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Full paper

Application of human induced pluripotent stem cell-derived cardiomyocytes sheets with microelectrode array system to estimate antiarrhythmic properties of multi-ion channel blockers

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ABSTRACT

We examined electrophysiological indices of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) sheets in order to quantitatively estimate Na^+, K^+ and Ca^{2+} channel blocking actions of bepridil and amiodarone using microelectrode array system in comparison with that of E-4031. We analyzed the field potential duration, effective refractory period, current threshold and conduction property using a programmed electrical stimulation protocol to obtain the post repolarization refractoriness and coefficient *a* of the relationship between the pacing cycle length and field potential duration. Electropharmacological profile of each drug was successfully characterized; namely, 1) the changes in the current threshold and conduction property provided basic information of Na^+ channel blocking kinetics, 2) the relationship between pacing cycle length and field potential duration reflected drug-induced inhibition of human ether-à-go-go-related gene (hERG) K^+ channel, 3) the post repolarization refractoriness indicated the relative contribution of these drugs to Na^+ and K^+ channel blockade, and 4) L-type Ca^{2+} channel blocking action was more obvious in the field potential waveform of the hiPSC-CMs sheets than that expected in the electrocardiogram in humans. Thus, this information may help to better utilize the hiPSC-CMs sheets for grasping the properties and net effects of drug-induced Na^+, Ca^{2+} and K^+ channel blockade.

1. Introduction

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are expected as a useful and reliable platform to assess the arrhythmogenicity of drug candidates, especially the risk of torsade de pointes following the prolongation of QT interval/action potential duration. The hiPSC-CMs express multiple cardiac ion channels, which are expressed in human intact heart.[1,2] Meanwhile, their action potential waveform and resting potential, which are determined by the expression levels of each ion channel, have been shown to be different from those in human/mammal intact cardiomyocytes.[2–4] In the hiPSC-CMs sheet, human ether-à-go-go-related gene (hERG) K^+ channel blockade prolongs the field potential duration and induces early afterdepolarization, which are expected as a sensitive surrogate marker of drug-induced torsade de pointes.[1,2,5,6] Inhibition of the other K^+ currents like I_{Ks} and I_{Kur} also prolongs the repolarization period in the hiPSC-CMs sheet,[7,8] reflecting their physiological contribution. Moreover, Ca^{2+} and Na^+ channels have been shown to play important roles in determining the field potential waveform and conduction property in the hiPSC-CMs sheet.[2,9] However, information is still limited for the currently utilized hiPSC-CMs sheets how to quantitatively estimate

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the antiarrhythmic properties and proarrhythmic potentials of multi-ion channel blockers except for detecting the onset of early afterdepolarization.

We hypothesized that a combination of the hiPSC-CMs sheets and microelectrode array system can be used to characterize antiarrhythmic properties of drugs having multiple Na^+, K^+ and Ca^{2+} channel blocking actions. For this purpose, we adopted multi-ion channel blockers bepridil and amiodarone along with a specific hERG K^+ channel blocker E-4031, and analyzed their effects on the field potential duration, effective refractory period, current threshold and conduction speed by using the programmed electrical stimulation protocol to evaluate both the post repolarization refractoriness and the relationship between the pacing cycle length and field potential duration.

We have already established an assay protocol of the hiPSC-CMs sheet by multi-site validation study with microelectrode array systems, which made it possible to assess the drug-induced changes of field potential duration followed by the onset of early afterdepolarization with high sensitivity and reliability.[5,6] Moreover, we have demonstrated how the blockade of I_{Kr}, I_{Ks}, I_{Kur}, I_{Na} and/or $I_{Ca,L}$ can impact the field potential waveform, effective refractory period or conduction property in the hiPSC-CMs sheets.[7–9] Importantly, we have analyzed the relationship between the pacing cycle length and field potential duration, showing that rate-dependent change in the repolarization period was significantly smaller in the hiPSC-CMs sheets than those reported in the human hearts due to their low gene expression levels of hKCNJ2 and hKCNE1.[8]

We propose that current strategy described in this paper may help to better utilize the hiPSC-CMs sheet for assessing the antiarrhythmic potential of drug candidates possessing multichannel blocking property with microelectrode array system.

2. Materials and methods

2.1. Culture of hiPSC-CMs sheets

Cryopreserved hiPSC-CMs (iCell® Cardiomyocytes; Cellular Dynamics International (CDI), Madison, WI, USA) were cultured as previously described.[5,9] Briefly, the cryopreserved cardiomyocytes were thawed, incubated for >2 days in the 6-well tissue-culture plates for recovery, dispersed with 0.25% trypsin–EDTA and resuspended in culture medium (Maintenance Media, CDI) at 1.5×10^4 cells/μL. A volume of 2 μL of the cell suspension was plated onto 64-microelectrode arrays (MED probe; MED-P515A, Alpha MED Scientific Inc., Osaka) after having coated with fibronectin. The culture medium around the probe was fully replaced with fresh one once a week. The cardiomyocytes were cultured for 3–5 days to form a cell sheet with spontaneous and synchronous electrical automaticity, which were used for experiments within 3 weeks.

2.2. Electrical stimulations, conductions and field potentials

The hiPSC-CMs sheet was incubated at 37 °C with gas mixture of 95% O_2 and 5% CO_2. Then, the MED probe was connected to the amplifiers (MED-A64HE1S and MED-A64MD1, Alpha MED Scientific Inc.) with a MED connector (MED-C03, Alpha Med Scientific Inc.). The hiPSC-CMs 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 ms duration, which were applied in cycle lengths of 600–1600 ms.

The rate-adapted field potential duration and conduction speed were assessed with a train of 15 stimuli at a cycle length of 600–1600 ms before and after the drug treatment. Field potentials of the hiPSC-CMs sheet at 62 microelectrodes were acquired with high- and low-pass filters of 0.1 and 5 kHz, respectively. Field potentials were digitized at a sampling rate of 20 kHz with a MED64-Basic system (Alpha MED Scientific Inc.). The effective refractory period of the cardiomyocyte sheet was assessed by the programmed electrical stimulation, consisting of 6 beats of basal stimuli at a cycle length of 600–1600 ms that was followed by an extra stimulus of various coupling intervals with a pause of 30 s between each sequence.

2.3. Drugs

We selected a specific hERG K^+ channel blocker E-4031,[10,11] and two multichannel blockers, bepridil and amiodarone. Bepridil is generally classified as a Vaughan Williams class IV agent,[12] which can block various K^+ channels including hERG K^+ channels in addition to Na^+ and Ca^{2+} channels.[13–16] Amiodarone is a Vaughan Williams class III agent,[12] which can inhibit multiple K^+ channels including I_{Kr}, I_{Ks}, I_{to}, I_{K1}, $I_{K,ACh}$ and $I_{K,Na}$ as well as Na^+ channels, Ca^{2+} channels and β-adrenoceptors,[12,17–23] while the agent has been known to hardly induce torsade de pointes.[24] The concentration of E-4031 (1–10 nM) was determined based on our previous studies[5,11] and the IC_{50} values for hERG K^+ channel of 8–570 nM (median 16.5 nM, n = 14).[25] Those of bepridil (0.1–1 μM) and amiodarone (1.5 μM) were chosen to achieve their effective therapeutic plasma concentrations, which were reported to be 0.55–1.6 μM and 1.5 μM, respectively at the steady-state.[23,26]

The following drugs were purchased: E-4031 (Sigma–Aldrich Japan K.K., Tokyo), bepridil hydrochloride (Sigma–Aldrich Japan K.K.), amiodarone hydrochloride (Sigma–Aldrich Japan K.K.), polyethylene glycol 400 (Wako Pure Chemical Industries, Ltd., Osaka) and ethyl alcohol (99.5%) (Wako Pure Chemical Industries, Ltd.). E-4031 and bepridil were dissolved in distilled water at a concentration of 1 mM, divided into aliquots, and frozen at −20 °C. Amiodarone was dissolved in the mixture of polyethylene glycol 400 and ethyl alcohol (99.5%) by 1:1 at a concentration of 1.5 mM, which was filtered and stocked at 4 °C. Drug solutions were diluted in distilled water on the day of experiment, which were added to the cultured medium in the ratio of 1:100 to prepare the desired final concentrations. Data acquisition was performed at 30 min after application of drugs. Gelatin and fibronectin were obtained from BD Biosciences (Franklin Lakes, NJ, USA). Trypsin–EDTA was purchased from Gibco®, Life Technologies Japan (Tokyo).

2.4. Data analyses

Field potential duration and conduction speed were analyzed with Mobius software (Alpha MED Scientific Inc.) as previously described.[9] Post repolarization refractoriness was calculated by the formula; [post repolarization refractoriness (ms)] = [effective refractory period (ms)] − [field potential duration (ms)].[27] A coefficient *a* in each cell sheet was calculated by fitting the relationship between field potential duration (FPD, ms) and cycle length (CL, ms) of electrical pacing into non-linear equations based on the standard correction formulae of QT interval[8];

$$\mathrm{FPD}_{CL=1000\ ms} = \frac{FPD}{\left(\frac{CL}{1000}\right)^a}$$

Statistical analysis was performed with the software GraphPad Prism 6 (ver 6.03, GraphPad Software, Inc., La Jolla, CA, USA). Statistical significances within a parameter were assessed with one-way repeated measures analysis of variance (ANOVA) followed by

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