Antiarrhythmic Effects of a New Drug, E-0747, on Canine Ventricular Arrhythmia Models

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Abstract—Antiarrhythmic effects of a new antiarrhythmic drug, E-0747, were examined using four canine ventricular arrhythmia models: digitalis-, adrenaline- and two-stage coronary ligation-induced arrhythmias and a newly developed locally-induced digitalis arrhythmia. The minimum effective plasma concentration of E-0747 was determined for the first three arrhythmia models. E-0747 suppressed those arrhythmias, and the minimum effective plasma concentrations for arrhythmias induced by digitalis, adrenaline, 24 hr coronary ligation, and 48 hr coronary ligation were 1.4±0.6, 1.8±0.4, 1.6±0.4 and 2.2±0.2 µg/ml, respectively (mean±S.D., n=5-10). The aforementioned minimum effective plasma concentrations of E-0747 for these arrhythmias were almost equal to the reported concentration in vitro to suppress the Na channels of isolated canine ventricular tissues. The class 1 property of E-0747 was also shown in a newly developed locally-induced digitalis arrhythmia. Thus, E-0747 suppresses arrhythmia by inhibiting Na channels of cardiac cells and is expected to become a clinically useful antiarrhythmic drug.

E-0747 is a newly developed antiarrhythmic drug (Fig. 1) having advantages of oral effectiveness and absence of cardiovascular depressing and anticholinergic effects (1, 2). Electrophysiologically, effects of E-0747 on isolated normal cardiac muscle have recently been reported to have strong rate-dependent effects in suppressing the maximum rate of rise (max dV/dt) of the normally polarized cardiac action potential (3), and it was designated to be a class 1c drug according to the Vaughan Williams classification system (4).

Previously, we examined effects of various antiarrhythmic drugs on three canine ventricular arrhythmias: i.e. digitalis-, adrenaline- and two-stage coronary ligation-induced arrhythmias (5–10), and we suggested that the efficacy of drugs on these arrhythmia models correlated well with the drug's electrophysiological action on cardiac action potentials. The above can be summarized as follows: All class 1 Na channel blockers were effective on the digitalis-induced arrhythmia (8) and also effective on coronary ligation-induced arrhythmia except lidocaine (5), whereas they showed variable effects on adrenaline arrhythmia (9). In the present study, we examined the effects of E-0747 on these arrhythmia models and compared them with other antiarrhythmic drugs. We also examined its effects on a newly developed digitalis arrhythmia model, termed "locally induced digitalis arrhythmia".
This arrhythmia is suppressed specifically by Na channel blocking class I antiarrhythmic drugs; therefore, it is useful for elucidating the antiarrhythmic mechanism of E-0747.

Materials and Methods

Production of digitalis-induced arrhythmia: Ten mongrel dogs of either sex, weighing 8–15 kg, were anesthetized with 30 mg/kg pentobarbital sodium. As reported earlier (8), 40 μg/kg ouabain was injected intravenously into the femoral vein and then followed by an additional dose of 10 μg/kg every 20 min until stable ventricular arrhythmia of more than 1 hr duration was produced. E-0747, 3 mg/kg, was injected as an intravenous bolus through a cannula in the femoral vein within seconds.

Lead II electrocardiograms (ECG), atrial electrograms from catheter tip electrodes in the right atrium, and blood pressure were continuously recorded. Venous blood samples were taken from the jugular vein 5 min before and 1, 3, 5, 10, 15, 30 and 60 min after E-0747 injection.

Production of adrenaline-induced arrhythmia: Six mongrel dogs of either sex, weighing 8–15 kg, were anesthetized initially with thiopental sodium. As reported earlier (9), 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 2.5–3.5 μg/kg/min for 18 min. After 3 min of adrenaline infusion and when stable and severe ventricular arrhythmia was produced, 3 mg/kg E-0747 was injected into the right femoral vein.

The lead II ECG and blood pressure were continuously recorded. Venous blood samples were taken from the jugular vein 1 min before and 1, 3, 5, 10 and 15 min after E-0747 injection.

Production of two-stage coronary ligation-induced arrhythmia: Six beagle dogs, weighing 8–11 kg, were anesthetized initially with 30 mg/kg of thiopental sodium. As reported earlier (5), the chest was opened, and the 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 the left atrial appendage, and blood pressure were recorded continuously using telemetry systems (Nihon Kohden and Nishimu). Three mg/kg E-0747 was injected through a cannula inserted into the jugular vein. Venous blood samples were taken from the cannula 5 min before and 1, 3, 5, 10, 15, 30 and 60 min after injection of E-0747.

Production of locally-induced digitalis arrhythmia: Mongrel dogs of either sex, weighing 8–15 kg, were anesthetized with 30 mg/kg pentobarbital sodium. After the chest was opened through the left 5th intercostal space, the left anterior descending coronary artery (LAD) was dissected free. As reported earlier (11), a polyethylene cannula was inserted into the artery, and the heart was then perfused by its own heparinized arterial blood from the carotid artery. Heparin, 500 units/kg, was initially given intravenously, and then 200 units/kg was supplemented every hour. Ouabain, 40 μg, was injected intraarterially and followed by 10 μg every 20 min until stable ventricular arrhythmia was produced. Arrhythmia usually occurred after a total dose of 60–70 μg. Probably due to the short exposure time of the perfused myocardium to ouabain, this arrhythmia was short-lived and occasionally showed a tendency to become less severe and disappear. In such cases, an additional 10 μg of ouabain was injected intraarterially, and the experiment could be continued unless ventricular fibrillation was produced. Antiarrhythmic drugs were injected intraarterially into LAD. Blood pressure was measured through the cannula inserted into the right femoral artery, and the atrial electrogram was recorded through bipolar electrodes attached to the left atrial appendage. Lead II ECG were recorded continuously along with the atrial electrogram, coronary flow and blood pressure.

Determination of plasma concentration of E-0747: Venous blood samples were centrifuged, and the plasma was stored in a freezer at about −25°C before plasma E-0747 concentration analysis.

E-0747 was determined using a high-performance liquid chromatograph (HPLC). 6-Chloro-2,2-dimethyl-1'-(5-(4-hydroxy-
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piperizino) penty1) spiro (chroman-4,4'-imidazolidine) 2',5'-dione was synthesized at Eisai Co., Ltd. and used as an internal standard.

The HPLC system consisted of a Shimadzu LC-5A pump, a Rheodyne 7125 injector and a Shimadzu SPD-2 variable-wavelength UV detector. Conditions were: column, YMC ODS, 150×6 mm, particle size, 5 μm; mobile phase, methanol-acetate buffer pH 5.6 (45:55), 1.2 ml/min; detection, UV at 225 nm.

To extract E-0747, 10 μl of internal standard (1 μg, methanol) and 1 ml of distilled water were added to a 15 ml screw-capped tube containing 1 ml of plasma sample. Four ml ethyl acetate was added to the tube and shaken for 10 min and then centrifuged for 5 min at 1000 g. The extraction procedure was repeated three times with each plasma sample. The combined ethyl acetate layer was evaporated at 40°C in a stream of nitrogen gas. The residue was dissolved in 100 μl of ethyl acetate by vortexing and sonicating, and 40 μl of the solution was injected into the HPLC system.

There was an excellent linearity (r=0.998) in the calibration curve. The limit of detection was 50 ng/ml, and the recovery was 99.4±1.2% (mean±S.E., n=3) when 1 ml of spiked plasma (500 ng) was determined.

Determination of the minimum effective plasma concentration: The severity of arrhythmia was expressed by the arrhythmic ratio: the number of ventricular ectopic beats divided by the total heart rate. For three types of arrhythmias (except locally-induced digita1is arrhythmia), the arrhythmic ratios before drug injection were almost 1, and there were no spontaneous improvements in these ratios. As reported earlier (5), the minimum effective plasma concentration of E-0747 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. Student’s t-test for paired data was used to determine the significance of difference. 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.

Results

Effects of E-0747 on digitalis-induced arrhythmia: After injection of a total dose of 70–80 μg/kg ouabain, almost all the beats were of ventricular origin. Preliminary experiments using 1 and 3 mg/kg E-0747, i.v., showed that the 1 mg/kg dose had almost no antiarrhythmic effect; therefore, the 3 mg/kg dose was used in this study. As shown in Fig. 2, the arrhythmic ratio was significantly (P<0.05) reduced for 16 min after E-0747 injection, accompanied by a reduction in the total heart rate and blood pressure for 60 min. The plasma concentration-time curves fitted well with that predicted by the two-compartment open model. The parameters of the E-0747 concentration-time equation, expressed as concentration=Ae^{-α}+Be^{-β}, were: A=14.3±6.8 μg/ml, alpha=0.84±0.15/min, B=1.8±0.6 μg/ml, and beta=0.01±0.01/min (mean±S.D., n=9); where A is the concentration at 0 time of the distribution curve and B is that of the elimination curve, and alpha is the time constant of the former curve and beta is that of the latter. The minimum effective plasma concentration was calculated as that at 16 min, 1.4±0.6 μg/ml.

Effects of E-0747 on adrenaline-induced arrhythmia: As reported previously (9), 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. The doses of E-0747, 3 and 5 mg/kg, were examined in a preliminary study. Since the 5 mg/kg dose either induced ventricular fibrillation or worsened this arrhythmia, a 3 mg/kg dose was used. As shown in Fig. 3, E-0747 decreased total heart rate, the number of ventricular beats, and arrhythmic ratio after E-0747 injection; and this antiarrhythmic effect lasted up to 6 min. The blood pressure was gradually reduced, but the change was not significant. The plasma concentration-time curves fitted well with that predicted by the one compartment open model theory. The parameters of the E-0747 concentration-time equation, expressed as concentration=Ae^{-α}, were: A=13.7±5.6 μg/ml and alpha=0.92±0.24 r/min (n=6); where A is the concentration at 0 time and alpha is the time constant. We estimated the minimum antiarrhythmic E-
Fig. 2. Summary of the effects of 3 mg/kg E-0747, i.v., on digitalis-induced arrhythmia. E-0747 suppressed the arrhythmia soon after injection and the antiarrhythmic effect lasted 16 min. *P<0.05, **P<0.01.

E-0747 plasma concentration for canine halothane-adrenaline-induced arrhythmia as that at 6 min, 1.8±0.4 μg/ml.

Effects of E-0747 on two-stage coronary ligation-induced arrhythmia: After 24–48 hr of coronary ligation, beagle dogs showed ventricular tachycardia, as indicated by the -5 and 0 time values in Fig. 4. While the 48 hr arrhythmia spontaneously became less severe, the 24 hr arrhythmia was so severe that there were almost no conducted beats at the onset of the experiment. The preliminary experiments using 3–6 mg/kg E-0747, i.v., showed that 6 mg/kg induced vomiting, while the antiarrhythmic effect of this dose was not stronger than that of 3 mg/kg. Therefore a 3 mg/kg dose was used, although this dose also produced vomiting within 4 min after adminis-
E-0747 decreased the total heart rate, increased the number of conducted beats, and decreased the arrhythmic ratio for up to 22 min. The blood pressure remained almost unchanged. The same dogs were used the next day for the study of E-0747 effects on the 48 hr arrhythmia. The plasma concentration of E-0747, just before the 48 hr experiments, was zero, even though 3 mg/kg E-0747 had been administered 24 hr before. The same 3 mg/kg dose of E-0747 similarly suppressed this 48 hr arrhythmia up to 9 min, but the arrhythmic ratio was decreased to a smaller value compared to that for the 24 hr experiments. The plasma concentration time curve fitted well with that predicted by the two compartment model. The parameters of the curve of the 24 hr experiments were: $A=7.5\pm9.2$ µg/ml, $\alpha=0.30\pm0.19$ /min, $B=1.9\pm0.69$ µg/ml, and $\beta=0.0083\pm0.0044$ /min ($n=6$). Those for the 48 hr experiments were: $A=8.9\pm10.0$ µg/ml, $\alpha=0.43\pm0.29$ /min, $B=2.3\pm0.23$ µg/ml, and $\beta=0.012\pm0.0023$ /min ($n=5$). The minimum antiarrhythmic plasma concentrations for the canine 24 hr and 48 hr coronary ligation-induced arrhythmias were calculated as $1.6\pm0.4$ µg/ml (at 22 min) and $2.2\pm0.2$ µg/ml (at 9 min).
Fig. 4. Effects of E-0747 on 24 hr (left) and 48 hr (right) two-stage coronary ligation-induced arrhythmias. E-0747 (3 mg/kg, i.v.) decreased the total heart rate and arrhythmic ratio without decreasing the blood pressure both in the 24 hr and 48 hr experiments.

Effects of E-0747 on locally-induced digitalis arrhythmia: After intracoronary injection of a total dose of 60–70 μg ouabain, stable ventricular arrhythmia occurred, which lasted long enough (at least 20 min) to examine the effects of intraarterial administration of the drugs. Though this arrhythmia spontaneously changed into ventricular fibrillation in rare cases, more than 85% (29 out of 33 cases) of the experiments could be conducted to examine antiarrhythmic effects of drugs. E-0747 (0.5–1 mg, i.a.) was effective in suppressing this arrhythmia soon after injection without marked effects on mean blood pressure or coronary flow, and the duration of these antiarrhythmic effects were dose-dependent. As shown in Fig. 5, after intracoronary injection of 0.5 mg E-0747, the arrhythmia was suppressed for 1 min; and then, after a stable and sustained arrhythmia reappeared, a higher dose of the drug, 1 mg, i.a., was administered. The antiarrhythmic effects of this higher dose lasted longer (19 min).

Discussion
The present study has demonstrated that E-0747 was effective in four canine ventricular arrhythmia models. In our previous studies, we examined the effects of class 1 antiarrhythmic drugs on canine ventricular arrhythmias (digitalis-, adrenaline- and two-stage coronary ligation-induced arrhythmias); and we showed that some of these class 1 drugs such as phenytoin, aprindine, mexiletine, propafenone and tocainide were effective on the three types of arrhythmias, while others were not (5–10). The plasma concentration data of E-0747 indicates that there are no significant quantitative differences between its effectiveness on different arrhythmias. Although the minimum antiarrhythmic plasma concentration for digitalis-induced arrhythmia, 1.4 μg/ml, was the lowest compared to those for adrenaline and coronary ligation-induced arrhythmias, they are not significantly different. Comparing E-0747 with those of
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Fig. 5. Effects of E-0747, 0.5 mg, i.a. and 1 mg, i.a., on locally-induced digitalis arrhythmia in a dog. E-0747 suppressed the arrhythmia soon after injection, and the antiarrhythmic effect lasted dose-dependently as shown in ECG. Two doses of E-0747 were injected at the arrows with an interval of about 10 min. BP = blood pressure, HR = heart rate, ECG = electrocardiogram, CF = coronary flow. In the upper ECG at a slower paper speed, A and B represent the ECG before and after E-0747 0.5 mg administration, respectively, and those at a faster paper speed are shown in the lower ECG. Note that the arrhythmia shown in line A was suppressed completely after administration of 0.5 mg E-0747, i.a.

the many previously investigated antiarrhythmic drugs, E-0747 resembles phenytoin (8) in that it showed uniform effectiveness in the three canine ventricular arrhythmias. Therefore, it can be expected that E-0747 is also effective on various kinds of clinical ventricular arrhythmias. It also may be predicted from a conclusion of our previous studies that canine and human antiarrhythmic concentrations of drugs are almost similar (8); 1–2 \( \mu \)g/ml may be the minimum clinical effective plasma concentration of E-0747.

An electrophysiological study by Sawada et al. (3) has indicated that E-0747 is an antiarrhythmic drug with class 1 properties and decreases the max dV/dt of cardiac action potential at concentrations higher than 30 \( \mu \)M, which corresponds approximately to our plasma concentrations of E-0747 used in the present study. Considering such a correspondence between effective concentrations obtained in vitro experiments and our plasma effective concentrations of E-0747, it is likely that the antiarrhythmic mechanism of E-0747 can be attributed to its Na channel blocking effect.

The class 1c property of E-0747 seems to be reflected in the relatively slow onset of antiarrhythmic action even after intravenous injection as shown in Figs. 2–4. Other class 1
antiarrhythmic drugs which we studied showed a maximum antiarrhythmic effect within 1 min of injection, but in the case of E-0747, it took about 3 to 5 min to reach its maximum effect. Thus the correlation of the E-0747 plasma concentration and antiarrhythmic effect (data not shown) was very low compared to other class 1 drugs (6, 7), because of the relatively low effectiveness of E-0747 when its plasma concentrations are high. Whether this is due to the class 1c property of E-0747 or to other physicochemical properties cannot be ascertained from our experimental design, because E-0747 is the first class 1c antiarrhythmic drug to be studied in our models.

On the new arrhythmia model, locally-induced digitalis arrhythmia, it was already shown that Na channel blockers have antiarrhythmic effects. As other class 1 drugs such as procainamide (5–10 mg, i.a.), disopyramide (0.5–3 mg, i.a.), lidocaine (1–2 mg, i.a.), phenytoin (2.5 mg, i.a.), mexiletine (1 mg, i.a.) and tetrodotoxin (0.001–0.003 mg, i.a.), E-0747 injected into the same myocardial area where ouabain was injected was effective in suppressing this digitalis arrhythmia. This again proved that E-0747 has a property common to class 1 drugs.

In summary, E-0747 seems to be a valuable class 1 antiarrhythmic drug worthy of further experimental studies and clinical trials. Side effects related to cardiovascular and central nervous systems need to be carefully observed in future clinical trials.

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