Evaluation of the Long-Term Exposure Effects of Compounds on Human iPS Cell-Derived Cardiomyocytes Using a Multi-Electrode Array

多点電極アレイを用いたヒトiPS細胞由来心筋細胞に対する化合物の長時間曝露による影響評価

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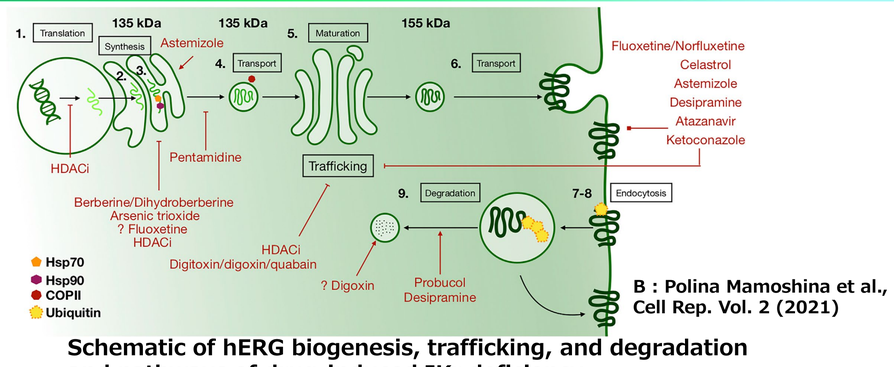
Background

■ **The importance of long-term exposure evaluation in cardiotoxicity assessment**

- Side effects on the cardiovascular system are one of the main reasons for terminating drug development. Therefore, it is crucial to accurately understand the effects of compounds on the cardiovascular system at an early stage.
- The functional inhibition of the hERG channel is commonly used for cardiovascular risk assessment. There are two types of mechanisms for hERG channel inhibition: direct inhibition and trafficking inhibition.
- Trafficking inhibition leading to hERG functional disruption can be detected by prolonged exposure of several hours or more. Therefore, it is common to evaluate the expression of hERG protein as an indicator.
- However, the expression of hERG protein does not directly evaluate hERG function and does not evaluate involvement of factors other than hERG channels.



B : Polina Mamoshina et al., Cell Rep. Vol. 2 (2021)

Schematic of hERG biogenesis, trafficking, and degradation and pathways of drug-induced IKr deficiency

Purpose of research

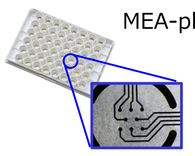
We investigated whether continuous long-term recording of extracellular potentials in iPSC-derived cardiomyocytes (iPSC-CMs) using a multi-electrode array (MEA) could be effective in evaluating the activity of hERG transport inhibitors.

Materials and Methods

Measuring equipment

✓MEA : Maestro Pro (Axion BioSystems)
✓MEA plate: Cytoview (Axion BioSystems)

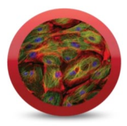
Multi-electrode array



MEA-plate

Reagents

✓Cell : iCell® Cardiomyocytes^2 (FUJIFILM Cellular Dynamics, Inc.)
✓Coating agent : Fibronectin (Roche Diagnostics Deutschland GmbH)
✓Media : iCell® CM Plating Medium , iCell® CM Maintenance Medium (FUJIFILM Cellular Dynamics, Inc.)



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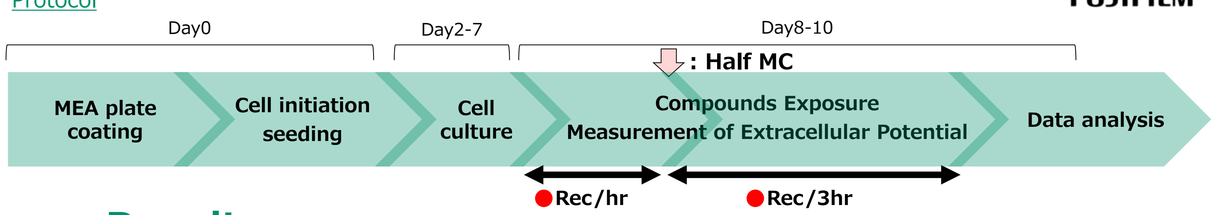
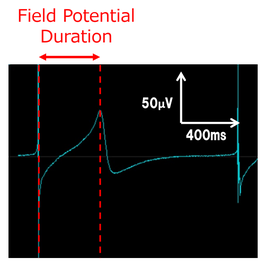
Protocol

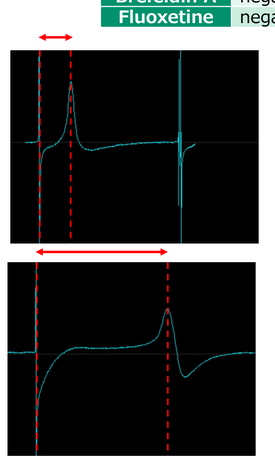


Analysis method



Extracellular waveform

Compound exposure



Calculate the change in Field Potential Duration after compounds exposure /before compounds exposure

Parameter

$$FPDcF = \frac{FPD}{\sqrt[3]{BeatPeriod}}$$

FPDcF : Field potential duration corrected by Fridericia's formula

dFPDcF/dBR (Beat rate):
the ratio of change relative to the baseline condition, defined as 0.1% DMSO

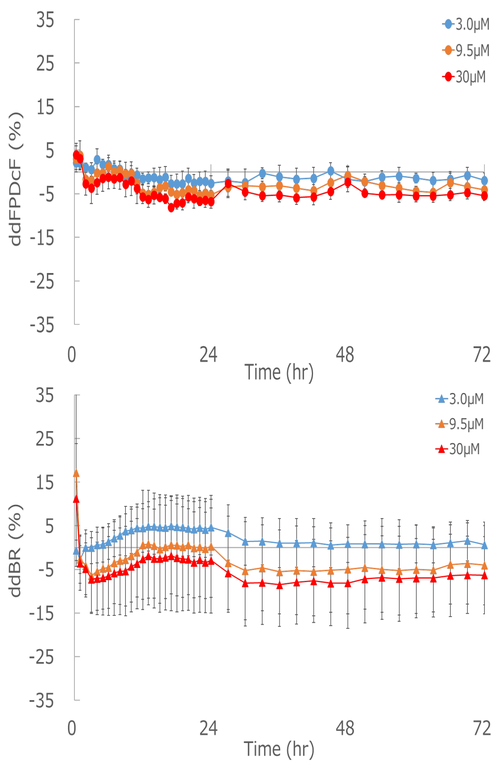
ddFPDcF/ddBR (Beat rate) :
The difference between dFPDcF/dBR and the mean dFPDcF for Vehicle-treated controls at the same time point

Test Compounds

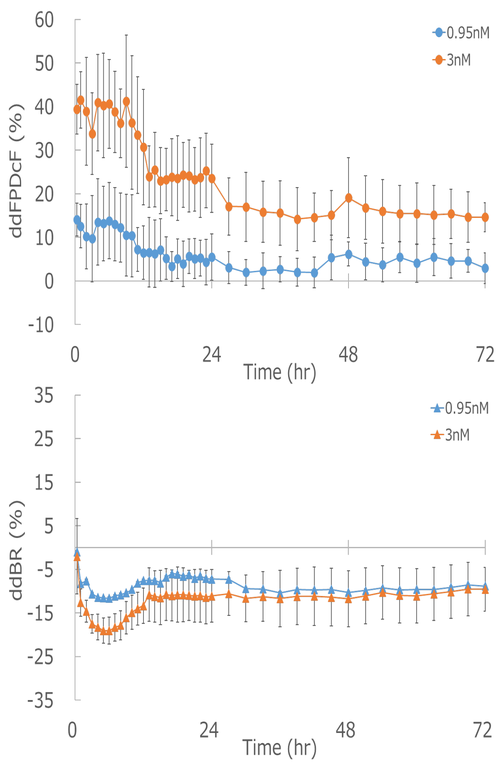
Compounds	Effect on hERG		Pharmacological effect/Mechanism
	Directly	Trafficking	
DMSO	negative	negative	Vehicle
Aspirin	negative	negative	NSAIDs
E-4031	positive	negative	experimental class III antiarrhythmic drug
Pentamidine	negative	positive	antimicrobial medication
Brefeldin A	negative	positive	lactone antiviral
Fluoxetine	negative	positive	antidepressant medication (SSRI)

Result

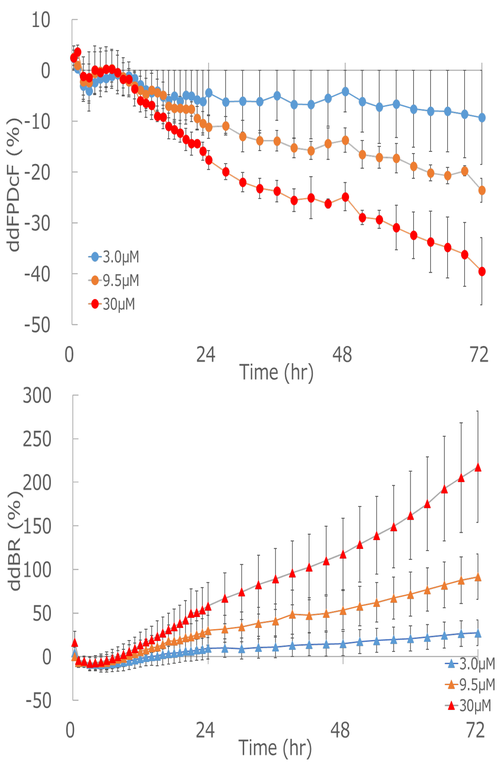
■ **Aspirin**



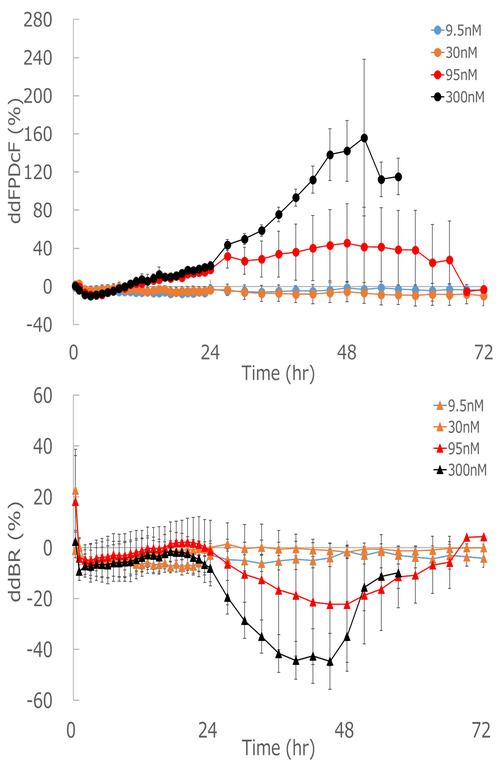
■ **E-4031**



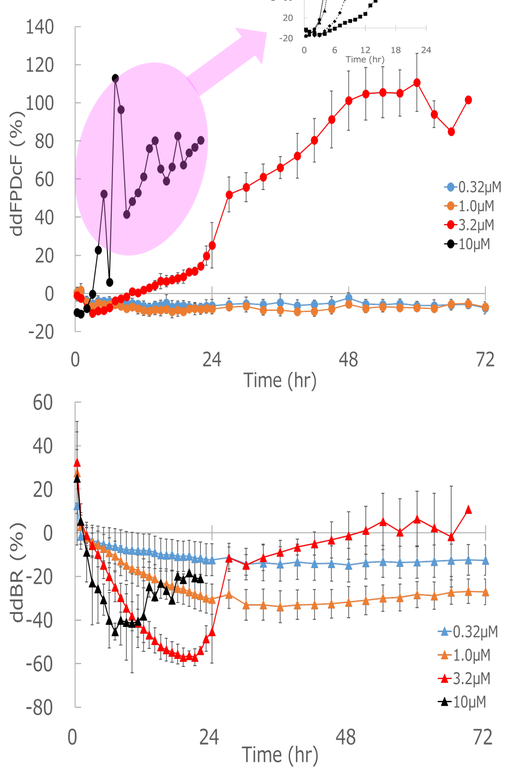
■ **Fluoxetine**



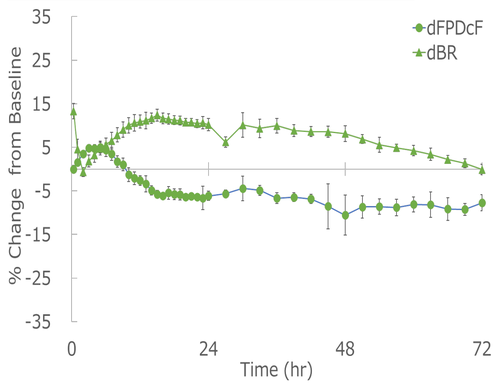
■ **Brefeldin A**



■ **Pentamidine**



■ **Vehicle-treated control**



- During the test period, no significant changes were observed in vehicle-treated control and aspirin in FPDcF and BeatRate.
- In the E-4031, the extent of FPDcF prolongation and BeatRate reduction began to decrease within several hours after compound administration and stabilized between 24 and 72 hr post-treatment.
- In the Fluoxetine, a dose- and time-dependent decrease in FPDcF and increase in BeatRate were observed.
- In Brefeldin A and Pentamidine, dose-dependent and time-dependent prolongation of FPDcF and decreases in heart rate were observed. At high doses, cardiac arrest occurred during the study period.

We confirmed delayed electrophysiological changes caused by long-term exposure (e.g., 72 hours) to compounds on iPS-CMs using MEA.

Discussion

We have confirmed that it is possible to measure extracellular potentials stably several days in the MEA assay. In studies using hERG-expressing cell lines, compounds that similarly inhibit hERG channel trafficking (such as Fluoxetine, Brefeldin A, and Pentamidine) produced contrasting results in this electrophysiological assay. This suggests that different mechanisms of action among these compounds lead to distinct effects on proteins other than hERG. Furthermore, it is necessary to evaluate compounds with diverse mechanisms of action and to investigate proteins, such as ion channels and transporters, that contribute to the formation of extracellular potentials. These studies will facilitate the precise assessment of the long-term effects of compounds on cardiomyocytes using the MEA assay.

Conclusion

The MEA assay of iPSC-CMs is a promising method for detecting the effects of long-term exposure to various compounds on cardiomyocytes.

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