Validating MARK4 Inhibitors as Novel Inotropes

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Introduction

Problem
- Myocardial infarctions (MI) severely compromise cardiac function and often result in chronic heart failure.

Identifying a Mechanism
- Previous studies[1] have identified an inverse relationship between microtubule detyrosination in cardiomyocytes and left ventricular ejection fraction.
- Microtubule affinity regulating kinase 4 (MARK4) modulates post-translational modifications in α-tubulin. More specifically, its activity is positively associated with microtubule detyrosination.

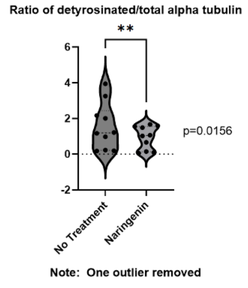
Solution
- Deliver a MARK4 inhibitor to preserve heart contractility.

Methodology
- Identifying potential inhibitors based on prior literature.
- Confirm that these inhibitors do lead to a reduction in microtubule detyrosination in rodent MI models.
- Confirm that these inhibitors do lead to improvements in contractility in rodent MI models.

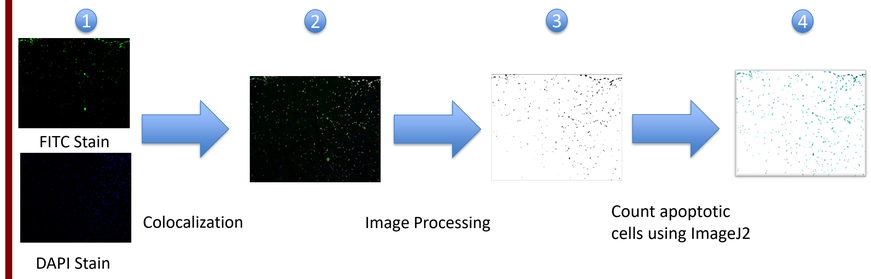
Methods

Protein Analysis
- Apex tissue from rodent hearts is first chemically and mechanically broken down to separate microtubule protein from other tissue.
- This protein is then Western blotted for detyrosinated α-tubulin and total α-tubulin.
- Western blot data is quantified in terms of ratios, with both the detyrosinated α-tubulin and total α-tubulin first normalized to each of their loading controls.

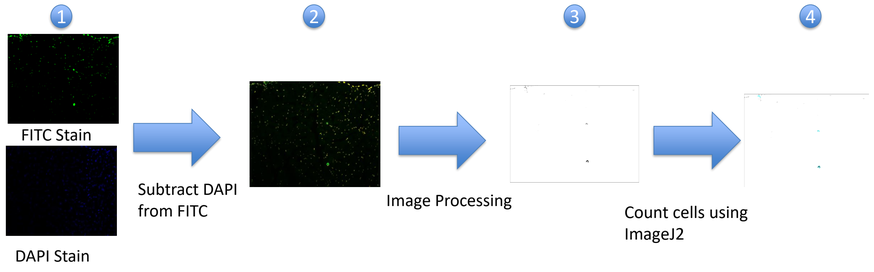
The graph to the right compares the proportions of detyrosinated to total α-tubulin in rodent hearts given Mis with no treatment versus those given naringenin.



TUNEL Staining
- To measure the toxicity of the MARK4 inhibitor, one metric we are using is the number of apoptotic/necrotic cells in lung, heart, kidney, and liver
- To streamline toxicity analyses, we developed software to process images to be analyzed using ImageJ2

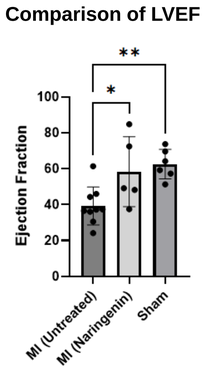


- Through TUNEL analysis, we also determined that we could further distinguish apoptosis from other forms of cell death by comparing the DAPI stain on the nucleus with the DNA breaks stained by FITC.

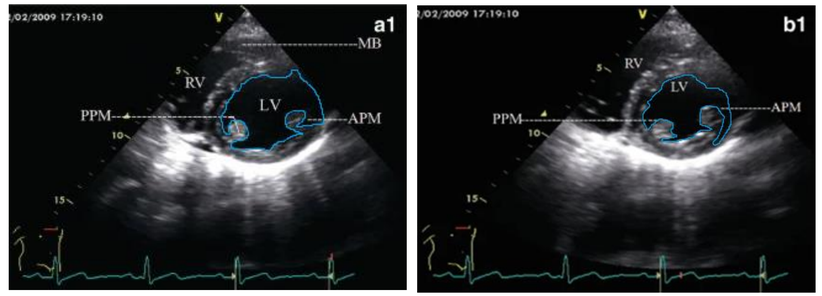


Invasive Hemodynamics
- We can insert a catheter through the left common carotid artery into the aorta, and past the aortic valve into the left ventricle to determine the pressure exerted and volume of blood contained in the left ventricle.

The graph to the right compares the ejection fractions of rodent hearts after various treatments. Under small sample sizes, an *in vivo* analysis assessing naringenin's ability to preserve contractility appears promising.



Echocardiography
- We directly measured contractility by comparing cross-sectional areas from the left ventricle during systole and diastole
- Ejection fraction is then calculated as the systolic area divided by the diastolic area



Images from He, S. (2018). Echocardiography. In: Zhang, M. (eds) Atlas of Human Body Ultrasound Scanning[2]

Above, example echo images are shown. Software on our echo machine allows us to trace out the cross-sectional area of the left ventricle. Then, it calculates an area, and we divide the systolic area(on the right) by the diastolic area (on the left) to calculate an ejection fraction.

Conclusions

While all metrics thus far show promising signs, there are numerous different techniques we have had to both develop and implement to determine the safety and efficacy of this treatment.
Next steps for this project will include testing on larger mammals and ensuring the therapeutic doesn't have any adverse effects on other organs.

References

[1] Yu, X., Chen, X., Amrute-Nayak, M. et al. MARK4 controls ischaemic heart failure through microtubule detyrosination. Nature 594, 560–565 (2021). https://doi.org/10.1038/s41586-021-03573-5
[2] He, S. (2018). Echocardiography. In: Zhang, M. (eds) Atlas of Human Body Ultrasound Scanning. Springer, Singapore. https://doi.org/10.1007/978-981-10-5834-9_6