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Lack of Osteopontin Induces Systolic and Diastolic Dysfunction in the Heart Following Myocardial Ischemia/Reperfusion Injury

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Caytlin James

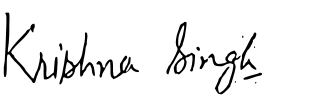

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Table of Contents

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Abstract

Ischemic heart disease is a leading cause of death worldwide. Osteopontin (OPN), a cell-secreted extracellular matrix protein, is suggested to play a cardioprotective role in mouse models of ischemic heart disease. The objective of this study was to examine the role of OPN in modulation of systolic and diastolic functional parameters of the heart following mouse ischemia/reperfusion (I/R) injury. For this, wild-type (WT) and OPN-knockout (KO) mice aged approximately 4 months were subjected to cardiac ischemia for 45 minutes by the ligation of the left anterior descending coronary artery (LAD) followed by reperfusion of LAD by snipping the ligature. Heart function was measured using echocardiography at baseline, 1, 3, 7, 14, and 27 days post-I/R injury. M-mode echocardiographic images were used to calculate % fractional shortening [%FS], % ejection fraction [%EF], end-systolic volume [ESV], and end-diastolic volume [EDV], while pulsed wave Doppler images were used to measure aortic ejection time [AET], isovolumic relaxation time [IVRT], and total systolic time [TST]. Velocity of circumferential fiber shortening (Vcf) was calculated using FS and AET. I/R injury significantly decreased %EF and %FS in both WT and KO groups at all time points (1, 3, 7, 14, and 27 days post-I/R) versus the baseline. However, the decrease in % EF and %FS was significantly greater in KO-I/R group versus WT-I/R at 3, 7, 14 and 27 days post-I/R. I/R-mediated increase in ESV and EDV were significantly greater in KO-MI group versus WT-MI 3 day post-I/R. AET was significantly higher in WT-I/R group 27 days post-I/R versus baseline. However, AET was significantly lower in KO-I/R group 3 and 27 days post-I/R versus WT-I/R. IVRT was significantly higher in KO-I/R group 27 days post-I/R vs baseline. However, IVRT was significantly lower in KO-I/R

group 1 day post-I/R vs WT-I/R. TST remained unchanged in WT and KO groups post-I/R versus their respective baseline groups. However, TST was significantly lower in KO-I/R group versus WT-I/R at 3 days post-I/R. Vcf was significantly higher at basal levels in the KO versus WT mice. I/R injury decreased Vcf in both groups versus their baseline at all time-points. These data provide evidence that lack of OPN deteriorates systolic and diastolic functional parameters of the heart following I/R injury, suggesting a cardioprotective role of OPN in myocardial remodeling post-IR.

Introduction

Heart Disease

Heart disease is a leading cause of death in the United States, responsible for approximately one in four deaths every year[1]. Over half of these deaths yearly are attributed to coronary heart disease, which entails a buildup of plaque along the walls of the coronary arteries causing narrowing and hardening of the arteries thereby partially or completely blocking the blood supply to the heart. This buildup can be caused by a variety of factors. Poor lifestyle habits, such as poor diet, lack of exercise, and smoking are described as major factors contributing to coronary heart disease.

The aforementioned occlusion of coronary arteries often leads to an insufficient supply of blood to the heart, known as ischemia. Ischemia results in damage to the heart muscle commonly referred to as heart attack or myocardial infarction (MI). According to the Centers for Disease Control and Prevention, one person suffers from a heart attack every 40 seconds, which amounts to approximately 790,000 incidents per year[1].

Myocardial Ischemia/Reperfusion Injury

The blockage of oxygen and nutrients during ischemia results in cardiac muscle damage and death. Following an ischemic event, early reperfusion of blood flow to the area of the infarct is the current standard of therapy to reduce infarct size, minimize myocardial damage, and preserve heart function[2]. However, the process of reperfusion, in some cases, lead to further damage to the cardiac muscle. The integrated damage

from the ischemia and reperfusion to the heart is known as myocardial ischemia-reperfusion (I/R) injury.

The complex pathophysiology of myocardial I/R injury results in chronic heart failure. During an ischemic event, blockage of the artery deprives the heart of oxygen and nutrients, resulting in an abrupt halt of oxidative phosphorylation and leading to depolarization of the mitochondrial membranes, depletion of ATP, and inhibition of the heart's contractile function[2]. This, in turn, forces the cell to switch to anaerobic respiration, resulting in lactate production and a decrease in intracellular pH. The increased acidity results in an overload of Na+ and Ca2+ in the cardiac cells and induces myocardial contractile dysfunction[2].

The damaging effects of reperfusion include reactivation of electron transport chain generating reactive oxygen species (ROS), an increase in oxidative stress, inflammation, and intracellular Ca2+ overload, leading ultimately to myocytes apoptosis, necrosis, hypertrophy and myocardial fibrosis[3,4]. Myocytes are vital for heart function, as they are the primary cells responsible for the rhythmic contraction and relaxation, and proper functioning of the heart. Importantly, myocytes are terminally differentiated and non-regenerative cells, therefore new myocytes may never grow to replace myocytes that have been lost from an injury[5]. This amplifies the severity of the myocardial I/R injury.

Additionally, increased levels of ROS impact different proteins involved in the excitation-contraction coupling mechanism, such as the sarcoplasmic reticulum Ca2+ release channel[4]. It is suggested that increased oxidative stress plays a primary role in

diminishing the systolic and diastolic functions of the heart, and contributing to heart failure following an I/R injury[4].

Systolic and Diastolic Functional Parameters of the Heart

The cardiac cycle consists of two phases: systole and diastole. Systole occurs during the period of contraction when the heart is pushing blood out of its chambers, followed by diastole, or the relaxation of the heart as it refills with blood. Left ventricular systolic and diastolic dysfunction are primary indicators of coronary heart disease[6]. Therefore, several measurements can be analyzed to detect myocardial dysfunction.

Systolic heart function can be measured using calculations such as % fractional shortening (%FS) and % ejection fraction (%EF). %FS is another term for the percentage of the size reduction of the left ventricle that occurs by the end of systole[7]. %EF denotes the amount of blood ejected from the left ventricle during a period of systole[8]. These calculations are a measure of the contractility of the heart's muscle. Diastolic function of the heart can be measured using calculations such as isovolumic relaxation time (IVRT) and E/A wave ratio, where E wave represents the peak velocity of early left ventricular filling and A wave represents the peak velocity of late left ventricular filling.

Other parameters which are also used to assess heart function include measurement of end diastolic volume (EDV), end systolic volume (ESV), aortic ejection time (AET), and total systolic time (TST). EDV refers to the volume of blood in the ventricles before the heart contracts, while ESV is the volume of the blood at the end of contraction[9]. AET measures the rate at which the left ventricle is pumping out blood[10].

IVRT is the time from aortic valve closure to the opening of the mitral valve[11]. In other words, AET and IVRT describe the periods of contraction and relaxation of the heart muscle. TST is the total amount of time from the beginning of heart muscle contraction to the end of relaxation. Velocity of circumferential fiber shortening (Vcf) is a measure of left ventricular mechanical function by examining the degree of fiber shortening and the duration of ejection[12].

Osteopontin

Osteopontin (OPN), also referred as cytokine Eta-1, is a cell-secreted extracellular matrix protein[5]. It was first identified in 1979 as being associated with malignant transformation but has since been shown to play a role in a range of biological processes. OPN is located on human chromosome 4q13 and has 314 amino acid residues, exhibiting high homology among many species such as humans, rats, and mice[13]. Due to the presence of RGD (Arg-Gly-Asp) amino acid residue, OPN is shown to interact with a variety of cell surface integrin receptors family. In cardiac myocytes, OPN is suggested to interact with a variant of hyaluronan receptor, CD44[14].

OPN is described as a protein with diverse biological functions such as bone resorption, immune cell activation, extracellular matrix remodeling, and inflammation[13,15]. The heart expresses low levels of OPN under basal conditions. Myocytes, fibroblasts and endothelial cells of the heart are shown to express OPN. A variety of stimuli such as angiotensin II, glucocorticoid hormone, cytokines, endothelin, norepinephrine are suggested to increase OPN expression in the heart and cardiac cells[5]. In the heart, increased OPN expression associates with the development of heart

failure[16]. Cardiac myocytes are identified as a source of OPN in models of myocardial hypertrophy[5]**.** OPN expression increases in both infarct and non-infarct regions of the heart post-MI in animal models[17], and in cardiac myocytes of patients with dilated cardiomyopathy[5,14].

A cardioprotective role of OPN has been demonstrated in a non-reperfused mouse model, MI, of myocardial remodeling. Mice lacking OPN exhibited exaggerated left ventricular chamber dilation following MI compared to wild-type mice due to increased expansion of the infarcted and remote myocardium[17]. OPN knock-out mice also exhibited decreased collagen accumulation and fibrosis deposition in the heart 28 days post-MI[17]. In a mouse model of repetitive myocardial I/R injury over a period of 7 days, OPN is shown to modulate contractile elements, antioxidative mediators, and inflammatory response[18]. These studies suggest a cardioprotective role of increased OPN expression during the development of ischemic heart disease. However, the role of OPN in systolic and diastolic functional parameters of the heart following an I/R injury remains to be fully explored.

Specific Aim

The objective of this study was to determine the role of OPN in the modulation of systolic and diastolic functional parameters of the heart following a myocardial ischemia/reperfusion injury in a time-dependent manner. It was hypothesized that lack of OPN would decrease the functional parameters of the heart following ischemia/reperfusion injury.

Materials and Methods

Animal Care

This study was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996). The animal protocols were approved by the East Tennessee State University Committee on Animal Care. Buprenorphine was administered subcutaneously 30 min prior to the surgery and once per day for 2 days after surgery to relieve pain. Mice were anesthetized using a mixture of 2.5% isoflurane and oxygen at a flow rate of 0.5 L/min. The study used both male and female mice, aged approximately 4 months, with 129X black Swiss hybrid background. After genotyping, wild-type (WT) mice and OPN-knockout (KO) mice were bred and maintained as separate colonies.

Myocardial Ischemia/Reperfusion (I/R) Injury

Myocardial I/R surgery was performed in WT and KO mice as described previously[19]. Briefly, mice were anesthetized using a mixture of 2.5% isoflurane and 0.5 L/min of oxygen and ventilated using a Harvard Apparatus rodent ventilator. Body temperature was maintained at approximately 37°C during surgery using a heating pad. The heart was exposed via a left thoracotomy. Cardiac ischemia was induced by ligating the left anterior descending artery (LAD) for 45 minutes using a 7-0 braided silk suture, which was tied around a piece of polypropylene tubing to allow easy reperfusion. After 45 minutes of ischemia, polypropylene tubing was removed and the ligature was snipped to allow reperfusion to occur. Ischemia was confirmed by a visible pale coloring of the heart and ST segment elevation in the EKG. Reperfusion was confirmed by

restoration of pink color of the heart. Functional parameters were measured using echocardiographic images at basal levels and 1, 3, 7, 14, and 27 days following I/R injury.

Echocardiography

Heart function was measured using transthoracic two-dimensional M-mode and pulsed-wave Doppler echocardiograms obtained using a VEVO 1100 imaging system (VisualSonics, Fujifilm) equipped with an MS550D 22-55MHz Microscan transducer. Echocardiography was performed at baseline, 1, 3, 7, 14, and 27 days following I/R surgery. Mice were anesthetized during echocardiography using a mixture of 2.5% isoflurane and 0.5 L/min of oxygen, and body temperature was maintained at approximately 37°C using a heating pad. Heart rate was maintained between 400-500 beats/min. M-mode images were used to measure end-systolic and end-diastolic diameters, which were then used to calculate %FS, %EF, ESV and EDV as described[19] (Fig.1). Pulsed-wave Doppler images were used to measure AET, IVRT, and TST (Fig.2). Vcf was calculated as a ratio of FS and AET.

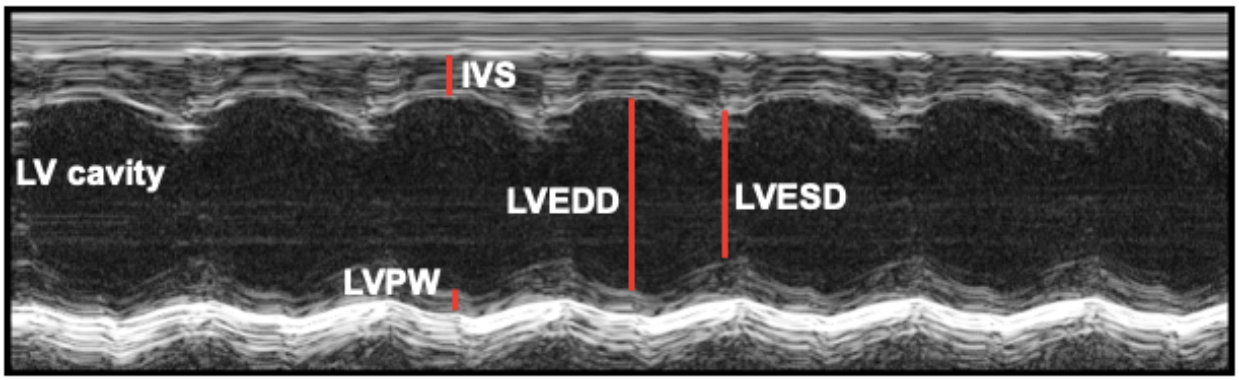

Figure 1. Representative M-mode image depicting left ventricular end diastolic volume (LVEDD), left ventricular end systolic volume (LVESD) and left ventricular cavity.

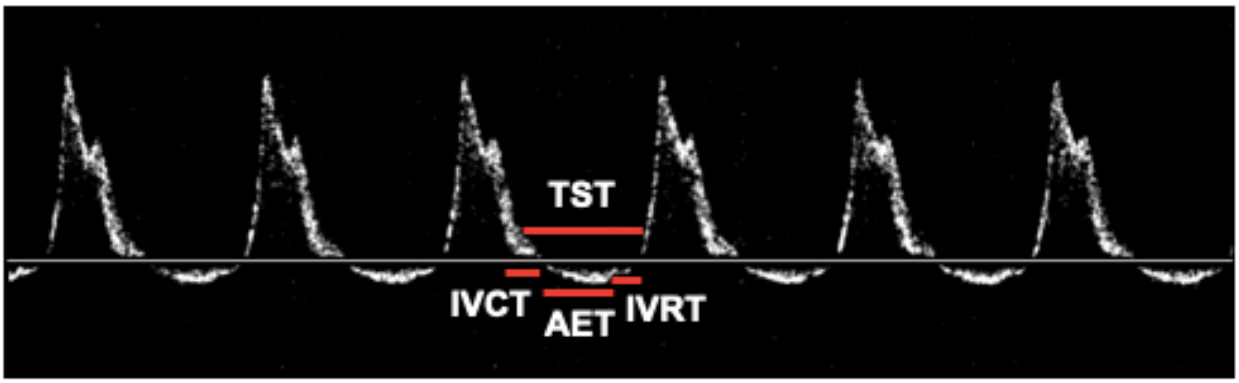

Figure 2. Representative pulsed-wave Doppler image depicting total systolic time (TST), isovolumic contraction time (IVCT), aortic ejection time (AET), and isovolumic relaxation time (IVRT).

Statistical Analysis

All data are expressed as mean ± SE. Data were analyzed using the 2-tailed Student's *t*-test. Probability (p) values of <0.05 were considered to be significant.

Results

Lack of OPN decreases percent fractional shortening (%FS) starting 3 days post-I/R

%FS (a measure of systolic function) is calculated as = (LVEDD-LVESD / LVEDD) x 100; where LVEDD represents left ventricular end diastolic diameter and LVESD represents left ventricular end systolic diameter. These parameters were measured using M-mode images as depicted in Figure 1. Calculation of %FS revealed no significant difference between the two genotypes (WT and KO) at basal levels (baseline). I/R injury significantly decreased %FS in both genotypes starting 1 day post-I/R, and remained significantly lower in both genotypes versus their baseline throughout the observation period ($^{\$}p<0.05$ vs baseline; n=7-16; Figure 3). Additionally, %FS was significantly lower at 14 days post-I/R in WT group versus 3 days post-I/R ($^{\#}p<0.05$ vs 3 days post-I/R; n=7-16; Figure 3). KO-I/R group also exhibited a decline in %FS with time, and was significantly lower at 7, 14 and 27 days post-I/R versus 3 days post-I/R ($^{@}p<0.05$ vs 3 days post-I/R; n=7-16; Figure 3). Interestingly, I/R-mediated decrease in %FS was significantly lower in KO-I/R group versus the WT-I/R group starting 3 days post-I/R and remained lower throughout the observation period (*p<0.05 vs WT-I/R; n=7-16; Figure 3).

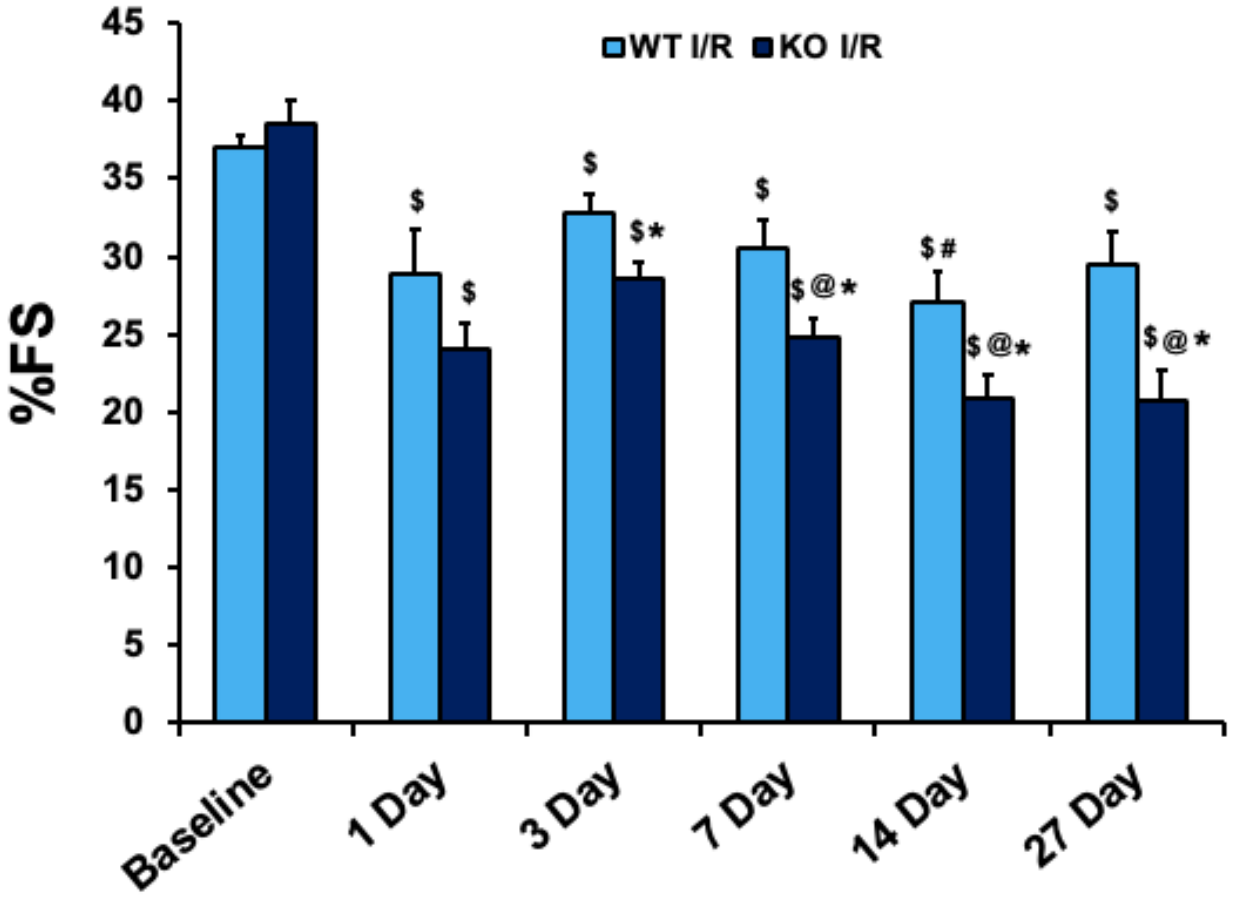

Figure 3. Lack of OPN decreases %FS post-I/R. $^{\$}$p<0.05 vs WT and KO baseline; $^{\#}$p<0.05 vs 3 days post-I/R (WT group); $^{@}$p<0.05 vs 3 days post-I/R (KO group); *p<0.05 KO-IR vs WT-I/R n=7-16.

Lack of OPN increases LV end systolic volume (ESV) starting 1 day post-I/R

ESV is the volume of blood remaining in the left ventricle at the end of systole. It was calculated using LVESD from M-mode images depicted in Figure 1 using a Fujifilm software on the VEVO 1100 echocardiographic system. ESV calculations showed no significant difference between WT and KO mice at baseline. The ESV was significantly higher at 7, 14, and 27 days post-I/R when compared to the baseline in WT group.

However, ESV was significantly greater in KO-I/R group 1 day post-I/R, and remained higher throughout the time course ($p<0.05 vs baseline; n=7-16; Figure 4). In WT-I/R group, ESV was significantly elevated at 14 and 27 days post-I/R versus 3 days post-I/R (#p<0.05 vs 3 days post-I/R; n=7-16; Figure 4). In KO-I/R group, ESV was significantly higher at 14 and 27 days versus 1, 3, and 7 days post-I/R (@p<0.05 vs 1, 3 and 7 days post-I/R; n=7-16; Figure 4). Notably, there was a significant increase in ESV in KO-I/R group vs WT-I/R at 3 days post-I/R (*p<0.05 vs WT I/R; n=7-16; Figure 4).

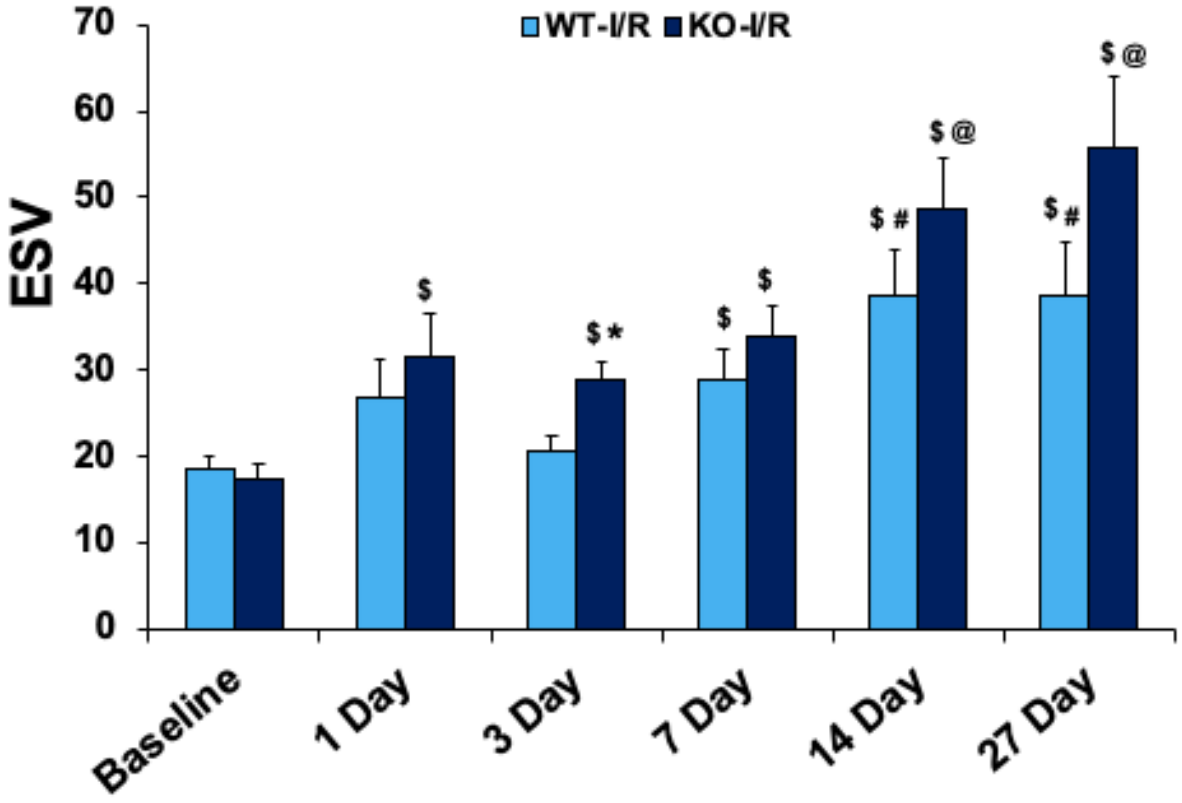

Figure 4. Lack of OPN increases ESV (in µl) starting 1 day post-I/R. $p<0.05 vs WT and KO baseline; #p<0.05 vs 3 days post-I/R (WT group); @p<0.05 vs 1, 3 and 7 days post-I/R (KO group); *p<0.05 KO-I/R vs WT-I/R; n=7-16.

Lack of OPN increases end diastolic volume (EDV) 3 days post-I/R

EDV is the volume of blood in the left ventricle right before the heart contracts. It was calculated using LVESD from M-mode image depicted in Figure 1 using Fujifilm software on the VEVO 1100. There was no significant difference in EDV between WT and KO mice at basal levels. I/R led to a significant increase in EDV in both genotypes 14 and 27 days post-I/R versus baseline ($p<0.05 vs baseline; n=7-16; Figure 5). Additionally, EDV was significantly higher in WT-I/R group at 14 and 27 days post-I/R versus 1 and 3 days post-I/R (#p<0.05 vs 1 and 3 days post-I/R; n=7-16; Figure 5). In KO-I/R group, EDV was significantly higher at 14 and 27 post-I/R versus 1, 3, and 7 days post-I/R (@p<0.05 vs 1, 3 and 7 days post-I/R; n=7-16; Figure 5). Interestingly, there was a significant increase in EDV in KO-I/R group vs WT-I/R at 3 days post-I/R (*p<0.05 vs WT I/R; n=7-16; Figure 5).

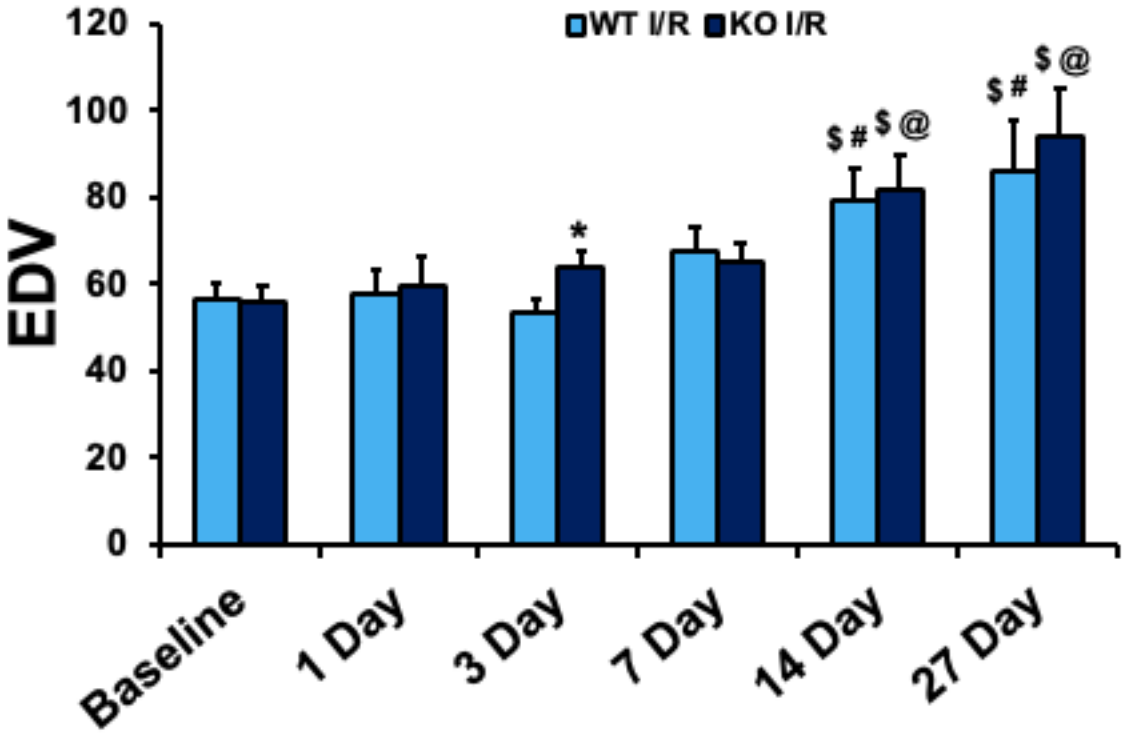

Figure 5. Lack of OPN increases EDV (in µl) 3 days post-I/R. $^{\$}$p<0.05 vs WT and KO baseline; $^{\#}$p<0.05 vs 1 and 3 days post-I/R (WT group); $^{@}$p<0.05 vs 1, 3 and 7 days post-I/R (KO group); *p<0.05 KO-I/R vs WT-I/R; n=7-16.

Lack of OPN decreases percent ejection fraction (%EF) starting 7 days post-I/R

%EF represents the percentage of blood leaving the left ventricle each time it contracts. It is calculated using ESV and EDV as (EDV-ESV)/EDVx100. Calculation of %EF revealed no significant difference between WT and KO mice at basal levels. I/R led to significant decrease in %EF in both genotypes versus their respective baseline controls starting 1 day post-I/R. %EF remained significantly lower in both I/R groups versus their baseline throughout the observation period ($^{\$}$p<0.05 vs baseline; n=7-16; Figure 6). Additionally, %EF was significantly lower WT-I/R group at 14 days post-I/R versus 3 days post-I/R ($^{\#}$p<0.05 vs 3 days post-I/R; n=7-16; Figure 6). In KO-I/R group, %EF was significantly lower at 14 and 27 post-I/R versus 3 and 7 days post-I/R ($^{@}$p<0.05 vs 3 and 7 days post-I/R; n=7-16; Figure 6). Importantly, I/R injury significantly decreased %EF in KO-I/R group versus WT-I/R group at 3, 7, 14, and 27 days post-I/R (*p<0.05 vs WT-I/R; n=7-16; Figure 6).

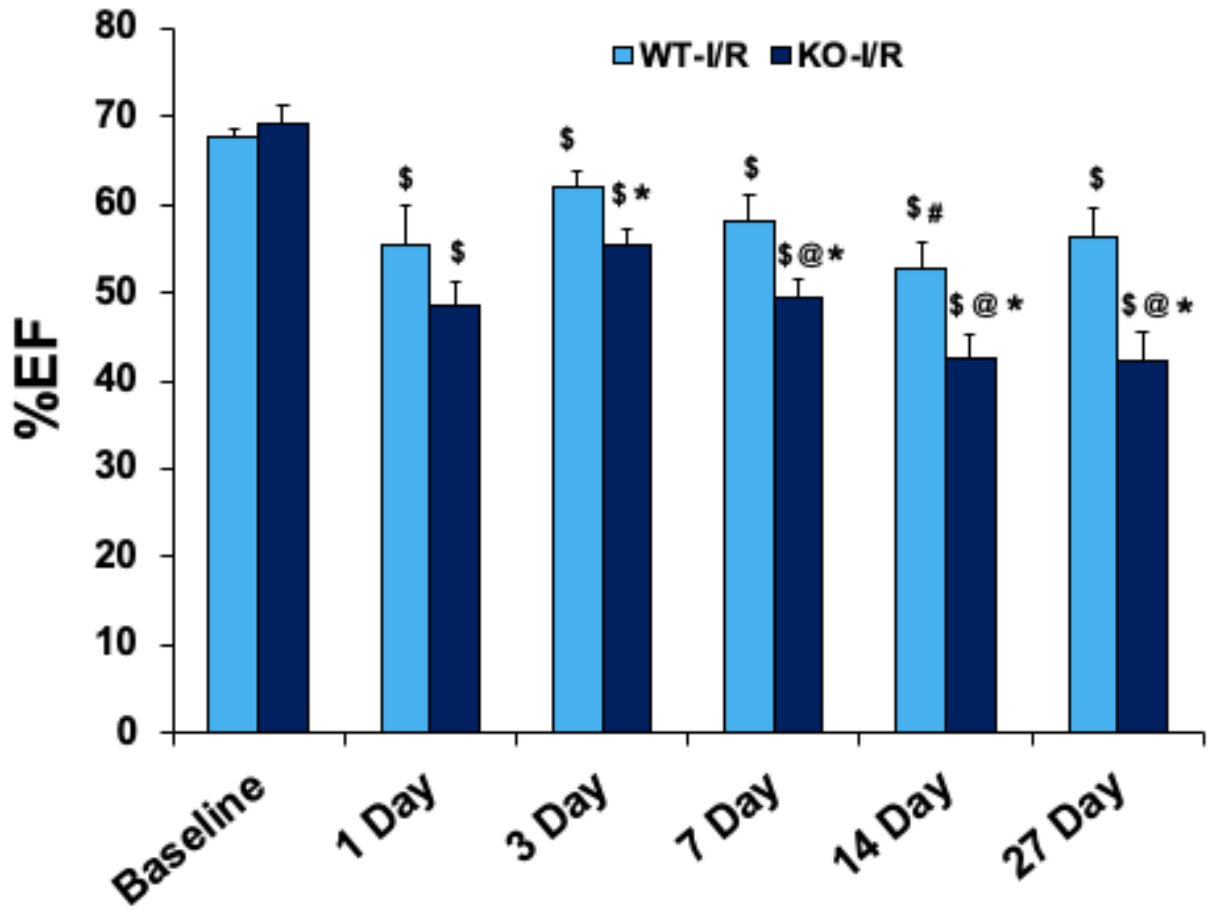

Figure 6. Lack of OPN decreases %EF starting 7 day post-I/R. $^{\$}p<0.05$ vs WT and KO baseline; $^{\#}p<0.05$ vs 3 days post-I/R (WT group); $^{@}p<0.05$ vs 3 and 7 days post-I/R (KO group); *p<0.05 KO-I/R vs WT-I/R; n=7-16.

Lack of OPN decreases aortic ejection time (AET) 3 and 27 days post-I/R

AET is the time between the opening and closing of the aortic valve. It is measured using the pulsed-wave Doppler images depicted in Figure 2. No significant difference in AET was observed at basal levels between the two genotypes. AET was found to significantly higher in WT-I/R group at 27 days post-I/R versus baseline ($^{\$}p<0.05$ vs baseline; n=6-10), while it remained unchanged in the KO-I/R group versus

baseline throughout the observation period. AET was significantly greater in WT-I/R group at 27 days post-I/R versus 1, 3 and 14 days post-I/R ($^{\#}p<0.05$ vs 1, 3 and 14 days post-I/R; n=6-10; Figure 7). In KO-I/R group, AET was significantly higher at 7 days post-I/R versus 1 and 3 days post-I/R ($^{@}p<0.05$ vs 1 and 3 days post-I/R; n=6-10; Figure 7). Interestingly, AET was significantly lower in KO-I/R group versus WT-I/R group at 3 and 27 days post-I/R (*p<0.05 vs WT I/R; n=6-10; Figure 7).

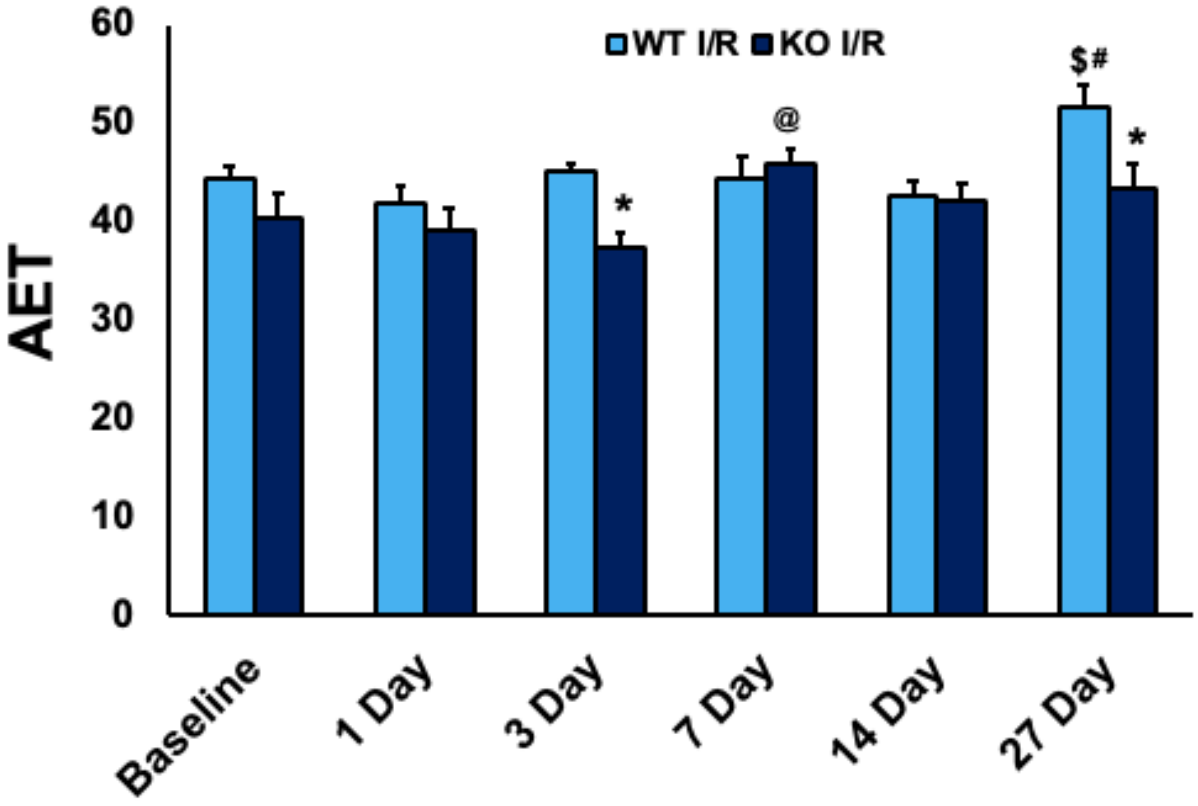

Figure 7. Lack of OPN decreases AET (in ms) 3 and 27 days post-I/R. $^{\$}p<0.05$ vs WT and KO baseline; $^{\#}p<0.05$ vs 1, 3 and 14 days post-I/R (WT group); $^{@}p<0.05$ vs 1 and 3 days post-I/R (KO group); *p<0.05 KO-I/R vs WT-I/R; n=6-10.

Lack of OPN decreases isovolumic relaxation time (IVRT) 1 day post-I/R

IVRT, a measure of diastolic function, represents the time interval in the cardiac cycle from the closing of the aortic valve to the opening of the mitral valve. It is measured using pulsed-wave Doppler images as depicted in Figure 2. IVRT was not found to be significantly different between the two genotypes at baseline. I/R injury significantly increased IVRT in KO group 27 days post-I/R versus baseline ($^{\$}p<0.05$ vs baseline; n=6-10; Figure 8). Additionally, IVRT was found to be significantly lower at 14 days post-I/R versus 1 day post-I/R in WT group ($^{\#}p<0.05$ vs 1 days post-I/R; n=6-10). In KO-I/R group, IVRT was significantly higher at 27 days post-I/R versus 1, 3 and 7 days post-I/R ($^{@}p<0.05$ vs 1, 3 and 7 days post-I/R; n=6-10; Figure 8). Interestingly, I/R injury resulted in a significant decline in IVRT in KO-I/R group versus WT-I/R group 1 day post-I/R (*p<0.05 vs WT I/R; n=6-10; Figure 8).

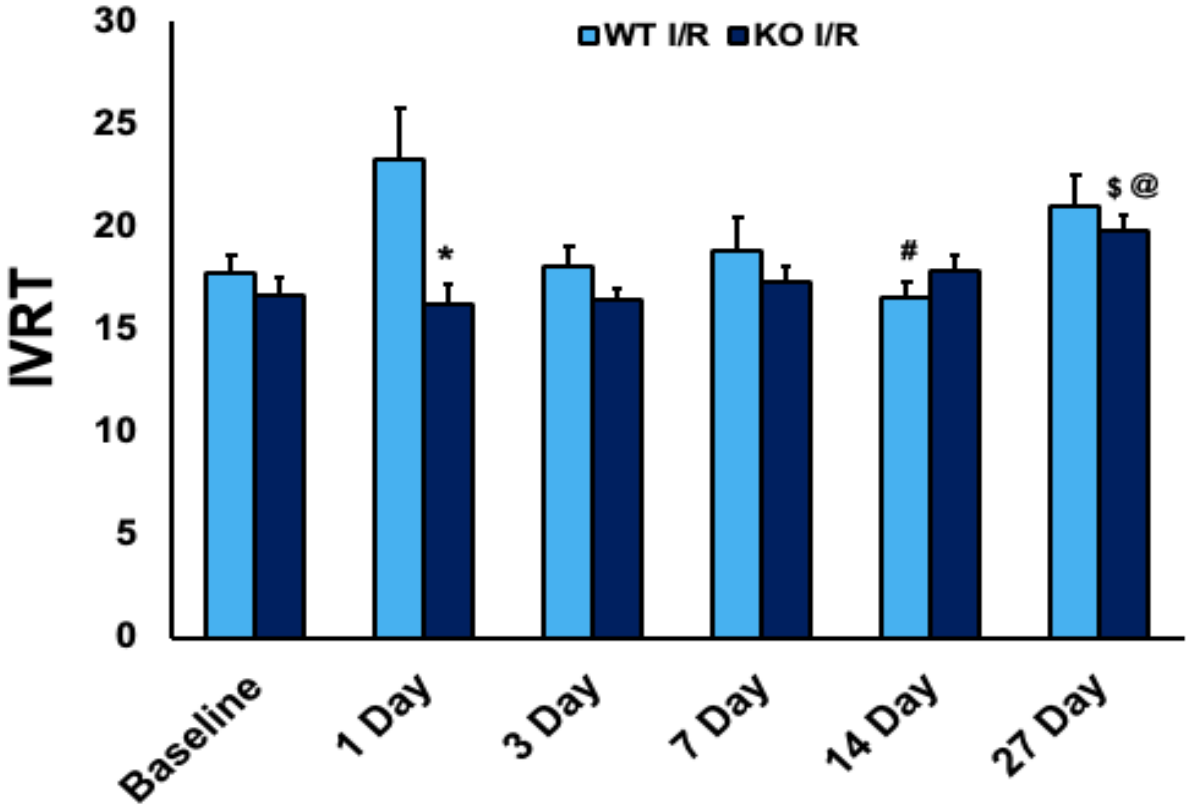

Figure 8. Lack of OPN decreases IVRT (in ms) 1 day post-I/R. $^{\$}p<0.05$ vs WT and KO baseline; $^{\#}p<0.05$ vs 1 day post-I/R (WT group); $^{@}p<0.05$ vs 1, 3 and 7 days post-I/R (KO group); *p<0.05 KO-I/R vs WT-I/R; n=6-10.

Lack of OPN decreases total systolic time (TST) 3 days post-I/R

TST represents the time interval from the onset of closing of the mitral valve to the opening of the mitral valve. It combines IVCT+AET+IVRT, where IVCT (isovolumic contraction time) is a time interval from the closure of the mitral valve to the opening of the aortic valve. IVCT is measured using pulsed-wave Doppler images as exhibited in Figure 2. There was no significant difference in TST between the two genotypes at basal levels. I/R injury did not result in a significant change at any point in the time course compared to the baseline in either group. However, TST was significantly lower in WT-I/R group at 14 days post-I/R versus 7 and 27 days post-I/R ($^{\#}p<0.05$ vs 7 and 27 days post-I/R; n=6-10; Figure 9). In KO-I/R group, TST was significantly lower at 3 days post-I/R versus 7 and 27 days post I/R ($^{@}p<0.05$ vs 7 and 27 days post-I/R; n=6-10; Figure 9). Interestingly, TST was significantly lower in KO-I/R group versus WT-I/R group 3 days post-I/R. (*p<0.05 vs WT-I/R; n=6-10; Figure 9).

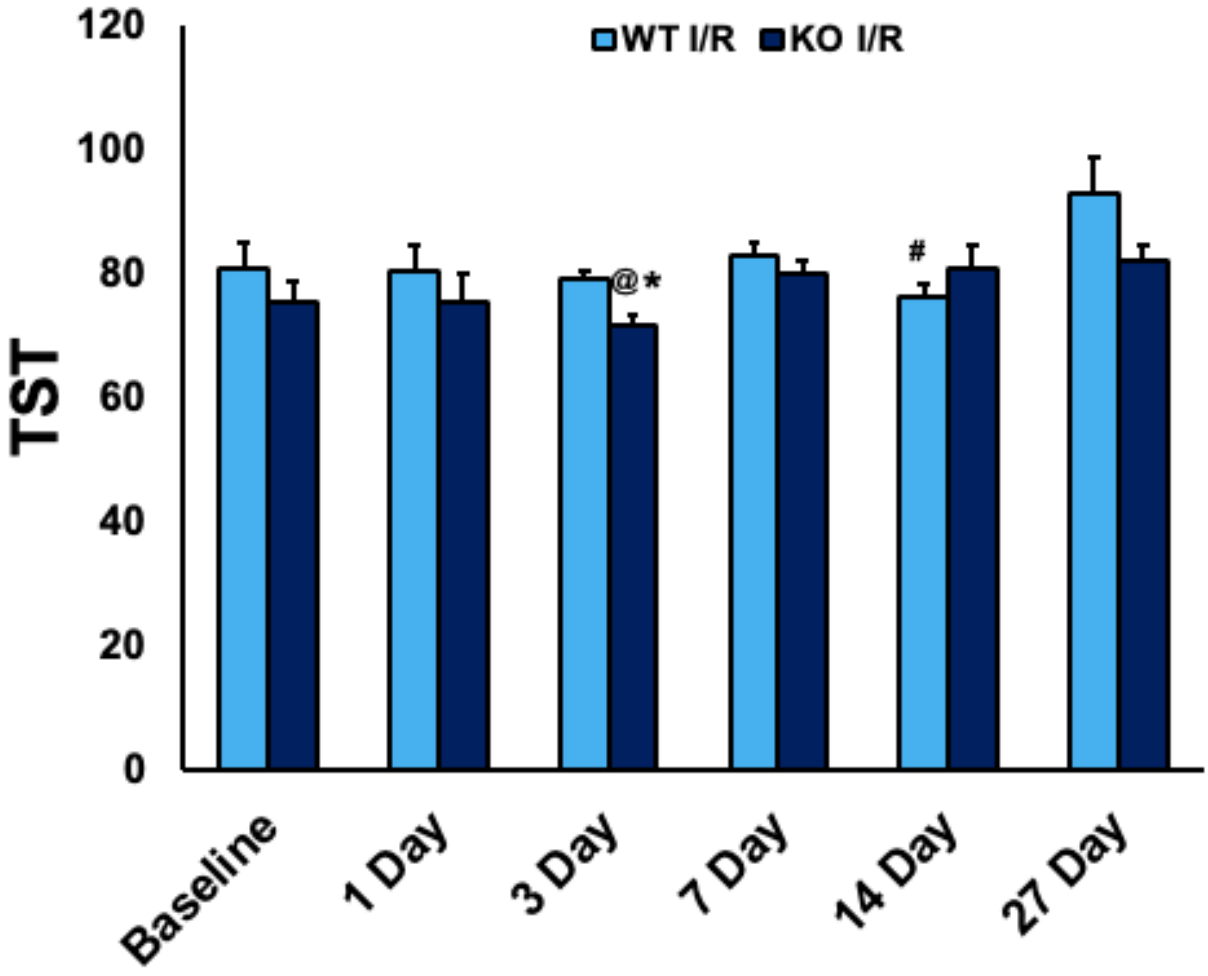

Figure 9. Lack of OPN decreases TST (in ms) 3 days post-I/R. $^{\#}p<0.05$ vs 7 and 27 days post-I/R (WT group); $^{@}p<0.05$ vs 7 and 27 days post-I/R (KO group); *p<0.05 KO-I/R vs WT-I/R; n=6-10.

Lack of OPN differentially affects velocity of circumferential fiber shortening (Vcf)

Vcf is a preload independent measurement of LV systolic function. It is calculated using M-mode and pulsed-wave Doppler images as (FS/AET) x 1000 in mm/sec. Calculations are generated using both M-mode and pulsed-wave Doppler images as shown in Figures 1 and 2. Results revealed that the Vcf is significantly higher in KO

group when compared to the WT group at basal levels (^p<0.05 vs WT baseline; n=6-10; Figure 10). I/R injury decreased Vcf in both groups 1 day post-I/R versus their respective baseline. Vcf was also found to be significantly lower at 27 days post-I/R versus baseline in WT-I/R group. In KO-I/R group, significant decrease in Vcf versus baseline was observed throughout the time course ($p<0.05 vs baseline; n=6-10; Figure 10). In addition, Vcf was significantly lower at 14 and 27 days post-I/R when compared to 3 days post-I/R in KO-I/R group (@p<0.05 vs 3 days; n=6-10; Figure 10).

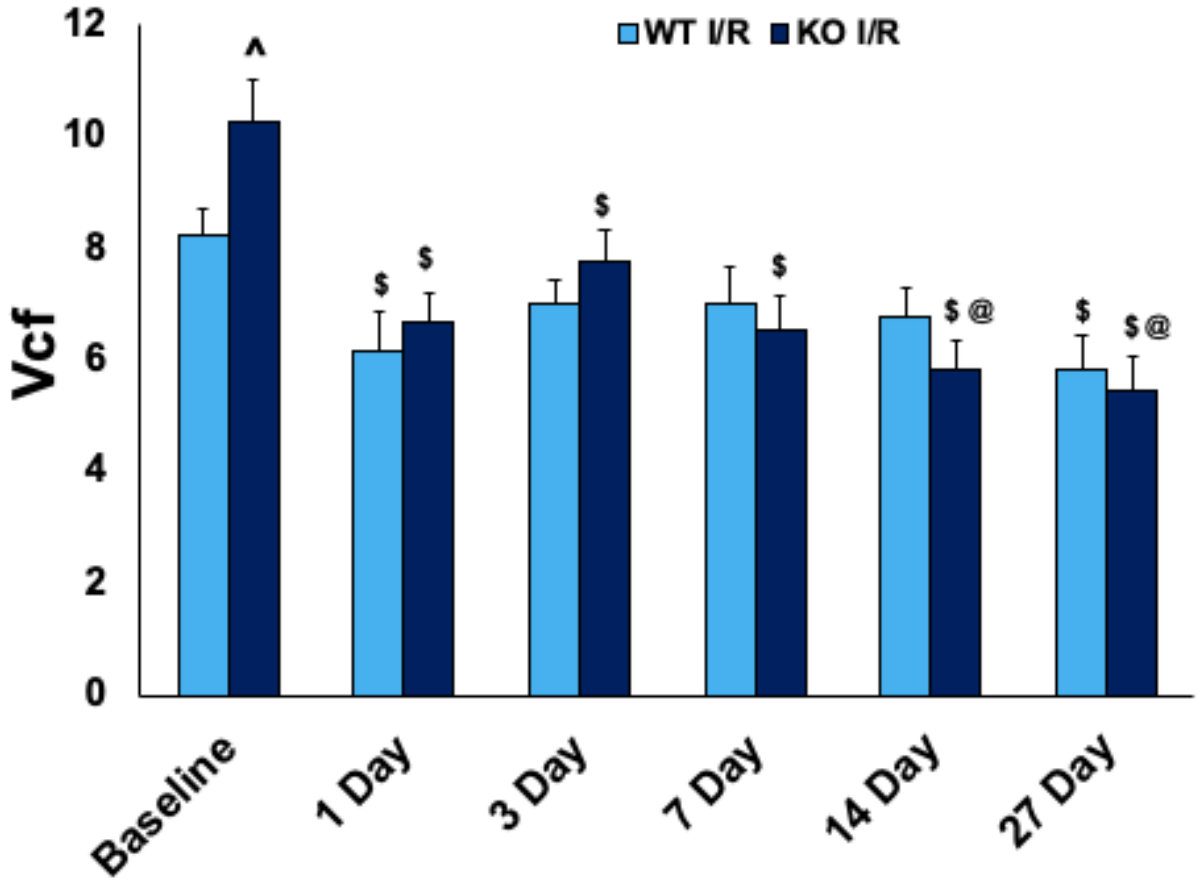

Figure 10. Lack of OPN differentially affects Vcf (in mm/sec). ^p<0.05 WT baseline vs KO baseline; $p<0.05 vs WT and KO baseline; @p<0.05 vs 3 days post-I/R (KO group). n=6-10.

Discussion

Previously, a cardioprotective role of OPN has been demonstrated in a non-reperfused MI mouse model, as well as in a mouse model of a repetitive I/R injury over 7 days[17,18]. This study investigated the role of OPN in systolic and diastolic functional parameters of the heart following an I/R injury in a time-dependent manner using WT and OPN-KO mice. The major findings of the study are that lack of OPN – 1) decreases %FS, %EF, AET, IVRT, and TST; and 2) increases ESV and EDV versus WT mice post-I/R. In addition, increased Vcf (a systolic index representing how fast the left ventricle shortens) was observed in mice lacking OPN at basal levels.

Myocardial I/R injury is known to induce systolic and diastolic dysfunction[6]. Systolic and diastolic functions are characterized by periods of contraction and relaxation as blood is being pumped in and out of the heart. M-mode images collected in this study were used to measure systolic heart function, such as %FS, %EF, ESV, and EDV. The %FS and %EF both decreased significantly throughout the time course post-I/R compared to the baseline in both OPN-KO and WT mice. However, the decrease in %FS and %EF was significantly greater in mice lacking OPN at 3, 7, 14, and 27 days following I/R injury, indicating that lack of OPN induces greater LV systolic dysfunction post-I/R. Previous work from our lab has shown that lack of OPN associates with decreased %FS and %EF 14 days post-MI[20]. Likewise, a significant decrease in %FS was observed in mice lacking OPN in a model of repetitive I/R injury 7 days post-I/R[18]. MI model represents a non-reperfused model of myocardial remodeling. The remodeling of the heart may occur differently between the non-reperfused (MI) versus the reperfused (I/R) models. Consistent with the non-reperfused MI and repetitive I/R

models, this study observed that I/R also leads to a significant decline in heart function as measured by %FS and %EF in mice post-I/R. Interestingly, mice lacking OPN exhibited greater LV dysfunction starting 3 days post-I/R. Together, these studies suggest that lack of OPN has an adverse effect on the development of cardiac dysfunction following a myocardial injury.

Remodeling of the heart following an injury due to MI or I/R induces LV chamber enlargement. This enlargement associates with increased systolic and diastolic volumes in the LV. Here, myocardial I/R injury increased both end systolic and diastolic volumes (ESV and EDV) in both WT and KO mice, specifically at later time points (14 and 27 days post-I/R). However, the increase in ESV in mice lacking OPN started at 3 days post-I/R, while increase in ESV in WT mice was observed 7 days post-I/R. Interestingly, both ESV and EDV were significantly higher in mice lacking OPN 3 days post-I/R. In a non-reperfused MI model, lack of OPN has been shown to associate with increased LV dilation and lower fibrosis 28 days post-MI[17]. The data presented here showed no difference in ESV and EDV between the WT and KO mice 27 days post-I/R. These data point towards the possibility that chamber dilation may be affected differently between the non-perfused (MI) and reperfused (I/R) models. Deposition of fibrosis following an injury affects heart function and remodeling processes. Therefore, analyses of components of fibrosis post-I/R may help explain lack of excessive LV dilation in mice lacking OPN 27 days post-I/R. However, early increase in ESV, and a significant increase in ESV and EDV together with decreased %FS and %EF in mice lacking OPN 3 days post-I/R support the notion that lack of OPN decreases systolic functional parameters of the heart following I/R injury.

Diastolic dysfunction is commonly represented by changes in IVRT and E/A wave ratio. Due to higher heart rates in mice (500 -700 beats/min), the E/A wave are usually merged on pulse wave Doppler images and making it difficult to draw conclusion. Therefore, this study did not analyze changes in E/A wave ratio. However, we were able to assess other functional parameters of the heart such as AET and TST using pulsed-wave Doppler images. Previous work from our lab has shown that IVRT is not significantly different between the WT and OPN-KO mice at basal levels or 30 days following induction of diabetes using streptozotocin[21]. However, this study did not measure AET or TST. Acute MI patients are suggested to display a significant decrease in AET and TST when compared to the normal patients[22]. Patients with dilated ischemic heart disease also exhibit shorter IVRT[23]. Consistent with the streptozotocin study, IVRT remained unchanged between WT and KO mice at basal levels. However, our study revealed that I/R injury increases IVRT in WT mice 1 day post-MI. In contrast, no such increase in IVRT was observed in mice lacking OPN. In fact, IVRT was significantly lower in mice lacking OPN when compared to WT mice 1 day post-I/R. AET was significantly shorter in mice lacking OPN when compared to WT mice at 3 and 27 days post-I/R, while TST was also significantly shorter in mice lacking OPN when compared to WT mice 3 days post-I/R. These findings suggest that lack of OPN decreases IVRT, AET and TST, although at different time points, following a myocardial I/R injury.

The Vcf is an index of myocardial performance calculated from FS and AET. Patients with myocardial disease are shown to exhibit lower mean Vcf when compared with the normal subjects[24]. In this study, the Vcf was significantly higher in mice lacking OPN at basal levels. While there was no significant difference in Vcf levels between KO

and WT mice throughout the time course post-I/R, there was a significant decrease in Vcf 1 day post-I/R in mice lacking OPN and remained lower in this group versus baseline throughout the observation period. In WT mice, Vcf decreased significantly 1 day post-I/R when compared to baseline. It remained unchanged (vs baseline) at 3, 7 and 14 days post-I/R. These data further suggests that lack of OPN affects myocardial contractility following I/R injury.

Conclusion

Heart disease is a leading cause of death worldwide, affecting thousands of individuals every year. More than half of these deaths are attributed to coronary artery disease, which causes a buildup of plaque along the walls of arteries, and can lead to an ischemia/reperfusion injury, or a heart attack. Myocardial I/R injuries are often linked to heart failure. This study investigated the role of OPN in systolic and diastolic heart function following an I/R injury. The major findings of this study were that lack of OPN decreases %FS, %EF, AET, IVRT and TST compared to WT mice post-I/R, increases ESV and EDV versus WT mice post-I/R, and increases Vcf versus WT mice at basal levels. Collectively, the data presented here provide evidence that lack of OPN exaggerates systolic and diastolic functional parameters of the heart following a myocardial I/R injury. However, further investigations are needed to understand the remodeling processes of the heart during OPN deficiency as OPN is described as a multifunctional protein with a potential to affect myocardial inflammation, apoptosis, hypertrophy, fibrosis etc. Understanding the role of OPN in heart function following an I/R injury may support the perspective of OPN as a possible target for therapeutic intervention in protection of the heart following a myocardial I/R injury.

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