of propranolol declined biexponentially and its mean concentration at the first sampling time, 3 min after the injection, was significantly lower in rats given propranolol with quinidine than in those given propranolol alone ($p<0.05$). In the coadministration of propranolol and quinidine, the plasma concentration of propranolol declined rather slower than in the case of propranolol alone. The drug concentration data were fitted to the biexponential equation and the solid lines in Fig. 2 represent computer-fitted biexponential curves. The estimated pharmacokinetic parameters of propranolol are given in Table I. The β values were significantly lower and the V_c and V_{ss} were higher for the combination than for propranolol alone. The CL_s of propranolol was very high and was not affected by quinidine. As shown in Table II, the availability of propranolol (AUC_{oral}/AUC_{iv}) was higher when propranolol was given with quinidine than when it was given alone (44.2 ± 4.6 *vs.* $13.8\pm2.2\%$, $p<0.01$).

To study the mechanism by which quinidine alters propranolol pharmacokinetics, the binding of propranolol to rat plasma in the presence and absence of quinidine (1.0 μg/ml) was determined over propranolol concentrations from 0.2 to 2.0 μg/ml. As shown in Fig. 3, the plasma protein binding of propranolol was inversely related to the plasma concentration of propranolol. Quinidine decreased the plasma protein binding at all concentrations. Under these experimental conditions, the mean of propranolol binding

TABLE I. Pharmacokinetic Parameters of Propranolol Administered Intravenously (2.5 mg/kg) to Rats with or without Quinidine (10 mg/kg)

Parameters	Propranolol alone ($n=12$)	Propranolol+quinidine ($n=9$)
A (μg/ml)	1.39 ± 0.20	$0.72\pm0.09^{b)}$
α (min^{-1})	0.328 ± 0.035	0.268 ± 0.040
B (μg/ml)	0.63 ± 0.08	0.46 ± 0.05
β (min^{-1})	0.020 ± 0.002	$0.013\pm0.002^{a)}$
V_c (l/kg)	1.53 ± 0.24	$2.32\pm0.25^{a)}$
V_{ss} (l/kg)	3.45 ± 0.43	$5.01\pm0.58^{a)}$
CL_s (ml/min/kg)	73.7 ± 5.0	67.7 ± 6.2

Each value is a mean±S.E. *a*) $p<0.05$, *b*) $p<0.01$.

TABLE II. Effect of Quinidine on the AUC and Availability of Propranolol in Rats

	AUC (μg·min/ml)		Availability (%)
	Oral	i.v.	
Propranolol alone	4.9 ± 0.8	35.6 ± 4.0	13.8 ± 2.2
Propranolol +quinidine	$17.6\pm1.9^{a)}$	39.9 ± 4.5	$44.2\pm4.6^{a)}$

Each value is a mean±S.E. *a*) $p<0.01$.

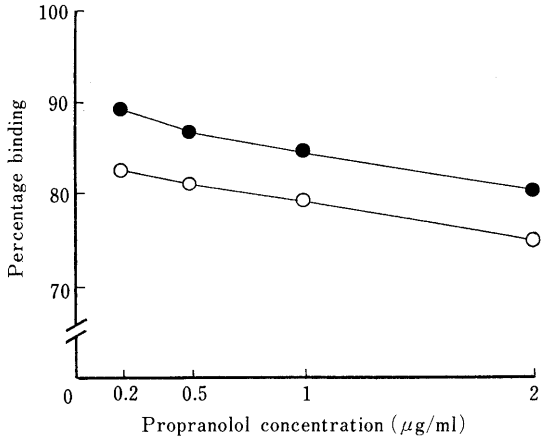

Fig. 3. Relation between Plasma Concentration of Propranolol (0.2—2 μg/ml) and Mean Percentage Binding of Propranolol, in the Presence (○) and Absence (●) of Quinidine (1 μg/ml)

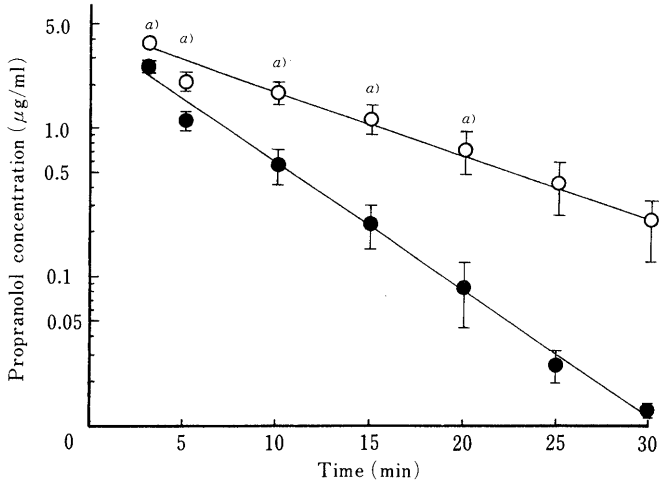

Fig. 4. Effect of Quinidine on the Elimination of Propranolol from the Perfused Rat Liver

The liver was perfused at a constant rate of 15 ml/min, and 2.5 mg/kg propranolol alone (●) or in combination with 10 mg/kg quinidine (○) was added to the 30 ml perfusate reservoir. Each point and vertical bar represents a mean ± S.E. of three experiments. *a)* $p<0.05$.

decreased from 85.1 ± 1.8 to 79.5 ± 1.7% in the presence of 1 μg/ml quinidine ($p<0.01$).

Effect of Quinidine on Hepatic Elimination of Propranolol Hepatic elimination of propranolol in the presence and absence of quinidine was examined by the liver recirculation method. The same dose used for the oral studies was injected into the 30 ml perfusate reservoir. As shown in Fig. 4, propranolol disappeared from the perfusate, apparently following first-order kinetics. The elimination rate constant was significantly lower for propranolol with quinidine than for propranolol alone (0.104 ± 0.017 *vs.* 0.197 ± 0.009 min^{-1}, $p<0.01$).

Discussion

The present study shows that the plasma concentration of propranolol after oral administration is significantly higher in rats given quinidine along with the propranolol (Fig. 1), which is consistent with our previous observations in man.[13] Thus, the propranolol–quinidine interaction observed in rats as well as in man may indicate the possible usefulness of the male Wistar rat as an animal model for investigation of the mechanisms of drug interaction in man.

Several mechanisms may account for this effect of quinidine on plasma concentrations of propranolol. One is an increase in the gastrointestinal absorption of propranolol. However, it has already been shown that propranolol is almost completely absorbed from the gastrointestinal tract in man[20] and in the rat,[17] and is not metabolized by gastrointestinal mucosa.[21,22] A more subtle mechanism may be related to the fact that the presystemic metabolism of propranolol is capacity-limited. The presystemic clearance of propranolol was found to be lower at higher rates of intraportal infusion, which is consistent with capacity-limited hepatic metabolism.[21,23] It is also conceivable that quinidine could cause propranolol to be absorbed more rapidly, which would lead to a higher concentration of propranolol in systemic circulation due to the capacity-limited presystemic metabolism. However, the fact that the time required to reach the peak plasma drug concentration was not affected by quinidine suggests that there was no acceleration of propranolol absorption by quinidine.

In the present study, the very high CL_s of propranolol was estimated from drug concentrations in plasma after intravenous administration and the values seemed to exceed the hepatic plasma flow. However, the physiological interpretation on the clearance process must be based on blood rather than plasma concentration, since drug distributed in the erythrocyte usually equilibrates rapidly with that in the plasma and is available for extraction.[24] The CL_s of propranolol obtained from the intravenous study was almost the same as our previous data, which was obtained at higher doses.[15,17] Iwamoto and Watanabe reported that the CL_s of propranolol is essentially assigned to the hepatic clearance and is constant from 1 to 10 mg/kg.[21] These data together with our present findings indicate that the hepatic clearance of propranolol in the rat is limited by the rate of blood flow to the liver. Because the CL_s of propranolol is rate-limited by hepatic blood flow, it is reasonable to expect little if any change in the clearance of this drug after intravenous administration, provided that splanchnic blood flow is not affected by quinidine. Thus, the effect of quinidine on the pharmacokinetics of propranolol depended on the route of administration. Comparing the oral to the intravenous data, we find that the average systemic availability of orally administered propranolol increased from approximately 14 to 44% when propranolol was given along with quinidine, but there was no significant change of the AUC when the drugs were given intravenously (Table II). Since there was no change in CL_s, the increase in the systemic availability of propranolol must reflect a decrease in hepatic presystemic clearance, *i.e.*, decreased removal by the liver during the initial transit from the intestine to the systemic circulation. The increased AUC of orally administered propranolol would reflect the decreased intrinsic clearance of propranolol by quinidine.

On the other hand, both the V_c and V_{ss} were significantly higher, and β was significantly lower, when the two drugs were given together (Table I). Since we measured plasma concentrations of propranolol in the present study, the changes in volume of distribution may include the changes of drug distribution to the blood cells. These differences

may be explained by the decrease in the plasma protein binding of propranolol.[24-26] As is evident from Fig. 3, quinidine increased the unbound fraction of propranolol in plasma from about 14.9 to 20.5%. There was no significant difference in the V_{ss} of unbound propranolol, which was calculated by dividing the V_{ss} of plasma propranolol by the average unbound fraction in plasma (propranolol alone: 23.2 ± 2.5 l/kg; propranolol with quinidine: 24.5 ± 2.5 l/kg). However, the decreased plasma protein binding of propranolol was not related to the change in availability of orally administered propranolol, because a displacement interaction at the plasma binding site would have resulted in an increase in presystemic clearance, rather than the reduction in presystemic clearance which was actually observed.

The effect of quinidine on the presystemic elimination of propranolol in the liver was directly examined in the liver recirculation experiment. As shown in Fig. 4, quinidine markedly delayed the elimination of propranolol from the perfused rat liver. This indicates that quinidine increases the systemic availability of propranolol by inhibiting presystemic elimination of propranolol in the liver. We have already reported that quinidine causes a marked increase in the plasma concentration of ajmaline in man.[27] Animal experiments have shown that giving quinidine and ajmaline together prevents the presystemic elimination of ajmaline.[28] These results suggest that quinidine can interact with various drugs which undergo extensive presystemic clearance in the liver.

In conclusion, we demonstrated a pharmacokinetic interaction between propranolol and quinidine in male Wistar rats. Quinidine significantly increased the systemic availability of orally administered propranolol, by reducing presystemic elimination.

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