High throughput quantification of inotropic compound effects on adult canine cardiomyocytes via Kinetic Image Cytometry®

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Abstract

Primary adult canine cardiomyocytes have similar electrophysiology and contractility to human cardiomyocytes, making them a preferred cell model for preclinical drug cardiosafety testing and for efficacy testing of therapeutics for heart failure. However, canine cardiomyocytes are expensive to prepare and must be used for testing within a few days after preparation. In this study, Vala researchers used Kinetic Image Cytometry® (KIC®)—Vala's automated, high throughput digital microscopy workstation—to develop more efficient testing methods for canine cardiomyocytes. To evaluate the methods' accuracy, Vala tested three known inotropic compounds: omecamtiv mecarbil, a cardiac myosin activator; isoproterenol, a β-adrenergic receptor agonist; and levosimendan, a troponin C calcium sensitizer. In canine cardiomyocytes treated with DMSO alone or one of the inotropic compounds, Vala's CyteSeer® image analysis software simultaneously measured cell contraction and calcium transients in cells loaded with Fluo-4, a fluorescent intracellular calcium indicator. CyteSeer® also detected contraction of individual sarcomeres in bright field images. Omecamtiv mecarbil and levosimendan had minimal effects on calcium transients but increased contractile motion in a dose-dependent manner. Omecamtiv mecarbil also slowed cardiomyocyte contraction and relaxation at high doses. Isoproterenol increased both contractile motion and the calcium transient amplitude. In bright field images, omecamtiv mecarbil and isoproterenol increased sarcomere shortening. The results demonstrate that KIC® can be used for automated, efficient, and accurate testing for inotropic effects of compounds on canine cardiomyocytes in preclinical drug discovery and cardiosafety screening.

Materials and Methods

Cardiomyocyte handling. Primary adult canine cardiomyocytes (AnaBios, San Diego, CA) were plated into a glass-bottom 96-well plate at a density of 4,400 rod-shaped cells per cm^2. Cells were allowed to settle for at least 5 minutes prior to further manipulation (staining, adding compounds, etc.). Some cells were labeled with Fluo-4 to detect intracellular calcium concentration. These cells were also labeled with Hoechst 33342 to detect nuclei. In certain experiments, cells were not labeled with Fluo-4 ("label-free") and were imaged in bright field.

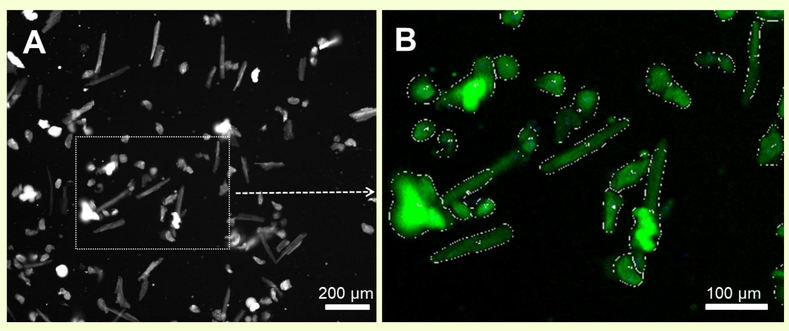
Cardiomyocyte imaging. The cells were rinsed and treated with DMSO alone or inotropic compounds at various concentrations. All cells were prepaced with a 15 V, 6 msec electrical stimulus for 60 seconds at 1 Hz, and then imaged for an additional 10 seconds, with pacing, at 30 frames per second using Vala's IC200 Kinetic Image Cytometer®. Fluo-4-labeled cells were imaged with a 10x NA 0.45 objective (field of view = 2.32 mm^2), and label-free cells were imaged in brightfield with a 20x NA 0.75 objective (field of view = 0.58 mm^2). Image acquisition was accomplished in a fully automated fashion and required approximately 75 seconds per well (60 seconds prepacing, 10 seconds image acquisition, 5 seconds to move to the next well).

Image analysis. In images of Fluo-4-labeled cells, CyteSeer® was used to create cell masks based on Fluo-4 signal and to measure contractile motion and intracellular calcium concentration simultaneously. Cells were selected for analysis if they displayed contractions in response to the stimulation and Fluo-4 intensity changes of >10% compared to the baseline (determined via gates incorporated into the data analysis workflow of CyteSeer®). In bright field images of label-free cells, sarcomere lengths were measured using an autocorrelation algorithm developed by Vala researchers. This algorithm, which is included in CyteSeer®, measures sarcomere length in each frame of an image acquisition at sub-pixel resolution and tracks changes in sarcomere spacing over time.

Vala's IC200-Kinetic Image Cytometer (KIC®)

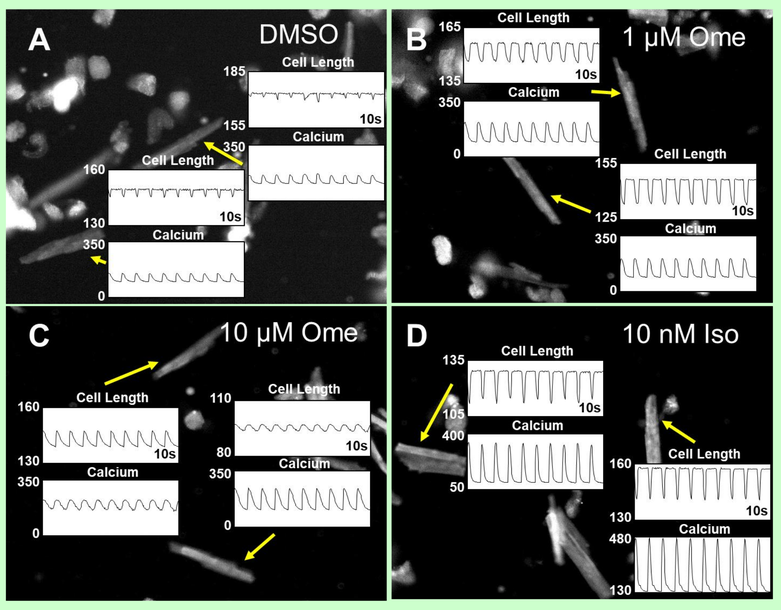
Acquisition frequency	Time-series acquisition at 0.1-100 full frames per second. Time-lapse acquisition for repeated sequence of an imaging protocol.
Stimulation options	Electrical stimulation: available with the automated electrode option. Optogenetics: optical stimulation using any of the available excitation lines.
Environmental control	Temperature (30-40°C) and CO_2 (5-10%) available. Hypoxia (5%, 10%, or 15%) available.
Cameras	Up to two 5.53 MP scientific CMOS cameras
Objectives	Plan APO 4x NA 0.20 (1.63 µm/pixel) Plan APO 10x NA 0.45 (0.64 µm/pixel) Plan APO 20x NA 0.75 (0.32 µm/pixel) Plan APO 40x NA 0.95 (0.16 µm/pixel) Plan APO 60x NA 0.95 (0.11 µm/pixel)
Well plates supported	Supports 1536, 384, 96, 48, 24, 12, and 6 well plates Ability to define custom formats.
Standard filter sets	DAPI, FITC, TRITC, Cy5, and Cy7 standard Other filer sets available
Dimensions	21" x 21" x 22" (53cm x 53cm x 56cm) WxDxH including light engine.

Generating cell masks of canine cardiomyocytes



Cardiomyocytes were seeded in a 96-well plate and labeled with Fluo-4. (A) Full field of view with Fluo-4 signal shown in grayscale. (B) Area in (A) at higher magnification with Fluo-4 signal in green. Cell masks generated by CyteSeer® shown by dashed white lines.

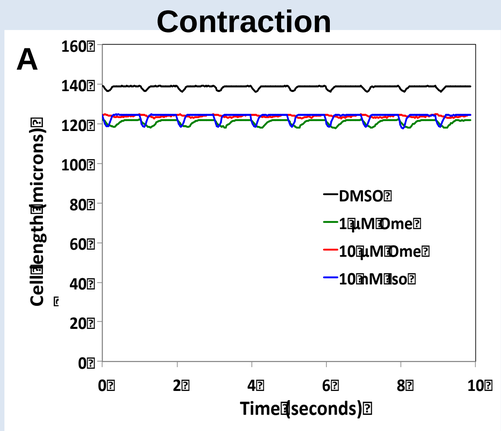
Contractility and calcium measured simultaneously in canine cardiomyocytes



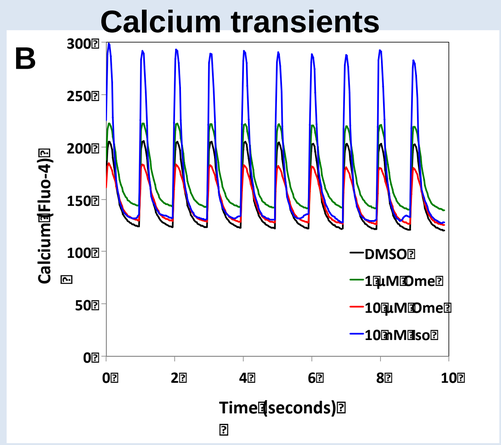
Representative cells treated with DMSO alone (A), 1 µM omecamtiv mecarbil (Ome) (B), 10 µM Ome (C), or 10 nM isoproterenol (Iso) (D). Plots show the cell contraction, measured in microns (top) and the calcium transients, measured by Fluo-4 fluorescence (bottom) for individual cells. Cells were paced at 1 Hz for 60 seconds prior to imaging and during the 10-second image acquisition period.

Omecamtiv mecarbil increases contractility but not calcium transients

Contraction



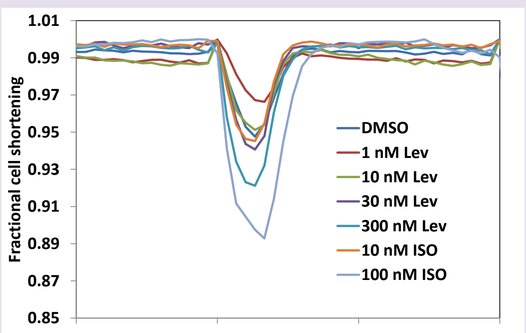
Calcium transients



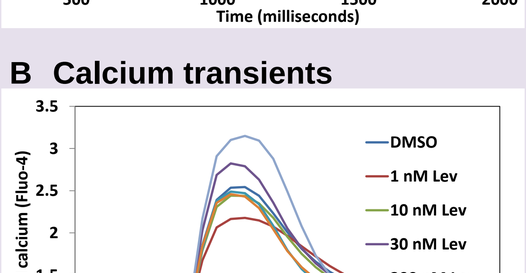
Only cells that had consistent calcium transients in response to the stimulator were included in the average. DMSO: n = 84 cells; 1 µM Ome: n = 60 cells; 10 µM Ome: n = 49 cells; 10 nM Ome: n = 49 cells.

Levosimendan increases contractile motion but not calcium transients

A Contraction

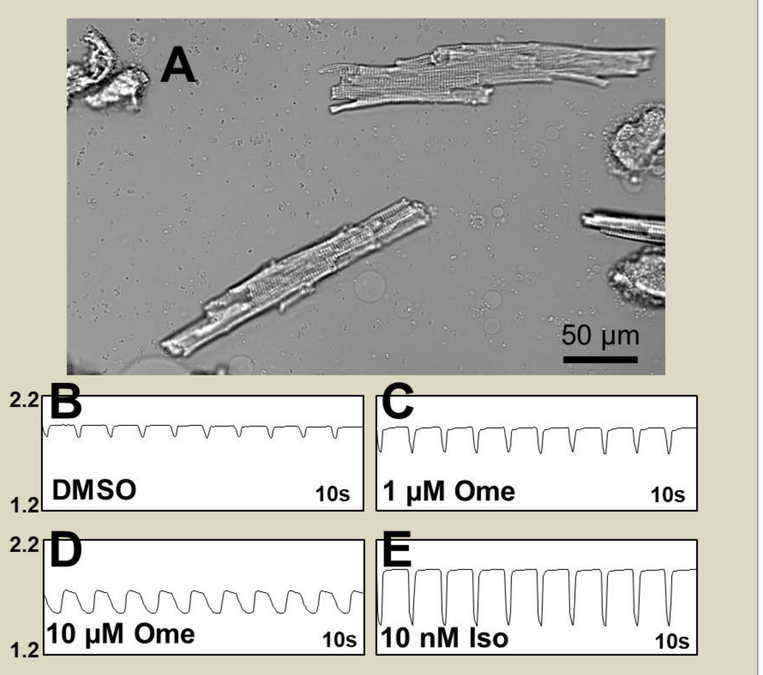


B Calcium transients



Only cells that had consistent calcium transients in response to the stimulator were included in the average. DMSO: n = 71 cells; 1 nM Lev: n = 52 cells; 10 nM Lev: n = 55 cells; 30 nM Lev: n = 38 cells; 300 nM Lev: n = 49 cells; 10 nM Iso: n = 33 cells; 100 nM Iso: n = 30 cells.

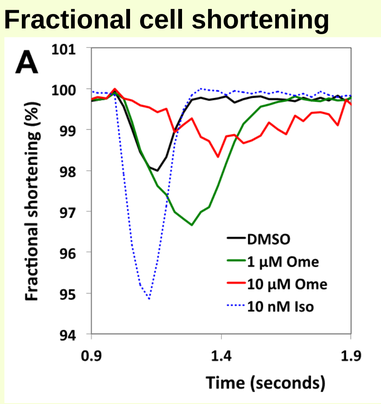
Omecamtiv mecarbil and isoproterenol increase sarcomere contraction



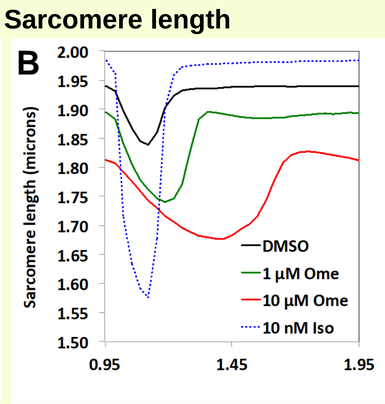
(A) Representative cells treated with DMSO alone and imaged in bright field to visualize sarcomeres. (B-E) Representative plots showing changes in sarcomere length (measured in microns) over time in cells treated with DMSO alone (A), 1 µM omecamtiv mecarbil (B), 10 µM omecamtiv mecarbil (C), or 10 nM isoproterenol (D).

Vala data matches published effects of omecamtiv mecarbil on cardiomyocyte contraction

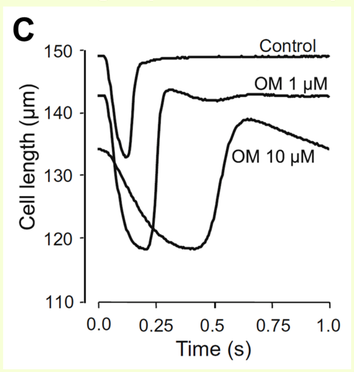
Fractional cell shortening



Sarcomere length



Cell length (Horvath, et al.)



Only cells that had consistent calcium transients in response to the stimulator were included. DMSO: n = 84 cells; 1 µM Ome: n = 60 cells; 10 µM Ome: n = 49 cells; 10 nM Ome: n = 49 cells. (B) Sarcomere length over time measured by CyteSeer for cells treated with DMSO alone (black), 1 µM Ome (green), 10 µM Ome (red), or 10 nM Iso (blue). DMSO: n = 11 cells; 1 µM Ome: n = 5 cells; 10 µM Ome: n = 7 cells; 10 nM Ome: n = 2 cells. (C) Cell length over time in canine cardiomyocytes treated with DMSO alone, 1 µM Ome, or 10 µM Ome (red), as reported by Horvath et al., 2017. Lines show representative records of a single cell for each condition.

Conclusions

Chronic heart failure resulting from reduced cardiac output is on the rise worldwide. Because many current therapies have dangerous side effects due to increased intracellular calcium concentration, development of new drugs is critical to improving patient prognosis. The data presented here demonstrate that Vala's instrumentation (the IC200 KIC®) and image analysis software (CyteSeer®) can simultaneously quantify the effects of inotropic compounds on the magnitude and kinetics of cell contraction and calcium transients in adult canine cardiomyocytes. Vala's technology can also detect sarcomere shortening, thus directly measuring the effects of omecamtiv mecarbil and other compounds on the contractile machinery within cardiomyocytes. Using FluoVolt™, a molecular voltage sensor that increases in fluorescence when cells depolarize, action potentials can also be quantified simultaneously with contractile motion. Vala's technology can also detect sarcomere shortening, thus directly measuring the effects of omecamtiv mecarbil and other compounds on the contractile machinery within cardiomyocytes. Vala's technology can evaluate the effects of potential therapeutics in cardiomyocytes isolated from normal canines or canine models of chronic heart failure, which have similar etiologies to human chronic heart failure. Thus, the IC200 KIC® is a versatile automated microscopy workstation with broad applications across biomedical and cell biology research.

References

Kaplinsky, E. & Mallarkey, G. Cardiac myosin activators for heart failure therapy: focus on omecamtiv mecarbil. *DIC* **7**, 1–10 (2018).

Horváth, B. *et al.* Frequency-dependent effects of omecamtiv mecarbil on cell shortening of isolated canine ventricular cardiomyocytes. *Naunyn-Schmiedeberg's Arch Pharmacol* **390**, 1239–1246 (2017).

Contact

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