Cardiohemodynamic and electrophysiological effects of a selective EP₄ receptor agonist ONO-AE1-329 in the halothane-anesthetized dogs

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Abstract

Cardiovascular effects of a highly selective prostaglandin E₂ type 4 (EP₄) receptor agonist ONO-AE1-329 were assessed with the halothane-anesthetized dogs (n=6).

ONO-AE1-329 was intravenously infused in three escalating doses of 0.3, 1 and 3 ng/kg/min for 10 min with a pause of 20 min between the doses. The low dose of 0.3 ng/kg/min significantly increased maximum upstroke velocity of left ventricular pressure by 18% at 20 min, indicating increase of ventricular contractility. The middle dose of 1 ng/kg/min significantly decreased total peripheral resistance by 24% and left ventricular end-diastolic pressure by 32% at 10 min, indicating dilation of arteriolar resistance vessels and venous capacitance ones, respectively; and increased cardiac output by 25% at 10 min in addition to the change induced by the low dose. The high dose of 3 ng/kg/min increased heart rate by 34% at 10 min; decreased mean blood pressure by 14% at 10 min and atrioventricular nodal conduction time by 13% at 5 min; and shortened left ventricular systolic period by 8% at 10 min and electromechanical coupling defined as an interval from completion of repolarization to the start of ventricular diastole by 39% at 10 min in addition to the changes induced by the middle dose. No significant change was detected in a ventricular repolarization period. These results indicate that ONO-AE1-329 may possess a similar cardiovascular profile to typical phosphodiesterase 3 inhibitors as an inodilator, and suggest that EP₄ receptor stimulation can become an alternative strategy for the treatment of congestive heart failure.

Keywords: ONO-AE1-329, EP₄, Positive inotropic effect, Vasodilation, Halothane-anesthetized dog, Electro-mechanical relationship

Chemical compound studied in this article

ONO-AE1-329 (PubChem CID: 5311230)

Abbreviations: AH: atrio-His; ANOVA: analysis of variance; EMc: electro-mechanical coupling; EMw: electro-mechanical window; EP1, 2, 3 and 4: prostaglandin E₂ type 1, 2, 3 and 4; HV: His-ventricular; LVdP/dt_{max}: maximum upstroke velocity of the left ventricular pressure; MAP: monophasic action potential; MAP₉₀: MAP duration at 90% repolarization level; MAP_{90(sinus)}: MAP₉₀ during sinus rhythm; MAP_{90(CL300)}: MAP₉₀ at a pacing cycle length of 300 ms; MAP₉₀₍₄₀₀₎: MAP₉₀ at a pacing cycle length of 400 ms; MBP: mean blood pressure; QTc: corrected QT interval; QTcB: QT interval corrected by Bazett's formula; and QTcV: QT interval corrected by Van de Water's formula

1. Introduction

Prostaglandin E₂ is the most widely produced within the body among the prostanoids, which is involved in a number of physiological and pathophysiological responses including fever, pain and inflammation (Yokoyama et al., 2013). Prostaglandin E₂ type 4 (EP₄) receptor is one of 4 receptor-subtypes for prostaglandin E₂, which belongs to the family of a G_sα protein-coupled, adenylyl cyclase-stimulating receptor (Nishigaki et al., 1995). EP₄ signaling has been shown to be involved in the pathophysiology of ischemic damage, hypertrophy, fibrosis and atherosclerosis in cardiovascular diseases (Yokoyama et al., 2013).

ONO-AE1-329 (Fig. 1) is a highly selective EP₄ receptor agonist, of which affinity for EP₄ receptor has been shown to be >100 times greater than those of the other subtypes of prostaglandin E₂ receptors like EP₁, EP₂ and EP₃ (Suzawa et al., 2000; Maruyama et al., 2002). EP₄ agonists including ONO-AE1-329 and EP4RAG have been shown to protect the heart from coronary ischemia-reperfusion injury in mice (Hishikari et al., 2009; Xiao et al., 2004), in addition to the attenuation of cardiovascular depression during the lipopolysaccharide-induced shock in rats (Sakamoto et al., 2004). Although the precise *in vivo* electropharmacological profile needs to be clarified before applying these new drugs as a clinically available therapeutic strategy, such information remains limited.

In this study, we adopted the halothane-anesthetized canine model to better examine the cardiohemodynamic and electrophysiological profile of ONO-AE1-329, since the model has been used to study the electropharmacological effects of various kinds of drugs (Ishizaka et al., 2008; Izumi-Nakaseko et al., 2014; Satoh et al., 1999, 2004; Sugiyama, 2008; Sugiyama et al., 2001a, 2001b; Takahara et al., 2003, 2005). To accurately analyze the 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 the durations of left ventricular systolic and diastolic periods separately to compare them with MAP duration, which will provide important information of the drug effects on the electro-mechanical relationship, one of the preclinical biomarkers of proarrhythmia (Izumi-Nakaseko et al., 2014; Vargas, 2010; Van der Linde et al., 2010).

2. Materials and Methods

Six male beagle dogs weighing 10 to 15 kg were used for experiments. Animals were obtained from Institute for Animal Reproduction (Ibaraki, 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. Experimental protocols

The aortic pressure, left ventricular pressure, electrocardiogram, His bundle electrogram and MAP signals were monitored with a polygraph system (RM-6000, Nihon Kohden, Tokyo, Japan) and analyzed with a real-time full automatic data analysis system (WinVAS3 ver 1.1R24v, Physio-Tech, Tokyo, Japan). Each measurement of electrocardiogram, MAP as well as atrio-His (AH) and His-ventricular (HV) intervals was the mean of three recordings of consecutive complexes. The cardiovascular variables were assessed in the following order. The electrocardiogram, His bundle electrogram, aortic and left ventricular pressures and MAP signals 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 assessment of the pre-drug control (C), ONO-AE1-329 in a low dose of 0.3 ng/kg/min was intravenously infused for 10 min, and each parameter was assessed at 5, 10, 15, 20 and 30 min after the start of administration. Then, ONO-AE1-329 in a middle dose of 1 ng/kg/min was intravenously infused for 10 min, and each parameter was assessed in the same manner as the low dose. Finally, ONO-AE1-329 in a high dose of 3 ng/kg/min was intravenously infused for 10 min, and each parameter was assessed at 5, 10,

15, 20, 30, 45 and 60 min after the start of administration. Plasma concentration of ONO-AE1-329 was not determined in this study, since the blood samplings might have some potential to affect the cardiohemodynamic effects of the drug particularly on the maximum upstroke velocity of the left ventricular pressure ($LVdP/dt_{max}$) and left ventricular end-diastolic pressure.

2.2. 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 Manufacturing, Tokyo, Japan). Tidal volume and respiratory rate were set at 20 ml/kg and 15 strokes/min, respectively.

Heparin calcium (100 IU/kg) was intravenously administered to prevent blood clotting. A clinically available catheter sheath (FAST-CATH™ 406108, St. Jude Medical Daig Division, Inc., Minnetonka, MN, USA) was inserted through the right femoral artery, and the aortic pressure was continuously monitored via a flush line connected to the catheter sheath by using a transducer (DX-100, Nihon Kohden). A thermodilution catheter (TC-504NH; Nihon Kohden) 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 placed at the right femoral artery to measure the left ventricular pressure. The $LVdP/dt_{max}$ and the left ventricular end-diastolic pressure were obtained during sinus rhythm to estimate the contractility and the preload to the left ventricle, respectively. The duration of left ventricular systolic period was measured as an interval (ms) from the time point of end-diastole to that of start of rapid decrease in left

ventricular pressure (end-systole), whereas that of left ventricular relaxation period was determined as an interval (ms) from the time point of the end-systole to that of completion of rapid decrease in left ventricular pressure as depicted in Fig. 2. The sum of the left ventricular systolic and diastolic periods indicates the duration of ventricular pressure cycle.

2.3. Electrophysiological parameters

The surface lead II electrocardiogram was obtained from the limb electrodes. Corrected QT intervals (QTc) were calculated by using Bazett's formula (QTcB) (Bazett, 1920), and Van de Water's formula (QTcV) (Van de Water et al., 1989). A quad-polar electrodes catheter (Cordis-Webster, Baldwin Park, CA, USA) 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 MAP₉₀.

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 MAP₉₀ 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-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 shown in Fig. 2: 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 the end-systole of left ventricular pressure. Second, electro-mechanical window (EMw) was obtained as an interval (ms) from the time point of MAP at 90% repolarization level to that of completion of the ventricular relaxation. Drug-induced reduction of EMc and/or EMw values has been shown to be a useful surrogate marker for risk of torsade de pointes in long QT type 1 canine model (Vargas, 2010; Van der Linde et al., 2010).

2.4. Drugs

ONO-AE1-329 (16-(3-Methoxymethyl)phenyl- ω -tetranor-3,7-dithia PGE_1) was obtained from Ono Pharmaceutical Co., Ltd. (Osaka, Japan). ONO-AE1-329 was dissolved in concentrations of 3, 10 and 30 ng/ml with 1% lactic acid (Sigma-Aldrich Co. LLC., Tokyo, Japan). Other drugs used were thiopental sodium (Mitsubishi Tanabe Pharma Corporation, Osaka, Japan), halothane (Takeda Pharmaceutical Company Limited, Osaka, Japan) and heparin calcium (Sawai Pharmaceutical Co., Ltd, Tokyo, Japan).

2.5. Statistical Analyses

Data are presented as the mean $\pm$ S.E.M. The statistically significant differences within a parameter were evaluated by one-way repeated-measures analysis of variance (ANOVA) followed by a post-hoc test Contrasts for mean values comparison. A *P* value

<0.05 was considered to be statistically significant.

3. Results

No animals showed cardiohemodynamic collapse or lethal ventricular arrhythmias leading to the animal's death during the experimental period.

3.1. Effects on the heart rate, mean blood pressure, cardiac output, total peripheral resistance, $LVdP/dt_{max}$ and left ventricular end-diastolic pressure

Typical tracings of the effects of ONO-AE1-329 on the aortic and left ventricular pressure are depicted in Fig. 2, whereas the time courses of changes in the heart rate, mean blood pressure, cardiac output, total peripheral resistance, $LVdP/dt_{max}$ and left ventricular end-diastolic pressure are summarized in Fig. 3 (n=6), of which pre-drug controls (C) were 114 ± 6 beats/min, 115 ± 2 mmHg, 1.67 ± 0.08 L/min and 72 ± 4 mmHg·min/L, $2,045 \pm 169$ mmHg/s and 11 ± 1 mmHg, respectively. The low dose of 0.3 ng/kg/min significantly increased the $LVdP/dt_{max}$ at 20 min, whereas no significant change was detected in the other variables. The middle dose of 1 ng/kg/min significantly increased the cardiac output for 5-20 min and $LVdP/dt_{max}$ for 5-20 min; but significantly decreased the total peripheral resistance for 5-20 min and left ventricular end-diastolic pressure for 5-20 min; whereas no significant change was detected in the heart rate or mean blood pressure. The high dose of 3 ng/kg/min significantly increased the heart rate for 5-20 min, cardiac output at 10 min and $LVdP/dt_{max}$ for 5-60 min; but significantly decreased the mean blood pressure for 5-15 min, total peripheral resistance for 5-15 min and left ventricular end-diastolic pressure for 5-20 min. The peak changes in the heart rate and mean blood pressure were observed at 10 min after the start of the high dose of 3 ng/kg/min, which were +34% and -14%, respectively; those in the cardiac output and total peripheral resistance were observed at 10 min after the middle dose of 1 ng/kg/min and the high dose, which were +25% and -26%, respectively; and those in the $LVdP/dt_{max}$ and left ventricular end-diastolic pressure were observed at 5 and 10 min after the high dose, which were

+35% and -60%, respectively.

3.2. Effects on the ECG

Typical tracings of the effects of ONO-AE1-329 on the ECG are depicted in Fig. 2, and the time courses of changes in the ECG parameters are summarized in Fig. 4 (n=6). The PR interval, QRS width, QT interval, QTcB and QTcV at the pre-drug control (C) were 106 ± 6 ms, 72 ± 3 ms, 257 ± 10 ms, 371 ± 20 and 326 ± 15 , respectively. No significant change was detected in any of the ECG parameters during the whole experimental period except that the high dose of 3 ng/kg/min significantly shortened the QT interval at 10 min by 5%.

3.3. Effects on the AH and HV intervals and MAP duration during sinus rhythm

Typical tracings of the effects of ONO-AE1-329 on the His bundle electrogram and MAP are depicted in Fig. 2, and the time courses of changes in the AH and HV intervals and $MAP_{90(\text{sinus})}$ are summarized in Fig. 4 (n=6), of which pre-drug controls (C) were 83 ± 4 ms, 26 ± 2 ms and 234 ± 7 ms, respectively. The low dose of 0.3 ng/kg/min or middle dose of 1 ng/kg/min did not affect the AH or HV interval, or $MAP_{90(\text{sinus})}$. The high dose of 3 ng/kg/min significantly shortened the AH interval for 5-20 min, whereas no significant change was detected in the HV interval or $MAP_{90(\text{sinus})}$. The peak change in the AH interval was observed at 5 min after the high dose of 3 ng/kg/min, which was -13%.

3.4. Effects on the MAP duration during the ventricular pacing, effective refractory period and terminal repolarization period

The time courses of changes in the MAP_{90} during the ventricular pacing at a cycle length of 300 and 400 ms, effective refractory period, and terminal repolarization period are summarized in Fig. 4 (n=6), of which pre-drug controls (C) were 222 ± 4 ms, 240 ± 5 ms, 211 ± 6 ms and 29 ± 7 ms respectively. The low dose of 0.3 ng/kg/min or middle dose of 1

ng/kg/min did not affect these variables. The high dose of 3 ng/kg/min significantly prolonged the effective refractory period only at 60 min by 6%, whereas no significant change was detected in the others.

3.5. Effects on the EMc and EMw

Typical tracings showing how to obtain the EMc and EMw are depicted in Fig. 2, and the time courses of changes in the EMc and EMw are summarized in Fig. 5 (n=6), of which pre-drug controls (C) were 45 ± 9 ms and 181 ± 12 ms, respectively. The high dose of 3 ng/kg/min significantly shortened the EMc and EMw for 5-10 min and at 10 min, respectively, whereas no significant change was detected in them after the low dose of 0.3 ng/kg/min or middle dose of 1 ng/kg/min. The peak changes in the EMc and EMw were observed at 10 min after the high dose of 3 ng/kg/min, which were -39% and -19% , respectively.

3.6. Effects on the left ventricular systolic/diastolic periods and duration of ventricular pressure cycle

The time courses of changes in the left ventricular systolic/diastolic periods and duration of ventricular pressure cycle are summarized in Fig. 5 (n=6), of which pre-drug controls (C) were 279 ± 9 / 136 ± 6 and 415 ± 14 ms, respectively. The low dose of 0.3 ng/kg/min or middle dose of 1 ng/kg/min did not change these variables. The high dose of 3 ng/kg/min significantly shortened the left ventricular systolic period and duration of ventricular pressure cycle for 10-15 min, whereas it tended to shorten the left ventricular diastolic period. The peak changes in the left ventricular systolic period and duration of ventricular pressure cycle were observed at 10 min after the high dose of 3 ng/kg/min, which were -8% and -12% , respectively.

4. Discussion

In the present study, we investigated the cardiohemodynamic and electrophysiological effects of ONO-AE1-329 together with its consequence to electro-mechanical relationship by using the well-established halothane-anesthetized *in vivo* canine model (Ishizaka et al., 2008; Izumi-Nakaseko et al., 2014; Satoh et al., 1999, 2004; Sugiyama, 2008; Sugiyama et al., 2001a, 2001b; Takahara et al., 2003, 2005). This is the first report describing that EP₄ receptor stimulation can induce the positive chronotropic, inotropic, dromotropic and vasodilator effects in addition to a reduction of electromechanical coupling/window in the non-rodent animal model.

4.1. Rationale for dose selection

In the extensive preliminary experiments using the halothane-anesthetized beagle dogs, ONO-AE1-329 at ≥ 1 ng/kg/min for 10 min significantly increased the heart rate but decreased the blood pressure, which let us start to assess the effects of ONO-AE1-329 in doses of 0.3, 1 and 3 ng/kg/min for 10 min under the monitoring of multiple cardiovascular variables. Meanwhile, EC₅₀ value of ONO-AE1-329 for mice EP₄ receptors expressed in Chinese hamster ovary cells has been shown to be 3.1 nmol/L (1.5 ng/ml), when assayed by the increase in the intracellular cyclic AMP production rate (Maruyama et al., 2002). Based on our previous experiences with the halothane-anesthetized beagle dogs (Ishizaka et al., 2008; Izumi-Nakaseko et al., 2014; Satoh et al., 1999, 2004; Sugiyama et al., 2001a, 2001b), administration of 1 ng/kg/min for 10 min (10 ng/kg/10 min) can be expected to provide maximum plasma drug concentration of around 10 pg/ml, which will be 150-fold smaller than EC₅₀ value for the mice EP₄ receptors (Maruyama et al., 2002). Since ONO-AE1-329 has been shown to be highly selective for EP₄ receptors according to the information from the manufacturer, its higher potency in dogs might at least in part suggest the presence of species difference in the affinity of EP₄ receptors to ONO-AE1-329.

4.2. Cardiohemodynamic effects

ONO-AE1-329 decreased the total peripheral resistance and left ventricular end-diastolic pressure together with the hypotensive action in a dose-related manner, reflecting a dilation of arteriolar resistance vessels and venous capacitance ones, respectively. EP₄ receptors have been shown to be expressed in the aorta (Bayston et al., 2003; Cao et al., 2012; Yokoyama et al., 2012), renal arteriole (Purdy and Arendshorst, 2000) and saphenous vein (Coleman et al., 1994), whereas similar vasodilator effects as observed in this study have been reported on the renal arteriole and saphenous vein with other EP₄ agonists (Coleman et al., 1994; Purdy and Arendshorst, 2000). EP₄ receptor has been shown to be coupled with stimulatory G protein and can activate adenylate cyclase to increase cyclic AMP production rate (Nishigaki et al., 1995), which may provide a rationale for the vasodilator effects of ONO-AE1-329 (Schaub and Kunz, 1986). In addition, it has been reported that EP₄ receptor-mediated, endothelium-dependent stimulation of eNOS activity may result in guanylyl cyclase-dependent vasorelaxation (Hristovska et al., 2007), which might be also involved in the mechanism of ONO-AE1-329-induced vasodilator effects.

ONO-AE1-329 exerted the positive chronotropic and inotropic effects in a dose-related manner. Since EP₄ receptors have been shown to be expressed in the cardiomyocytes (Birkenmeier et al., 2008), EP₄ receptor-dependent stimulation of adenylate cyclase activity (Nishigaki et al., 1995) may increase the inward Ca²⁺ current via increase of cyclic AMP production (Hartzell HC and Fischmeister R, 1987), which can explain the positive chronotropic and inotropic effects of ONO-AE1-329. Thus, the effect of the drug on the intracellular dynamics of Ca²⁺ needs to be analyzed to confirm this hypothesis. In addition, the decrease of the mean blood pressure may increase reflex-mediated sympathetic tone, which would have enhanced the positive chronotropic and inotropic effects of the drug.

The cardiohemodynamic profile of ONO-AE1-329 was qualitatively similar to those of phosphodiesterase 3 inhibitors in previous studies assessed in the halothane- and pentobarbital-anesthetized dogs (Pagel et al., 1996; Sugiyama et al., 2001a). Such current observation together with previous knowledge including EP₄ receptor-dependent anti-inflammatory effect and stimulation of eNOS activity (Hristovska et al., 2007; Yokoyama et al., 2013) may suggest that ONO-AE1-329 could become a candidate of an alternative to phosphodiesterase 3 inhibitors for the treatment of congestive heart failure. In addition, it should be noted that the cardiac output tended to be depressed below its basal control at 120 min together with an increase of the total peripheral resistance that appears to be even slightly above its basal control, which may become important information to better understand how to use ONO-AE1-329 in patients with congestive heart failure.

4.3. Electrophysiological effects

ONO-AE1-329 did not affect the electrophysiological parameters except for the QT or AH interval after the high dose of 3 ng/kg/min. Shortening of the QT interval after the high dose of 3 ng/kg/min may be secondary induced by an increase of the heart rate, since there was no significant change in the QTc or the MAP₉₀ during the ventricular pacing. The positive dromotropic effect after the high dose of 3 ng/kg/min may be induced through increases of cyclic AMP via EP₄ receptor stimulation. In addition, reflex-mediated increase in the sympathetic tone may also enhance the positive dromotropic effect of ONO-AE1-329. On the other hand, ONO-AE1-329 did not prolong the ventricular repolarization period, effective refractory period or terminal repolarization period except that effective refractory period was slightly prolonged only at 60 min after the high dose of 3 ng/kg/min, indicating a lack of proarrhythmic potential leading to the onset of torsade de pointes.

4.4. Electro-mechanical relationship

The duration of ventricular pressure cycle and left ventricular systolic period were shortened in a dose-related manner possibly due to the enhancement of intracellular Ca^{2+} removal by sarco/endoplasmic reticulum Ca^{2+} -ATPase through EP_4 receptor stimulation-induced cyclic AMP increase (Schaub and Kunz, 1986). ONO-AE1-329 did not affect the repolarization period, but shortened the duration of left ventricular pressure cycle, leading to the shortening of the EMc and EMw. When ONO-AE1-329 is used with drugs that delay the repolarization, the EMc and/or EMw will reduce and become negative, predicting an increase of proarrhythmic risk (Vargas, 2010; Van der Linde et al., 2010).

4.5. Study limitation

The electrostatic charge of an ionized molecule attracts water dipoles and results in a polar, relatively water-soluble and lipid insoluble complex. Although ONO-AE1-329 is weak acid with carboxyl group having less ability to permeate the blood-brain barrier, there may be still some possibilities that cardiovascular effects of ONO-AE1-329 in this study might be at least in part mediated through direct neuronal mechanisms besides the hypotension-induced, reflex-mediated increase of sympathetic tone. In order to eliminate such possibilities, further experiments would be needed including pharmacological blockade of autonomic nervous system and/or use of isolated cardiac preparations.

Use of an anesthetic also deserves a comment. We have already assessed various types of drugs with/without proarrhythmic potential by using the same halothane-anesthetized canine model as used in this study (Ishizaka et al., 2008; Izumi-Nakaseko et al., 2014; Satoh et al., 1999, 2004; Sugiyama, 2008; Sugiyama et al., 2001a, 2001b; Takahara et al., 2003, 2005). This information helps us better analyze the currently observed cardiovascular effects and anti/pro-arrhythmic potentials of ONO-AE1-329. However, there might be some possibilities that anesthetics drop the

heart rate, can suppress neuronal cardiovascular pathways, and might modify anti/pro-arrhythmic potentials, which would have altered normal physiological responses to ONO-AE1-329.

Another concern may be the stability of the halothane-anesthetized canine model during the experimental period. In a previous study using the same halothane-anesthetized canine model together with the same experimental protocol as used in this study (Sugiyama and Hashimoto, 1998), we have confirmed that each of the cardiovascular variables was stable for ≥ 2.5 h. However, it may be possible that there has been some damage to the heart or altered physiological responses by dosing every animal with all three doses in combination with pacing, which would partly explain that variables such as the $LVdP/dt_{max}$ or effective refractory period did not return to their control levels by the 120 min point.

5. Conclusion

ONO-AE1-329 exerted positive chronotropic, inotropic, dromotropic and vasodilator effects, which may be induced through an increase of cyclic AMP production by EP_4 receptor stimulation and reflex-mediated sympathetic tone; whereas the drug by itself did not induce the ventricular arrhythmias or QT-interval prolongation. Since the estimated plasma concentration at doses attained in this study would be 50- to 500-fold smaller than EC_{50} value for EP_4 receptor of mice, dogs might be more sensitive to EP_4 agonist than mice. The duration of ventricular pressure cycle as well as left ventricular systolic period was shortened, suggesting that uptake rate of intracellular Ca^{2+} by sarco/endoplasmic reticulum Ca^{2+} -ATPase may be enhanced by ONO-AE1-329. Although ONO-AE1-329 will not affect the repolarization period, caution has to be paid on concomitant use of QT-interval prolonging drugs during the ONO-AE1-329 treatment, which may have potential to make the EMc and/or EMw negative. Further studies may be needed to clarify the utility of ONO-AE1-329 in a model of heart failure together with

analysis of intracellular Ca^{2+} dynamics in comparison with those of phosphodiesterase 3 inhibitors.

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Figure legends

Fig. 1

Chemical structure of ONO-AE1-329

(16-(3-Methoxymethyl)phenyl- ω -tetranor-3,7-dithia PGE₁).

Fig. 2

Typical tracings showing the His bundle electrogram (His), lead II surface electrocardiogram (ECG), aortic pressure (AoP), left ventricular pressure (LVP) and monophasic action potential (MAP) recorded from the right ventricle during sinus rhythm at pre-drug control (C) and 10 min after the start of 3 ng/kg/min of ONO-AE1-329 infusion. Note that heart rate was increased after the infusion of 3 ng/kg/min of ONO-AE1-329, and that electro-mechanical coupling (EMc) and window (EMw) were shortened.

Fig. 3

Summary of cardiohemodynamic effects of ONO-AE1-329. The time courses of the heart rate (HR), mean blood pressure (MBP), cardiac output (CO), total peripheral vascular resistance (TPR), maximum up stroke velocity of left ventricular pressure (LVdP/dt_{max}) and left ventricular end-diastolic pressure (LVEDP). Data are presented as the mean $\pm$ S.E.M. (n=6). The closed symbols represent significant differences from pre-drug control (C) by $P<0.05$.

Fig. 4

Summary of electrophysiological effects of ONO-AE1-329. The time courses of the PR interval (circles), QRS width (triangles), QT interval (squares), QT corrected by Bazett's formula (QTcB, circles) and QT corrected by Van de Water's formula (QTcV, triangles); atrio-His interval (AH, circles), His-ventricular interval (HV, triangles) and MAP₉₀ during

sinus rhythm ($MAP_{90(sinus)}$, squares); the MAP_{90} at a pacing cycle length of 300 ms ($MAP_{90(CL300)}$) and 400 ms ($MAP_{90(CL400)}$); and the effective refractory period at a basic pacing cycle length of 400 ms (ERP); and terminal repolarization period ($TRP=MAP_{90(CL400)}-ERP$). Data are presented as the mean $\pm$ S.E.M. (n=6). The closed symbols represent significant differences from pre-drug control (C) by $P<0.05$.

Fig. 5

Summary of electro-mechanical relationship of ONO-AE1-329. The time courses of the electro-mechanical coupling (EMc, triangles) and window (EMw, circles), left ventricular systolic (circles) and diastolic (triangles) periods, and duration of ventricular pressure cycles (squares). Data are presented as the mean $\pm$ S.E.M. (n=6). The closed symbols represent significant differences from pre-drug control (C) by $P<0.05$.

Fig. 1

**10 min after Start of
3 ng/kg/min ONO-AE1-329 Infusion**

Fig. 3

Fig. 4

Fig. 5