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Originals

A Novel Cardioprotective Drug, K201 (JTV519), Induces Prolongation of QT and QTc Intervals, but not Torsades de Pointes

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SUMMARY

K201 (JTV-519 ; 4-[-3{1-(4-benzyl) piperidinyl} propionyl]-7-methoxy-2, 3, 4, 5-tetrahydro-1, 4-benzothiazepine), is a multi-channel blocker and a ryanodine receptor stabilizer that exhibits strong cardioprotective and antiarrhythmic effects. In this study, we examined how intravenous infusion of K201 without an α-agonist prolongs the QT interval in chloralose-anaesthetized rabbits, and whether the maximum dose of K201 given concomitantly induces torsades de pointes. The QT interval was significantly prolonged in the group receiving 6-hour infusion of K201 at 20 μg/kg/min, but the QTc interval was not prolonged (n = 5). With infusion of K201 at 0 (vehicle ; n = 5), 40 (n = 6), 100 (n = 6), 200 (n = 6) and 400 μg/kg/min (n = 5), blood pressure and HR were decreased, and prolongation of PQ, QRS complex, QT and QTc intervals occurred dose-dependently. The QTc interval was significantly prolonged from 211.5 ± 11.9 ms of the baseline to 319.9 ± 31.1 ms at a concentration of 400 μg/kg/min, which is the maximum dose of K201, but torsades de pointes was not induced at this dose. These results show that K201, which has a suppressive effect on Ca^{2+} leakage from the sarcoplasmic reticulum and an α_1-adrenoceptor-blocking effect, does not induce torsades de pointes, although it causes prolongation of the QT interval.

Key Words : K201 (JTV-519), QT interval, QTc interval, torsades de pointes, ryanodine receptor

INTRODUCTION

Prolongation of the QT interval is sometimes, but not always, associated with the occurrence of fatal arrhythmias, such as torsades de pointes and ventricular fibrillation. Torsades de pointes is a severe form of polymorphic ventricular tachycardia and a cause of sudden death[1]. Certain drugs that prolong the QT interval, such as class I and class Ⅲ antiarrhythmic agents, can also lengthen the action potential duration and induce torsades de pointes[2].

K201 (JTV519), a 1, 4-benzothiazepine derivative, was first developed as a drug for effective suppression of sudden cardiac cell death and for treatment of myocardial infarction[3], and has subsequently been shown to have a strong cardioprotective effect against ischemia-reperfusion-induced myocardial injury[4, 5]. The drug exhibits multi-channel blocking effects, inhibiting the fast sodium current (I_{Na}), potassium current (I_K) and L-type calcium current ($I_{Ca, L}$) in atrial and ventricular cardiomyocytes from rabbits, rats and guinea pigs[5～8]. Nevertheless, some discrepancies remain con-

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cerning the effects of K201 on ion currents and action potential duration in cardiomyocytes, and recently it was shown that K201 prevents diastolic calcium leak and ventricular tachyarrhythmia by increasing the affinity of calstabin2 for the ryanodine receptor[9, 10]. In this study, we investigated whether K201 prolongs the PQ, QRS, QT and corrected QT (QTc) intervals, and examined whether K201 induces torsades de points when the maximum dose is given to chloralose-anaesthetized rabbits.

METHODS

Animal preparation

Forty-one male New Zealand white rabbits (2.5 to 3.5 kg) were housed in plastic cages in a temperature - and light-controlled room for several days, with free access to standard chow and water. Tracheotomy was performed after anesthesia. The rabbits were artificially ventilated with room air using a ventilator (SN-480 -7 ; Shinano, Japan). Tidal volume (10 ml/kg) and respiratory rate (30 strokes/min) were adjusted to maintain blood gases and pH within their normal physiological ranges. A double-lumen catheter (14G, Medicut UKII, UNITIKA Ltd., Japan) was inserted into the right femoral vein for administration of K201, and a 5F Millar catheter (MPC-500, Millar Instruments Inc.) was placed in the right carotid artery for recording blood pressure.

Needle electrodes were applied to the surface of the limbs for recording the standard surface electrocardiogram, and lead II was used to measure electrocardiographic parameters. All the experiments were conducted after an equilibration period of 10 min. The electrocardiographic parameters were measured from at least five complexes in lead II . When there was an interruption of a T wave with a U wave, the QT interval was measured to the end of the U wave, as described in a previous report[11]. A polygraph (GE Marquette Medical Systems Japan) was connected via a computer to a data analysis system (MP100WS for MAC ; BIOPAC System). The experiments were performed in accordance with the guidelines for animal experimentation established by the ethics committee of Dokkyo University School of Medicine and Environment Biological Life Science. Methohexital sodium, and α-chloralose were obtained from Sigma (St. Louis, MO, USA), and K201 was donated by Aetas Pharma (Tokyo, Japan). K201 was dissolved in 5% mannitol adjusted to pH 3.5 with 0.2% citric acid, and clofilium was dissolved in saline.

Experimental protocol

Infusion of K201 for 6 h : After a bolus infusion of methohexital sodium (5 mg/kg), K201 was administered, followed by administration of α-chloralose (90 mg/kg infused in 20 min) via a marginal ear vein. K201 was initially infused at 100 μg/kg/min (n = 4) or 200 μg/kg/min (n = 5) for 2 min at 0.1 ml/min. Subsequently, 10 μg/kg/min or 20 μg/kg/min K201 was infused for 358 min at 0.01 ml/min. The vehicle group received continuous administration of the vehicle solution (saline) without K201 (n = 4). Two hours later, α-chloralose (20 mg/kg infused every 1 h) was administered again to maintain anaesthesia.

Infusion of K201 for 30 min : Initial anaesthesia was induced using thiopental sodium (20 mg/kg), followed by administration of α-chloralose (90 mg/kg infused in 20 min) via a marginal ear vein. K201 was infused at concentrations of 0 (vehicle, n = 5), 40 (n = 6), 100 (n = 6), 200 (n = 6) and 400 μg/kg/min (n = 5) for 30 min.

In each group, mean blood pressure (MBP), RR or heart rate (HR), and PQ, QRS complex, QT and QTc intervals determined by Bazett's formula (QT/$\sqrt{\text{RR}}$ interval)[12] were measured every 10 min for the first 60 min and then every 30 min for the next 5 h in the animals receiving K201 for 6 h, and every 5 min in the animals receiving K201 for 30 min. The occurrence of torsades de pointes and polymorphic ventricular arrhythmia for fewer than 6 consecutive beats (2-5 beats) and atrioventricular block was investigated. Torsades de pointes was defined as at least 6 consecutive beats of polymorphic ventricular tachycardia, as reported previously[13].

In the statistical analysis, each data point represents the mean ± standard error (SE). Data analysis was performed using repeated measures analysis of variance (ANOVA) and differences with $P < 0.05$ were considered significant.

RESULTS

In the groups given K201 for 360 min (Fig. 1), the

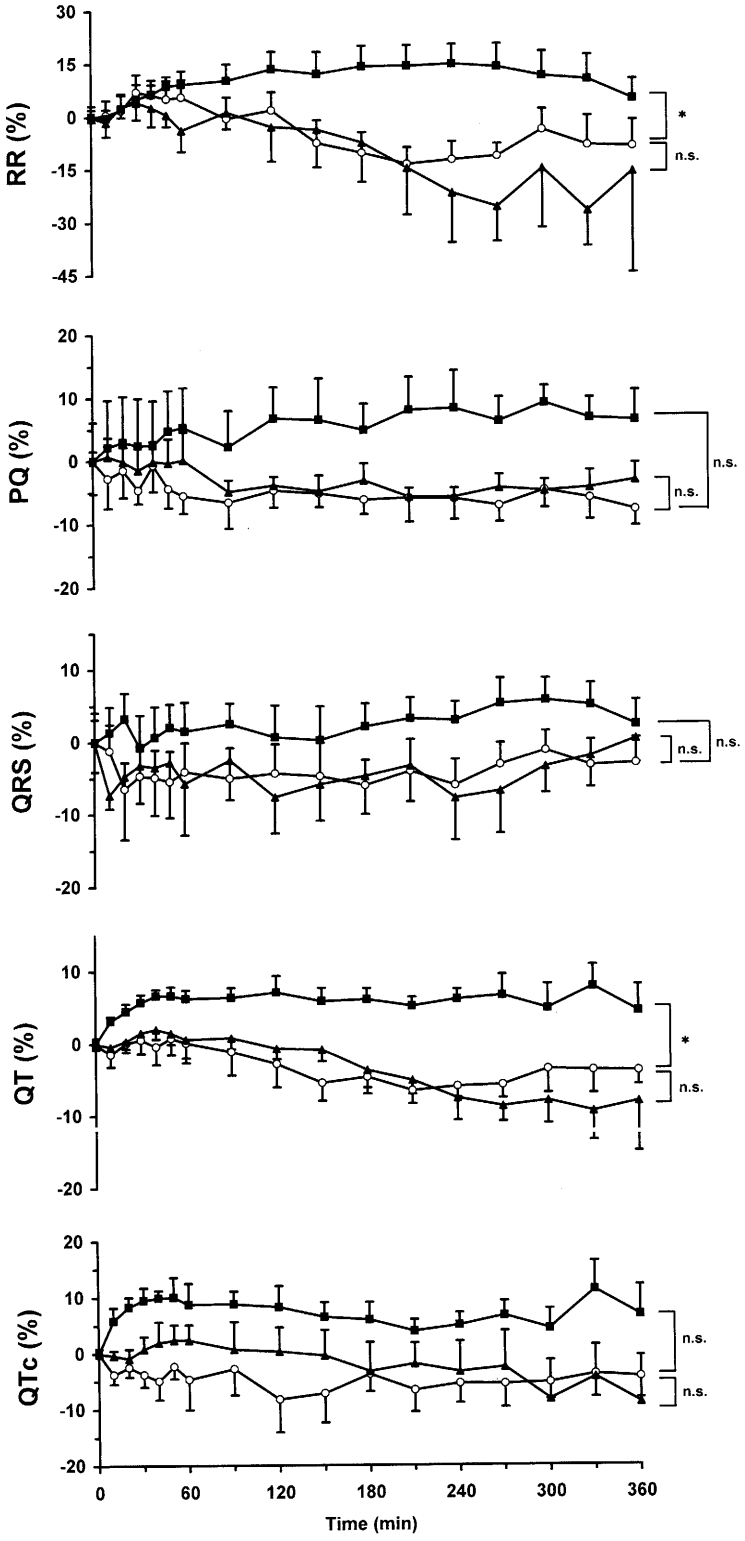

Fig. 1 Effects of administration of K201 for 6 h.

The rate of change of the RR, PQ, QRS complex, QT, and QTc intervals is expressed as the percentage of each value before drug infusion (baseline values). RR and QT intervals in the 20 μg/kg/min treatment group (■) were significantly prolonged compared to the vehicle group (○). There was no significant difference between the 10 μg/kg/min group (▲) and the vehicle group (○) in the RR, PQ, QRS complex, QT, and QTc intervals.

Each point represents the mean ± S.E. $^{*}P < 0.05$ vs. vehicle ; n.s. : not significant.

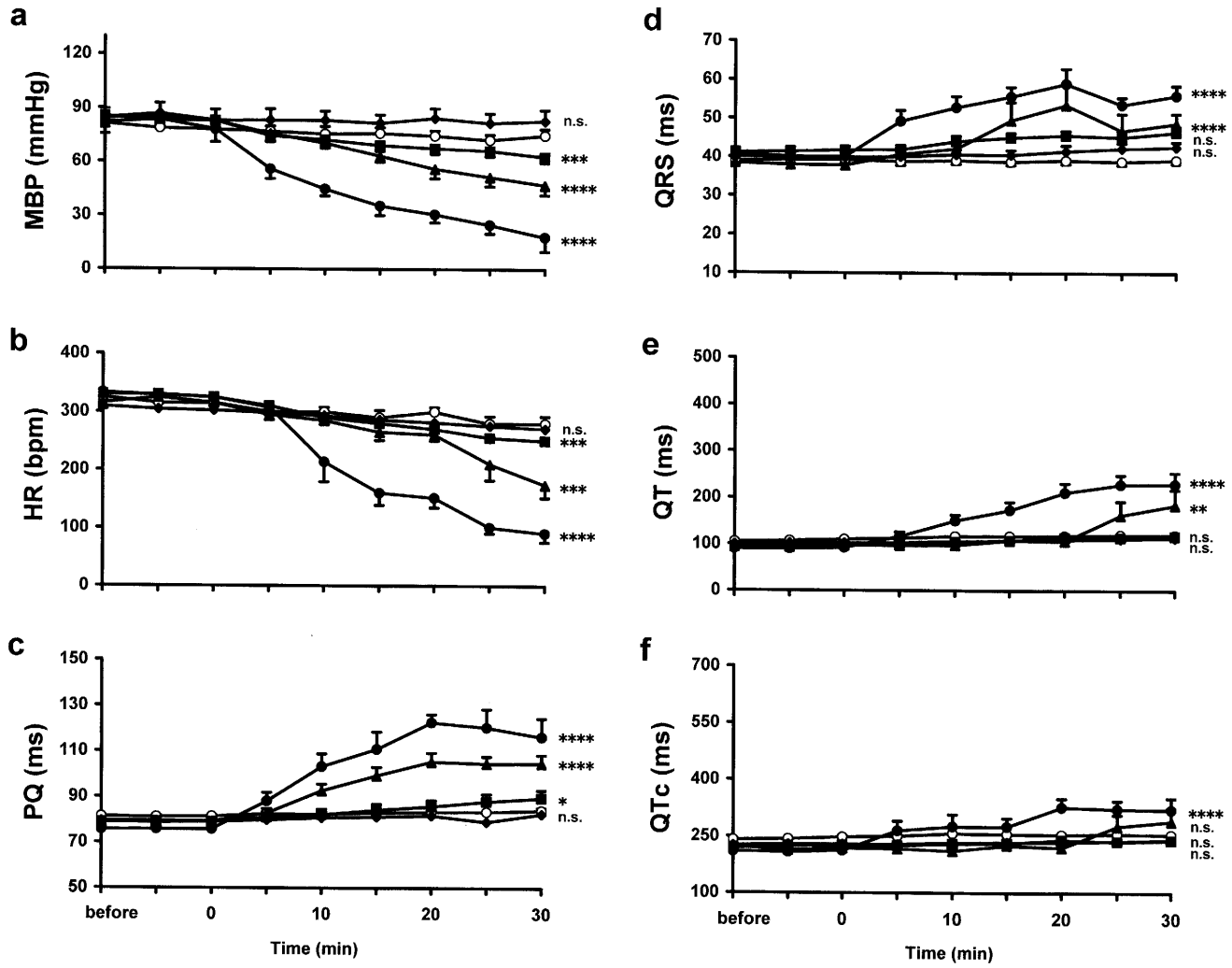

Fig. 2 Effects of administration of K201 for 30 min.

K201 at 40 (◆), 100 (■), 200 (▲) and 400 μg/kg/min (●) was infused intravenously for 30 min. Vehicle group (5% mannitol and 0.2% citric acid, ○). In the 400 μg/kg/min group, MBP and HR were significantly decreased and PQ, QRS, QT and QT intervals were significantly prolonged compared to the vehicle group.

a, Mean blood pressure (MBP) ; b, Heart rate (HR) ; c, PQ interval ; d, QRS interval ; e, QT interval ; f, QTc interval. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$ vs. vehicle ; n.s. : not significant.

RR, PQ, QRS complex, QT and QTc intervals were 192 ± 6, 69.4 ± 3.6, 30.6 ± 1.3, 110 ± 3 and 250 ± 6 ms before drug infusion, respectively, where each mean value is expressed as a percentage increase above the baseline value.

The RR, PQ, QRS complex, QT and QTc intervals were not significantly prolonged in the 10 μg/kg/min K201 group. The RR interval in the 20 μg/kg/min K201 group was significantly increased ($P<0.05$ vs. vehicle group), while there was no significant difference in the PQ and QRS complex intervals between the 20 μg/kg/min K201 group and the vehicle group. The QT interval was significantly prolonged in the group receiving K201 at 20 μg/kg/min ($P<0.05$ vs. vehicle group), but the QTc interval was not significantly prolonged.

In the groups that received K201 at concentrations of 0 (vehicle), 40, 100, 200 and 400 μg/kg/min for 30 min, MBP decreased in a dose-dependent manner. In the group receiving K201 at 400 μg/kg/min, MBP was less that 30 mmHg and 2 of the 5 rabbits died due to the fall in blood pressure, but not due to arrhythmia during infusion (Fig. 2a). There was no change in HR at 40 μg/kg/min, but a significant decrease of HR occurred at 100, 200 and 400 μg/kg/min (Fig. 2b). Significant prolongation of the PQ interval occurred at

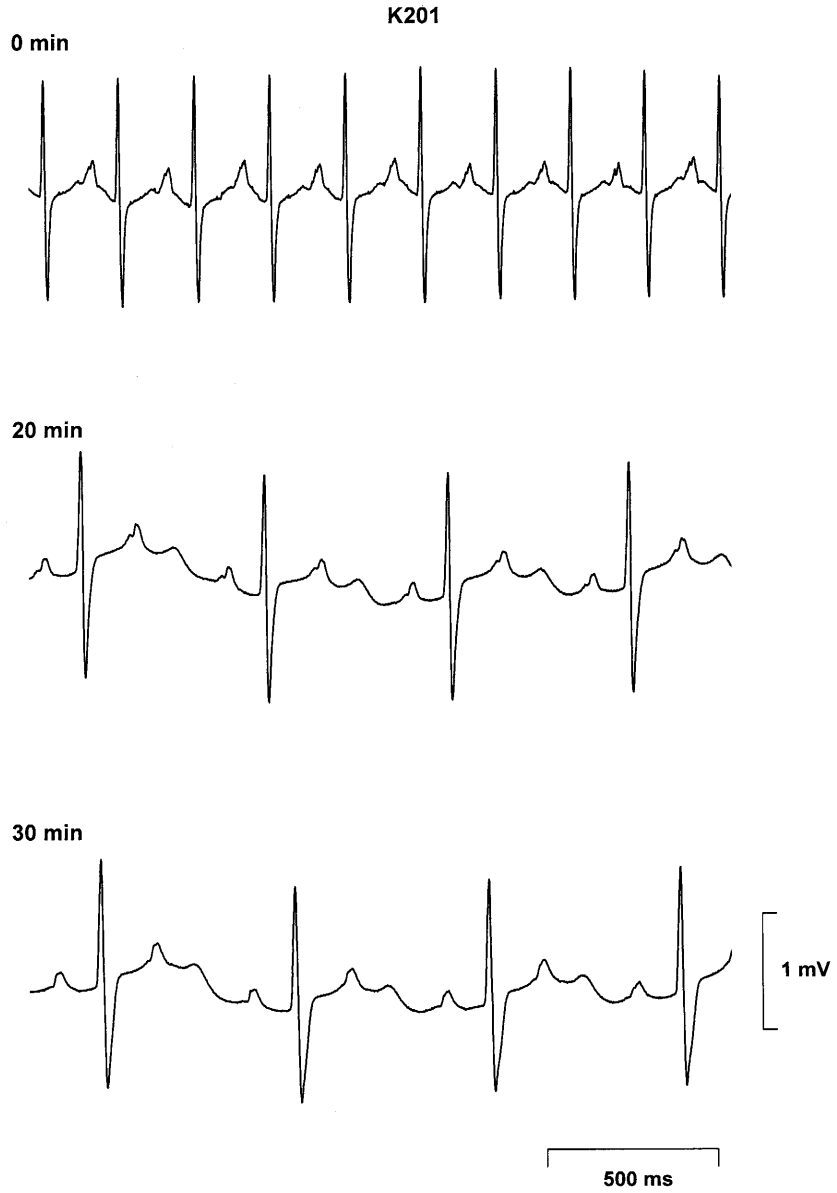

Fig. 3 ECG.
2 : 1 atrioventricular block (A-V block) was appeared from 20 min after administration of 400 μg/kg/min of K201.
Upper ; 0 min, before the start of administration of 400 μg/kg/min of K201. middle ; 20 min after the start. lower ; 30 min after the start.

100, 200 and 400 μg/kg/min (Fig. 2c), and the QRS interval was significantly prolonged at 200 and 400 μg/kg/min (Fig. 2d). A significant prolongation of the QT interval occurred at 200 and 400 μg/kg/min, being to 183.8 ± 33.0 and 228.6 ± 24.8 ms, respectively (Fig. 2e), but the QTc interval was prolonged only at 400 μg/kg/min, being from 211.5 ± 11.9 ms of the baseline to 319.9 ± 31.1 ms (Fig. 2f). Neither torsades de pointes nor polymorphic ventricular arrhythmia was induced at any concentration of K201. In the group receiving K201, a minimum dose of prolongation of the PQ, QRS and QTc intervals was 100, 200 and 400 μg/kg/min, respectively (Fig. 2c, d, f).

An ECG from a rabbit infused with 400 μg/kg/min of K201 showed 2 : 1 atrioventricular block and marked QT prolongation after 20 min of infusion (Fig. 3).

2 : 1 atrioventricular block occurred in all animals in the K201 group (400 μg/kg/min). Although, no these arrhythmia of the polymorphic ventricular arrhythmia

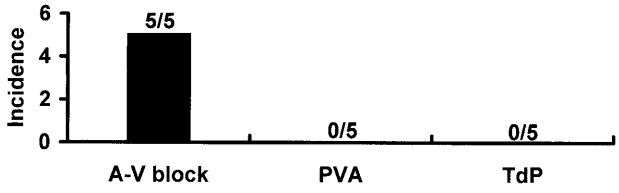

Fig. 4 Incidence of various arrhythmia.
2 : 1 atrioventricular block (A-V block), polymorphic ventricular arrhythmia (PVA) for fewer than 6 consecutive beats (2-5 beats), and torsades de pointes (TdP) in each animal in the K201 group (400 μg/kg/min). The A-V block was induced all cases in the K201 group, however, PVA and TdP were not induced.

(PVA) and torsades de pointes was observed in the K201 group at all (Fig. 4).

DISCUSSION

K201 was initially discovered as an effective protective agent against sudden death due to Ca^{2+}-overload, and has a strong cardioprotective effect[3]. In the present study, K201 induced about 10 % prolongation of the QT interval for 6 h at 20 μg/kg/min. K201 induced significantly prolonged the QT interval for 30 min at doses of 200 and 400 μg/kg/min. K201 at 400 μg/kg/min, which is the maximum dose, decreased MBP and HR, and prolonged the PQ, QRS, and induced hypotension. Although QT and QTc intervals were prolonged, torsades de pointes did not develop.

We have previously reported that K201 has an α_1-adrenoceptor blocking effect[3], and Carlsson et al. reported that the incidence of clofilium-induced ventricular tachycardia was significantly increased by concomitant continuous infusion of the α_1-agonist methoxamine[14]. It has also been shown that the α_1-adrenoceptor blocker prazosin is effective for suppression of ventricular tachycardia. Hence, in the present study, we investigated whether K201, which is known as a ryanodine receptor stabilizer, can also suppress polymorphic ventricular arrhythmia and torsades de pointes.

Studies have shown that K201 is a non-specific blocker of sodium, potassium and calcium channels. K201 exhibits voltage- and frequency-dependent blocking effects on I_{Na} and inhibits I_{Na}, I_K and $I_{Ca,L}$ to 65 %, 70 % and 80 % of normal levels at a concentration of 1 μM ; however, it is difficult to explain the shortening of the action potential duration by 50 % solely on the basis of K201 inhibition of these ion channels[6]. In contrast, Kiriyama et al. have reported that K201 lengthens the action potential duration due to selective inhibition of the delayed rectifier I_K, rather than $I_{Ca,L}$, in guinea pig ventricular myocytes[7]. K201 also inhibits I_{Kr} and $I_{K,ACh}$ in guinea pig atrial cells, slightly prolongs the action potential duration, and reduces atrial fibrillation[8]. Consistent with this, K201 has been shown to exhibit a significant protective effect against atrial fibrillation in an animal model, by increasing the effective refractory period while not affecting the intra-atrial conduction time[15].

K201 also possesses multiple pharmacological properties, and the effects of K201 may be related to the opening of mitochondrial K_{ATP} channels[5], activation of protein kinase C-δ[4], and inhibition of annexin V-actin binding and annexin V-Ca^{2+} channel activity[16]. K201 has a multi-channel blocker effect, and inhibits diastolic leak of Ca^{2+}, which may explain the lack of induction of torsades de pointes, despite the potency of the K^+ channel blocking effect of K201. Since K201 possesses multiple pharmacological properties, the prolongation effects on the PQ, QT and QTc intervals may be helpful as markers following K201 administration. Following infusion of K201, prolongation of the PQ interval occurred at 100 μg/kg/min, but prolongation of the QRS and QTc intervals first occurred at 200 μg/kg/min and 400 μg/kg/min, respectively. Prolongation of the PQ, QRS and QT (QTc) intervals generally reflects a blocking effect of Ca^{2+}, Na^+ and K^+ channels, respectively. Presumably, an overdose of K201 escalates the cardiodepressant effect more than the cardioprotective effect caused by blocking of Ca^{2+} channels. Amiodarone[17] and bepridil[18] are other compounds with complex multi-channel blocking effects, and these antiarrhythmic agents are known to cause lengthening of the action potential duration[19] and to induce prolongation of the QT interval. In fact, a prolonged QT interval has been suggested as a possible marker for the antiarrhythmic therapeutic effect of amiodarone[20].

It has recently been shown that K201 improves defective channel gating of the ryanodine receptor in heart failure[21]. K201 also suppresses sudden cardiac death and ventricular arrhythmia by stabilizing the in-

teraction between the ryanodine receptor-2 and calstabin2, and prevents Ca^{2+} leakage from the sarcoplasmic reticulum that triggers arrhythmia[9, 10]. In summary, the present study demonstrates that K201 induces prolongation of the QT and QTc intervals, but not torsades de pointes. These properties of K201 may be related to an inhibitory effect of diastolic Ca^{2+} leakage from the sarcoplasmic reticulum, and an α_1-adrenoceptor blocking effect despite exerting a multi-channel blocking effect.

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