Effects of KT-362, a New Na and Ca Influx and Ca Release Inhibitor, on Canine Ventricular Arrhythmias

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Abstract—Antiarrhythmic effects of the new drug KT-362, which was reported to suppress Na and Ca currents of cardiac cells and also to suppress intracellular Ca release in isolated smooth muscle preparations, were examined using two-stage coronary ligation-, digitalis- and adrenaline-induced ventricular arrhythmias in the dog. Intravenous KT-362 at 10 mg/kg suppressed coronary ligation arrhythmia both at 24 and 48 hr after ligation, and the minimum effective plasma concentrations for arrhythmias induced by 24 hr coronary ligation and 48 hr coronary ligation were 6.1±1.7 and 8.6±2.7 μg/ml, respectively. Antiarrhythmic effects were accompanied by transient hypotension. Oral administration of 70–100 mg/kg was also effective on 24 hr coronary ligation arrhythmia. However, there was no prominent hypotension in these experiments. Intravenous KT-362 at 3 mg/kg suppressed digitalis arrhythmia; and the minimum effective plasma concentration was 3.3±1.2 μg/ml, which was lower than the effective plasma concentrations for coronary ligation arrhythmias. Intravenous KT-362 at 1 mg/kg also suppressed adrenaline arrhythmia; and the minimum effective plasma concentration was 1.0±0.1 μg/ml, the lowest among the effective plasma concentrations. These pharmacological profiles of KT-362 are quite different from those of class 4 Ca antagonists, but similar to those of class 1 drugs such as propafenone. Though KT-362 has a hypotensive effect, it is effective on canine ventricular arrhythmias; thus its clinical usefulness for supraventricular and ventricular arrhythmias is expected.

KT-362, 5-(3-((2-(3,4-dimethoxyphenyl)ethyl)amino)-1-oxopropyl)-2,3,4,5-tetrahydro-1,5-benzothiazepine fumarate, is a new synthetic antiarrhythmic agent with vasodilator effects that was reported to be an intracellular Ca antagonist (1). A preliminary study demonstrated its efficacy in animal models of arrhythmia (2), and electrophysiologically, it is also a Na channel and Ca channel blocker (3). The electrophysiological study demonstrated that KT-362 decreased the maximum rate of rise of the action potential (Vmax) of normally polarized and also of depolarized cardiac cells. Since KT-362 is not only a blocker of both the depolarizing ionic channels, but also an inhibitor of intracellular release of Ca, it does not fit in the well-known Vaughan Williams classification of antiarrhythmic drugs. Thus, it may have quite different pharmacological effects on animal and clinical arrhythmias.

We have recently reported the effects of various antiarrhythmic drugs using canine ventricular arrhythmia models (4–8) and classified antiarrhythmic drugs based on their pharmacological effectiveness. Using exactly the same experimental methods that we employed before, the present canine experiment was designed to examine the antiarrhythmic effects of KT-362 qualitatively and quantitatively in comparison with other antiarrhythmic drugs.

Materials and Methods

Production of two-stage coronary ligation-induced arrhythmia: Thirteen female beagle
dogs, weighing 7–9 kg, were anesthetized initially with intravenous thiopental sodium at 30 mg/kg and then intubated. As reported earlier (4), the chest was opened and a two-stage coronary ligation was performed under halothane anesthesia.

Experiments were done without anesthesia 24 and 48 hr after coronary ligation. The lead II ECG, atrial electrogram from implanted electrodes sutured on the left atrial appendage, and the instantaneous and mean blood pressure were recorded continuously using telemetry systems (Nihon Kohden, Tokyo and Nishimu Electronics Industries, Fukuoka, Japan). In the figures, data on the mean blood pressure are shown. For intravenous studies, 10 mg/kg KT-362 was injected through a cannula in the jugular vein over a period of 3 to 5 sec. Venous samples were drawn from the cannula 5 min before and 1, 3, 5, 10, 15, 30 and 60 min after injection of KT-362. For oral administration studies, 70–100 mg/kg KT-362 in a gelatine capsule was administered to dogs 24 hr after coronary ligation. Venous samples were drawn up to 24 hr after oral administration.

Production of digitalis-induced arrhythmia: Eleven mongrel dogs of either sex, weighing 8–15 kg, were anesthetized with intravenous pentobarbital sodium at 30 mg/kg. As reported earlier (5), 40 µg/kg ouabain was injected intravenously and then followed by an additional 10 µg/kg every 20 min until a stable ventricular arrhythmia was produced. KT-362 at 3 mg/kg was injected intravenously through a cannula in the femoral vein over a period of 3 to 5 sec.

The lead II ECG, atrial electrogram from catheter tip electrodes in the right atrium, and instantaneous and mean blood pressure were continuously recorded. Venous blood samples were drawn from the jugular vein 5 min before and 1, 3, 5, 10, 15, 30 and 60 min after KT-362 injection.

Production of adrenaline-induced arrhythmia: Five mongrel dogs of either sex, weighing 7–15 kg, were anesthetized initially with thiopental sodium. As reported earlier (6), after intubation, 1.0% halothane, vaporized with 100% oxygen, was administered with a volume-limited ventilator. Adrenaline was infused through the left femoral vein at a rate of 2.5 µg/kg/min. If multifocal ventricular tachycardia was not induced, a higher infusion rate was employed. After 3 min of adrenaline infusion, 1 mg/kg KT-362 was injected into the right femoral vein over a period of 3 to 5 sec.

The lead II ECG, atrial electrogram from catheter tip electrodes in the right atrium and blood pressure were continuously recorded. Venous samples were drawn from the jugular vein 1 min before and 1, 3, 5, 10 and 15 min after injection.

Plasma KT-362 assay: A rapid, sensitive and specific method for the determination of KT-362 in plasma using a high-performance liquid chromatograph (HPLC) was developed. A plasma volume of 0.5 ml was shaken for 10 min with 3.0 ml of ethylacetate/triethylamine (9:1 v/v). The mixture was centrifuged at 3000 rpm for 10 min. The extraction was performed twice. The organic phase was evaporated under reduced pressure. After a 0.1 ml of beta-naphthol solution (2.0 µg/ml in ethylacetate) was added as an internal standard, the mixture was shaken for 10 min, and it was centrifuged at 3000 rpm for 10 min. The organic phase was evaporated under reduced pressure. The residues were dissolved in 0.2 ml of acetonitrile/20 mM sodium phosphate buffer, pH 6.0 (4:6 v/v) which was the mobile phase.

Thirty ml of this solution was injected into the column (4.6 mm i.d.×15 cm length, Toyo Soda, TSK-gel ODS-80TM) of a HPLC (Hitachi 655A). The limit of detection was 5 ng/ml plasma. Recovery from the plasma was over 92% (16 µg/ml), and the calibration range was 0.5–32 µg/ml plasma.

Evaluation of the antiarrhythmic effects: The severity of ventricular arrhythmia was expressed by the arrhythmic ratio: number of ventricular ectopic beats divided by the total heart rate. The total heart rate is the number of all beats counted from the 5 sec strip of ECG (i.e., the number of ventricular ectopic beats plus the number of conducted beats), and ventricular beats were judged by the different shape of the ventricular complex from the normal QRS complex. For the three arrhythmias, the arrhythmic ratios before drug injection was almost 1, and there were no spontaneous improvements in these ratios. If
the arrhythmic ratio after drug administration was decreased significantly from the 0 time value, as determined by the Chi square test \((P<0.05)\), the drug was judged as having an antiarrhythmic effect. For other parameters, Student’s \(t\)-test for paired data were used to evaluate statistical significance as compared to the 0 time value. As reported earlier \((4–8)\), the minimum effective plasma concentration of KT-362 was determined as follows: The last minute of statistically significant decrease \((P<0.05)\) in the arrhythmic ratio compared with that at 0 time was determined. Then the corresponding plasma concentration was calculated from the experimentally derived plasma concentration-time equations, and this was regarded as the minimum effective plasma concentration. The plasma concentration-time equations and their curve parameters were analyzed by a non-linear regression program “MULTI” \((9)\) and an NEC PC-9801Vm computer (Tokyo, Japan).

**Results**

**Effects of KT-362 on two-stage coronary ligation-induced arrhythmia:** After 1–2 days of coronary ligation, beagle dogs showed multifocal ventricular tachycardia, as indicated by the arrhythmic ratios of nearly 1 at −5 and 0 min of Fig. 1. The preliminary experiments using 3–10 mg/kg of KT-362, i.v., showed that 3 and 5 mg/kg had no or only a weak antiarrhythmic effect; therefore, a 10 mg/kg dose was used. This dose suppressed the 24 hr arrhythmias gradually after injection, and a statistically significant decrease in the arrhythmic ratio was observed at 4 and 5 min. Because the blood pressure transiently decreased, higher doses could not be tested. As shown in the lower values of arrhythmic ratio of about 0.8 at −5 and 0 time in Fig. 2, the 48 hr arrhythmia was less severe. The plasma concentration of KT-362, just before the 48 hr experiment, was zero, although 10 mg/kg had been administered 24 hr before.

![Graph](image)

**Fig. 1.** Summary of the effects of KT-362 on 24 hr two-stage coronary ligation-induced arrhythmias. KT-362 at 10 mg/kg, i.v., increased the number of conducted beats, and it decreased the blood pressure and arrhythmic ratio. Vertical bars represent the standard deviation. *\(P<0.05\).*
The same 10 mg/kg dose of KT-362 showed a stronger and longer antiarrhythmic effect lasting from 6 to 10 min after injection. However, other effects were qualitatively similar to those at 24 hr after coronary ligation. The plasma concentration-time curve of each experiment fitted well with that predicted by the two-compartment open model. The average values of the parameters of the curves of the 24 hr experiment, expressed as concentration = \( A e^{-\alpha t} + B e^{-\beta t} \), were: \( A = 35.4 \pm 27.6 \mu g/ml, \alpha = 1.27 \pm 1.10/min, B = 6.25 \pm 2.00 \mu g/ml, \beta = 0.012 \pm 0.006/min \) (mean \pm S.D., n=6). where A and B are plasma concentrations of KT-362 at 0 time of distribution and the elimination phase, respectively; and \( \alpha \) and \( \beta \) are the rate constants of the two curves. Those for the 48 hr experiments were: \( A = 17.0 \pm 14.5 \mu g/ml, \alpha = 0.49 \pm 0.39/min, B = 11.4 \pm 1.9 \mu g/ml, \beta = 0.014 \pm 0.005/min \) (n=7). The minimum antiarrhythmic plasma concentrations for the canine 24 and 48 hr coronary ligation-induced arrhythmias were calculated as 6.1 \pm 1.7 \mu g/ml (at 5 min and 62% decrease in the arrhythmic ratio) and 8.6 \pm 2.7 \mu g/ml (at 10 min and 60% decrease in the arrhythmic ratio), respectively.

Fig. 2. Effects of KT-362 at 10 mg/kg, i.v., on 48 hr two-stage coronary ligation-induced arrhythmia. *P<0.05.
Fig. 3. Effects of orally administered KT-362, 70–100 mg/kg, on 24 hr two-stage coronary ligation-induced arrhythmia. Only the changes in the plasma concentrations and arrhythmic ratio are shown. There were wide individual variations in the responses to KT-362 and its time course.

KT-362 required to decrease the arrhythmic ratio to 60%, as in the cases of the intravenous experiments, was calculated as 13.0 μg/ml from the regression line of the correlation between the plasma concentration and the arrhythmic ratio in this p.o. study.

Effects of KT-362 on digitalis-induced arrhythmia: After injection of a total dose of 70–90 μg/kg ouabain, almost all the beats were of ventricular origin, as shown in the 0 time value of the arrhythmic ratio of Fig. 4. Different doses of KT-362, 0.1–3 mg/kg, were examined in the preliminary experiments. Doses up to 1 mg/kg showed only a weak antiarrhythmic effect; therefore, a dose of 3 mg/kg was chosen for this study. As shown in Fig. 4, 3 mg/kg transiently decreased the atrial rate and increased the number of conducted beats. The arrhythmic ratio decreased continuously up to 3 min. The plasma KT-362 concentration-time curves fitted well with that predicted by the two-compartment open model. The parameters of the curves were: A=2.72±1.89 μg/ml, α=0.25±0.31/min, B=1.95±1.44 μg/ml, β=0.014±0.006/min (n=7). The calculated minimum antiarrhythmic plasma concentration of KT-362 for canine digitalis-induced arrhythmia (at 3 min and 77% decrease in the arrhythmic ratio) was 3.3±1.2 μg/ml and was significantly lower than those of 24 and 48 hr coronary ligation arrhythmias (P<0.01), when compared using Student's
Fig. 4. Summary of the effects of KT-362 at 3 mg/kg, i.v., on digitalis-induced arrhythmia. KT-362 decreased the arrhythmic ratio and blood pressure, and it increased the number of conducted beats. *P<0.05, **P<0.01.

t-test for unpaired data.

Effects of KT-362 on adrenaline-induced arrhythmia: Adrenaline infusion at rates of 2.5–3.5 μg/kg/min induced ventricular tachycardia. Various doses of KT-362, 0.5–1 mg/kg, were examined in the preliminary study; as the 0.5 mg/kg dose did not completely suppress the arrhythmia, a 1 mg/kg dose was used. As shown in Fig. 5, after injection of KT-362, the number of ventricular ectopic beats decreased transiently, and the arrhythmic ratio declined to about 0.25, 30 sec after KT-362 injection. Antiarrhythmic effects reappeared after 10 min, even though the KT-362 plasma concentration was decreasing. The plasma concentration-time curves fitted well with that predicted by the one compartment, open model theory because of the short experimental duration and thus consisted of only 5 data points. The parameters of the curves, expressed as concentration=Ae^{-\alpha t}, were: A=1.03±0.35 μg/ml, \alpha=0.077±0.042/min (n=5). We estimated the minimum antiarrhythmic KT-362 plasma concentration for canine halothane-adrenaline induced arrhythmia (at 1 min and 43% decrease in the arrhythmic ratio) as 1.0±0.1 μg/ml. This concentration was significantly lower than the concentrations obtained using other arrhythmia models (P<0.01).

Discussion

The present experiment using the three canine ventricular arrhythmia models confirmed previous preliminary reports that KT-362 is effective in animal experimental arrhythmias (2). In our experiment on coronary ligation arrhythmia, intravenous KT-362 suppressed the 24 and 48 hr arrhythmias, but the onset of the antiarrhythmic effect was delayed and was very short lasting. This delayed onset of action by intravenous administration is not a common finding for other class 1 drugs except for the so-called slow kinetic drugs, like E-0747 (10) and SUN 1165 (5). We estimated the minimum effective plasma concen-
Fig. 5. Summary of the effects of KT-362 at 1 mg/kg, i.v., on halothane-adrenaline-induced arrhythmia. KT-362 showed transient hypotensive and antiarrhythmic effects. *P<0.05.

KT-362 also suppressed adrenaline arrhythmia at the significantly lower concentration of 1.0 μg/ml. We have reported that Ca channel block is one of the mechanisms of many antiarrhythmic drugs to suppress this arrhythmia (6), even though the role of Na influx might be more important in the generation of ventricular automaticity. An electrophysiological study reported the Ca channel blocking concentration of KT-362 to be higher than that for suppressing the Na channel, about $3 \times 10^{-6}$ to $10^{-5}$ M or 1.5 to 5 mg/l, which decreased the $V_{\text{max}}$ of the guinea pig ventricular fiber action potential (3). In addition, KT-362 was effective on digitalis induced arrhythmia at the minimum concentration of 3.3 μg/ml. This plasma concentration is also within the Na channel blocking concentration; thus we speculate that KT-362 suppressed those arrhythmias by the Na channel blocking effect.

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Compared with our previous studies using the same canine arrhythmia models and determining the minimum effective plasma
concentrations of antiarrhythmic drugs (4–8, 11, 12). KT-362 shows the same antiarrhythmic profile as propafenone, where the two drugs were effective on three canine ventricular arrhythmias and were most potent on adrenaline arrhythmia, followed by digitalis and coronary ligation arrhythmias. Unlike KT-362, other class 4 Ca channel blocking drugs that we have tested showed only effectiveness on adrenaline arrhythmia (11, 12). Those other Ca channel blockers include verapamil and bepridil which are known to have a Na channel blocking effect in high concentrations. Therefore, KT-362 is more like a Na channel blocking class 1 drug with an effect on adrenaline arrhythmia. It is interesting that KT-362 resembles propafenone (5), which is known to have β-adrenergic blocking activity, but KT-362 has no β-blocking activity (our unpublished data).

KT-362 has been shown to be a unique drug that has a blocking effect on intracellular Ca mobilization in smooth muscle preparations (1). As stated earlier, it may be of interest to know whether such an effect occurs in cardiac tissues and whether it may provide some beneficial mechanism to especially suppress adrenaline arrhythmia. However, this effect inevitably produces hypotensive effects after intravenous administration. However, the hypotensive effect was minimal in the oral administration experiment in 24 hr coronary ligation arrhythmia, thus it may become a clinically useful antiarrhythmic drug for both supraventricular and ventricular arrhythmias as in the case of propafenone.

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