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Appraisal of state-of-the-art

Points to consider for a validation study of iPS cell-derived cardiomyocytes using a multi-electrode array system

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ABSTRACT

Human induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) provide a novel assay system to assess cardiac safety in drug development to overcome a problem of species difference in non-clinical testing during drug development. Using the multi-electrode array (MEA) platform, electrophysiological activities of iPS-CMs can be recorded easily to assess QT prolongation and proarrhythmic potential of drug candidates. Here we have established a standardized protocol to evaluate the possibility of iPS-CMs, and shared the protocol with an international consortium. To obtain reproducible and reliable experimental data from these cells, we determined the optimal experimental conditions, such as cell density, MEA coating, culture conditions, high-pass filter frequency, definition of early afterdepolarization or triggered activity, and calibration compounds. Based on the protocol, our validation study using 60 compounds is in progress. Thus, MEA-based experiments using iPS-CMs would be a standard testing method to evaluate QT prolongation and proarrhythmic potentials.

1. Introduction

Torsade de pointes (TdP) associated with QT prolongation is a significant factor for the high attrition rate of new-drug candidates in clinical studies and withdrawal of products from the market. Since the QT prolongation potential is a surrogate marker of TdP, drug candidates are evaluated non-clinically by in vitro hERG assays and in vivo QT assays (ICH S7B guideline) and clinically by tQT/QTc study (ICH E14 guideline) (Anonymous, 2005a; Anonymous, 2005b; Chi, 2013; Sager, Gintant, Turner, Pettit, and Stockbridge, 2014). While there have been no withdrawals by TdP from the market, after the implementation of S7B and E14 guidelines, drug-induced proarrhythmia has still been a major safety concern in the development of new drugs (Redfern et al., 2003; Lacerda et al., 2008; Gintant, 2011). Thus, we need a new paradigm for non-clinical test methods to predict the proarrhythmic risk of the drugs. Human iPS cell-derived cardiomyocytes (iPS-CMs) might be useful to establish the new paradigm, because of their expression of multiple ion channels (Ma et al., 2011).

To realize the new paradigm, we first performed survey regarding for availability of iPS-CMs. We found that there were inter-laboratory variations in experimental conditions such as iPS cell lines, differentiation methods, types of experimental preparation, culture conditions, testing methods for cell function, drugs, and endpoints (Tanaka et al., 2009; Zhang et al., 2009; Itzhaki et al., 2011; Mehta et al., 2011; Harris et al., 2013; Ohno et al., 2013). Thus, it was difficult to evaluate reproducibility and reliability of results using iPS-CMs. For the robustness of pharmacological and toxicological testing methods using iPS-CMs, we need a standardized protocol using the same cell preparations, measurement method, positive and negative compounds, and have to decide endpoints to predict the proarrhythmic risk.

In this manuscript, we will review our activities for the development of a standardized protocol using iPS-CMs for safety assessment toward international acceptance.

2. Our activity

Fig. 1 shows the timeline of our activities from 2010 to 2017. The division of pharmacology in National Institute of Health Sciences (NIHS) has designed the timeline toward international acceptance of the proposed methods using iPS-CMs according to the general validation process as a lead laboratory.

The first step of the validation study was to survey the scientific basis and regulatory needs of iPS-CMs for the safety pharmacology. Then, we defined the details for the methods such as experimental preparation, methodology, calibration compounds, and endpoints to be measured,

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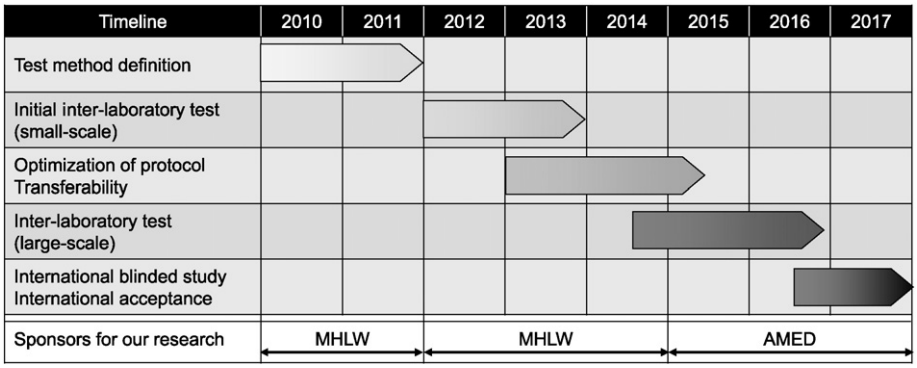

Fig. 1. The timeline of our activities. The timeline of our activities from 2010 to 2017 shows key validation processes including test method definition, small-scale inter-laboratory test, large-scale inter-laboratory test, international blinded test, and international acceptance. The reference to MHLW and AMED indicates sponsors in our research.

based on results of the surveys. The process of the definition of test method described in section 3 was supported by a grant from the Ministry of Health, Labour, and Welfare (MHLW) from 2010 to 2011. The next step of the validation study was a small-scale initial inter-laboratory test supported by a grant from MHLW from 2012 to 2014. We checked intra- and inter-laboratory reproducibility and transferability at participating laboratories. Then, we demonstrated that the test method can be reliable in detecting the drug-induced repolarization delay and arrhythmias with high reproducibility in inter-laboratory (Nakamura et al., 2014).

We presented the validation of protocol at ILSI-Health and Environmental Sciences Institute (HESI) workshop on 'Pluripotent Stem Cell: Applications for Cardiovascular Risk Assessment' held in March 2013. We organized the first think-tank meeting in Japan on non-clinical and clinical cardiac safety assessment (Kirishima meeting, Jan 2014), where Dr. Sager gave a presentation about Comprehensive in vitro Proarrhythmia Assay (CiPA) activity with a special focus on a comprehensive assessment of multi ion channel effects (Chi, 2013; Colatsky et al., 2016).

From our results of the initial inter-laboratory test and international activities toward proarrhythmic risk assessment, the Japanese government decided to support our validation study. A management team for the validation study was organized to establish subgroups; we included other participates from academia, industry and society. We determined a minimum list of reference chemicals, optimized protocol with a help of Japan Safety Pharmacology Society (JSPS), evaluated relevance and reproducibility. We also checked transferability of the protocol with JSPS and a consortium of Japan Pharmaceutical Manufacturers Association (JPMA Task force for iPS cells). To conduct the large-scale validation study, NIHS organized a cpmsprtoim, Japan iPS Cardiac Safety Assessment (JiCSA), as a task group with experts from academia, industry, and government, supported by MLHW in Aug 2014 (http://jicsa.org/en/). We have finalized the MEA-based protocol described in section 4 with participating laboratories (Asakura et al., 2015). JiCSA shared our standardized protocol with CiPA myocyte work stream. In addition to our protocol, JiCSA shared the pilot study data using selective ion channel blockers (E-4031 for hERG, JNJ303 for I_{Ks}, mexiletine for I_{Na}, and nifedipine for I_{CaL}) and mixed channel blockers (flecainide, moxifloxacin, ranolazine, and quinidine). We will describe the test method definition and 'points to consider' to minimize the variability of the protocol in details.

3. Test method definition

3.1. Type of cell preparation

There are some variations of action potential waveform from single cell iPS-CM (Fig. 2), as previously reported (Zhang et al., 2009; Fatima et al., 2011; Matsa et al., 2014). In the case of an embryoid-body cluster and 3-dimensional model of CM, it was difficult to perform experiments with reproducible preparation in regarding to its size and cell density. For these reasons, we selected a monolayer sheet as the type of cell preparation.

3.2. Methodology, calibration compounds and endpoints to be measured

To evaluate the function of the monolayer sheet, there are several protocols, such as MEA system, optical measurement system, motion vector and propagation (Tanaka et al., 2009; Itzhaki et al., 2011; Asakura et al., 2015; Ren et al., 2011; Kadota et al., 2013; Hayakawa et al., 2014; Kitaguchi et al., 2016). MEA technology can easily provide extracellular FP duration (FPD) that is similar to QT duration of human ECG. Based on these considerations, a small scale inter-laboratory test evaluated the effects of E-4031 using iPS-CMs (iCell; Cellular Dynamics International) and one MEA platform (MED64; Alpha Med Scientific) at multiple test sites (Nakamura et al., 2014). We have shown that FPD and incidence of early afterdepolarization (EAD) or triggered activity (TA) are which are reproducible and reliable endpoints at these sites under our common protocol.

4. 'Points to consider' to minimize the variability of iCell/MEA assay

Before JiCSA started the large-scale inter-laboratory test using iCell and one MEA platform (MED64), we listed 'points to consider' to minimize the variability. Fig. 3 shows the workflow of the MEA assay using iCell. To obtain reproducible and reliable data, there are many critical parameters that may unexpectedly change during the experiment and influence the electrical activity. Table 1 shows the factors that may affect reproducibility and reliability of the MEA data. Details are below.

4.1. Cell density

We customized the cell number of the monolayer sheet on the MED probe(Asakura et al., 2015), which is known to affect pharmacological responses (Uesugi, Ojima, Taniguchi, Miyamoto, and Sawada, 2014). When 15,000, 20,000, or 25,000 cells were plated on the MEA probe, the number of electrodes that fulfilled the criteria (Fig. 4, first peak amplitude ≥ ± 200 μV, second peak amplitude ≥15 μV) increased with

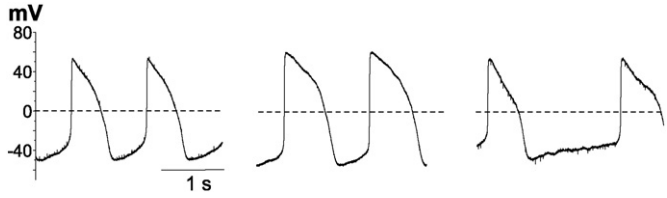

Fig. 2. Variations of action potential waveform from single iCell. Action potential waveforms are obtained by patch-clamp techniques using iCell cardiomyocytes.