Electropharmacological Effects of a Spironolactone Derivative, Potassium Canrenoate, Assessed in the Halothane-Anesthetized Canine Model

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Abstract. While aldosterone receptor blockers improve survival of patients with congestive heart failure, spironolactone and its derivatives were recently shown to block ether-a-go-go-related gene (HERG) channels and native I_{Kr} and I_{Ks} currents in guinea pig ventricular myocytes. In this study, we examined in vivo electropharmacological effects of an active derivative of spironolactone, potassium canrenoate, using a halothane-anesthetized canine model. Potassium canrenoate was intravenously administered in three doses of 1, 10, and 100 mg/kg per 10 min with a pause of 20 min between doses (n = 5). The low dose hardly affected any of the cardiovascular parameters. The middle dose, a clinically recommended daily maximum i.v. dose, slightly inhibited the intraventricular conduction. The high dose decreased the heart rate, ventricular contraction and blood pressure, delayed the atrioventricular and intraventricular conduction, and prolonged the ventricular repolarization and refractory period. Increment in the refractoriness by the high dose was greater than that in the repolarization, resulting in the reduction of ventricular electrical vulnerability. This unique electrophysiological profile of potassium canrenoate may in part contribute to the favorable clinical results, whereas caution has to be paid on the cardiohemodynamic actions, particularly for patients with risk of elevated plasma drug concentration.

Keywords: spironolactone, canrenoate, monophasic action potential, QT prolongation, torsades de pointes

Introduction

The Randomized Aldactone Evaluation Study (RALES) showed that addition of spironolactone to a standard multidrug therapy substantially reduced the risk of both morbidity and mortality among patients with severe heart failure (1). Based on this observation, spironolactone has been suggested to act on non-renal tissue; namely the heart, aorta together with its major branches, and autonomic nervous system, besides the natriuretic effect (1). Also, antiarrhythmic potential of spironolactone and its derivatives has been indicated in basic experiments (2, 3) as well as in a clinical study (4). However, these drugs were recently shown to block ether-a-go-go-related gene (HERG) channels and native rapid (I_{Kr}) and slow (I_{Ks}) components of the delayed rectifier K+ currents in guinea pig ventricular myocytes (5), indicating proarrhythmic potential via excessive QT interval prolongation (6 – 9).

Since the drug-induced long QT syndrome is a hot topic of concern for the pharmaceutical companies as well as clinicians, in this study we simultaneously assessed the cardiohemodynamic and electrophysiological effects of a spironolactone active derivative potassium canrenoate, using the halothane-anesthetized in vivo canine model (9 – 15). To better analyze the electrophysiological effects of the drug on the depolarization and repolarization phases, we recorded the His bundle electrograms and monophasic action potentials (MAPs), respectively, in addition to the standard lead II 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 (9–15). Assessment of these actions would provide a better understanding of the efficacy and adversity of spironolactone in clinical practice (1).

**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 the systemic blood pressure through the right femoral artery. A thermodilution catheter (TC-704; 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 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\(_{90}^{\text{sinus}}\)) and at a pacing cycle length of 400 ms (MAP\(_{90}^{\text{CL400}}\)) and 300 ms (MAP\(_{90}^{\text{CL300}}\)). The ERP of the right ventricle was assessed by the programmed electrical stimulation. The pacing protocol consisted of 8 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, namely, phase 3 repolarization of the action potential, was calculated by the difference between the MAP\(_{90}^{\text{CL400}}\) and ERP at the same site, which reflects the extent of electrical vulnerability of the ventricular muscle (18).

**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 (MP/VAS 3 for Macintosh ver 1.0; 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 twice. 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 assessment, potassium canrenoate 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 potassium canrenoate in a middle dose of 10 mg/kg, which is a clinically recommended daily maximum i.v. dose, was administered over 10 min, and...
each parameter was observed in the same manner. Finally, potassium canrenoate in a high dose of 100 mg/kg was administered over 10 min, and each parameter was observed 5, 10, 15, 20, 30, 45, and 60 min after the start of the infusion.

**Plasma drug concentration**

A volume of 3 ml of blood was drawn from the left femoral artery to measure the plasma drug concentration. The blood samples were centrifuged at 1,500 x g for 30 min at 4°C. The plasma was stored at –80°C until the drug concentration was measured. Sensitive and specific determinations of the concentrations of potassium canrenoate were performed using a spectrofluorometric method of Gochman and Gantt (19).

**Drugs**

Potassium canrenoate (monopotassium 17-hydroxy-3-oxo-17α-pregna-4,6-diene-21-carboxylate) (Soldac-tone Inj™; Pharmacia, Tokyo) was dissolved with saline in concentrations of 0.5, 5, and 50 mg/ml. Other drugs used were thiopental sodium (Tanabe, Osaka), halothane, and heparin calcium (Nihon Schering, Osaka).

**Statistical analyses**

Data are presented as the mean ± 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 Contrasts for mean values comparison, and a P value <0.05 was considered significant.

**Results**

Two animals out of five died from cardiohemo-dynamic collapse during the experimental period, which occurred at 16 and 37 min after the start of the 100 mg/kg of potassium canrenoate infusion, respectively.

**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). The heart rate and mean blood pressure at the pre-drug control (C) were 124 ± 7 beats/min and 108 ± 5 mmHg, respectively. The heart rate and mean blood pressure decreased for 15–60 and 5–60 min after the start of the high dose of 100 mg/kg infusion, respectively.

**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 1.41 ± 0.12 L/min and 79 ± 7 mmHg · min/L, respectively. The CO and TPR decreased for 10–20 and 5–60 min after the start of the high dose infusion, respectively.

**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 1,849 ± 229 mmHg/s and 14.0 ± 2.7 mmHg, respectively. The LVdP/dt\_max decreased for 10–30 min after the start of the high dose infusion, whereas no significant change was detected in the LVEDP during the experimental period.

**Plasma drug concentration**

The time course of the plasma drug concentration are summarized in Fig. 1 (n = 5). The decrease of the plasma concentration followed a pattern predicted by the two-compartment theory of pharmacokinetics. The peak plasma concentrations of the drug after 1, 10, and 100 mg/kg infusion were 11 ± 2, 127 ± 13, and 2,360 ± 374 µg/ml, respectively.

**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 corrected by Bazett’s and Van de Water’s formulas at the pre-drug control (C) were 107 ± 4, 67 ± 6, 268 ± 21, 383 ± 26, and 313 ± 20 ms, respectively. The QT interval and QTc corrected by Van de Water’s formula were prolonged for 15–60 min after the start of the high-dose infusion. The PR interval, QRS width, and QTc corrected by Bazett’s formula tended to be prolonged, but did not achieve statistical significance.

**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 MAP\_max\_\,(sinus) during the sinus rhythm are summarized in Fig. 3 (n = 5). The AH and HV intervals and MAP\_max\_\,(sinus) at the pre-drug control (C) were 76 ± 4, 31 ± 2, and
Fig. 1. Cardiohemodynamics effects of potassium canrenoate on the halothane-anesthetized dogs. Time courses of the heart rate (HR), mean blood pressure (MBP), total peripheral resistance (TPR), cardiac output (CO), maximum upstroke velocity of the left ventricular pressure (LVdP/dt\text{\textsubscript{max}}), left ventricular end-diastolic pressure (LVEDP), and plasma drug concentration (Plasma Conc.). Data are presented as the mean ± S.E.M. (n = 5). The asterisks represent significant differences from each control value 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 recorded from the right ventricle (MAP) during the sinus rhythm at pre-drug control (Control) and 30 min after starting potassium canrenoate (100 mg/kg) infusion.
243 ± 14 ms, respectively. The AH and HV intervals and MAP_{90(sinus)} were all prolonged. Significant changes were observed in the AH interval for 45 – 60 min after the start of high-dose infusion, in the HV interval for 20 – 30 min after the middle-dose infusion and for 10 – 60 min after the high-dose infusion, and in the MAP_{90(sinus)} for 15 – 60 min after the high-dose infusion.

**Effects on the MAP_{90} during the ventricular pacing**

The time courses of changes in MAP_{90} 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-drug control (C) were 245 ± 13 and 225 ± 10 ms, respectively. Both the MAP_{90(CL400)} and MAP_{90(CL300)} were prolonged. Significant changes were observed in the MAP_{90(CL400)} for 15 – 60 min after the start of high-dose infusion and in the MAP_{90(CL300)} for 15 – 20 min and at 45 min after the start of high-dose infusion. It should be noted that the time course of QTc corrected by Van de water well correlated with that of MAP_{90(CL400)}. The time courses of the increments in the MAP_{90(CL400)} and MAP_{90(CL300)} were also calculated (not shown in the figure). Increment in the MAP_{90(CL400)} was greater than that in the MAP_{90(CL300)} at 15 min after the start of the high-dose infusion, indicating reverse use-dependent prolongation of the repolarization period.

**Effects on the ERP and TRP**

The time courses of changes in the ERP and TRP are summarized in Fig. 3 (n = 5). The ERP and TRP at the pre-drug control (C) were 225 ± 12 and 20 ± 4 ms. The ERP was prolonged for 5 – 60 min after the start of the
Cardiohemodynamic effects

The low dose of 1 mg/kg as well as the middle dose of 10 mg/kg hardly affected any of the cardiohemodynamic parameters. On the other hand, the high dose of 100 mg/kg decreased the TPR and CO, which induced a severe hypotension in some animals. Previous studies have indicated that the hypotensive action could be induced through an inhibition of voltage-dependent Ca\(^{2+}\) channels on the smooth muscle cells (21 – 23) in addition to re-activation of the Na\(^+\)/K\(^+\) ATPase inhibited by high levels of endogenous ouabain-like factor (24, 25). While the hypotensive effect should increase the reflex-mediated adrenergic tone, the sinus automaticity and ventricular contraction were both suppressed in this study, suggesting that higher dose of potassium canrenoate may also exert direct negative chronotropic and inotropic effects on the heart. These cardiohemodynamic effects were essentially in accordance with the previous in vitro and in vivo observations (3, 4, 25). Also, it should be noted that the extent of changes in these variables largely paralleled the time course of the plasma drug concentration.

Antiarrhythmic/proarrhythmic potential

It is well known that impulses that reach the ventricles during the middle and terminal portions of the T wave can initiate the ventricular tachycardias and fibrillation, since the repolarization is most heterogeneous and sodium channels are in different phases of recovery during this period (18). In the halothane-anesthetized animal model, such an electrically vulnerable period can be estimated by TRP (9 – 15) and drug-induced prolongation of TRP has been known to increase the potential for slow conduction and reentry that allows perpetuation of torsades de pointes (9, 14, 18, 26, 27). Moreover, prolongation of this phase could generate early afterdepolarizations by spending too much time in the window voltage for Ca\(^{2+}\) channel reactivation (28). In this study, we found that the high dose of potassium canrenoate shortened the TRP, supporting its previously reported antiarrhythmic potential (2, 3, 4). It should be noted that similar electrophysiological profile have been confirmed for amiodarone i.v. in our previous study using the same canine experimental model system (13).
Study limitations

Hypotension observed after the high dose administration of the parent compound spironolactone in the treatment of chronic heart failure. However, caution must be paid to its cardiohemodynamic actions, particularly for patients with risk of elevated plasma drug concentration.

Conclusions

Currently observed unique electrophysiological profile of potassium canrenoate may at least in part contribute to the clinically demonstrated therapeutic efficacy of the parent compound spironolactone in the treatment of chronic heart failure. However, caution must be paid to its cardiohemodynamic actions, particularly for patients with risk of elevated plasma drug concentration.

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