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Promising approach for the preclinical assessment of cardiac risks using left ventricular pressure-volume loop analyses in anesthetized monkeys

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ABSTRACT

Introduction: Load-independent cardiac parameters obtained from the ventricular pressure-volume relationship are recognized as gold standard indexes for evaluating cardiac inotropy. In this study, for better analyses of cardiac risks, load-independent pressure-volume loop parameters were assessed in addition to load-dependent inotropic, hemodynamic and electrocardiographic changes in isoflurane-anesthetized monkeys.
Methods: The animals were given milrinone (a PDE 3 inhibitor), metoprolol (a β-blocker), or *dl*-sotalol (a $\beta + I_{Kr}$ blocker) intravenously over 10 min at two dose levels including clinically relevant doses ($n = 5$/drug).
Results: Milrinone and metoprolol produced positive and negative inotropy, respectively. These effects were detected as changes in the slope of the preload-recruitable stroke work, which is a load-independent inotropic parameter. However, *dl*-sotalol did not alter the slope of the preload-recruitable stroke work. That means *dl*-sotalol produced no inotropy, although it decreased load-dependent inotropic parameters, including maximal upstroke velocity of left ventricular pressure, attributable to decreased heart rate and blood pressure. Other typical pharmacological effects of the compounds tested were also detected. Both β-blockers produced PR prolongation, decreased left ventricular end-systolic pressure, increased left ventricular end-diastolic pressure, and increased maximal descending velocity of left ventricular pressure and time constant for isovolumic relaxation. *dl*-Sotalol also prolonged heart-rate-corrected QT interval. Milrinone induced reflex tachycardia, PR shortening, and decreased left ventricular end-diastolic pressure.
Discussion: The overall assessment by not only load-dependent inotropic parameters but also load-independent parameters obtained from the ventricular pressure-volume loop analysis using monkeys can provide further appropriate information for the assessment of drug-induced cardiac risks.
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1. Introduction

Drug-induced cardiotoxicity is still a major concern endangering patient health during clinical trials for new chemical entities. Accurate and effective assessment leading to selection of promising candidates without cardiotoxic potential is one of the most important strategies at the preclinical phase of drug development (Piccini et al., 2009; Pettit, Berridge, & Sarazan, 2010; Sarazan et al., 2011). In recent years, preclinical in vitro, in vivo, or in silico cardiac electrophysiological safety assessments, typified by predicting drug-induced long QT syndrome, have been enhanced (Ando et al., 2005; Beattie et al., 2013; Elkins et al., 2013; Holzgrefe et al., 2014; Tashibu, Miyazaki, Aoki, Akie, & Yamamoto, 2005). An additional consideration to drug effects on heart rhythm involves cardiac contractility. However, other cardiovascular adverse effects including congestive heart failure are often difficult to detect during the preclinical stage (Ferri et al., 2013), and the methods for assessment of the effects of the candidates on cardiac function, like their cardiac inotropic and lusitropic potential, still have room for development (Sarazan, Kroehle, & Main, 2012). Diagnostic imaging methods such as magnetic resonance imaging or ultrasound echocardiography have been suggested as the most useful tools for the cardiac functional evaluation; however, these methods have some inherent drawbacks including requirement for a large-scale installations, differences in technique between facilities, or difficulties in obtaining a load-independent index of cardiac contractility. The end-systolic pressure-volume (PV) relationship is known as a gold standard for evaluating the inotropic potential (Suga, Sagawa, & Shoukas, 1973). Recent improvement of PV catheter technology has made possible the accurate measurement of the left ventricular (LV) PV by the admittance method. This method incorporates elements of the conductance of blood flow and cardiac muscle combined with the capacitance of the myocardium (Pearce & Feldman, 2009; Porterfield et al., 2009; Clark & Marber, 2013). The measurement of PV using this method has been conducted mainly in rodents (Kijtawornrat et al., 2014; Pacher, Nagayama, Mukhopadhyay, Bátkai, & Kass, 2008), but not in non-rodents. Preclinical studies in

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monkeys still occupy an important position in the toxicological field (Mecklenburg & Romeike, 2016). In addition, the evaluation of cardiovascular safety profile for promising drugs is carried out using monkeys as one of the non-rodent species in safety pharmacology testing (Omata, Kasai, Hashimoto, Hombo, & Yamamoto, 2005). However, the evaluation for cardiac contractility still depends on load-dependent indexes (e.g., maximal upstroke velocity of left ventricular pressure, LVdP/dt_{max}) (Markert et al., 2007). The LVdP/dt_{max} is suitable as an index of cardiac contractility only when loading conditions are unchanged (Hamlin & Del Rio, 2012). The QA interval, the time between the onset of the QRS complex of the ECG and the onset of the systemic arterial pressure pulse, is also used as an inotropic index (Adeyemi et al., 2009; Johnson, Geys, Lissens, & Guns, 2012; Norton, Iacono, & Vezina, 2009). However, this parameter is affected by electro-pressor latency, contraction time during isovolumic systole, and pulse wave propagation (Hamlin & Del Rio, 2010). Since many factors other than cardiac contractility are involved in the LVdP/dt_{max} and the QA interval, interpretation of inotropic and lusitropic properties being examined only by these parameters remains elusive. Therefore, there is a need to increase the knowledge and awareness of available and reliable non-rodent models by adopting the PV loop method in order to identify the potential cardiac functional toxicity of new chemical entities. Despite the enormous preclinical importance of assessment of cardiac function, there are no published direct assessments using monkeys.

To establish a LV PV loop method in cynomolgus monkeys for preclinical evaluation of drug-induced cardiac inotropic changes, milrinone, a phosphodiesterase 3 inhibitor, metoprolol, a representative selective β_1-adrenergic blocker, or *dl*-sotalol, a non-selective competitive β-adrenergic receptor blocker with Class III antiarrhythmic properties, was administered intravenously over 10 min to animals and cardiac inotropic and lusitropic activities were assessed simultaneously with the hemodynamic and electrocardiographic parameters.

2. Materials and methods

2.1. Experimental animals

Cynomolgus monkeys were obtained from CLEA Japan, Inc. (Tokyo, Japan) or Hamri Co., Ltd. (Ibaraki, Japan). The present study was carried out according to the Research Standard Operation Procedures for animal experiments approved by the Ethics Review Committee for Animal Experimentation of Daiichi Sankyo Co., Ltd. for compliance with regulations. Experiments were performed using a total of twenty monkeys of either sex weighing approximately 3 to 5 kg at ages of 4 to 10 years, which were divided into 4 groups: vehicle control, milrinone, metoprolol, and *dl*-sotalol groups ($n = 5$/group).

2.2. Induction and maintenance of anesthesia and surgical preparation

Monkeys were initially anesthetized with intramuscular administration of ketamine hydrochloride (Ketalar® Intramuscular 500 mg, Daiichi Sankyo Co., Ltd.) at 10 mg/kg, and intubated with a cuffed endotracheal tube. After that, 1 to 3% isoflurane (Escain®, Pfizer Japan Inc., Tokyo, Japan) vaporized with 100% oxygen was inhaled with a volume-cycled animal ventilator (Anesthetic ventilator PRO-55S combined with PRO-55V, Acoma Medical Industry Co., Ltd., Tokyo, Japan), and the respiratory rate and tidal volume were set at 12 to 20 breaths/min and 12.5 to 30 mL/kg, respectively. Body temperature, oxyhemoglobin saturation measured by pulse oximetry, and end tidal carbon dioxide were continuously monitored by a multi-functional physiologic monitoring system (BioScope AM130, Fukuda M-E Kogyo Co., Ltd., Tokyo, Japan) and were sustained within the physiological range throughout the experiment period via warming the animals with a forced-air warming system (3M™ Bair Hugger™ Warming Unit Model 750, 3M Company, MN, USA) and adjusting the volume-cycled animal ventilator. The isoflurane concentration during both inhalation and expiration was also monitored using the multi-functional physiologic monitoring system. After a proper and stable anesthetic condition was established, the animals were paralyzed with intravenous administration of rocuronium bromide (ESLAX® Intravenous 25 mg/2.5 mL, MSD K.K., Tokyo, Japan) at 1 to 3 mg/body to arrest spontaneous respiration in order to create stable PV loops during occlusion. A heparinized catheter (Hemostasis Introducer, Nihon Kohden, Tokyo, Japan) was inserted through the right femoral artery for continuous monitoring of arterial blood pressure. A PV catheter (FTH-5018B-E248B, Transonic Scisense Inc., Ontario, Canada) was positioned in the left ventricle via the carotid artery to monitor left ventricular pressure and volume. A venous occlusion catheter (NOK-3F080-W, Nipro Corporation, Tokyo, Japan) was positioned in the caudal vena cava via a catheter inserted in the left femoral vein.

2.3. Data acquisition

Pressure and volume of the left ventricle were monitored through another system (ADV500 Admittance Pressure Volume Control Unit, Transonic Scisense Inc.). Standard limb lead II electrocardiogram (ECG) obtained from limb electrodes was monitored via a multi-functional ECG monitoring system (Cardisuny D700, Fukuda M-E Kogyo Co., Ltd.), and arterial blood pressure was obtained using a polygraph system (RM-6000; Nihon Kohden Corporation). The LVdP/dt_{max}, maximal rate of fall of left ventricular pressure (LVdP/dt_{min}), left ventricular end-systolic pressure (LVESP), and left ventricular end-diastolic pressure (LVEDP) were recorded via the PV catheter. LVEDP was measured at the end of the atrial "kick" before the rapid rise of LV pressure. Families of left ventricular PV loops were generated via the PV catheter during acutely decreased preload produced by occlusion of the caudal vena cava for approximately 5 to 8 s. All these parameters were continuously recorded into a physiological data acquisition system (Ponemah Physiology Platform, Data Science International., MN, USA).

2.4. Experimental protocol

The cardiovascular variables were assessed in the following order. The electrocardiogram, arterial blood pressure, and pressure and volume of the left ventricle were recorded under non-occlusion and sinus rhythm conditions. Families of LV PV loops were obtained during brief occlusions of the vena cava conducted in duplicate or triplicate, allowing the electrophysiological or hemodynamic parameters to return to the pre-occlusion status between occlusions. After a pre-dose control assessment, 0.05 mg/kg milrinone was administered intravenously over 10 min and the cardiovascular variables were assessed 5, 10, 15 and 20 min after the initiation of dosing. Then, additional milrinone at 0.5 mg/kg was administered and each parameter was assessed 5, 10, 15, and 20 min after the initiation of the second dosing. Effects of metoprolol at 0.05 and 0.15 mg/kg and *dl*-sotalol at 0.3 and 3 mg/kg were assessed in a similar manner. All doses chosen were expected to cover the plasma therapeutic level of each drug in human. Effects of 0.9% physiological saline (as vehicle control) were also assessed as another series.

2.5. Data analysis

Each hemodynamic and electrophysiological parameter was represented as the mean of ten consecutive heart beats. PR interval, QRS width, and QT interval were measured manually from the ECG trace, supported by an ECG waveform recognition software (Ponemah Physiology Platform, Data Science International, St. Paul, MN, USA). QT interval corrected for heart rate (QTc) was calculated using Bazett's formula [$QTcB = QT/(60{,}000/RR)^{1/2}$] (Bazett, 1920), Fridericia's formula [$QTcF = QT/(60{,}000/RR)^{1/3}$] (Fridericia, 1920), and Van de Water's formula [$QTcV = QT - 0.087 \times (RR - 1000)$] (Van de Water, Verheyen, Xhonneux, & Reneman, 1989), where the unit is given in msec. QA

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