Effects of a New Antiarrhythmic Drug, SD-3212, on Canine Ventricular Arrhythmia Models

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SUMMARY

The antiarrhythmic effects of a new antiarrhythmic agent, SD-3212, (-)-(S)-3,4-Dihydro-2-[5-methoxy-2-[3-[N-methyl-N-[2-[(3,4-methylene dioxy)phenoxy]ethyl]amino]propoxy]phenyl]-4-methyl-3-oxo-2H-1,4-benzothiazine hydrogen fumarate, were investigated using canine models of ventricular arrhythmias, i.e. spontaneously occurring digitalis-, two-stage coronary ligation- and adrenaline-induced arrhythmias. SD-3212 suppressed adrenaline-induced arrhythmia and showed some antiarrhythmic effect on digitalis- and 48 hr coronary ligation-arrhythmias. These results indicate that SD-3212 has antiarrhythmic effects common among class IV antiarrhythmic drugs and also has additional efficacy common among class I antiarrhythmic drugs, thus when considering the level of experimental arrhythmias it somewhat resembles propafenone. It may therefore become a clinically useful antiarrhythmic drug among typical class I or class IV antiarrhythmic drugs.

Key Words:
SD-3212 Ventricular arrhythmia Effective plasma concentration

SD-3212, (-)-(S)-3,4-Dihydro-2-[5-methoxy-2-[3-[N-methyl-N-[2-[(3,4-methylene dioxy)phenoxy]ethyl]amino]propoxy]phenyl]-4-methyl-3-oxo-2H-1,4-benzothiazine hydrogen fumarate is a new synthetic antiarrhythmic drug which was reported to have antiarrhythmic effects in rat and canine models.1)-4) This compound is a stereoisomer of SD-3211, which is an investigational non-dihydropyridine type Ca²⁺ antagonist.5) Electrophysiological studies demonstrated that SD-3212 decreased the maximum rate of rise (Vmax) of the normally polarized action potential at a concentration range of 10⁻⁶-10⁻⁵ M and decreased the action potential duration at a concentration range of 10⁻⁶-10⁻⁵ M in isolated guinea pig ventricular muscle preparations, while SD-3211 only decreased the action potential duration at a con-

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centration range of 10⁻⁶-10⁻⁵ M. These data suggest that SD-3212 has Ca channel blocking effects and additional Na channel blocking effects, thus they may be expected to act as class I and IV antiarrhythmic drugs according to the Vaughan Williams' classification system.

We have been reporting the effects of various antiarrhythmic drugs using canine ventricular arrhythmia models, i.e. spontaneously occurring digitalis-, (two-stage coronary ligation- and adrenaline-induced ventricular arrhythmias, and classified antiarrhythmic drugs based on their pharmacological effectiveness: those effective on three arrhythmias such as many of the class I drugs including aprindine, mexiletine, cibenzoline, flecainide, and propafenone, those effective on coronary ligation- and digitalis-induced arrhythmias such as disopyramide, procainamide, pilscainide, and those effective on adrenaline-induced arrhythmia such as class II β-blockers and class IV Ca channel blockers. Using the same experimental methods, the present experiments were designed to examine the antiarrhythmic effects of SD-3212 qualitatively and quantitatively compared to other antiarrhythmic drugs.

**Methods**

**Production of digitalis-induced arrhythmia**

Six mongrel dogs of either sex, weighing 9–10 kg, were anesthetized with intravenous pentobarbital sodium, 30 mg/kg. As reported earlier, 40 µg/kg ouabain was injected intravenously and then followed by an additional 10 µg/kg every 20 min until stable ventricular tachycardia was produced. In the preliminary experiments using bolus injections of up to 3 mg/kg SD-3212, a transient but severe hypotensive effect that might obscure the antiarrhythmic action of SD-3212 occurred, thus a constant rate infusion was used in this experiment. Infusion of SD-3212 was performed for 10 min using a syringe pump (Terumo, Tokyo, Japan), and arterial blood samples were drawn from one lumen of the arterial double lumen cannula at 0, 1, 3, 5, 7, 9, 13, 15, 30 and 60 min after the start of the infusion.

The lead II ECG, atrial electrogram from catheter tip electrodes in the right atrium, and the instantaneous and mean blood pressure were continuously recorded.

**Production of two-stage coronary ligation-induced arrhythmia**

Six female beagle dogs, weighing 7–11 kg, were anesthetized initially with intravenous thiopental sodium, 30 mg/kg, and intubated. As reported earlier, the chest was opened and the two-stage coronary ligation of the left anterior descending artery (LAD) 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 a telemetry system (Nihon Kohden, WEB-5000, Tokyo). The same constant rate infusion of SD-3212 was performed for 10 min using a syringe pump, and arterial blood samples were drawn from one lumen of the arterial double lumen cannula at 0, 1, 3, 5, 7, 9, 13, 15, 30 and 60 min after the start of the infusion.

### Production of adrenaline-induced arrhythmia

Six mongrel dogs of either sex, weighing 7–16 kg, were anesthetized initially with thiopental sodium, 30 mg/kg. As reported earlier, after intubation, 1.0% halothane, vaporized with 100% oxygen, was administered with a volume-limited ventilator (20 ml/kg, 15 strokes/min). Adrenaline was infused through the left femoral vein at a rate of 2.5 μg/kg/min using a syringe pump. If multifocal ventricular tachycardia was not induced, a higher infusion rate was employed. At 3 min after the start of adrenaline infusion, 2 mg/kg SD-3212 was injected as a bolus into the right femoral vein within seconds. Arterial blood samples were drawn from one lumen of the arterial double lumen cannula 1 min before and 1, 3, 5, 7, 9, 13 and 15 min after the injection.

The lead II ECG, atrial electrogram from catheter tip electrodes in the right atrium and the instantaneous and mean blood pressures were continuously recorded.

### Determination of plasma concentration of SD-3212

A sensitive and specific determination of SD-3212 in plasma was performed at Santen Pharmaceutical Co., Ltd. (Osaka, Japan) using a high performance liquid chromatographic method.

### Statistics

One way analysis of variance (ANOVA) was used to compare treatment groups with the control group. If the ANOVA value was significant, comparisons between the control and treatment groups were performed using a one way ANOVA followed by Dunnett’s t-test to localize the significant difference. A p value of less than 0.05 was considered significant. All statistics were run with Stat View 512+ statistical software (Brainpower Inc., CA, USA) on a Macintosh personal computer.
Evaluation of the antiarrhythmic effects

The severity of ventricular arrhythmia was expressed by the arrhythmic ratio: the 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 the ventricular ectopic beats were judged by the different shape of the ventricular complex from the normal QRS complex. The arrhythmic ratio before drug injection was almost 1 as shown in the control values of the figures, and there were no spontaneous improvements in these ratios. If the values after drug administration were decreased significantly from the 0 time value, the drug was judged to have antiarrhythmic effects. The antiarrhythmic plasma concentrations of drugs were determined as follows: simple linear regression was calculated between the data of plasma concentrations of a drug and the data of arrhythmic ratio when the plasma were obtained using the computer program Stat View 512+, and the plasma concentration which decreased the arrhythmic ratio to 50% of that at 0 concentration was calculated as the 50% inhibitory concentration, IC_{50}.

Results

Digitalis-induced arrhythmia

After intravenous injection of a total dose of about 70–90 µg/kg ouabain, almost all the beats became of ventricular origin. In the preliminary experiments using various infusion rates from 0.3 to 0.6 mg/kg/min, SD-3212 at the 0.6 mg/kg/min infusion rate was found to produce a significant antiarrhythmic effect with minimum cardiovascular depression. Thus this rate of infusion was chosen in the present study. As shown in Fig. 1, SD-3212 significantly decreased the total heart rate, atrial rate and blood pressure and increased the number of conducted beats 3–4 min after the start of infusion. The arrhythmic ratio started to decrease from the control value of 0.84±0.19 (mean±S.D., n=6) to 0.34±0.45 after 4 min as the plasma concentration of SD-3212 increased, but then even though the plasma concentration continued to increase the number of conducted beats decreased and the arrhythmic ratio increased. This may be due to the appearance of the proarrhythmic effect seen in 1 dog and the disappearance of atrial beats seen in 1 dog, thus the plasma concentration decreasing the arrhythmic ratio 50% was somewhat close to the toxic concentration. After stopping the infusion, the antiarrhythmic effect reappeared and remained decreased thereafter and even at 45 min after stopping the infusion. There was a statistically significant linear regression between the plasma concentration of SD-3212 and the arrhythmic
Fig. 1. Summary of the effects of intravenous infusion of SD-3212 on digitalis-induced arrhythmia. SD-3212 decreased the arrhythmic ratio and increased the number of conducted beats. SDs are shown only at control and 0 time. Shadowed marks represent significant changes from 0 time values (p<0.05).

Fig. 2. Correlation between plasma SD-3212 concentration and its antiarrhythmic effects in the digitalis-induced arrhythmia model. Dashed line represents the 95% confidence bands for the true mean.
ratio data \( r = -0.47, n=24, p<0.05 \), and the calculated antiarrhythmic plasma concentration for digitalis arrhythmias was 7.8 µg/ml \( (4.9-\infty, 95\% \) confidence range, Fig. 2).

**Two-stage coronary ligation-induced arrhythmia**

After 1–2 days of coronary ligation, all dogs showed continuously occurring multifocal ventricular ectopic beats. The arrhythmic ratios after 24 and 48 hr of ligation were 0.96±0.08 (mean±S.D., n=6) and 0.80±0.27 (n=6), respectively (Figs. 3 and 4), showing that the 48 hr arrhythmia was less severe. The same infusion rate as that used in the digitalis arrhythmia experiment was used to examine the arrhythmic effect of SD-3212 in this arrhythmia model.

For 24 hr arrhythmia, SD-3212 decreased the total heart rate, and increased the number of conducted beats, but the arrhythmic ratio did not decrease significantly as shown in Fig. 3. One dog vomited during the drug infusion. For 48 hr arrhythmia, SD-3212 at the same infusion rate showed an antiarrhythmic effect starting 7 min after the start of infusion, and this

![Fig. 3. Summary of the effects of intravenous infusion of SD-3212 on the 24 hr coronary ligation arrhythmia. SDs are shown only at control and 0 time. Shadowed marks represent significant changes from 0 time values \( p<0.05 \).](image-url)
Fig. 4. Summary of the effects of intravenous infusion of SD-3212 on the 48 hr coronary ligation arrhythmia. SD-3212 decreased the arrhythmic ratio and increased the number of conducted beats. SDs are shown only at control and 0 time. Shadowed marks represent significant changes from 0 time values (p<0.05).

The linear regression between the plasma concentration of SD-3212 and the arrhythmic ratio data in the 48 hr experiment (r=−0.47, n=29, p<0.05) yielded calculated antiarrhythmic plasma concentrations of 18.3 (9.8−∞) μg/ml for 48 hr coronary ligation arrhythmias.

Adrenaline-induced arrhythmia

As reported previously, adrenaline infusion for 3 min at a rate of 2.5–3.5 μg/kg/min induced tachycardia with almost all the beats consisting of ventricular ectopic beats. Three doses of SD-3212, 1, 2 and 3 mg/kg, were examined in the preliminary experiments. At a dose of 1 mg/kg, SD-3212 did not show any antiarrhythmic effect, and at a dose of 3 mg/kg it showed a severe hypotensive effect; therefore a dose of 2 mg/kg was chosen for this study. As shown in Fig. 5, the number of ventricular ectopic beats decreased only transiently at 0.5 and 1 min after SD-3212 injection, and arrhythmia reap-
Fig. 5. Summary of the effects of intravenous injection of SD-3212 on adrenaline arrhythmia. SD-3212 showed a transient antiarrhythmic effect. Vertical bars show SD, *p<0.05.

peared. The linear regression between the plasma concentration of SD-3212 and the arrhythmic ratio data ($r = -0.37, n=23, p<0.08$) yielded calculated antiarrhythmic plasma concentrations of 1.8 (9.8−∞) μg/ml for adrenaline arrhythmias.

**DISCUSSION**

The present experiment using the 3 canine ventricular arrhythmia models confirmed previous reports that SD-3212 is effective in animal experimental arrhythmias. In 48 hr coronary artery ligation induced ventricular arrhythmia, SD-3212, 6 mg/kg/10 min, showed some antiarrhythmic effect with slightly decreasing effects on the atrial rate and blood pressure, and also showed some central nervous system stimulating effect, such as vomiting. The effective antiarrhythmic plasma concentration data of SD-3212 obtained in this study may only provide a rough measure of the drug’s potency as judged by the wide confidence limit, however, since there were significant correlations between the plasma concentrations and the antiarrhythmic effects, they allowed us to discuss the following. We calculated the IC$_{50}$ for 48 hr ar-
rhythmias to be around 18 μg/ml. However, in 24 hr arrhythmias, the same 6 mg/kg/10 min SD-3212 only decreased the total heart rate and did not show any antiarrhythmic effect, and the higher dose showed a hypotensive effect without suppressing arrhythmia. This may indicate that the 24 hr coronary ligation arrhythmia is more severe compared to the 48 hr arrhythmia and less susceptible to antiarrhythmic drugs, though we have shown that most class I drugs showed antiarrhythmic effects on both 24 and 48 hr arrhythmias and the effective plasma concentrations did not differ significantly. The reason for the ineffectiveness of SD-3212 on the 24 hr arrhythmia was not determined, but it is clear that SD-3212 is not a potent antiarrhythmic drug for these arrhythmias. In digitalis induced arrhythmia, the antiarrhythmic plasma concentration was around 9 μg/ml, and was 4 times higher than the concentrations in vitro suppressing Vmax of action potentials of guinea pig ventricular muscle, namely about 2 μg/ml. This drug concentration in crystalline solution is that of a free drug and naturally the concentration of a free drug in plasma is lower than the plasma concentration by the fraction bound to plasma protein, thus the present result suggests that regardless of the mechanism of generation of coronary ligation and digitalis arrhythmias, the antiarrhythmic effects on these arrhythmias occurred when the Na channels were blocked by the drug, probably suppressing automaticity in the ventricular tissue or decreasing conduction velocity. In adrenaline induced arrhythmias, a lower dose of 2 mg/kg SD-3212 was effective and the IC50 was low at about 1.8 μg/ml. We have reported that Ca channel block is one mechanism by which drugs suppress this arrhythmia, and electrophysiologically that the Ca channel blocking concentration of SD-3212 in vitro was about 2 μg/ml, almost the same as the plasma concentration of SD-3212 on adrenaline induced arrhythmia. We speculate that SD-3212 suppressed this arrhythmia by both Ca and Na channel blocking effects.

Compared with our previous studies using the same canine arrhythmia models and determining the antiarrhythmic plasma concentration of antiarrhythmic drugs, SD-3212 exhibited an antiarrhythmic profile somewhat similar to that of propafenone and KT-362, in that these drugs were effective on 3 canine ventricular arrhythmias, except for 24 hr coronary ligation arrhythmia, and were most potent on adrenaline arrhythmia as judged by the lowest effective plasma concentrations as compared to that on the digitalis and coronary ligation arrhythmias. Propafenone is a class I drug having a β blocking effect, and KT-362 is a Na channel blocker with an additional Ca channel blocking effect and an intracellular Ca release blocking effect in smooth muscles. It is interesting that SD-3212, which is an equipotent blocker of Na and Ca channels, showed a similar pattern of effectiveness. Unlike SD-
3212, other class IV Ca channel blocking drugs that we have tested, including those with an additional Na channel blocking effect at higher concentrations such as verapamil and bepridil, only showed effectiveness on adrenaline arrhythmia. Therefore, our in vivo study indicates that SD-3212 is a Na and Ca channel blocking drug not like its stereoisomer, SD-3211, which is a potent Ca channel blocker with little Na channel blocking effect.\textsuperscript{4)–6)}

In conclusion, the result of the present investigation indicates that SD-3212 is an antiarrhythmic agent possessing efficacy in 3 canine arrhythmia models; digitalis-, 48 hr coronary ligation- and adrenaline-induced arrhythmias, especially for adrenaline arrhythmia, and though it has a significant blood pressure lowering effect, it may become a clinically useful antiarrhythmic drug for both supraventricular and ventricular arrhythmias as in the case of propafenone.

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