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Cardiohemodynamic and electrophysiological effects of a selective EP_4 receptor agonist ONO-AE1-329 in the halothane-anesthetized dogs

Hiroaki Nomura [a], Yuji Nakamura [a], Xin Cao [a], Atsushi Honda [a], Jun Katagi [a], Hiroshi Ohara [a,b], Hiroko Izumi-Nakaseko [a], Yoshioki Satoh [c], Kentaro Ando [a], Atsushi Sugiyama [a,c,*]

[a] Department of Pharmacology, Faculty of Medicine, Toho University, 5-21-16, Omori-nishi, Ota-ku, Tokyo 143-8540, Japan
[b] Division of Cardiovascular Medicine, Department of Internal Medicine, Faculty of Medicine, Toho University, 6-11-1 Omori-nishi, Ota-ku, Tokyo 143-8541, Japan
[c] Yamanashi Research Center of Clinical Pharmacology, 73-5 Hatta, Isawa-cho, Fuefuki-city, Yamanashi 406-0023, Japan

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ABSTRACT

Cardiovascular effects of a highly selective prostaglandin E_2 type 4 (EP_4) 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 EP_4 receptor stimulation can become an alternative strategy for the treatment of congestive heart failure.

1. Introduction

Prostaglandin E_2 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 E_2 type 4 (EP_4) receptor is one of 4 receptor-subtypes for prostaglandin E_2, which belongs to the family of a $G_s\alpha$ protein-coupled, adenylyl cyclase-stimulating receptor (Nishigaki et al., 1995). EP_4 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 EP_4 receptor agonist, of which affinity for EP_4 receptor has been shown to be >100 times greater than those of the other subtypes of prostaglandin E_2 receptors like EP_1, EP_2 and EP_3 (Suzawa et al., 2000; Maruyama et al., 2002). EP_4 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

Abbreviations: AH, atrio-His; ANOVA, analysis of variance; EMc, electro-mechanical coupling; EMw, electro-mechanical window; $EP_{1,2,3}$ and $_4$, prostaglandin E_2 type 1, 2, 3 and 4; HV, His-ventricular; $LVdP/dt_{max}$, maximum upstroke velocity of the left ventricular pressure; MAP, monophasic action potential; MAP_{90}, MAP duration at 90% repolarization level; $MAP_{90(sinus)}$, MAP_{90} during sinus rhythm; $MAP_{90(CL300)}$, MAP_{90} at a pacing cycle length of 300 ms; $MAP_{90(400)}$, MAP_{90} 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

* Corresponding author at: Department of Pharmacology, Faculty of Medicine, Toho University, 5-21-16, Omori-nishi, Ota-ku, Tokyo 143-8540, Japan.
Fax: +81 3 5493 5413.
E-mail address: atsushi.sugiyama@med.toho-u.ac.jp (A. Sugiyama).

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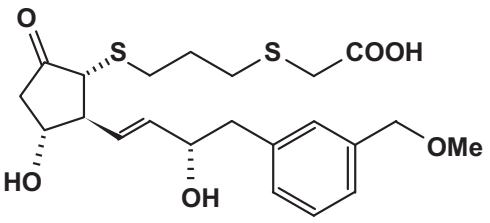

Fig. 1. Chemical structure of ONO-AE1-329 (16-(3-Methoxymethyl)phenyl-ω-tetranor-3,7-dithia PGE_1).

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 10–15 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 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