MECHANISMS OF PHARMACOKINETIC INTERACTION BETWEEN AJMALINE AND QUINIDINE IN RATS

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In order to elucidate the mechanism of ajmaline–quinidine interaction previously observed in humans, the effects of quinidine on pharmacokinetics of ajmaline were investigated in rats. Concurrent oral administration of 10 mg/kg of quinidine markedly increased the plasma concentration of ajmaline at a dose of 2 mg/kg. On the other hand, it did not affect the pharmacokinetics of ajmaline after intravenous dose. The availability of ajmaline after oral dose showed an increase from 13% to nearly 100% by the presence of quinidine, which suggests a change in the presystemic clearance of ajmaline. In fact, when ajmaline was administered into the intestinal loop, its concentration in mesenteric venous plasma increased approximately 5-fold by the combination with quinidine. Furthermore, quinidine delayed the elimination rate of ajmaline from the perfused rat liver. These results indicate that quinidine prevents presystemic elimination of ajmaline in the intestine and liver, and increases the systemic availability of ajmaline.

Keywords — ajmaline; quinidine; drug interaction; pharmacokinetics; presystemic elimination; availability; combined therapy; rat

INTRODUCTION

Most of the currently available antiarrhythmics have a narrow therapeutic index, necessitating careful dose titration. In spite of their frequent use in combination, little is known about the pharmacokinetics in combined therapy of antiarrhythmic drugs. Recently, we found that quinidine induced a marked rise in the plasma concentration of ajmaline after oral administration to humans. Plasma concentration of ajmaline was almost negligible in normal volunteers receiving ajmaline alone, but a high plasma concentration, sufficient for antiarrhythmic therapy, was obtained when 50 mg of ajmaline was administered with 200 mg of quinidine.11 These observations suggest the presence of pharmacokinetic drug interaction between ajmaline and quinidine in the combination therapy.

In the present study, the effects of quinidine on the pharmacokinetics of ajmaline were investigated in rats in order to clarify the interaction mechanism(s). A preliminary report in abstract form has been published elsewhere.22

MATERIALS AND METHODS

Materials — Ajmaline was purchased from Sigma (St. Louis, USA). Quinidine was obtained from Nakarai Chem. Ltd. (Kyoto, Japan). All other chemicals were of the finest grade available.

Animal Experiments in Vivo — Male Wister albino rats, weighing 180—210 g, were fasted for about 18 h prior to experiments with free access to water. 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 rat’s neck to obtain blood samples.

The incisions were closed with sutures. Heparin (1000 units/kg) was administered intravenously. After recovery from ether anesthesia, drugs were administered to rats. In the oral administration study, the rats received either a single dose of ajmaline (2 mg/kg) alone or in combination with quinidine (10 mg/kg) by gastric intubation. In a separate experiment, ajmaline alone or in combination with quinidine was injected into a femoral vein in the same dose as in the oral studies. Additionally, ajmaline (2 mg/kg) in combination with quinidine (20 mg/kg) was administered intravenously. Each animal was kept in an individual cage without any constraints. Blood samples were obtained at 30, 50, 90, 180 min for oral studies, at 2, 10, 30, 50, 90, 180 min for intravenous studies in the

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same dose as in the oral studies and at 2, 30, 50, 90, 180 min for intravenous studies in a dose of 2 mg/kg ajmaline with 20 mg/kg quinidine. Plasma was separated immediately by centrifugation and stored at −20 °C until assayed.

**Intestinal Absorption** — The absorption rate of ajmaline from the rat small intestine was determined by the in situ loop method. The isotonic sodium phosphate buffer solution (5 ml, pH 6.5) containing ajmaline with or without quinidine in the same dose used for oral studies was injected into the loop of small intestine. At the end of a prescribed period, the luminal content was withdrawn and the intestinal lumen was washed with an isotonic saline. The washings were combined with the luminal content, the volume adjusted to 100 ml with saline and the amount of drug remaining in the loop was determined.

**Determination of Ajmaline Amount in Mesenteric Venous Plasma** — Cumulative amount of ajmaline in mesenteric venous plasma was determined by the method of Barr and Riegelman with slight modification. Under anesthesia with ether, about 20 cm of closed ileal loop was prepared and heparin was administered intravenously (1000 units/kg). For complete collection of venous blood draining from the region of absorption, the mesenteric vein of the loop was cannulated with a PE 10 polyethylene tubing. Immediately, 2 ml of isotonic sodium phosphate buffer solution (pH 6.5) containing ajmaline (0.4 mg) with or without quinidine (2 mg) was injected into the loop. All draining venous blood was collected in calibrated centrifuge tubes at certain intervals. The blood lost from the mesenteric vein was replaced with an equal volume of blood–10% sodium citrate mixture (92:8) previously prepared by an intravenous infusion via the femoral vein.

**Liver Perfusion Studies** — A closed perfusion of rat liver was carried out according to the method of Mortimore et al. as previously described. Rats weighing 240–260 g were used in this experiment. The liver was isolated under pentobarbital anesthesia and perfused via the hepatic portal vein with 20% (v/v) bovine blood cell, 5% (w/v) bovine serum albumin in Krebs-Henseleit buffer solution equilibrated with 95% O₂ plus 5% CO₂ to maintain a pH of 7.4 at 37 °C. A flow rate of the circulating perfusate was constantly maintained at 15 ml/min. After a stabilization period of about 10 min, 0.5 ml of a drug solution containing 0.4 mg ajmaline with or without 2 mg quinidine was added to the 30 ml perfusate reservoir, and 0.2–0.5 ml sample of reservoir solution was obtained at 3, 5, 10, 15, 20 and 30 min. The total sampling volume was less than 2 ml. After separation of blood cells, perfusate plasma was assayed in the same manner as the plasma.

**Analytical Method of Ajmaline** — The analysis of ajmaline was performed using a slight modification of our method previously reported. Plasma and other samples (0.1–1 ml) of in vivo, in situ and liver perfusion experiments were adjusted to pH 10 with 1 ml of 0.1 M glycine buffer. Then, 5 ml of ether was added. The mixture was mixed with a vortex mixer for 2 min. After centrifugation at 2000 × g (4°C) for 10 min, 4 ml of organic layer was withdrawn, to the organic fraction 0.5–2 ml of 2% (v/v) acetic acid was added and the mixed solution was centrifuged at 2000 × g (4°C) for 5 min. The upper layer was discarded. A 50 μl of aqueous layer was injected on high-performance liquid chromatography system (combination into a Shimadzu Model LC-3A and a Shimadzu Model RF-500LC). After separation with a Zorbax CN column using a mobile phase of acetonitrile–2.5% acetic acid (44:56, v/v), ajmaline was detected by fluorescence using an excitation wavelength of 295 nm and an emission wavelength of 375 nm.

**Data Analysis** — The area under the plasma drug concentration–time curve (AUC) was calculated by the trapezoidal rule for the observed blood levels from 0 to 180 min after administration. Mean values are reported with standard error. Statistical analyses were performed with the Student’s t-test with p = 0.05 as the minimal level of significance.

**RESULTS**

**Effect of Quinidine on the Plasma Concentration of Ajmaline in Vivo**

Figure 1 shows the plasma concentration of ajmaline after oral administration at a dose of 2 mg/kg with or without 10 mg/kg of quinidine. Quinidine induced a marked rise in the plasma concentration of ajmaline although a large individual variation was observed at 30 min after oral administration. The plasma peak concentration was elevated about 10-fold from
0.021 ± 0.004 μg/ml by the single administration to 0.235 ± 0.024 μg/ml by the combination with quinidine (p < 0.001). The AUC of ajmaline after oral dose increased from 2.38 ± 0.16 μg·min/ml for ajmaline alone to 18.29 ± 1.85 μg·min/ml for ajmaline with quinidine (p < 0.001). These results are in agreement with our previous observations in humans.\(^1\) On the other hand, as shown in Fig. 2, there was no significant difference in the plasma concentration of ajmaline after intravenous administrations of ajmaline alone or ajmaline coadministered with 10 mg/kg quinidine. The AUCs of ajmaline after intravenous dose were 17.80 ± 1.41 and 17.80 ± 1.57 μg·min/ml for ajmaline alone and in combination with quinidine, respectively. The availability of ajmaline, calculated by the AUCs obtained from the oral and intravenous studies, showed an increase from 13.4 ± 0.9% by the single administration to 102.7 ± 10.4% by the coadministration with quinidine (p < 0.001). Additionally, when ajmaline (2 mg/kg) and quinidine (20 mg/kg) were coadministered intravenously, plasma concentration of ajmaline was high compared with the intravenous studies at the same dose used for the oral studies.

**Effect of Quinidine on the Absorption and Metabolism of Ajmaline in Rat Small Intestine**

In order to investigate the effect of quinidine on the intestinal absorption rate of ajmaline, ajmaline was administered into the loop of rat small intestine with or without quinidine. As shown in Fig. 3, about 50% of dose disappeared from the intestinal lumen at 30 min after the ad-

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**FIG. 1.** Effect of Quinidine (10 mg/kg) on the Plasma Concentrations of Ajmaline (2 mg/kg) after Oral Administration in Conscious Rats

Each point and vertical bar represents the mean ± S.E., respectively, of five (○, ajmaline alone) and ten (●, ajmaline with quinidine) rats. a) p < 0.001 compared with ajmaline alone.

**FIG. 2.** Effect of Quinidine (10 or 20 mg/kg) on the Plasma Concentrations of Ajmaline (2 mg/kg) after Intravenous Administration in Conscious Rats

Each point and vertical bar represents the mean ± S.E., respectively, of three (○, ajmaline alone), four (●, ajmaline with 10 mg/kg of quinidine) and three (▲, ajmaline with 20 mg/kg of quinidine) rats. a) p < 0.01, b) p < 0.025 and c) p < 0.05 compared with ajmaline alone.

**FIG. 3.** Effect of Quinidine on the Absorption Rate of Ajmaline in Rats

Five ml of buffer solution (pH 6.5) containing ajmaline (2 mg/kg) with or without quinidine (10 mg/kg) was injected into the intestinal loop. At the end of absorption period, the luminal content was recovered and the residual amount of the drug in the lumen was determined. Each point and vertical bar represents the mean ± S.E., respectively, of at least three experiments. ○, ajmaline alone; ●, ajmaline with quinidine.
administration and the absorption rate of ajmaline was not affected by the coadministration of quinidine. The contribution of intestinal metabolism to the overall pre-systemic elimination of ajmaline was examined by the complete collection of mesenteric venous blood after drug administration into the intestinal loop. Since no detectable amount of ajmaline was found in the systemic plasma obtained from carotid artery, it can be considered that all venous blood draining from the region of absorption was completely collected in the present experiment. As shown in Fig. 4, quinidine induced about a five- to six-fold increase in the cumulative amount of ajmaline recovered in the mesenteric venous plasma.

**Effect of Quinidine on the Hepatic Elimination of Ajmaline**

Hepatic elimination of ajmaline was examined by the liver recirculation method. The dose to the 30 ml perfusate reservoir was adjusted, referring to the plasma concentration of ajmaline in the mesenteric vein. As shown in Fig. 5, at 3 min after the administration of 0.4 mg ajmaline into the reservoir, the perfusate plasma concentrations of ajmaline ranged from 4.0 to 6.1 \( \mu \text{g/ml} \), which were much higher than those observed in the *in vivo* intravenous experiments. Ajmaline disappeared from the perfusate following apparent first-order kinetics. The elimination rate constant was 0.338 ± 0.056 min\(^{-1}\) for ajmaline alone and 0.104 ± 0.002 min\(^{-1}\) for ajmaline with 2.0 mg quinidine (\( p < 0.005 \)).

**DISCUSSION**

The basic observation reported here is that the plasma concentrations in rats are increased by oral quinidine after oral dosing with ajmaline, which is in agreement with our previous observations in humans.

The increased plasma concentrations of ajmaline could result either from (1) increased absorption from the intestine, (2) decreased removal by the liver or intestinal mucosa during the initial transit through the intestine to the general circulation (pre-systemic clearance), or (3) decreased rate of removal after entry into the general circulation (systemic clearance).

The absorption studies by the *in situ* loop method confirmed that ajmaline was well absorbed from the rat small intestine and the absorption rate was not enhanced by the coadministration with quinidine. Furthermore, the plasma concentrations of ajmaline after intravenous dosing were not affected by the presence of quinidine at the same dose used for the oral stud-
ies. However, a combination with 20 mg/kg of quinidine and ajmaline induced a significant increase in the plasma concentration of ajmaline and the AUC. This indicates that quinidine combination at the large dose reduced the systemic clearance of ajmaline. These data may come from route- and dose-dependent differences in the behavior of quinidine. By exclusion of changes in absorption and systemic clearance at the same dose used for the oral studies, the change in systemic availability of ajmaline must reflect a change in presystemic clearance.

Kleinsorge and Gaida reported that only 3.9% of dose was excreted in the urine as an intact drug after intravenous administration of ajmaline at a dose of 50 mg in man. They also found that the mean urinary recoveries of ajmaline following oral administration of plain tablets and enteric coated tablets were 0.36 and 0.14% of dose, respectively. These findings suggest the elimination of ajmaline by non-renal mechanisms and the low availability of ajmaline after oral administration. In the present study, the availability of ajmaline after a single oral dosing was 13.4 ± 0.9% in spite of nearly complete absorption from the rat small intestine. Thus, ajmaline after oral administration must undergo avid presystemic elimination.

The effect of quinidine on the presystemic elimination of ajmaline in the small intestine and the liver was examined separately. When ajmaline was administered into the intestinal loop, its concentration in mesenteric venous plasma increased about five-fold by the combination with quinidine. Since no significant difference was found in the absorption rate of ajmaline from the intestinal loop (Fig. 3), it can be considered that quinidine prevents the presystemic metabolism of ajmaline in the intestinal mucosa. Furthermore, quinidine delayed the elimination rate of ajmaline from the perfused rat liver. These results indicate that quinidine increases the systemic availability of ajmaline by preventing the presystemic elimination of ajmaline in both the small intestine and the liver.

It is of interest to consider the implications of the interaction we found for the clinical use of ajmaline. The antiarrhythmic effect of ajmaline has been known to be satisfactory only after parenteral administration. We have already reported that plasma concentrations after ajmaline alone by mouth were less than 0.03 µg/ml, while plasma concentrations of ajmaline sufficient for antiarrhythmic therapy were obtained when 50 mg of ajmaline were coadministered orally with 200 mg of quinidine. Thus, quinidine combination would appear to have potential in the treatment of arrhythmias by oral dosing with ajmaline. At the same time, careful dose titration would be necessary for the proper use of combination drug therapy especially in such patients with liver disease, since the drug interaction takes place, at least in part, at the hepatic drug elimination process.

REFERENCES