The novel myotrope, AMG 594, is a small-molecule cardiac troponin activator that increases cardiac contractility *in vitro* and *in vivo*

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INTRODUCTION

Heart failure (HF) is the final pathway for many diseases that affect the heart. It is a clinical syndrome marked by impaired cardiac function and characterized by an imbalance between tissue demand and cardiac output (Roger *et al*, 2012; Hilfiker-Kleiner *et al,* 2006). Presently, there are approximately 5.7 million cases in the United States (Mozaffarian *et al,* 2016). HF requires lifelong treatment, and despite all different modalities available, morbidity and mortality remain high. Currently, all available chronic therapies target only the compensatory neurohormonal cascades, without treating the primary pathophysiologic problem in HF. Improvement in cardiac contractility through direct activation of the cardiac sarcomere is, thus, a potential strategy to treat HF. Recently, a new class of drugs known as sarcomere activators has been developed as an alternative therapeutic approach for the failing heart muscle (Liu *et al,* 2016; Malik *et al,* 2011). The first sarcomere activator, *omecamtiv mecarbil*, a cardiac myosin activator, is currently being evaluated in a Phase 3 clinical study. AMG 594 is a potent and selective activator of cardiac troponin and acts on the sarcomere by sensitizing cardiac troponin to existing intracellular calcium, leading to more myosin heads engaging actin filaments, and more contractile force being generated.

OBJECTIVES

The pharmacology of AMG 594 was profiled in a series of *in vitro* biochemical assays to define the molecular target and mechanism of action. *In vivo* proof of concept was demonstrated in two species, rat and dog.

METHODS

Animals were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals*; all research protocols were approved by an Institutional Animal Care and Use Committee.

Preparation of Reagents: Myofibrils were prepared from flash-frozen bovine cardiac, bovine masseter, and rabbit psoas tissue as described in Hwee *et al,* (2015). Bovine cardiac myosin subfragment-1 (S1), actin, troponin, and tropomyosin were prepared as described in Malik *et al,* (2011).

ATPase Assays: Steady-state ATPase activity was measured using a pyruvate kinase and lactate dehydrogenase-coupled enzyme system as described in Hwee *et al.* (2015) and Malik *et al,* (2011). Non-myosin ATPase activity was subtracted from cardiac and slow skeletal myofibril assays in Figure 2 by subtracting the ATPase activity in the presence of a saturating concentration of the non-selective myosin II inhibitor (-)-blebbistatin.

Skinned Cardiac Muscle Fiber Assay: Adult rat ventricular tissue bundles were skinned using 0.5% Brij 58 followed by storage in 50% glycerol as per Lynch and Faulker (1998). Muscle fibers were dissected from larger segments of tissue in rigor buffer at 4ºC (20 mM MOPS, 5 mM $MgCl_2$, 120 mM potassium acetate, 1 mM EGTA, pH 7.0) and subsequently attached to a force transducer (403A, Aurora Scientific, Ontario, Canada) and a fixed post using a 5% solution of methylcellulose in acetone. Isometric tension was measured at 10ºC in a relaxing buffer (20 mM MOPS, 5.5 mM $MgCl_2$, 132 mM potassium acetate, 4.4 mM ATP, 22 mM creatine phosphate, 1 mg/ml creatine kinase, 1 mM DTT, 44 ppm antifoam , pH 7.0) supplemented with $CaCl_2$ to produce appropriate concentrations of free Ca^{2+}.

Measurement of Cardiomyocyte Contractility and Calcium Transients: Adult rat ventricular cardiomyocytes were isolated and loaded with Fura-2 as described in Malik *et al.* (2011). Cardiomyocyte contractility and calcium transients were measured by edge-detection video microscopy and fluorescence photometry (IonOptix, Milton, MA) as described in Malik *et al,* (2011).

In Vivo Rodent Pharmacodynamics: Normal (healthy) CD rats were purchased from CRL (Hollister, 9-10 wks of age). Myocardial infarcted (MI) SD rats with permanent ligation of the left descending coronary artery (LAD) were purchased from Envigo (Indianapolis; 9-11 wks of age at time of dosing; baseline ejection fraction (EF)< 45%). Echocardiography (Vevo 2100) was conducted in isoflurane-anesthetized normal (SAX M-mode) or MI rats (PLAX B-mode) during a single, continuous IV administration of AMG 594 or vehicle. The dose delivered was escalated by increasing the infusion rate in a stepwise manner with the aid of a programmable syringe pump. IV infusion of the vehicle was delivered at the same infusion rates used for the AMG 594 infusion for time-matched comparison. Every 5 minutes, 5 µL of whole blood (expressed from a small nick in the tail) was collected for subsequent bioanalytical determination of test article exposure.

In Vivo Canine Pharmacodynamics: Cardiovascular effects during IV infusion of AMG 594 were evaluated in an exploratory study using α-chloralose-anesthetized normal healthy beagle dogs (n = 6 males). Using a cumulative dose escalation design, dogs were administered IV infusion doses of vehicle or AMG 594 in a step-wise infusion. IV infusion of the vehicle was delivered at the same infusion rates used for the AMG 594 infusion for time-matched comparison. Echo images (SAX M-mode) and plasma samples for exposure assessment were collected every 10 minutes during each infusion period.

RESULTS

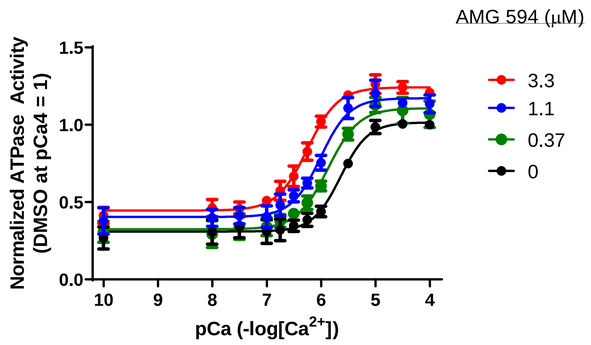


Figure 1. AMG 594 sensitizes the ATPase activity of bovine cardiac myofibrils to activation by calcium. The ATPase activity of Triton X-100-skinned bovine cardiac myofibrils was measured as described in the *Materials and Methods*. Data shown are mean values ± SD (n=4).

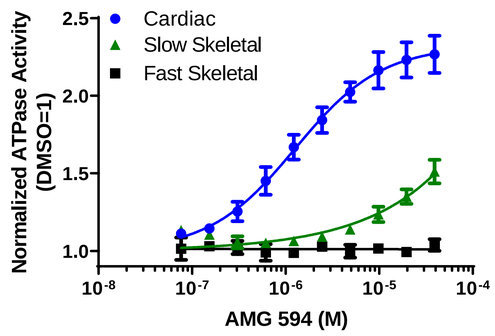


Figure 2. AMG 594 selectively activates the ATPase activity of cardiac myofibrils. Dose-response analysis was performed with cardiac (bovine, n=9), slow skeletal (bovine, n=5), and fast skeletal (rabbit, n=4) detergent-skinned myofibrils as described in the *Materials and Methods*. Data shown are mean values ± SD.

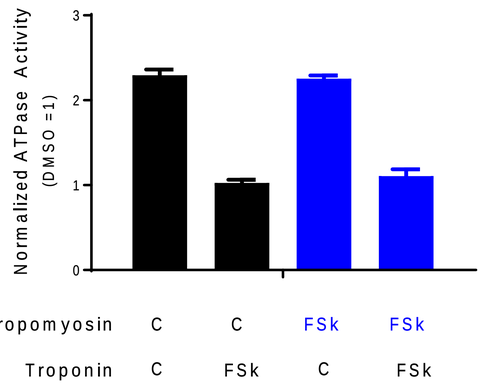


Figure 3. AMG 594 selectively activates hybrid reconstituted sarcomere assays containing cardiac troponin. Thin filament activation (as indicated by an increase in myosin ATPase activity) was observed with thin filaments containing cardiac (C) troponin but not fast skeletal (Fsk) troponin. The tropomyosin isoform did not substantially affect the myosin ATPase, indicating the cardiac troponin complex is required for compound activity. Reactions were performed as described in the *Materials and Methods*. Data shown are mean values ± SD (n=8 replicate reactions).

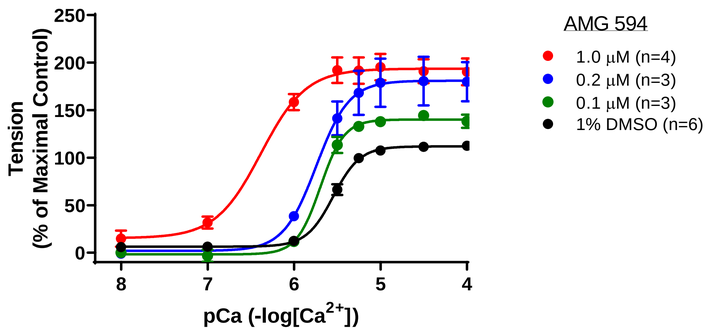
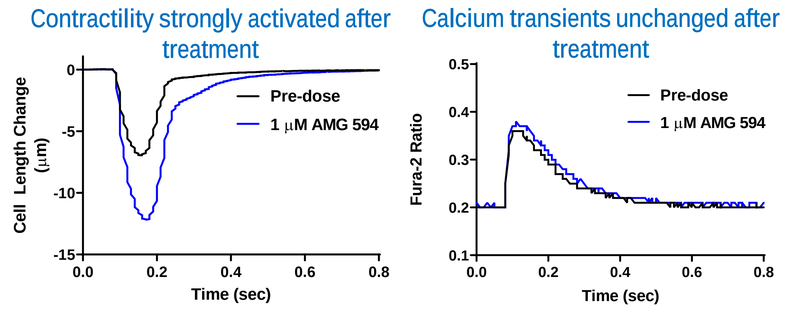


Figure 4. AMG 594 increases calcium sensitivity and isometric tension at saturating calcium in permeabilized adult rat cardiac muscle fibers. Force production by detergent-skinned adult rat cardiac muscle fibers was measured at 10ºC as described in the *Material and Methods*. Data shown are mean values ± SEM.



Treatment	N	Fractional Shortening (% of basal)	Fura-2 Ratio		
			Diastolic	Systolic	Time to Baseline 75% (T_{75}, seconds)
Pre-dose	6	100	0.183 ± 0.015	0.345 ± 0.015	0.242 ± 0.015
1.0 µM AMG 594	6	139.3 ± 11.3*	0.194 ± 0.011	0.344 ± 0.020	0.274 ± 0.017

Figure 5. AMG 594 increases shortening of isolated adult rat ventricular myocytes without increasing calcium transients. Shown is a representative time course of cell shortening (left) and calcium transients (right) measured using the ratio of Fura-2 fluorescence when excited at 340 and 380 nm. Data shown are for one cell before (pre-dose) and after treatment with 1 µM AMG 594. The table shows compiled results from six cells. AMG 594 (1 µM) significantly increases fractional shortening (* = $p < 0.05$) without increasing any calcium transient parameter (p = NS). Data are presented as mean values ± SEM.

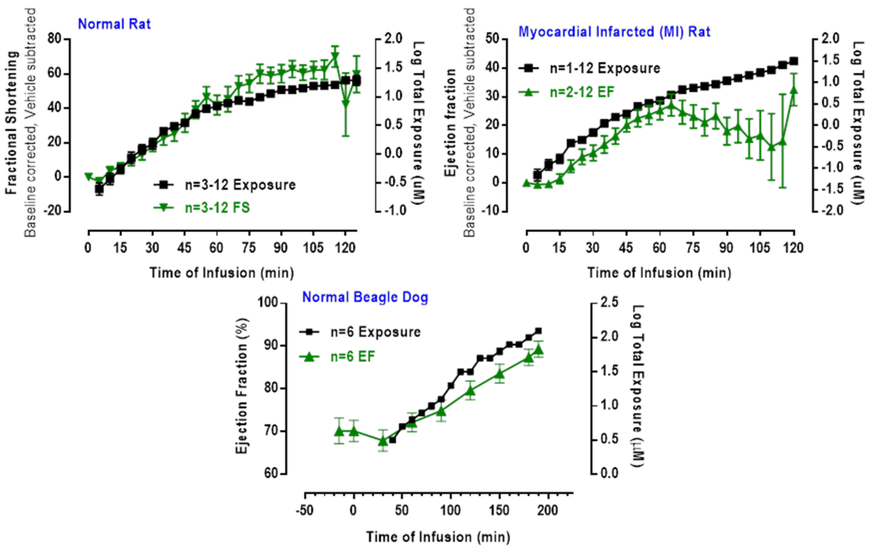


Figure 6. Acute pharmacodynamics of AMG 594 in rat and canine models. Echocardiographic assessment was conducted in normal rats, myocardial infarcted rats, and normal beagle dogs during a single, continuous IV administration of AMG 594 under anesthesia. Intravenous infusion of an escalating dose of AMG 594 was associated with a time-dependent increase in the total concentration of AMG 594 in whole blood. Exposure-dependent increases in measures of fractional shortening (FS) or ejection fraction (EF) were observed, and were driven primarily by a reduction in left ventricular end systolic dimensions. Data are presented as mean values ± SEM.

CONCLUSIONS

AMG 594 is a first-in-class, selective, small-molecule cardiac troponin activator that increases cardiac contractility, potentially providing a viable approach to augment cardiac contractility in numerous disease states marked by reduced cardiac function.

REFERENCES

Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics-2012 update: a report from the American Heart Association. *Circulation*. 2012;125:e2-e220.

Hilfiker-Kleiner D, Landmesser U, Drexler H. Molecular mechanisms in heart failure. Focus on cardiac hypertrophy, inflammation, angiogenesis, and apoptosis. *J Am Coll Cardiol*. 2006;48(9):A56-A66.

Mozaffarian D, Benjamin EJ, Go AS, et al. American Heart Association Statistics Committee; Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2016 Update: A report from the American Heart Association. *Circulation.* 2016;133:e38-e360.

Liu LC, Dorhout B, van der Meer P, Teerlink JR, Voors AA. Omecamtiv mecarbil: a new cardiac myosin activator for the treatment of heart failure. *Expert Opin Investig Drugs*. 2016;25(1):117-127.

Malik FI, Hartman JJ, Elias KA, et al. Cardiac myosin activation: a potential therapeutic approach for systolic heart failure. *Science.* 2011;331(6023):1439-1443.

Hwee, Darren T., et al. "The small-molecule fast skeletal troponin activator, CK-2127107, improves exercise tolerance in a rat model of heart failure." *Journal of Pharmacology and Experimental Therapeutics* 353.1 (2015): 159-168.

Lynch, G. S., & Faulkner, J. A. (1998). Contraction-induced injury to single muscle fibers: velocity of stretch does not influence the force deficit. *American Journal of Physiology-Cell Physiology*, *275*(6), C1548-C1554.

DISCLOSURES

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