Quantitative Analysis of the Antiarrhythmic Effect of Drugs on Canine Ventricular Arrhythmias by the Determination of Minimum Effective Plasma Concentrations

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Antiarrhythmic effects and minimum antiarrhythmic plasma concentrations (min PC) were examined using three canine ventricular arrhythmia models, i.e., two-stage coronary ligation arrhythmia (Cor), halothane-adrenaline arrhythmia (Adr) and digitalis arrhythmia (Dig). Disopyramide suppressed these three arrhythmias and its min PCs were 5.3 ± 1.8 (Cor), 4.2 ± 1.1 (Adr) and 1.7 ± 0.4 $\mu\text{g/ml}$ (Dig). Procainamide suppressed coronary ligation arrhythmia and its min PC was 27.1 ± 4.0 $\mu\text{g/ml}$, but was not effective on adrenaline arrhythmia. Aprindine was effective on three arrhythmias and its min PCs were 1.6 ± 0.3 (Cor), 1.0 ± 0.4 (Adr) and 0.8 ± 0.4 $\mu\text{g/ml}$ (Dig). Lidocaine was not effective on coronary ligation arrhythmia, but was effective on adrenaline arrhythmia and its min PC was 15 $\mu\text{g/ml}$. Phenytoin was effective on three arrhythmias and its min PCs were 9.8 ± 2.0 (Cor), 12.1 ± 3.1 (Adr) and 11.3 ± 3.0 $\mu\text{g/ml}$ (Dig). It was not specifically effective on canine digitalis arrhythmia. Propranolol and other β -blockers were effective on adrenaline arrhythmia, but very high doses were necessary for suppressing coronary ligation and digitalis arrhythmias. Verapamil was only effective on adrenaline arrhythmia and its min PC was 0.03 ± 0.01 $\mu\text{g/ml}$. Canine min PCs of drugs were almost the same as those reported in man except for procainamide and lidocaine. Membrane stabilizing effects seem to be important for suppressing canine ventricular arrhythmias.

RECENT developments in new antiarrhythmic drug research have given clinicians the opportunity to select the most appropriate drug for arrhythmic patients. However, it is still difficult to find the mechanism of arrhythmia generation in each patient, therefore, the pharmacological characteristics of drugs and the action mechanisms of these drugs are an impor-

tant basis for selecting the proper drug. This information is usually obtained from animal experiments. We think that in animals the electrophysiological methods to evaluate drug effects on cardiac action potential characteristics or cardiac ionic channels have already been well established, but the pharmacological differences in drug effects for correlating their action mechanism have not yet been clarified. Therefore, we have tried to study the pharmacological effects of antiarrhythmic drugs qualitatively and quantitatively using three canine ventricular arrhythmia models with the simultaneous exami-

Key Words:

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nation of drug plasma concentrations. This data is useful in selecting drug concentrations in electrophysiological studies of isolated cardiac muscle of the dog. The three arrhythmia models used in the present study were: 1) two-stage coronary ligation arrhythmia (also called Harris arrhythmia), 2) halothane-adrenaline arrhythmia and 3) digitalis arrhythmia. These three arrhythmias are considered to be generated by different mechanisms.

Experiments on two-stage coronary ligation¹ and halothane-adrenaline arrhythmias² have already been reported, but the drug plasma concentrations were reanalyzed to obtain average plasma concentration data for these two arrhythmias.

METHODS

Production of Two-stage Coronary Ligation Arrhythmia

Beagle dogs, weighing 6 to 12 kg, were anesthetized initially with 30 mg/kg of thiopental sodium. After intubation, 1.5% halothane, vaporized with 95% O₂ + 5% CO₂, was administered using a volume-limited ventilator. Using an aseptic technique, the chest was opened and a two-stage coronary ligation was performed as reported previously.¹

Experiments were done without anesthesia. The lead II ECG, atrial electrogram and the blood pressure were recorded continuously using telemetry systems. Antiarrhythmic drugs were injected through a cannula inserted into the jugular vein. Venous samples were taken from this cannula 5 min before and 1, 3, 5, 10, 15, 30 and 60 min after the injection of the drugs.

Production of Adrenaline Arrhythmia

Mongrel dogs of either sex, weighing 6 to 15 kg, were anesthetized initially with thiopental sodium and after intubation the 1.0% halothane, vaporized with 95% O₂ + 5% CO₂, was administered using a volume-limited ventilator. After 30 min, adrenaline was infused into the femoral vein using a Harvard infusion/withdrawal pump as reported previously.² If ventricular arrhythmia was induced by adrenaline infusion within 2 min, infusion was continued up to 15 min. The same procedure was repeated more than 20 min after stopping the control adrenaline infusion, and after 3 min antiarrhythmic drugs were injected into the femoral vein.

The lead II ECG and blood pressure were

recorded continuously. Venous blood samples were taken from the jugular vein 5 min before and 1, 3, 5 and 10 min after the injection.

Production of Digitalis Arrhythmia

Mongrel dogs of either sex, weighing 8 to 16 kg, were anesthetized with 30 mg/kg of pentobarbital sodium. Forty µg/kg ouabain was injected intravenously which was followed by an additional 10 µg/kg every 20 min until stable ventricular arrhythmia was produced. Arrhythmia produced by the total doses of 70 to 80 µg/kg usually lasted more than one hour. Antiarrhythmic drugs were injected intravenously through the cannula inserted into the femoral vein.

Lead II ECG, the atrial electrogram and blood pressure were recorded continuously. Venous blood samples were taken from the jugular vein 5 min before and 1, 3, 5, 10, 15, 30 and 60 min after the drug injection.

Plasma Drug Assays

Appropriate chemical methods for each drug were used to determine plasma drug concentrations as reported previously.^{1,2}

Determination of the Minimum Effective Plasma Concentrations

Severity of arrhythmia was expressed by the arrhythmic ratio, the number of ventricular ectopic beats divided by the total heart rate. The last minute, up to which a statistically significant decrease of the arrhythmic ratio was observed as compared to that at time 0, was determined using the paired *t*-test. This minute was substituted for the time of the experimentally derived plasma concentration-time equations obtained using a Canon BX-1 calculator for each experiment and the resulting plasma concentrations were judged as the minimum effective plasma concentrations.

RESULTS

Our methods of analysis used in the present study are illustrated in Fig. 1, using disopyramide for the digitalis arrhythmia as an example. At time 0, just before the injection of the drug, the total heart rate was high, about 200 beats/min, and there were almost no conducted beats (QRS complexes preceded by P waves) and almost all the beats were of ventricular origin. Atrial rate, which reflects sinoatrial node activity and

Fig. 1. Effects of disopyramide, 3 mg/kg i.v., on canine digitalis arrhythmia. Disopyramide increased the number of conducted beats and decreased the arrhythmic ratio. Minimum effective plasma concentration was $1.7 \pm 0.4 \mu\text{g/ml}$. Each point represents mean value and bar standard deviation. * $p < 0.05$, ** $p < 0.01$ from the values of 0 min. See text for explanation.

counted by the sharp deflection from the left atrium or the right atrium, was lower than the total heart rate. The arrhythmic ratio at time 0 was almost 1. After drug injection, in this case 3 mg/kg of disopyramide, i.v., the total heart rate decreased as well as the atrial rate, but there was no hypotensive response to disopyramide. In the digitalis arrhythmia, the blood pressure gradually decreased after about 30 min regardless of the antiarrhythmic drugs used. Disopyramide showed antiarrhythmic effect as shown by a statistically significant increase in the number of conducted beats and a significant decrease of the arrhythmic ratio. This decrease of the ratio lasted up to 16 min after injection. Although 28 min after the injection there was still a statistically significant reduction of the arrhythmic ratio, we considered the 16th min as the last minute of

continuous antiarrhythmic effects. The minimum effective plasma concentration was $1.7 \pm 0.4 \mu\text{g/ml}$ ($n = 6$).

Table I shows minimum effective plasma concentrations of drugs thus obtained; the doses administered intravenously; statistically significant reduction of the arrhythmic ratio (∇) or worsening of arrhythmia (Δ) or no effect ($-$); statistically significant reduction of the total heart rate (∇), reduction (∇) or increase (Δ) of the atrial rate; reduction (∇) or increase (Δ) in the mean blood pressure. This table also shows the reported data on human effective plasma concentrations and membrane stabilizing concentration in the artificial solution used in isolated canine ventricular tissues.^{1,3-6}

Disopyramide: On all three arrhythmia models, disopyramide showed antiarrhythmic effects. The minimum plasma concentration for digitalis arrhythmia was lower than those for the other two arrhythmias. Although in digitalis arrhythmia the atrial rate was decreased, there were almost no depressant effects on the cardiovascular system as shown by the absence of hypotensive effect on these three arrhythmia dogs.

Procainamide: It was examined in the coronary ligation and halothane-adrenaline arrhythmias and was effective only on the former. The ventricular tachycardia induced by adrenaline often developed fatal ventricular fibrillation. Procainamide had no hypotensive effect.

Aprindine: It was effective for all three arrhythmias and the mean minimum plasma concentrations for adrenaline and digitalis arrhythmias were lower than that for coronary ligation arrhythmia. Aprindine had hypotensive effects and in unanesthetized dogs with coronary ligation arrhythmia it showed a central excitatory action.

Lidocaine: It was studied in coronary-ligation and adrenaline arrhythmias. In the coronary ligation arrhythmia, a high dose, such as 10 mg/kg, which was enough to induce central excitation with convulsions, caused atrial tachycardia and only during this atrial tachycardia did it suppress the arrhythmia. As stated in a previous paper,¹ we judged this antiarrhythmic effect to be due to the atrial overdriving action on the ventricle. The two-stage coronary ligation arrhythmia was suppressed by atrial pacing at a rate higher than the total heart rate or by tachycardia-inducing agents like isoproterenol.⁷ Lidocaine suppressed adrenaline arrhythmia only at a very high con-

TABLE I ANTIARRHYTHMIC EFFECTS AND MINIMUM EFFECTIVE PLASMA CONCENTRATIONS OF ANTIARRHYTHMIC DRUGS

Drugs	Arrhythmia	Dose (mg/kg)	Arrhythmic ratio	Minimum plasma concentration (μg/ml)	Total heart rate	Atrial Rate	Blood pressure	Human effective concentration (μg/ml)	Canine in vitro membrane stabilizing concentration (μg/ml)
Disopyramide	Cor	5	▽	5.3 ± 1.8	▽	—	Δ	(3-8)	(5)
	Adr	3	▽	4.2 ± 1.1	▽	—	—	—	—
	Dig	3	▽	1.7 ± 0.4	▽	▽	—	—	—
Procainamide	Cor	60	▽	27.1 ± 4.0	▽	—	—	(4-10)	(30)
	Adr	10-60	Δ	—	—	—	—	—	—
Aprindine	Cor	3	▽	1.6 ± 0.3	▽	—	▽	—	—
	Adr	3	▽	1.0 ± 0.4	▽	—	▽	(1.4-2.7)	(0.5)
	Dig	1.5	▽	0.8 ± 0.4	▽	▽	▽	—	—
Lidocaine	Cor	10	—	—	—	Δ	▽→Δ	(2-6)	(10)
	Adr	10	▽	15	▽	—	—	—	—
Phenytoin	Cor	10	▽	9.8 ± 2.0	▽	Δ	▽	—	—
	Adr	5	▽	12.1 ± 3.1	▽	—	▽	(10-16)	(5-10)
	Dig	10	▽	11.3 ± 3.0	▽	▽	▽	—	—
Mexiletine	Cor	5	▽	—	▽	—	▽	—	—
	Adr	5	▽	—	▽	—	▽	(0.5-2)	(2)
	Dig	3	▽	1.8 ± 0.6	▽	▽	—	—	—
Propranolol	Adr	0.03	▽	—	▽	—	Δ	(0.02-1)	(3)
	Dig	3	▽	—	—	—	—	—	—
Verapamil	Cor	0.3	—	—	▽	—	▽	—	—
	Adr	0.1	▽	0.03 ± 0.01	▽	—	▽	(0.05-0.2)	(10)
	Dig	1	—	—	▽	▽	▽	—	—

Human effective plasma concentration and canine in vitro membrane stabilizing concentration in ventricular tissues were obtained from published papers. 1,6,8-10

Cor = coronary ligation arrhythmia; Adr = adrenaline arrhythmia; Dig = digitalis arrhythmia; ▽ = reduction; Δ = increase;

— = no change

centration, such as 15 $\mu\text{g}/\text{ml}$.

Phenytoin: It suppressed all three arrhythmias, but as compared to other drugs the degree of the arrhythmic ratio reduction was weaker. Higher doses could not be used due to its strong hypotensive action. The minimum plasma concentrations for three arrhythmias were almost the same, indicating no specific effect of phenytoin on digitalis arrhythmia.

Mexiletine: It also suppressed three arrhythmias. The plasma concentration data was only available at the present time for digitalis arrhythmia. It was almost similar to the concentration data obtained in human subjects. Mexiletine produced transient hypotension in dogs with coronary ligation and adrenaline arrhythmias.

Propranolol and Other β -blockers: As has already reported,¹ many β -blockers had no antiarrhythmic effect on coronary ligation arrhythmia except for a new β -blocker, N-696. However, on adrenaline arrhythmia they were effective in small doses, such as a range from 10 to 100 $\mu\text{g}/\text{kg}$.^{8,9} The plasma concentration must be far below the membrane-stabilizing concentration of β -blockers. For digitalis arrhythmia, a high dose of propranolol, 3 mg/kg, was effective but the effect lasted for only few minutes. In this case, suppression of arrhythmia may not be explained by its β -blocking action.

Verapamil: It was effective only on adrenaline arrhythmia and the minimum effective plasma concentration was 30 ng/ml. In coronary ligation and digitalis arrhythmias, verapamil did not suppress the arrhythmic ratio. The doses used for the latter two arrhythmias were enough to produce profound hypotension, atrial arrest or atrioventricular block.

DISCUSSION

The final aim of our study is to clarify the mechanism of the antiarrhythmic drug action. Although there has been great progress in understanding arrhythmias and drug actions on them in the human heart electrophysiologically, many of the studies on the mechanism of antiarrhythmic drug actions have been done using animal tissues, especially using isolated animal cardiac muscle preparations. However, there have been few studies which have clarified the drug effects on animal arrhythmias quantitatively and qualitatively. Because canine isolated cardiac preparations are one of the most commonly used tissues for such studies, we designed our present study

to compare antiarrhythmic drugs using three canine ventricular arrhythmias and obtained minimum effective plasma concentrations for comparing the drug effects qualitatively. We found that most of the clinically effective drugs were also effective on canine ventricular arrhythmias and their human effective plasma concentrations are similar to those obtained in dogs, but lidocaine and procainamide required higher concentrations for canine arrhythmias. Lidocaine is known to be effective on post myocardial infarction arrhythmia, but it did not show antiarrhythmic effect on the two-stage coronary ligation arrhythmia. This may be due to the species difference or the difference in the clinical and the animal model arrhythmias. These results indicate that care must be taken in correlating animal electrophysiological data to clinical antiarrhythmic effects.

In comparing our animal models and the drug effects on them, two-stage coronary ligation and digitalis arrhythmias seem to be useful models of clinical ventricular arrhythmias for predicting drug effectiveness in humans, while halothane-adrenaline arrhythmia seems to be different from the commonly observed clinical arrhythmia, because the drug effects of procainamide, low doses of β -blockers and verapamil on the adrenaline arrhythmia are quite different from those on clinical ventricular arrhythmias.

Our results on phenytoin, which is not specifically effective on digitalis arrhythmia, are not consistent with the general consensus, but there is not enough clinical data to show that phenytoin is especially effective on digitalis arrhythmia³ and there has been no quantitative comparison with other drugs.

As for the mechanism of antiarrhythmic action of drugs on the canine ventricular arrhythmia, Na channel blocking or membrane-stabilizing effect seems to be important, because the membrane stabilizing concentrations are close to the minimum effective plasma concentrations.¹ However, before any definite conclusions can be reached, more precise electrophysiological studies must be done using both the normal and abnormal or pathological cardiac tissues. Ca-channel and its regulator, β -receptor, does not seem to play a significant role in generating ventricular arrhythmias because verapamil and β -blockers were only effective on the halothane-adrenaline arrhythmias. If the recently proposed Ca-channel relating arrhythmogenic mechanism¹⁰ plays significant roles in the generation of ven-

tricular arrhythmia, the Ca-channel blocker should be effective, but in our study they act more strongly on vascular, SA nodal and AV nodal Ca-channels.

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