APPLICATION NOTE

VALA SCIENCES
Unlocking the Cellular Secrets of Disease

Measuring contractility, calcium transients, and sarcomere shortening in adult canine cardiomyocytes treated with omecamtiv mecarbil

INTRODUCTION

Chronic heart failure currently affects 26 million people worldwide, and its frequency is expected to increase in the coming decades.[1] The primary symptom of this disease is reduced cardiac output resulting from impaired contraction of cardiac muscle cells (cardiomyocytes). Inside cardiomyocytes, the motor protein myosin harnesses the chemical energy in ATP to trigger cyclical cell contractions. Myosin motor domains hydrolyze ATP to ADP and release inorganic phosphate, allowing myosin to bind and pull on actin filaments (Figure 1).[1,2] Following the force-generating power stroke, ATP binds myosin motor domains, releasing them from actin and restarting the cycle. In cardiomyocytes, as in skeletal muscle, myosin and actin are organized into repeating structures called sarcomeres that coordinate the force production of individual myosin molecules, thereby causing the entire cell to contract.[3]

In healthy hearts, action potentials (transient electrical depolarizations) trigger increases in intracellular calcium concentration. Calcium changes the conformation of the actin-binding protein troponin, allowing myosin motor domains to access actin and initiate sarcomere and cardiomyocyte contraction.[4,5] Inotropic compounds, which are commonly used to treat chronic heart failure, stimulate cardiomyocytes to beat harder or faster.[6,7] Many inotropic agents increase intracellular calcium concentration, thus increasing access of myosin to actin and leading to greater force production. However, increased intracellular calcium can also cause excess oxygen consumption, cell toxicity, atrial fibrillation, hypotension, and/or myocardial ischaemia.[1,7] Measuring the effects of potential therapies for chronic heart failure on cardiomyocyte contractility and calcium concentration can predict and mitigate negative side effects.

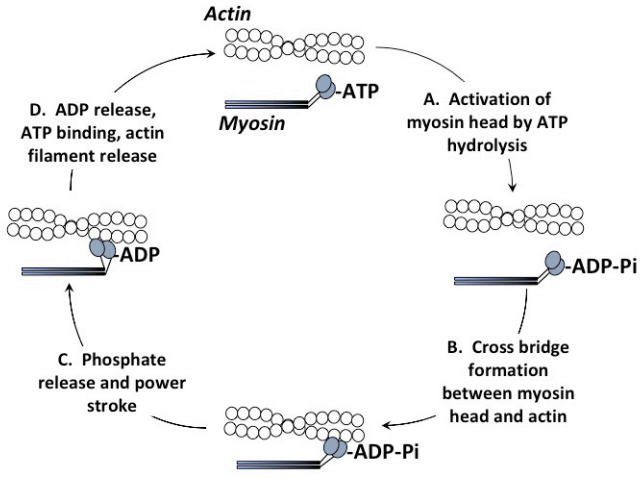

Figure 1. *Myosin force production cycle. ADP, adenosine diphosphate; ATP, adenosine triphosphate; Pi, inorganic phosphate. (adapted from Kaplinsky and Mallarkey 2018).*

In 2010, Cytokinetics, Inc. discovered omecamtiv mecarbil, a first-in-class myosin activator that targets cardiac myosin without affecting smooth or skeletal muscle myosin.[8] Omecamtiv mecarbil increases the proportion of myosin motor domains that bind to actin by increasing myosin's rate of ATP hydrolysis and phosphate release (step C in Figure 1). Omecamtiv mecarbil can therefore improve cardiac myosin force production without changing intracellular calcium levels.[1,8,9] Early clinical trials showed omecamtiv mecarbil to be safe and well tolerated,[1] and current Phase 3 clinical trials are testing the efficacy of omecamtiv mecarbil in patients with chronic heart failure.[10]

In this pilot study, we tested if Vala's IC200 Kinetic Image Cytometer® (KIC®)—an automated, high-throughput digital microscopy workstation designed to record images at high frame rates—can quantify the effects of omecamtiv mecarbil on cardiomyocyte contraction. We used cardiomyocytes from adult dogs, which have similar heart rates and cardiac electrophysiology to humans[11] and are more sensitive to omecamtiv mecarbil than rodents.[12] Using Vala's CyteSeer® image analysis software, we simultaneously measured cell contraction and calcium transients in cells loaded with Fluo-4, a fluorescent intracellular calcium indicator. We also detected contraction of individual sarcomeres in bright field images. We used these data to quantify the effects of omecamtiv mecarbil and the inotropic compound isoproterenol, an adrenaline-related β-adrenergic receptor agonist. This method enables accurate, high-throughput measurements of changes in cell length and calcium transients or sarcomere shortening from large numbers of adult canine cardiomyocytes, an important model system for discovery and development of therapeutics for human heart failure.

METHODS

Isolated adult canine cardiomyocytes (AnaBios, San Diego, CA) were plated into a glass-bottom 96-well plate (Cat. No. 655892, Greiner, Monroe, NC) 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.). Cells were labeled and imaged in a modified Tyrode's solution (145 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 11.1 mM glucose, 10 mM HEPES, 1.8 mM $CaCl_2$, pH 7.4). Some cells were labeled with Fluo-4 (at room temperature for 1 hour in the dark) to detect intracellular calcium concentration. These cells were also labeled with Hoechst 33342 at a final concentration of 2.5 µg/mL to detect nuclei. In certain experiments, cells were not labeled with Fluo-4 ("label-free") and were imaged in bright field.

The cells were rinsed and treated with DMSO alone, 1 µM omecamtiv mecarbil, 10 µM omecamtiv mecarbil, or 10 nM isoproterenol for 15 minutes at 37°C prior to imaging (also at 37°C). The final DMSO concentration in all wells was 0.1%. 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).

Images were analyzed with CyteSeer®. In images of Fluo-4-labeled cells, CyteSeer® was used to create cell masks based on Fluo-4 signal (Figure 2) and to measure contractile motion and intracellular calcium concentration simultaneously. Cells were selected for analysis if

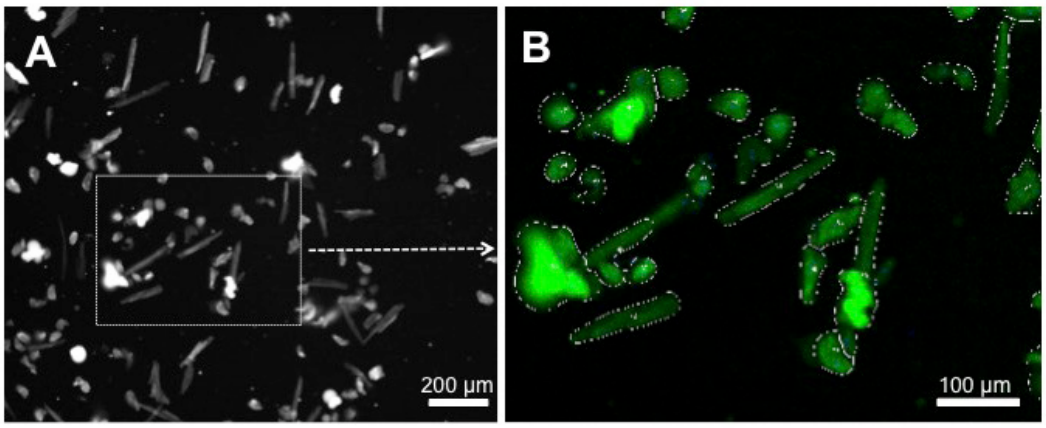

Figure 2. *Generating cell masks of canine cardiomyocytes in the field of view. Cardiomyocytes were seeded in a 96-well plate and labeled with Fluo-4. (A) Full field of view with Fluo-4 signal shown in grayscale. (B) Area in (A) at higher magnification with Fluo-4 signal in green. Cell masks generated by CyteSeer® shown by dashed white lines.*

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.

RESULTS

DMSO-treated canine cardiomyocytes displayed consistent cell contractions and Fluo-4-detected intracellular calcium transients (Figures 3 and 4). Omecamtiv mecarbil had little or no effect on calcium transients. Treatment with 1 µM omecamtiv mecarbil increased the magnitude of cell shortening during contraction. In contrast, cells treated with 10 µM omecamtiv mecarbil exhibited less fractional shortening vs. the DMSO control and the cells contracted and relaxed more slowly. Treatment with 10 nM isoproterenol caused large changes in cell length, and cells contracted and relaxed rapidly. Compared with the other treatment groups, isoproterenol-treated cells have larger calcium transients with quicker upstroke and decay. Also, cells exposed to omecamtiv mecarbil and isoproterenol had shorter maximum cell lengths compared to controls, as previously observed for omecamtiv mecarbil.[9] This effect may be related to increased actomyosin contractility

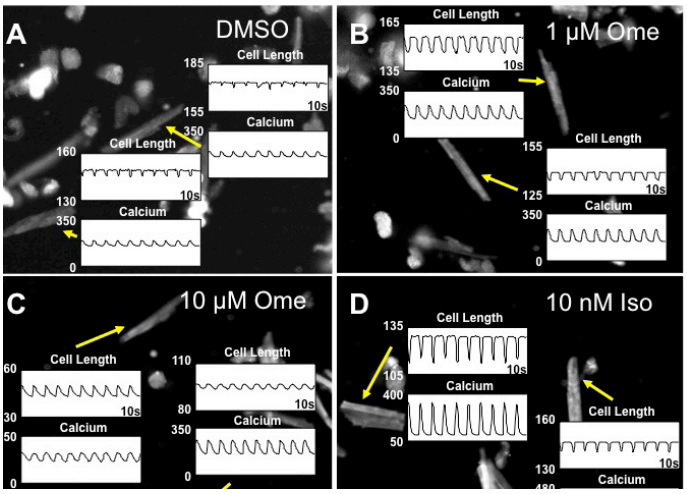

***Figure 3. Contractile motion and calcium transients measured simultaneously from cardiomyoctes**. Representative cells treated with DMSO alone (A), 1 µM omecamtiv mecarbil (Ome) (B), 10 µM Ome (C), or 10 nM isoproterenol (Iso) (D). Plots show the cell contraction, measured in microns (top) and the 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.*

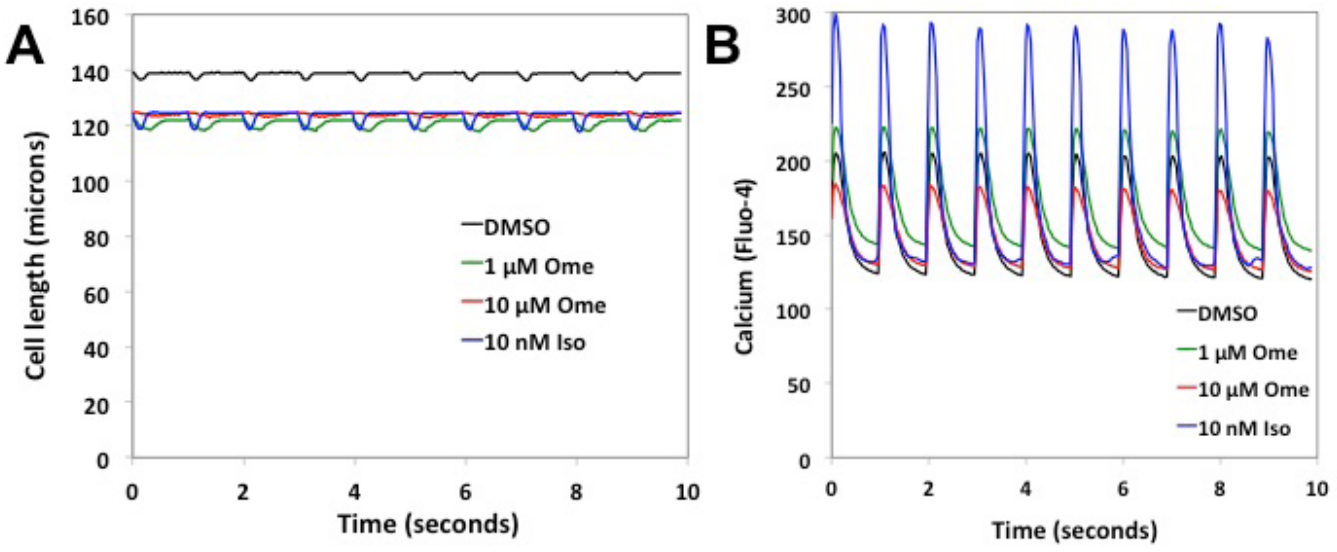

***Figure 4. Averages of cell-by-cell traces from each treatment condition.** Average cell length (A) and intracellular calcium concentration (B) over time for cells treated with DMSO alone (black), 1 µM Ome (green), 10 µM Ome (red), or 10 nM Iso (blue). Only cells that had consistent calcium transients 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.*

during diastole, which may prevent full relaxation in cardiomyocytes paced at 1 Hz, as in these experiments.

Analysis of sarcomere length in bright field images revealed shortening similar in proportion to the shortening of cell length (Figure 5). As in whole cells, treatment with omecamtiv mecarbil increased the magnitude of sarcomere contractions, but 10 µM omecamtiv mecarbil slowed contraction and relaxation kinetics. Treatment with isoproterenol increased the magnitude and speed of sarcomere contractions, as expected for this β-adrenergic receptor activator.

Vala's data on fractional cell shortening and sarcomere contractions are similar to published data on the effects of omecamtiv mecarbil on cell contractions (Figure 6).[9] These results demonstrate that Vala's IC200 KIC® and CyteSeer® analysis software can quantify the effects of inotropic compounds on sarcomere contraction or both cell contraction and intracellular calcium transients in canine cardiomyocytes, which are widely used in preclinical research in the search for effective therapeutics for heart failure.

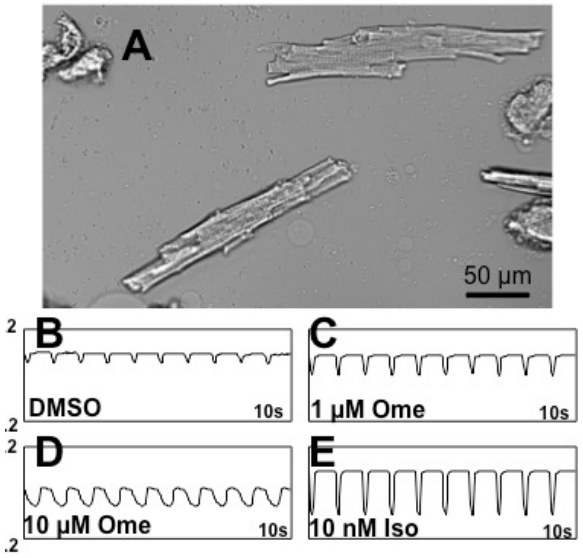

Figure 5. Sarcomere contraction measured from cardiomyoctes. *(A) Representative cells treated with DMSO alone and imaged in bright field to visualize sarcomeres. (B-E) Representative plots showing changes in sarcomere length (measured in microns) over time in cells treated with DMSO alone (A), 1 µM Ome (B), 10 µM Ome (C), or 10 nM Iso (D).*

DISCUSSION

Chronic heart failure resulting from reduced cardiac output is on the rise worldwide.[1] Because many current therapies have dangerous side effects due to increased intracellular calcium concentration, development of new drugs is critical to improving patient prognosis. Measuring the effects of potential therapies on canine cardiomyocytes, which have similar electrophysiology to human cardiomyocytes, can help predict and mitigate side effects. The data above 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 canine cardiomyocytes. Using FluoVolt™, a molecular voltage sensor that increases in fluorescence when cells depolarize,[13] 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.

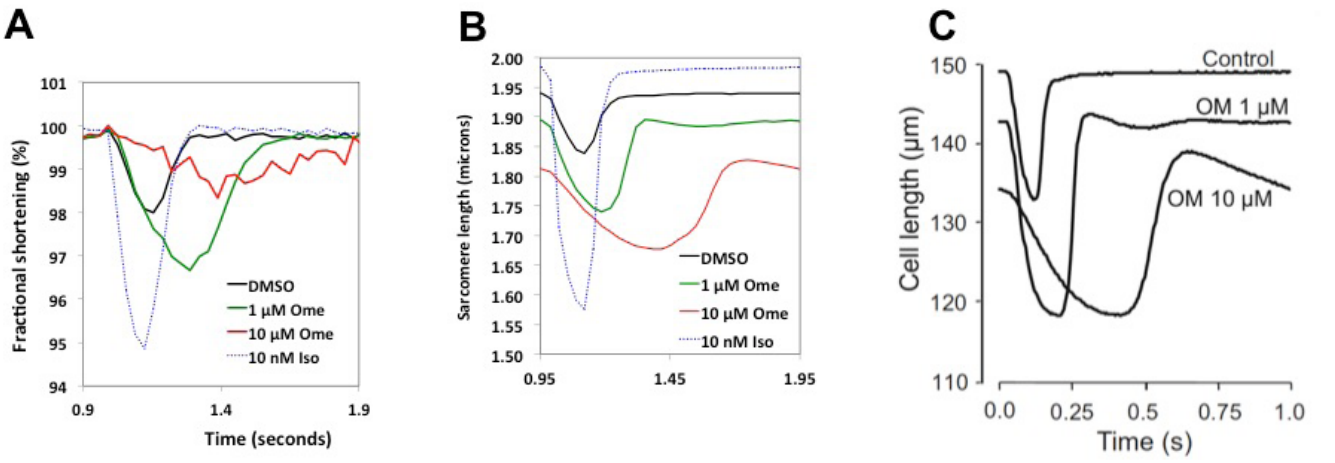

Figure 6. Comparison of Vala data with published effects of omecamtiv mecarbil on cardiomyocyte contraction. *(A) Fractional shortening (current cell length/maximum cell length) measured be CyteSeer for cells treated with DMSO alone (black), 1 µM Ome (green), 10 µM Ome (red), or 10 nM Iso (blue). Only 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

This report demonstrates that Vala's IC200 KIC® and CyteSeer® can detect cell contractility, calcium transients, and sarcomere shortening in adult canine cardiomyocytes. Vala's technology can evaluate the effects of cardiac myosin activators or other potential therapeutics in cardiomyocytes isolated from normal canines or canine models of chronic heart failure, which have similar etiologies to human chronic heart failure.[14,15] Vala's IC200 KIC® can also measure contractility and/or calcium transients in cardiomyocytes differentiated from human induced pluripotent stem cells and other excitable cells such as neurons or skeletal muscle to produce data relevant to chronic heart failure, cardiomyopathies, neurodegeneration, or muscular dystrophies. Thus, the IC200 KIC® is a versatile automated microscopy workstation with broad applications across biomedical and cell biology research.

CONTACT US

Please contact Vala Sciences, Inc at www.valasciences.com/contact, info@valasciences.com, or toll-free at (858) 742-8252 for more information about purchasing an instrument, which includes a copy of CyteSeer®. We also provide drug discovery or safety screening services using our technologies.

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