Use of the local extracellular action potential (LEAP) determined with MEA to enhance prediction of cardiotoxicity

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Abstract

Microelectrode array (MEA) has been shown to be a key assay in predicting toxicity issues at early stages and thus in reducing the cost and time caused by candidate compound failure at a later stages of drug development.

The regular and synchronous contraction of the heart requires periodic activation and inactivation of various cardiac ion channels. Any discrepancies in the cardiac ion channel signaling network could result in a change of membrane potential and in the shape of cardiac action potential (AP). These changes can modify cardiac excitation-contraction coupling which may lead to arrhythmias and eventually could cause patient death.

The local extracellular action potential (LEAP) technique enables non-invasive, label-free monitoring of cardiac action potential in a high-throughput real-time format.

We used the Axion LEAP MEA assay to study the effect of various selective and non-selective ion channel inhibitors on cardiac AP by quantification of action potential morphology, repolarization irregularities, and arrhythmic risk factors such as triangulation. All experiments were done in FujiFilms CDI iPSC-derived cardiomyocytes using AXION Biosystems Maestro Pro research platform.

Regular MEA assay provides the following endpoint parameters of cardiomyocytes: 1) the beat period, 2) fast Na+ slope, 2) fast Na+ amplitude and 4) field potential duration (FPD) per well. These parameters represent inhibition of hERG and fast sodium current and activation of late sodium current. LEAP study can provide additional information such as about calcium channel inhibition and detect toxic effects of the drugs on multiple ion channels. We tested specific and non-specific cardiac ion channels TTX, E-4031, dofetilide, verapamil, nifidepine, late sodium channel activator ATX-II and beta-adrenergic agonist isoproterenol.

The results demonstrated that that we can separate effects of drugs on different ion channels in LEAP assay. Activation of the late sodium current by ATX-II prolonged LEAP duration by extension of the LEAP plateau, whereas dofetilide did not change the plateau but slow down the rate of repolarization. Modulation of calcium channels activity changed the shape and duration of LEAP.

Our data showed that LEAP study of iPSC-derived cardiomyocytes coupled to MEA platforms is a suitable tool for identifying the cardiotoxic effects of studied articles. Because of the wide variety of molecular targets and off-targets of reference drugs with cardiotoxic risk, LEAP assay has advantages compared to single cell electrophysiological assays. We were able to identify the possible cytotoxic mechanisms of investigated drugs by analyzing the results of LEAP.

Overall, we have demonstrated that this *in vitro* approach represents a promising translational value in identifying the cardiotoxicity of drug candidates at the early stages of development. LEAP MEA assay can serve as a useful link between *in vitro*, *in vivo*, and clinical studies.

Methods

Cell culture:

FUJIFILM Cellular Dynamics, Inc. (FCDI) CDI iCell® cardiomyocytes[2] (iPSC-CM) were thawed according to the manufacturer's specifications. Cells were plated onto a fibronectin coated BioCircute 48-well microelectrode array plate (Figure 1A) at a density of 50,000 cells per well in 5µl iCell® plating medium.

After 2 hours, 300µl of maintenance medium was added to each well. The plate was incubated for 2 days and then the medium was completely changed to 500µl of maintenance medium. While iCell Cardiomyocytes[2] begin spontaneous beating in culture by day 4. For our experiments we used cells on day 7, when cells establish regular beating in all wells. The medium was changed the day before dosing. The cells were treated with vehicle (0.2% DMSO) and test compounds.

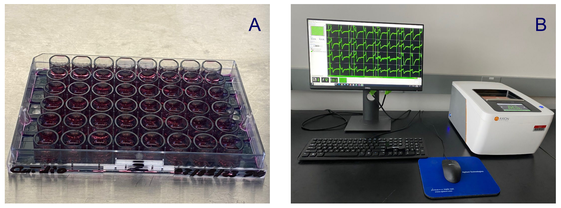

Figure 1: A. Biocircute 48-well/16 electrodes plate. B. AXION MAESTRO PRO research platform.

Experimental Procedure - eCiphrCardio:

All experiments were done on AXION Biosystems Maestro Pro research platform (Figure 1B). MEA assays with iPSC-derived cardiomyocytes enable label-free detection of extracellular field potentials from cardiomyocytes. Analysis of the recordings yields metrics such as spontaneous beat rate, sodium spike slope and amplitude and FPD (Figure 2A). Local extracellular action potential (LEAP) assay was developed to measure the cardiac action potential on MEA. Induction of LEAP increases coupling between the cells and the electrodes, resulting in a larger assay signal (millivolts vs. microvolts) that is more robust against pharmacological modulation. Spontaneous activity of cells in a 48-well microelectrode array was recorded prior to treatment (baseline) and at 1 hour post-treatment using the Axion Biosystems Maestro Pro MEA research platform. After the spontaneous basic activity recorded LEAP function was initiated and LEAPs were recorded using the same research platform. We analyzed the following LEAP endpoints: the beat period, APD30, APD50, APD90, triangulation ratio and rise time (Figure 2B).

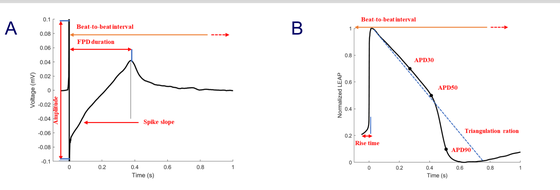

Figure 2: Endpoint parameters for field potential (A) and LEAP (B) studies.

The recording conditions were at 37°C with 5% CO2 using the standard settings on the Axion Biosystems Maestro Axis software version 2.1.

Results

Below we compare the results recorded from field potential and LEAP experiments. The field potential traces shown as baseline (before drug application, black) and 1 hour after the drug application (red). Quantified drug response was normalized first to baseline and then calculated as a percent of the vehicle treated wells.

After the LEAP induction traces represent recordings from vehicle-treated wells (black) and drug-treated wells (red). Quantified drug response was compared to the vehicle treated wells.

Sodium channel modulators

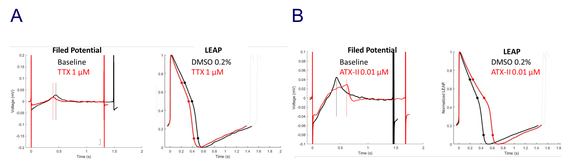

Figure 3: Effects of sodium channel blocker TTX (A) and late sodium current activator ATX-II (B)

Calcium channel blockers

Figure 4: Effects of calcium channel blockers verapamil (A) and nifedipine (B)

Potassium Kr channel (hERG) blockers

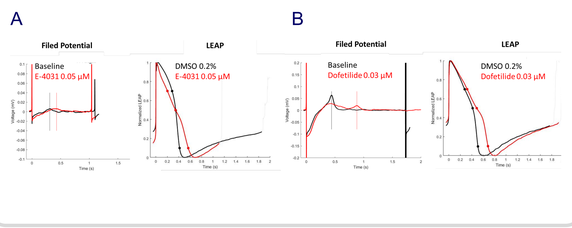

Figure 5: Effects of E-4031 (A) and dofetilide (B).

ß-Adrenergic stimulation

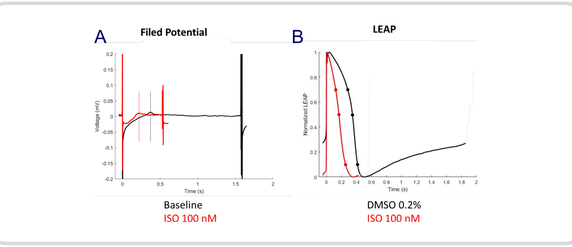

Figure 6: Effect of isoproterenol (ISO) on electrical activity in iPSC-CM.

Drug-induced early afterdepolarizations (EADs) in iPSCC-CMs. LEAP detection:

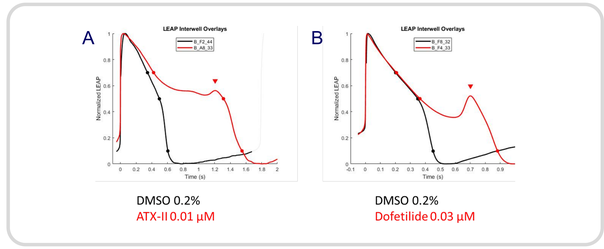

Figure 7: Detection of ED in cells treated with ATX-II (A) and dofetilide (B).

Tables

Treatment	Concentration (µm	Beat Period Mean (s	Spike Slope Mean (V/s	Spike Amplitude (mV	FPD (ms)	Plate #
TTX	1	79 ± 37.4%	90 ± 16.4%	88 ± 14.7%	35 ± 5.0%	Plate 2
ATX-II	0.01	115 ± 14.6%	97 ± 6.1%	98 ± 0.9%	122 ± 17.5%	Plate 2
Nifedipine	0.03	46 ± 6.6%	114 ± 56.1%	115 ± 19.3%	40 ± 26.3%	Plate 1
Verapamil	0.03	46 ± 6.6%	114 ± 56.1%	115 ± 19.3%	40 ± 26.3%	Plate 3
E-4031	0.05	112 ± 2.7%	98 ± 1.8%	96 ± 6.16%	151 ±9.8%	Plate 2
Dofetilide	0.03	112 ± 0.2%	159± 3.0%	156 ± 6.8%	147 ± 0.6%	Plate 2
ISO	0.1	51 ± 18.5%	131 ± 28.6%	107 ± 34.6%	65 ± 5.2%	Plate 1
DMSO 0.2%		100 ± 9.6%	100 ± 1.8%	100 ± 2.3%	100 ± 9.3%	Plate 1
DMSO 0.2%		100 ± 9.6%	100 ± 1.8%	100 ± 2.3%	100 ± 9.3%	Plate 2
DMSO 0.2%		100 ± 9.6%	100 ± 1.8%	100 ± 2.3%	100 ± 9.3%	Plate 3

Table 1: Effects of tested compounds on the MEA field potential assay.

	Test Article	Concentration (µM)	Beat period (s)	APD30 (s)	APD50 (s)	APD90 (s)	Triangulation rat	Rise time (ms)	
Sodium Channel Modulators	TTX	1	1.27 ± 0.03	0.076 ± 0.007	0.113 ± 0.005	0.182 ± 0.005	0.664 ± 0.011	3.3 ± 0.14	Plate 2
	ATX-II	0.01	1.59 ± 0.28	0.292 ± 0.008	0.434 ± 0.030	0.538 ± 0.032	0.806 ± 0.007	10 ± 2.8	Plate 2
Calcium Channel Blockers	Nifedipine	0.03	0.67 ± 0.24	0.137 ± 0.117	0.179 ± 0.139	0.207 ± 0.100	0.693 ± 0.144	3.2	Plate 1
	Verapamil	0.03	0.63 ± 0.03	0.071 ± 0.002	0.110 ± 0.006	0.195 ± 0.010	0.566 ± 0.001	2.3 ± 0.14	Plate 3
Kr Channel Blockers	E-4031	0.05	0.19 ± 0.05	0.225 ± 0.032	0.358 ± 0.019	0.569 ± 0.003	0.595 ± 0.141	2.5 ± 0.14	Plate 2
	Dofetilide	0.03	1.92 ± 0.08	0.257 ± 0.016	0.438 ± 0.004	0.671 ± 0.018	0.651 ± 0.041	5.1 ± 1.56	Plate 2
ß-Adrenergic Stimulation	ISO	0.1	0.568697	0.1248	0.1672	0.256	0.653125	4	Plate 1
	DMSO 0.2%		1.91 ± 0.05	0.282 ± 0.004	0.344 ± 0.006	0.418 ± 0.04	0.841 ± 0.005	2 ± 0.57	Plate 1
	DMSO 0.2%		1.83 ± 0.05	0.330 ± 0.10	0.456 ± 0.025	0.535 ± 0.003	0.853 ± 0.001	6.8 ± 1.7	Plate 2
	DMSO 0.2%		1.31 ± 0.02	0.239 ± 0.005	0.350 ± 0.003	0.487 ± 0.010	0.735 ± 0.0.03	3.1 ± 0.34	Plate 3

Table 2: Effects of tested compounds on MEA LEAP assay.

Conclusions

LEAP method assay offers a reliable *in vitro* assay for cardiac safety/toxicity electrophysiological studies. LEAP assay provides additional information to the field potential assay through the analysis of action potential morphology and detection of proarrhythmic action potential irregularities such as early afterdepolarizations (EADs). Implementation of the LEAP assay provides a robust alternative solution for pharmacological modulation and could be beneficial for certain studies in cardiac electrophysiology.

References

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- Millard *et al.* Cross-Site Reliability of Human Induced Pluripotent stem cell-derived Cardiomyocyte Based Safety Assays Using Microelectrode Arrays: Results from a Blinded CiPA Pilot Study. *Toxicol Sci.* 2018 Aug 1;**164(2)**:550-562.
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