The Role of Autophagy During Myocardial Ischemia and Reperfusion Injury in Isolated Rat Hearts

Aloysius Ibe, Anahi McIntyre, Alisa Kim, Hannah Kim, Marquese Daniels, Lindon Young, Robert Barsotti, and Qian Chen
Biomedical Science Department, Philadelphia College of Osteopathic Medicine (PCOM)
aloysiusib@pcom.edu, qianch@pcom.edu

Introduction

Background

Coronary Heart Disease (CHD) is the leading cause of death and disability worldwide, claiming about 600,000 deaths in the United States each year (1). The most severe problem occurring in CHD is caused by plaque accumulation that leads to ischemia, chest pain, and ultimately myocardial infarction (1). The best treatment is timely and effective reperfusion via angioplasty to salvage heart tissue and decrease infarct size.

However, reperfusion can cause additional injury to the heart, which is termed "reperfusion injury". The strategy to reduce reperfusion injury to preserve more cardiac function of the patients is under active investigation. Our research focuses on understanding the relationship between autophagy and myocardial ischemia/reperfusion (I/R) injury.

Autophagy is a housekeeping process used to degrade protein aggregates and damaged cytoplasmic constituents when cells go through a nutrient stress (2). Research has shown that autophagy enhancement before ischemia (i.e. pre-treatment) is beneficial to cardiac function (3). However, a debate persists on whether autophagy is beneficial to attenuate or promote cell damage at the beginning of reperfusion (i.e. post-treatment) (see figure 1). This study tested the effects of an autophagy enhancer (e.g. rapamycin and trehalose) and inhibitor (e.g. 3-methyladenine) on heart function and infarct size after global I/R in isolated rat hearts.

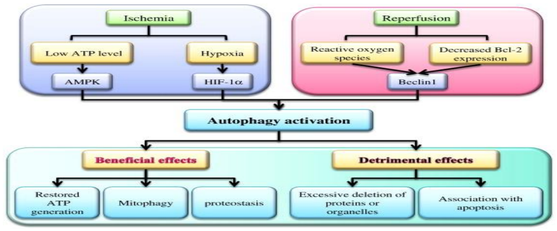

Figure 1. The possible role of autophagy during I/R (1).

Objective

To investigate if autophagy is beneficial or detrimental to myocardial I/R injury by measuring post-reperfused cardiac function and infarct size when giving autophagy enhancers or inhibitor as either pre-treatment or post-treatment compared to control I/R group.

Hypothesis

Autophagy enhancement would be beneficial to cardiac function and decrease infarct size when given as post-treatment similar to pre-treatment when compared to control I/R. By contrast, autophagy inhibition would exhibit compromised cardiac function and similar infarct percentage as control I/R.

Methods

Figure 2a. Flow Diagram of experimental protocol

Experimental groups consisted of:

- Control I/R (n=9)
  - No drug infused
- Autophagy Enhancer
  - 25nM Rapamycin (Rapa.) (n=5)
  - 5mM Trehalose (Treh.)
    - Pre-treatment (n=6)
    - Post-treatment (n=5)
- Autophagy Inhibitor
  - 1mM 3-Methyladenine (3-MA)
    - Pre-treatment (n=6)
    - Post-treatment (n=5)

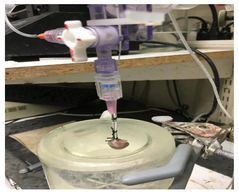

Figure 2b. Langendorff Apparatus used for isolated heart.

All animal procedures were in accordance with Philadelphia College of Osteopathic Medicine rules and regulations. Cardiac function was measured by placing a pressure transducer into the left ventricle of the isolated rat heart and the data was recorded using Powerlab Station acquisition software. The cardiac parameters of importance included: final left ventricular end systolic pressure (LVESP), final left ventricular end diastolic pressure (LVEDP), final left ventricular developed pressure (LVDP=LVESP-LVEDP) and the minimal rate of LVDP relaxation (-dP/dt min).

All data in the text and figures are presented as means ± standard error. Cardiac function and TTC staining data was analyzed by ANOVA using post hoc analysis with Student-Newman-Keuls test. Probability values of $p<0.05$ were considered statistically significant and represented by an asterisk (*) as previously described (4).

Results

Key Findings

•Autophagy enhancement improved cardiac function and decreased infarct size when given as a form of post-treatment, similar to pre-treatment.

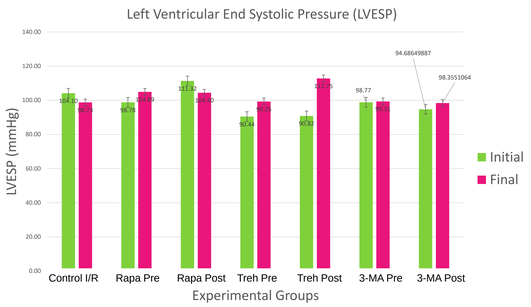

Figure 3. **Initial and final levels of LVESP among all groups**. Initial LVESP among all groups were similar. Compared to final LVESP in control I/R group, autophagy enhancer and inhibitor did not show significant difference.

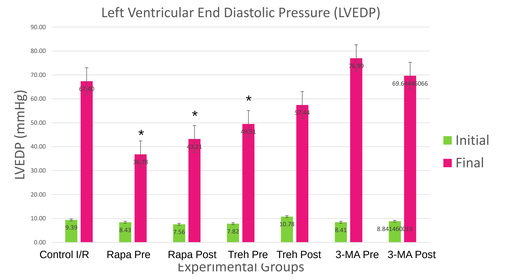

Figure 4. **Initial and final LVEDP levels among all groups**. Control I/R group showed a very high final LVEDP level. By contrast, autophagy enhancement as pre or post-treatment showed a decrease in final LVEDP compared to that of control I/R; rapamycin pre-treatment showed the lowest final LVEDP ($p<0.05$). However, autophagy inhibition as pre or post-treatment (3-MA) did not reduce final LVEDP compared to control I/R group.

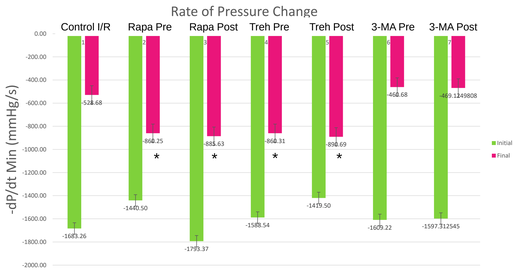

Figure 5. **Initial and final levels of –dP/dt min among all groups**. Control I/R group showed a very low final -dP/dt min level. By contrast, autophagy enhancement as pre or post-treatment showed significantly better rate of pressure change when compared to control I/R ($p<0.05$). However, autophagy inhibition as pre or post-treatment did not improve final -dP/dt min when compared to control I/R.

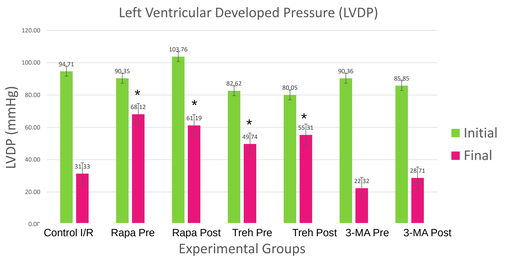

Figure 6. **Initial and final levels of LVDP among all groups**. Control I/R showed a decrease in final LVDP. By contrast, autophagy enhancement as pre or post-treatment showed an increase in final LVDP when compared to control I/R ($p<0.05$). Rapamycin pre-treatment had the highest final LVDP value. However, autophagy inhibition as pre or post-treatment did not improve final LVDP when compared to control I/R.

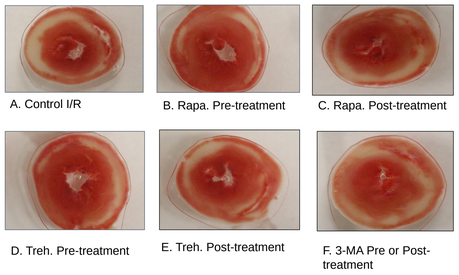

Figure 7. **Representative sectional slice of heart after 1% TTC staining among all groups.** The brick red staining indicated viable cardiac tissue whereas the white area indicated non-viable cardiac tissue. By contrast to control I/R and 3-MA treatment (autophagy inhibitor), the autophagy enhancers reduced infarct area when given as pre or post-treatment.

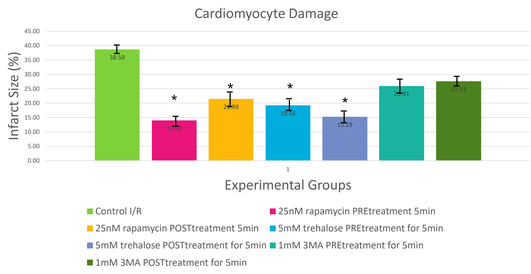

Figure 8. **Infarction percentage among all groups**. Control I/R had a high infarct percentage of 39 ± 4%. By contrast, autophagy enhancement as pre or post-treatment showed a significant decrease in infarct percentage compared to control I/R ($p<0.05$). Rapamycin pre-treatment had the lowest infarct percentage. Autophagy inhibition as pre or post-treatment also showed a similar infarct percentage as control I/R.

Conclusions & Implications

- Hearts treated with autophagy enhancers as pre-treatment or post-treatment both showed a decrease in infarct size percentage and an increase in cardiac function when compared to control I/R group.
- Hearts treated with an autophagy inhibitor as pre-treatment or post-treatment did not show a significant decrease in infarct size, and cardiac function was compromised similar as control I/R group.

The results suggest enhancement of autophagy may mitigate reperfusion injury when given as either pre or post-treatment.

Future Research

Future investigations will include:

- Evaluation of the synergistic effects of autophagy enhancers on cardiac function and infarct size when given as both pre and post-treatment.
- Evaluate the change of the autophagy induction markers by measuring LC3-2 and Beclin-1 (see figure 9) in an isolated hypoxia/re-oxygenation cardiomyocyte model.
- Evaluate the effects of autophagy enhancers or inhibitors in isolated hypoxia/ re-oxygenation cardiomyocytes model.

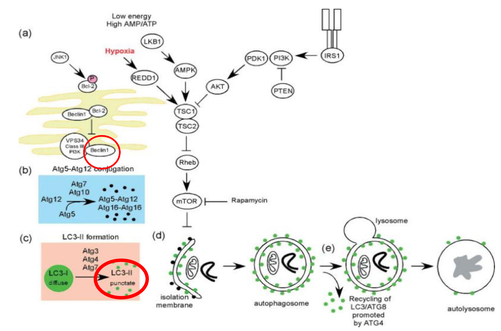

Figure 9. **Signaling pathway involved upregulation of Beclin-1 and LC3-2 leading to autophagy induction.**

References

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Acknowledgements

- This study was supported by the Division of Research, Center for Chronic Diseases of Aging and the Department of Bio-Medical Sciences at Philadelphia College of Osteopathic Medicine (PCOM).
- We would also like to thank PCOM alumni Andrew Castellano.