PROJECT DESCRIPTION

Project Title:

Identifying the Coronary Metabolic Dilator

Principal Investigator:

Adam G. Goodwill, PhD, FCVS; Assistant Professor Integrative Medical Sciences

Research Location:

Northeast Ohio Medical University College of Medicine
RGE-300
RGE-308
Comparative Medicine Unit

Abstract:

Owing to the need of the heart to constantly oscillate between contraction and relaxation, the metabolic demands of the heart are amongst the highest of any tissue in mammalian physiology. To meet these demands, cardiac tissues rely almost exclusively on aerobic metabolism. Accordingly, the heart must have mechanisms in place to rapidly increase coronary blood flow (oxygen delivery) in response to increases in myocardial demand. While this need is known, the specific mechanism linking these processes remains undefined. In collaboration with other NEOMED faculty including Dr. William Chilian and Dr. Xinwen Wang, we believe that we have developed an experimental approach to allow for novel insights into metabolically driven coronary dilator responses. Our hypothesis is that any metabolic dilator must be initially secreted by cardiac tissue and that increases in myocardial demand will result in proportional increases in this secreted compound. Using a large animal model, we intend to place custom biocompatible catheters directly into the left ventricular free wall of an anesthetized swine model and vary myocardial demand through alterations in cardiac electrical activity and/or chemical stimulation of contractility. We will collect samples of extracellular fluid at specific and carefully controlled levels of myocardial oxygen consumption. This extracellular fluid will then be submitted for metabolomic analyses (Dr. Wang's group). It is our belief that these analyses will allow for initial identification of plausible, physiologically relevant metabolic dilators.

Significance:

The treatment of all diseases is predicated on the notion that we understand how a tissue/organ works under normal conditions. Identifying what mechanism tie cardiac metabolism to coronary blood flow is central to identifying pathways for pharmacologic interventions in cardiac ischemic disease.

Goals and Objectives for the Research Project

The goal of this research is to create a dataset that will allow for identification of extracellular compounds that increase/decrease in proportion to myocardial demand.

Research Methods

All sample collections will be performed in a large animal model. Studies will be performed in the comparative medicine unit. All animal procedures will be performed under the direct supervision of Adam Goodwill PhD. In brief, animals will be anesthetized using an induction cocktail prior to intubation. Once intubated, general anesthesia will be maintained using a combination of α-chloralose and an approved schedule II analgesic. While under general anesthesia, arterial and venous access will be obtained and the animal rotated into lateral recumbency and a left lateral thoracotomy performed. After breaching the body wall, the pericardium will be opened and perivascular flow probes placed around the left anterior descending and left circumflex coronary arteries. An intraventricular venous catheter will be placed, a left ventricular pressure-volume catheter may be

placed and a custom micro-renethane catheter will be passed through the epicardium. This combination of instrumentation will allow for real-time measurement of all left ventricular coronary flow, myocardial oxygen consumption and myocardial mechanics. Systemic parameters (ECG, blood pressure, respiration) will also be monitored through the procedure. Finally, an epicardial cardiac pacing lead may be placed to allow for external pacing of the heart.

Upon completion of instrumentation, baseline measurements will be taken and extracellular fluid collected. Myocardial demand will then be increased in a step wise manner using the external pacing device, and/or pharmacologic intervention (commonly dobutamine). Myocardial demand may also be stepwise decreased via direct electrical stimulation of the Vagus nerve. Functional parameters, blood gasses and extracellular fluid will be collected at each level of demand. All samples will be snap frozen for later molecular analyses.

At the conclusion of the protocol, one final metabolic challenge may be administered and cardiac biopsies obtained at each level of demand. All above procedures are performed under general (surgical grade) anesthesia. Once all studies are completed, each animal will be humanely euthanized by direct application of a 9v battery to the heart.

Samples will be provided to our collaborator at the conclusion of each individual experiment. Data from *in vivo* studies will be integrated with molecular data for analyses of relevant molecular pathways.

Methods of Data Analysis

Data analysis will be performed using commercially available software (SigmaPlot & GraphPad). At this time no specific analysis has been designated as singularly appropriate. Based on the study design, the vast majority of data will be analyzed using repeated measures ANOVA and multivariate regression analyses.

Anticipated Findings

We anticipate that we will be able to simulate the intact condition using our experimental approach. Accordingly, we anticipate increases in coronary flow to occur in proportion to myocardial demand. In systems that match well, coronary venous oxygen content remains relatively constant across a range of demand (the same amount remains after what is needed is extracted). Since we use a large animal model, we are uniquely able to monitor this parameter to assure that there is not an experimental perturbation that results in demand:perfusion mismatching. We assert that the metabolomics will likely reveal one or more compounds that increase in proportion to myocardial demand, each of which can/will serve as avenues of future investigation.

STUDENT FELLOW TRAINING/MENTORING PLAN

Training Plan

All learners will participate in necessary CITI training and CMU training in accordance with IACUC protocols. Additionally, all animal studies will be performed under the instruction and supervision of Adam G. Goodwill Ph.D. Every member of the laboratory will cross-train in every function of the laboratory from data acquisition to analysis to dissemination. Dr. Goodwill intends to work with every member of the laboratory on a daily basis. Learners will participate in daily informal meetings with Dr. Goodwill and in weekly joint lab meetings with Dr. William Chilian's research group. Dependent on the number of summer laboratory members, we tentatively plan to host a student led cardiovascular journal club for the duration of the summer training experience. These journal clubs will be open to all other NEOMED faculty, staff and students. Trainee's will be encouraged to assist our collaborators in any studies, at the discretion of those laboratories, and will be encouraged to explore the techniques and approaches employed by other faculty in the cardiovascular center. Trainee's will be encouraged to actively participate in any available seminars.

Data analysis software will be made available to all trainees. This software will be used in a see one, do one, teach one approach wherein students will be guided through the software and then encouraged to explore data

analyses and visualizations. Dr. Goodwill will be available for questions and guidance through this process and will verify all analyses before any presentation.

All data presentations will be developed in coordination with Dr. Goodwill and rehearsed first with the laboratory group, then our cardiovascular center before any public presentation.

Finally, trainees will be encouraged to disengage from research at the end of the day. Our laboratory believes that mental health is health and it should be safeguarded accordingly. While we assert that our work is important, we recognize that it will be there in the morning.

Available Resources

- Assorted Surgical Equipment
- ADInstruments Powerlab C with 16 inputs
- Dell XPS Computer for data acquisition
- Grass Amplifier (Multiple)
- Harvard Apparatus Perfusion Servo Controller (2)
- Harvard Apparatus Syringe Pumps (Multiple)
- Harvard Apparatus Transducer Amplifier (8)
- Haake K20 Heated/Cooled Circulating Water Bath
- iWorx Biopotential Amplifier for ECG
- Lifepak 20 Defibrillator with Internal/External Paddles
- Masterflex L/S Peristaltic Pumps (2)
- Stryker 810 Autopsy Saw
- Transonic ADV-550 Admittance Pressure Volume System
- Transonic TS410 Tubing Flow Module (2)
- Transonic TS420 Transit Time Perivascular Flow Module (3)
- Werfen Gem Premier 5000 Blood Gas Analyzer

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