In Vitro Toxicology Testing Using Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes to Predict Tobacco Smoking-Related Cardiovascular Diseases

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Abstract

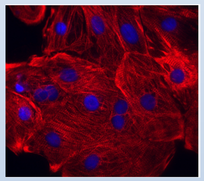
Cigarette smoking is associated with many cardiovascular dysfunctions. However, while there are reliable biomarkers of exposure to cardiovascular toxicants, there are no reliable biomarkers of harm that can be used to predict future onset of disease. Induced pluripotent stem cells (iPS) derived cardiomyocytes recapitulate physiological characteristics of human cardiac myocytes, providing a novel path for cardiotoxicity prediction. In this study, the toxicity of mainstream cigarette smoke condensates (CSCs) was assessed in iPS derived cardiomyocytes with cellular function assays and some cardiomyocyte-specific endpoints. The CSC treatments reduced cell viability as evidenced by established cytotoxicity assays (e.g., lactate dehydrogenase release) and ATP measurement. Treatment of cardiomyocytes with CSCs resulted in dose-dependent-beat rate changes as assessed by real-time cellular impedance measurements. Intermediate doses (e.g., 25 µg/ml) of CSC resulted in irregular beating that models arrhythmia and the highest dose (50 µg/ml) resulted in a cessation of beating. Global gene expression analysis of cardiomyocytes treated with CSCs using Next Generation Sequencing identified dysregulation of genes for multiple cardiac ion channels, including major genes from potassium and calcium channels. The results suggest that the inhibitory effects of CSCs on cardiomyocytes beating might be associated with multiple ion channels. The human iPS-derived cardiomyocytes model represents a novel *in vitro* approach that could potentially assess tobacco-induced cardiac harm including arrhythmias.

Background

While the effect of cigarette smoking on coronary artery diseases is established, the association between cigarettes and cardiac arrhythmia is less clearly defined. Based on the literature, cigarette smoking may be a risk factor for arrhythmia through mitochondrial damage, oxidative stress and cardiomyocyte necrosis. For example, one clinical trial reported that the risk of life-threatening ventricular tachyarrythmia is higher in current smokers than non-smokers.

Induced pluripotent stem cells (iPS) derived cardiomyocytes are a mixture of spontaneously electrically active atrial, nodal, and ventricular-like myocytes that possess typical electrophysiological characteristics. The toxicity of mainstream cigarette smoke condensates on cardiac arrhythmia was assessed using iPS derived cardiomyocytes in the study.

(A) cTnT /DAPI



(B) Cell population with positive cTnT

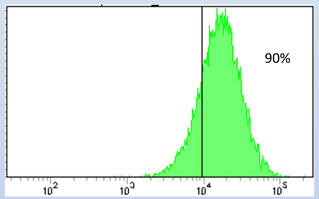


Figure 1. Structural organization and purity of human induced pluripotent stem cells (iPS) derived cardiomyocytes (A) Immunostaining of iPS-Cardiomyocytes. (B) Purity (~ 90%) of cells.

Materials & Methods

Cigarette smoking condensates (CSC)

CSC generated by two reference cigarettes (3R4F and CM6) were provided and quantified (as µg/ml total particulate matter (TPM)) by the Centers for Disease Control and Prevention (CDC).

Adapted from Adamson et al. (2011)

MEA

Multielectrode array (MEA) measures electrical field potential rather than physical beating by impedance measurement.

xCELLigence

This cardio system is able to sensitively and quantitatively detect cellular impedance; therefore, it monitors real-time beating pattern of cardiomyocytes in 96-well format.

Next-Gen Sequencing

Measure the gene expression levels of whole genome, including cardiac specific genes.

MEA Results

Figure 3. Time (5min, 0.5, 2,and 24 hr)-, concentration (0, 1, 10, 30 µg/ml CSC) -dependent amplitude decreases in iPS-derived cardiomyocytes.

(A) Baseline

(B) 24h post treatment

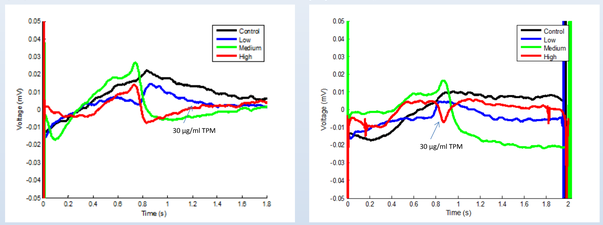


Figure 4. One day post treatment, some early after depolarization (EAD)-like feature was observed at 30 µg/ml TPM after 24h using MEA.

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Cell Viability

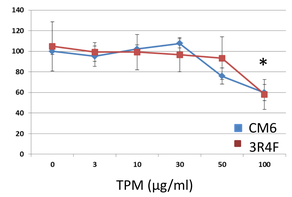

Figure 2. Cell viability decreased to 60% at 100 µg/ml TPM after 24h. No significant cell death was observed at 30 µg/ml by cell index assay.

xCELLigence Results

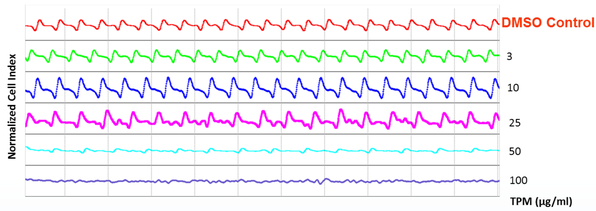

Figure 5. CSC treatment with > 25 µg/ml total particulate matter (TPM) significantly affected the beating of iPS-derived human cardiomyocyte. The DMSO control (0.1%) had no effect; however, CSC treatment caused arrhythmia-like feature at 25 µg/ml or complete arrest of beating at 100 µg/ml .

(A) Beating Rhythm Irregularity

(B) Normalized Amplitude

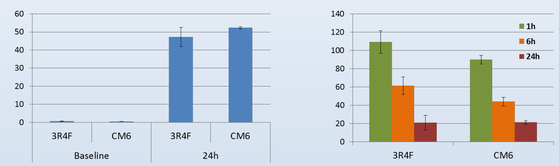

Figure 6. CSC treatment resulted in irregular beating pattern at 30 µg/ml TPM (A) Irregular beating rhythm increased to ~50% at 30 µg/ml TPM after 24h. (B) Time dependent decrease in normalized beating amplitude.

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Gene Expression Results

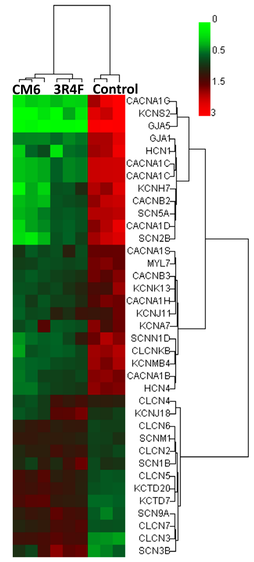

Figure 7. CSC 24h treatment with 30 µg/ml TPM significantly affected the gene expression of cardiac ion channel. Heat Map showing the altered patterns of gene expression, with green color indicating the down-regulation and red as up-regulation.

Summary

- ❑ Treatment dependent changes in cellular cytotoxicity were identified. 100 µg/ml TPM caused significant cell death. The difference between 3R4F and CM6 is not significant.
- ❑ CSC treatments have strong effect on amplitude decrease at 30 µg/ml.
- ❑ Some EAD-like features presented at 30 µg/ml 1 day post-treatment. EAD can result in torsades de pointes, tachycardia, and other arrhythmias.
- ❑ Treatment with CSCs resulted in concentration-dependent changes in the beating rhythm irregularity.
- ❑ Treatment dependent changes in patterns of multiple cardiac ion channel genes were identified:
  - Calcium voltage-dependent channel (Decrease)
  - Potassium voltage-gated channel (Decrease)
  - Sodium voltage-gated channel (Increase)

Acknowledgements

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