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Development of correction formula for field potential duration of human induced pluripotent stem cell-derived cardiomyocytes sheets

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ABSTRACT

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been used in many studies to assess proarrhythmic risks of chemical compounds. In those studies, field potential durations (FPD) of hiPSC-CMs have been corrected by clinically used Fridericia's and/or Bazett's formulae, however, the rationale for the use of these formulae has not been well established. In the present study, we developed a correction formula for experiments using hiPSC-CMs. First, we analyzed the effect of beating rate on FPD in the hiPSC-CMs sheets with electrical stimuli and a HCN channel inhibitor zatebradine. Next, we examined the relationship between the electrophysiological properties and the expression levels of ion channel genes in the cell sheets. Zatebradine slowed the beating rate and allowed to analyze FPD changes at various pacing cycle lengths. Rate-dependent change in the repolarization period was smaller in the cell sheets than that reported on the human hearts, which can be partly explained by lower gene expression level of hKCNJ2 and hKCNE1. Thus, non-linear equation for correcting FPD in the cell sheet; $FPDc = FPD/RR^{0.22}$ with RR given in second was obtained, which may make it feasible to assess net repolarization delay by various chemical compounds with a chronotropic action.

1. Introduction

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been used in numerous studies to assess the potential risks of chemical compounds for inducing fatal arrhythmias like torsade de pointes.[1–4] In order to precisely analyze the electrophysiological and safety pharmacological profiles of various chemical compounds, the extracellular field potential recording on a platform of microelectrode array technology has been adopted as the alternative methodology to action potential recording and/or QT-interval measurement.

Although the field potential duration (FPD) in those studies has been commonly corrected with Fridericia's and/or Bazett's formulae,[1–4] the rationale for using these formulae has not been well established. Recently, Yamamoto W, et al[3] studied the FPD-interspike interval relationship using 96 samples of hiPSC-CM sheets under spontaneous automaticity rate, and found that the obtained regression coefficient was close to that in the Framingham Heart Study.[5] Since they clarified the FPD-interspike interval relationship only for the sample population,[3] there may be limitation to apply their formula to correct the FPD changes within a hiPSC-CM sheet.

In the present study, we developed a correction formula that can overcome the current limitation related to the use of conventional correction formulae, and could be flexibly applied for experiments with hiPSC-CMs sheets. First, we analyzed the effect of beating rate on FPD in cell sheets derived from three lots of iCell® cardiomyocytes by using both the programmed electrical stimulation and a HCN channel inhibitor zatebradine. Next, we examined the relationship between the electrophysiological properties of the hiPSC-CMs and the gene expression levels. Finally, we proposed a

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new non-linear equation for correcting FPD for the cell sheets, which may make it feasible to assess the electropharmacological profile of various compounds possessing chronotropic action.

2. Materials and methods

2.1. Culture of hiPSC-CMs

Cryopreserved hiPSC-CMs (iCell® Cardiomyocytes; Cellular Dynamics International (CDI), Madison, WI, USA) were cultured as previously described.[1,6,7] Three lots of the cells (#1093227, #1096654 and #1094831) were used. A volume of 2 μL of the cell suspension at 1.5×10^4 cells/μL was plated onto 64-microelectrode arrays (MED probe; MED-P515A, Alpha MED Scientific Inc., Osaka) after coating 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, and were used within 3 weeks after re-plating.

2.2. Electrical stimulations, conductions and field potentials

The cell 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 an MED connector (MED-C03, Alpha Med Scientific Inc.). The cardiomyocyte 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. In this study, we selected cycle lengths of 600–2000 ms to mimic the range of normal sinus rhythm in humans. Meanwhile, the inter-spike interval of the cardiomyocyte sheet during spontaneous excitation was recorded 10 s prior to the stimulation.

The conduction speed and FPD were assessed before and after the loading of an I_f inhibitor, zatebradine hydrochloride (Boehringer Ingelheim Japan, Inc., Tokyo). The concentration of the drug was determined for each lot separately that could make the inter-spike interval >2 s during the spontaneous excitation. The rate-adapted FPD and conduction speed were assessed with a train of 15 stimuli at each cycle length of 1,000, 800, 700 and 600 ms before the drug treatment, whereas those were assessed at each cycle length of 2,000, 1,500, 1000 and 800 ms after the treatment. Field potentials of the cell 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.).

2.3. Quantitative PCR assays

Total RNA was isolated from iCell® Cardiomyocytes using TRIzol reagent (Life Technologies Life Technologies, Carlsbad, CA, USA). Quantitative real-time reverse transcription (RT)-PCR of hKCNH2, hKCNQ1, hKCNE1, hKCNJ2, hSCN5A, hGJA1 and hGAPDH was performed with a QuantiTect SYBR Green RT-PCR Kit (Qiagen, Valencia, CA, USA) on an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA, USA), as previously reported.[8] Relative changes in transcript levels were normalized to mRNA levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primer sequences used for real-time PCR analysis are shown in Table 1.

Table 1
List of primer sequences for quantitative PCR assays.

gene	forward	reverse
hKCNH2	TCAACTGCGAGATACCAACATG	CTGGCTGCTCCGTGTCCTT
hKCNQ1	CGCCTGAACCGAGTAGAAGA	TGAAGCATGTCGGTGATGATGAG
hKCNE1	GGAGGAAGGCATTATCTGTATCC	TCCTGGGCATTAAGGTTCCA
hKCNJ2	GTGTCCGAGGTCAACAGCTT	GGTTGTCTGGGTCTCAATGG
hSCN5A	CATGGAACTGGCTGGACTTTA	GAAGGTGCGTAAGGCTGAGA
hGJA1	TCAATCACTTGGCGTGACTTCA	GCGCTCCAGTCAACCCATGT
hGAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

2.4. Measurement of membrane potential

Each cell sheet was formed on a cover glass in the same manner as described above. Tyrode solution in the bath contained (mM); NaCl, 137; KCl, 5.4; $CaCl_2$, 2.0; $MgCl_2$, 1.0; $NaHCO_3$, 11.9; NaH_2PO_4, 0.4 and glucose, 5.6, which was equilibrated with gas mixture of 95% O_2 and 5% CO_2 at 37 °C in a water bath. Pipette solution contained (mM); L-glutamic acid potassium salt monohydrate, 120; tetramethylammonium chloride, 20; adenosine 5′-triphosphate magnesium salt, 5; phosphocreatine disodium salt hydrate, 5; guanosine 5′-triphosphate sodium salt hydrate, 0.2 and HEPES, 10; which was titrated to pH = 7.3 with KOH. Perfusing bath solution was kept at 37 °C by an automatic temperature controller (TC-324C, Warner Instrument, LLC., Hamden, CT, USA). Internal membrane potential of the cell sheet was measured by whole cell clamp configuration with current clamp mode, amplified by MultiClamp 700B (Molecular Devices, LLC., Sunnyvale, CA, USA), and acquired by Clampex 10.4 (Molecular Devices, LLC.) with Axon™ Digidata®1550 (Molecular Devices, LLC.).

2.5. Data analyses

FPD and conduction speed were analyzed with Mobius software (Alpha MED Scientific Inc.) as previously described.[7] 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 a repeated measures one-way analysis of variance (ANOVA) followed by the uncorrected Fisher's LSD test for mean value comparison. Statistical significances in the FPD and conduction speed before and after the zatebradine treatment at a cycle length of 1000 ms were evaluated with paired *t*-test. Statistically significant differences of the FPD and conduction speed among the lots were assessed with one-way ANOVA followed by the uncorrected Fisher's LSD test for mean value comparison at a cycle length of 1000 ms. Statistically significant differences in averaged relative expression levels of mRNA among the ion channels and gap junction protein were determined with one-way ANOVA followed by the uncorrected Fisher's LSD test for mean value comparison. Data were presented as the mean ± S.E.M. *P*-values <0.05 were considered to be statistically significant.

The relationships in each lot between FPD and cycle length (CL, ms) of electrical pacing was fitted into linear and non-linear equations based on the standard correction formulae of QT interval:

1) linear equation:

$$\mathrm{FPD}_{1{,}000\ ms} = \mathrm{FPD} - a(\mathrm{CL} - 1{,}000)$$

$$\mathrm{FPD} - \mathrm{FPD}_{1{,}000\ ms} = a(\mathrm{CL} - 1{,}000)$$

2) non-linear equation:

$$\mathrm{FPD}_{1{,}000\ ms} = \frac{FPD}{\left(\frac{CL}{1{,}000}\right)^a}$$

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