Effects of selective I_{Kr} channel blockade by E-4031 on ventricular electro-mechanical
relationship in the halothane-anesthetized dogs

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Abstract

An inversion of electro-mechanical coupling; namely, mechanical relaxation which precedes electrical repolarization, has been proposed as a surrogate marker to predict the occurrence of drug-induced arrhythmias. The present study was designed to qualitatively and quantitatively clarify the effects of rapidly activating delayed rectifier K^+ current (I_{Kr})-selective blockade by E-4031 on the electro-mechanical relationship *in vivo*. We adopted the halothane-anesthetized canine model (n=4). E-4031 in doses of 0.01 and 0.1 mg/kg that can provide the plasma concentrations effectively to inhibit I_{Kr} *in vitro* significantly delayed the repolarization beyond the initiation of diastole, resulting in the inversion of electro-mechanical coupling, which provides an ideal proarrhythmic substrate, while the durations of left ventricular systole and diastole remained the same. Since these observed changes were solely caused by the repolarization delay, the inversion of electro-mechanical coupling may have a similar extent of sensitivity to QT-interval prolongation as a surrogate marker in predicting the onset of I_{Kr} inhibitor-induced arrhythmias.

Keywords: E-4031, I_{Kr} , Positive inotropic action, Halothane-anesthetized dog, QT prolongation, Electro-mechanical relationship

Chemical compound studied in this article

E-4031 (PubChem CID: 3087190)

Abbreviations: EMc: electro-mechanical coupling; EMw: electro-mechanical window;

I_{Kr} : rapidly activating delayed rectifier K^+ current; $LVdP/dt_{max}$: maximum upstroke velocity of the left ventricular pressure; MAP: monophasic action potential; MAP_{90} : interval from MAP upstroke to 90% repolarization level; $MAP_{90(sinus)}$: MAP_{90} during sinus rhythm; $MAP_{90(CL300)}$: MAP_{90} at pacing cycle length of 300 ms; $MAP_{90(CL400)}$: MAP_{90} at pacing cycle length of 400 ms; QTc: corrected QT interval

1. Introduction

Drug-induced proarrhythmia has been a major safety concern for pharmaceutical companies and regulatory authorities as well as academia on the development of new drugs, leading to issues of ICH E14 and S7B guidelines (Food and Drug Administration, 2005a, 2005b). After the guidelines became adopted, the number of new compounds with the risk causing torsades de pointes has been substantially reduced, but they identify many drugs as being “positive” despite a lack of demonstrable proarrhythmic risk (Darpo, 2010; Giorgi et al., 2010; Food and Drug Administration, 2012; Sugiyama et al., 2014). Thus, there remain strong needs for reliable surrogate markers that can determine actual proarrhythmic risk of drugs.

The electro-mechanical window (EMw), which is the interval between the end of T wave of electrocardiogram and end of the ventricular pressure curve, has been shown to be a useful surrogate marker of torsades de pointes in long QT type 1 canine model induced by a slowly activating delayed rectifier K^+ current inhibitor HMR1556 and a β -agonist isoprenaline (Vargas, 2010; Van der Linde et al., 2010). Although a hypothesis that the EMw may also have a potential to predict the occurrence of arrhythmias in long QT type 2 was proposed in their report (Van der Linde et al., 2010), it remains uncertain whether EMw may become a reliable surrogate marker of torsades de pointes in the rapidly activating delayed rectifier K^+ current (I_{Kr}) inhibitor-induced long QT syndrome. Indeed, in a recent study using a chronic atrioventricular block canine model, negative EMw values; namely, mechanical relaxation which precedes electrical repolarization, induced by I_{Kr} blocker dofetilide lacks the specificity to predict dofetilide-induced torsades de pointes (Stams et al., 2014).

The present study was designed to precisely clarify the effects of selective I_{Kr} blockade induced by E-4031 on the electro-mechanical relationship *in vivo* (Tamargo et al., 2004; Wettwer et al., 1991). We adopted the halothane-anesthetized beagle dog model, since most of the attacks of torsades de pointes have been clinically reported to occur during sinus rhythm, and the model has been used to examine the cardiovascular response of drugs that caused QT-interval prolongation in humans (Kimura et al., 2007; Merot et al., 1999; Sugiyama, 2008; Tabo et al., 2006; Weissenburger et al., 2000). To better analyze the electrophysiological effects of the drug on the depolarization and repolarization process, we recorded the His-bundle electrogram and monophasic action potential (MAP), respectively, in addition to assessing the standard lead II electrocardiogram. Moreover, we measured ventricular systolic and relaxation periods in addition to the duration of ventricular pressure cycle to directly analyze the effects of I_{Kr} channel blockade on the electro-mechanical coupling.

2. Materials and Methods

Experiments were carried out by using beagle dogs of either sex weighing approximately 10 kg (n=4). Animals were obtained from Kitayama Labes Co., Ltd. (Ina, Nagano, Japan). All experiments were approved by the Animal Research Committee for Animal Experimentation of Toho University (No. 13-53-151) and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Toho University.

2.1. Cardiohemodynamic parameters

Dogs were anesthetized initially with thiopental sodium (30 mg/kg, i.v.). After intubation with a cuffed endotracheal tube, 1% halothane vaporized with 100% oxygen was inhaled with a volume-limited ventilator (SN-480-3; Shinano, Tokyo, Japan). Tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively. To prevent blood clotting, heparin calcium (100 IU/kg) was intravenously administrated.

A clinically available catheter sheath (FAST-CATHTM 406108, St. Jude Medical Daig Division, Inc., Minnetonka, MN, USA) was inserted through the right femoral artery, and the aortic pressure was measured via a flush line connected to the catheter sheath. A thermodilution catheter (TC-504NH; Nihon Kohden, Tokyo, Japan) was positioned at the right side of the heart through the right femoral vein. The cardiac output was measured by a standard thermodilution method with a cardiac output computer (MFC-1100, Nihon Kohden). The total peripheral resistance was calculated with the basic equation: total peripheral resistance = mean blood pressure/cardiac output.

A pig-tail catheter was positioned at the left ventricle through the catheter sheath at the right femoral artery to measure the left ventricular pressure. The maximum upstroke velocity of the left ventricular pressure ($LVdP/dt_{max}$) and the left ventricular end-diastolic pressure were obtained during sinus rhythm to estimate the contractility and the preload to the left ventricle, respectively. Left ventricular systolic period was measured as an interval (ms) from the time point of end-diastole to that of peak of left ventricular pressure, whereas left ventricular diastolic period was determined as an interval (ms) from the time point of peak of left ventricular pressure to that of completion of ventricular relaxation. The sum of the left ventricular systolic and diastolic periods indicates the duration of ventricular pressure cycle.

2.2. Electrophysiological parameters

The surface lead II electrocardiogram was obtained from the limb electrodes. Corrected QT interval (QTc) was calculated with Van de Water's formula (Van de Water et al., 1989). A quad-polar electrodes catheter was positioned at the non-coronary cusp of the aortic valve through the left femoral artery to obtain the His bundle electrogram. A bidirectional steerable MAP recording/pacing combination catheter (1675P; EP Technologies, Inc., Sunnyvale, CA, USA) was positioned at the endocardium of the right ventricle through the left femoral vein to obtain MAP signals. The signals were amplified with a DC preamplifier (model 300; EP Technologies, Inc.). The duration of the MAP signals was measured as an interval, along a line horizontal to the diastolic baseline, from the MAP upstroke to the desired repolarization level. The interval (ms) at 90% repolarization level was defined as MAP_{90} .

The heart was electrically driven by using a cardiac stimulator (SEC-3102,

Nihon Kohden) with the pacing electrodes of the combination catheter placed in the right ventricle. The stimulation pulses were rectangular in shape, 1-2 V (about twice the threshold voltage), and of 1-ms duration. The MAP_{90} was measured during sinus rhythm ($MAP_{90(sinus)}$) and at a pacing cycle length of 400 ms ($MAP_{90(CL400)}$) and 300 ms ($MAP_{90(CL300)}$). The effective refractory period of the right ventricle was assessed by the programmed electrical stimulation. The pacing protocol consisted of 5 beats of basal stimuli in a cycle length of 400 ms followed by an extra stimulus of various coupling intervals. Starting in late diastole, the coupling interval was shortened in 5- or 10-ms decrements until refractoriness occurred. **The duration of the terminal repolarization period in the right ventricle was calculated by the difference between the $MAP_{90(CL400)}$ and effective refractory period at the same site, which has been known to reflect the duration of phase 3 repolarization, an electrically vulnerable period (Sugiyama, 2008; Sugiyama and Hashimoto, 2002).**

Electro-mechanical relationship was analyzed during sinus rhythm as follows: First, the extent of electro-mechanical coupling (EMc) was estimated by calculating an interval (ms) from the time point of MAP at 90% repolarization level to that of peak of left ventricular pressure. Second, EMw was obtained as an interval (ms) from the time point of MAP at 90% repolarization level to that of completion of ventricular relaxation.

2.3. Experimental protocols

The systemic blood pressure, left ventricular pressure, electrocardiogram, His bundle electrogram and MAP signals were monitored with a polygraph system (RM-6000, Nihon Kohden) and analyzed with a real-time full automatic data analysis system (WinVAS3 ver1.1R24v, Physio-Tech, Tokyo, Japan). Each measurement of

electrocardiogram, MAP as well as atrio-His and His-ventricular intervals was the mean of three recordings of consecutive complexes. The cardiovascular variables were assessed in the following order. The electrocardiogram, His bundle electrogram, systemic and left ventricular pressures and MAP signal were recorded under sinus rhythm. Then, the cardiac output was measured three times. Next, MAP signals were recorded during the ventricular pacing at a cycle length of 400 and 300 ms. Finally, the effective refractory period was measured. All parameters described above were usually obtained within 1 min at each time point.

After the basal assessment, E-4031 in a low dose of 0.01 mg/kg was intravenously infused over 10 min, and each parameter was assessed at 5, 10, 15, 20 and 30 min after the start of administration. Then, E-4031 in a high dose of 0.1 mg/kg was intravenously infused over 10 min, and each parameter was observed at 5, 10, 15, 20, 30, 45 and 60 min after the start of administration. A volume of 3 ml of blood was drawn from the left femoral artery at 5, 10, 15 and 30 min after the start of the low-dose administration, and at 5, 10, 15, 30 and 60 min after the start of the high-dose administration to measure the plasma concentration of E-4031. The blood samples were centrifuged at 1,500 g for 30 min at 4°C. The plasma was stored at –80°C until the drug concentration was measured.

2.4. Plasma drug concentration

Sensitive and specific determinations of the concentration of E-4031 were performed with a liquid chromatography/tandem mass spectrometry method (API3000; Applied Biosystems/MDS SCIEX, Foster City, CA, USA).

2.5. Drugs

E-4031 (N-[4-[[1-[2-(6-Methyl-2-pyridinyl)ethyl]-4-piperidinyl]carbonyl]phenyl]methanesulfonamide dihydrochloride) (Sigma-Aldrich Co. LLC., Tokyo, Japan) was dissolved in saline in concentrations of 0.01 and 0.1 mg/ml. Other drugs used were thiopental sodium (Mitsubishi Tanabe Pharma Corporation, Osaka, Japan), halothane (Takeda Pharmaceutical Company Limited, Osaka, Japan) and heparin calcium (Sawai Pharmaceutical Corporation Limited, Tokyo, Japan).

2.6. Statistical Analyses

Data are presented as the mean $\pm$ S.E.M. The statistical differences within a parameter were evaluated by one-way repeated-measures analysis of variance (ANOVA) followed by post-hoc test (Contrasts) for mean values comparison, whereas those of paired data within a parameter were evaluated with paired *t*-test. A *P* value <0.05 was considered significant.

3. Results

3.1. Plasma drug concentration

The time course of the plasma concentration of E-4031 is summarized in Fig. 1 (n=4). The decrease in the plasma concentration followed a pattern that could be predicted by the two-compartment theory of pharmacokinetics. The peak plasma concentrations of E-4031 after 0.01 and 0.1 mg/kg infusion were 24 ± 7 ng/ml (=59 nM) and 182 ± 48 ng/ml (=453 nM), respectively.

3.2. Effects on the heart rate, blood pressure, cardiac output and total peripheral resistance

The time courses of changes in the heart rate and mean blood pressure are summarized in Fig. 1 (n=4) and typical tracings of the effects of E-4031 on the aortic blood pressure are depicted in Fig. 2. The heart rate and mean blood pressure at the pre-drug control (C) were 98 ± 6 beats/min and 107 ± 6 mmHg, respectively. After the start of the low and high doses of E-4031 infusion, no significant change was detected in the heart rate or mean blood pressure during the observation period. The time courses of changes in the cardiac output and total peripheral resistance are also summarized in Fig. 1 (n=4). The cardiac output and total peripheral resistance at the pre-drug control (C) were 1.53 ± 0.08 L/min and 72 ± 8 mmHg (L/min)⁻¹, respectively. The low and high doses of E-4031 infusion tended to increase the cardiac output but decrease the total peripheral resistance, either of which did not achieve the statistical significance.

3.3. Effects on the LVdP /dt_{max} and left ventricular end-diastolic pressure

The time courses of changes in the LVdP/dt_{max} and left ventricular end-diastolic pressure are summarized in Fig. 1 (n=4) and typical tracings of the effects of E-4031 on the left ventricular pressure are depicted in Fig. 2. The LVdP/dt_{max} and left ventricular end-diastolic pressure at the pre-drug control (C) were 2,327±150 mmHg/s and 10±2 mmHg, respectively. The LVdP/dt_{max} increased for 10-30 min after the low dose and for 5-60 min after the high dose, whereas no significant change was detected in the left ventricular end-diastolic pressure during the whole experimental period.

3.4. Effects on the electrocardiogram

Typical tracings of the electrocardiogram are depicted in Fig. 2, and the time courses of changes in the electrocardiogram variables are summarized in Fig. 3 (n=4). The pre-drug control values (C) of the PR interval, QRS width, QT interval and QTc were 99±7, 65±2, 280±5 and 313±8 ms, respectively. The QT interval and QTc were prolonged for 5-30 min and for 5-20 min after the low dose, respectively; and those were prolonged for 5-60 after the high dose. The longest QT and QTc were observed at 10 min after the start of the drug infusion of the respective doses, but any torsade de pointes was not detected. No significant change was detected in the PR interval or QRS width during the observation period.

3.5. Effects on the atrio-His and His-ventricular intervals and MAP duration during sinus rhythm

Typical tracings of the His bundle electrogram and MAP are depicted in Fig. 2, and the time courses of changes in the atrio-His and His-ventricular intervals and $MAP_{90(sinus)}$ during sinus rhythm are summarized in Fig. 3. The atrio-His and His-ventricular intervals and $MAP_{90(sinus)}$ at the pre-drug control (C) were 76 ± 6 , 24 ± 2 and 234 ± 1 ms, respectively. $MAP_{90(sinus)}$ was prolonged for 5-20 min after the low dose and for 5-60 min after the high dose, whereas no significant change was detected in the atrio-His or His-ventricular interval during the observation period. The longest $MAP_{90(sinus)}$ was observed at 10 min after the start of the drug infusion of the respective doses, which were 290 ± 4 and 330 ± 12 ms.

3.6. Effects on the MAP_{90} during the ventricular pacing

The time courses of changes in the MAP_{90} during the ventricular pacing at a cycle length of 300 and 400 ms are summarized in Fig. 3. The $MAP_{90(CL300)}$ and $MAP_{90(CL400)}$ at the pre-drug control (C) were 203 ± 2 and 225 ± 4 ms, respectively. The $MAP_{90(CL300)}$ and $MAP_{90(CL400)}$ were prolonged for 5-30 min and for 5-20 min after the low dose, respectively, and those were prolonged for 5-60 min after the high dose. The time courses of the increment in the $MAP_{90(CL300)}$ and $MAP_{90(CL400)}$ from the respective pre-drug control (C) were also calculated (data not shown in the figure). The maximum increments in the $MAP_{90(CL300)}$ and $MAP_{90(CL400)}$ were +51 and +65 ms, respectively, which were both observed at 10 min after the high dose. Increment in the $MAP_{90(CL400)}$ was significantly greater than that in the $MAP_{90(CL300)}$ for 5-20 min after the high dose, indicating a reverse use-dependent prolongation of repolarization.

3.7. Effects on the effective refractory period and terminal repolarization period

The time courses of changes in the effective refractory period and terminal repolarization period are summarized in Fig. 3. The effective refractory period and terminal repolarization period at the pre-drug control (C) were 201 ± 5 and 24 ± 3 ms, respectively. The effective refractory period was prolonged for 5-30 min after the low dose and for 5-60 min after the high dose. The terminal repolarization period tended to be prolonged, but it did not achieve a statistical significance.

3.8. Effects on the EMc and EMw

Typical tracings showing the change of the EMc and EMw are depicted in Fig. 2, and the time courses of changes in the EMc and EMw are summarized in Fig. 4A. The EMc and EMw at the pre-drug control (C) were 56 ± 9 and 199 ± 18 ms, respectively, both of which decreased for 5-30 min after the low dose, and further decreased for 5-60 min after the high dose.

3.9. Effects on the left ventricular systolic and diastolic periods

Typical tracings showing the change of wave forms of the left ventricular pressure before and after the drug administration are depicted in Fig. 4B, and the time courses of changes in the left ventricular systolic and diastolic periods, and the duration of ventricular pressure cycle are summarized in Fig. 4C. The left ventricular systolic and diastolic periods, and the duration of ventricular pressure cycle at the pre-drug control (C) were 265 ± 7 , 143 ± 9 and 409 ± 16 ms, respectively. No significant change was detected in these variables during the experimental period.

4. Discussion

In the present study, the cardiohemodynamic and electrophysiological effects of E-4031 together with its pharmacokinetic profile were simultaneously examined to better understand how the prolonged repolarization period affects the mode of ventricular contraction/relaxation by using the well-established halothane-anesthetized *in vivo* canine model with sinus rhythm (Ishizaka et al., 2008; Satoh et al., 2004, 1999; Sugiyama, 2008; Sugiyama and Hashimoto, 2002; Sugiyama et al., 2001; Takahara et al., 2005).

4.1. Plasma drug concentration

E-4031 was administered at two escalating i.v. doses, and its $C_{\max}$ was 24 ± 7 (59 nM) after 0.01 mg/kg infusion and 182 ± 48 ng/ml (453 nM) after 0.1 mg/kg infusion as shown in Fig. 1. E-4031 has been reported to inhibit the cloned human ether-a-go-go-related gene channel expressed in human embryonic kidney 293 cells with IC_{50} of 79 nM (Davie et al., 2004). The IC_{50} values obtained from canine and human ether-a-go-go-related gene channels expressed in human embryonic kidney 293 cells were comparable for each of MK-499, astemizole, terfenadine and cisapride (Wang et al., 2003), whereas there has been no report describing a significant difference in the IC_{50} values between these two species. These observations may indirectly suggest that E-4031 would have a similar extent of inhibitory effects on both the canine and human ether-a-go-go-related gene channels, although IC_{50} value of E-4031 against canine ether-a-go-go-related gene channel has not been reported. Since the plasma protein binding of E-4031 has been reported to be 69% in dogs (Webster et al., 2001),

the doses of E-4031 used in this study can be considered to effectively inhibit I_{Kr} .

4.2. Cardiohemodynamic effects

In the present study, E-4031 did not affect the heart rate, which is consistent with previous reports using the conscious dogs (Toyoshima et al., 2005), thiopental-anesthetized open-chest dogs (Izumi et al., 2010) and chloralose-anesthetized dogs (Wallace et al., 1991). Moreover, E-4031 in doses of 0.1 to 0.4 $\mu\text{g/kg}$ did not affect the heart rate in humans (Katritsis et al., 1997), suggesting that the halothane-anesthetized model can mimic the chronotropic response in humans. Meanwhile, other selective I_{Kr} blockers such as dofetilide and sotalolol have exerted the negative chronotropic effect in the halothane-anesthetized canine model in our previous studies (Satoh et al., 1999; Takahara et al., 2005), although these drugs exerted a similar extent of QT-interval prolongation to E-4031, indicating that the chronotropic effect of E-4031 can be much weaker than those of dofetilide and sotalolol. On the other hand, bradycardic effect of E-4031 has been reported in pentobarbital-anesthetized dogs (Nagashima et al., 1998; Yamazaki et al., 1996). Pentobarbital has been reported to have anti-vagal effects (Chiba and Tsuboi, 2001; Takahara et al., 2005), increasing sensitivity of the model for detecting the negative chronotropic effects of drugs, which may partly explain why E-4031-induced bradycardic effect was observed in those previous studies.

As shown in the results, E-4031 significantly increased the ventricular contractile force from the low dose. The ventricular contractile force is generally determined by the dynamics of Ca^{2+} -induced Ca^{2+} release in addition to the Ca^{2+} sensitivity of the microfilament (Bers, 2001). The prolongation of action potential

duration; i.e., MAP₉₀ in this study, could increase net Ca²⁺ influx via voltage-dependent L-type Ca²⁺ channels, and enhance excitation-contraction coupling (Wood et al., 1969), resulting in an increase in the ventricular contraction, which can partly explain the currently observed inotropic response. On the other hand, neither sematilide nor dofetilide increased the ventricular contraction at all in the same experimental model (Satoh et al., 1999; Takahara et al., 2005). One can speculate that the bradycardic action of dofetilide and sematilide may counteract their positive inotropic effects in a framework of the concept of positive staircase phenomenon.

4.3. Electrophysiological and proarrhythmic effects

E-4031 prolonged the repolarization period during sinus rhythm, which is in good accordance with other class III agents including *dl*-sotalol, sematilide, nifekalant, amiodarone and dofetilide (Ishizaka et al., 2008; Satoh et al., 2004, 1999; Sugiyama et al., 2001; Takahara et al., 2005). In addition, E-4031 prolonged the MAP duration in a reverse use-dependent manner, which has been observed by other I_{Kr} blockers (Ishizaka et al., 2008; Satoh et al., 2004, 1999; Sugiyama, 2008; Sugiyama and Hashimoto, 2002; Sugiyama et al., 2001; Takahara et al., 2005). E-4031 did not affect the atrioventricular or intraventricular conductions, indicating a lack of effect on Ca²⁺ or Na⁺ channel. Thus, the electrophysiological effects of E-4031 can be explained only by I_{Kr} blockade *in vivo*. Also, E-4031 significantly prolonged the repolarization period with modest increase in the terminal repolarization period, indicating that E-4031 may have some potential to induce torsades de pointes (Sugiyama, 2008).

4.4. Electro-mechanical relationship

E-4031 delayed the repolarization beyond the initiation of diastole to the midway of isovolumic relaxation phase after the infusion of 0.1 mg/kg, since EMc was negative and EMw was positive values (Figs. 2 and 4A). The situation, in which the ventricular muscle was relaxed but still electrically depolarized, provides an ideal proarrhythmic substrate, since L-type Ca^{2+} window current and/or late inward Na^{+} current are the most likely responsible for the initiation of early afterdepolarization-induced triggered activity (Remme and Wilde, 2013; Van der Linde et al., 2010). E-4031 increased the left ventricular contractile force ($\text{LVdP}/\text{dt}_{\text{max}}$) in a dose-related manner (Fig. 4B), whereas it did not affect the duration of left ventricular systole or diastole (Fig. 4C). Therefore, the changes in EMc and EMw can be considered to be mirror images to the QT-interval prolongation. Also, these results suggest that the repolarization delay could enhance both the net Ca^{2+} influx at the initiation of systole and the removal of intracellular Ca^{2+} for the relaxation. Thus, I_{Kr} inhibitor-induced alterations of electro-mechanical relationship may be a less specific surrogate marker of proarrhythmia, since their sensitivity will be equal to that of the QT-interval prolongation.

5. Study limitations

The use of halothane may have affected the electromechanical coupling and its alteration by E-4031, since halothane is a potent blocker of a number of different ionic channels, and halothane has significant effects on excitation-contraction coupling in the mammalian myocardium. Meanwhile, we needed to use the anesthesia to perform detailed analysis of electro-mechanical coupling and its alteration by E-4031 *in vivo* in this study. We assessed the cardiac effects of E-4031 under the same anesthetic

condition during the experiment; however, we could not totally rule out the influences of halothane on the analysis of the present results. **Another limitation would be the site where the monophasic action potentials were recorded. In this study the monophasic action potentials were recorded at the right ventricle, suggesting that the information of the monophasic action potentials indirectly reflects the electrical activity of the left ventricle.** Experiments are now on-going as another series of experiment by using cell motion imager with two-dimensional sheet of human induced pluripotent stem cell-derived cardiomyocytes to directly assess the electro-mechanical relationship.

6. Conclusions

In summary, E-4031 in doses that can provide the plasma concentrations to inhibit I_{Kr} *in vitro* exerted the positive inotropic action and repolarization delay together with the prolongation of effective refractory period without significantly affecting the other electropharmacological variables *in vivo*. Alternation in EMc and/or EMw may have a similar extent of sensitivity to QT-interval prolongation as a surrogate marker in predicting the onset of I_{Kr} inhibitor-induced arrhythmias, since the observed changes solely depended on the repolarization delay. The information described in this paper can become a guide to better understand the electro-mechanical actions of compounds with I_{Kr} blocking activity *in vivo*.

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Figure legends

Fig. 1

Summary of cardiohemodynamic effects of E-4031. Time courses of the plasma drug concentration (Plasma Conc.), heart rate (HR), mean blood pressure (MBP), cardiac output (CO), total peripheral resistance (TPR), maximum upstroke velocity of the left ventricular pressure (LVdP/dt_{max}) and left ventricular end-diastolic pressure (LVEDP). Data are presented as the mean±**S.E.M.** (n=4). The closed symbols represent significant differences from the control value (C) by $P < 0.05$.

Fig. 2

Typical tracings showing the His bundle electrogram (His), lead II surface electrocardiogram (ECG), aortic blood pressure (AoP), left ventricular pressure (LVP) and monophasic action potential (MAP) recorded from the right ventricle during sinus rhythm at pre-drug control (Control) and 10 min after the start of 0.1 mg/kg of E-4031 infusion. Note that repolarization was delayed beyond the initiation of diastole after the infusion of 0.1 mg/kg of E-4031, resulting in the inversion of electro-mechanical coupling (EMc) and the shortening of electro-mechanical window (EMw).

Fig. 3

Summary of electrophysiological effects of E-4031. Time courses of the PR interval (circles), QRS width (triangles), QT interval (squares) and QT corrected by Van de Water's formula (QTc, squares) (left upper panel); the MAP₉₀ (MAP_{90(sinus)}), atrio-His interval (AH, circles) and His-ventricular interval (HV, triangles) during sinus rhythm

(left lower panels); the MAP_{90} at a pacing circle length of 300 ms ($MAP_{90(CL300)}$) and 400 ms ($MAP_{90(CL400)}$) (right upper panels); and the effective refractory period at a basic pacing circle length of 400 ms ($ERP_{(CL400)}$) and terminal repolarization period ($TRP=MAP_{90(CL400)}-ERP_{(CL400)}$) (right lower panels). Data are presented as the mean $\pm$ S.E.M. (n=4). MAP_{90} represents the duration of monophasic action potential at 90% repolarization level. The closed symbols represent significant differences from each control value (C) by $P<0.05$.

Fig. 4

Effects of E-4031 on the electro-mechanical relationship. (A) The time courses of electro-mechanical coupling (EMc, triangles) and electro-mechanical window (EMw, circles). (B) Typical tracing of the left ventricular pressure curves at pre-drug control (C) (a), 10 min after the start of the low-dose infusion (0.01 mg/kg) (b) and 10 min after the start of the high-dose infusion (0.1 mg/kg) (c). (C) The time courses of the effects of E-4031 on the left ventricular systolic (circles) and diastolic (triangles) periods, and the duration of ventricular pressure cycle (squares) (ms). Data are presented as the mean $\pm$ S.E.M. (n=4). The closed symbols represent significant differences from each control value (C) by $P<0.05$.

Figure 1

Figure 2

Figure 3

Figure 4