In Vivo Antiarrhythmic Profile of AP-792 Assessed in Different Canine Arrhythmia Models

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ABSTRACT—The antiarrhythmic effects of a novel antiarrhythmic drug AP-792, 4-(5H-dibenzo[a,d]cyclohepten-5-yldiene)-1-[4-cyclohexylbutyl]piperidine hydrochloride, were analyzed using the epinephrine-, digitalis- and two-stage coronary ligation-induced canine ventricular arrhythmia models. Intravenous administration of AP-792 (0.3 or 1.0 mg/kg) effectively suppressed each of the ventricular arrhythmias, an action that resembles that of a typical cardioselective Ca²⁺ channel blocker, AH-1058. The antiarrhythmic action of AP-792 was slow in onset and longer-lasting than those in our previous studies using more than 50 antiarrhythmic drugs, including Na⁺ and Ca²⁺ channel blockers. These results suggest that AP-792 can become a unique long-acting antiarrhythmic drug.

Keywords: AP-792, Ventricular arrhythmia

Cyproheptadine and its derivatives have been reported to exert antiarrhythmic action in canine digitalis- or coronary ligation-induced arrhythmia models (1–3), in which class I antiarrhythmic drugs are effective (4). Whereas most of the antiarrhythmic drugs have a negative dromotropic action, one of the compounds, cyproheptadinium methiodide, hardly affects AV nodal and intraventricular conduction in antiarrhythmic doses (3), which appears to be unique as an antiarrhythmic compound. Recently, a cyproheptadine-derived compound, 4-(5H-dibenzo[a,d]cyclohepten-5-yldiene)-1-[4-cyclohexylbutyl]piperidine hydrochloride (AP-792), as shown in Fig. 1, has been developed as an antiarrhythmic drug that can suppress serotonin (5-HT) induced platelet aggregation by inhibition of 5-HT₂ receptor binding (H. Dohmoto et al., unpublished data). In our preliminary study, an antiarrhythmic effect of AP-792 in doses of 0.3 to 1 mg/kg has already been investigated in the guinea pig digitalis-induced arrhythmia model.

The purpose of the present study is to assess the antiarrhythmic effects of AP-792, using three well-established types of canine in vivo arrhythmia models: namely, epinephrine-, digitalis- and two-stage coronary ligation-induced arrhythmia models (5–7). In our previous studies with more than 50 antiarrhythmic drugs (4, 5, 8–10), the epinephrine-induced ventricular arrhythmia was suppressed by drugs possessing a Ca²⁺ channel blocking property, while digitalis- and two-stage coronary ligation-induced arrhythmias were inhibited by Na⁺ channel blockers or a cardioselective Ca²⁺ channel blocker. In the present study, we compared the effects of AP-792 with those of Na⁺ and

Fig. 1. Chemical structure of AP-792, 4-(5H-dibenzo[a,d]cyclohepten-5-yldiene)-1-[4-cyclohexylbutyl]piperidine hydrochloride and cyproheptadine.
Ca²⁺ channel blockers previously obtained in our studies to characterize the in vivo antiarrhythmic profile of AP-792.

**MATERIALS AND METHODS**

All experiments were performed according to Guidelines for Animal Experiments, Yamanashi Medical University. We used mongrel and Beagle dogs of either sex weighing 10 to 17 kg.

*Epinephrine-induced arrhythmia*

Six dogs (group 1) were anesthetized initially with thiopental sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, 1.0% halothane vaporized with 100% oxygen was inhaled with a volume-limited ventilator (SN-408-3; Shinano, Tokyo). Epinephrine was infused into the left femoral vein at a rate of 2.5 μg/kg per min for 18 min using a syringe pump (5). It has already been reported that the ventricular arrhythmias were observed for more than 20 min when an antiarrhythmic drug was not used (11). Three minutes after the start of epinephrine infusion, AP-792 in a dose of 0.3 mg/kg was intravenously administered into the right femoral vein. The lead II electrocardiogram (ECG), the right atrial electrogram and mean blood pressure were recorded for 15 min using a polygraph system (RM-6000; Nihon Kohden, Tokyo).

*Digitalis-induced arrhythmia*

Five dogs (group 2) were anesthetized with pentobarbital sodium (30 mg/kg, i.v.) and intubated with a cuffed endotracheal tube. Ouabain in a dose of 40 μg/kg was intravenously injected, and 10 μg/kg of ouabain was supplemented every 20 min until stable ventricular tachycardia was produced (6). It has already been reported that this arrhythmia was stable at least for 1 h when an antiarrhythmic drug was not used (11). After a stable ventricular tachycardia was induced, AP-792 in a dose of 0.3 mg/kg was intravenously administered via the right femoral vein. The lead II ECG, the right atrial electrogram and mean blood pressure were recorded for 15 min using a polygraph system (RM-6000, Nihon Kohden). For example, the arrhythmic ratio is zero during the sinus rhythm, while it is one during ventricular tachycardia. The ventricular ectopic beats were evaluated from the shape of the QRS complex and its sequential relation to the atrial electrogram. The arrhythmic ratio was almost one before the drug injection in each arrhythmia model. When the ratios after drug administration decreased significantly from the 0 time value, the antiarrhythmic effects of the drug were judged as significant, as previously reported (5–7).

*Two-stage coronary ligation-induced arrhythmia*

Five dogs (group 3) were initially anesthetized with thiopental sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, 1.0% halothane was inhaled with a volume-limited ventilator (SN-408-3, Shinano). The chest was opened and the left anterior descending artery was ligated in a two-stage manner to induce continuous and multifocal ventricular beats, as previously reported (4, 7–10). Furthermore, it has already been reported that the arrhythmia lasted for at least 2 days after coronary ligation when an antiarrhythmic drug was not used (11). AP-792 in a dose of 1 mg/kg was intravenously administered without anesthesia at 24 and 48 h after the coronary ligation. The lead II ECG, the left atrial electrogram from implanted electrodes sutured on the left atrial appendage, and mean blood pressure were recorded every 2 min (0 to 30 min after the drug administration) and 5 min (30 to 60 min after the drug administration) using a telemetry system (WEB-5000, Nihon Kohden).

**Evaluation of antiarrhythmic effects**

The severity of ventricular arrhythmias was expressed as the arrhythmic ratio: the number of ventricular ectopic beats (/min) divided by that of total QRS complexes (/min). For example, the arrhythmic ratio is zero during the sinus rhythm, while it is one during ventricular tachycardia. The ventricular ectopic beats were evaluated from the shape of the QRS complex and its sequential relation to the atrial electrogram. The arrhythmic ratio was almost one before the drug injection in each arrhythmia model. When the ratios after drug administration decreased significantly from the 0 time value, the antiarrhythmic effects of the drug were judged as significant, as previously reported (5–7).

**Drugs**

The following drugs were used: AP-792 (Ajinomoto Co. Inc., Tokyo), thiopental sodium (Tanabe Seiyaku, Osaka), halothane (Takeda Chemical Industries, Osaka), pentobarbital sodium (Tokyo Kasei, Tokyo), heparin calcium (Mitsui Pharmaceuticals, Tokyo), epinephrine (Dai-ichi Seiyaku, Tokyo) and (−)-ouabain octahydrate (Aldrich Chemical, Milwaukie, WI, USA). Since AP-792 is chemically hydrophobic as shown in Fig. 1, AP-792 was dissolved in polyethylene glycol 400/saline (70:30, vol/vol) for intravenous administration.

**Statistics**

All values are expressed as means ± S.E.M. Analysis of variance (ANOVA) for repeated measures was employed for overall statistical analysis, followed by Contrasts for statistical analysis comparing basal values (zero time) and other values. Statistical analysis was performed using a commercially available software, SuperANOVA (Abacus Concepts, Inc., Berkeley, CA, USA). Differences with a P value of less than 0.05 were considered to be statistically significant.
RESULTS

Effects of AP-792 on epinephrine-induced arrhythmia model (group 1)

Three minutes after the start of the intravenous infusion of epinephrine, ventricular tachyarrhythmia was induced, as shown in Fig. 2. When AP-792 in a dose of 0.3 mg/kg was intravenously administered at time 0, epinephrine-induced ventricular arrhythmias were completely suppressed within 5 min. The summary of antiarrhythmic effects of AP-792 on the epinephrine-induced arrhythmias is shown in Fig. 3A. Basal values (at 0 min) of the total heart rate, atrial rate, conducted beats, mean blood pressure and arrhythmic ratio were 225 ± 22 beats/min, 197 ± 11 beats/min, 44 ± 20 beats/min, 164 ± 8 mmHg and 0.76 ± 0.11, respectively. AP-792 in a dose of 0.3 mg/kg significantly decreased the total heart rate from 6 min and blood pressure from 2 min and increased the number of conducted beats from 3 min after drug administration, while the atrial rate was not affected by the drug. The arrhythmic ratio decreased significantly from 3 min after drug administration. These significant changes lasted throughout the 15-min observation period.

Effects of AP-792 on digitalis-induced arrhythmia model (group 2)

After intravenous injection of a total dose of 70–90 μg/kg of ouabain, almost all the beats became of ventricular origin. The effects of AP-792 on the digitalis-induced arrhythmia model are summarized in Fig. 3B. Basal values (at 0 min) of the total heart rate, atrial rate, conducted beats, mean blood pressure and arrhythmic ratio were 226 ± 5 beats/min, 199 ± 13 beats/min, 0 ± 0 beats/min, 188 ± 18 mmHg and 1.00 ± 0.00, respectively. AP-792 in a dose of 0.3 mg/kg significantly decreased the total heart rate from 6 min, atrial rate from 8 to 40 min and blood pressure from 4 min, and increased the number of conducted beats from 6 min after drug administration. The arrhythmic ratio decreased significantly from 6 min after drug administration. These significant changes lasted throughout the 60-min observation period.

Effects of AP-792 on two-stage coronary ligation-induced arrhythmia model (group 3)

One to two days after the coronary ligation, all dogs showed continuously occurring multifocal ventricular ectopic beats. The effects of intravenous administration of AP-792 on the coronary ligation-induced arrhythmia model are summarized in Fig. 4. In the 24-h arrhythmia model, basal values (at 0 min) of the total heart rate, atrial rate, conducted beats, mean blood pressure and arrhythmic ratio were 171 ± 22 beats/min, 170 ± 23 beats/min, 19 ± 19 beats/min, 99 ± 15 mmHg and 0.88 ± 0.12, respectively. AP-792 in a dose of 1 mg/kg significantly increased the number of conducted beats at 24, 28, 30, 35 and 40 min, and decreased the arrhythmic ratio at 12, 16, 20, 24–45 and 55 min, while little change was observed in the total heart rate, atrial rate and mean blood pressure (Fig. 4A). In the 48-h arrhythmia model, basal values (at 0 min) of the total heart rate, atrial rate, conducted beats, mean blood pressure and arrhythmic ratio were 170 ± 9 beats/min, 148 ± 19 beats/min, 12 ± 12 beats/min, 102 ± 9 mmHg and 0.92 ± 0.08, respectively. AP-792 in a dose of 1 mg/kg significantly increased the number of conducted beats at 10, 14–18 and 22–60 min and decreased the arrhythmic ratio from 10 min to 60 min (except for 22 min) with little change in the total heart rate, atrial rate and mean blood pressure (Fig. 4B).

![Fig. 2.](image-url) Typical tracing showing the effect of AP-792 on epinephrine-induced ventricular arrhythmias. AP-792 in a dose of 0.3 mg/kg was intravenously administered at 0 min. BP, blood pressure; ECG, electrocardiogram.
DISCUSSION

The present study was designed to characterize the antiarrhythmic profile of AP-792 using well-established canine arrhythmia models (5–7). As clearly shown by the results, AP-792 effectively suppressed the epinephrine-, digitalis- and two-stage coronary ligation-induced ventricular arrhythmias. Although a relatively higher dose of AP-792 was administered in the two-stage coronary ligation-induced ventricular arrhythmia model, even clinically available class I antiarrhythmic drugs have been shown to require higher doses or plasma drug concentration to effectively suppress ventricular arrhythmias induced by two-stage coronary ligation among the three arrhythmia models (4). Antiarrhythmic spectrum of AP-792 in these models, as well as its cardiohemodynamic effects in the two-stage coronary ligation model that the drug hardly affects mean blood pressure and atrial rate, resemble those of a cardioselective Ca\(^{2+}\) channel blocker AH-1058 (10). Since protection of the ischemic heart is important in many patients with ventricular arrhythmias, a drug with anti-serotonergic activity such as AP-792 may be desirable (12), and add a new category of antiarrhythmic drugs.

Time course of antiarrhythmic action of AP-792 was unique as an antiarrhythmic drug. Whereas most of the clinically available antiarrhythmic drugs exert their maximum antiarrhythmic effects within 5 min after their administration in these canine arrhythmia models (5–7, 13–16), the antiarrhythmic action of AP-792 was slow in onset; namely, the peak effect appeared more than 5 min after administration and was longer-lasting than that of other antiarrhythmic drugs. Slow onset and long-lasting action of AP-792 might be due to its high lipophilicity, as shown in Fig. 1, which allows it to be readily distributed to the cardiac tissues. However, this hypothesis must be further elucidated by measuring the drug concentration in the cardiac tissue as well as plasma. In addition, since duration of drug’s action in addition to antiarrhythmic profile is important information for clinical usage, further experiments will be needed using conscious animal models such as dogs.
Antiarrhythmic Action of AP-792

The present study clearly shows that the antiarrhythmic spectrum of AP-792 resembles that of class I antiarrhythmic drugs and the cardioselective \( \text{Ca}^{2+} \) channel blocker AH-1058 (4, 10). To clarify the antiarrhythmic mechanism of AP-792, electrophysiological studies including effects on \( \text{Na}^+ \) and \( \text{Ca}^{2+} \) channels should be carried out. On the other hand, our preliminary examination showed that its mother compound cyproheptadine in a dose of 1 mg/kg (i.v.) can also suppress digitalis-induced arrhythmias in dogs, and the IC\textsubscript{50} against cardiac L-type \( \text{Ca}^{2+} \) channel currents is 69.4 \( \mu \text{M} \) using guinea pig cardiomyocytes, which is about tenfold less effective than those by the other cyproheptadine-derived drug AH-1058 (17). These observations suggest that the antiarrhythmic action of cyproheptadine-derived compounds including AP-792 may correlate with their inhibitory action on cardiac L-type \( \text{Ca}^{2+} \) channels.

In a previous study, serotonin antagonism has been reported to suppress ventricular arrhythmias using serotonin receptor antagonists such as cinanserin, methysegide and cyproheptadine (2). In the rat platelet, AP-792 has been demonstrated to effectively suppress serotonin-dependent platelet aggregation (IC\textsubscript{50} 1.4 \( \mu \text{M} \)), the potency of which is the same as that of AH-1058 (H. Dohmoto et al., personal communication). Since the antiarrhythmic potential of AP-792 is 3- to 10-fold less than that of AH-1058 in the same canine ventricular arrhythmia models (10), the anti-serotonergic activity of the two drugs might not positively explain the difference in their antiarrhythmic potency. To further clarify the contribution of anti-serotonergic activity to antiarrhythmic mechanisms, antiarrhythmic action of cyproheptadine-derived serotonin antagonists lacking effects on \( \text{Na}^+ \) and \( \text{Ca}^{2+} \) channels should be studied.

In summary, AP-792 suppressed the epinephrine-, digitalis- and two-stage coronary ligation-induced ventricular arrhythmias with a minor hypotensive action, and the antiarrhythmic effects were slow in onset and long-lasting. The results of this experimental study suggest that AP-792 can become a unique antiarrhythmic compound.

**Fig. 4.** Effects of intravenous administration of AP-792 (1 mg/kg) on the arrhythmias induced by two-stage coronary ligation in dogs (group 3). AP-792 was administered at 24 h (A, \( n = 5 \)) or 48 h (B, \( n = 5 \)) after coronary ligation. Data are expressed as means ± S.E.M. Closed symbols represent significant differences from the pre-administration values (\( P < 0.05 \)).
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