A Human Cardiomyocyte-Based Platform for the Profiling of Positive Inotropes with Potential to Treat Heart Failure

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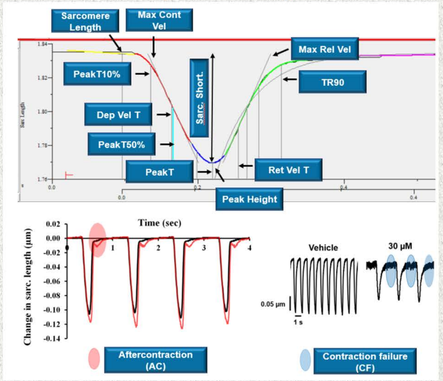
Introduction and Methods

Heart failure remains a major unmet medical need. From a therapy development standpoint, a key challenge originates from the lack of a relevant model to aid the selection of the best candidates for clinical development. Over the last few years, we have focused on the development of strategies and tools to bridge the translational gap by enabling large scale utilization of human primary cells and tissue. Access to cardiac tissue and cardiomyocytes from healthy as well as heart failure hearts, obtained from organ donors, allows for functional, biochemical- and omics-based investigation of the pathophysiology to a level unattainable in the past. To facilitate the identification of molecules with the most desirable efficacy profile, we developed a human cardiomyocyte contractility assay for the identification of positive inotropes with potential to correct contractility deficit in heart failure. Adult human primary ventricular myocytes isolated from ethically consented donor's hearts were used to measure fractional sarcomere shortening induced by field-stimulation and recorded using the IonOptix™ system[1]. The stability of sarcomere shortening was assessed by continuous recording for 2 min. in Tyrode's solution with control vehicle (0.1% DMSO). The test articles were applied for a maximum of 250 s period or when a steady-state effect was achieved. Four ascending concentrations were tested. We modulated excitation-contraction coupling with a panel of well characterized inotropes (17 positives and 9 negatives) with diverse mechanisms of action.

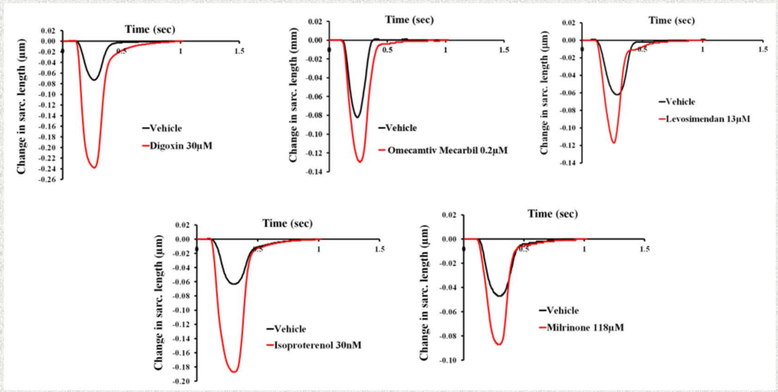
Panel of inotropes with diverse mechanisms of action

Inotropic Effect	Mechanism of Action	Drug
Positive	Na^+/K^+ pump inhibition	Digoxin
Positive	Na^+/K^+ pump inhibition	Ouabain
Positive	Na^+/Ca^{2+} exchanger inhibition	SEA-0400
Positive	Myosin activation	Omecamtiv Mecarbil
Positive	Myosin activation	EMD-57003
Positive	Ca^{2+} sensitization	Levosimendan
Positive	Non-selective b-adrenoceptor activation	Isoproterenol
Positive	Non-selective b-adrenoceptor activation	Epinephrine
Positive	b1-adrenoceptor activation	Dobutamine
Positive	PDE3 inhibition	Milrinone
Positive	PDE inhibition	IBMX
Positive	Ca^{2+} channel activation	Bay-K 8644
Positive	Adenylyl cyclase activation	Forskolin
Positive	Adenylyl cyclase activation	NKH-477
Positive	Calcemia	CaCl2
Positive	SERCA activation	N106
Positive	RyR activation	Caffeine
Negative	SERCA inhibition	Thapsigargin
Negative	RyR inhibition	Ryanodine
Negative	Ca^{2+} channel inhibition	Nitrendipine
Negative	Ca^{2+} channel inhibition	Nifedipine
Negative	Ca^{2+} channel inhibition	Diltiazem
Negative	Ca^{2+} channel inhibition	Mibefradil
Negative	Ca^{2+} channel inhibition	Verapamil
Negative	Na+ channel inhibition	Mexiletine
Negative	Na+ channel inhibition	Flecainide

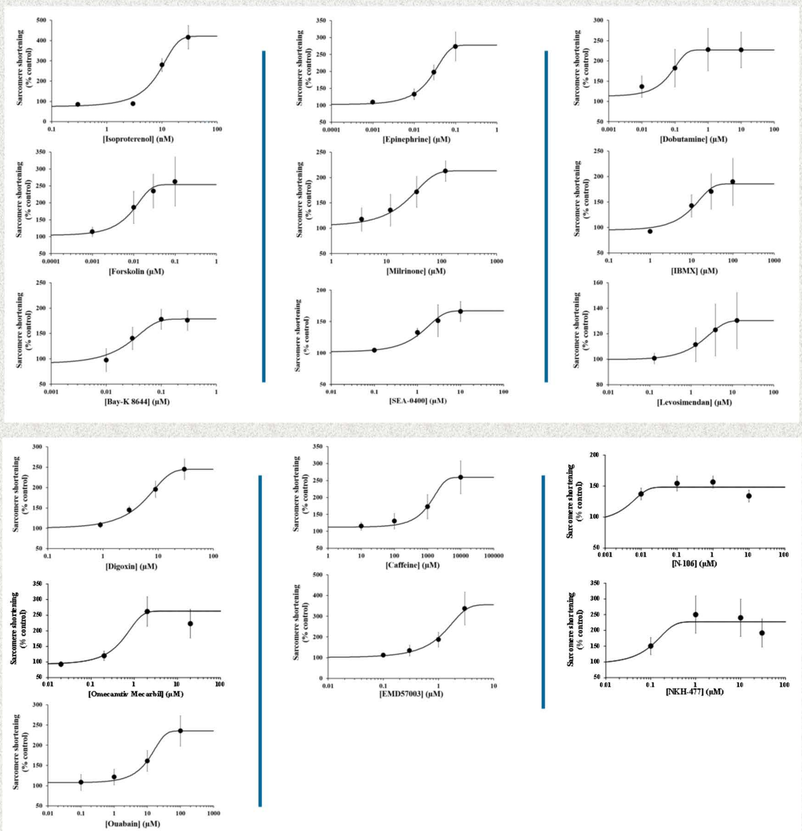
Contractility transient parameters



Identification of drug-induced positive inotropic effects

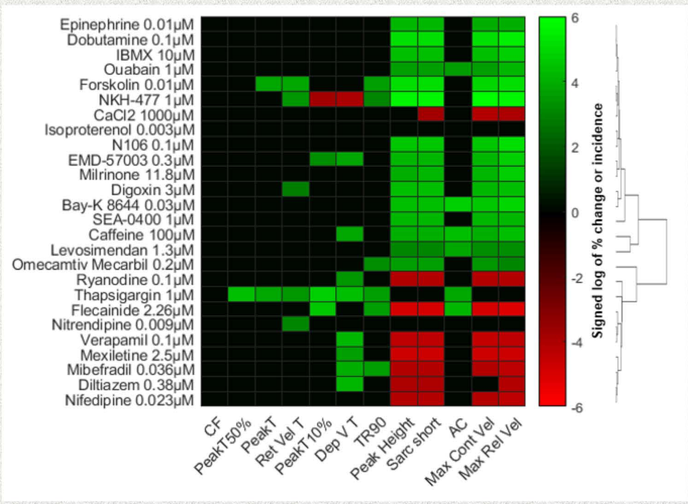


Concentration-Effect curves

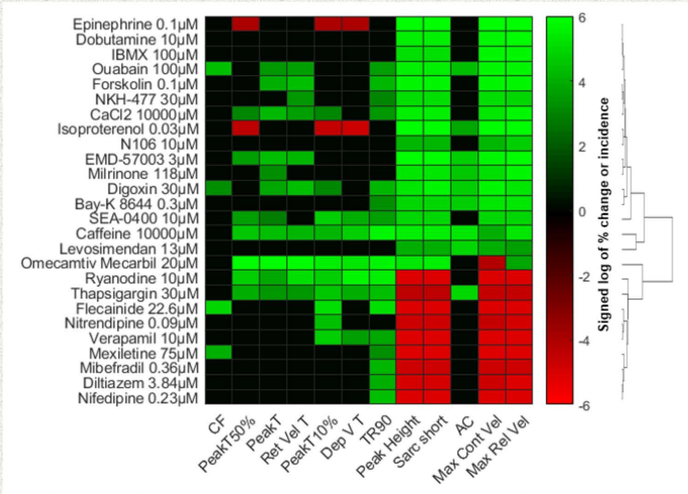


Differentiation of drugs operating via distinct mechanisms

Heatmap generated from 2nd concentration data



Heatmap generated from 4th concentration data



Right-skewed percentage data of incidence (AC and CF) and change (all other transient parameters) were normalized with a signed log transformation. Heatmap representations were created with a cluster analysis using dendrograms and partitions given the elbow criterion. Red and green colors indicate decrease and increase of >25% and >10% change, respectively. **Black** colors indicate no effect (<-25% < % change < 10%). Treatment % effects were expressed relatively to the myocyte's specific baseline control period. AC and CF were expressed as incidence: number of cells showing events normalized by the total number of cardiomyocytes.

Summary

The adult human primary cardiomyocyte-based platform:

1. Can identify inotropic potential of molecules and enable the classification of inotropes in a mechanism-related mode
2. Will facilitate the identification of molecules with the most desirable pharmacological profile for the correction of forms of contractility deficit
3. Scalable, efficient and predictive

1- Nguyen N et al., Front Physiol 8 (2017) 1073 doi: 10.3389/fphys.2017.01073. eCollection 2017