PROJECT DESCRIPTION

Project Title:

Coronary Hypoxemia is a Sufficient Stimulus for Cardiac Effects of Sodium Glucose Cotransporter Type 2 Inhibitors

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:

Since their initial FDA approval in 2013, sodium-glucose cotransporter type 2 inhibitors (SGLT2i) have become an increasingly prescribed drug category for glycemic control in patients with type 2 diabetes mellitus. Through therapeutically induced glycosuria, SGLT2i have been demonstrated to be well tolerated and efficacious in lowering a long-term measure of glucose regulation. Interestingly, numerous outcome studies have demonstrated unexpected and potent decreases in major adverse cardiac effects (MACE) with SGLT2i therapy independent of effects on glucose levels. Understanding of SGLT2i mediated cardioprotection is confounded by the consistent observation that neither SGLT2 mRNA nor protein are measurable in cardiac tissue. Although myriad molecular mechanisms of cardioprotection have been proposed, no mechanism has received general support. Work from our research group has provided compelling data that SGLT2i can act directly on the heart and these actions specifically only occur when the heart is in a condition of disease/damage. It is our assertion that SGLT2i act through a mechanism that is dependent on factors released during myocardial ischemia. We propose a study design that will allow us to better understand the differential role of ischemia vs hypoxemia and stands to provide a dataset for identification of the molecular mechanisms at play in SGLT2i mediated cardioprotection.

Significance:

The cardioprotective effects of SGT2 inhibitors are well documented while the mechanisms remain fully undefined. Insight into mechanism will provide insight into more targeted therapies specific to enhancing cardioprotection.

Goals and Objectives for the Research Project

The goal of this research is to identify the relative contributions of coronary oxygen delivery vs delivery of other humoral factors in mediating SGLT2 inhibitor associated cardioprotection.

Research Methods

All studies will be performed in a large animal model. Studies will be performed in the comparative medicine unit. Animals will be divided into two groups: Control & Treatment. 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. Large bore arterial catheters will be advanced through the arterial access points into the aorta to provide an aortic blood supply. The animal will then be rotated into lateral recumbency and a left lateral thoracotomy performed. After breaching the body wall, the pericardium will be opened. The left anterior descending and left circumflex coronary arteries will be carefully isolated from the surrounding tissue. Each of the left ventricular

epicardial coronary arteries will be cannulated and blood will be supplied from the arterial via a custom servo-controlled peristaltic pump drive extracorporeal perfusion system (similar to bypass equipment). A custom extracorporeal oxygenation/deoxygenation device will be connected in series to the perfusion circuit.

An intraventricular venous catheter will be placed, and a left ventricular pressure-volume catheter may be placed. This combination of instrumentation will allow for real-time pressure-clamp control of all left ventricular coronary flow, measurement of myocardial oxygen consumption and myocardial mechanics. Systemic parameters (ECG, blood pressure, respiration) will also be monitored through the procedure.

Upon completion of instrumentation, baseline measurements (coronary perfusion pressures clamped at 100mmHg) will be taken. Following collection of baseline data, coronary autoregulatory responses will be assessed by adjusting servo-control settings step wise, assessing flow at coronary perfusion pressures from 50-150 mmHg. Samples will be collected at each stage.

Using the extracorporeal membrane oxygenator/deoxygenator, plasma oxygen content will be modulated to create hypoxemic and hyperoxemic conditions. Autoregulatory responses will again be assessed with the heart in hypoxemic conditions and hyperoxemic conditions. Functional parameters and blood gasses will be collected at each step.

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.

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

Historic data has demonstrated that the onset of acute coronary ischemia is associated with a near instantaneous decrease in cardiac efficiency in control animals whereas efficiency is maintained in treated animals. While interesting, this response only allows for binary analyses: total regional myocardial ischemia vs control conditions. We believe that the response to therapy will be proportional to the magnitude of supply:demand imbalance and that this will specifically be an oxygen sensitive response. As we are one of the few research groups in the world trained and equipped to perform these studies, we are in the unique position to be able to create coronary specific hypoxemia at normal perfusion pressures. By comparing the effects of ischemia (inadequate perfusion) vs non-ischemic hypoxemia, we will be able to establish whether effects are mediated by plasma oxygen concentrations or some other humoral factor. Moreover, while our hypothesis is centered on the notion that effects are oxygen specific, the study design should allow us to identify the relative contribution of oxygen delivery. Even if oxygen does not play a role, that data is informative and could guide us to future studies, aided by analyses of samples collected at each level of oxygen tension and coronary perfusion pressure.

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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