Myocardial Edema Attenuation and Sphingosine-1-Phosphate

David Straus

Rush University Medical Center, Chicago

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Abstract:

Introduction: *Myocardial edema contributed to cardiac dysfunction in different clinical circumstances. The precise pathophysiology of myocardial edema and therapeutic interventions that target it have remained largely unexplored. The lysophospholipid, Sphingosine-1-Phosphate (S1P) has been shown to decrease edema in the lung through modulation of pulmonary endothelial barrier function. We apply this agent to an isolated rat heart model of ischemia-reperfusion injury and examine its effects on subsequent myocardial edema formation.*

Methods: *18 isolated male Sprague-Dawley rat hearts were used in this experiment. 3 served as non-ischemic controls, 7 served as ischemic controls and 8 served as the intervention group. A 20 minute ischemic period was applied to all groups except the non-ischemic controls. In the intervention group, the rat hearts were given a 30mL bolus of 10nM S1P prior to ischemia. After completion, heart were histologically analyzed to evaluate the extent of myocardial edema.*

Results: *In the non-ischemic controls there was 13.65% (+/- 0.73%) extracellular area and 84.56% (+/- 0.89%) intracellular area. In the ischemic controls there was 24.50% (+/- 3.92%) extracellular area and 74.11% (+/- 3.90%) intracellular area. In the S1P treatment group, there was 21.55% (+/- 2.6%) extracellular area and 76.77% (+/- 2.70%) intracellular area. These differences did not reach statistical significance (p>0.05).*

Conclusion: *In this experimental design we observed a non-significant trend in histologic myocardial edema in the S1P treatment group. We also observed a correlated trend in improved myocardial function in the S1P treatment group.*

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Introduction:

Myocardial edema contributes to cardiac dysfunction and has various iatrogenic and pathologic etiologies. Iatrogenic causes include cardiopulmonary bypass (CPB), cardioplegia and orthotropic heart transplantation.[1] Pathologic causes include myocardial ischemia/infarction, arterial hypertension, pulmonary hypertension and sepsis.[1] Preventing fluid collection in the myocardial interstitium is particularly relevant to cardiac surgery since common surgical interventions (CPB and cardioplegia) are major iatrogenic sources of myocardial edema. Compromising cardiac function in this high-risk population may mute the benefits of cardiac surgical interventions by retarding post-operative recovery, increasing post-operative morbidity and increasing post-operative mortality. Defending the myocardial interstitium from edematous fluid overload offers a means to preserve cardiac function from various pathophysiological and iatrogenic insults.

The precise pathophysiological mechanisms of myocardial edema-induced cardiac dysfunction remain poorly defined. However, numerous studies using various models have demonstrated both systolic and diastolic dysfunction linked to myocardial edema. Preload recruitable stroke work (PRSW), an index of myocardial contractility independant of preload and afterload, is found to be decreased in edematous hearts. This decrease in PRSW responds in a dose-dependant fashion to increases in myocardial water content.[2] Diastolic dysfunction in myocardial edema is seen as an increase in the isovolumic relaxation time constant (ô)[2] and an increase in myocardial stiffness (i.e. a decrease in compliance effecting both the active and passive phases of ventricular relaxation. These combined effects result in decreased

Address of Correspondence : David Straus, Rush University Medical Center, Department of Neurological Surgery, Chicago, IL, USA. Email: david_straus@rush.edu

cardiac output (CO): a 3.5% increase in myocardial water content decreases CO by 40% at a given preload.[3] Decreased ventricular compliance and increased water content may also reduce cardiac efficiency as myocytes are forced to contract against increased intra- myocardial/ interstitial pressures and as the mass (inertia) of the myocardium increases with increasing water content. Ultimately, myocardial edema increases cardiac energy requirements.[4] Moreover, edema may lead to myocardial ischemia as diffusion distances increase with expanding interstitial space[4] and as coronary vascular resistance is increased[5] with elevated interstitial pressures and the resulting capillary bed compression. Finally, myocardial edema may trigger interstitial cardiac fibrosis,[3] thus chronically impeding cardiac function.

Sphingosine-1-phosphate, a lysophospholipid acting at various endothelial differentiation gene (EDG) receptors, has been shown to increase endothelial barrier function in human pulmonary artery endothelial cells, reducing both inflammation, microvascular leak and ultimately producing a protective effect against pulmonary edema formation.[6] The increase in endothelial cell barrier function is mediated by the EDG1 (S1PR1) receptor. This S1P receptor induces cell-to-cell adherens junction formation, cell-to-extracellular matrix focal adhesion formation and a resultant decrease in vascular permeability.[6] Since the EDG1 receptor is abundantly expressed in cardiac endothelial cells[7] we hypothesized that S1P would, similarly, be protective against edema formation in the myocardium.

Study Methods

Animals, Groups and Heart Harvest

Male Sprague-Dawley rats weighing 350-450 grams (Harlan, *Indianapolis, IN*) were housed in facilities at the University of Chicago until experimentation. Animals were randomized to control or treatment groups by coin toss.

Three experimental groups were used. One group served as a non-ischemic control (Control, n=3), another served as an ischemia-reperfusion control (IR, n=7) and a final group served as the treatment group (S1P, n=8).

Rats are anesthetized using sodium pentobarbital (30mg bolus). Rats were anesthetized, intubated through a tracheostomy and then heparinized (500 IU bolus) via the IVC. 3 minutes after the heparin bolus the heart is removed from the chest through a sternotomy incision and placed in cold Krebs-Henseleit buffer.

Isolated Heart Prep and Working Heart Model

The aorta was cannulated and the hearts were perfused for 5 minutes in retrograde fashion (Langendorff prep) with a modified Krebs-Henseleit solution (37°C, pH 7.4). The hearts were switched to antegrade flow (working heart prep) and perfused with modified Krebs-Henseleit solution in a closed recirculating system at 37 °C. Fresh perfusate was given every 15 minutes. In the working heart prep, perfusate entered the cannulated left atrium at a pressure of 8 mmHg (preload), and passed into the left ventricle (LV) from which it was spontaneously ejected through the aortic cannula against a pressure of 80 mmHg (afterload) (Figure 1). After an equilibration period of 5 min, hearts were electrically paced at 290-320 BPM via the metal cannula in the left atrium (LA). Heart rate and left atrial, aortic systolic and aortic diastolic pressures were recorded. Cardiac output (flow into the LA) and aortic flow were also measured. Coronary flow was calculated at the difference between cardiac output and aortic flow.

Fig.-1: *Isolated working rat heart model demonstrating cannulation of left atrium and aorta.*

Experimental protocol

Control hearts were mounted on the perfusion apparatus in a Langendorff prep for a 10 minute initiation period. After 10 minutes in the Langendorff prep these hearts were switched into the working heart prep for the remainder of the experiment. IR hearts were mounted on the perfusion apparatus in the Langendorff prep for 10 minutes and were subsequently switched into the working heart prep, as with the Control group. After a 15 minute stabilization period in the working heart prep, however, the IR hearts received a retrograde 30mL bolus of perfusate over 3 minutes and were then subjected to global, no-flow ischemia for 20 minutes. Following the ischemic period, IR hearts were reperfused in the Langendorff prep for 5 minutes and then switched back into the working heart prep for the remainder of the experiment. S1P hearts followed a protocol identical to the IR hearts, however the 30mL bolus of perfusate administered to the S1P hearts prior to ischemia contained 10nM S1P (Sigma Chemical, *St. Louis, MO*). All experiments ended at systolic failure, defined as the loss of antegrade aortic flow. In the working heart prep hearts were paced at 290-320 BPM via the left atrium.

Quantification of Myocardial Edema

LV tissue samples were stained with H&E. The ratio of intracellular area to total area and the ratio of interstitial area to total area were measured using Chromavision ACIS software (Clarient, *Aliso Viejo, CA*).

Statistics

Data are presented as mean (SEM). Error bars on graphs indicate SEM. Either one-way or two-way ANOVA was used to compare differences among groups when appropriate. Student's *t*-test was used to compare two groups. Significance was taken at $p < 0.05$.

Results

Edema

Histological analysis of cardiac tissue showed increased extracellular area in the IR group compared to the Control group (p=0.12) and increased extracellular area in the S1P group compared to the Control group (p=0.12). Additionally, the IR group had decreased intracellular area compared to the Control group (p=0.13), as did the S1P group (p=0.14). In relation to the IR group, the S1P group showed non-significant decreases in extracellular area (p>0.05) and increases intracellular area (p>0.05).

Table-I

Histological measurements. Data are presented as a percentage of the total area (±SEM).

	% Extracellular Area	% Intracellular Area
Control	13.65% (±0.73%)	84.56% (±0.89%)
IR	24.50% (±3.92%)	74.11% (±3.90%)
S1P	21.55% (±2.6%)	76.77% (±2.70%)

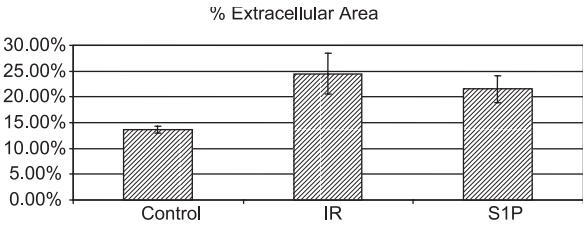

Fig.-2: *Extracellular area as a percentage of total area. Error bars represent SEM.*

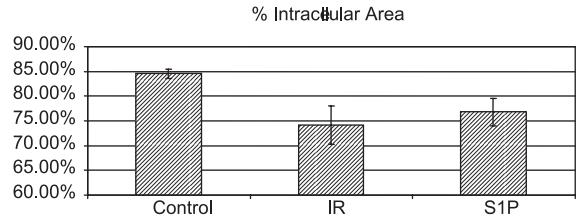

Fig.-3: *Intracellular are as a percentage of total area.*

Hemodynamics

Hemodynamic data were recorded every 15 minutes throughout the experiment to monitor cardiac function. The Control group remained hemodynamically stable until the 105 min time-point, at which point their function steadily declined until failure. Neither the IR group nor the S1P group stabilized hemodynamically. They both demonstrated a post-ischemic loss of systemic flow and a hyperemic increase in coronary flow within the first 15 minutes of reperfusion. These effects offset each other to

produce a less pronounced decrease in post-ischemic cardiac output. The hyperemic response seen in the early reperfusion period abated within 30 minutes post-ischemia and coronary flow remained stable until 105 minutes post-ischemia. Functional recovery in both IR and S1P groups peaked at 45 minutes post-ischemia, followed by a steady decline in function until failure.

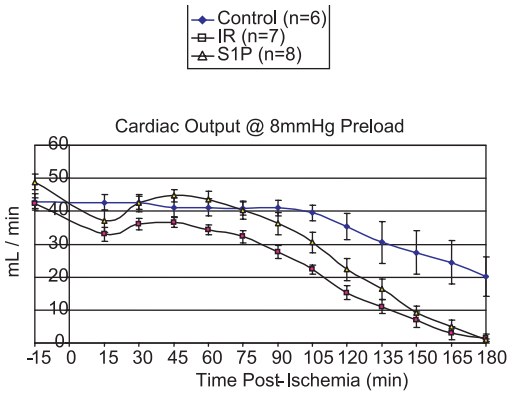

Fig.-4: *Cardiac output, calculated as flow through the left atrial cannula.*

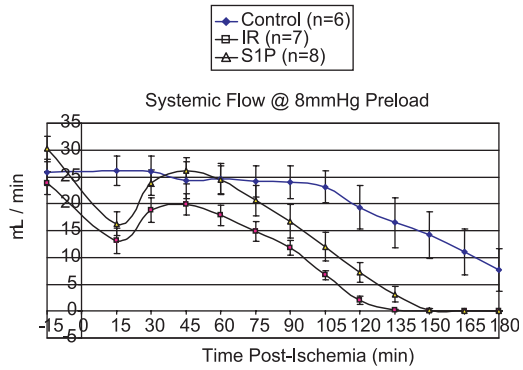

Fig.-5: *Systemic flow, calculated as flow through the aorta.*

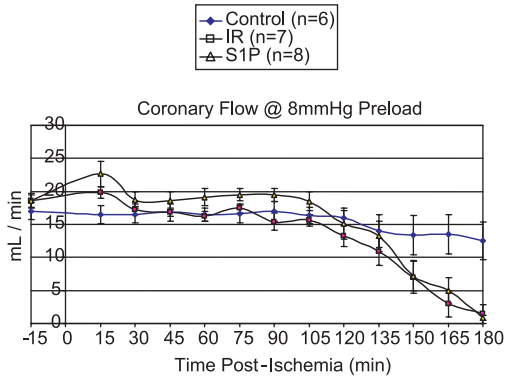

Fig.-6: *Coronary flow, calculated as the difference between cardiac output and systemic flow.*

Discussion:

Our data show indications that S1P may act to decrease interstitial edema in cardiac tissue following ischemia-reperfusion injury. Increases in extracellular area indicate fluid collection within the interstitium. As the interstitial space expands, intracellular area (as a percentage of total area) decreases. Both of these effects are seen in the S1P group and the IR group. Moreover, these effects are more severe in the IR group compared to the S1P group. These measures show a consistent, albeit non-significant, tendency for S1P treated hearts to recover towards Control values. Moreover, the higher post-ischemic coronary flows (both during the hyperemic recovery period and throughout the experiment) in the S1P hearts compared to IR hearts may be an additional indicator of decreased edema in S1P treated hearts. As fluid collects in the myocardium, interstitial pressures rise causing an increase in vascular resistance (increased interstitial pressure compresses the microvasculature). Despite trends towards improved functionality of S1P treated hearts, definitive conclusions about functional differences are hindered by the differing baseline characteristics of the S1P and IR groups. Although coronary flows were nearly identical at baseline, S1P hearts had noticeably higher systemic flows (and, consequently, higher cardiac outputs) at baseline.

This study gives some indication that S1P may be acting to reduce post-ischemic edema formation in the heart. Despite the observed trends, significance was not obtained in our primary measures of edema and our hemodynamic data is confounded by baseline differences in systemic flows between S1P and IR hearts. While these experiments may have picked up on some faint signals of edema attenuation, more sensitive methods will be required to further clarify the role of S1P in myocardial edema.

Currently, we have altered some of our methods in order to re-examine our hypothesis. To measure microvascular permeability directly, we will use Evans-Blue dyed albumin to quantify colloid protein extravasation. We will also add albumin to our modified krebs-henseleit solution

in order to avoid the use of a crystalloid perfusate. This should serve as a more sensitive marker of edema. Although sustained recovery of systemic flow is the most clinically relevant parameter, we will include hemodynamic measures that are more sensitive to myocardial edema (e.g. PRSW, dP/dt, tau, -dP/dt and LVEDP). Finally, instead of ending the experiments at systolic failure, we will use a standard endpoint relative to the end of ischemia, where functional differences are more pronounced.

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