Cardiovascular Assessment of ER-118585, a Selective Phosphodiesterase 5 Inhibitor

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The aim of this study was to assess the cardiovascular effects of a selective phosphodiesterase 5 inhibitor ER-118585, 4-[(3-chloro-4-methoxybenzyl)amino]-1-(2-hydroxy-7-azaspiro[3.5]non-7-yl)-6-phthalazinecarbonitrile monohydrochloride. The present results indicated that 1) ER-118585 significantly inhibited the human ether-a-go-go related gene (HERG) tail current at 10 nM and above with an IC_{50} value of 40.7 nM in human embryonic kidney 293 cells transfected with HERG cDNA; 2) ER-118585 at 100 and 1000 nM significantly increased the action potential duration (APD) at 50% and 90% repolarization in isolated papillary muscles of guinea pig; and 3) intravenous infusion of ER-118585 at 10 μg/kg/min significantly prolonged the QT interval by 10.5±1.6% from 281±2 ms to 311±6 ms in six anesthetized dogs subjected to atrial pacing. In consideration of both the plasma concentration of ER-118585 (984±78 nM, n=3) and its protein binding fraction (99.0±0.1%, n=5), the free plasma concentration was estimated at 9.8±0.8 nM, which is consistent with the minimum concentration of HERG current inhibition. In conclusion, these evaluation methods demonstrated that ER-118585 could prolong the QT interval *via* APD prolongation, attributable to the inhibition of the HERG potassium current.

Key words QT prolongation; human ether-a-go-go related gene; action potential duration

A number of drugs that prolong the electrocardiographic QT interval are known to cause polymorphous ventricular tachycardia commonly referred to as "torsades de pointes (TdP)." TdP may terminate in ventricular fibrillation and sudden death. These ligands interact with the rapidly activating delayed rectifier potassium current (I_{Kr}), and this interaction is thought to be important in the mediation of TdP. The human ether-a-go-go related gene (HERG) codes for this potassium channel, and mutations in this gene are associated with congenital long QT syndrome.[1—5)]

In the present study, we examined the cardiovascular characteristics of a selective phosphodiesterase 5 inhibitor, ER-118585, (4-[(3-chloro-4-methoxybenzyl)amino]-1-(2-hydroxy-7-azaspiro[3.5]non-7-yl)-6-phthalazinecarbonitrile monohydrochloride), using two *in vitro* electrophysiological assays and an *in vivo* canine model. While the inhibition of I_{Kr} is thought to be a commonly related cellular mechanism for QT interval prolongation in humans, a delay in repolarization as a result of actions on other ion channels could also be involved. Thus, studies on I_{Kr} potassium current[6)] and action potential parameters[7,8)] should provide useful information on the effects of ER-118585 on the integrated activity of multiple ion channels in the heart. Hemodynamic effects were evaluated in an anesthetized canine model simultaneously subjected to sinus node ablation and right atrial pacing.[9)] This model enables examination of the effects of the compound on the electrocardiograms (ECGs), including the QT interval, at a constant heart rate during intravenous infusion with continuous monitoring of plasma concentrations.

The objectives of the present study were to assess the electrophysiological risk of ER-118585 and to verify an evaluation program for the selection of chemical entities that could delay ventricular repolarization or potentially cause QT interval prolongation.

MATERIALS AND METHODS

This study was approved by the Eisai Laboratory Animal Care and Use Committee. All experiments were carried out in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, adopted by the Japanese Pharmacological Society, and Eisai's guidelines on animal experimentation (Eisai Tsukuba Research Laboratories, Ibaraki, Japan). Every effort was made to reduce the number of animals used in the study and to minimize suffering.

Effect on HERG Tail Current (Experiment 1) This study was conducted at Quintiles (Riccarton, U.K.). HEK293 cells stably transfected with HERG cDNA were obtained from the University of Wisconsin. In the whole-cell patch clamp experiment, the cells were plated onto sterile glass cover slips in 35 mm^2 dishes (containing 3 ml of medium, without geneticin) at a density of 1.5×10^5 cells per dish in order to isolate cells.[6)] The dishes were stored in a humidified, gassed (5% CO_2) incubator at 37 °C.

In the experiments, a bath solution (mM; NaCl: 137, KCl: 4, $CaCl_2$: 1.8, $MgCl_2$: 1.0, D-glucose: 10, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid: 10, pH 7.4 with 1 M NaOH) and a pipette solution (mM; KCl: 130, $MgCl_2$: 1.0, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, 5; Mg-ATP, 5; *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid: 10, pH 7.2 with 1 M KOH) were used.

The cells were transferred to the recording chamber and continuously perfused at approximately 1—2 ml/min using the bath solution at room temperature. Gigaohm seals formed between the patch electrodes (actual resistance range: 1.7—3.8 MΩ; seal resistance range: 1—80 GΩ) and individual cells. The membrane on the electrode tip was ruptured, and the whole-cell patch-clamp configuration was established. Once a stable patch had been obtained, recording was begun

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in the voltage-clamp mode by initially clamping the cell at −80 mV. The steps from −80 mV to +20 mV resulted in an outward current, and the steps from +20 mV to −50 mV resulted in a tail current. The voltage was held at +20 mV for 4.8 s, −50 mV for 5 s and −80 mV for 5.2 s, for a total pulse length of 15 s.

A control recording was done, and ER-118585 was then perfused through the bath. The compound was allowed to equilibrate for at least 5 min. During the equilibration phase, the voltage protocol was run and recorded. Increasing concentrations of ER-118585 (1, 3, 10, 100, 1000 nM) were then applied to 4 cells, and a recording was made as described above. In 7 cells, the effects of the vehicle (dimethyl sulfoxide, DMSO) were examined. After exposure to the vehicle for 10—15 min, a representative class III antiarrhythmic and potassium channel blocking agent, E-4031,[7] at a concentration of 100 nM, was applied to 5 cells.

Effects on Action Potential Parameters (Experiment 2) Male 3-week-old Hartley-strain guinea pigs were purchased from Japan SLC, Inc. (Shizuoka, Japan). Throughout the study, including the quarantine/acclimation period, the animal rooms were maintained under the following conditions: temperature 20—26 °C, relative humidity 40—70%, and 12-h lighting (07:00—19:00).

In this experiment, 12 guinea pigs (weight: 307—562 g) were used. The animals were anesthetized by ether inhalation and exsanguinated to remove the heart. Immediately thereafter, the papillary muscle was isolated from the right ventricle and placed in Tyrode's solution (mM; NaCl: 123.8, $NaHCO_3$: 25.0, Glucose: 11.2, KCl: 5.0, $CaCl_2 \cdot 2H_2O$: 2.0, $MgCl_2 \cdot 6H_2O$: 1.2, $NaH_2PO_4 \cdot 2H_2O$: 1.2) that was aerated with a gas mixture (95% O_2+5% CO_2). The specimens were electrically stimulated by square waves (voltage: 1.5 times the threshold, 3—4.5 V; pulse duration: 1 ms; frequency: 1 Hz) generated by an electrical stimulation apparatus (SEN-3301, Nihon Kohden, Tokyo, Japan) and applied *via* an isolator (SS-403J, Nihon Kohden).

The isolated papillary muscles of guinea pigs were tested as follows: A glass microelectrode filled with 3 M KCl (resistance: 20—30 MΩ) was inserted into a heart-muscle cell, and the action potential was recorded using an amplifier (MEZ-8301; Nihon Kohden). The preparations were perfused with Tyrode's solution at 5 ml/min during a control period of 30—60 min, followed by perfusion of the solution containing 10, 100 or 1000 nM of ER-118585 every 30 min in a concentration-escalating fashion. Likewise, E-4031 (10—1000 nM) was also examined in 6 other preparations. The measured parameters were analyzed using computer software (WinCAPA, Physio Tech, Tokyo): data were collected before, and at 30, 60 and 90 min after treatment with a test substance or the vehicle.

The measurement parameters included resting membrane potential (RMP), action potential amplitude (APA), maximal rising velocity (V_{max}) and action potential durations at 50% (APD_{50}) and 90% (APD_{90}) of repolarization.

Hemodynamic Experiments *in Vivo* (Experiment 3) Twelve beagle dogs (Marshall Farms USA, Inc., NY, U.S.A.), that weighed 8.7 to 12.4 kg (11—25 months), were divided into vehicle-treated and ER-118585-treated groups (three males and three females in each group).

On the day of the experiment, each animal was sedated by intramuscularly injecting 100 mg/dog of ketamine hydrochloride (Sankyo, Tokyo). The animals were anesthetized by intravenous injection of 25 mg/kg sodium thiopental (Tanabe Pharmaceutical, Osaka, Japan), intubated, and ventilated using a mixture of enflurane (2—3%; Dainippon Pharmaceutical, Osaka), nitrous oxide and oxygen (2 : 1).

An intravenous catheter (6 Fr, Atom Medical Industry, Tokyo) was inserted into an exposed cephalic vein to infuse drugs. Two micro-tip catheter transducers (MPC-500, Millar Instruments Inc., TX, U.S.A.) were inserted bilaterally into the femoral arteries. One transducer was advanced to the aorta to measure aortic blood pressure using a pressure amplifier (AP-601G, Nihon Kohden) and a pressure measurement unit (AP-611G, Nihon Kohden), while the other transducer was positioned in the left ventricle to measure left ventricular pressure (LVP). The maximum rate of increase in left ventricular pressure ($LVdP/dt_{max}$) was measured based on the differentiation of left ventricular pressure using an electric differentiation unit (EQ-45764, Nihon Kohden). Heart rate was measured by analyzing left-ventricular-pressure waveforms using an instantaneous heart rate counter (AT-601G, Nihon Kohden). Heart rate, aortic blood pressure, LVP and $LVdP/dt$ were continuously recorded on a thermal array recorder (RTA-3200, Nihon Kohden).

Standard limb lead II ECG was recorded using needle electrodes and recorded on a thermal array recorder (RTA-3200, Nihon Kohden) *via* an electrocardiograph (ECG-6303, Nihon Kohden). ECG parameters (PQ interval, QRS duration, and QT interval) were measured for three consecutive waveforms and expressed as mean values.

A right thoracotomy was performed in the fourth intercostal space, and after cutting through the pericardium, the heart was elevated and suspended in a pericardial cradle. Sinus node ablation was induced by injecting a 37% formalin solution (0.05—0.15 ml) into the sinus node artery. Effective ablation was confirmed by the absence of P waves on ECGs.[9] A bipolar electrode was attached to the right atrial appendage, and the right atrium was paced with 2—6 V (approximately twice the threshold voltage) and a duration of 1 ms at 600 ms intervals (heart rate: 100 beats/min) using an electric stimulator (SEN-7203, Nihon Kohden) and an isolator (SS-202J, Nihon Kohden). After the condition stabilized, ECG parameters and hemodynamic parameters (systolic and diastolic blood pressure, and $LVdP/dt_{max}$) were measured at 0 (baseline), 30, 60 and 90 min from the start of infusion.

ER-118585 (0.1, 1, 10 μg/kg/min) was infused continuously (0.3 ml/min) into the cephalic vein for 30 min at each dose, in stepwise increments. In the control group, 5% glucose solution containing 5 mM phosphoric acid was infused for 90 min in the same manner.

Blood samples (1.5 ml) were collected from the femoral artery using a heparinized syringe to measure the plasma concentration of ER-118585 immediately after measuring the ECGs and hemodynamic parameters. The blood samples were stored by placing them in an ice bath until the end of the experiment. They were then centrifuged at 3000 rpm for 10 min at 4 °C to isolate the plasma. The plasma samples were stored at −20 °C. The plasma concentration of ER-118585 was measured by a validated high-performance liquid chromatography (HPLC) assay at Eisai Tsukuba Research Laboratories.

Measurement of Plasma Concentration and *in Vitro* Plasma Protein Binding Fraction After mixing 0.1 ml of a plasma sample, 0.1 ml of internal standard, 1.0 ml of a 1/15 M phosphate buffer (pH 7.0), and 4 ml of diethylether for 10 min, the resulting solution was centrifuged (2800 rpm for 5 min, at 4 °C), and the resulting organic layer was collected. This extraction procedure was repeated, and 0.2 ml of 0.1 M HCl was added to the organic layer. This resulting solution was stirred for 10 min and then centrifuged at 2800 rpm for 5 min at room temperature. The organic layer was removed, and the aqueous layer was subjected to HPLC under the following conditions: column; Wakosil-II 5C18 RS, 4.6× 250 mm; column temperature, 40 °C; mobile phase, 5 mM sodium dodecyl sulfate (pH 2.5)/acetonitrile (50 : 50, v/v); wave length, 215 nm; flow rate, 1.0 ml/min; injection volume, 30 μl.

Plasma samples were obtained fresh from five additional dogs. The plasma protein binding fraction of ER-118585 was determined by the equilibrium dialysis method[10] using a phosphate buffer containing 50 mM NaCl (buffer A, pH 7.4). The concentration of ER-118585 was measured by the HPLC method shown above. Its plasma protein binding fraction (%) was calculated by the following formula:

$$\text{protein binding (\%)} = 100 - (\text{Cs in buffer A}/\text{Cs in plasma}) \times 100$$

Where Cs is the concentration of ER-118585 in sample.

Chemicals ER-118585 (molecular weight: 500.4) was synthesized at the Department of Eisai Kashima Organic Chemistry (Ibaraki). E-4031 was obtained from Wako Pure Chemical Industries (Osaka). In the *in vitro* electrophysiological studies, ER-118585 was dissolved in DMSO to prepare a 100 mM stock solution. Also, E-4031 was dissolved in sterile water to prepare a 100 μM stock solution. The final concentration of DMSO used was 0.1%: this concentration of DMSO was found to have no effect on the resting membrane potentials of guinea pig papillary muscle.[7] In the *in vivo* hemodynamic study, the concentration of ER-118585 was adjusted using the vehicle (5% glucose solution containing 5 mM phosphoric acid) to 10 μg/kg/min (high dose solution). Also, medium and low dose solutions (1 μg/kg/min and 0.1 μg/kg/min, respectively) were prepared by serial dilution of the high dose solution using the vehicle.

Statistical Analysis All data were expressed as means±S.E.M. In the HERG current study, the residual current (% of baseline value) for each ER-118585 concentration was calculated. The groups were compared through the use of one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. To calculate the IC_{50} value of ER-118585, tail currents in the presence of the compound were first corrected for the mean vehicle rundown observed using the normalization process. A concentration–response curve was drawn, and an IC_{50} value was calculated using a sigmoidal function fit to the data. In the action potential study, the results for each specimen were expressed as a percentage of the baseline values. An unpaired Student's *t*-test was used to analyze the significance between the vehicle- and ER-118585- or E-4031-treated groups. In the *in vivo* canine study, the values obtained from the ER-118585-treated group were compared with those obtained from the vehicle-treated group using an unpaired Student's *t*-test. p values of less than 0.05 (two-sided) were considered significant.

RESULTS

Experiment 1: Effect on HERG Tail Current When the vehicle-treated cells (n=7) were exposed to a bath solution containing 0.1% DMSO for 10 min, the residual tail current was reduced to 79.4±1.2% of the baseline value (Table 1). When exposed to the five vehicle-treated cells, E-4031 inhibited the HERG tail current to 21.2±7.6% of the baseline value (Table 1).

The effect of ER-118585 on the HERG tail current was examined at concentrations of 1, 3, 10, 100 and 1000 nM (n=4/concentration). ER-118585 inhibited the HERG tail current in a concentration-dependent manner. Statistically significant inhibition of the tail current was seen with concentrations of 10 nM and above (Table 1, Fig. 1A). The IC_{50} value of ER-118585, corrected for the mean vehicle rundown observed using the normalization process, was 40.7 nM (Fig. 1B).

Experiment 2: Effects on Action Potential Parameters A representative example of the effects of ER-118585 on the action potential profile in guinea pig papillary muscles is illustrated in Fig. 2. It clearly showed that ER-118585 at concentrations of 10, 100, or 1000 nM produced a concentration-dependent prolongation of the action potential duration.

Table 2 summarizes the effects of ER-118585 and E-4031 on the action potential parameters recorded in six experiments. The vehicle, 0.1% DMSO, showed no effects on the action potential parameters. E-4031 did not cause any changes in RMP, APA or V_{max}, but it significantly prolonged both APD_{50} and APD_{90} at concentrations of 10 nM and above.

Like E-4031, ER-118585 caused significant increases in APD_{50} and APD_{90}, indicating prolongation of the repolarization phase in the ventricular myocardium, at concentrations of 100 and 1000 nM when compared to the vehicle-treated group. However, ER-118585 had no effects on RMP, APA or V_{max} (Table 2). ER-118585 at a concentration of 100 nM increased APD_{50} and APD_{90} to 112.0±1.4% and 111.8±1.3% of the baseline value, respectively.

Experiment 3: Effects on Canine Electrocardiograms and Hemodynamics The effects of the vehicle and ER-118585 on the ECGs and cardiohemodynamic parameters are shown in Fig. 3. The vehicle had no effect on the ECGs or hemodynamic parameters during the study period of 90 min in anesthetized dogs subjected to atrial pacing at 100 beats/min.

Table 1. Inhibitory Effects of ER-118585 and E-4031 on HERG Tail Current

Drug	Concentration (nM)	Number of cells	Tail current (% of baseline)
Vehicle		7	79.4±1.2
E-4031	100	5	21.2±7.6*
ER-118585	1	4	79.6±1.9
	3	4	73.8±5.9
	10	4	60.2±1.5*
	100	4	26.1±3.0*
	1000	4	2.6±0.2*

Vehicle: bath solution containing 0.1% dimethyl sulfoxide. All values are expressed as a percentage of the baseline value and are means±S.E.M. for seven cells in the vehicle-treated group, for five cells after treatment with vehicle in the E-4031-treated group, and for four cells in each group of ER-118585. *p<0.05 *vs.* vehicle-treated group (ANOVA followed by Dunnett's *t*-test).

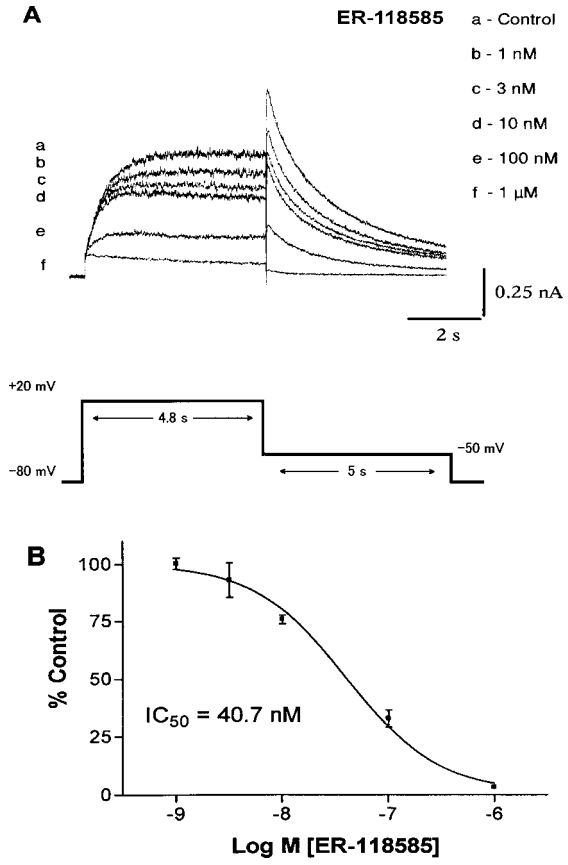

Fig. 1. Effect of ER-118585 on Human Ether-a-go-go Related Gene (HERG) Potassium Current in HEK293 Cells

(A) Patch-clamp recordings of currents from HERG channels starting 5 min after exposure to the control solution (0.1% DMSO) and 1, 3, 10, 100 and 1000 nM of ER-118585. Voltage protocol is shown below example current traces with recovery studied at −80 mV. (B) Concentration-dependent inhibition of HERG tail currents by ER-118585. Tail currents in the presence of ER-118585 were corrected for the mean vehicle rundown observed using the normalization process. All values are expressed as a percentage of the control value before the treatment of ER-118585. Each point and bar represents the mean±S.E.M. for four cells.

ER-118585 at a dose of 10 μg/kg/min significantly prolonged the QT interval by 10.5±1.6%, from 281±2 ms in the baseline to 311±6 ms ($p<0.05$ *vs.* vehicle-treated group), without affecting the PQ interval or QRS duration (Fig. 3). In addition, it caused a slight decrease in diastolic blood pressure, from 71±2 mmHg at the baseline to 56±3 mmHg ($p<0.05$), without affecting LV$dP/dt_{max}$.

The plasma concentration of ER-118585, just after the completion of infusing 10 μg/kg/min of ER-118585, was 984±78 nM ($n=3$). ER-118585 was highly bound to plasma protein, independent of concentration, with 99.0±0.1% ($n=5$) bound at 3 or 10 μg/ml. Accordingly, the free plasma concentration of ER-118585 was estimated to be 9.8±0.8 nM.

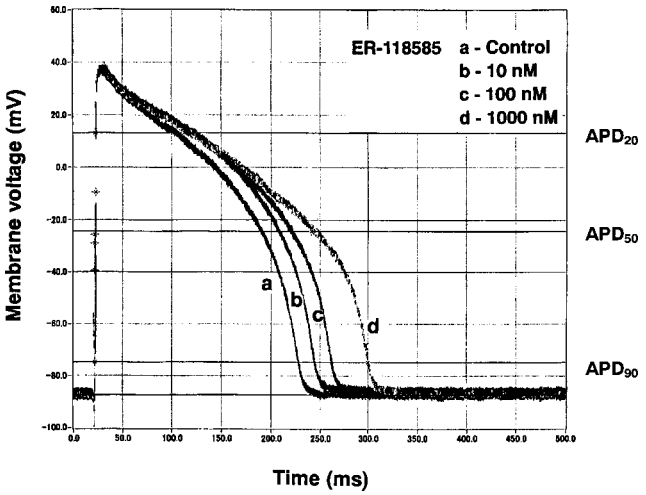

Fig. 2. Effect of ER-118585 on the Action Potential of a Papillary Muscle from a Guinea Pig

The preparation was stimulated at 1 Hz, then perfused with increasing concentrations of ER-118585 (10, 100, 1000 nM). Each concentration was applied for 30 min before the recording was made. The action potentials shown were recorded in the control situation (a), and after ER-118585 10 nM (b), 100 nM (c) and 1000 nM (d). APD: action potential duration.

Table 2. Effects of ER-118585 and E-4031 on Action Potential Parameters in Isolated Papillary Muscles of Guinea Pigs

	Drug	Baseline	Concentration		
			10 nM (%)	100 nM (%)	1000 nM (%)
RMP	Vehicle	−84±1 mV	98.9±2.6	96.9±1.1	97.6±2.9
	ER-118585	−86±1 mV	98.3±0.5	98.4±0.7	98.5±0.4
	E-4031	−85±1 mV	99.2±0.4	99.1±0.4	99.6±0.3
APA	Vehicle	128±1 mV	100.8±0.4	100.6±0.4	100.7±0.4
	ER-118585	126±1 mV	100.3±0.3	99.4±1.0	99.8±0.9
	E-4031	124±1 mV	99.7±0.5	100.3±0.5	98.7±0.9
V_{max}	Vehicle	236±18 V/s	102.1±2.1	103.0±2.4	103.5±2.8
	ER-118585	248±6 V/s	98.4±3.8	97.6±5.0	99.4±4.8
	E-4031	226±26 V/s	99.7±2.1	101.4±3.7	100.6±2.3
APD_{50}	Vehicle	178±5 ms	101.7±1.2	102.0±1.1	101.8±0.9
	ER-118585	165±5 ms	106.5±1.1	112.0±1.4*	125.0±2.3*
	E-4031	166±5 ms	114.7±2.3*	130.1±3.0*	134.9±3.1*
APD_{90}	Vehicle	205±5 ms	101.3±1.0	101.6±0.8	101.4±0.8
	ER-118585	202±4 ms	105.4±0.8	111.8±1.3*	127.0±2.5*
	E-4031	202±4 ms	116.6±2.4*	135.7±3.2*	142.7±3.6*

RMP: resting membrane potential, APA: action potential amplitude, V_{max}: maximal rising velocity, APD_{50} and APD_{90}: action potential duration at 50% and 90% repolarization, respectively, vehicle: Tyrode's solution containing 0.1% dimethyl sulfoxide. All values are expressed as a percentage of the baseline value and are means±S.E.M. for 6 preparations. $*p<0.05$ *vs.* vehicle-treated group (unpaired Student's *t*-test).

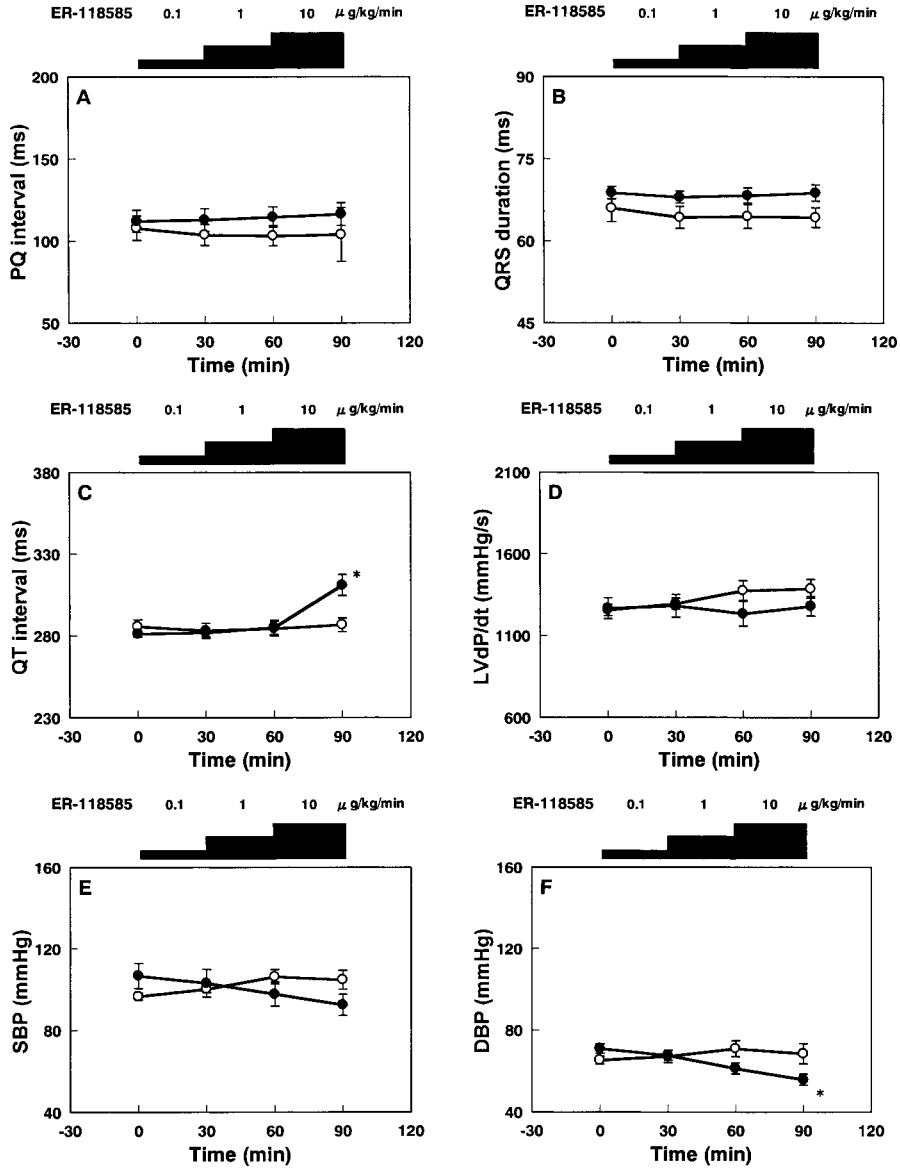

Fig. 3. Effects of ER-118585 on Electrocardiograms and Cardiohemodynamics in Anesthetized Dogs Subjected to Atrial Pacing

The control (vehicle: 5% glucose containing 5 mM phosphoric acid, $n=6$, open circles) or ER-118585 (0.1, 1, 10 μg/kg/min, $n=6$, closed circles) was continuously infused into the cephalic vein for 30 min in stepwise increments. Each point and bar represents the mean±S.E.M. A: PQ interval, B: QRS duration, C: QT interval, D: maximum rate of increase in left ventricular pressure, E: systolic blood pressure, F: diastolic blood pressure. *: $p<0.05$ *vs.* vehicle-treated group (unpaired Student's *t*-test).

DISCUSSION

The present study showed that the electrophysiological features of ER-118585 resembled those of class III antiarrhythmic agents lengthening cardiac repolarization, as determined by using the evaluation method for pre-clinical screening of new chemical entities with a QT prolonging action. The results were as follows: 1) in the whole-cell patch clamp experiment using HEK293 cells transfected with HERG cDNA, ER-118585 showed an inhibitory effect on the HERG potassium current with an IC_{50} value of 40.7 nM; 2) 100 and 1000 nM of ER-118585 significantly prolonged the action potential duration of guinea pig papillary muscles in a concentration dependent manner; 3) these effects may contribute to the prolonged QT interval observed in anesthetized dogs subjected to atrial pacing at a constant heart rate; and 4) the free plasma concentration of ER-118585 (9.8±0.8 nM) estimated based on the QT prolongation *in vivo* was consistent with a statistically significant inhibition of HERG current (10 nM).

TdP is a malignant ventricular arrhythmia associated with syncope and sudden death. It is known that drugs evoking TdP prolong the QT interval and inhibit the HERG potassium channel, but the relationships are unclear, as drugs that inhibit HERG do not cause TdP. QT interval prolongation is likely to indicate a potential risk of TdP and sudden death. Not all drugs that prolong the QT interval evoke TdP and sudden death,[1—5] but it is thought that a drug having no effect on the QT interval would be clinically safer. Recently, several marketed products, including antihistamines (astemizole and terfenadine) and a gastric prokinetic agent, cisapride, have been withdrawn due to acquired long QT syndrome.[3] Therefore, pharmaceutical companies are very concerned about the QT interval prolongation and TdP induced by non-antiarrhythmic drugs.

A pharmacological evaluation program for the cardiac safety of drug candidates has been proposed as part of the

guidelines on safety pharmacology studies. Safety pharmacology studies are conducted to predict the potential risks associated with new chemical entities prior to first clinical use.[11] In fact, the prolonged cardiac repolarization and QT interval can be accurately predicted by extrapolating the dose-dependent effect of a drug to the highest dose. In the present study, we have assessed a newly synthesized phosphodiesterase 5 inhibitor, ER-118585 (IC_{50} value: 0.53 nM), using two *in vitro* electrophysiological techniques and an *in vivo* canine model.

In the mechanism of inhibition of HERG potassium current or prolongation of action potential duration by ER-118585, neither the cGMP elevation in HEK293 cells or papillary muscles is likely to be related to its *in vitro* actions. Unlike ER-118585, a representative phosphodiesterase 5 inhibitor sildenafil, used clinically for the treatment of erectile dysfunction, does not prolong cardiac repolarization in guinea pig papillary muscles[12] and has a weakly inhibitory effect on HERG potassium current with an IC_{50} value of 100 μM.[13]

An evaluation system based on HERG channel current may be a valuable and reliable tool in predicting the proarrhythmic potential of drugs. Although verapamil inhibits the HERG channel (IC_{50} value: 143 nM) as well as the voltage-dependent Ca^{2+} channel, this calcium antagonist does not cause TdP as an exception, because the two activities could produce opposing effects on the cardiac action potential, presumably canceling each other out.[14] However, when a drug has greater affinity towards the HERG channel, in general it is more likely to prolong the QT interval.[15] In this study, ER-118585 significantly inhibited this current at concentrations of 10 nM and higher, and its IC_{50} value was 40.7 nM. On the other hand, E-4031 at a concentration of 100 nM also potently decreased the currents. The potency of ER-118585 in blocking I_{Kr} was approximately one-fifth the previously reported value for E-4031 (IC_{50} value: 7.7 nM).[6] Thus, the data presented here show that ER-118585 influences the human cardiac potassium current, suggesting that the underlying molecular mechanism associated with the QT prolongation of ER-118585 might be related to high affinity blocking of the HERG potassium channel.

Ventricular repolarization involves ion currents through calcium, sodium, and potassium channels, including the HERG potassium channel. It is therefore important to clarify the effects of a drug on the action potential parameters constituted by multiple ion currents. Our results also showed that E-4031 at concentrations of 10—1000 nM prolonged APD_{50} and APD_{90} in a concentration dependent fashion, without changing V_{max}, RMP or APA. This agrees with the results of the previous study using guinea pig papillary muscles.[7] ER-118585 at concentrations of 100 and 1000 nM also significantly increased APD_{50} and APD_{90}, but its effect was weaker than that of E-4031. This result suggests that ER-118585 prolonged cardiac repolarization due to its inhibitory effect on the HERG potassium current.

We employed a canine model with atrial pacing to evaluate the propensity of ER-118585 to induce acquired long QT syndrome. This model could control the heart rate through electrical-stimulation-induced atrial pacing after sinus node ablation by the injection of formalin.[9,16,17] As it is well known that the duration of the QT interval is influenced by changes in the heart rate, this model has the advantage of excluding this fluctuation during attempts to estimate the effects of drugs on the QT interval.[9]

ER-118585 at a dose of 10 μg/kg/min evoked QT prolongation at a plasma concentration of 984$\pm$78 nM in this anesthetized dog model. As the plasma protein binding fraction of the compounds was 99.0$\pm$0.1% in the dog, the free drug concentration (9.8 nM) is consistent with the minimum inhibitory concentration (10 nM) for blocking I_{Kr}. On the other hand, the free plasma concentration in dogs was not close to the minimum concentration of 100 nM for prolongation of APD_{50} and APD_{90} recorded from the papillary muscles of guinea pigs. It is difficult to explain why there is one order of difference between the inhibitory concentration for HERG potassium current and the prolonging concentration for cardiac repolarization, but species sensitivity[18] and tissue sensitivity[19] could play a major role. In addition, reverse frequency dependency[20] of the APD prolongation, being characteristic of class III antiarrhythmic agents, between $<$0.1 Hz in the HERG current study and 1 Hz in the action potential study, might be observed in the present study.

At present, our greatest concern in the development of drugs has been the identification of candidates with a potential risk of inducing QT prolongation, TdP and sudden death. The results of the present study suggest that ER-118585 may prolong the QT interval similarly to some non-antiarrhythmic agents.[5] Furthermore, ER-118585 caused a slight decrease in diastolic blood pressure due to the potent inhibition of phosphodiesterase 5 in dogs. The exact proarrhythmic risk of ER-118585 in clinical settings is unknown because a decision not to conduct further preclinical and clinical investigations has been made based on the findings obtained from the three studies described here, and because of the narrow safety margins, 19 or 77 times, between the minimum inhibitory concentration or the IC_{50} value for blocking I_{Kr} and the IC_{50} value for inhibiting phosphodiesterase 5, respectively.

CONCLUSION

In conclusion, the present study demonstrated that ER-118585 prolongs ventricular repolarization by blocking I_{Kr}, and that this effect may be related to QT prolongation caused by the intravenous infusion of ER-118585 to anesthetized dogs. Therefore, the pharmacological evaluation methods used in the present study could be useful for clarifying whether new chemical entities have a potential risk of inducing QT prolongation followed by TdP.

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