Acute Electropharmacological Effects of Intravenously Administered Amiodarone Assessed in the In Vivo Canine Model

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ABSTRACT—Acute hemodynamic and electrophysiological effects of amiodarone were assessed simultaneously, using the halothane-anesthetized, closed-chest in vivo canine model in comparison with those of solvent ethanol alone. Intravenous administration of the solvent (n = 8) induced no significant change in any of the cardiovascular parameters. On the other hand, intravenous amiodarone in the canine antiarrhythmic dose of 3.0 mg/kg (n = 6) exerted negative chronotropic, inotropic and dromotropic effects in addition to the transient hypotensive action followed by an increase of the total peripheral vascular resistance. Amiodarone also prolonged both the ventricular repolarization phase and the effective refractory period, where the increment was greater in the latter than in the former, indicating the shortening of the electrical vulnerable period of the ventricle. More importantly, appearance of the electrophysiological effect on repolarization took more time and higher dose compared with the effect on refractoriness, which could be detected at a one tenth the dose. These results support the previous knowledge that intravenously administered amiodarone possesses class I, III and IV actions and suggest that shortening of the electrical vulnerable period may be one of the unique antiarrhythmic properties of intravenous amiodarone against re-entry type arrhythmias.

Keywords: Amiodarone, Monophasic action potential, I_{Ks}, I_{Kr}, Repolarization

Amiodarone is generally classified as a Vaughan-Williams class III agent that prolongs repolarization by inhibiting outward potassium currents (1). In addition, previous experimental studies (2 – 7) have shown that amiodarone effectively blocks the sodium and calcium channels in addition to the β-adrenoceptors, and they demonstrated that intravenous amiodarone is effective against the standard laboratory models of ventricular arrhythmias acutely induced by epinephrine, digitalis and coronary ligation. Thus, intravenous amiodarone is now clinically being used for the treatment of frequently recurring ventricular fibrillation and hemodynamically unstable ventricular tachycardia refractory to other therapies (1). However, which action or combination of actions of intravenous amiodarone is fundamental and salutary for its potent antiarrhythmic activity remains incompletely understood because of mutual complex interactions of its multiple pharmacological effects on the cardiovascular system.

The present study was designed to simultaneously assess the acute hemodynamic and electrophysiological effects of intravenous amiodarone using the halothane-anesthetized, closed-chest in vivo canine model and to provide a link between basic observations in vitro and clinical use of the drug (8 – 11). To better analyze the electrophysiological effects on the depolarization and repolarization process, we recorded His bundle electrograms and monophasic action potentials (MAPs), respectively, in addition to the standard lead II surface ECG. Moreover, a MAP recording/pacing combination catheter was used to simultaneously measure both MAP and effective refractory period (ERP) at the same site and directly compare the drug effects on the repolarization and refractoriness (12 – 14).

MATERIALS AND METHODS

All experiments were performed in accordance with Guidelines for Animal Experiments, Yamanashi Medical University. Experiments were carried out using beagle dogs weighing approximately 10 kg. Animals were obtained through the Animal Laboratory for Research of Yamanashi Medical University. Dogs 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-480-3; Shinano, Tokyo). Tidal volume and respiratory
Cardiovascular Effects of Amiodarone

rate were set at 20 ml/kg and 15 strokes/min, respectively. To prevent blood clotting, heparin calcium (100 units/kg, i.v.) was administered.

**Cardiohemodynamic and electrophysiological parameters**

The surface lead II ECG was obtained from the limb electrodes. Corrected QT interval (QTC) was calculated using Bazett’s formula (15). The systemic blood pressure was measured at the left femoral artery. A pig tail catheter was positioned at the left ventricle through the left femoral artery to measure the left ventricular pressure. The maximum upstroke velocity of the left ventricular pressure (LVdP/dt\text{max}) and the left ventricular end-diastolic pressure (LVEDP) were obtained to estimate the contractility and the preload of the left ventricle, respectively. A quad-polar electrodes catheter was positioned at the non-coronary cusp through the right femoral artery to obtain a His bundle electrogram. A thermodilution catheter (TC-704; Nihon Kohden, Tokyo) was positioned at the right side of the heart through the right femoral vein. The cardiac output (CO) was measured by a standard thermodilution method using a cardiac output computer (MFC-1100, Nihon Kohden). Total peripheral vascular resistance (TPR) was calculated using the basic equation:

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TPR = \frac{mean\ blood\ pressure}{cardiac\ output}
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A bi-directional steerable MAP recording/pacing combination catheter (1675P; EP Technologies Inc., Sunnyvale, CA, USA) was positioned at the endocardium of the right ventricle through the left femoral vein to obtain MAP signals. The signals were amplified with a DC preamplifier (model 300, EP Technologies Inc.). The amplitude of the MAP was measured as the distance from the diastolic baseline to the crest of the MAP plateau phase as reported previously (13). The duration of the MAP signal was measured as an interval, along a line horizontal to the diastolic baseline, from the MAP upstroke to the desired repolarization level. The interval at 90% repolarization was defined as MAP\text{90}.

The heart was electrically driven via the pacing electrodes of the combination catheter using a cardiac stimulator (SEC-3102, Nihon Kohden). The stimulation pulses were rectangular in shape, 1–2 V of amplitude (about twice the threshold voltage) and of 1-ms duration. The MAP\text{90} was measured during the sinus rhythm (MAP\text{90(sinus)}) and at the pacing cycle length of 400 ms (MAP\text{90(CL400)}) and at 300 ms (MAP\text{90(CL300)}). In addition, ERP of the ventricle was assessed by the programmed electrical stimulation via the pacing electrodes of the combination catheter. The pacing protocol consisted of 5 beats of basal stimuli in a cycle length of 400 ms followed by an extrastimulus of various coupling intervals. Starting in the late diastole, the coupling interval was shortened in 5–10-ms decrements until refractoriness occurred. The final repolarization phase of the ventricle, i.e., relative refractory period, was analyzed by the difference between the ERP and the MAP\text{90(CL400)} at the same site. In this study, the postrepolarization refractoriness, PRR = ERP – MAP\text{90(CL400)}, was calculated to estimate the extent of the electrical vulnerability of the heart (8–14).

**Experimental protocol**

The systemic blood pressure, left ventricular pressure, ECG, His bundle electrogram and MAP signals were continuously monitored using a polygraph system (RM-6000, Nihon Kohden), and analyzed using a real time full automatic data analysis system (MP/VAS 3 for Macintosh ver 1.0; Physio-Tec, Tokyo). The cardiovascular variables were assessed in the following order at each time point. The cardiac output was measured twice. The ECG, His bundle electrogram, systemic and left ventricular pressure and MAP signal were analyzed during sinus rhythm. Then, MAP signals were analyzed during the ventricular pacing at the cycle length of 400 and 300 ms, while the left ventricular pressure was examined during the ventricular pacing at the cycle length of 400 ms. Finally, ERP of the ventricle was measured. These data were usually obtained within one minute at each time point.

The solvent of commercially available amiodarone injection (Cordarone™) consists of Tween 80, benzyl alcohol and saline. Before starting the current study protocol, we first confirmed the cardiovascular effects of the solvent using the same model (n=2), since the cardiovascular inhibitory actions of amiodarone injection have been reported to be largely due to Tween 80 (1, 16, 17). Typical tracings demonstrating its hypotensive and negative inotropic effects are depicted in Fig. 1. Based on this observation together with the previous knowledge, we dissolved amiodarone with ethanol in this study.

After each basal control cardiovascular value was assessed, amiodarone in a low dose of 0.3 mg/kg was intravenously administered over 30 s. The effects of the drug on each cardiovascular parameter were assessed at 5, 10, 15, 20 and 30 min after the start of injection. Then, amiodarone in a high dose of 3.0 mg/kg, which has been demonstrated to exert an antiarrhythmic action on the canine ventricular arrhythmia models (2), was additionally administered over 30 s. The effects on each parameter were observed at 5, 10, 15, 20, 30, 45 and 60 min after the start of the drug injection. The same protocol was used for analyzing the cardiovascular effects of 15 μl/kg and 150 μl/kg of solvent ethanol.

A volume of 3 ml of blood was drawn from the right femoral artery at each time point to measure the plasma drug concentration. The blood samples were centrifuged at 1,500 × g for 30 min at 4°C. The plasma was stored at −80°C until the drug concentration was measured. Sensi-
tive and specific determinations of the concentrations of amiodarone were performed at the laboratory of SRS Co., Ltd. (Tokyo) using a standard high performance liquid chromatographic (HPLC) method. The limit of quantification was 50 ng/ml.

Drugs
The following drugs were used: amiodarone (Sigma Chemical Company, Tokyo), thiopental sodium (Tanabe Seiyaku, Osaka), halothane (Takeda Chemical Industries, Tokyo) and heparin calcium (Mitsui Pharmaceuticals, Tokyo). Amiodarone was dissolved with 100% ethanol in a concentration of 20 mg/ml.

Statistics
Data are presented as the mean ± S.E.M. The statistical differences of paired data within a group were evaluated by paired t-test or one-way repeated-measures analysis of variance (ANOVA) followed by Contrasts for mean values comparison, while those of unpaired data between the groups were evaluated by the unpaired t-test. A P-value of less than 0.05 was considered significant.

RESULTS
Neither the spontaneously occurring ventricular premature beat nor cardiovascular collapse was observed in any dog during the whole experimental period. After the administration of solvent ethanol, no significant change was detected in any of the cardiovascular parameters during the experimental period.

Effects on the heart rate, mean blood pressure and TPR
The time courses of the heart rate, mean blood pressure and TPR are summarized in Fig. 2. At the pre-drug control, the heart rate (beats/min), mean blood pressure (mmHg)
Cardiovascular Effects of Amiodarone 77

and TPR (mmHg·min/l) were 120 ± 6, 119 ± 4 and 92 ± 10 in the amiodarone group (n = 6) and 120 ± 5, 112 ± 4 and 85 ± 8 in the vehicle group (n = 8), respectively. Each control value was not significantly different between the amiodarone- and vehicle-administered groups. In the amiodarone group, the heart rate decreased, and significant changes were detected for 5–60 min after the high dose of 3.0 mg/kg of amiodarone administration. The mean blood pressure also decreased, and significant changes were detected for 5–15 min after the high dose administration. On the other hand, the TPR increased, and significant changes were detected for 30–60 min after the high dose administration.

Effects on the LVdP/dt\textsubscript{max}

The time courses of the LVdP/dt\textsubscript{max} are summarized in Fig. 3. At the pre-drug control, the LVdP/dt\textsubscript{max} values during the sinus rhythm (LVdP/dt\textsubscript{max(sinus)}) and during the ventricular pacing at a cycle length of 400 ms (LVdP/dt\textsubscript{max(CL400)}) (mmHg/s) were 2,067 ± 240 and 1,844 ± 84 in the amiodarone group (n = 6) and 2,088 ± 88 and 2,025 ± 125 in the vehicle group (n = 8), respectively. Each control value was not significantly different between the amiodarone- and vehicle-administered groups. In the amiodarone group, the LVdP/dt\textsubscript{max(sinus)} as well as LVdP/dt\textsubscript{max(CL400)} decreased, and significant changes were detected in these parameters for 5–60 min after the high-dose administration.

Effects on the LVEDP and CO

The time courses of the LVEDP and CO are summarized in Fig. 3. The LVEDP (mmHg) and CO (l/min) at the pre-drug control were 11.8 ± 1.5 and 1.35 ± 0.09 in the amiodarone group (n = 6) and 11.8 ± 1.2 and 1.39 ± 0.08 in the vehicle group (n = 8), respectively. Each control value was not significantly different between the amiodarone- and vehicle-administered groups. In the amiodarone group, no significant change was observed in the LVEDP during the experimental period. On the other hand, the CO decreased and significant changes were observed for 10–60 min after the high-dose administration.
Effects on the ECG

Typical traces of ECG during the sinus rhythm are shown in Fig. 4, and the time courses of the parameters of ECG are summarized in Fig. 5. The PR interval, QRS width, QT interval (ms) and QTc (ms/s) at the pre-drug control were 103 ± 2, 66 ± 4, 268 ± 10 and 376 ± 11 in the amiodarone group (n = 6) and 97 ± 2, 71 ± 1, 265 ± 8 and 374 ± 7 in the vehicle group (n = 8), respectively. Each control value was not significantly different between the amiodarone- and vehicle-administered groups. In the amiodarone group, the PR interval, QRS width and QT interval were prolonged. Significant increases were observed in the QRS width for 10–60 min and in the PR and QT intervals for 5–60 min after the high-dose administration. On the other hand, QTc was slightly shortened, and a significant decrease was detected only at 60 min after the high-dose administration.

Fig. 3. Time courses of the maximum upstroke velocity of the left ventricular pressure (LVdP/dt_{max}) during the sinus rhythm (LVdP/dt_{max(sinus)}) and the ventricular pacing at a cycle length of 400 ms (LVdP/dt_{max(CL400)}), left ventricular end-diastolic pressure (LVEDP) and cardiac output (CO) after administration of amiodarone (left panels) or solvent ethanol (right panels). Data are presented as the mean ± S.E.M. The closed symbols represent the significant differences from each control value (C) at P<0.05.

Fig. 4. Typical tracings of lead II surface ECG (ECG), His bundle electrogram (His) and monophasic action potentials (MAP) recorded from the right ventricle during sinus rhythm at pre-drug control (Control), 15 min after 0.3 mg/kg of amiodarone administration and 15 min after 3.0 mg/kg of amiodarone administration. Prolongation of QT interval and MAP duration were observed.
Typical traces of the His bundle electrogram and MAP during the sinus rhythm are shown in Fig. 4, and the time courses of the AH and HV intervals and MAP<sub>90(sinus)</sub> are summarized in Fig. 5. The AH and HV intervals and MAP<sub>90(sinus)</sub> (ms) at the pre-drug control were 77 ± 3, 25 ± 1 and 264 ± 8 in the amiodarone group (n = 6) and 73 ± 3, 21 ± 1 and 262 ± 8 in the vehicle group (n = 8), respectively. Each control value was not significantly different between the amiodarone- and vehicle-administered groups. In the amiodarone group, the AH and HV intervals and MAP<sub>90(sinus)</sub> were prolonged. Significant increases were observed in the HV interval from 20 min after the low dose administration to the end of the experiment and in the AH interval and MAP<sub>90(sinus)</sub> for 5 – 60 min after the high dose administration. The maximum prolongations of the AH and HV intervals were +17 ms and +4 ms, respectively, both of which were attained at 10 min after the start of the second dose. The early afterdepolarization potential, i.e., defined as a hump in the last phase of the repolarization in the MAP recording (8, 9), was not observed during the experimental period.

Effects on the AH and HV intervals and MAP<sub>90(sinus)</sub>

The time courses of the MAP<sub>90(CL300)</sub>, MAP<sub>90(CL400)</sub>, ERP and PRR are summarized in Fig. 6. The MAP<sub>90(CL300)</sub>, MAP<sub>90(CL400)</sub>, ERP and PRR (ms) at the pre-drug control were 233 ± 4, 254 ± 5, 226 ± 5 and -28 ± 3 in the amiodarone group (n = 6) and 230 ± 4, 252 ± 8, 230 ± 4 and -22 ± 6 in the vehicle group (n = 8), respectively. Each control value was not significantly different between the amiodarone- and vehicle-administered groups. In the amiodarone group, the MAP<sub>90(CL300)</sub>, MAP<sub>90(CL400)</sub> and ERP were prolonged. Significant increases were observed in the MAP<sub>90(CL300)</sub> at 10 min and for 30 – 45 min after the high dose administration, in the MAP<sub>90(CL400)</sub> for 10 – 60 min after the high-dose administration, and in the ERP from 5 min after the low-dose administration to the end of the experiment. The increment of MAP<sub>90(CL400)</sub> by the amiodarone administration was comparable to those of MAP<sub>90(CL300)</sub> except that the former was greater than the latter only at 60 min after the high-dose administration. On the other hand, PRR was negative during the study. After the low-dose administration, PRR shifted to the positive direction, and significant changes were detected from 5 min after the low-dose administration to 20 min after the high-dose administration. Then, PRR was returned to the basal level for 30 – 60 min after the high-dose administration.

Effects on the MAP<sub>90(CL300)</sub>, MAP<sub>90(CL400)</sub>, ERP and PRR
DISCUSSION

In this study, we assessed the acute electropharmacological effects of amiodarone using the halothane-anesthetized, closed-chest in vivo canine model (8–11). Amiodarone was dissolved in ethanol solvent and intravenously administered in two escalating doses of 0.3 and 3.0 mg/kg. As shown in the results, amiodarone exerted the negative chronotropic, inotropic and dromotropic effects in addition to the transient hypotensive action followed by an increase of the total peripheral vascular resistance, while it prolonged the ventricular refractory period and repolarization process. These results are essentially in accordance with the most of the previous knowledge from animal and clinical studies (1, 18, 19).

Relation between plasma amiodarone concentration and cardiovascular effects

The results of plasma drug concentrations suggest that a clinically and experimentally expected antiarrhythmic plasma concentration of amiodarone can be obtained by the intravenous administration of 3.0 mg/kg (1, 20). However the time course of the plasma drug concentration did not necessarily parallel the extent of each of the cardiovascular effects of amiodarone. Moreover, the appearance of an electrophysiological effect of amiodarone on repolarization took more time and a higher dose when compared with the effect on refractoriness, reflecting the complexity in its mode of ion channel inhibition. In addition, the QTc was slightly shortened after the high dose of amiodarone when Bazett’s formula was used (15), while the MAP90 was significantly prolonged during the constant heart rate of 150 and 200 beats/min. This observation demonstrates the inherent limitation of the formula, by which the QT interval is overcorrected at the canine normal heart rate.

Cardiohemodynamic action of amiodarone

Amiodarone has been reported to have a calcium channel blocking effect and non-competitive weak β-blocking action at plasma concentrations reached in the present experiments (1, 4, 5), which would be the mechanism of the currently observed negative chronotropic, inotropic and dromotropic effect. On the other hand, amiodarone significantly decreased the mean blood pressure, but this effect was transient compared with its cardiac effects; moreover, amiodarone increased TPR. These results are in sharp contrast with our previous observation (2), where long-lasting and severe hypotensive action was induced soon after the injection of clinically available amiodarone injection. Since the solvent of amiodarone injection alone
induced the cardiovascular inhibitory action in this study (Fig. 1) as well as in the previous reports (1, 16, 17), a large part of the previously observed marked hypotensive effect can be considered to be induced by the solvent of amiodarone injection.

Effects on the sodium channels

Currently observed prolonging effects of amiodarone on the HV interval and QRS width suggest its inhibitory effect on the sodium channels, since the intraventricular conduction solely depends on the sodium current (21). This observation supports the previous in vitro reports describing that amiodarone blocks the sodium channels in a use- and voltage-dependent manner (3, 6, 7). More importantly, the prolonging effect on the ERP as well as the HV interval was observed after the administration of low dose of 0.3 mg/kg, while no significant change was detected in other electrophysiological parameters. These results suggest that amiodarone can inhibit the ventricular sodium channels more selectively compared with other cardiac ion channels in vivo.

Effects on the potassium channels

The effects of acute amiodarone on action potential duration have been conflicting; namely, a moderate prolongation has been shown by some investigators, but others have reported no substantial change or shortening (22). Since in this study, intravenously administered amiodarone prolonged the repolarization period, the inhibitory action of amiodarone on the outward currents will be greater than that on the inward currents in the canine ventricular muscle. It should be noted that only a small degree of the reverse use-dependent prolongation of the MAPs was observed by intravenous amiodarone when compared with a specific I_K blocker dofetilide in our previous report (11). This result supports the recent concept of acute effects of amiodarone (22), in which amiodarone can block both I_K and I_Kr. Moreover, in the present in vivo model, ß-blocking action of amiodarone may have enhanced the prolongation of the repolarization period, since I_Kr is regulated by adrenergic tone (23).

Effects on the final repolarization phase

The potential mechanisms of the anti- and pro-arrhythmic effects of amiodarone were analyzed by measuring the ventricular MAP and ERP at the same site to precisely assess the drug effects on the final repolarization phase (relative refractory period) (8 – 14). The prolongation of the relative refractory period has been reported to provide an ideal substrate for slow conduction and re-entry that allows perpetuation of torsades de pointes (14). This is the rationale for using PRR as a marker of the antiarrhythmic effect of drugs. However, PRR cannot be used for estimating the antiarrhythmic potency against the canine ventricular arrhythmia models induced by digitalis, adrenaline and coronary ligation, in which abnormal automaticity, rather than re-entry, plays a major role in the onset (2). As demonstrated in this study, amiodarone prolonged both the ventricular repolarization phase and the effective refractory period, where the increment was greater in the latter than in the former, indicating the shortening of the electrical vulnerable period of the ventricle. Thus, the protection against re-entry type arrhythmias by acute amiodarone can be considered to depend on the balance between its class I and class III actions on the ventricular muscle, both of which may be influenced differently by heart rate, drug doses and time after the drug administration. In addition, PRR of the ventricle would be shifted to a positive direction more effectively during tachycardia by amiodarone, since sodium channel blocking action by amiodarone has been reported to be frequency-dependent (6, 7) and the attenuation of the prolongation of repolarization would be minimal as described above. Experiments are now on-going to demonstrate this hypothesis using the canine in vivo complete atroventricular conduction block models, of which heart rate can be controlled by the ventricular pacing over 40 – 200 beats/min.

In summary, the cardiovascular effects of intravenously administered amiodarone may be due to its class I, III and IV actions, which can be related to its unique spectrum of antiarrhythmic effects in clinical circumstances. Among them, shortening of the electrical vulnerable period would be one of the important antiarrhythmic properties of amiodarone against re-entry type arrhythmias. These results confirmed the complex nature of the in vivo electrophysiological actions of intravenous amiodarone and may provide a possible rationale for its clinical effectiveness.

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