Acute Cardio-Selective Functional Modulation via a Small-Molecule Direct Myosin-Attenuator (MYK-581) Preserves *in vivo* Diaphragmatic Function in rats: Comparison versus Disopyramide

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INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a progressive cardiac disease characterized by sarcomeric dysfunction, hyperdynamic contraction, hindered ventricular relaxation/filling, and impaired exercise capacity. Conventional negative inotropes can alleviate the inotropic burden in HCM, but do not improve ventricular filling and have the potential to further decrease exercise capacity due to their off/on-target effects. Recently, a novel small molecule cardiac-myosin attenuator, mavacamten, has been shown to normalize hyper-contractility in patients with obstructive HCM, while improving cardio-pulmonary exercise indices (e.g., Heitner et al., 2019)

This study evaluated and compared the *in vivo* and *ex vivo* functional selectivity of MYK-581, a mavacamten analog, with those of disopyramide (DISO), a negative inotrope commonly used to treat HCM patients..

METHODS

In vivo: acute cardiac (left ventricular pressures, LVP) and neuro-muscular (force of diaphragmatic and skeletal muscle contractions) responses to MYK-581 (up to 0.5 mg/kg IV, n = 8) and DISO (up to 10 mg/kg IV, n = 8) were simultaneously evaluated *in situ* using anesthetized (isoflurane) and mechanically-ventilated Sprague-Dawley rats; diaphragmatic (both intrinsic and phrenic nerve stimulated) and skeletal contractions (stimulated quadriceps) were measured via strain gauges. In another set of rats, cardiac effects were evaluated by echocardiographic and LVP assessments before/after MYK-581 (n = 5) and DISO (n = 5) administration.

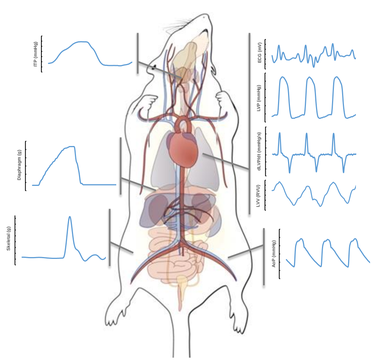

Figure 1. Schematic of the *in vivo* rat preparation, showing representative tracings for cardiac (right: ECG, LVP, dLVP/dt, LVV, and AoP), respiratory (intratracheal pressure, ITP), diaphragmatic and skeletal force signals

In vitro: the functional activity /selectivity of MYK-581 was evaluated both *ex vivo* via vascular preparations and cardiac (papillary) skinned fiber Ca^{2+}-sensitivity assays, as well as *in vitro* with biochemical assays (ATPase activity) using rat myofibrils (cardiac and diaphragmatic) and myosin-S1s with actin (14 µM). Tension changes in aortic and mesenteric artery rings were studied.

RESULTS

MYK-581: selective direct cardiac functional modulation

in vitro & ex vivo

MYK-581 dose-dependently decreased cardiac myofibrillar ATPase rate (IC_{50}: 0.42 µM @pCa 6.25) showing lower (P < 0.05) potency in diaphragmatic myofibrils (IC_{50}: 1.48 µM, @pCa 6.5) (see Fig. 2A).

Similarly, preferential cardiac activity was seen with clean myosin-S1/actin from cardiac (bovine @0.25 µM, IC_{50}: 0.32 µM), skeletal (rabbit @0.1 µM, IC_{50}: 2.23 µM), and smooth muscles (chicken gizzard @0.5 µM, IC_{50}> 30µM) (see Kawas et al., 2017). In line with these observations, MYK-581 showed negligible effects in aortic (-13.5 ± 2.9% at 30 µM) and mesenteric artery preparations.

In rat permeabilized papillary fibers (Fig 2B), MYK-581 dose-dependently decreased maximal tension, while blunting length-dependent activation (LDA) between short (2.0 µm) and long (2.3 µm) sarcomere lengths (SL). (Green et al., 2016, Anderson et al., 2018, Ma et al., 2019)

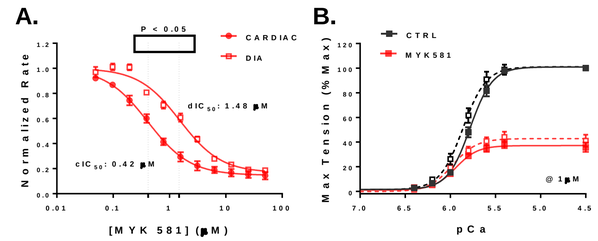

Figure 2. LEFT: DMSO normalized steady-state ATPase rates in cardiac (closed circles) and diaphragmatic (open squares) rat myofibrils exposed to MYK-581. **RIGHT:** Effect of MYK-581 (at 1 µM) treatment in tension-pCa curves at short (closed) and long (open) SL.

MYK-581 decreased indices of systolic function, such as dP/dt_{max} (-24.9 ± 3.3, n = 13; P < 0.05 vs. PRE) and ejection fraction (EF: -21.5 ± 1.5%, P < 0.05 vs. PRE); reductions in LV systolic function were predicted by plasma exposures. Functional depression with MYK-581 was accompanied by end-diastolic volume increases (EDV: +14.3 ± 3.5%, P < 0.05 vs. PRE), but preserved end-diastolic pressures (EDP: -4.5 ± 6.7%, from 5.7 ± 0.4 to 5.6 ± 0.5 mmHg) suggesting improved ventricular distensibility/compliance (See Fig. 3A) (as supported by a blunted LDA in fibers).

Despite these marked cardiac effects (see Fig. 4), MYK-581 preserved both diaphragmatic (23.7 ± 2.0 to 23.3 ± 2.2 g) and skeletal developed force (14.9 ± 1.4 to 13.9 ± 0.8 g). In contrast, DISO at matched levels of negative inotropy (dP/dt_{max}: -20.2 ± 2.5, n=13; P < 0.05 vs. PRE; see Fig. 4A/B) depressed diaphragmatic force (-16 ± 3%, 23.8 ± 1.9 to 19.8 ± 1.2 g, P < 0.05)

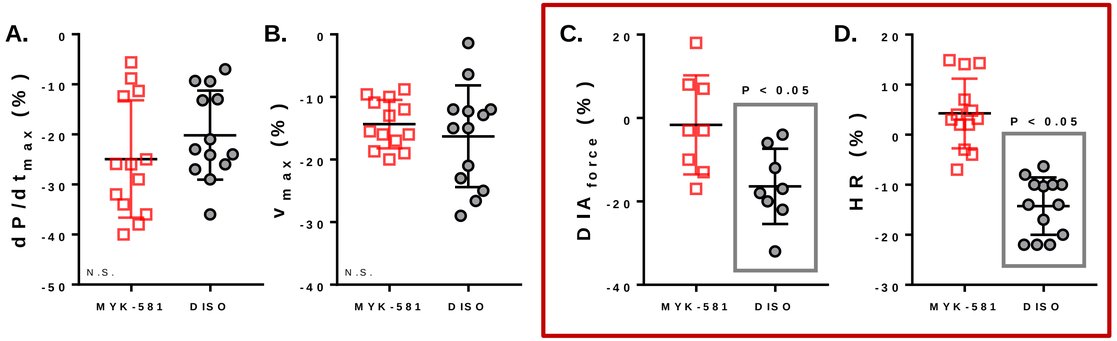

Figure 4. Relative changes in dP/dt_{max} (A) and (estimated) v_{max} (B), two inotropic indices derived from the LVP, as well as in diaphragmatic developed force (C) and heart rate (D) in response to either MYK-581 (red) or DISO (gray)

In addition, MYK-581 increased the LV diastolic filling rate (DFR: +69 ± 16%, from 4.0 ± 0.4 to 6.9 ± 1.3 µL/ms; P < 0.05) while preserving the end-diastolic wall stress (σ_{ed}: +5.4 ± 0.9%). DISO, on the other hand, maintained DFR (-4 ± 8%, P < 0.05 vs. MYK-581), despite slowing heart rate (HR, see Fig4D) and prolonging the diastolic filling period. DISO also increased σ_{ed} (+80 ± 26%) due to elevations in EDP (+62 ± 27%, from 4.2 ± 0.9 to 6.4 ± 1.0 mmHg, P < 0.05 vs. PRE), likely reflecting decreased distensibility.

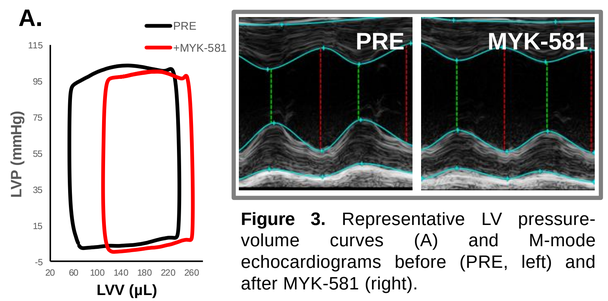

Figure 3. Representative LV pressure-volume curves (A) and M-mode echocardiograms before (PRE, left) and after MYK-581 (right).

CONCLUSIONS

Direct myosin modulation with MYK-581, a mavacamten analog, is characterized *in vivo* by reductions in systolic function with preserved filling pressures and improved LV compliance.

Unlike disopyramide, MYK-581's cardiac actions were observed in the setting of preserved diaphragmatic and skeletal force, and were free of vascular effects. confirming its biochemical cardio-selectivity. This novel pharmacological profile could have salutary effects in patients with HCM and/or impaired ventricular filling presenting with decreased exercise capacity.

REFERENCES

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DISCLOSURES

All authors, except Roof SR, are employees and have significant financial interests with MyoKardia