Utilization of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes on an MEA Platform for Prediction of Liabilities with Chronic Drug Treatment

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Abstract

The use of microelectrode array technology (MEA) has proven to be a very powerful tool for prediction of proarrhythmic liabilities in iPSC derived cardiomyocytes. This work has focused primarily on the prominent cardiac ion channels with an emphasis on hERG. To this end, the Comprehensive In Vitro Proarrhythmia Assay (CIPA) initiative was established and has been tasked with prediction of proarrhythmic compounds in an in vitro setting with acute short term dosing. Although most of the focus on the early work has been on arrhythmias driven primarily by hERG block, the real power of iPSC-derived cardiomyocytes on an MEA platform is predicting unexpected liabilities and long term chronic effects in vitro. These type of studies are currently performed in telemerized dogs at great expense. Here we show examples of compounds whose liabilities were identified with chronic dosing of compounds in an MEA assay. Due to the ability to measure and maintain cells over long periods of time, this platform is ideal for evaluating short and long term exposures early in drug development. Examples of hERG trafficking effects are demonstrated at 24 and 48 hours. The hepatitis C drug BMS-986094, which failed in clinical trials, is shown to progressively deteriorate cardiomyocyte health as compared to safe hepatitis C drugs such as sofosbuvir in 14-day studies. We also show examples of compounds that have other unexpected responses such as delayed onset arrhythmias and Na amplitude effects that are not observed acutely and cannot be predicted by ion channel screening. These results demonstrate that iPSC derived cardiomyocytes on an MEA platform are an effective tool for screening compounds to identify unexpected long term liabilities before expensive preclinical animal experiments are performed, thus improving the chances of moving forward with a safe compound.

Introduction

- Late stage failures of drugs in clinical trials have significant costs as well as significant safety risk to patients.
- Identification of liabilities early will save money and allow for prioritization of better compounds.
- Identification of ion channel liabilities will only identify specific acute liabilities for cardiac risk
- Telemerized dog studies are expensive and limit the amount of compounds against a target that can be screened. In Vitro testing allows for multiple compounds and scaffolds to be tested to identify a safe compound at affordable prices.
- Other than animal studies, there is no effective way to identify chronic dosing effects
- IPSC cardiomyocytes can remain healthy for at least two weeks plated on an MEA plate. Due to the fact that it measures electrical activity without addition of any reagents, extended multi-timepoint assays can be run.
- Timepoints for the screening assay need to be determined by the customer depending on the mechanism or the observed phenotype in animal testing. For unknown compounds, a broad multi-timepoint assay would be suggested.
- Compounds to be tested in this poster
  - Pentamidine – Known hERG trafficker
  - BMS-986094 (INX-08189) – Hepatitis C drug that caused death in clinical trial after multiple weeks
  - Compound X – Caused loss of Na amplitude and slope after 24 hours with arrhythmias
  - Compound Y – Caused delayed arrhythmias after 6 days with delay in repolarization but no hERG effect

Methods

- 48-well MEA plates were pre-coated with 5µl of fibronectin directly over the electrode grid and incubated at 37°C one hour before plating cells. Alternatively the cells were also seeded in a 96 well and 384 well fibronectin coated plate.
- iCell2 cardiomyocytes were then resuspended in CDI Cardiomyocyte plating medium and dot plated in 5µl at a density of 50,000 viable cells per well.
- The cells were incubated, humidified at 37°C in 5% CO2 for 6 days..
- 100% of the medium was changed every 2 days.
- Compounds were serially diluted in DMSO at 500X the concentrations to be tested. Compound is diluted 50 fold in an intermediate plate followed by addition of compound in medium from the intermediate plate at a 10 fold dilution into the MEA plate (500 fold dilution).
- MEA recordings were acquired before compound treatment (baseline) and after dosing (1 hour). Readings were also taken at timepoints designated in the experiment. Medium with compound was changed every two to three days.

Results: Pentamidine and hERG Trafficking

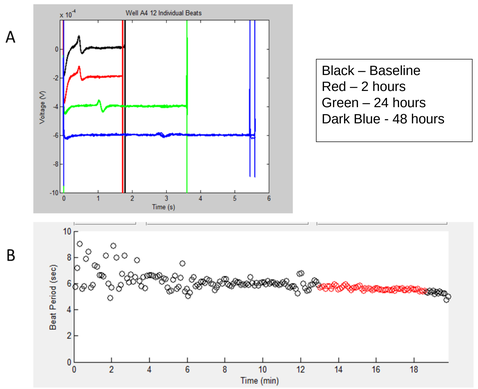

Figure 1. iCell Cardiomyocytes treated with Pentamidine. A. Time course of the MEA traces of Pentamidine. The Baseline and the 2 hour trace show no effect acutely. The green trace shows the 24 hour results. This shows a significant delay in the repolarization as well as in the beat length. There also is a slight decrease in the amplitude of the repolarization amplitude. At 48 hours, the effects are increased with a greater delay in the repolarization and a further decrease in the amplitude. B. The beat plot shows a loss in the regulation of the beat rate with arrhythmias beginning.

Results: 24 hour dosing of Compound X – Decreases Na Amplitude

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Test Article	Time Point	Test Conc. (µM)	% of Vehicle: Average Beat Period	Average Na+ Slope	Average Na+ Amplitude	Average FPD	Comment
Compound X	1 hour	150	76 ± 3.1%	62 ± 1.8%	65 ± 1.4%	93 ± 1.3%	
		75	85 ± 0.2%	72 ± 10.0%	76 ± 5.9%	97 ± 1.0%	
		30	92 ± 0.2%	82 ± 12.3%	83 ± 9.9%	99 ± 0.3%	
		15	96 ± 0.1%	95 ± 6.2%	95 ± 4.6%	100 ± 1.4%	
		7.5	98 ± 0.1%	85 ± 15.4%	92 ± 5.4%	101 ± 0.5%	
		MEC (µM)	30	75	75	NA	
	24 hours	150	86 ± 0.5%	12 ± 3.4%	22 ± 2.9%	72 ± 1.0%	
		75	94 ± 0.2%	23 ± 0.0%	30 ± 0.9%	83 ± 0.2%	A,C
		30	132 ± 17.1%	17 ± 0.4%	25 ± 0.9%	TND	A,C
		15	144 ± 21.4%	20 ± 2.3%	29 ± 2.7%	TND	A,C
		7.5	157 ± 1.3%	21 ± 1.8%	29 ± 0.9%	92 ± 6.9%	A,C
		MEC (µM)	150	7.5	7.5	15	

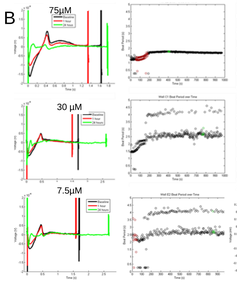

Figure 2. MEA data for compound X. **A.** At 1 hour, compound X caused some effect at 150 and 75 µM. There is a decrease in Na amplitude and slope with an increase in beat rate (decrease in beat length). At 24 hours, all of the concentrations have a significant decrease in Na amplitude and slope at all concentrations. This effect does not appear to be dose dependent at these concentrations as all of the concentrations have roughly the same decrease This suggests the target for this effect is below the lowest dose tested. There does appear to be a dose effect for other components such as beat rate which has longer beats at lower doses. At lower concentrations, the cells also become more arrhythmic. B. MEA traces show that the compound has significant effects on the trace. The repolarization peaks are hard to identify as there may be two peaks of reduced amplitude at all concentrations. The beat plots show that the compound causes more arrhythmias at the lower concentrations than at the higher concentration. The beat traces at the lowest concentration show an abnormal beat plot with duplicate depolarization peaks and an unidentifiable repolarization peak.

Results: 24 hour dosing of Compound Y – Delayed unexplained arrhythmic activity

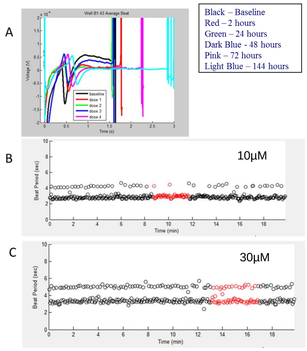

Figure 3. MEA data for compound Y. **A.** Traces show the time course of the compound Y. The traces show that the repolarization becomes more delayed over time with the largest increase occurring at 6 days. It does not appear to be a hERG effect as we see no decrease in the repolarization peak amplitude. You can see multiple depolarization peaks showing arrhythmias. **B and C**. At 10 and 30µM, arrhythmias are observed at 6 days as can be observed in the beat plot.

Results: Chronic testing of INX-08189

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Test Article	Time (hr)	Test Conc. (µM)	% of Vehicle: Average Beat Period	Average Na+ Slope	Average Na+ Amplitude	Average FPD
INX-08189	1	10	106 ± 5.4%	78 ± 10.8%	80 ± 11.7%	105 ± 6.2%
		2	106 ± 2.7%	112 ± 9.6%	111 ± 7.3%	107 ± 5.5%
		0.4	105 ± 1.8%	91 ± 23.1%	93 ± 26.2%	99 ± 8.5%
		0.08	106 ± 3.4%	122 ± 18.9%	129 ± 16.1%	105 ± 5.3%
		0.016	106 ± 4.6%	115 ± 16.7%	115 ± 16.3%	107 ± 5.9%
		MEC (µM)	NA	10	10	NA
	120	10	ND	ND	ND	ND
		2	100 ± 4.8%	57 ± 26.0%	41 ± 24.3%	102 ± 8.0%
		0.4	85 ± 2.0%	93 ± 35.1%	96 ± 36.0%	82 ± 10.6%
		0.08	88 ± 3.2%	185 ± 47.5%	203 ± 60.5%	86 ± 8.3%
		0.016	96 ± 3.3%	121 ± 50.7%	125 ± 53.1%	92 ± 4.7%
		MEC (µM)	10	0.08	0.08	10
	288	10	ND	ND	ND	ND
		2	ND	ND	ND	ND
		0.4	70 ± 5.1%	33 ± 13.1%	7 ± 4.6%	ND
		0.08	66 ± 3.0%	130 ± 19.5%	138 ± 24.2%	72 ± 6.5%
		0.016	91 ± 6.4%	107 ± 9.9%	107 ± 12.2%	89 ± 6.9%
		MEC (µM)	0.08	0.08	0.08	0.08
	336	10	ND	ND	ND	ND
		2	ND	ND	ND	ND
		0.4	ND	ND	ND	ND
		0.08	62 ± 5.5%	128 ± 11.7%	136 ± 19.1%	68 ± 7.0%
		0.016	93 ± 6.9%	108 ± 2.9%	110 ± 3.1%	90 ± 5.5%
		MEC (µM)	0.08	0.08	0.08	0.08

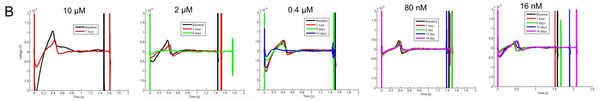

Figure 4. MEA data for INX-08189. A. Cells were treated with compound for 14 days with fresh medium and drug added every 3 days. The experiment consisted of a 5 point dose curve with 4 replicates at each dose. The 4 replicates consisted of 2 replicates on 2 separate plates. MEA measurements were taken at multiple timepoints during the 14 day experiment. All data is reported as a percent of the baseline corrected to the vehicle controls. The correction to the vehicle controls allows the experiment to correct for any maturation changes or condition differences that may exist over the 2 week long experiment. The table shows the changes that occur over the dose curve over the time course of the experiment. As the experiment progresses to the later time points, the lower concentrations continue to become more effected and stop beating. At 14 days, even the 80nM concentration has a significant change in the beat rate as well a reproducible and elevated increase in the Na amplitude. B. Traces of the different drug concentrations over time show the changes in the beat length as well as the overall change in the amplitude of the T-wave at the different doses and later time points.

Conclusions

- Use of an MEA cardiomyocyte assay is an effective way of identifying chronic liabilities.
- Pentamidine, a known hERG trafficking inhibitor, was identified using this assay
- The chronic MEA assay was effective at identifying compound X which decreased Na amplitude at 24 hours.
- The assay identified a compound which caused delayed arrhythmias (5-6 days) of unknown mechanism in telemerized dogs.
- The assay also identified INX-08189, a compound which caused a death in clinical trial, as a significant liability over the course of 14 days.

References

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