Cardiac Contractility Modulation Stimulation During Systole Enhances Contraction and Calcium Handling Properties in Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes

T.K. Feaster[1], **Maura Casciola**[1], **Akshay Narkar**[1], **Ayda SoltanianTiranchi**[1], **Ksenia Blinova**[1]

1 Center for Devices and Radiological Health, US Food and Drug Administration, Silver Spring Maryland, USA

Abstract

Background: Cardiac contractility modulation (CCM) is a cardiac therapy whereby non-excitatory electrical simulations are delivered during the absolute refractory period of the cardiac cycle. CCM is indicated for patients with heart failure (Class III/IV) and reduced ejection fraction. We previously evaluated the effects of CCM and found isolated adult rabbit cardiomyocytes display a transient increase in calcium and contractility. Purpose: In the present study, we sought to extend these results to human cardiomyocytes using human stem-cell cardiomyocytes (hiPSC-CMs) as a model. Methodology: hiPSC-CMs (iCell Cardiomyocytes[2], Fujifilm Cellular Dynamic Inc) were studied in monolayer format plated on Matrigel mattress substrate. Contractility, calcium handling and electrophysiology were evaluated by image and fluorescence analysis (CellOPTIQ, Clyde Biosciences). Standard clinical CCM pulse parameters were applied with an A-M Systems 4100 pulse generator. Pacing threshold was determined and stimulation performed at 1.5 times the diastolic capture threshold. Results: Robust CCM response was observed at 5 V/cm (64 mA) for pacing and 10 V/cm (120 mA) for CCM. Under these conditions hiPSC-CMs displayed a sustained increase in contraction amplitude. Furthermore, the first CCM beat displayed an increase in contraction amplitude relative to control that reached steady-state within 5 - 6 beats. CCM stimulation resulted in faster contraction kinetics and relaxation kinetics. Likewise, calcium transient amplitude increased, and action potential duration was shortened, relative to control. The CCM effects subsided when the signal was turned off. We also observed, by modulation of extracellular calcium concentration, that lower levels of extracellular calcium results in increased CCM induced contraction amplitude relative to control. Conclusion: This study provides a comprehensive characterization of the effects of CCM on hiPSC-CMs. These data suggest, CCM exerts its effects by, at a minimum, two calcium centric mechanisms including 1) modulation of L-type calcium channels and 2) increased myofilament calcium sensitivity. These data provide the first study of CCM in hiPSC-CMs and demonstrates the potential utility of hiPSC-CMs to assess physiologically relevant mechanisms and evaluate safety and effectiveness of cardiac medical devices.

Introduction

Cardiac contractility modulation (CCM) is a cardiac therapy whereby non-excitatory electrical stimulations are delivered to the heart during the absolute refractory period of the cardiac cycle. Recently, the first CCM device was approved in the U.S. to treat heart failure (HF) patients (NYHA II – IV), with reduced ejection fraction (i.e., < 50 %) and preserved sinus rhythm, an important cause of morbidity and mortality. While pre-clinical models have provided potential mechanistic insight into our understanding of CCM a major hinderance to the detailed study of CCM biology has been the lack of appropriate in vitro human cardiac models. In this work we demonstrate that hiPSC-CMs exposed to clinical CCM pulse parameters exhibit distinct features. This work, the first hiPSC-CM CCM device studies, elucidates the effects of CCM on human cardiomyocyte biology, and provides important insights and evidence of CCM mechanisms including 1) modulation of L-type Ca channels and 2) increased myofilament Ca sensitivity. This work provides a scientific basis for mechanistic regulatory assessment of the performance of current and novel cardiac medical devices using hiPSC-CMs as a model.

Materials and Methods

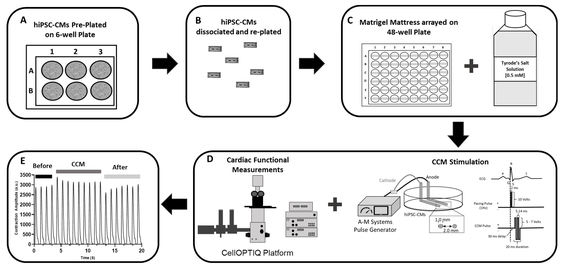

Figure 1. Schematic of human in vitro CCM Model A, hiPSC-CMs are pre-plated in monolayer format on gelatin (0.1%) coated 6-well plates. B, After 2 – 28 days in culture hiPSC-CMs are dissociated and prepared for plating on Matrigel mattress substrate. C, Isolated hiPSC-CMs are plated at high density on Matrigel mattress arrayed in 48-well format (left) and assayed in [0.5 mM] $[Ca]_0$ Tyrode solution (right). D, Commercial pulse generator and standard clinical CCM pulse parameters (right) are used stimulate hiPSC-CMs (right), cardiac function is assessed by video and florescence measurements (left). E, Representative contraction recording before CCM (5V), CCM (10V) and after (5V).

Human iPSC-CM Derivation and Culture:

Cryopreserved Human iPSC Cardiomyocytes (iCell Cardiomyocytes[2], Fujifilm Cellular Dynamic Inc) were thawed and plated according to the manufacture's instruction. Briefly 1,500,000 cells were plated per well of a 6-well plate on 0.1% gelatin and allowed to recover from cryopreservation at least 2 days at 37 °C. Cells were then dissociated, each well was washed twice with 2X volume (i.e., 4 ml) with DPBS then 1 ml of TrypLE™ Express was added and cells we incubated for 15 minutes at 37°C to dissociate. M3 medium consisting of RPMI 1640 with glucose (Invitrogen, cat# 11875); 2% B27 with insulin (Invitrogen, cat#17504-044); 1 % Pen-Strep (Invitrogen, cat#17504) was used to quench the TrypLE™ Express and cells were collected in a 15 ml conical tube and centrifuged at 200 g for 5 minutes at 25°C. Cells were then resuspended in M3 (e.g., 2 ml), counted, and plated on Matrigel mattress substrate as previously described. Matrigel mattresses were arrayed horizontally in 48-well glass bottom plates 1 mattress (i.e., ~ 1 µl) per well and allowed to incubate for 8 - 10 minutes at which point 30,000 cells in 100 µl volume were added directly to the Matrigel Mattress. After 10 – 15 minutes incubation at room temperate the volume was adjusted to 300 µl per well. M3 medium was changed every day thereafter and cells well allowed 2 - 5 days before experiments.

Electrical Field (CCM) Stimulation:

The 48-well glass bottom plate (MatTek) was equipped with a pair of removable platinum wire electrodes placed on parallel sides of the well. Both pacing and CCM electrical pulses were delivered through the same pair of electrodes, resulting in field stimulation of the hiPSC-CMs. Square wave electrical pulses (i.e., monophasic) were generated using AM-Systems software through commercial pulse generator (Model4100, World Precision Instruments, Sarasota, FL). hiPSC-CMs were paced at 1 Hz (2 ms stimulus pulse duration), 11 V/cm (64 mA) and CCM stimulation was delivered as four 5.14 ms biphasic pulses (20.56 ms total duration), 22 V/cm (128 mA). The delay between pacing pulses and CCM stimulation was 30 ms. CCM pulse parameters were comparable to the setting typically used in clinical practice. Pacing and CCM stimulus amplitudes were optimized to obtain consistent cardiac response and maximize the CCM effect.

Results and Discussion

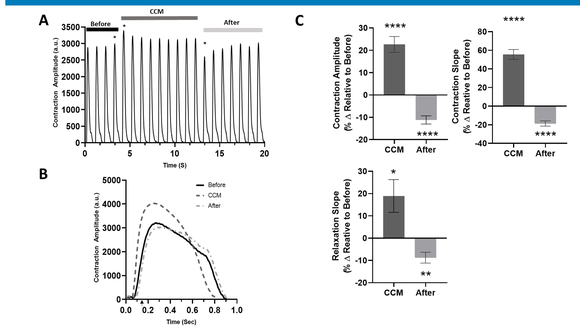

Figure 2. CCM Increases Contraction Amplitude and Kinetics. A. Representative contraction recording for Before (5V), CCM (10V), and After (5V). B. Representative contraction traces of immediate effects. Summary bar graphs. Percent change, data are mean ± SEM. n = 23. *P < 0.05.

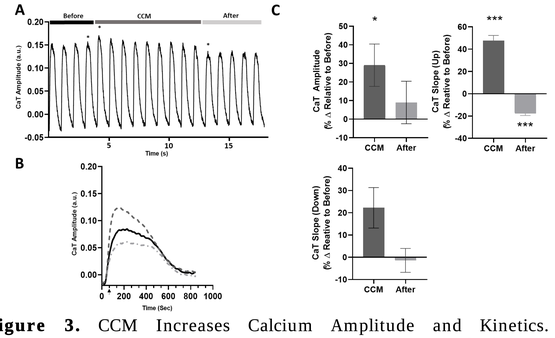

Figure 3. CCM Increases Calcium Amplitude and Kinetics. A. Representative Ca recording for Before (5V), CCM (10V), and After (5V). B. Representative Ca transients of immediate effects. Summary bar graphs. Percent change, data are mean ± SEM. n = 13. *P < 0.05.

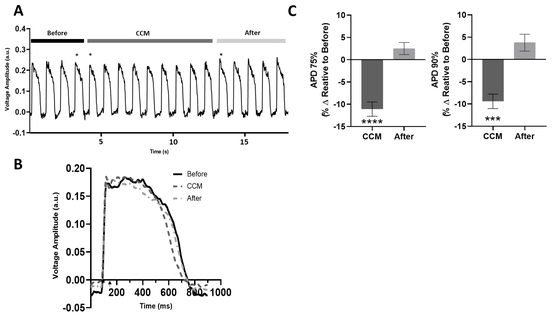

Figure 4. CCM Shortens Action Potential Duration. A. Representative action potential recording for Before (5V), CCM (10V), and After (5V). B. Representative action potential morphology of immediate effects Summary bar graphs. Percent change, data are mean ± SEM. n = 12. *P < 0.05.

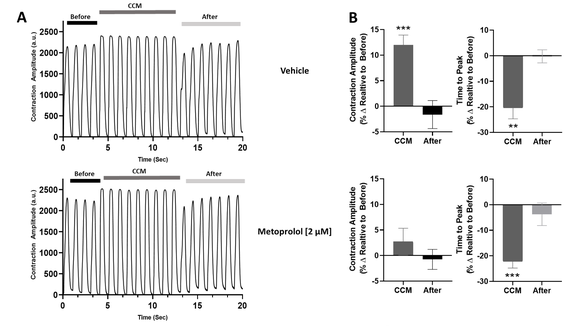

Figure 5. CCM Response is Independent of β-adrenergic signaling. A. Representative contraction traces for each group, Before (5V), CCM (10V), After (5V), hiPSC-CMs were pretreated with Vehicle or Metoprolol [2 µM]. B. Summary bar graphs. Percent change, data are mean ± SEM. n = 9 per group. *P < 0.05.

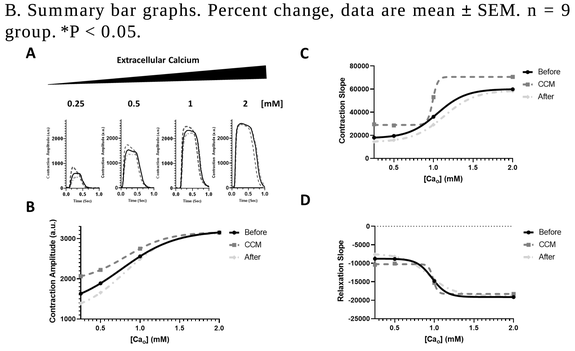

Figure 6. CCM Increases Myofilament Ca Sensitivity. A. Representative contraction traces for each group, Before (5V), CCM (10V), After (5V), hiPSC-CMs were exposed to increasing concentrations of extracellular Ca $[Ca_o]$ 0.25 – 2 mM. B-D. Transformed data demonstrating the effect of CCM on Ca sensitivity of contractile properties (i.e., amplitude and kinetics). n = 6 - 8 per group.

Conclusion

In this study, we establish a robust in vitro method, to quantify the effect of CCM stimulation on human cardiomyocytes and improve regulatory decision-making capabilities. We demonstrate acute CCM stimulation results in significant enhancement of contraction, Ca handling, and electrophysiological properties. Moreover, CCM exerts its effects by, at a minimum, two calcium centric mechanisms including 1) modulation of L-type calcium channels and 2) increased myofilament calcium sensitivity. These data provide the first study of CCM in hiPSC-CMs and demonstrates the potential utility of hiPSC-CMs to assess physiologically relevant mechanisms and evaluate safety and effectiveness of cardiac medical devices. **Disclaimer:** The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services