Nanoparticle Delivery of Antioxidant-Response Activating Drug to Atherosclerotic Plaque

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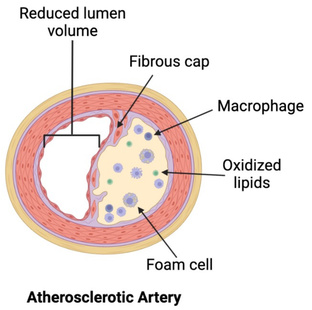
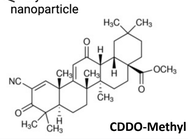
Introduction

- Atherosclerosis is a leading cause of death worldwide, as it precedes acute coronary syndromes, myocardial infarction, and strokes[4].
- This disease is driven by synergistic effects of low-density lipoprotein (LDL) accumulation in the arteries and chronic inflammation that modulate the formation of fibrous lesions/plaque in the arterial wall[4].
- Reactive oxygen species (ROS), are prominent activators of inflammatory response in the vascular wall[2]. Under normal conditions, ROS are cleared by cellular antioxidant mechanisms, however, redox imbalances can advance the progression of atherosclerosis.
- The canonical Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway is responsible for the cell's response to oxidative stress[3].
- At basal levels of oxidative stress, Nrf2 is bound to Kelch-like ECH-associated protein 1 (Keap1) where it is ubiquitinated for proteasomal degradation. Under increased oxidative stress, Keap1 dissociates from Nrf2, promoting nuclear translocation of Nrf2 which initiates transcription of antioxidant enzymes.
- We employ a novel technique for drug delivery of a Nrf2-activating drug, CDDO-Methyl, by flash nanoprecipitation to synthesize nanoparticle polymers that encapsulate the antioxidant-response-activating drug.





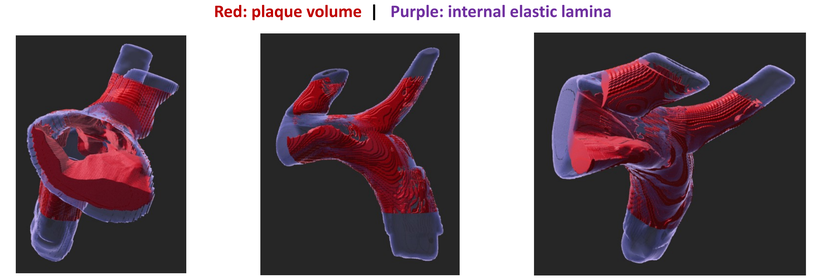
Objectives

- Conduct in vitro and in vivo studies to determine Nrf2 activation by CDDO-Me ARAPas treatment
- Investigate the therapeutic efficacy of CDDO-Me ARAPas in progression of atherosclerosis in an atheroprone murine model using traditional two-dimensional histological methods and novel three-dimensional analysis of the entire murine aorta via light sheet fluorescent microscopy

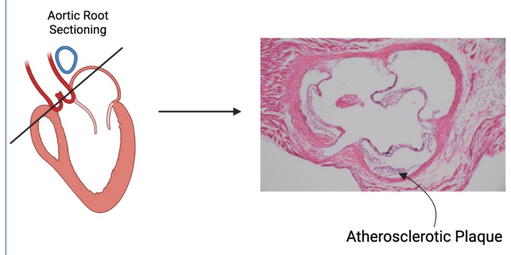
Methods

Induction of Atherosclerosis in a Murine Model and Treatment with ARAPas

Atheroprone mice were placed on a high-fat diet for a total of 16 weeks. 8 weeks into the high-fat diet, mice were injected once per week with CDDO-Me ARAPas. Mouse aortas and hearts were surgically harvested.



Analysis of Aortic Atherosclerotic Plaque by Tissue Sectioning and Staining

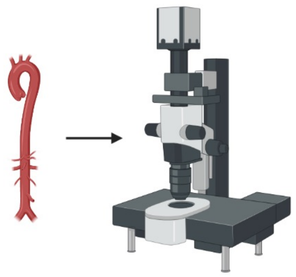


Harvested mouse hearts were frozen and aortic roots were sectioned using a cryostat. Tissue sections were stained to conduct immunofluorescence, histological analysis via hematoxylin and eosin, and Oil Red O for visualization of fatty plaques. Atherosclerotic plaque in the aortic root was quantified by tracing its area in ImageJ.

Analysis of Aortic Atherosclerotic Plaque by Light Sheet Fluorescent Microscopy

Light sheet fluorescence microscopy (LSFM) is used to excite fluorophores in one plane of visualization at a time. Imaging of entire mouse aortas via LSFM allows for rendering of three-dimensional surfaces to conduct quantitative analysis of plaque volumes in the CDDO-Me treated vessels. Benefits of LSFM[1]:

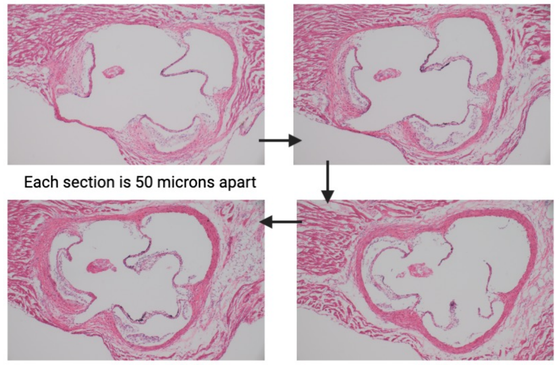
- 3D surface reconstruction reduces analysis bias that may be typical in two-dimensional histological techniques
- Allows for accurate remodeling of the entire vessel where 2D tissue sections capture incomplete morphologies
- Immunostaining of entire organs/tissues supports volumetric analysis of plaque in the mouse aorta



Red: plaque volume | Purple: internal elastic lamina

Results

Representative Images of Mouse Aortic Roots



Hematoxylin and eosin staining was performed on frozen tissue sections of mouse aortic roots. Quantification of plaque area using traditional histology is in progress.

Aortas were stained for endothelium and macrophages to delineate between the internal elastic lamina and atherosclerotic plaque. Light sheet fluorescence microscopy analysis of entire mouse aortas is in progress.

Plaque volumes of high fat-diet atheroprone mice treated with CDDO-Me ARAPas will be compared to those without CDDO-Me treatment to evaluate therapeutic efficacy of CDDO-Me ARAPas in treatment of atherosclerosis.

Conclusions

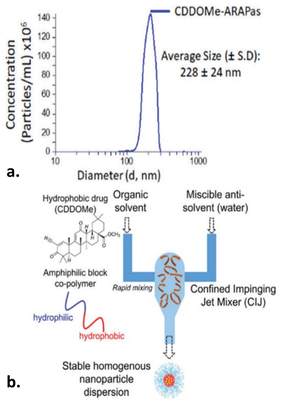
- CDDO-Me ARAPas localize to atherosclerotic plaque and locally activate Nrf2 which may serve as a therapeutic to reduce progression of atherosclerosis
- Light sheet fluorescent microscopy serves as a powerful tool for volumetric analysis of entire organs, including mouse aortas, and will be used to assess the therapeutic efficacy of CDDO-Me ARAPas in treatment of atherosclerosis by volumetric measurement of aortic plaque reduction in mice

Future Directions

- Complete analysis and quantification of mouse aortic root plaque areas by traditional histological techniques
- Complete analysis and quantification of atherosclerotic plaque volume in entire mouse aortas by LSFM
- Assess local Nrf2 activation in atherosclerotic blood vessels by immunofluorescent staining for antioxidant enzymes that are under transcriptional control by Nrf2

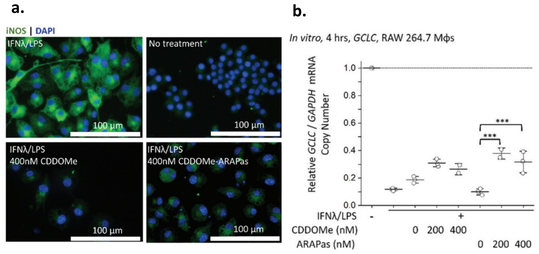
Background/Preliminary Data[3]

Figure 1: *Creation of CDDO-Me Nanoparticles*



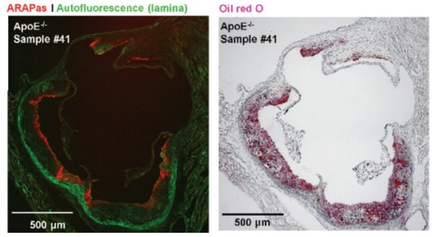
(A) Nanotracking analysis of CDDO-Me ARAPas reveals particle sizes that are 228 nm in diameter on average. **(B)** Flash Nanoprecipitation was used to create the nanoparticles encapsulating the Nrf2-activating drug CDDO-Me. This technique allows for scalable and efficient generation of homogenous nanoparticles[3].

Figure 2: *Nrf2 Activation*



(A) Murine macrophages were classically stimulated with IFNλ and LPS. Cells were stained with an anti-iNOS primary antibody and a secondary antibody conjugated to a fluorophore to detect iNOS protein expression. CDDO-Me-treated macrophages are noted to have decreased expression of the pro-inflammatory iNOS protein. **(B)** mRNA extraction from stimulated macrophages treated with CDDO-Me ARAPas demonstrates an increase in GCLC, an antioxidant enzyme under transcriptional control by Nrf2. Together these data suggest that CDDO-Me ARAPas activate Nrf2 to induce an antioxidant response, highlighting the potential for redox balance and therapeutic benefit.

Figure 3: *Nanoparticles Localize to Plaque*



Murine aortic roots were sectioned and imaged with fluorescent microscopy for visualization of ARAPas and lipids in atherosclerotic plaque were then stained with Oil Red O. This reveals that CDDO-Me ARAPas localize to atherosclerotic plaque, prompting further investigation of their therapeutic potential in treatment of atherosclerosis.

References

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