

Antiarrhythmic Effects of MS-551, a New Class III Antiarrhythmic Agent, on Canine Models of Ventricular Arrhythmia

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ABSTRACT—The antiarrhythmic effects of MS-551, which prolongs cardiac action potential duration without affecting the maximum upstroke velocity of the action potential, were assessed in three different canine ventricular arrhythmia models: 1) ventricular tachycardia (VT) induced by electrical stimuli 3–5 days after myocardial infarction, 2) spontaneous ventricular tachyarrhythmias 24–48 hr after two-stage coronary ligation and 3) ventricular tachyarrhythmias induced by digitalis. Intravenous administration of MS-551 (0.1–1 mg/kg) decreased the susceptibility in 10 dogs out of 13 to VT or ventricular fibrillation evoked by programmed electrical stimulation (PES) delivered to the ventricular septum 3–5 days after myocardial infarction. Oral administration of MS-551 (3 mg/kg) also decreased the susceptibility to VT evoked by PES in 7 out of 10 conscious postinfarction dogs. Concurrently, intravenous (0.1–1 mg/kg) or oral (3 mg/kg) administration of MS-551 produced increases in the ventricular effective refractory periods (ERP) by $7 \pm 1\%$ – $17 \pm 3\%$ or $13 \pm 2\%$, respectively. Similarly, *d*-sotalol (0.3–3 mg/kg, i.v. and 10 mg/kg, p.o.) decreased the susceptibility to VT with increased ERP. However, MS-551 (1 and 10 mg/kg, i.v.) failed to inhibit both canine two-stage coronary ligation arrhythmia and digitalis arrhythmia. These results suggest that MS-551 is a pure class III antiarrhythmic drug which may be effective in the treatment of life-threatening reentrant tachyarrhythmias, but not in automaticity arrhythmias.

Recently, the Cardiac Arrhythmia Suppression Trial (CAST) showed that class I antiarrhythmic drugs (encainide and flecainide) could not reduce the mortality in patients with ventricular arrhythmias after myocardial infarction (1). Moreover, these class I drugs rather increased the risk of cardiac death in these patients. Therefore, more attention has been focused on class III antiarrhythmic drugs which may prevent the development of lethal

arrhythmias more efficiently than the class I drugs (2).

MS-551, 1,3-dimethyl-6-[(2-[*N*-(2-hydroxyethyl)-3-(4-nitrophenyl)propylamino]ethylamino)-2,4(1*H*,3*H*)-pyrimidinedione hydrochloride, is a new class III antiarrhythmic agent having electrophysiologically similar properties to sotalol, although MS-551 has no beta-adrenergic blocking action. In an in vitro study (3), MS-551 at concentrations above 1

μM selectively prolonged the cardiac action potential duration of canine Purkinje fibers, but had no effect on the maximum upstroke velocity of the action potential even at 100 μM . In an *in vivo* study (4), MS-551 (0.1–30 mg/kg, *i.v.*) decreased the heart rate accompanied by QTc prolongation and increased atrial and ventricular ERP in a dose-dependent manner. Thus, it was suggested that MS-551 might be effective in cardiac arrhythmias by a different mechanism from class I antiarrhythmic drugs. Lucchesi et al. suggested that ventricular tachycardia (VT) in response to programmed electrical stimulation (PES) delivered to the ventricular septum of dogs with myocardial infarction is an useful method for evaluating pharmacological interventions for the prevention of life-threatening reentrant tachyarrhythmias (5–8). They showed that a number of class III antiarrhythmic drugs prevented such VT. In the present study, we examined the efficacy of MS-551 on VT induced by PES in conscious or anesthetized dogs during the subacute phase of myocardial infarction.

Furthermore, we studied the antiarrhythmic effect of MS-551 on two-stage coronary ligation arrhythmia and digitalis arrhythmia, the most commonly used canine experimental ventricular arrhythmia models for preclinical assessment of class I antiarrhythmic drugs. Although it was pointed out that abnormal automaticity resulted from infarcted lesion (9) and oscillatory afterpotential (10) may be the main mechanisms of these two arrhythmias, respectively, the possible contributions of other mechanisms, such as reentry, have not yet been excluded. Therefore, we thought it would be of interest to assess the effect of MS-551, a pure class III antiarrhythmic drug, on these two canine models of ventricular arrhythmias.

MATERIALS AND METHODS

VT model

Animal preparation: Mongrel dogs, weighing between 8 and 14 kg, were anesthetized with sodium pentobarbital (30 mg/kg, *i.v.*). A

cuffed endotracheal tube was inserted and the animals were ventilated with room air in a tidal volume of 20 ml/kg at 18 breaths/min using a Harvard respirator. Using an aseptic technique, a left thoracotomy was performed in the fourth intercostal space and the anterior surface of the heart was exposed. The heart was then suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was isolated at the proximal portion and occluded by a suture passed around the artery. After 2 hr of regional ischemia, the blood flow was restored. A bipolar plunge electrode (25 gauge insulated stainless steel wire, 5 mm in length, 2 mm apart) was inserted into the interventricular septum near the right ventricular outflow tract adjacent to the site of the LAD occlusion. The chest incision was closed and the animals were allowed to recover from surgical anesthesia, and given benzyl penicillin procaine (20,000 U/kg, *i.m.*; Meiji, Tokyo, Japan).

VT induction: Three to five days after myocardial infarction, the animals were returned to the laboratory. Some of them were used in the conscious state and the rest were anesthetized again with sodium pentobarbital (30 mg/kg, *i.v.*). Basic electrical stimuli (S1: 400 msec cycle length, 4 msec duration, $2 \times$ diastolic threshold) were delivered to the ventricle via a bipolar plunge electrode inserted into the interventricular septum, using a programmable cardiac stimulator (DHM-230, DIA Medical) and an isolation unit (DPS-105, DIA Medical). The PES procedure was performed under ventricular pacing according to the method described by Lynch et al. (5). Single (S2), double (S2, S3), and then triple (S2, S3, S4) premature ventricular stimuli were introduced as follows: 1) Single ventricular extra-stimuli were introduced at intervals descending in 10-msec steps from 350 msec after S1 until ventricular refractoriness occurred. The ventricular effective refractory period (ERP) was determined by this procedure. 2) In the double premature ventricular stimulation, S2 coupled with S3 in various coupling intervals (200, 180, 170, 160, 150, 140, 135 and 130 msec)

was introduced at intervals decreasing in 10-msec steps from 350 msec until ventricular refractoriness occurred. 3) In the triple premature ventricular extrastimulation, S2 coupled with S3 and S4 was introduced at intervals decreasing in 10-msec steps from 350 msec until ventricular refractoriness; coupling intervals of S3–S4 were the same as that of S2–S3 which varied as stated above. In conscious dogs sedated in a cage, the PES procedure was performed under the controlled heart rate by ventricular overdrive pacing. In anesthetized dogs, sinus pacemaker activities were eliminated by injecting ethanol (0.2 ml) into the sinus node artery or crushing the area, if necessary.

In the present study, ventricular tachyarrhythmias were defined as follows: “nonsustained”, when there were five or more repetitive ventricular responses, which terminate spontaneously within 30 sec, and “sustained”, when the response persisted longer than 30 sec or required ventricular burst pacing for the termination to prevent hemodynamic deterioration. Once ventricular fibrillation occurred, the hearts were electrically defibrillated by a defibrillator (Senko Medical).

Experimental procedures: 1) In anesthetized dogs, the aortic arterial pressure was measured with a Statham P50 transducer. The lead II ECG and arterial pressure were recorded continuously on a polygraph (RM-6000, Nihon Kohden). After the determination of predrug electrophysiological values and reproducibility of VT or VF by means of the PES procedure, MS-551 (0.1 mg/kg) was administered intravenously. Electrophysiological testing was repeated at 5 min after MS-551 administration. Additional cumulative doses of MS-551 (0.3, 1 and 3 mg/kg) were administered in an identical manner and the electrophysiological testing was repeated. 2) In conscious dogs, the lead II ECG was monitored by a telemetry system (ZB-141G, ZR-601G, Nihon Kohden). After predrug electrophysiological parameters and reproducibility of VT were determined, MS-551 (3 mg/kg) was administered orally. The electrophysiological testing and PES were re-

peated every 15 min after MS-551 administration.

Two-stage coronary ligation arrhythmia model

Mongrel dogs of either sex, weighing 9–13 kg, were anesthetized initially with thiopental sodium (30 mg/kg, i.v.). According to the method described by Hashimoto et al. (11), the chest was opened and the two-stage coronary ligation was performed under halothane anesthesia (1–1.5%).

Experiments were done without anesthesia at 24 and 48 hr after coronary ligation. The lead II ECG, the atrial electrogram from the left atrial appendage, and aortic blood pressure were recorded continuously using a telemetry system (Nihon Kohden) on a recorder (WT-685G, Nihon Kohden). MS-551 was injected through a cannula into the jugular vein.

Digitalis arrhythmia model

Mongrel dogs of either sex, weighing 8–13 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.). As reported earlier (12, 13), 40 μ g/kg of ouabain was injected intravenously and then followed by an additional 10 μ g/kg every 20 min until stable ventricular arrhythmia was produced. MS-551 was injected intravenously through a cannula in the femoral vein within several seconds. The lead II ECG, atrial electrogram from a catheter tip electrode in the right atrium and blood pressure were recorded continuously as described above.

Drugs

MS-551 and *d*-sotalol were synthesized at Mitsui Toatsu Chemicals, Inc., Japan, and they were dissolved in 0.9% NaCl.

Statistics

Data are expressed as means \pm S.E.M. The significance of differences between mean values was assessed by Student's *t*-test for paired samples, and a value of $P < 0.05$ was considered significant.

RESULTS

Effects of MS-551 and d-sotalol on ventricular tachyarrhythmias induced by PES

VT models in anesthetized dogs: Fifty-five dogs were subjected to PES 3–5 days after myocardial infarction. In 32 of the dogs, VT was not inducible or not reproducible in response to PES, and these dogs were not used for further study. The remaining 23 dogs responded to predrug programmed stimulation with reproducible nonsustained or sustained VT or VF. Typical tracings of such arrhythmias are shown in Fig. 1. The occurrence of ventricular arrhythmias in 13 postinfarction dogs before and after the administration of MS-551 are illustrated in Fig. 2. The cumula-

tive intravenous administration of MS-551 lessened the severity of ventricular tachyarrhythmias induced by PES in 10 out of 13 dogs. MS-551 was effective in converting sustained VT and VF to nonsustained VT or the noninducible one, except for one dog. Nonsustained VT was relatively resistant to MS-551. The suppression of inducible VT by MS-551 appeared to be closely associated with increased ventricular ERP, as shown in Table 1. The cumulative administration of up to 1 mg/kg of MS-551 had no significant effect on the mean arterial pressure and excitation threshold of ventricular myocardium. The excitation threshold of ventricular myocardium was not changed even at the highest dose of MS-551 (1 mg/kg).

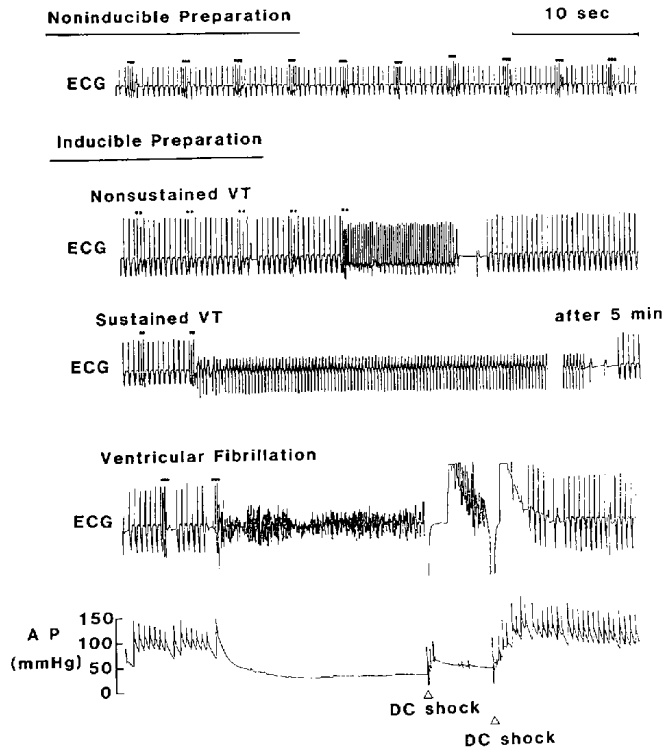


Fig. 1. Typical ECG (II) tracings of ventricular tachycardias (VT) induced by double or triple premature ventricular extrastimuli (denoted by dots) in anesthetized postinfarction dogs. Sinus pacemaker activities were extinguished with ethanol injection into the sinus artery. When ventricular fibrillation occurred, DC shock was immediately delivered to the heart using a defibrillator.

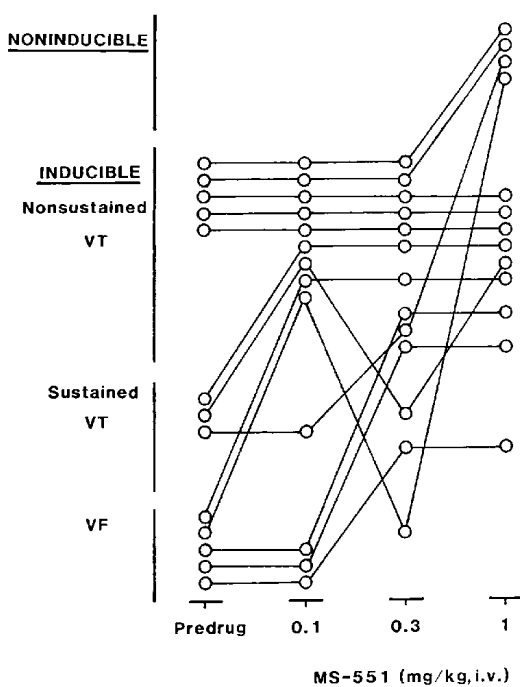


Fig. 2. Effects of MS-551 on ventricular tachycardia (VT) induction by programmed electrical stimulation in 13 anesthetized postinfarction dogs. Each symbol represents one animal, with the nature of the response of each animal to programmed stimulation listed on the y-axis.

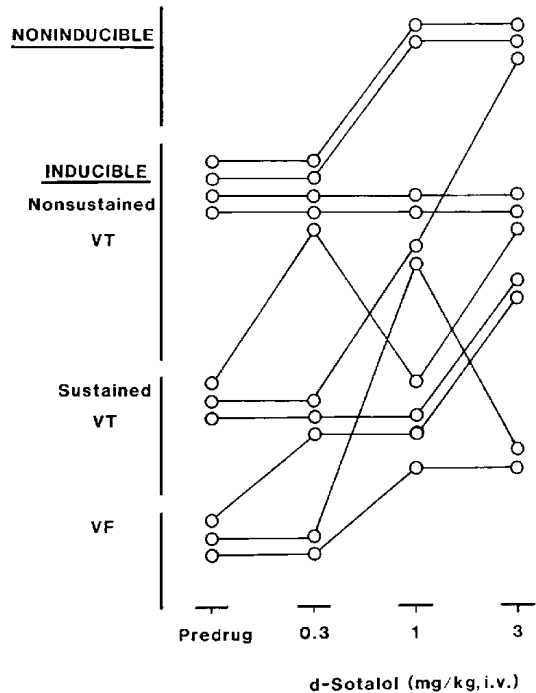


Fig. 3. Effects of *d*-sotalol on ventricular tachycardia (VT) induction by programmed electrical stimulation in 10 anesthetized postinfarction dogs. Each symbol represents one animal, with the nature of the response of each animal to programmed stimulation listed on the y-axis.

Table 1. Effect of MS-551 and *d*-sotalol on ventricular effective refractory periods (ERP) in anesthetized postinfarction dogs

MS-551 (mg/kg, i.v.)					<i>d</i> -Sotalol (mg/kg, i.v.)					
	N	Predrug	0.1	0.3	1	N	Predrug	0.3	1	3
ERP (msec)	13	149 ± 4	159 ± 4*	166 ± 4**	175 ± 5**	10	145 ± 4	156 ± 4*	161 ± 3**	167 ± 4**

Values shown are the means ± S.E.M. *P < 0.05, **P < 0.01, as compared to the predrug values.

Similarly, *d*-sotalol (0.3–3 mg/kg, i.v.) decreased the susceptibility to the induction of VT by PES with increased ERP as shown in Fig. 3 and Table 1. Arterial pressure was increased slightly and transiently by higher doses of *d*-sotalol (1 and 3 mg/kg).

VT models in conscious dogs: Thirty-seven postinfarction dogs were subjected to PES. In 14 dogs, VTs were not inducible. Four dogs responded to predrug PES with a rapid VT which quickly degenerated into VF that could not be defibrillated. The remaining 19 dogs responded to PES with reproducible sustained

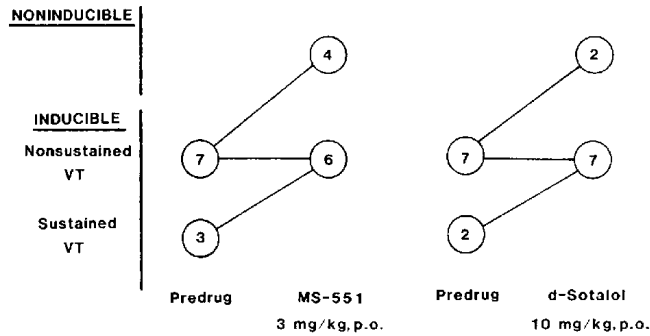


Fig. 4. Effects of oral administration of MS-551 (3 mg/kg) and *d*-sotalol (10 mg/kg) on ventricular tachycardia (VT) induction by programmed electrical stimulation in conscious postinfarction dogs. The number in the circle represents the number of animals, and the nature of the response of each animal to programmed stimulation is listed on the y-axis.

or nonsustained VT. The responses of these dogs to PES before and after oral administration of MS-551 (3 mg/kg) and *d*-sotalol (10 mg/kg) are illustrated in Fig. 4. Both MS-551 and *d*-sotalol lessened the severity of VT with increased ventricular ERP ($13 \pm 2\%$ and $15 \pm 2\%$). The inhibitory action of MS-551 and *d*-sotalol on VT appeared within 60 min after drug administration, and drug action persisted for longer than 2 hr except for one dog (30 min).

At the end of the experiments, the anatomic locations of stimulating electrode sites were confirmed to be in the non-infarct area of the interventricular septum.

Effects of MS-551 on two-stage coronary ligation arrhythmia

After 24 hr of coronary ligation, dogs showed stable ventricular arrhythmia. MS-551 (1 and 10 mg/kg, i.v.) did not produce any significant changes in the number of ectopic beats, atrial rate, blood pressure and also the behavior. Summarized data from dogs treated with 10 mg/kg of MS-551 (i.v.) are shown in Fig. 5. On the next day, the same dogs were used for the evaluation of the efficacy of MS-551 on 48-hr arrhythmia, which is less severe than 24-hr arrhythmia. Again, MS-551 at the same doses did not cause any appreciable changes in the parameters described above.

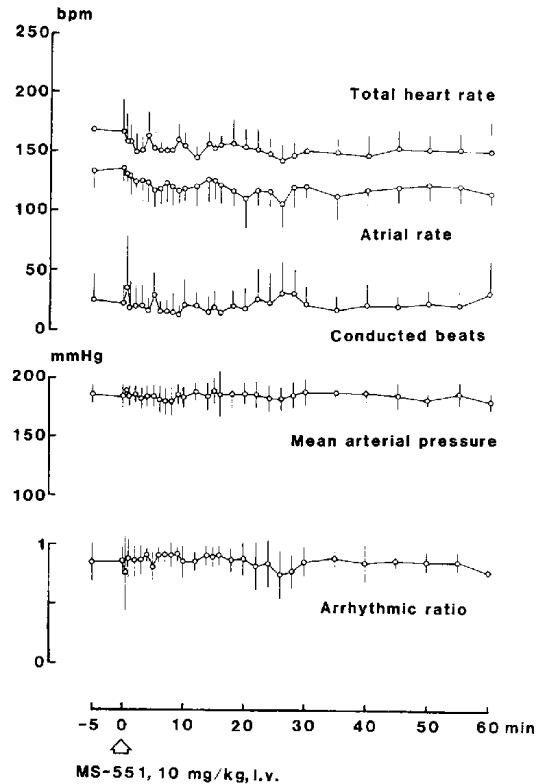


Fig. 5. Effect of MS-551 (10 mg/kg, i.v.) on canine two-stage coronary ligation arrhythmia (N = 4). Each point represents the mean value and vertical bars show the standard deviation. Arrhythmic ratio: the ratio of the number of ventricular ectopic beats divided by the total number of beats counted from the ECG (total heart rate).

Effects of MS-551 on digitalis arrhythmia

After injection of a total dose of 60–90 $\mu\text{g/kg}$ ouabain, almost all the beats were of ventricular origin. Intravenous administration of 1 and 10 mg/kg of MS-551 did not decrease the number of ectopic beats, but a high dose of MS-551 (10 mg/kg, i.v.) tended to reduce atrial rate, as shown in Fig. 6. At least, MS-551 did not aggravate digitalis arrhythmia. After the intravenous administration of 10 mg/kg of MS-551, a slight increase followed by a significant decrease in blood pressure was observed. The mechanism of the decrease in blood pressure is not clear, but might reflect the decrease in the concentration of ouabain.

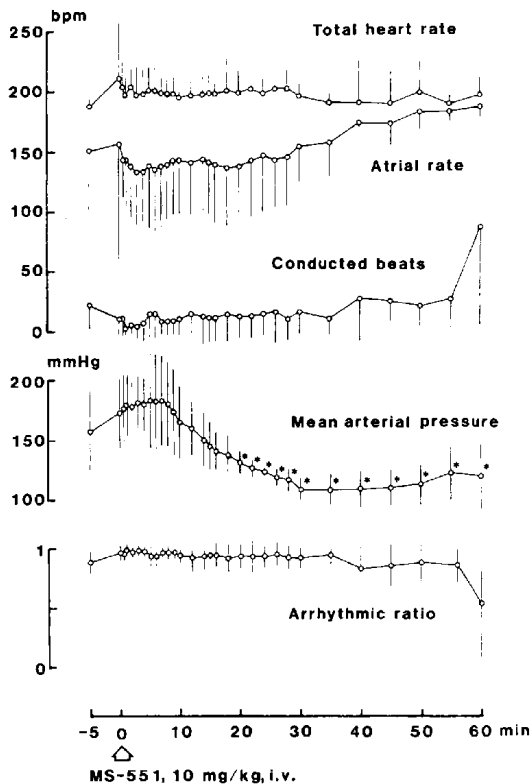


Fig. 6. Effect of MS-551 (10 mg/kg, i.v.) on canine digitalis arrhythmia ($N = 4$). Each point represents the mean value and vertical bars show the standard deviation. Arrhythmic ratio = ventricular ectopic beats/total heart rate. * $P < 0.05$; compared with values of the predrug control.

DISCUSSION

In the present study, VT could be induced repeatedly by PES in 30–40% of postinfarction dogs. The mechanism of VT evoked by PES in postinfarction dogs has been proposed to be reentry (14). It was demonstrated that the electrophysiologic substrates for reentry such as marked heterogeneity of ERP are present inside and at the periphery of the infarct zone in this model. Premature ventricular stimulation might further enhance the heterogeneity of ERP and thereby promote the development of slow conduction and block which are necessary for the initiation and the maintenance of reentry, as suggested by El-Sherif et al. (15, 16). Both intravenous and oral administration of MS-551 decreased the susceptibility to ventricular arrhythmias, concomitantly with increases in ventricular ERP. Similarly, *d*-sotalol decreased the susceptibility to the VT, although the effective doses of *d*-sotalol were about 3 times higher than those of MS-551. Increases in ventricular ERP observed in this study suggest that the class III effect might play an important role in the antiarrhythmic and antifibrillatory actions of these two drugs. MS-551 and *d*-sotalol were effective in lessening the severity of the arrhythmias in all animals with sustained VT and VF, but were less effective in those with nonsustained VT in spite of increasing ERP. The reason why these drugs were less effective in nonsustained VT is not clear, but it might be that a mechanism other than reentry might be partly involved in the nonsustained VT. It is well-known that patients resuscitated from cardiac arrest can easily show reentrant ventricular tachyarrhythmias on PES, which are apparently identical to the patients' previous spontaneously occurring arrhythmias (17–19). Moreover, it has been demonstrated that antiarrhythmic drugs, which could suppress the induction of VT in responses to PES, are successful in preventing the recurrence of the ventricular tachyarrhythmias during chronic therapy (17, 19). Therefore, MS-551, which can suppress the induction of VT in canine VT

models, is expected to be a useful antiarrhythmic and antifibrillatory drug.

Early reports indicated that *dl*-sotalol was ineffective in suppressing ventricular arrhythmias 48 hr after coronary artery occlusion or after the administration of toxic doses of ouabain (20). However, it does not mean that pure class III drugs are ineffective in these putative automaticity arrhythmias, because *dl*-sotalol has not only a class III effect but also beta-blocking action. In the present study, therefore, antiarrhythmic actions of two doses of MS-551 (1 and 10 mg/kg, i.v.) on these canine ventricular arrhythmias were examined. MS-551 did not show any significant effect on these ventricular arrhythmias even when a ten or a hundred times higher dose of the drug than that needed to suppress the PES-induced VT was given. Thus, MS-551 could not suppress ventricular arrhythmias resulting from enhanced automaticity in infarcted lesions or digitalis-intoxicated myocardium. These findings indicated that the reentrant mechanism is of little significance in the genesis of ventricular arrhythmias observed 24–48 hr after myocardial infarction or after the digitalis intoxication. In addition, these two canine models of ventricular arrhythmias, which can be readily suppressed by class I drugs, may not be useful in assessing the efficacy of pure class III drugs.

In conclusion, MS-551 may be an effective class III drug for the treatment of life-threatening reentrant tachycardias, but not for automaticity arrhythmias.

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