Elucidating the mechanism of Danicamtiv on force, kinetics, and myosin structure and function

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Myosin modulators are a novel class of small molecules that alter cardiac contractility. Omecamtiv mecarbil, the first identified myosin activator, showed only modest clinical benefits in systolic heart failure patients. Thus, there is an urgency to develop alternative myosin activators. Danicamtiv (Dani) has emerged as a potential candidate; however, a detailed mechanism is not known. Here, we aim to elucidate the mechanism of Dani on contractile function in pig cardiac muscle. Demembranated ventricular tissues show a significant 0.1 pCa unit increase in calcium sensitivity and 10% increase in maximal force after incubation in 1 µM Dani. The most potent effects occur in submaximal calcium concentrations, leading to a flattening of the force-calcium relationship, suggesting decreased cooperativity. Maximal rates of tension redevelopment are decreased by approximately 60% with Dani. Isolated cardiac myofibrils provide details about contractile kinetics. Experiments with 1 µM Dani show a 49% decrease in fast-phase relaxation kinetics. Slow-phase isometric relaxation exhibits 47% slower crossbridge detachment rate and 34% longer thin filament deactivation. Next, we assess ATP utilization in the crossbridge cycle. Filament sliding velocity slows 55% on addition of 0.5 µM Dani, similar to the effect of ADP on velocity. The effects of Dani and ADP are not additive suggesting a similar mode of action. ATP binding is unaltered up to 10 µM Dani using stopped flow spectroscopy. Results of X-ray diffraction studies of porcine myocardium at rest show an increase in equatorial intensity ratio (I1,1/I1,0) in response to 50 µM Dani, reflecting an increased proximity of myosin heads to the thin filament. In conclusion, we hypothesize that Dani primes the thick filament for activation and alters relaxation through inhibited ATP hydrolysis product release. Future studies will test these hypotheses.