Characterization of human iPS cell-derived cardiomyocyte sheets as a model to detect drug-induced conduction disturbance

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ABSTRACT — In order to characterize human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) sheets as a model for detecting drug-induced conduction disturbance, we examined their electrophysiological and electropharmacological properties by using the multi-electrode array system with a programmed electrical stimulation protocol. At pre-drug control, the conduction speed, effective refractory period and field potential duration were 0.14 ± 0.01 m/sec, 453 ± 10 msec and 361 ± 9 msec, respectively at a cycle length of 1,000 msec (n = 18). Shortening the pacing cycle length from 1,000 to 600 msec decreased the conduction speed and field potential duration, but prolonged the effective refractory period. Disopyramide, lidocaine and flecaainide decreased the conduction speed but prolonged the effective refractory period and field potential duration, whereas the reverse was true for verapamil. Thus, conduction properties of the cell sheet may largely depend on the extent of Na+ channel availability as is the case in the human ventricle. Importantly, there was no relationship between the conduction delay and 1st spike amplitude reduction after the treatment of Na+ channel blockers. These findings may provide crucial guide on future application of this new technology for early phase safety pharmacological screening of new chemical entities.

Key words: iPS, Cardiomyocytes, Conduction, Effective refractory period, Field potential duration

INTRODUCTION

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are known to express multiple cardiac ion channels, profiles of which can be similar to those in human intact heart (Ma et al., 2011; Navarrete et al., 2013). In order to evaluate the utility of hiPSC-CMs as a tool for detecting drug-induced electrophysiological modifications, the effects of various drugs and chemical compounds have been assessed on the action potential waveform of single hiPSC-CM (Gibson et al., 2014; Peng et al., 2010) as well as the field potential configuration of two-dimensional cell sheets of hiPSC-CMs (Harris et al., 2013; Mehta et al., 2013; Nakamura et al., 2014; Nozaki et al., 2014; Uesugi et al., 2014). Thus, hiPSC-CMs have been well recognized as a new strategy that can provide reliable information for predicting drug-induced repolarization delay in the human heart.

Drug-induced intraventricular conduction delay has been known to cause dys-synchrony of the ventricular contraction, enhancing pump failure, whereas it may develop a substrate for re-entrant excitation circuit, resulting in the onset of ventricular tachyarrhythmias (Miller and Zipes, 2008). At present, the drug-induced intraventricular conduction delay has been largely assessed with the isolated papillary muscle, Langendorff perfused in vitro heart and electrocardiogram in the in vivo animals, of which throughput is not necessarily so high as that needed for early screening of drug candidates. Although hiPSC-CMs sheet has been expected to become an alternative for such classical methods, electropharmacological information particularly on its conduction properties is still limited (Kadota et al., 2013; Lee et al., 2012; Mehta et al., 2013).
Here, we examined the conduction property, field potential duration and effective refractory period of the hiPSC-CMs sheet by using the multi-electrode array system with programmed electrical stimulation protocol in the presence and absence of Na\(^+\) and Ca\(^{2+}\) channel blockers (Task Force of the Working Group on Arrhythmias of the European Society of Cardiology, 1991; Vaughan Williams, 1992). In addition, we assessed the effect of the drugs on 1st spike amplitude of the field potential to analyze whether it can be used for estimating the drug-induced conduction changes. This is the first report that precisely characterized the electrophysiological and electropharmacological profile of the hiPSC-CMs sheet within the range of the human heart rate.

**MATERIALS AND METHODS**

**Culture of hiPSC-CMs**

Cryopreserved hiPSC-CMs (iCell Cardiomyocytes; Cellular Dynamics International (CDI), Madison, WI, USA) were cultured as previously described (Nakamura et al., 2014; Uesugi et al., 2014). The 64-microelectrode array (MED probe; MED-P515A, Alpha MED Scientific, Inc., Osaka, Japan) was placed into a 25-mm-diameter chamber. An area of 1 mm\(^2\) of the surface of the array was coated with 2 μL of fibronectin (50 μg in 1 mL of D-PBS(−) (1×, nacalai tesque, Inc., Kyoto, Japan) before incubating at 37°C for ≥1 hr. The cultured cardiomyocytes in the 6-well tissue-culture plates were dispersed with 0.25% trypsin-EDTA and resuspended in culture medium (Maintenance Media, CDI) at 1.5 x 10\(^4\) cells/μL. A volume of 2 μL of the cell suspension was plated onto the coated area of each MED probe according to the previous study (Nakamura et al., 2014). The cells were incubated overnight at 37°C with 5% CO\(_2\) in humidified conditions to promote cell attachment onto the microelectrode array, and then 1 mL of the culture medium was added into each chamber. The culture medium around the cardomyocyte sheet was also recorded 10 sec prior to the stimulation.

**Electrophysiological analysis of hiPSC-CMs sheets**

Prior to recording, we replaced the culture medium immersing the cardiomycyte sheet on the MED probe. The cell sheet was re-incubated at 37°C in 5% CO\(_2\) for >30 min, and the MED probe connected to the amplifiers (MED-A64HE1S and MED-A64MD1, Alpha MED Scientific, Inc.) using an MED connector (MED-C03, Alpha MED Scientific, Inc.). It was then equilibrated with a gas mixture of 95% O\(_2\) and 5% CO\(_2\), for 30 min at 37°C on a heater (MED-CP02H, Alpha MED Scientific, Inc.) placed inside a plastic box. The cardomyocyte sheet was electrically driven through a pair of neighboring electrodes. The stimulation pulses were biphasic, rectangular in shape, 12-50 μA in amplitude (about three times the threshold current), and of 0.4 msec duration. In this study, we selected a cycle length of 450 to 1,000 msec (60 to 133 beats/min) to mimic normal sinus rhythm in the human heart. The field potential duration and conduction speed were assessed with a train of 15 stimuli at a pacing cycle length of 1,000, 800, 700, 600, 500 and 450 msec with a pause to recover the rate of spontaneous excitation between the trains. The effective refractory period of the cardomyocyte sheet was assessed by the programmed electrical stimulation, which consisted of 6 beats of basal stimuli at a cycle length of 1,000, 800, 700, 600, 500 and 450 msec that was followed by an extra stimulus of various coupling intervals with a pause of 30 sec between each sequence. The coupling interval was shortened in 5-msec decrements until refractoriness occurred. In addition, the spontaneous excitation and its propagation across the cardomyocyte sheet were also recorded 10 sec prior to the stimulation.

**Pharmacological analysis of hiPSC-CMs sheets**

The conduction speed, field potential duration, effective refractory period and 1st spike amplitude were assessed before and after the treatment of Na\(^+\) channel blockers: disopyramide (class Ia), lidocaine (class Ib) and flecainide (class Ic); and a Ca\(^{2+}\) channel blocker: verapamil (class IV) (Task Force of the Working Group on Arrhythmias of the European Society of Cardiology, 1991; Vaughan Williams, 1992). The amplitude of 1st spike was obtained as the sum of the positive and negative sharp deflections. The drugs were cumulatively administered, and the drug effects on each parameter were examined 30 min after each treatment. Each cardomyocyte sheet received only one drug out of 4. Each field potential at 64 microelectrodes in the cell sheet were acquired. A high-pass filter of 1 Hz was used for assessing the effects of disopyramide, lidocaine and flecainide to stabilize the baseline, and 0.1 Hz was selected for analyzing that of verapamil to better examine the morphological changes in the plateau phase, whereas a low-pass filter of 5 kHz was applied for each drug. Field potentials were digitized at a sampling rate of 20 kHz using MED64-Basic system (Alpha MED Scientific, Inc.).
Conduction property of hiPSC-CMs sheets

Drugs

The following drugs were purchased: disopyramide phosphate salt (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan), lidocaine (Xylocaine® Injection 2%, AstraZeneca K.K., Osaka, Japan), flecainide acetate (Eisai Co., Ltd., Tokyo, Japan) and verapamil hydrochloride (Vasolan® for intravenous injection 5 mg, Eisai Co., Ltd.). Disopyramide and flecainide were dissolved in distilled water at a concentration of 100 mmol/L, divided into aliquots, and frozen at −20°C. Drug solutions were diluted in distilled water on the day of experiment, and added to the cultured medium in the ratio of 1:100 to prepare the desired final concentrations. Gelatin and fibronectin were obtained from BD Biosciences (Franklin Lakes, NJ, USA). Trypsin-EDTA was purchased from Gibco®, Life Technologies Japan (Tokyo, Japan).

Data analyses

Data were analyzed with Mobius software (Alpha MED Scientific, Inc.) and presented as the mean ± S.E. The conduction speed was obtained by the following calculation: conduction speed (m/sec) = [distance between the stimulus electrode and a recording electrode (m)] / [elapsed time from an artifact of the stimulus to a negative field potential of a recording electrode (sec)]. Each conduction speed was normalized to that for the pre-drug control at a cycle length of 1,000 msec. The normalized conduction speed was obtained by dividing the distance between the electrodes by the duration at pre-drug control, respectively, indicating small variability among the cell sheets.

RESULTS

We prepared 27 hiPSC-CMs sheets for this experiment. We selected 18 hiPSC-CMs sheets out of 27 based on their ability to be paced at control and after drug treatment; namely, 27 (100%), 26 (96%) and 20 (74%) out of the 27 cell sheets were able to be electrically paced at cycle lengths of 1,000-800, 700 and 600 msec, respectively at control. We initially used 20 cell sheets which could be paced at cycle lengths of ≤ 600 msec, but 2 of them were withdrawn because of pacing failure after the administration of higher drug concentrations.

Electrophysiological analysis of hiPSC-CMs sheets

The pre-drug cycle length of spontaneous automaticity was 1,350 ± 43 msec (n = 18) and the pre-drug conduction speed at a pacing cycle length of 1,000 msec was 0.14 ± 0.01 m/sec (n = 18). Representative activation maps are shown in Fig. 1A (upper). The normalized conduction was significantly slowed by shortening the pacing cycle length from 1,000 to 800, 700 and 600 msec (Fig. 2A). The pre-drug effective refractory period at a cycle length of 1,000 msec was 453 ± 10 msec (n = 18) (Fig. 2B). The effective refractory period was prolonged by shortening the pacing cycle length, and a significant difference was observed at cycle lengths between 1,000 and 600 msec. The pre-drug field potential duration at a cycle length of 1,000 msec was 361 ± 9 msec (n = 18) (Fig. 2C). The field potential duration was significantly shortened by decreasing the pacing cycle length from 1,000 to 800, 700 and 600 msec.

The cell sheets were randomized into 4 groups; namely, disopyramide group (n = 5), lidocaine group (n = 4), flecainide group (n = 5) and verapamil group (n = 4). There was no significant difference among the 4 groups in any of the pre-drug control values of conduction speed, effective refractory period or field potential duration at cycle lengths of 1,000, 800, 700 and 600 msec. In addition, coefficient of variance was 8~16, 0~12, 2~15 and 2~20 in the cycle length of spontaneous automaticity, conduction speed, effective refractory period and field potential duration at pre-drug control, respectively, indicating small variability among the cell sheets.

Electropharmacological analysis of hiPSC-CMs sheets

Spontaneous automaticity

Effects of Na+ and Ca2+ channel blockers on the cycle length of the spontaneous automaticity of the cell sheets are summarized in Table 1. Disopyramide tended to prolong the cycle length, which did not achieve statistical significance. Lidocaine and flecainide prolonged it in a concentration-related manner, and significant changes were detected at 3 and 0.1-1 μmol/L, respectively. In contrast, verapamil shortened it in a concentration-related manner, and significant changes were detected at 0.03-1 μmol/L.

Conduction

Representative activation maps before (Control) and after the drug treatment are depicted in Fig. 1A. Disopyramide and lidocaine slowed the conduction in a concentration-related manner, and a significant change was detected at 3 μmol/L for both drugs (Fig. 3 top). Flecainide slightly accelerated the conduction at 0.03-0.1 μmol/L,
Fig. 1. Representative activation maps and field potential waveforms during electrical stimulation. A: Representative activation maps of human induced pluripotent stem cell-derived cardiomyocytes sheets are shown at pre-drug control (Control, upper) and after the treatment of the drugs (Drug-treated, lower): namely, 3 of disopyramide and lidocaine, 1 of flecainide and 0.3 μmol/L of verapamil. Asterisks on each map show the region where field electrical stimulation was applied through a pair of electrodes. The numbers on isochrones indicate the elapsed time (msec) from an artifact of the 15th electrical stimulus to a negative deflection of 1st spike of the field potential of each recording electrode at a basic cycle length of 800 msec for disopyramide, lidocaine and flecainide and 600 msec for verapamil. It took 8-12 msec to conduct across the cardiomyocyte sheet in an area of 1.05 × 1.05 mm. B: Representative waveforms of field potential of the last excitation during a train of 15 stimuli at a cycle length of 800 msec for disopyramide, lidocaine and flecainide and 600 msec for verapamil are shown before and after the treatment of drugs.
but slowed it at 1 μmol/L. Verapamil enhanced the conduction in a concentration-related manner, and significant changes were detected at 0.03-0.3 μmol/L. We could not pace the cell sheets at a cycle length of 600 msec after 1-3 μmol/L of disopyramide, at cycle lengths of 600-700 msec after 1 μmol/L of flecainide, and at cycle lengths of 600-1,000 msec after 1 μmol/L of verapamil.

**Effective refractory period**

Disopyramide, lidocaine and flecainide prolonged the effective refractory periods in a concentration-related manner, and significant changes were detected at 0.1-3 of disopyramide, 1-3 of lidocaine and 0.03-1 μmol/L of flecainide (Fig. 3 middle upper). Meanwhile, verapamil shortened the effective refractory period in a concentration-related manner, and significant changes were detected at 0.03-0.3 μmol/L.

**Field potential duration**

Representative traces of field potential changes before and after the drug treatment were overlaid as shown in Fig. 1B. Disopyramide, lidocaine and flecainide prolonged the field potential duration in a concentration-related manner, and significant changes were detected at 1-3 of disopyramide, 0.1-3 of lidocaine and 0.1-1 μmol/L of flecainide (Fig. 3 middle lower). Meanwhile, verapamil shortened the field potential duration in a concentration-related manner, and significant changes were detected at 0.03-0.3 μmol/L.

**First spike amplitude**

Disopyramide increased the normalized 1st spike amplitude at 0.1-1 μmol/L at each pacing cycle length, but decreased it at 3 μmol/L at a pacing cycle length of 700 msec (Fig. 3 bottom). Lidocaine increased it at 0.1-3 μmol/L. Flecainide increased it at 0.1 μmol/L at a pacing cycle length of 1,000 msec, but decreased it at 600 msec. Similarly, flecainide increased it at 1 μmol/L at a pacing cycle length of 1,000 msec, but decreased it at 800 msec. Verapamil increased it at 0.03-0.1 μmol/L at pacing cycle lengths of 700-600 msec.

**DISCUSSION**

By using the programmed electrical stimulation protocol, in this study we precisely analyzed the basal electrophysiological properties of the hiPSC-CMs sheet as well as the electropharmacological effects of Na+ and Ca2+ channel blockers on them within the physiological human heart rate for the first time. We found the cell sheet exerted essentially the same responses during electrophysiological and pharmacological interventions as those expected in the human heart; although there were some differences between them as discussed below.
Table 1. Effects of Na$^+$ and Ca$^{2+}$ channel blockers on the spontaneous automaticity in the human induced pluripotent stem cell-derived cardiomyocytes sheets.

<table>
<thead>
<tr>
<th>μM</th>
<th>Cycle length of spontaneous automaticity (msec)</th>
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<tbody>
<tr>
<td></td>
<td>Disopyramide (n = 5)</td>
</tr>
<tr>
<td>0</td>
<td>1,379 ± 112</td>
</tr>
<tr>
<td>0.03</td>
<td>--</td>
</tr>
<tr>
<td>0.1</td>
<td>1,407 ± 121</td>
</tr>
<tr>
<td>0.3</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>1,401 ± 136</td>
</tr>
<tr>
<td>3</td>
<td>1,448 ± 128</td>
</tr>
</tbody>
</table>

Data are represented as the mean ± S.E. *p value < 0.05, compared with the respective pre-drug control value (= 0 μM) within a group.

Fig. 3. The effects of disopyramide, lidocaine, flecainide and verapamil on the normalized conduction speed, effective refractory period (ERP), field potential duration (FPD) and normalized 1st spike amplitude. Conduction speed and 1st spike amplitude were normalized by the pre-drug controls at a pacing cycle length (CL) of 1,000 msec. Closed symbols indicate that the value is significantly different from the pre-drug control at the same pacing cycle length. Data are presented as the mean ± S.E.
**The field potential duration of hiPSC-CMs sheets**

The field potential duration of the cell sheet was 361 ± 9 msec at the pacing cycle length of 1,000 msec (n = 18) (Fig. 2C). The shortening of pacing cycle length from 1,000 to 600 msec abbreviated the field potential duration, which was similar to that reported in the *in situ* intact humans heart (Josephson, 1993). Ma et al. (2011) analyzed the membrane potential of single iCell Cardiomyocytes (CDI) by using perforated patch-clamp techniques to classify the cells according to their action potential waveforms, in which the average action potential duration at 90% repolarization was 255 msec in nodal-type, 286 msec in atrial-type and 415 msec in ventricular-type. Asakura et al. (2015) simultaneously recorded the field potential and membrane potential of hiPSC-CMs, and found that end of the field potential duration corresponded to the action potential duration at 50% repolarization level. Since the field potential duration observed in this study was close to the action potential duration of the ventricular-type single iCell Cardiomyocyte (Ma et al., 2011), a large population of cells in our sheets can be considered to be ventricular-type.

The action potential duration of ventricular muscle cells in humans was reported to be 200-300 msec (Fozzard and Arnsdorf, 1992), which would be much shorter than that estimated from our study. It was reported that expression levels of genes of KCNH2, KCNE2 and KCNQ1 were higher in hiPSC-CMs than those in the human adult heart, whereas that of KCNE1 was similar between them (Uesugi et al., 2014). In addition, the extent of expression of a gene SLC8A1 encoding Na+/Ca2+ exchanger 1 (NCX1) has been reported to be similar between adult heart tissue and structured hiPSC-CMs (Rao et al., 2013). Meanwhile, inward rectifier K+ current (I_K1) was shown to be hardly expressed in the cell sheet unlike the human heart. Inward rectifier K+ channels have been reported to explain the difference in the extent of prolongation of field potential duration. It should be noted that the result for lidocaine was directionally different from that in the *in situ* human heart, in which the QT interval was shortened via late Na+ current inhibition (Johannesen et al., 2016). Thus, the constitutively active late Na+ current may be hardly expressed in the cell sheet unlike the human heart.

Contrary to our expectation based on the previous knowledge; namely, the 1st spike amplitude of field potentials reflects the Na+ influx during the depolarization phase of the ventricular action potential (Fozzard and Arnsdorf, 1992), there was no relationship between the conduction delay and 1st spike amplitude reduction after the treatment of Na+ channel blockers (Fig. 3 bottom).

**Effects of verapamil on the electrophysiological variables**

Verapamil at 0.03 μmol/L shortened the field potential duration by 23 msec in the cell sheet, at a cycle length of 1,000 msec (Fig. 3 middle lower). This result is quite different from a previous report in humans by Johannesen et al. (Johannesen et al., 2014), describing that verapamil hardly altered the corrected QT interval by Fridericia’s formula at a peak plasma concentration of 130 ± 76 ng/mL (0.270 μmol/L). The IC_{50} values of verapamil for Na+, L-type Ca2+ and *hERG* K+ channels have been reported to be 4.272, 0.333 and 0.201 μmol/L (Okada et al., 2015), respectively. Moreover, a selective L-type Ca2+ channel blocker nifedipine and a specific *hERG* K+ channel blocker E-4031 have been demonstrated to shorten and prolong the field potential duration, respectively in hiPSC-CMs sheets (Harris et al., 2013). In addition, verapamil has been reported to block cardiac L-type Ca2+ chan-
nels in a frequency- and use-dependent manner (Nawrath and Wegener, 1997), whereas drug-induced inhibition of the rapidly activating delayed-rectifier K+ current (I_{Kr}) is known to be enhanced in a reverse use-dependent manner (Yang and Roden, 1996). Since verapamil increased the automaticity rate of the cell sheet, the effects of verapamil on the field potential duration were assessed at relatively faster beating rate. It might have enhanced inhibition of L-type Ca\(^{2+}\) channels, but reduced the extent of I_{Kr} inhibition, explaining the verapamil-mediated abbreviation of field potential duration.

Verapamil shortened the effective refractory period and accelerated conduction (Fig. 3 top and middle upper), latter of which has been reported in the accessory pathway in patients with Wolff-Parkinson-White syndrome (Harper et al., 1982). The shortening of the field potential duration by verapamil (Fig. 3 middle lower) may not only abbreviate the effective refractory period but also enhance the recovery period of Na\(^{+}\) channels, leading to the acceleration of conduction, which could be reflected in the increase of the 1st spike amplitude (Fig. 3 bottom). Moreover, these results suggest that the conduction may not depend on L-type Ca\(^{2+}\) channel availability in the cell sheet.

**Relationship between Na\(^{+}\) channel availability and conduction**

At the pacing cycle length of 1,000 msec (n = 18), the conduction speed of excitations in the cell sheets (0.14 ± 0.01 m/sec) was approximately half of that in the intact human ventricle (0.2-0.3 m/sec) (Levy and Pappano, 2007). Also, the effective refractory period of the cell sheet (453 ± 10 msec) was longer than that in the human right ventricle (170-290 msec) (Josephson, 1993). Conduction speed of two-dimensional cell sheet consisting of hiPSC-CMs has been reported to be 0.041-0.056 m/sec (Kadota et al., 2013), 0.22 ± 0.02 m/sec (Lee et al., 2012) and ~0.15 m/sec (Nunes et al., 2013), indicating that conduction speed in our cell sheets can be comparable to others’. Shortening of the pacing cycle length from 1,000 down to 600 msec prolonged the effective refractory period but slowed the conduction in the cell sheet at the pre-drug control (Figs. 2A and 2B), which were opposite to the previous observation in the intact human heart (Josephson, 1993; Ward et al., 1979). V_{1/2} for steady-state inactivation of cardiac Na\(^{+}\) channel (Nav1.5) has been reported to be −84 mV (Wang et al., 2015). Maximum diastolic potential in single hiPSC-CM ranged from −75 to −60 mV (Ma et al., 2011), whereas that in the human ventricle was −87 mV (Trautwein et al., 1962), suggesting that steady-state inactivation of Na\(^{+}\) channels may be enhanced in the cell sheet. In addition, since a long depolarization pulse could delay the recovery of Nav1.5 channels from inactivation (Ramos and O’Leary, 2004), the long action potential duration in the cell sheet might have slowed the recovery of Na\(^{+}\) channels; thus, facilitating Na\(^{+}\) channel inactivation, which may explain the frequency-dependent conduction delay and effective refractory period prolongation in this study. This hypothesis can be indirectly supported by the results of verapamil, in which conduction was accelerated concomitantly with the shortening of field potential duration and effective refractory period.

**Automaticity of the cell sheet**

Verapamil enhanced the automaticity of the cell sheet by 120%, whereas flecainide and lidocaine suppressed it by 12 and 9%, respectively; and disopyramide tended to suppress it by 5%, extent of which was in parallel with the IC_{50} value of I_{Na} for open state; namely, 0.63 ± 0.06 for flecainide, 35.3 ± 2.7 for lidocaine and 52.5 ± 2.8 μmol/L for disopyramide (Wang et al., 2013). Thus, upstroke of action potentials in the cell sheet during spontaneous automaticity is considered to be mediated by Na\(^{+}\) channel current rather than L-type Ca\(^{2+}\) channel current like in the idiioventricular rhythm in Purkinje fibers (Abete et al., 1991; Sugiyama et al., 1994), although iCell Cardiomyocytes (CDI) have been reported to contain a small number of nodal-type cells (Ma et al., 2011).

**Limitation**

Calcium imaging, membrane potential recording with voltage sensitive dye and field potential recording have respective advantage and disadvantage in testing conduction speed. Calcium imaging can show the intracellular Ca\(^{2+}\) dynamics, which may be directly linked to contraction of the cells; however, the contractile movement of cell sheet is usually restricted to obtain better images, and the breaching of fluorescence dye limits the observation period. Membrane potential recording with voltage sensitive dye can record the action potential waveform changes, and map the macroscopic conduction wavefront, but the breaching of fluorescence dye limits the observation period like calcium imaging. The field potential recording can obtain indirect information on electrical excitation of cells, but it may monitor the conduction properties continuously for minutes to days without any modification.

It should be also noted that some differences in drug responses between the cell sheets and the human ventricle were observed. For example, lidocaine prolonged the field potential duration; and verapamil shortened the field potential duration and effective refractory period and
enhanced the conduction in the cell sheets. Thus, caution should be taken in the evaluation of multi-channel blockers with the cell sheet.

In conclusion, the cell sheets exerted essentially the same responses to those expected in the human ventricle during electrophysiological and electropharmacological interventions; namely, conduction as well as spontaneous automaticity of the cell sheet may largely depend on the extent of Na⁺ channel availability. Importantly, there was no relationship between the conduction delay and 1st spike amplitude reduction after the treatment of Na⁺ channel blockers. These findings may provide crucial guidance on future application of this new technology for early phase safety pharmacological screening of new chemical entities.

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

REFERENCES


