Conduction and contraction properties of human iPS cell-derived cardiomyocytes: analysis by motion field imaging compared with the guinea-pig isolated heart model

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ABSTRACT — We used motion field imaging to characterize the conduction and contraction of a sheet of cardiomyocytes derived from human induced pluripotent stem cells (hiPS-CMs). A hiPS-CMs sheet of 2.8 mm × 2.8 mm allowed us to simultaneously measure the conduction and the contraction properties in the same cells. Pharmacological responses in the hiPS-CMs of four typical cardiac functional modulators, Na⁺ channel blocker (lidocaine), Ca²⁺ channel blocker (diltiazem), gap-junction inhibitor (carbenoxolone), and β-adrenergic stimulator (isoproterenol), were investigated, and the results were compared to those found using the isolated guinea-pig heart model perfused by the Langendorff method. The conduction speed of excitation waves in hiPS-CMs was decreased by lidocaine, diltiazem, and carbenoxolone, and increased by isoproterenol, and these results were in accordance with the changes in the conduction parameters of electrocardiogram (QRS duration, PR interval, and P duration) in the Langendorff guinea-pig heart model. The maximum speeds for contraction and relaxation, which respectively represent the contraction and relaxation kinetics of hiPS-CMs, were decreased by lidocaine and diltiazem, and increased by isoproterenol. These results also corresponded to alterations in the contractile and relaxation parameters found by measuring left ventricular pressure (LVdP/dt max and LVdP/dt min) in the Langendorff guinea-pig heart model. From these lines of evidence, it was suggested that hiPS-CMs enable us to evaluate the cardiac toxicities associated with conduction disturbance or contractile dysfunction, and thereby would be useful as an integrated assessment of cardiac function.

Key words: iPS, Cardiomyocytes, Conduction, Contraction, Motion field imaging, Langendorff

INTRODUCTION

Human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) have been applied to cardiac safety assessment in the non-clinical stages of drug development. Human iPS-CMs express fundamental cardiac ion channels and can generate action potential (Honda et al., 2011; Ma et al., 2011), which makes them a useful non-clinical tool for assessing the proarrhythmic potential of drugs (Ando et al., 2017; Kitaguchi et al., 2017; Takasuna et al., 2017; Nozaki et al., 2016). Moreover, the hiPS-CMs can be formed into a two-dimensional sheet that is electrically coupled and contracts synchronously as a functional cluster, which are properties that allow us to evaluate cardiac contraction and conduction (Izumi-Nakaseko et al., 2017; Pointon et al., 2015). Hence the hiPS-CMs may enable us to evaluate diverse cardiac functional toxicities and are expected to be a novel non-clinical tool for cardiac safety assessment.

Cardiac toxicities are a major cause of drug attrition in the non-clinical and clinical phases of drug development (Cook et al., 2014; Laverty et al., 2011) and cover a wide variety of adverse effects, including life-threatening arrhythmia, dysfunction of contractility, or conduction disturbances. To mitigate various kinds of cardiac safety liabilities, it is important to evaluate the whole cardiac function in an integrated system. The in-vivo animal model, which uses a telemetry transmitter, and the ex-vivo isolated animal heart model are valuable integrated systems to evaluate cardiac function and have been widely utilized (Guth et al., 2015; Komatsu et al., 2010; Honda et al., 2010; Tabo et al., 2010; Guo et al., 2009). However, the drawbacks of these animal models are the low through-put, the limitations of species difference,
and the 3Rs. The hiPS-CMs sheet can be applied to evaluate the integrated cardiac function at a higher throughput than the animal models and, as a human relevant cell, is expected to reflect human cardiac physiology, and contributes to the 3Rs.

Motion field imaging (MFI), which uses phase-contrast video imaging at a high spatiotemporal resolution, can analyze the contractile motion of hiPS-CMs and the two-dimensional conduction property of an excitation wave on a hiPS-CMs sheet (Hayakawa et al., 2014, 2012). Furthermore, it is notable that the MFI system is able to measure and analyze the motion of cardiomyocytes with multi-well plates in a label-free and non-invasive manner. These features mean that the contraction property and the conduction property can be simultaneously measured in the same cells at a moderate to high throughput, which supports the potential that MFI has to allow us to use hiPS-CMs in an integrated cardiac safety assessment. Recent studies demonstrated that the cardiac proarrhythmic potential, alterations in contractility, and chronic cardiotoxicity can be evaluated by analyzing the contractile motion obtained by MFI (Kopoljar et al., 2017; Hayakawa et al., 2014). However, the conduction property remains to be characterized and used to evaluate cardiac toxicities.

The purpose of this study, therefore, was to characterize the conduction property and the contraction property of a hiPS-CM sheet which were simultaneously measured with MFI. To investigate the effect of positive and negative modulations on the contraction and propagation properties, the study tested four reference compounds that affect these properties by a different pharmacological action: a Na+ channel blocker (lidocaine), a Ca2+ channel blocker (diltiazem), a gap-junction inhibitor (carbenoxolone), and a β-adrenergic stimulator (isoproterenol). To validate the results from hiPS-CMs, the pharmacological responses of the hiPS-CMs were compared to those in the isolated guinea-pig heart model perfused by the Langendorff method, which is a well-established whole heart model for cardiac safety assessment.

**MATERIALS AND METHODS**

**Culture of hiPS-CMs**

Cryopreserved cardiomyocytes derived from human iPSCs (iCell® Cardiomyocytes; lot nos. 1091313 and 1093227; Cellular Dynamics International, Madison, WI, USA) were obtained and prepared according to the manufacturer’s protocol. The cells were immediately thawed in the iCell Cardiomyocytes Plating Medium (Cellular Dynamics International) and plated onto 6-well culture plates coated with 0.1% gelatin (Becton Dickinson, Bedford, MA, USA), where the cells were cultured for 48 hr at 37°C under an atmosphere of 5% CO2. Then, the culture medium was replaced by the iCell Cardiomyocytes Maintenance Medium (Cellular Dynamics International), in which the cells were cultured for an additional 2 days. The cells were dispersed with 0.25% trypsin-EDTA (Invitrogen, Carlsbad, CA, USA), and 30 μL of cell suspensions were re-plated on a small area (approximately 3 mm in diameter) coated with fibronectin (Corning; New York, NY, USA) on 12-well plates (Thermo Fisher Scientific, Waltham, MA, USA) at a density of 3.9 × 10⁴ cells/30 μL/well. Each well was then filled with the maintenance medium at 2 mL. The cells were incubated at 37°C under an atmosphere of 5% CO₂ for 7 to 12 days until recording, during which time the medium was replaced every 2 to 3 days. On the days of recording, the culture medium in the wells of a 12-well plate was exchanged at a volume of 2 mL before starting experiments. The plate was then set into a mini-incubator attached to the stage of the video-microscope system, in which cells were maintained at 37°C under an atmosphere of 5% CO₂. The cells were cultured in the mini-incubator for more than 30 min for stabilization.

**Video microscopy and motion vector analysis of hiPS-CMs**

Video images of beating hiPS-CMs were recorded as sequential phase-contrast images with a 4 × objective at a frame rate of 150 frames/sec and a resolution of 2048 × 2048 pixels using the SI8000 cell motion imaging system (Sony Corporation, Tokyo, Japan). Following stabilization, video images of each well were recorded three times at intervals of 5 min for a duration of 5 sec each time, to assess whether the contractile motion of cells was stable. We analyzed the beating rate and confirmed that the coefficient of variance between the measurements taken in the three recordings was less than 5% for all the preparations in this study.

The culture medium was first treated for 15 min to obtain a baseline value of each well. Then, vehicle (0.1%-0.4% dimethyl sulfoxide: DMSO) or test articles (lidocaine: 5, 15, 45, 135 μM; diltiazem: 0.1, 0.3, 1, 3 μM; isoproterenol: 0.003, 0.01, 0.03, 0.1 μM; carbenoxolone: 3, 10, 30, 100 μM) were applied from the lowest to the highest concentration in a cumulative manner with each concentration of test article treated for 15 min. Analysis points were 15 min (for the vehicle, lidocaine, diltiazem, and carbenoxolone groups) or 5 min (for the isoproterenol group) after treatment of the medium or of each concentration of the test articles, and video images
were recorded for 10 sec.

Motion vectors of beating hiPS-CMs were analyzed with the SI8000 system using a block matching algorithm, the details of which are described in a previous report (Hayakawa et al., 2014). A series of parameters were obtained and analyzed from the motion waveforms: maximum contraction speed (MCS) and maximum relaxation speed (MRS) — which represent the contraction property of the hiPS-CMs sheet —, contraction-relaxation duration (CRD), and beating rate (BR) (Fig. 1). CRD corrected by Fridericia’s formula (CRDcF) was calculated as follows: CRDcF = CRD/([mean inter-beat interval (msec)/1000]^{1/3}). In this formula, the mean inter-beat interval was calculated from the BR. Fridericia’s formula was applied in this study because it was effective for the correction of field potential duration of the hiPS-CMs (Yamamoto et al., 2016).

Propagation analysis of hiPS-CMs

The conduction speed of an excitation wave on the hiPS-CMs sheet was calculated by the SI8000 system. In the microscopic view, the field of the hiPS-CMs sheet (2.8 mm x 2.8 mm) is divided into 64 (8 x 8) square compartments of equal size. The time when a contraction wave is elicited in each compartment is measured. Based on the time, the conduction speed was determined by the following calculation: conduction speed (m/sec) = [distance between A and B (m)]/[elapsed time from C to D (sec)]. A (or B) represents the center point of a compartment on the cell sheet where an excitation wave was first (or last) elicited during a synchronous contraction. C (or D) represents the time when an excitation wave was elicited in the A (or B) compartment.

Animals in the Langendorff study

Male guinea pigs ([Slc: Hartley], Japan SLC, Inc., Shizuoka, Japan) weighing 542.8-806.3 g were used in this study. The animals were provided with food and water ad libitum, and were pair-housed or group-housed on woodchip bedding in stainless steel cages (pair housing) or modified polyphenylene oxide cages (group housing). In each cage, the animals were provided with a dumbbell shaped toy made of polypropylene as an enrichment. The room was HEPA-filtered and environmentally controlled with a temperature range of 23 ± 3°C, a relative humidity of 50% ± 20%, and a 12:12-hr dark/light cycle. Animals were acclimatized for at least 1 week before experiments started. All procedures of animal use were reviewed and approved by the Institutional Animal Care and Use Committee in Chugai Pharmaceutical Co., Ltd., which is an institute accredited by AAALAC international.

Preparation of guinea-pig Langendorff hearts

The experimental method was described previously (Tabo et al., 2010). Briefly, guinea pigs were pre-treated with heparin (1,000 unit/kg, i.p.) and anesthetized by pentobarbital (100 mg/kg, i.p.). With the animals restrained in a supine position, a tracheotom was performed, and the animals were artificially ventilated with room air. Then the heart was quickly removed and its aorta was cannulated for perfusion of the coronary artery according to the Langendorff method with constant pres-
sure (60-70 mmHg). Throughout the experiments, the hearts were perfused at a temperature of 36.3-37.3°C and a flow rate of 15 ± 2 mL/min with Krebs-Henseleit (KH) solution containing (in mM) NaCl (120.0), KCl (4.7), Na-Pyruvate (2.0), CaCl₂ (1.8), MgSO₄ (1.2), KH₂PO₄ (1.2), NaHCO₃ (25.0), and glucose (11.1), which was continuously aerated with a mixture of 95% O₂ and 5% CO₂. The negative electrocardiogram (ECG) electrode was connected to the perfusion cannula, and the positive electrode was softly attached to the apex of the left ventricle. A water-filled latex balloon was placed into the left ventricle cavity to measure left ventricular pressure (LVP). The heart was stabilized for more than 30 min before recording started.

**Experimental protocol and data analysis of Langendorff study**

Following stabilization, lidocaine (20, 60, 180 μM), diltiazem (0.1, 0.3, 1 μM), carbenoxolone (1, 3, 10 μM), or isoproterenol (1, 3, 10, 30 nM) were applied to the isolated heart from low to high concentration. Each concentration of lidocaine, diltiazem, and carbenoxolone was applied for 30 min, at the end of which the data of 10 consecutive waveforms were analyzed. Each concentration of isoproterenol was applied for 15 min. The pharmacological responses of isoproterenol reached maximum strength 2 to 3 min after perfusion of each concentration had started, at which point the data of 10 consecutive waveforms were analyzed. Data of 5 animals were obtained for each test article. Vehicle (0.1% DMSO) was applied for 90 min to obtain time-matched control data. Data of 6 animals were obtained for the vehicle. The ECG and LVP signals were amplified with an ECG amplifier (Nihon Kohden, Tokyo, Japan, AC-601G) and a carrier amplifier (Nihon Kohden, AP-601G), respectively. The rate of change in LVP (LVdP/dt) was measured with a pressure processor (Nihon Kohden, EQ-601G). All the signals were digitized and recorded with a software (Win VAS3 version 1.1, Physio-Tech Co., Ltd., Tokyo, Japan) at a sampling rate of 1 kHz. The software was used to analyze a series of ECG parameters: PR interval, P duration, QRS duration, QT interval, and heart rate (HR) as the reciprocal of RR interval. QT interval corrected by Fidericia’s formula (QTcF) was calculated as follows: QTcF = QT/(RR/1000)³. As a contractility parameter, the maximum rate of change in LVP (LVdP/dtₘₐₓ) and minimum rate of change in LVP (LVdP/dtₘᵦ₂ₜ) were analyzed with the software.

**Drugs**

Lidocaine and carbenoxolone were purchased from Sigma-Aldrich (St. Louis, MO, USA). Diltiazem was purchased from Wako Pure Chemical Industries (Osaka, Japan). Isoproterenol was purchased from Nacalai Tesque Inc. (Kyoto, Japan) or Sigma-Aldrich (St. Louis, MO, USA). The test articles were dissolved in the DMSO to prepare stock solutions that were 1000-fold the final concentrations to be applied. As for the hiPS-CMs experiments, the stock solutions at a volume of 2 μL were applied to each well containing the medium at 2 mL. In the guinea-pig Langendorff experiments, the stock solutions were diluted 1000-fold with the KH solutions immediately before use.

**Statistical analysis**

Data are presented as mean ± S.E. The F-test was used between the test article group and the vehicle control group to test for homogeneity of variance. Then, Student’s t-test and Welch’s t-test were applied when the variance between the two groups were homogenous and heterogeneous, respectively. The statistical analysis was performed using the time-matched vehicle control data for hiPS-CMs data and Langendorff data of lidocaine, diltiazem, and carbenoxolone. As for isoproterenol, the statistical analysis was performed using the vehicle control data, of which the analysis points were 15 min after treatment of each concentration of vehicle for hiPS-CMs data, or 15, 30, 45, and 60 min (to compare the isoproterenol at 1, 3, 10, 30 nM, respectively) after the start of application of vehicle (0.1% DMSO) for Langendorff data. Values of P < 0.05 were considered statistically significant. These statistics were calculated with JMP® (SAS Institute Inc., Cary, NC, USA).

**RESULTS**

**Contraction and conduction profiles of the hiPS-CM sheet and of the guinea-pig isolated heart**

A phase-contrast image of the hiPS-CM sheet obtained by the 4 × objective is shown in Fig. 2A. The motion waveforms obtained from the same microscopic view are represented in Fig. 2C as two-peak profiles that reflect the contraction and relaxation process, which are similar to those obtained using 10 × objective (Hayakawa et al., 2014). Hence a series of parameters derived from the motion waveforms were analyzable. Baseline values of the contractility parameters were 12.2 ± 1.1 μm/sec for MCS and 6.7 ± 1.9 μm/sec for MRS (mean ± S.D., N = 25). The baseline value of the spontaneous BR was 40.9 ± 4.9 bpm, and CRD was 0.66 ± 0.07 sec (mean ± S.D., N = 25). As shown in Figure 2B, a representative isochronal map obtained from the same microscopic view as...
Fig. 2A demonstrates that the excitation wave was propagated in a concentric fashion at a steady speed. The baseline conduction speed of the excitation wave in the hiPS-CM sheet was 0.149 ± 0.038 m/sec (mean ± S.D., N = 25) in this study, which was close to the conduction speed measured using a multi-electrode array system (Izumi-Nakaseko *et al.*, 2017). The vehicle treatment had no effect on the parameters or the conduction speed up to 0.4% DMSO (Fig. 2D).

Regarding the baseline values of the guinea-pig isolat-
ed heart model, the contractility parameters were 1126 ± 247 mmHg/sec for LVdP/dt max, and −1051 ± 214 mmHg/sec for LVdP/dt min. Baseline values of the conduction parameters were 21 ± 2 msec for QRS duration, 30 ± 2 msec for P duration, and 65 ± 5 msec for PR interval. In addition, the HR was 183 ± 15 bpm and the QT interval was 190 ± 13 msec. All these parameters are represented as the mean ± S.D. (N = 26).

**Effect of the reference compounds on the conduction of excitation waves in the hiPS-CM sheet and in guinea-pig isolated heart**

The isochronal maps of the hiPS-CMs sheet before and after treatment with each compound are represented in Figs. 3A-D. The intervals between isochronal lines were shortened by lidocaine (Fig. 3A), diltiazem (Fig. 3B), and carbenoxolone (Fig. 3D), which indicates that the conduction speed of an excitation wave decreased. The intervals were slightly prolonged by isoproterenol (Fig. 3C), demonstrating that the conduction speed increased.

In the guinea-pig isolated heart, lidocaine caused dose-dependent QRS widening, PR prolongation, and a widening of P duration, which represent decreases in the conduction speed in ventricle, AV node, and atrium, respectively (Fig. 4A). These conduction delays in the guinea-pig isolated heart correspond with the decreases by lidocaine in the hiPS-CMs sheet, which were dose-dependent and statistically significant compared to vehicle control. In the guinea-pig isolated heart, diltiazem caused PR prolongation without altering QRS duration or P duration, which signifies a conduction delay in the AV node (Fig. 4B), while in the hiPS-CMs diltiazem dose-dependently decreased the conduction speed, with a statistically significant decrease at the highest concentration (Fig. 4B). In the guinea-pig isolated heart, isoproterenol slightly shortened the QRS duration, PR interval, and P duration, which signifies the increases in the conduction speed in ventricle, AV node, and atrium, respectively (Fig. 4C), and correspondingly in the hiPS-CMs isoproterenol slightly but significantly increased the conduction speed (Fig. 4C). In the guinea-pig isolated heart, carbenoxolone caused QRS widening, PR prolongation, and a widening of P duration, which respectively represent conduction delays in the ventricle, AV node, and atrium (Fig. 4D), and in the hiPS-CMs, carbenoxolone decreased the conduction speed, with a statistically significant decrease at the highest concentration (Fig. 4D). Therefore, the positive and negative modulations in the conduction speed of hiPS-CMs were in accordance with the alterations in the cardiac conduction parameters of the guinea-pig isolated heart model.

**Effect of the reference compounds on the contractility in the hiPS-CM sheet and in guinea-pig isolated heart**

Representative motion waveforms of the hiPS-CM sheet before and after treatment of lidocaine, diltiazem, isoproterenol, and carbenoxolone are shown in
Fig. 4. Effects of lidocaine (A), diltiazem (B), isoproterenol (C), and carbenoxolone (D) on the conduction speed in a hiPS-CMs sheet and on the conduction parameters of ECG (QRS duration, P duration, and PR interval) in the guinea-pig Langendorff heart model. Data represent the mean ± S.E. (N = 5). Statistically significant differences compared to vehicle control are represented with the following symbols: (*) conduction speed in hiPS-CMs or QRS duration in Langendorff heart; (†) P duration in Langendorff heart; (‡) PR interval in Langendorff heart. (p < 0.05).
Figs. 5A-D.

In the guinea-pig isolated heart, lidocaine dose-dependently decreased the LVdP/dt_{max} and LVdP/dt_{min}, which respectively suggest decreases in cardiac contraction and relaxation performance (Fig. 6A), and these changes correspond to the changes in the hiPS-CM sheet, in which lidocaine dose-dependently decreased MCS and MRS. Consistent with the results of lidocaine, diltiazem dose-dependently decreased the LVdP/dt_{max} and LVdP/dt_{min} in the guinea-pig isolated heart (Fig. 6B), and decreased MCS and MRS in the hiPS-CM sheet. Isoproterenol dose-dependently increased the LVdP/dt_{max} and LVdP/dt_{min} in the isolated guinea-pig heart (Fig. 6C), and increased MCS and MRS in the hiPS-CM sheet. Carbenoxolone had no effect on the LVdP/dt_{max} or LVdP/dt_{min} up to the middle concentration and slightly decreased those parameters at the highest concentration in the guinea-pig isolated heart (Fig. 6D), but had no effect on the MCS and MRS in the hiPS-CM sheet. Though statistically significant differences compared to vehicle control were observed in MRS at 3 and 30 μM, these were considered to have physiologically no meaning because the changes were very small and were not dose-dependent. Therefore, positive and negative modulations in the contractility of hiPS-CMs were almost in accordance with those in the guinea-pig isolated heart.

Effect of the reference compounds on the BR and CRDCF in the hiPS-CM sheet, and the HR and QTcF in guinea-pig isolated heart

HR was decreased by lidocaine in the isolated guinea-pig heart, which was consistent with a decrease in the BR of the hiPS-CM sheet (Fig. 7A). The decrease in the BR of the hiPS-CMs by Na⁺ channel blockers was observed in a previous study (Izumi-Nakaseko et al., 2017). Diltiazem decreased the HR in the guinea-pig isolated heart, but increased the BR in the hiPS-CM sheet (Fig. 7B). An increased BR was considered to be a characteristic response to Ca²⁺ channel blockers of the hiPS-CMs used in this study (iCell® cardiomyocytes), as already observed in other studies (Harris et al., 2013; Guo et al., 2011). Isoproterenol increased the HR of the guinea-pig isolated heart and the BR of the hiPS-CM sheet (Fig. 7C). Carbenoxolone did not alter the HR of the guinea-pig isolated heart, whereas statistically significant differences compared to vehicle control in the BR of the hiPS-CMs were observed, though the changes were slight. Therefore, the alterations in the BR of hiPS-CMs were generally in accordance with the alterations in the HR of the guinea-pig isolated heart model. However, a recent study showed that \( I_{K1} \), an inward-rectifier potassium current, is a determinant of spontaneous beating activity of the hiPS-CMs (Li et al., 2017), suggesting that the mechanisms regulating the BR of hiPS-CMs and the HR
Conduction and contraction properties of hiPS-CMs analyzed by motion field imaging

Fig. 6. Effects of lidocaine (A), diltiazem (B), isoproterenol (C), and carbenoxolone (D) on the contraction parameters (MCS and MRS) in the hiPS-CMs sheet, and on the LVdP/dt_{max} and LVdP/dt_{min} in the guinea-pig Langendorff heart model. Data represent the mean ± S.E. (N = 5). Statistically significant differences compared to vehicle control were represented by the following symbols: (*) MCS in hiPS-CMs or LVdP/dt_{max} in Langendorff heart; (†) MRS in hiPS-CMs or LVdP/dt_{min} in Langendorff heart (p < 0.05).
Fig. 7. Effects of lidocaine (A), diltiazem (B), isoproterenol (C), and carbenoxolone (D) on the BR and CRDcF in the hiPS-CMs sheet, and on the HR and QTcF interval in the guinea-pig Langendorff heart model. Data represent the mean ± S.E. (N = 5). Statistically significant differences compared to vehicle control were represented by the following symbols: (*) BR in hiPS-CMs or HR in Langendorff heart; (†) CRDcF in hiPS-CMs or QTcF in Langendorff heart (p < 0.05).
of an isolated heart are different.

CRD is known to be prolonged by several hERG channel blockers and be shortened by a Ca$^{2+}$ channel blocker, verapamil (Takasuna et al., 2017; Hayakawa et al., 2014), representing that CRD is an index of QT interval. In this study, lidocaine dose-dependently decreased CRDcF in the hiPS-CMs. The shortening of CRDcF was in accordance with the previous reports which showed that lidocaine shortened action potential duration of Purkinje fibers, which is a typical effect of class 1b antiarrhythmic drugs (Goineau et al., 2012; Lu et al., 2010). Lidocaine did not alter QTcF at 20 μmol/L and 180 μmol/L. Statistically significant difference in QTcF compared to the time-matched vehicle control was observed at 60 μmol/L, but the change was slight (Fig. 7A). Diltiazem and isoproterenol dose-dependently decreased the CRDcF (Figs. 7B and 7C), which was in accordance with the results of a previous study which showed that CRD was decreased by isoproterenol or verapamil (Hayakawa et al., 2014). Consistently, diltiazem and isoproterenol decreased QTcF interval in the guinea-pig isolated heart. Carbenoxolone did not alter the CRDcF. Statistically significant differences compared to the time-matched vehicle control were observed at 10 and 100 μmol/L, but the changes were slight (Fig. 7D). Consistent with the results of the hiPS-CMs, carbenoxolone did not alter QTcF interval in the guinea-pig isolated heart. Based on the results of this study, the alterations of CRDcF in the hiPS-CMs were generally in accordance with the alterations of QTcF interval in the guinea-pig isolated heart. However, further investigations would be necessary to characterize CRD of the hiPS-CMs because the effect of any compounds that elicit QT prolongation were not tested in this study.

**DISCUSSION**

In this study, we characterized the conduction and contraction properties of hiPS-CMs sheet which were simultaneously measured by MFI. We analyzed 2.8 mm × 2.8 mm area of cardiomyocyte sheet, which allowed us to measure the conduction speed and the contractile motion in the same cells in a stable manner. The pharmacological responses of four typical cardiac functional modulators on the conduction speed and the contractility in the hiPS-CMs corresponded to those in the guinea-pig isolated heart model. These results represent the potential that the hiPS-CMs can be applied to evaluate cardiac conduction and contraction, and can be a useful model to assess integrated cardiac functions.

Positive and negative pharmacological modulations caused by the reference compounds in the conduction speed of the hiPS-CMs were in accordance with the alterations in the cardiac conduction parameters of the guinea-pig isolated heart (QRS duration, P duration, and PR interval), which indicates that hiPS-CMs can be used to evaluate the effects of these drugs on cardiac conduction velocity. Several points of interest about the reference compounds can be drawn from the results in hiPS-CMs in this study. Firstly, the Na$^+$ channel blocker (lidocaine) dose-dependently decreased the conduction speed of hiPS-CMs. Consistent results were observed with other Na$^+$ channel blockers, disopyramide and flecainide (Izumi-Nakaseko et al., 2017). The hiPS-CMs functionally express cardiac Na$^+$ channel, and consist of mainly ventricular-like cells and atrial-like cells, of which the action potential upstroke are mediated by I$_{Na}$. These lines of evidence suggest that the I$_{Na}$ may mediate the conduction of an excitation wave in the hiPS-CMs. Secondly, the Ca$^{2+}$ channel blocker (diltiazem) dose-dependently decreased the conduction speed of hiPS-CMs in this study. It is possible that the decreased conduction speed in the hiPS-CMs sheet by diltiazem could be due to the impaired conduction in the nodal-like cells contained in the hiPS-CMs (Ma et al., 2011). However, a previous study showed that the conduction speed of hiPS-CMs was negatively correlated with the BR (Izumi-Nakaseko et al., 2017), suggesting that the decreased conduction speed by diltiazem was at least partly dependent on the accompanied increase in BR in this study. Therefore, the pharmacological effect of diltiazem on the conduction speed of hiPS-CMs needs to be further investigated under pacing at a stable BR. Thirdly, the conduction speed of hiPS-CMs was dose-dependently decreased by a connexin43 inhibitor (carbenoxolone). Connexin43, the predominant connexin in the heart, is responsible for intercellular conduction of excitation waves in myocardium and is also expressed in hiPS-CMs (Kadota et al., 2013); the result in this study suggests that connexin43 mediates conduction in the hiPS-CMs. Finally, the conduction speed was slightly but significantly increased by a β adrenergic stimulator (isoproterenol) in the hiPS-CMs. Activation of the β adrenergic receptor induces augmentation of the cardiac I$_{Na}$ and I$_{Ca,L}$ and also increases the expression of connexin43 in the heart (Frohnwieser et al., 1997; Kamp and Hell, 2000; Salameh et al., 2009). In fact, the I$_{Ca,L}$ in hiPS-CMs was reported to be enhanced by isoproterenol (Zhang et al., 2013). Hence it can be speculated that isoproterenol increased the conduction speed of the hiPS-CMs by enhancing the functions of I$_{Na}$, I$_{Ca,L}$ and connexin43.

Positive and negative modulations by the reference compounds in the contraction and relaxation kinetics of hiPS-CMs, however, were generally in accordance with the alterations of QTcF interval in the guinea-pig isolated heart. Therefore, further investigations would be necessary to characterize CRD of the hiPS-CMs, and the conduction properties of the hiPS-CMs.
(MCS and MRS) of the hiPS-CMs corresponded to alterations in the respective contractile and relaxation parameters of left ventricular pressure (LVdP/dt_{max} and LVdP/dt_{min}) in the guinea-pig isolated heart, which suggests that hiPS-CMs can be used to evaluate the effects of drugs on the cardiac contractile property. Contractility of the hiPS-CMs was decreased by a Na+ channel blocker in this study. In the heart, the decrease in cardiac contractility by a Na+ channel blocker would probably be caused by inhibiting the Ca2+ entry via Na+-Ca2+ exchanger (NCX). NCX is known to be functionally expressed in the hiPS-CMs (Ma et al., 2011), and thereby would be responsible for the decreased contractility of the hiPS-CMs by a Na+ channel blocker. Contractility in the hiPS-CMs was decreased by a Ca2+ channel blocker and increased by a β adrenergic stimulator in this study. These results are consistent with those shown in a previous study, which demonstrated that the decreased contractility by a Ca2+ channel blocker (verapamil) and the increased contractility by a β adrenergic stimulator (isoproterenol) were respectively accompanied by a decrease and increase in the intracellular concentration of Ca2+ (Hayakawa et al., 2014). Notably, the increase in MRS by isoproterenol was prominent compared to the slight increase in MCS, which was also consistent with a finding in the previous study just mentioned. The relaxation process may reflect the Ca2+ uptake into sarcoplasmic reticulum (SR), which is mediated by SERCA. In hiPS-CMs, the expression level of the gene encoding SERCA is significantly lower than that in adult human cardiomyocytes, while the expression level of phospholamban, a key regulator of SERCA activity, is comparable to that in adult human cardiomyocytes (Rao et al., 2013). These expression profiles could account for the low basal activity of Ca2+ uptake into SR and its strong enhancement by β adrenergic stimulation in hiPS-CMs, which led to the marked increase in MRS. The connexin43 inhibitor did not affect the contractility of the hiPS-CMs, which was equivalent to the results of the isolated heart, though the contractility parameters decreased slightly in the heart model at the highest concentration.

There are limitations in this study. Firstly, consistent with the findings in other studies (Izumi-Nakaseko et al., 2017; Kadota et al., 2013), the conduction speed of the excitation wave in hiPS-CMs was much lower than the ventricular conduction velocity of the actual heart. The hiPS-CMs contain nodal-like cells, in which the action potential upstroke would be mediated by the Ca2+ current. Because conduction mediated through the Ca+ channel is slow, conduction velocity in the hiPS-CM sheet, which consists of different types of cells, may become slower in total compared to the cardiac ventricular conduction mediated by the Na+ channel (Sakakibara et al., 1993). Furthermore, the gap-junction of hiPS-CMs is located in the circumference of the cells, while the gap-junction of adult cardiomyocytes is accumulated at the intercalated disc (Denning et al., 2016), which may also account for the slower conduction of the hiPS-CMs. However, despite being low, the conduction speed in the hiPS-CM sheet was stable under the vehicle treatment in this study, which means it is feasible to evaluate the effects of compounds on the conduction velocity of hiPS-CMs. A second limitation of this study is that, when the pharmacological responses of the hiPS-CMs are compared with those of the guinea-pig isolated heart, the concentration of the test compounds cannot be discussed. A recent study demonstrated that the concentration of an unbound form of compound in the culture medium of hiPS-CMs is relevant to the clinical concentration associated with the proarrhythmic risk (Ando et al., 2017). In this study, although the compounds in the guinea-pig Langendorff heart study were perfused as unbound forms, we did not measure the concentration of the unbound form of the compounds in the medium of hiPS-CMs. Hence we considered it inappropriate to discuss the concentration of the test compounds when comparing the pharmacological responses between the hiPS-CMs and the guinea-pig isolated heart model. Thirdly, there was no experiment that investigated the molecular mechanism of MFI parameters in this study, though the pharmacological responses in the hiPS-CMs were in accordance with those of the isolated whole heart model. Because the conduction and contraction properties analyzed by MFI are the outcome of electrical activity of cardiomyocytes, the internal Ca2+ concentration, the speed of Ca2+ influx/exflux, and the functions of proteins regulating Ca2+, simultaneous measurement of multi-electrode array and/or calcium imaging with MFI would be expected to clarify the underlying mechanism of the conduction and contraction properties of hiPS-CMs.

In conclusion, the conduction of an excitation wave and the subsequent contraction of myocardium are fundamental properties underlying the function of the heart, and thereby conduction disturbance or contractile dysfunction may lead to serious cardiac functional toxicities. Using the MFI system, we could analyze the conduction property and the contraction property of hiPS-CMs simultaneously in the same cells, and the pharmacological responses of hiPS-CMs were in accordance with those of the guinea-pig isolated heart model. Therefore, hiPS-CMs can be applied to evaluate cardiac conduction and contraction, and can be a useful model to assess integrated cardiac functions when their functional properties are

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analyzed using the MFI system.

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Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES


