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Antiarrhythmic effects of an aconitine-like compound, TJN-505, on canine arrhythmia models

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Abstract

We examined the effects of an aconitine-like compound, TJN-505 (1α-16β-dimethoxy-20-ethyl-14α-(4-methoxybenzoyloxy)-aconitan-8,13-diol hydrochloride), on canine arrhythmias provoked by digitalis, two-stage coronary ligation, adrenaline, programmed electrical stimulation, or aconitine. TJN-505 (2–2.5 mg/kg i.v.) suppressed digitalis-, two-stage coronary ligation- and adrenaline-induced ventricular arrhythmias. The antiarrhythmic plasma concentrations (IC_{50}) of TJN-505 for these arrhythmia models were 1.26, 0.94 and 1.31 μg/ml, respectively. TJN-505 (2 mg/kg i.v. followed by the infusion of 0.1 mg/kg per min) prolonged PR, QRS, QTc and JTc intervals and the ventricular effective refractory period and reduced the incidence of programmed electrical stimulation-induced arrhythmias in dogs with 7-day-old myocardial infarction ($P < 0.05$). TJN-505 (2 mg/kg i.v.) also suppressed the aconitine-induced atrial arrhythmias. In conclusion, TJN-505 suppressed various canine ventricular and atrial arrhythmias and seems to act as a blocker of multiple channels.

Keywords: TJN-505; Aconitine; Antiarrhythmic drug; Arrhythmia model, canine

1. Introduction

The roots of some *Aconitum* plants have been used as a component of Chinese herbal recipes. Overdoses of these materials, however, often induce various arrhythmias, because they contain the potent arrhythmogenic, aconitine (Tai et al., 1992). In order to treat aconitine intoxication, the root of *Aconitum contortum*, another *Aconitum* plant, has been used empirically as an antidote. TJN-505 (1α-16β-dimethoxy-20-ethyl-14α-(4-methoxybenzoyloxy)-aconitan-8,13-diol hydrochloride) isolated from *Aconitum contortum* is a compound structurally related to aconitine (Fig. 1) (Niitsu et al., 1990), and is thought to be one of the active substances responsible for the antiarrhythmic effects of *Aconitum contortum*. However, it remains unclear whether TJN-505 experimentally has antiarrhythmic effects like those of other antiarrhythmic drugs. We have reported on effects of various antiarrhythmic drugs and determined effective plasma concentrations using canine digitalis-, two-stage coronary ligation- and adrenaline-induced arrhythmia models (Hashimoto et al., 1991a,b; Haruno and Hashimoto, 1993; Matsuzaki et al., 1993). Our studies indicated that class I drugs, Na^+ channel blockers, were effective on digitalis- and two-stage coronary ligation-induced arrhythmia models. Class II drugs, β-adrenoceptor antagonists, and class IV drugs, Ca^{2+} channel blockers, were effective on the adrenaline-induced arrhythmia model. Class III drugs, K^+ channel blockers, were ineffective on these arrhythmia models, but were effective on the programmed electrical stimulation-induced arrhythmia model.

In the present study, we examined the profile of effects of TJN-505 using these four arrhythmia models and compared them with those of other antiarrhythmic drugs which have already been reported on. In addition, we examined

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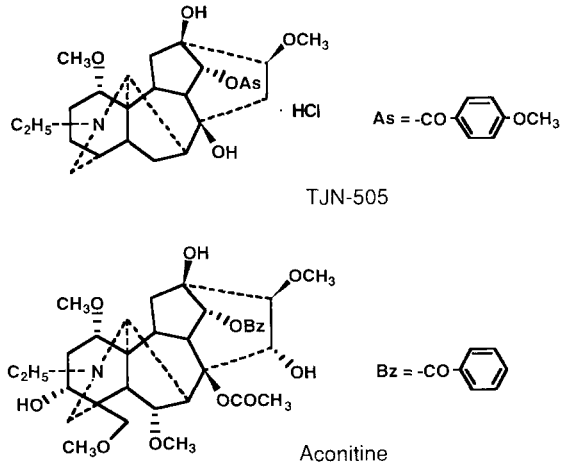

Fig. 1. Chemical structure of TJN-505 and aconitine.

the aconitine antagonistic effect of TJN-505 on the aconitine-induced atrial arrhythmia model.

2. Materials and methods

2.1. Production of digitalis-induced arrhythmia

The study was approved by the Animal Care and Use Committee of Yamanashi Medical University.

Six beagle dogs of either sex, weighing 9–10 kg, were anesthetized with sodium pentobarbital 30 mg/kg intravenously (i.v.) and intubated. Anesthesia was maintained with a supplemental infusion of sodium pentobarbital 3–5 mg/kg/h. Catheter tip electrodes were introduced into the right atrium from the left jugular vein to record the atrial electrogram and atrial rate. The lead II electrocardiogram (ECG), total heart rate, atrial electrogram and blood pressure were recorded continuously. Both vagi were cut at the cervical region.

As described earlier (Lucchesi and Hardman, 1961; Hashimoto et al., 1991a), 40 μg/kg ouabain was injected i.v. followed by an additional 10 μg/kg every 20 min until stable ventricular arrhythmia developed. A total of 60–80 μg/kg of ouabain was usually needed to induce stable ventricular arrhythmias. In the absence of drug administration, the resulting arrhythmia remained stable for more than 60 min. TJN-505 was injected i.v. within 10 s as a bolus after stable ventricular arrhythmias were established. We chose the bolus injection, because not only the antiarrhythmic effect with plasma concentrations of TJN-505 but also immediate toxic effects can be observed by this route of administration. To measure plasma concentrations of TJN-505, arterial blood samples were drawn from one lumen of the arterial double lumen catheter 0, 1, 3, 5, 7, 10, 15, 30 and 60 min after injection of TJN-505.

2.2. Production of two-stage coronary ligation-induced arrhythmia

Six beagle dogs of either sex, weighing 8–10 kg, were anesthetized initially with sodium thiopental 30 mg/kg i.v. and intubated. Anesthesia was maintained with 1.0% halothane. Using an aseptic technique, a left thoracotomy was performed at the 5th intercostal space and two-stage ligation of the left anterior descending coronary artery was performed according to the method of Harris (1950). Bipolar electrodes for measuring atrial electrogram were sutured on the left atrial appendage and the chest was closed. The left carotid artery and jugular vein were cannulated to record blood pressure and to inject TJN-505. The lead II ECG, total heart rate, atrial electrogram and blood pressure were recorded continuously using a telemetry system (Nihon Kohden, WEB-5000, Tokyo, Japan).

TJN-505 was injected i.v. within 10 s as a bolus 24 and 48 h after coronary artery ligation in conscious dogs. For measuring plasma concentrations of TJN-505, arterial blood samples were collected 0, 1, 3, 5, 7, 10, 15, 30, 45 and 60 min after injection of TJN-505. Animals showing an arrhythmic ratio of less than 0.8 were discarded.

2.3. Production of adrenaline-induced arrhythmia

Six beagle dogs of either sex, weighing 10–11 kg, were anesthetized initially with sodium thiopental 30 mg/kg i.v. and anesthesia was maintained with 1.0% halothane. The lead II ECG, total heart rate, atrial electrogram and blood pressure were recorded continuously. As reported earlier (Hashimoto et al., 1991a), adrenaline was infused through the left femoral vein at a rate of 2–2.5 μg/kg per min for 18 min using a syringe pump. In the absence of drug administration, the resulting arrhythmia remained stable during adrenaline infusion. At 3 min after the start of adrenaline infusion, TJN-505 was injected i.v. within 10 s as a bolus. For measuring plasma concentrations of TJN-505, arterial blood samples were collected 0, 1, 3, 5, 7, 10 and 15 min after injection of TJN-505.

2.4. Production of programmed electrical stimulation-induced arrhythmia

Five beagle dogs of either sex, weighing 8–11.5 kg, were used. Seven days after left anterior descending coronary artery ligation, the dogs were anesthetized with sodium pentobarbital 30 mg/kg i.v. and intubated. Anesthesia was maintained with a supplemental infusion of sodium pentobarbital 3–5 mg/kg/h. The chest was opened and bipolar electrodes were sutured on the epicardial surface of the ventricle. Programmed electrical stimulation was performed with a cardiac stimulator (Nihon Kohden, SEC-3102, Tokyo, Japan). The lead II ECG and blood pressure were recorded continuously. Total heart rate, blood pressure and electrocardiographic parameters were measured

before and after drug administration. The QTc interval was calculated from Bazett's formula [QTc = QT ms/$(RRs)^{1/2}$]. The JTc interval was calculated as QTc–QRS interval. The ventricular effective refractory period was defined as the longest S1–S2 interval not eliciting a propagated ventricular responses.

Programmed electrical stimulation was performed with pulses of 3 ms duration, twice the diastolic threshold voltage through bipolar electrodes. The basic pacing interval (S1–S1) was set at 250 or 300 ms, which was shorter than the cycle length of the spontaneous heart rate. After a train of 15 pacing stimuli, a single extrastimulus (S2) was delivered with a progressive decrease in 5-ms steps from the S1–S1 interval until they failed to elicit propagated ventricular responses. If S2 failed to induce arrhythmias, the procedure was repeated with double (S2 and S3) extrastimuli. We performed a triple extrastimulus for all dogs. The 2-mg/kg dose of TJN-505 was injected i.v. within 10 s as a bolus and was followed by the infusion at a rate of 0.1 mg/kg per min for 5 min. At 3 min after the start of TJN-505 infusion, the same stimulation protocol was applied after adjustment of the threshold voltage.

Ventricular premature contraction was defined as a single identified premature QRS complex. Ventricular tachycardia was three or more consecutive ventricular premature contractions and non-sustained ventricular tachycardia was ventricular tachycardia lasting less than 30 s.

2.5. Production of aconitine-induced atrial arrhythmia

Eight beagle dogs of either sex, weighing 7–10 kg, were anesthetized with sodium pentobarbital 30 mg/kg i.v. and intubated. As reported earlier (Mitsuhashi and Hashimoto, 1988), after the chest was opened through the right 4th intercostal space, a 5 × 5 mm filter paper soaked with 1% aconitine was placed on the right atrium for 18 min. Within 1 min after the topical application of aconitine, atrial premature contraction started to occur and the atrial rhythm changed to flutter (250–400 beats/min) or fibrillation (> 400 beats/min). The atrial rate was recorded through bipolar electrodes attached to the right atrial appendage. The atrial rate and the lead II ECG were recorded continuously. TJN-505 was injected i.v. within 10 s as a bolus 3 min after topical application of aconitine.

2.6. Drugs

TJN-505 (1α,16β-20-ethyl-14α-(4-methoxybenzoyloxy)-dimethoxyaconitan-8,13-diol hydrochloride, a gift from Tsumura Central Laboratories, Tsumura, Ibaraki, Japan) was dissolved in saline. The following drugs were used: aconitine and ouabain (Sigma, St. Louis, MO, USA); adrenaline (Bosmin Inj, Daiichi Seiyaku, Japan); halothane (Fluothane, Takeda, Japan); pentobarbital sodium salt (Tokyo Kasei, Japan). Adrenaline, ouabain and pentobarbital sodium salt were dissolved in saline.

2.7. Determination of plasma concentrations of TJN-505

The arterial blood samples were collected into heparinized syringes and centrifuged at 3000 × *g* for 30 min. The plasma was separated and stored in a freezer at about −80°C until analysis. The plasma concentrations of TJN-505 were measured at Tsumura Central Laboratories, Tsumura & Co., Ibaraki, Japan. After the plasma sample was pretreated, the sample was injected into a Schimadzu LC-6A high performance liquid chromatography system. The conditions were as follows: column, CAPCELL PAK C_{18} (4.6 × 100 mm, 3 mm, Shiseido, Japan); mobile phase, 8% tetrahydrofuran/100 mM NaH_2PO_4 (pH 3.0), 1.0 ml/min; detection, UV at 259 nm.

2.8. Evaluation of antiarrhythmic effects

Values were expressed as means ± S.E.M. Conducted beats were identified by their normal P and QRS morphology of the ECG and atrial electrogram. The antiarrhythmic effects of TJN-505 were evaluated from the arrhythmic ratio, which was calculated by dividing the number of ventricular premature contractions by the total heart rate. The arrhythmic ratio before drug administration was 0.9–1.0 as shown in the control 0 time values of the figures 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 one-way analysis of variance (ANOVA) followed by a paired Dunnett's test ($P < 0.05$), the drug was assumed to have a significant effect.

The antiarrhythmic plasma concentrations of TJN-505 were determined as the relation between the plasma concentrations of TJN-505 and the arrhythmic ratio as obtained by linear regression analysis. The plasma concentration which decreased the arrhythmic ratio to 50% of that at 0 concentration was calculated as the 50% inhibitory concentration, IC_{50}. With the programmed electrical stimulation-induced arrhythmia model, paired Student's *t*-test was used to compare electrocardiographic parameters before and after drug administration. The incidence of arrhythmia was compared by chi-squared test and when $P < 0.05$, the drug was assumed to have a significant effect.

3. Results

3.1. Effects of TJN-505 on digitalis-induced arrhythmia

After injection of a total dose of 60–80 μg/kg ouabain, stable ventricular arrhythmias occurred and the arrhythmic ratios in the untreated group ($n = 3$) were 0.97–1.0 for more than 60 min. The 2- and 3-mg/kg doses were tested

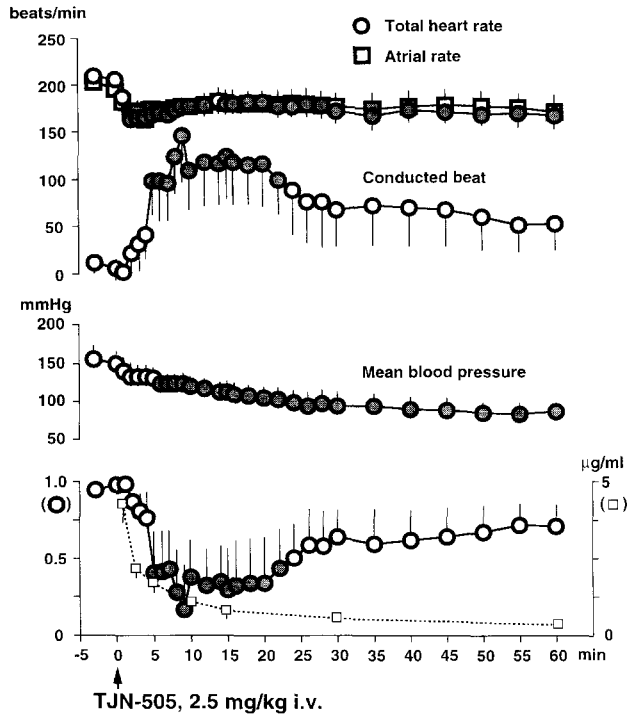

Fig. 2. Effects of TJN-505 on digitalis-induced arrhythmia. Values are means ± S.E.M of 6 experiments. Lower panel: arrhythmic ratio (open circles), plasma concentration of TJN-505 (open squares). Shaded symbols represent significant changes ($P < 0.05$) from time 0 values.

in preliminary experiments. The 2-mg/kg dose ($n = 3$) showed no antiarrhythmic effect and 5 min after injection of TJN-505, the average arrhythmic ratio remained 1.0. The 3-mg/kg dose ($n = 6$) decreased the average arrhythmic ratio from 0.97 to 0.17 and the effect lasted more than 60 min. Therefore, to determine the effective plasma concentrations, the 2.5-mg/kg dose was chosen (Fig. 2). In the 6 experiments, 5 min after injection of TJN-505, the average arrhythmic ratio decreased from 0.97 to 0.41 and the effect lasted for up to 22 min after injection ($P < 0.05$). TJN-505 also decreased the total heart rate, atrial rate and mean blood pressure ($P < 0.05$). Linear regression analysis between the plasma concentrations of TJN-505 and the arrhythmic ratio gave an IC_{50} of 1.26 μg/ml and the 95% confidence range was 0.35–5.80 μg/ml ($r = -0.469$, $n = 35$, $P = 0.0045$), when values at 5 to 60 min after injection of TJN-505 were used.

3.2. Effects of TJN-505 on two-stage coronary ligation-induced arrhythmia

Fig. 3a shows effects of TJN-505 on arrhythmias occurring 24 h after coronary ligation. In the untreated group ($n = 3$), the arrhythmic ratio remained 0.98–1.00 until 48 h after coronary ligation. The 1- and 3-mg/kg doses were tested in preliminary experiments. The 1-mg/kg dose

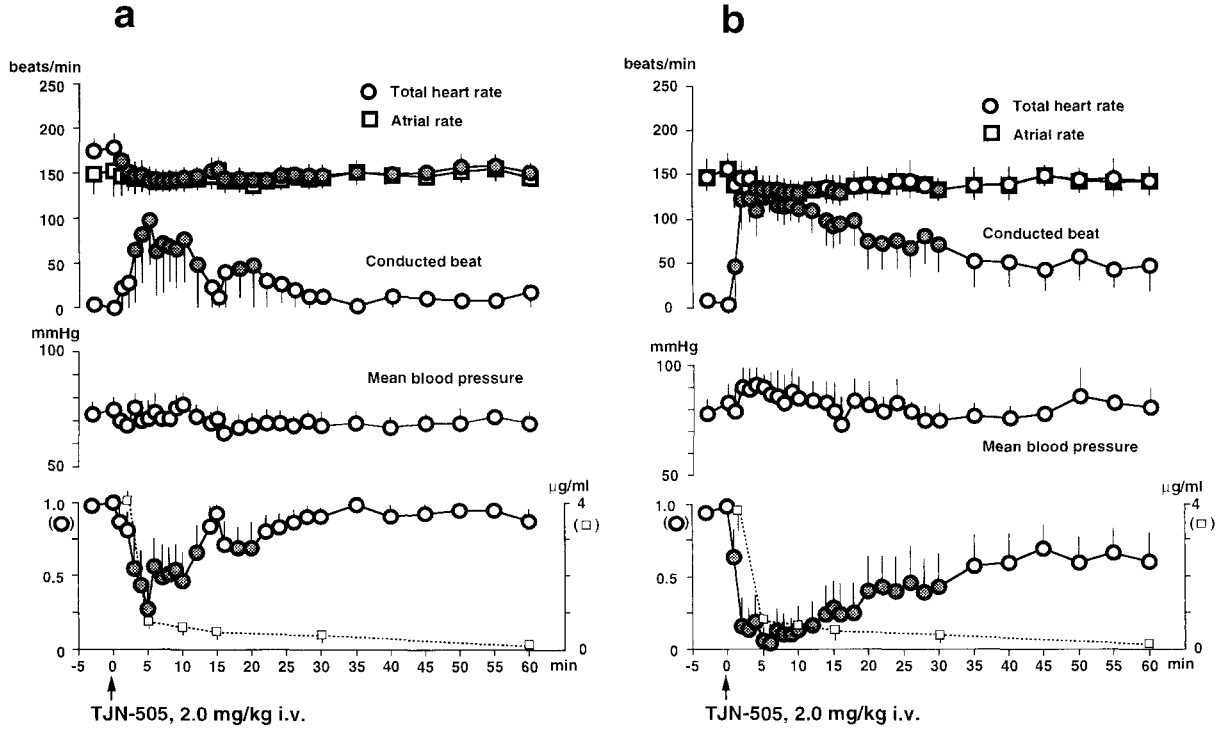

Fig. 3. Effects of TJN-505 on 24 h (a) and 48 h (b) two-stage coronary ligation-induced arrhythmia. Values are means ± S.E.M of 6 experiments. Lower panel: arrhythmic ratio (open circles), plasma concentration of TJN-505 (open squares). Shaded symbols represent significant changes ($P < 0.05$) from time 0 values.

($n = 2$) showed a weak antiarrhythmic effect and 5 min after injection of TJN-505, the average arrhythmic ratio decreased from 1.00 to 0.87. The 3-mg/kg dose ($n = 2$) decreased the average arrhythmic ratio from 0.92 to 0.00 and the effect lasted more than 60 min. To determine the effective plasma concentrations, the 2-mg/kg dose was chosen. In the 6 experiments, 5 min after injection of TJN-505, the average arrhythmic ratio decreased from 1.00 to 0.27 and the effect lasted for up to 12 min after injection ($P < 0.05$). TJN-505 decreased the total heart rate ($P < 0.05$), but did not change the atrial rate and mean blood pressure.

Fig. 3b shows effects of TJN-505 on arrhythmias occurring 48 h after coronary ligation. The average arrhythmic ratio was 0.98 at the control time 0. In the 6 experiments, 5 min after injection of TJN-505, the average arrhythmic ratio decreased from 0.98 to 0.06 and the effect lasted for up to 30 min after injection ($P < 0.05$). TJN-505 decreased the total heart rate and atrial rate ($P < 0.05$), but it did not change mean blood pressure. Linear regression analysis gave an IC_{50} of 0.94 μg/ml and the 95% confidence range was 0.53–2.12 μg/ml ($r = -0.547$, $n = 42$, $P = 0.0002$) in the 24 h two-stage coronary ligation-induced arrhythmia model, where the values for 0 to 60 min after injection of TJN-505 were used.

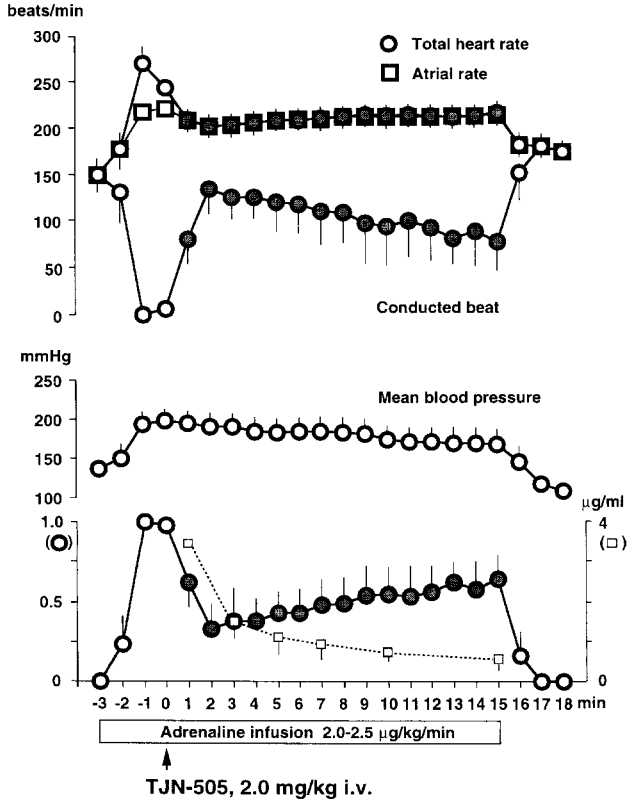

Fig. 4. Effects of TJN-505 on adrenaline-induced arrhythmia. Values are means ± S.E.M of 6 experiments. Lower panel: arrhythmic ratio (open circles), plasma concentration of TJN-505 (open squares). Shaded symbols represent significant changes ($P < 0.05$) from time 0 values.

Table 1
Effects of TJN-505 on electrocardiographic parameters and programmed electrical stimulation-induced arrhythmia

Parameter	TJN-505 ($n = 5$)	
	5 min before the injection	3 min after start of the infusion
THR (beats/min)	177 ± 27	165 ± 26 [a]
SBP (mmHg)	133 ± 19	122 ± 19
DBP (mmHg)	84 ± 14	76 ± 5
PR (ms)	86 ± 10	128 ± 25 [a]
QRS (ms)	37 ± 4	56 ± 10 [a]
QTc (ms/s$^{1/2}$)	281 ± 24	343 ± 28 [a]
JTc (ms)	240 ± 28	287 ± 29 [a]
ERP (ms)	146 ± 14	174 ± 12 [a]
PES-induced arrhythmia	VPC ($n = 3$)	NI ($n = 5$) [a]
	NSVT ($n = 2$)	

The 2 mg/kg dose of TJN-505 was injected i.v. and followed by the infusion of 0.1 mg/kg per min for 5 min. At 3 min after the start of TJN-505 infusion, all the parameters were measured. THR = total heart rate; SBP and DBP = systolic and diastolic blood pressures; QTc = corrected QT interval, calculated during sinus rhythm; JTc = QTc − QRS; ERP = ventricular effective refractory period; PES = programmed electrical stimulation; VPC = ventricular premature contraction; NSVT = non-sustained ventricular tachycardia; NI = non-inducible.
Values represents means ± S.E.M. [a] $P < 0.05$ compared with before value.

IC_{50} was calculated as 0.54 μg/ml and the 95% confidence range was 0.32–0.97 μg/ml ($r = -0.693$ $n = 42$, $P = 0.0001$) in the 48 h two-stage coronary ligation-induced arrhythmia model. The 2- to 3-mg/kg doses did not produce adverse effects such as convulsion, sedation and vomiting in conscious dogs.

3.3. Effects of TJN-505 on adrenaline-induced arrhythmia

Fig. 4 shows the effects of TJN-505 on adrenaline-induced arrhythmia. Adrenaline infusion at rates of 2–2.5 μg/kg per min induced ventricular tachycardia and in the untreated group ($n = 3$), the arrhythmic ratio remained 1.0 during adrenaline infusion. The 1- and 3-mg/kg doses were tested in preliminary experiments; the 1-mg/kg dose ($n = 2$) did not completely suppress the arrhythmia, the 3-mg/kg dose ($n = 2$) decreased the average arrhythmic ratio from 1.00 to 0.00 and the antiarrhythmic effect occurred during adrenaline infusion. Therefore, the 2-mg/kg dose was used. In the 6 experiments, TJN-505 decreased the average arrhythmic ratio immediately after injection and the antiarrhythmic effect lasted during the 15 min of adrenaline infusion ($P < 0.05$). Five minutes after injection of TJN-505, the average arrhythmic ratio decreased from 0.98 to 0.43. TJN-505 decreased the total heart rate and atrial rate ($P < 0.05$), but did not change the mean blood pressure. Linear regression analysis gave an IC_{50} of 1.31 μg/ml and the 95% confidence range was 0.40–6.29 μg/ml ($r = -0.582$, $n = 20$, $P = 0.007$), where the values for 0 to 15 min after injection of TJN-505 were used.

3.4. Effects of TJN-505 on electrocardiographic parameters and programmed electrical stimulation-induced arrhythmia

Table 1 shows the effects of TJN-505 on the electrocardiographic parameters and programmed electrical stimulation-induced arrhythmias in anesthetized dogs with an old myocardial infarction. The 2-mg/kg dose was injected i.v. within 10 s as a bolus followed by infusion at a rate of 0.1 mg/kg per min for 5 min. At 3 min after the start of TJN-505 infusion, all the parameters were measured and programmed electrical stimulation was performed. TJN-505 decreased the total heart rate and prolonged PR, QRS, QTc and JTc intervals ($P < 0.05$), but did not change the systolic and diastolic blood pressures. TJN-505 also prolonged the effective refractory period of the non-infarcted myocardium ($P < 0.05$). Before injection of TJN-505, 5 dogs exhibited either ventricular premature contraction ($n = 3$) or non-sustained ventricular tachycardia ($n = 2$) on programmed electrical stimulation with up to S1–S4 stimulation. After the administration of TJN-505, programmed electrical stimulation did not induce arrhythmia ($P < 0.05$).

3.5. Effects of TJN-505 on aconitine-induced atrial arrhythmia

Fig. 5 shows the effects of TJN-505 on aconitine-induced atrial arrhythmia. The topical application of aconitine on the right atrium induced atrial flutter or fibrillation within 1–2 min and 3 min after application, and the atrial rate increased from 138 ± 13 beats/min to 500 ± 12 beats/min. In the untreated group ($n = 3$), atrial fibrillation lasted for more than 30 min. The 1-mg/kg dose was tested in preliminary experiments; the 1-mg/kg dose ($n = 2$) decreased the atrial rate for a few minutes, thus the 2-mg/kg dose was used. In the 5 experiments, TJN-505 immediately decreased the atrial rate that was increased by

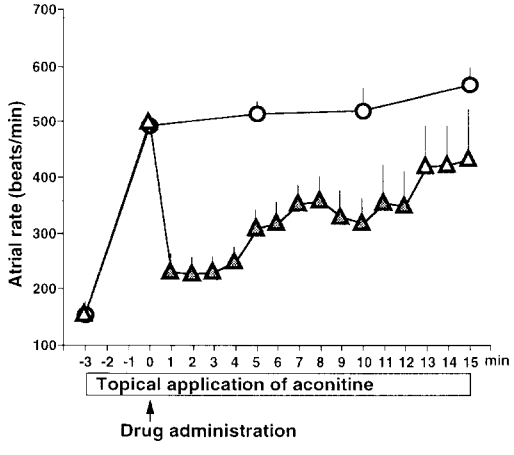

Fig. 5. Effects of TJN-505 on aconitine-induced atrial arrhythmia. Control (circles) and TJN-505 2 mg/kg (triangles). Values are means ± S.E.M of 3–5 experiments. Shaded symbols represent significant changes ($P < 0.05$) from time 0 values.

aconitine from 498 ± 146 beats/min to 229 ± 11 beats/min and decreased the average atrial rate for up to 12 min ($P < 0.05$).

4. Discussion

Aconitine has been widely used as a tool for induction of cardiac arrhythmias in various animals (Winslow, 1980; Lu and Clerck, 1993). Aconitine has been reported to prolong the open state of cardiac Na^+ channels (Peper and Trautwein, 1967; Schmidt and Schmitt, 1974; Honerjäger and Meissner, 1983) and to increase automaticity (Tanz et al., 1973). Thus aconitine must elevate the intracellular Na^+ concentrations and induce Ca^{2+} overload via a Na^+/Ca^{2+} exchange mechanism. This Ca^{2+} overload also may induce various cardiac arrhythmias (Sawanobori et al., 1987; Lu and Clerck, 1993). In China, *Aconitum contortum*, *Aconitum* plants have been used as an antidote against aconitine-induced arrhythmia. However, it is not known whether TJN-505 isolated from *Aconitum contortum* is only effective on aconitine-induced arrhythmia or is also effective on various other arrhythmias.

In the present study, we examined the effects of TJN-505, which was isolated from *Aconitum contortum*, on models of canine arrhythmia provoked by digitalis, two-stage coronary ligation, adrenaline, programmed electrical stimulation and aconitine. Digitalis-induced arrhythmia is thought to be induced by enhanced automaticity and triggered activity due to the accumulation of intracellular Na^+ and Ca^{2+}, resulting from inhibition of Na^+/K^+-ATPase. We reported that class I drugs, Na^+ channel blockers, were effective on this arrhythmia, and the effective plasma concentrations were almost similar to the in vitro concentrations that blocked the Na^+ channels of the normal canine or guinea-pig ventricular tissues (Hashimoto et al., 1991a).

Two-stage coronary ligation-induced arrhythmia is thought to be a consequence of abnormal automaticity originating from surviving subendocardial Purkinje fibers. But reentry or triggered activity in the ventricular muscle of the border from the infarcted zone may also play some role (Friedman et al., 1973; El-Sherif et al., 1982). Two-stage coronary ligation-induced arrhythmia according to the method of Harris is currently used to identify class I drugs (Hashimoto et al., 1991a; Haruno and Hashimoto, 1993). In the present study, TJN-505 suppressed digitalis- and two-stage coronary ligation-induced arrhythmias and prolonged PR and QRS intervals, thus TJN-505 may have the usual property of class I drugs to block cardiac Na^+ channels. Adrenaline-induced arrhythmia is thought to be induced by enhanced automaticity and triggered activity via the opening of cardiac Ca^{2+} channels by β-adrenoceptor stimulation. We reported that class II drugs, β-adrenoceptor antagonists, and class IV drugs, Ca^{2+} channel blockers, were effective on this arrhythmia (Hashimoto et al.,

1991a; Matsuzaki et al., 1993), and most of the class I drugs, flecainide and propafenone, also suppressed this arrhythmia (Hashimoto et al., 1991a). Flecainide has been shown to inhibit the Ca^{2+} current, in addition to the Na^+ current (Schulze and Knops, 1982). Propafenone has a β-adrenoceptor-blocking effect, in addition to the block of Na^+ current (Ledda et al., 1981; Dukes and Vaughan Williams, 1984). These additional effects, along with the blocking of Na^+ channels, may explain the antiarrhythmic effects of flecainide and propafenone on the three arrhythmia models mentioned above. These antiarrhythmic profiles of TJN-505 indicate that it may also block cardiac Na^+ and Ca^{2+} channels.

We have reported on the effects of various antiarrhythmic drugs and determined the plasma concentrations of drugs effective on arrhythmia models. The canine antiarrhythmic plasma concentrations of class I drugs on digitalis-induced arrhythmia were almost similar to the human antiarrhythmic plasma concentrations (Hashimoto et al., 1991a) and thus may also predict human effective or toxic plasma concentrations of newly developed drugs. Therefore, we also examined whether the effect of TJN-505 was correlated with the plasma concentrations. Since plasma concentrations of TJN-505 correlated with antiarrhythmic effects, we used linear regression analysis and estimated IC_{50} values of TJN-505. As these values were low in comparison with those for flecainide and propafenone (Hashimoto et al., 1991a), TJN-505 may become a potent antiarrhythmic drug.

Programmed electrical stimulation-induced arrhythmias in dogs with myocardial infarction are thought to be induced mainly by the reentry mechanism. Class III drugs, K^+ channel blockers, have been reported to suppress this reentrant arrhythmia due to the prolongation of the repolarization phase and effective refractory period (Katoh et al., 1990; Ogawa et al., 1993; Kondoh et al., 1994). Since TJN-505 prolonged the QTc and JTc intervals and the effective refractory period, and suppressed the programmed electrical stimulation-induced arrhythmia, TJN-505 may also block K^+ channels. However, some class I drugs (disopyramide, aprindine and flecainide) have been reported to prevent the ventricular tachycardia induced by programmed electrical stimulation (Ogawa et al., 1993). Flecainide has, however, been reported to fail to prevent the programmed electrical stimulation-induced arrhythmia (Kou et al., 1987), and flecainide, to facilitate the induction of sustained ventricular tachycardia (Lynch et al., 1987). The proarrhythmic mechanisms of class I drug effects by programmed electrical stimulation may be due to an increased dispersion of the local effective refractory period and/or marked reduction in conduction velocity (Miyazaki et al., 1988; Ogawa et al., 1993).

In the present study, although TJN-505 prolonged the effective refractory period in the non-infarcted myocardium and reduced conduction velocity, it did not have any proarrhythmic effect.

From the similarities between the chemical structure of TJN-505 and aconitine, the effect of TJN-505 may partly contribute to the antiarrhythmic effect of *Aconitum contortum* on aconitine intoxication. However, class I drugs have been reported to be effective on the aconitine-induced atrial canine arrhythmia model (Mitsuhashi and Hashimoto, 1988). Therefore it is difficult to determine whether the antiarrhythmic effects of TJN-505 on this arrhythmia model are due to unspecific Na^+ channel blocking effects or to aconitine antagonistic effects. The present in vivo experiments indicate possible multiple cardiac channel blocking effects of TJN-505 along with aconitine antagonistic effects. Cardiac electrophysiologic experiments and receptor analysis should throw light on the question. The effects of TJN-505 on the autonomic nervous system should also be clarified, because lappaconitine, which is structurally related to TJN-505, has been reported to activate the cardiac vagal nerve (Chiao et al., 1995).

In conclusion, TJN-505 is an active substance responsible for the antiarrhythmic effect of *Aconitum contortum* and it suppresses various canine ventricular and atrial arrhythmias due to multiple channel blocking effects.

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