

Full Paper

Effects of Acute Intravenous Administration of Pentamidine, a Typical hERG-Trafficking Inhibitor, on the Cardiac Repolarization Process of Halothane-Anesthetized DogsHirofumi Yokoyama^{1,2}, Yuji Nakamura¹, Hiroshi Iwasaki¹, Yukitoshi Nagayama^{1,2,3}, Kiyotaka Hoshiai^{1,2,3}, Yoshitaka Mitsumori¹, and Atsushi Sugiyama^{1,2,*}¹Department of Pharmacology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo, Yamanashi 409-3898, Japan²Yamanashi Research Center of Clinical Pharmacology, Fuefuki, Yamanashi 406-0023, Japan³Sugi Institute of Biological Science, Co., Ltd., Hokuto, Yamanashi 408-0044, Japan

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Abstract. Although acute treatment of pentamidine does not directly modify any ionic channel function in the heart at clinically relevant concentrations, its continuous exposure can prolong QT interval. Recent in vitro studies have indicated that hERG trafficking inhibition may play an important role in the onset of pentamidine-induced long QT syndrome. In this study, we examined acute in vivo electropharmacological effects of pentamidine using the halothane-anesthetized canine model (n = 5). The clinically relevant total dose of 4 mg/kg of pentamidine (namely, 1 mg/kg, i.v. over 10 min followed by 3 mg/kg, i.v. over 10 min with a pause of 20 min) decreased the mean blood pressure, ventricular contraction, preload to the left ventricle, and peripheral vascular resistance. Pentamidine also enhanced the atrioventricular conduction in parallel with its cardiohemodynamic actions, but it gradually prolonged both the ventricular repolarization period and effective refractory period, whereas no significant change was detected in the intraventricular conduction. Thus, acute administration of a clinically relevant dose of pentamidine can suppress cardiac function and vascular tone with reflex-mediated increase of sympathetic activity, whereas it may delay the repolarization process, suggesting that inhibition of potassium-channel trafficking might be induced more acutely in vivo than those previously expected in vitro.

Keywords: pentamidine, monophasic action potential, QT prolongation, torsades de pointes, trafficking

Introduction

The antiprotozoal agent pentamidine has been widely used for the treatment of *Pneumocystis carinii* pneumonia, a common opportunistic infection (1, 2). The drug is also used in developing countries for the treatment of parasitic diseases such as trypanosomiasis and antimony-resistant visceral leishmaniasis (3, 4). Meanwhile, pentamidine has been clinically shown to induce QT-interval prolongation, leading to the onset of

lethal ventricular arrhythmias, namely, torsades de pointes (5–9). The mechanism of pentamidine-induced long QT syndrome has been extensively investigated in vitro (10, 11). Acute treatment of therapeutically relevant concentrations of pentamidine hardly affected human ether a-go-go related gene (hERG) current, but its continuous exposure for >16 h can decrease hERG channel current together with the reduction of hERG channel protein of cell surface membrane (11). Since pentamidine did not affect the other cardiac ion channels either by acute or continuous exposure (11), hERG trafficking inhibition is considered to play a major role for the pentamidine-induced long QT syndrome (10, 11). Similar inhibitory action on hERG trafficking has

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been reported with some other drugs, including arsenic trioxide, an anticancer agent for acute promyelocytic leukemia, and ketoconazole, an antifungal agent (12, 13).

A previous experiment indicated that about 50% of initially synthesized immature hERG channel protein was converted into the mature form within the first 4 h, which decayed with a half-life of about 11 h (14). Since drug-induced inhibition of trafficking or turnover of hERG-channel protein has been studied only by *in vitro* experiments, *in vivo* information is still lacking regarding the time course of inhibitory effect of a hERG-trafficking inhibitor on the cardiac repolarization process. In the present study, we clarified such a profile using the halothane-anesthetized *in vivo* canine model, whose electrophysiological properties have been shown to be similar to those in humans (15–17). 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 the standard lead II electrocardiogram (ECG) (15–17).

Materials and Methods

Experiments were carried out using five beagle dogs of either sex weighing approximately 10 kg. Animals were obtained through the Animal Laboratory for Research of University of Yamanashi. All experiments were performed according to Guidelines for Animal Experiments, University of Yamanashi.

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, Tokyo). 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 heparinized catheter was placed in the aorta for continuous monitoring of systemic blood pressure through the right femoral artery. 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). The total peripheral resistance (TPR) was calculated using the basic equation: $TPR = \text{mean blood pressure} / \text{cardiac output}$. A pig-tail catheter was positioned at the left ventricle through the right femoral artery to measure the left ventricular pressure. The maximum upstroke velocity of the ventricular pressure

($LVdP/dt_{max}$) and the left ventricular end-diastolic pressure (LVEDP) were obtained during sinus rhythm to estimate the contractility and the preload to the left ventricle, respectively.

Electrophysiological parameters

The surface lead II ECG was obtained from the limb electrodes. Corrected QT interval (QTc) was calculated using Van de Water's formula (18). A quad-polar electrodes catheter was positioned at the non-coronary cusp of the aortic valve through the left femoral artery to obtain the His bundle electrogram. A bi-directional steerable monophasic action potential (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 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 90% repolarization level was defined as MAP_{90} .

The heart was electrically driven using a cardiac stimulator (SEC-3102, Nihon Kohden) with the pacing electrodes of the combination catheter placed in the right ventricle. The stimulation pulses were rectangular in shape, 1–2 V (about twice the threshold voltage) and of 1-ms duration. The MAP_{90} was measured during sinus rhythm ($MAP_{90(sinus)}$) and at a pacing cycle length of 400 ms ($MAP_{90(CL400)}$) and 300 ms ($MAP_{90(CL300)}$). The effective refractory period (ERP) of the right ventricle was assessed by the 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 in 5–10 ms decrements until refractoriness occurred. The duration of the terminal repolarization period (TRP) of the ventricle was calculated by the difference between the $MAP_{90(CL400)}$ and ERP at the same site, which reflects the extent of electrical vulnerability of the ventricular muscle (19).

Experimental protocol

The systemic blood pressure, left ventricular pressure, ECG, His bundle electrogram, and MAP signals were monitored using a polygraph system (RM-6000, Nihon Kohden) and analyzed using a real-time full automatic data analysis system (WinVAS3 ver 1.1R03; Physio-Tech, Tokyo). Each measurement of ECG, 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 CO was measured three times. The ECG, His bundle electrogram, systemic and left ventricular pressure, and MAP signal were recorded under sinus rhythm. In addition, MAP signals were recorded during the ventricular pacing at a cycle length of 400 and 300 ms. Then, ERP was measured. All parameters described above were usually obtained within 1 min at each time point.

After the basal control assessment, pentamidine in a low dose of 1 mg/kg was administered over 10 min; and each parameter was assessed 5, 10, 15, 20, and 30 min after the start of the infusion. Then, pentamidine in a high dose of 3 mg/kg was administered over 10 min; and each parameter was assessed 5, 10, 15, 20, 30, 45, and 60 min after the start of the infusion. The dose of pentamidine was determined based on the clinically recommended dose; namely, 4 mg/kg, i.v. over 1–2 h a day. After the cessation of the study protocol, the animals were moved to the animal intensive care unit for proper care of survival.

Drugs

Pentamidine isetionate [4,4'-(pentamethylenedioxy) dibenzamidine bis(2-hydroxy-ethanesulfonate)] (Benambax[®]; Sanofi Aventis, Tokyo) was dissolved with distilled water in a concentration of 50 mg/mL and diluted with saline in concentrations of 1 and 3 mg/mL. Other drugs used were thiopental sodium (Tanabe, Osaka), halothane (Takeda, Tokyo), and heparin calcium (Sawai, Osaka).

Statistical analysis

Data are presented as the mean \pm S.E.M. 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, statistical significance between the pre-drug control (C) and a value at a particular time point after the drug administration was determined by Contrast for mean values comparison, and a P value <0.05 was considered significant.

Results

Three animals out of five transiently fell in a cardiohemodynamic collapse after the start of the high-dose infusion; however, no animals died during the experimental period.

Effects on the heart rate and blood pressure

The time courses of changes in the heart rate and mean blood pressure are summarized in Fig. 1 ($n=5$) and typical tracings of the blood pressure are depicted in Fig. 2. The heart rate and mean blood pressure at the pre-drug control (C) were 102 ± 7 beats/min and 107 ± 5 mmHg, respectively. The heart rate transiently increased for 5–15 min after the start of the low-dose (1 mg/kg) infusion, but no significant change was detected after the start of the high-dose (3 mg/kg) infusion. The mean blood pressure decreased for 5–60 min after the start of the high-dose infusion.

Effects on the CO and TPR

The time courses of changes in the CO and TPR are summarized in Fig. 1 ($n=5$). The CO and TPR at the pre-drug control (C) were 1.44 ± 0.18 L/min and 81 ± 13 mmHg \cdot (L/min)⁻¹, respectively. The CO

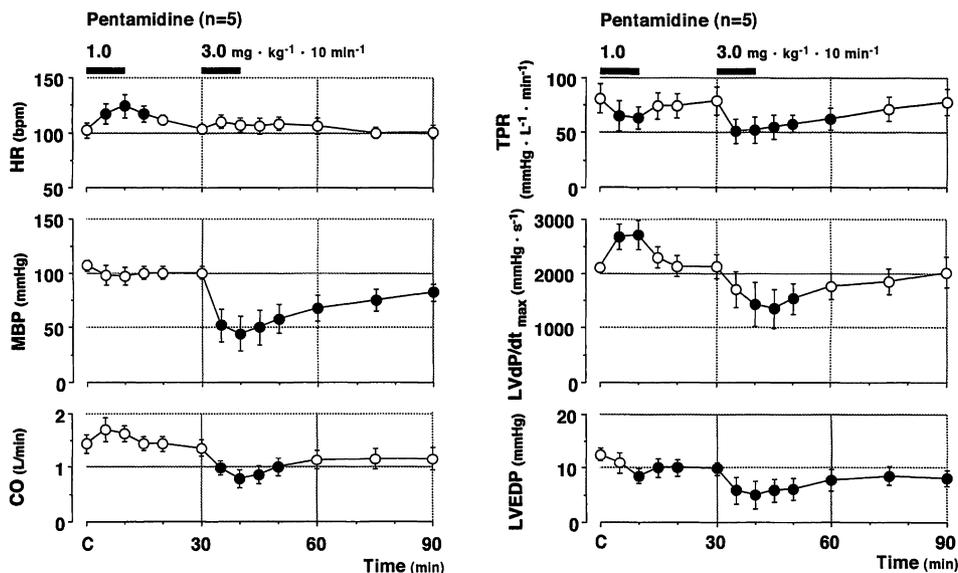


Fig. 1. Cardiohemodynamic effects of pentamidine in the halothane-anesthetized dogs. Time courses of the heart rate (HR), mean blood pressure (MBP), cardiac output (CO), total peripheral resistance (TPR), maximum upstroke velocity of the left ventricular pressure (LVdP/dt_{max}), and left ventricular end-diastolic pressure (LVEDP). Data are each presented as the mean \pm S.E.M. ($n=5$). Closed symbols represent significant differences from each control value (C) by $P < 0.05$.

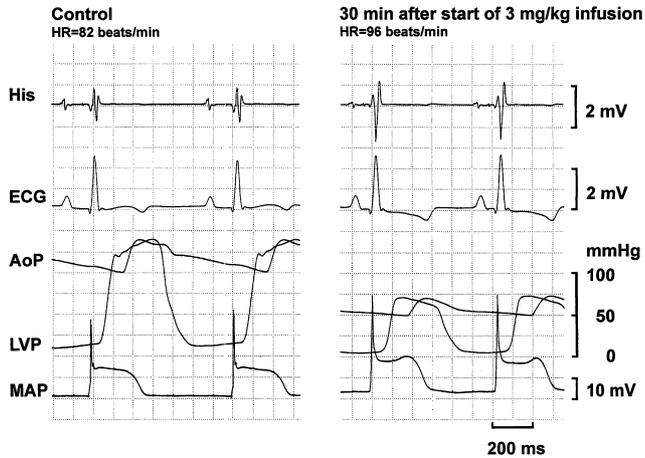


Fig. 2. Typical tracings of the His Bundle electrogram (His), lead II surface electrocardiogram (ECG), aortic pressure (AoP), left ventricular pressure (LVP), and monophasic action potential (MAP) recorded from the right ventricle during the sinus rhythm at pre-drug control (Control) and 30 min after the start of 3 mg/kg of pentamidine infusion.

decreased for 5–20 min after the start of the high-dose infusion. The TPR decreased for 5–10 min after the start of the low-dose infusion and for 5–30 min after the start of the high-dose infusion.

Effects on the LVdP/dt_{max} and LVEDP

The time courses of changes in the LVdP/dt_{max} and LVEDP are summarized in Fig. 1 (n = 5), and typical tracings of the left ventricular pressure are depicted in Fig. 2. The LVdP/dt_{max} and LVEDP at the pre-drug control (C) were 2,103 ± 71 mmHg/s and 12 ± 1 mmHg, respectively. The LVdP/dt_{max} increased for 5–10 min after the start of the low-dose infusion, whereas it decreased for 10–20 min after the start of the high-dose infusion. The LVEDP decreased for 10–30 min after the start of the low-dose infusion and for 5–60 min after the start of the high-dose infusion.

Effects on the ECG

Typical tracings of the ECG are depicted in Fig. 2, and the time courses of changes in the PR interval, QRS width, QT interval, and QTc are summarized in Fig. 3 (n = 5). The PR interval, QRS width, QT interval, and QTc at the pre-drug control (C) were 111 ± 8, 68 ± 3, 273 ± 15, and 308 ± 12 ms, respectively. The PR interval was shortened for 5–15 min after the start of the low-dose infusion and for 5–60 min after the start of the high-dose infusion. Both the QT interval and QTc were prolonged. Significant changes were observed in the QT interval at 10 min and for 30–60 min after the start of the high-dose infusion and in the QTc for 5–60

min after the start of the high-dose infusion. No significant change was detected in the QRS width during the experimental period. Prominent morphological changes of T wave were observed in two animals out of 5; one was flattening, and the other was negative inversion (data not shown).

Effects on the AH and HV intervals and MAP duration during the sinus rhythm

Typical tracings of the His bundle electrogram and MAP are depicted in Fig. 2, and the time courses of changes in the AH and HV intervals and MAP₉₀ during the sinus rhythm are summarized in Fig. 3 (n = 5). The AH and HV intervals and MAP_{90(sinus)} at the pre-drug control (C) were 87 ± 8, 28 ± 2 and 234 ± 14 ms, respectively. The AH interval was shortened for 5–15 min after the start of the low-dose infusion and 5–60 min after the start of the high-dose infusion. MAP_{90(sinus)} was prolonged for 20–60 min after the start of the high-dose infusion. No significant change was detected in the HV interval during the experimental period.

Effects on the MAP₉₀ during the ventricular pacing

The time courses of changes in MAP₉₀ during the ventricular pacing at a cycle length of 400 and 300 ms are summarized in Fig. 3 (n = 5). The MAP_{90(CL400)} and MAP_{90(CL300)} at the pre-dose control (C) were 227 ± 12 and 205 ± 10 ms, respectively. Both the MAP_{90(CL400)} and MAP_{90(CL300)} were prolonged. Significant changes were observed in the MAP_{90(CL400)} for 10–60 min after the start of the high-dose infusion and in the MAP_{90(CL300)} at 10 min and for 20–60 min after the start of the high-dose infusion. The time courses of the increments from the pre-drug control (C) in the MAP_{90(CL400)} and MAP_{90(CL300)} were also calculated (not shown in the figure), of which peak prolongations were both +10% of the control at 45 min after the high-dose infusion.

Effects on the ERP and TRP

The time courses of changes in ERP and TRP are summarized in Fig. 3 (n = 5). The ERP and TRP at the pre-drug control (C) were 201 ± 8 and 26 ± 5 ms, respectively. The ERP was shortened at 5 min after the start of the low-dose infusion, whereas it was prolonged for 15–60 min after the start of the high-dose infusion. No significant change was detected in the TRP during the experimental period.

Discussion

In the present study, we examined the effects of a single i.v. administration of pentamidine, a hERG trafficking inhibitor (10, 11), on the cardiac repolariza-

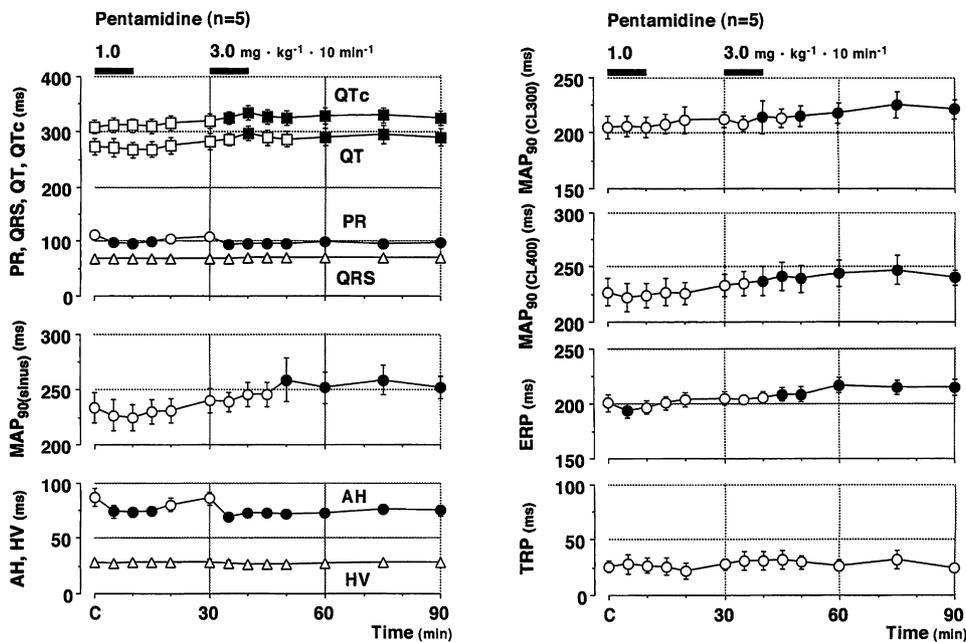


Fig. 3. Electrophysiological effects of pentamidine in the halothane-anesthetized dogs. Time courses of the PR interval (circles), QRS width (triangles), QT interval (squares), and QTc corrected by Van de Water's formula (QTc, squares) (left upper panel); those of the atrio-His interval (AH, circles), His-ventricular interval (HV, triangles), and MAP₉₀ during sinus rhythm (MAP_{90(sinus)}) and 400 ms (MAP_{90(CL400)}) and 300 ms (MAP_{90(CL300)}) (left lower panels); those of the MAP₉₀ at a pacing cycle length of 300 ms (MAP_{90(CL300)}) and 400 ms (MAP_{90(CL400)}) (right upper panels); and the effective refractory period (ERP) and terminal repolarization period (TRP = MAP_{90(CL400)} - ERP) (right lower panels). MAP₉₀ represents the duration of monophasic action potential at 90% repolarization level. Data are each presented as the mean ± S.E.M. (n = 5). Closed symbols represent significant differences from each control value (C) by $P < 0.05$.

tion process using the well-established halothane-anesthetized *in vivo* canine model (15–17). Pentamidine is clinically administered by intramuscular injection and intravenous infusion over 2 h in a dose of 4 mg/kg a day, of which peak plasma concentrations have been reported to be 209 ng/mL (0.4 μ M) and 612 ng/mL (1 μ M), respectively (20). In this study, we administered 1 mg/kg of pentamidine over 10 min followed by 3 mg/kg over 10 min with a pause of 20 min to better compare the cardiovascular effects of pentamidine with those of previously assessed drugs under the same experimental protocol as used in this study (15–17). Thus, the peak plasma concentration of pentamidine in this study may be transiently higher than those expected in clinical practice, although totally administered doses were essentially the same.

Cardiohemodynamic effects

The low dose of 1 mg/kg of pentamidine decreased the preload to the left ventricle and peripheral vascular resistance, but it increased the heart rate and ventricular contraction, whereas it hardly affected the mean blood pressure, suggesting that reflex-mediated increase of sympathetic tone may play some role. Meanwhile, the high dose of 3 mg/kg of pentamidine decreased the mean blood pressure and ventricular contraction in addition to further reducing the preload to the left ventricle and peripheral vascular resistance, suggesting that pentamidine can directly suppress the cardiac function and vascular tone. Similar hypotensive action with reflex-mediated tachycardia has been reported in

urethane-anesthetized rats (21, 22) and in patients (23–25), whereas the other cardiohemodynamic effects of pentamidine described above have not been reported before.

It should be noted that in this study, three animals out of five fell in cardiohemodynamic collapse after the start of the high-dose infusion. In spite of such an episode, all animals recovered after the experiment without any neurological deficit. In a previous study (17), we have reported that the suprathreshold dose of potassium canrenoate can cause cardiohemodynamic collapse, resulting in the animal's death in two out of five experiments, and moreover, the other three animals did not recover after the experiment because of the brain damage. The extent of suppressive effects on the cardiovascular system was similar between pentamidine in this study and canrenoate in the previous study (17) except that pentamidine decreased LVEDP, which was hardly affected by canrenoate. Thus, reduction of the preload to the left ventricle might be at least in part related to the better neurological prognosis after the severe hypotension.

Electrophysiological effects

Pentamidine enhanced the atrioventricular conduction in a dose-related manner, which might be explained by reflex-mediated increase of sympathetic tone, also supporting a previous report that pentamidine did not directly inhibit L-type Ca²⁺ channels in ventricular myocytes of guinea pigs (10, 11). On the other hand, pentamidine hardly affected intraventricular conduction

time at either dose, indicating that pentamidine may have no effect on cardiac Na^+ channels, since the intraventricular conduction solely depends on the Na^+ -channel activity (26). This observation is in accordance with a previous *in vitro* report that pentamidine did not modify Na^+ current in HEK293 cells stably expressing the cardiac Na^+ channel gene *SCN5A* (11).

As clearly shown in the results, acute *i.v.* administration of pentamidine gradually produced repolarization delay, which has not been demonstrated *in vivo* before. The peak response was obtained 45 min after the start of the high-dose administration. Meanwhile in the *in vitro* study, pentamidine has been reported to inhibit hERG current with an IC_{50} of 252 μM (150 $\mu\text{g}/\text{mL}$) (10), which is far above the therapeutic plasma concentration of 1 μM (612 ng/mL) (20). In this study, we administered a clinically relevant dose of pentamidine, which would provide a peak plasma concentration of about 1 to 3 $\mu\text{g}/\text{mL}$ based on our previous experience with this *in vivo* model (15–17, 27–29), indicating the presence of a large difference between the *in vitro* IC_{50} and estimated plasma concentration in this study. Thus, currently observed *in vivo* repolarization delay could not be explained by its direct I_{Kr} -channel blockade.

It has been reported that 48-h treatment with 3 μM (1.8 $\mu\text{g}/\text{mL}$) of pentamidine resulted in 44% reduction of the hERG polypeptide (10), and 16–20 h (overnight) treatment of pentamidine in concentrations of 0.01–10 μM reduced cell surface expression of hERG protein in a concentration-dependent manner with an IC_{50} of 7.8 μM (4.8 $\mu\text{g}/\text{mL}$) (11). So, the estimated peak concentration of pentamidine in our study would be close to the IC_{50} value for hERG trafficking *in vitro*. In addition, it has been shown that overnight treatment with pentamidine did not affect the expression of $\text{Kv}1.5$ (ultra-rapidly activating delayed rectifier current I_{Kur}) channel or current density of $\text{KvLQT1}/\text{minK}$ (slow component of the delayed rectifier current I_{Ks}) and $\text{Kv}4.3$ (transient outward current I_{to}) (11). Although possibilities, including that unknown metabolites of pentamidine might have inhibited I_{Kr} channels or pentamidine by itself could have inhibited the hERG protein synthesis or increased the turnover rate of membrane protein, cannot be totally excluded by this study, a more plausible explanation for the current observation would be that inhibition of I_{Kr} channel trafficking by pentamidine can be induced more acutely than previously expected.

It should be also noted that the repolarization period was gradually prolonged after the low-dose infusion, although it did not achieve statistical significance, suggesting that I_{Kr} -channel trafficking inhibition might have appeared soon after the treatment with the low dose of

pentamidine. This speculation may at least in part explain why pentamidine did not induce tachycardia at the high dose in spite of induction of sympathetic reflex as reflected by shortening of AH interval.

The effective refractory period was transiently shortened during the low-dose infusion of pentamidine, whereas it was prolonged at the high dose. The former could be explained by the increased adrenergic tone, whereas the latter might depend on the class III effect. On the other hand, as shown in the results, pentamidine slightly but significantly prolonged the ventricular repolarization period and effective refractory period to a similar extent, leading to backward shift of the terminal repolarization period in the cardiac cycle length. Based on our previous studies with QT-prolonging drugs, this type of electrophysiological effect suggests proarrhythmic potentials (30).

Conclusions

The present *in vivo* experimental study indicates that acute single *i.v.* administration of a clinically relevant dose of pentamidine can delay the repolarization process possibly via I_{Kr} channel-traffic inhibition besides suppressing the cardiac function and vascular tone.

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