Electropharmacological effects of amantadine on cardiovascular system assessed with J-T<sub>peak</sub> and T<sub>peak</sub>-T<sub>end</sub> analysis in the halothane-anesthetized beagle dogs

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ABSTRACT — Since amantadine-induced long QT syndrome has been clinically reported, we investigated its electropharmacological effects to estimate the extent of proarrhythmic risk by using the halothane-anesthetized beagle dogs (n = 4). Amantadine in doses of 0.1, 1 and 10 mg/kg was infused over 10 min with a pause of 20 min under the monitoring of multiple cardiovascular variables. J-T<sub>peak</sub> and T<sub>peak</sub>-T<sub>end</sub> were separately measured on the lead II electrocardiogram to precisely analyze the net balance between inward and outward current modulations by amantadine. The low dose increased the ventricular contractile force, but suppressed the intraventricular conduction. The middle dose prolonged the QT interval besides enhancing the changes induced by the low dose. The high dose increased the mean blood pressure, left ventricular end-diastolic pressure and total peripheral resistance, and accelerated the atrioventricular nodal conduction, but decreased the cardiac output besides enhancing the changes induced by the middle dose. A reverse use-dependence was confirmed in the repolarization delay. Amantadine hardly affected the J-T<sub>peak</sub>, but prolonged the T<sub>peak</sub>-T<sub>end</sub>. Amantadine can be considered to stimulate Ca<sup>2+</sup> channel but inhibit Na<sup>+</sup> and K<sup>+</sup> channels in the in situ heart. J-T<sub>peak</sub> and T<sub>peak</sub>-T<sub>end</sub> analysis suggests that amantadine may possess modest risk for arrhythmia.

Key words: Amantadine, Early repolarization, Late repolarization, QT prolongation, Torsade de pointes

INTRODUCTION

Amantadine was initially approved in October 1966 by the Food and Drug Administration as a prophylactic agent specially against Asian influenza, and then it was coincidentally found to improve the symptoms of Parkinson’s disease, and thus has been widely used for treating Parkinson’s disease (Hubsher et al., 2012). The anti-parkinsonian action of amantadine has been explained by the effects on central dopamine systems in animal studies (Takahashi et al., 1996). Amantadine has been shown to block K<sup>+</sup> channels (Freeman et al., 1985) as well as Ca<sup>2+</sup> channels in the in vitro study (Hiraoka et al., 1989), whereas in clinical practice, large acute dosage of amantadine has been reported to exert the cardiovascular toxicity manifested as widening of the QRS complex, QT prolongation and torsade de pointes (Manini et al., 2007; Schwartz et al., 2008). However, information regarding integrated in vivo electropharmacological analysis of amantadine still remains limited.

In order to bridge the gap between in vitro information of amantadine and its clinically reported cardiovascular adverse events, in the present study we used the halothane-anesthetized in vivo beagle dogs (Sugiyama, 2008). To better analyze the electrophysiological effects of the drug on the depolarization and repolarization phases, we recorded the His bundle electrogram and monophasic action potential (MAP), respectively, in addition to analyzing the standard lead II electrocardiogram. Moreover, a MAP recording/pacing combination catheter (1675P; EP Technologies, Inc., Sunnyvale, CA, USA) was used to simultaneously measure both MAP and effective refractory period at the same site and directly to compare the drug effects on the repolarization and refractoriness (Sugiyama, 2008). Importantly, early repolarization (J-T<sub>peak</sub>) and late repolarization (T<sub>peak</sub>-T<sub>end</sub>) were measured on the electro-
cardiovascular system. The ventricular pressure was recorded by a catheter through a space between the inside of the sheath and outside of the aortic pressure. The aortic pressure was measured at the non-coronary cusp of the aortic valve through the left femoral artery to obtain the His bundle electrogram. A bidirectional steerable MAP recording/pacing combination catheter (Cordis-Webster, Baldwin Park, 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 direct-current preamplifier (model 300; EP Technologies, Inc.). The duration of the MAP signals was measured as an interval, along a line horizontal to the diastolic baseline, from the MAP upstroke to the desired repolarization level. The interval (ms) at a 90% repolarization level was defined as MAP90.

The heart was electrically driven by using a cardiac stimulator (SEC-3102; Nihon Kohden) through the pacing electrodes of the combination catheter placed in the right ventricle. The stimulation pulses were rectangular in shape, 1-2 V of amplitude (about twice the threshold voltage) and of 1-ms duration. The MAP90 was measured during sinus rhythm (MAP90(sin)) and at a pacing cycle length of 400 ms (MAP90(CL400)) and 300 ms (MAP90(CL300)). The effective refractory period of the right ventricle was assessed with programmed electrical stimulation. The pacing protocol consisted of 5 beats of basal stimuli in a cycle length of 400 ms followed by an extra stimulus of various coupling intervals. Starting in late diastole, the coupling interval was shortened by 5-ms decrement until refractoriness occurred. The duration of the terminal repolarization period of the ventricle was calculated by the difference between the MAP90(CL400) and effective refractory period at the same site, which reflects the extent of electrical vulnerability of the ventricular muscle (Sugiyama, 2008).

J-Tpeak and Tpeak−Tend were measured separately. When the end of T wave was obscure, we used MAP signal as a guide to estimate it as shown in Fig. 1. Since the second peak of biphasic T wave was usually consistent with the end of MAP based on our experience with this method, the second peak may also become an alternative guide to determine Tend when MAP signals were not available (Izumi-Nakaseko et al., 2014; Sugiyama et al., 1994; Sugiyama, 2008). J-Tpeak was corrected for heart rate by using a coefficient as previously described (J-Tpeakcorr=J-Tpeak/RR0.58 with RR in seconds) (Johannesen et al., 2014; Smetana et al., 2003). Correction was not performed on

**MATERIALS AND METHODS**

Experiments were performed with 4 beagle dogs of either sex weighing approximately 10 kg. Animals were obtained from Kitayama Labes Co., Ltd. (Nagano, Japan). All experiments were approved by the Animal Research Committee for Animal Experimentation of Toho University (No. 12-52-151, No. 13-53-151) and performed in accordance with the Guidelines for the Care and Use of Laboratory Animal of Toho University.

**Cardiohemodynamic parameters**

Dogs were anesthetized initially with thiopental sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, 1% halothane vaporized with 100% oxygen was inhaled with a volume-limited ventilator (SN-480-3; Shinano Manufacturing Co., Ltd., Tokyo, Japan). Tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively. To prevent blood clotting, heparin calcium (100 IU/kg) was intravenously administered. A clinically available catheter-sheath set (FAST-CATH™ 406108, St. Jude Medical, Inc., Minnetonka, MN, USA) was inserted into the right femoral artery to introduce a pig-tail catheter for measuring the left ventricular pressure. The aortic pressure was measured at a space between inside of the sheath and outside of the catheter through a flush line. A thermodilution catheter (TC-504NH; Nihon Kohden Corporation, Tokyo, Japan) was positioned at the right side of the heart through the left femoral vein to obtain MAP signals. The signals was measured as an interval, along a line horizontal to the diastolic baseline, from the MAP upstroke to the desired repolarization level. The interval (ms) at a 90% repolarization level was defined as MAP90.

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the Tpeak-Tend, since previous QT studies have shown that the Tpeak-Tend exhibited minimal heart rate dependency at resting heart rate (Johannesen et al., 2014; Smetana et al., 2003).

**Experimental protocol**

The aortic and left ventricular pressures, electrocardiogram, His bundle electrogram and MAP signals were monitored by using a polygraph system (RM-6000; Nihon Kohden) and analyzed with a real time full automatic data analysis system (MP/VAS 3 for Macintosh ver 1.1R24; Physio-Tech Co., Ltd., Tokyo, Japan). Each measurement of electrocardiogram, MAP as well as atrio-His (AH) and His-ventricular (HV) intervals was the mean of three recordings of consecutive complexes. The cardiovascular variables were assessed in the following order. The electrocardiogram, His bundle electrogram, aortic and left ventricular pressures and MAP signals were recorded under sinus rhythm. Next, the cardiac output was measured three times. Then, MAP signals were recorded during the ventricular pacing at a cycle length of 400 and 300 ms. Finally, the effective refractory period was measured. All parameters described above were usually obtained within 1 min at each time point.

After the basal assessment, amantadine in a low dose of 0.1 mg/kg was intravenously infused over 10 min and each parameter was assessed at 5, 10, 15, 20 and 30 min after the start of infusion. Then, amantadine in a middle dose of 1 mg/kg was intravenously infused over 10 min, and each parameter was observed in the same manner. Finally, amantadine in a high dose of 10 mg/kg was intravenously infused over 10 min, and each parameter was assessed at 5, 10, 15, 20, 30, 45 and 60 min after the start of infusion.

**Drugs**

Amantadine hydrochloride was obtained from Sigma-Aldrich (St. Louis, MO, USA), and was dissolved
in saline to a concentration of 10 mg/mL, and then diluted with saline to obtain 1 and 0.1 mg/mL solution. The other drugs used were thiopental sodium (Ravonal® 0.5 g for Injection, Mitsubishi Tanabe Pharma Corporation, Osaka, Japan), halothane (Fluothane®, Takeda Pharmaceutical Co., Ltd., Osaka, Japan) and heparin calcium (Caprocin®, Sawai Pharmaceutical Co., Ltd., Osaka, Japan).

**Statistical analyses**

Data are presented as the mean ± S.E. Differences within a parameter were evaluated by one-way repeated-measures analysis of variance (ANOVA). When a p value was < 0.05 by ANOVA, the drug was judged as having affected the parameter. In this case, statistically significant differences between the pre-drug control (C) and a value at a particular time point after the drug administration were determined by Contrasts as a post-hoc test for mean values comparison, and a p value < 0.05 was considered to be significant.

**RESULTS**

No animals exerted any lethal ventricular arrhythmias or hemodynamic collapse, leading to the animals’ death during the experiment.

**Effects on the cardiohemodynamic variables**

Typical tracings of the aortic and left ventricular pressure are depicted in Fig. 1, and the time courses of changes in the cardiohemodynamic variables are summarized in Fig. 2. The pre-drug basal control values (C) of the heart rate, mean blood pressure, LVdP/dt\text{max}, left ventricular end-diastolic pressure, cardiac output and total peripheral resistance were 99 ± 4 beats/min, 104 ± 6 mmHg, 1,604 ± 78 mmHg/s, 13 ± 1 mmHg, 1.27 ± 0.10 L/min and 84 ± 10 mmHg/(L/min), respectively. The low and middle doses of 0.1 and 1 mg/kg increased the LVdP/dt\text{max} for 20-30 min and 15-30 min after the start of infusion, whereas no significant change was detected in the other variables. The high dose of 10 mg/kg

![Fig. 2](image-url) Time courses of changes in the heart rate (HR), mean blood pressure (MBP), maximum upstroke velocity of the left ventricular pressure (LVdP/dt\text{max}), left ventricular end-diastolic pressure (LVEDP), cardiac output (CO) and total peripheral vascular resistance (TPR) after the administration of amantadine (n = 4). Data are presented as the mean ± S.E. Closed symbols represent significant differences from the corresponding pre-drug basal control value (C) by p < 0.05.
increased the mean blood pressure for 5-60 min, LVdP/dt_{max} for 10-60 min, left ventricular end-diastolic pressure for 10-15 min and total peripheral resistance for 5-60 min, but decreased the cardiac output at 5 min and for 45-60 min after the start of infusion, whereas no significant change was detected in the heart rate.

**Effects on the electrocardiogram during sinus rhythm**

Typical tracings of the electrocardiogram are depicted in Fig. 1 and the time courses of changes in the electrocardiogram variables are summarized in Fig. 3 (left upper panel). The pre-drug basal control values (C) of the PR interval, QRS width, QT interval and QTcV for 5-60 min, but shortened the PR interval for 15-30 min.

The dose-related changes in the J-T_{peakc} and T_{peakc}-T_{endc} are summarized in Fig. 4. The pre-drug basal control values (C) of the J-T_{peakc} and T_{peakc}-T_{endc} for amantadine were 170 ± 2 and 91 ± 7 ms. The low and middle doses hardly affected the J-T_{peakc} or T_{peakc}-T_{endc}. The high dose prolonged the T_{peakc}-T_{endc}, whereas no significant change was detected in the other variables. The high dose prolonged the QRS for 5-60 min, QT and QTcV for 5-60 min, but shortened the PR interval for 15-30 min.

Effects on the AH and HV intervals, and MAP_{90} during sinus rhythm

Typical tracings of the His bundle electrogram and MAP are depicted in Fig. 1 and the time courses of changes in the AH and HV intervals, and MAP_{90} during sinus rhythm are summarized in Fig. 3 (left lower panels), of which pre-drug basal control values (C) were 84 ± 3, 23 ± 1 and 242 ± 14 ms, respectively. The low and middle doses prolonged the HV interval at 15 and 30 min, and for 5-30 min, respectively, whereas no significant change was detected in the other variables. The high dose prolonged the HV interval and MAP_{90(totum)} for 5-60 min, but it shortened the AH interval for 10-60 min.

Effects on the MAP_{90} during the ventricular pacing, effective refractory period and terminal repolarization period

The time courses of changes in the MAP_{90(CL400)}, MAP_{90(CL300)}, effective refractory period and terminal repolarization period are summarized in Fig. 3 (right panels), of which pre-drug basal control values (C) were 238 ± 12, 214 ± 9, 204 ± 6 and 34 ± 6 ms, respectively. The MAP_{90(CL400)} and MAP_{90(CL300)} were tended to be prolonged, which did not achieve a statistical significance at any dose of amantadine unlike the MAP_{90(totum)}. No significant change was detected in the effective refractory period or terminal repolarization period during the experiment.

**DISCUSSION**

Given limited information on the in vivo electrophysiological profile of amantadine, we assessed it by using well-established halothane-anesthetized canine model (Sugiyama, 2008). We found that amantadine shortened the PR interval but prolonged the QRS width and QT interval in vivo, and that it hardly affected early repolarization but prolonged late repolarization.

**Rationale for drug dose**

Three escalating i.v. doses of 0.1, 1 and 10 mg/kg were selected, since clinically recommended oral daily dose is described to be 100-300 mg/body in Japan in drug information from the manufacturer. The steady-state trough concentrations after 50, 200 and 300 mg/day, p.o. were reported to be 110 ± 39 (0.7), 302 ± 80 (2.0) and 572 ± 207 ng/mL (3.8 μM), respectively in healthy young adult subjects (Aoki et al., 1985). Meanwhile, according to our previous experience with the same halothane-anesthetized canine model as used in this study (Izumi-Nakaseko et al., 2014; Kise et al., 2010), C_{max} after the administration of 0.1, 1 and 10 mg/kg of amantadine could be estimated to be about 0.1 (0.5), 1 (5.3) and 10 μg/mL (53 μM), respectively. Thus, the doses of amantadine assessed in this study would be considered to provide subtherapeutic to supratherapeutic levels of the plasma drug concentrations.

**Cardiohemodynamic effects**

The low and middle doses hardly affected any of the cardiohemodynamic parameters except for the increase of the LVdP/dt_{max}, indicating that amantadine can exert a positive inotropic effect at clinically relevant concentrations, which is in good accordance with the previous in vitro (Freeman et al., 1985) and in vivo reports (Vernier et al., 1969). Amantadine has been shown to block Ca^2+ channels in a previous in vitro study (Hiraoaka et al., 1989). Also, when propranolol was added prior to amantadine, force of contraction was not increased in the
isolated guinea-pig atria (Freeman et al., 1985). Thus, currently observed positive inotropic effect of amantadine can be considered to be caused by not only the release of catecholamines from sympathetic nerve terminals (Farnebo et al., 1971), but also the inhibition of cardiac K⁺ channels leading to the prolongation of action potential duration, both of which may increase inward Ca²⁺ current (Freeman et al., 1985).

The high dose increased both the ventricular contraction and the total peripheral resistance, resulting in the increase in blood pressure. The increase of the afterload may be induced by the release of noradrenaline from the peripheral nerve terminals into the vessels (Lechin et al., 2010), and this vasoressor effect was consistent with previous in vivo study of rats (Ikeda et al., 2015). Meanwhile, the cardiac output decreased, but the preload to the ventricle increased, which may suggest that extent of increase in the afterload was greater than that of the ventricular contraction. No statistically significant change was detected in the heart rate, which is in accordance with

**Fig. 3.** Time courses of changes in the PR interval, QRS width, QT interval and QTcV; the atrio-His (AH) and His-ventricular (HV) intervals, and monophasic action potential duration at 90% repolarization level during sinus rhythm (MAP₉₀ms(sinus)); the monophasic action potential duration at a cycle length of 400 ms (MAP₉₀ms(CL400)) and 300 ms (MAP₉₀ms(CL300)); and the effective refractory period of the right ventricle (ERP) and terminal repolarization period (TRP) after the administration of amantadine (n = 4). Data are presented as the mean ± S.E. Closed symbols represent significant differences from the corresponding pre-drug basal control value (C) by p < 0.05.
a previous study of mongrel dogs which were anesthe-
tized with a mixture of barbital sodium and pentobarbital (Vernier et al., 1969). The lack of change in the heart rate may suggest that reflex-mediated increase of vagal tone following the amantadine-induced vasopressor response might have counteracted its noradrenaline release-de-
dependent direct positive chronotropic effect.

Electrophysiological effects
Amantadine delayed the intraventricular conduction in a dose-related manner, indicating that amantadine may inhibit the Na+ channel in vivo (Sugiyama et al., 1994), which is in accordance with an in vitro study (Freeman et al., 1985). However, the effective refractory period tended to be prolonged, which did not achieve a statistical significance, whereas dose-dependent prolongation was observed in HV interval. Based on our experience with the halothane-anesthetized canine model (Izumi-Nakaseko et al., 2014; Sugiyama et al., 1994; Sugiyama, 2008), HV interval can be more sensitive than effective refractory period in detecting the drug-induced Na+ channel inhibition, since detection power was 1 ms for HV interval and 5 ms for effective refractory period. The high dose shortened the PR and AH intervals, suggesting a stimulatory effect on Ca2+ channel, which is in sharp contrast to an in vitro study showing that amantadine blocked the calcium current at the concentration of 25 μM in guinea-pig hearts (Hiraoka et al., 1989). The discrepancy between in vivo and in vitro results on the dromotropic effect can be explained as follows: the extent of Ca2+-channel stimulation in the atrioventricular node via noradrenaline release from the peripheral nerve terminals induced by amanta-
dine may be greater than that of its direct Ca2+-channel inhibition.

The high dose delayed the repolarization at the heart rate of about 100 beats/min, which is directionally in accordance with previous studies of guinea pig, frog and rabbit (Freeman et al., 1985; Salata et al., 1982). How-
ever, such significant repolarization delay was not detect-
ed during the ventricular pacing at a heart rate of 150 or 200 beats/min, indicating that amantadine delayed the repolarization more potently at slower ventricular rate; namely, reverse use-dependent prolongation of repo-
larization, which may reflect I_K (rapid component of the delayed rectifier potassium current) inhibitory property of a drug in vivo (Sugiyama, 2008).

Effects on the J-Tpeakc and Tpeak-Tend variables
Amantadine prolonged the late repolarization, which may be induced by the inhibition of inward rectifier K+ current (I_{IK}) (Hiraoka et al., 1989) and I_{IK}, but it did not affect the early repolarization. As discussed above, aman-
tadine may suppress I_{IK} and Na+ channels, but indirectly stimulate Ca2+ channel in vivo, suggesting that the impact of Na+ channel inhibition might have been counteracted by the sum of those of K+ channel suppression and Ca2+ channel stimulation during the early repolarization, that may explain the lack of change in the J-Tpeakc.

Antiarrhythmic/proarrhythmic potential
In order to better characterize the effects of amanta-
dine on the net balance between the inward and outward

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![Fig. 4. Time courses of changes in the increments of J-Tpeakc (ΔJ-Tpeakc: left) and Tpeak-Tend (ΔTpeak-Tend: right) from the corresponding pre-drug basal control value (C) after the infusion of amantadine (circles), E-4031 (squares) and amiodarone (triangles). Data are presented as the mean ± S.E. Closed symbols represent significant differences from the corresponding pre-drug basal control value (C) by p < 0.05.](image-url)
currents, we reanalyzed the data of our previous studies (Izumi-Nakaseko et al., 2014; Ohara et al., 2014), in which cardiovascular effects of E-4031 in doses of 0.01 and 0.1 mg/kg and amiodarone hydrochloride in doses of 0.3 and 3 mg/kg were assessed with the same experimental protocol as used in this study. We calculated the $J$-$T_{peakc}$ and $T_{peakc}$-$T_{end}$ for E-4031 at the end of infusion at each dose, and for amiodarone at 10 min after the end of infusion at each dose. The pre-drug basal control values (C) of the $J$-$T_{peakc}$ and $T_{peakc}$-$T_{end}$ for E-4031 were $167 \pm 9$ and $84 \pm 14$ ms, and those for amiodarone were $168 \pm 8$ and $84 \pm 18$ ms, respectively. E-4031 prolonged both $J$-$T_{peakc}$ and $T_{peakc}$-$T_{end}$ in a dose-related manner, whereas amiodarone tended to shorten the $J$-$T_{peakc}$ but its high dose prolonged the $T_{peakc}$-$T_{end}$, as shown in Fig. 4. Meanwhile, amantadine hardly affected the $J$-$T_{peakc}$, but prolonged the $T_{peakc}$-$T_{end}$. These results suggest that proarrhythmic potential of amantadine will be in the middle between amiodarone and E-4031 (Johannesen et al., 2014). This finding is further supported by a lack of change in the terminal repolarization period by amantadine, which is known to be a selective IKr channel blockade by E-4031 on ventricular electrocardiogram: randomized study of dofetilide, quinidine, ranolazine, and verapamil. Clin Pharmacol Ther., 96, 549-558. (in Japanese).

In conclusion, the present study indicates that clinically-relevant doses of amantadine increased the ventricular contractile force and delayed the intraventricular conduction, whereas its supratherapeutic dose exerted unique cardiovascular profile, including the contraction of resistance vessels, enhancement of atrioventricular nodal conduction and reverse frequency-dependent prolongation of the ventricular repolarization in addition to the effects observed by clinically-relevant doses. Thus, amantadine may directly inhibit Na+ and K+ channels and indirectly enhance the Ca2+ channel in the in situ heart in vivo besides the dopamine-dependent peripheral vasoconstriction. Analysis of the early and late repolarizations together with the terminal repolarization period suggests that the extent of proarrhythmic potential of amantadine will be in the middle between E-4031 and amiodarone.

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Conflict of interest——The authors declare that there is no conflict of interest.

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