

# VO-OHpic Treatment Reduces Cardiac Remodeling in Doxorubicin-Induced Cardiomyopathy

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VO-OHpic TREATMENT REDUCES CARDIAC REMODELING IN DOXORUBICIN-  
INDUCED CARDIOMYOPATHY

by

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A thesis submitted in partial fulfillment of the requirements  
for the degree of Master of Science  
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## ABSTRACT

Doxorubicin (Doxo) is one of multiple anthracycline drugs used to effectively treat various forms of cancer. Unfortunately, Doxo treatment, as a side effect, induces cardiomyopathy and subsequent heart failure. We have previously demonstrated that transplanted embryonic stem (ES) cells and their conditioned medium (CM) modulate the PTEN pathway and reduce apoptosis, fibrosis and hypertrophy in a Doxo induced cardiomyopathy (DIC) model. However, mechanisms of inhibited apoptosis mediated through PTEN pathway are completely unknown. Therefore, we used VO-OHpic (VO), a potent PTEN inhibitor to understand the mechanism of apoptosis as well as its effect on cardiac remodeling in DIC. Animals were divided into three groups; Group 1: Control (Saline), Group 2: Doxo (12 mg/kg, cumulative dose) and Group 3: Doxo+VO (30ug/kg cumulative dose). Animals were studied at one week and eight weeks post-DIC. Mice were subjected to echocardiography to examine cardiac function, sacrificed and hearts were harvested for further analysis. Immunohistochemistry staining revealed a significant ( $p < 0.05$ ) decrease in apoptotic cardiomyocytes in Doxo+VO treated hearts compared with Doxo group. Furthermore, Hematoxylin and Eosin (H&E) and Masson's Trichrome histological stains confirmed reduced hypertrophy and fibrosis in Doxo+VO treated subjects compared to Doxo group. Western Blotting confirmed the reduction of p-PTEN levels and the increase in p-AKT cell survival protein expression in Doxo+VO subjects. In addition, VO-OHpic administration was shown to reduce the number of pro-inflammatory macrophages and increase the number of anti-inflammatory M2 macrophages that may further be involved in reduced apoptosis and fibrosis. Finally, heart function was improved in mice treated with VO compared to Doxo group. Collectively, our data suggests that

VO-OHpic treatment reduces apoptosis, cardiac fibrosis and the process is mediated through the PTEN/AKT and inflammatory mechanisms with improved heart function in the DIC heart.

First and foremost, I am thankful for my Lord and Savior, Jesus Christ. I am humbled by this experience and am thankful for the opportunities presented and the memories that are made. I dedicate this to my incredible family; who have been supportive, encouraging and loving throughout this entire journey. This is not the achievement of only one person, but everyone who has been involved through mentorship and guidance. For that, I will always be gracious for my parents and brother for everything they do.

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## LIST OF ABBREVIATIONS

AKT	Adenosine triphosphate-dependent tyrosine kinase
A.U.	Arbitrary Units
CD206	Cluster of Differentiation 206
CM	Conditioned Medium
DAPI	4',6-diamidino-2-phenylindole
DIC	Doxorubicin-Induced Cardiomyopathy
Doxo	Doxorubicin
ES	Embryonic Stem
EF	Ejection Fraction
FS	Fractional Shortening
H&E	Hematoxylin and Eosin
iNOS	inducible Nitric Oxide Synthase
IP	Intraperitoneal
LV	Left Ventricle
MMP9	Matrix Metalloproteinase 9
p-Akt	Phosphorylated Akt
PFA	Paraformaldehyde
PI3K	Phosphoinositide-3 Kinase
p-PI3K	Phosphorylated PI3K
p-PTEN	Phosphorylated PTEN
PTEN	Phosphatase and tensin homolog
SAA	Sarcomeric- $\alpha$ -Actin

TUNEL

Terminal Deoxynucleotidyl Transferase dUTP Nick End  
Labeling Assay

VO

VO-OHpic

## CHAPTER 1: INTRODUCTION

### *Doxorubicin Induced Cardiomyopathy*

Doxorubicin (also termed Adriamycin) is an anti-cancer drug widely used for a variety of cancer therapies. As of June 2016, there are over 550 open trials worldwide exploring doxorubicin usage or usage of one of its variants daunorubicin or idarubicin (ClinicalTrials.gov). Unfortunately, clinical applications are limited due to the severe, dose-dependent cardiotoxic events that can occur as a side effect. These events may contribute to the development of Doxorubicin-Induced Cardiomyopathy (DIC) and congestive heart failure. DIC is characterized by contractility and rhythmic dysregulation as well as restructuring of the heart tissue (Singal, Iliskovic, Li, & Kumar, 1997; Minotti, Menna, Salvatorelli, Cairo, & Gianni, 2004).

DIC is the result of multiple, interconnected complex disease mechanisms; unfortunately, at this time, the exact mechanisms are still being researched. Some proposed mechanisms of action include the regulation of reactive oxygen species and antioxidants, myofibril/muscle degradation and changes in genetic expression (Singal & Iliskovic, 1998; Chatterjee, Zhang, Honbo, & Karliner, 2010). DIC results in physiological changes to the heart, especially the left ventricle, ultimately resulting in reduced heart rate and impaired cardiac function. In addition, the heart will undergo physical, structural changes, through a series of adverse events that are collectively known as cardiac remodeling.

## ***Cardiac Remodeling***

Cardiac remodeling is classified by a series of multiple events that occur to and within the heart during tissue damage. A normal, healthy heart is comprised of compact cardiomyocytes that beat in synchrony to allow for blood to rush into either the lungs for oxygenation or to the rest of the body through the aorta. A remodeling heart will undergo apoptosis (cell death) of cardiomyocytes and subsequently stimulate inflammation. Monocytes, in particular, will travel to the site of injury and undergo reprogramming into either pro-inflammatory M1 macrophages or anti-inflammatory M2 macrophages. In fact, the balance of M1:M2 macrophages is viewed as a determination factor for severity of inflammatory pathologies (Fujiu, Wang, & Nagai, 2014).

Hereafter, existing cardiomyocytes will undergo hypertrophy (cell size expansion) in order to maintain muscular foundation of the heart. In addition, fibrosis (collagen synthesis) will commence in order to provide additional structural support. Our lab has previously shown collagen build-up within the heart between muscle and around arteries and documented an increased expression of MMP9, a mediator of extracellular matrix decomposition and subsequent fibrosis accumulation (Merino & Singla, 2014; Singla, 2015). Collectively, due to the apoptosis of cardiomyocytes, hypertrophy of existing cells and accumulation of collagen, the newly remodeled heart's function diminishes, ultimately resulting in congestive heart failure or stimulate development of another cardiovascular disease.

Our lab has previously evaluated different mechanisms at which doxorubicin stimulates cardiac remodeling, as shown here (Singla, Ahmed, Singla, & Yan, 2012; Merino & Singla, 2014; Singla et al., 2012; Singla & Abdelli, 2015).

### ***VO-OHpic***

VO-OHpic is a vanadium based compound that has been established in literature as the most potent known inhibitor of the cell death protein PTEN (Phosphatase and Tensin Homolog) (Rosivatz et al., 2006). Vanadium mimics phosphoesters and phosphates, allowing them to effectively inhibit phosphatases (Rosivatz et al., 2006). Recently, VO-OHpic has been evaluated as a potential therapeutic for cardiovascular diseases. Zu et al reported that VO administration in a myocardial infarction model to have reduced infarction size and improve cardiac function post ischemia-reperfusion (Zu, Shen, Wesley, & Cai, 2011). A separate study conducted by Li et al demonstrated how VO-OHpic administration prolongs survival and improves cardiac function in a mouse sudden cardiac arrest model (Li et al., 2015). Recent studies have supported the idea of VO-OHpic use in diseased heart and cardiovascular tissues to improve cell survival, as evidenced by the upregulation of p-AKT both *in vivo* (Zu et al., 2011; Li et al., 2015) and in cardiac tissues *in vitro* (Zhu et al., 2014). p-AKT is the phosphorylated version of AKT, an essential protein found within the PTEN-PI3K pathway.

### ***PTEN-PI3K Pathway***

The PTEN-PI3K pathway is an essential cell survival and inflammatory pathway. This pathway has been shown to influence the release and activation of pro- and anti-inflammatory cytokines. PTEN functions to dephosphorylate PIP3, which prevents the

phosphorylation of PI3K, subsequent activation of AKT and ultimately inhibiting cell survival. The converse also occurs; if PTEN is inactive, PI3K is phosphorylated, thus phosphorylating AKT and other downstream proteins to stimulate cell survival. Our lab has previously shown the increase of p-PTEN and subsequent decrease of p-AKT in cardiac diseases (Merino & Singla, 2014; Singla, 2015; Yan, Singla, Abdelli, Singal, & Singla, 2013). The PI3K-AKT pathway has also shown to be influential in the polarization of monocytes, driving them away from becoming pro-inflammatory M1 macrophages and instead becoming anti-inflammatory M2 macrophages (Rocher & Singla, 2013).

The benefits of stem cell based therapeutics in driving cell survival have been shown in a DIC model (Singla, 2015; Singla et al., 2012), however the potential efficacy of the PTEN inhibitor VO-OHpic has not been explored.

### ***Hypothesis***

In this current study, we hypothesize that:

- I. Intraperitoneal (IP) delivery of VO-OHpic blunts p-PTEN levels and protects the heart from DIC.
- II. VO-OHpic administration promotes anti-inflammatory macrophage activity and simultaneously decreases pro-inflammatory macrophage activity

### ***Aims***

Aim 1. Evaluate the cardioprotective effects of VO-OHpic in acute and chronic DIC.

Aim 2. Determine whether VO-OHpic influences pro- and anti-inflammatory macrophages, in particular promote anti-inflammatory M2 macrophage activity.



## CHAPTER 2: MATERIALS AND METHODS

### ***Animal Model and Drug Preparation***

All animal protocols were approved by the University of Central Florida Institutional Animal Care and Use Committee as per United States National Institute of Health guidelines. C57BL/6J male and female mice of 8-12 weeks of age were separated into groups of 7-8 animals. Mice were administered cumulative doses of 1) saline (Control group), 2) Doxo only (12 mg/kg) or 3) Doxo (12 mg/kg) and VO-OHPic (30ug/kg). Solutions were administered via intraperitoneal injection (IP) every other day (i.e. Monday, Wednesday, Friday) to achieve cumulative dose. VO-OHPic was administered 30 minutes prior to same day Doxo injections, as adapted by previous studies in literature.

### ***TUNEL staining***

Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL) assay was performed as previously reported. The ventricles were embedded in increasing ethanol dilutions (up to 100% ethanol) and subsequently blocked in paraffin. 5 micron sections were obtained and heat fixed on microscope slides. Slides were then deparaffinized and rehydrated using xylene, 50% xylene/50% 100% ethanol and decreasing ethanol solutions. Slides were incubated in proteinase K (Sigma-Aldrich) at a dose of 25 ug/ml in 100 mM Tris-HCL for 15 minutes. Thereafter, TUNEL staining was performed according to manufacturer's instructions. Sections incubated in TUNEL reaction mixture for one hour and washed with 1x PBS accordingly. Finally, VECTASHIELD mounting media with DAPI (4', 6-diamidino-2-phenylindole) (VECTOR Labs) was applied to identify nuclei (blue). Under an Olympus fluorescent microscope, 4

representative regions at 20X magnification were taken and the percentage of apoptotic nuclei (red TUNEL marker) over total nuclei. Representative images were taken using a confocal microscope.

### ***Immunohistochemistry staining***

Ventricle slides were deparaffinized and rehydrated as previously stated for immunohistochemistry staining. Mouse antibodies for Sarcomeric- $\alpha$ -actin (SAA) (Sigma Aldrich) and inducible Nitric Oxide Synthase (iNOS) (Abcam) were stained appropriately using MOM kit as instructions prescribe (VECTOR Labs). Slides for CD206 antibody were incubated in 10% Goat serum and Arginase-1 antibody was incubated in 10% Donkey serum for 1 hour. Rabbit antibody for CD206 (Abcam) and Goat antibody for Arginase-1 (Santa Cruz) were diluted in 10% goat serum or donkey serum and applied for overnight incubation at 4°C. Slides were then washed with 1X PBS and incubated with appropriate secondary for 1 hour at room temperature. Sections were counterstained with DAPI mounting medium and analyzed. An Olympus microscope was utilized for data quantification and a confocal microscope for representative images. Areas of atrophic tissue were imaged for quantification of % monocytes or macrophages over the total nuclei. One representative image from each quarter of the heart (4 regions) was quantified for apoptotic data.

### ***Remodeling Histological Staining***

Ventricle sections were deparaffinized and rehydrated, as stated previously, prior to Masson's trichrome or Hematoxylin and Eosin (H&E) staining. Masson's trichrome was performed as previously described. In brief, sections were incubated in Bouins fixative for

45 minutes, washed and stained with Weigert Iron Hematoxylin, Biebrich Scarlet Acid Fuschin and Aniline Blue. H&E stain was conducted using hematoxylin, acid alcohol, bluing reagent and eosin solutions as previously described.

Sections were mounted in permount thereafter and analyzed using a light microscope. In Masson's trichrome, cardiomyocytes were stained in red, nuclei in black and fibrotic areas in blue. In H&E staining, nuclei are stained in blue/purple and cardiomyocytes in pink. Image J software was utilized to quantify vascular fibrosis (over vascular area) and cardiomyocyte size ( $\text{mm}^2$ ) at 20x magnification.

### ***Western Blot Analysis***

Heart samples were prepared, supernatants were collected and gel electrophoresis was performed. Proteins from gels were transferred onto polyvinylidene difluoride membranes and blocked with 5% milk. Membranes were incubated with desired antibody (phosphorylated (p-) AKT, MMP9, p-PTEN, total PTEN and  $\beta$ -Actin (as loading control)) for 3 hours at room temperature or overnight at 4°C. Membranes were then washed and incubated with appropriate secondary antibody for 1 hour at room temperature. Membranes were then washed, incubated in chemiluminescent substrate and exposed using developing film. Band intensities were measured using ImageJ software and represented as arbitrary units (A.U.)

### ***Echocardiographic Analysis***

At day (D) 7 and 56 post final injection, two dimensional (2D) echocardiography was performed under 2% isoflurane via nose cone to evaluate heart function, as previously described. In brief, M-Mode images of the left ventricle (LV) during diastole

and systole were visualized and recorded for measurement of LV fraction shortening (FS) and LV ejection fraction (EF). Hereafter, animals were sacrificed under isoflurane and confirmed using cervical dislocation. Hearts were harvested, cleansed with 1X PBS and sectioned transversely. The top portion (atria) was kept in RNA later and the bottom portion (ventricles) in 4% paraformaldehyde (PFA).

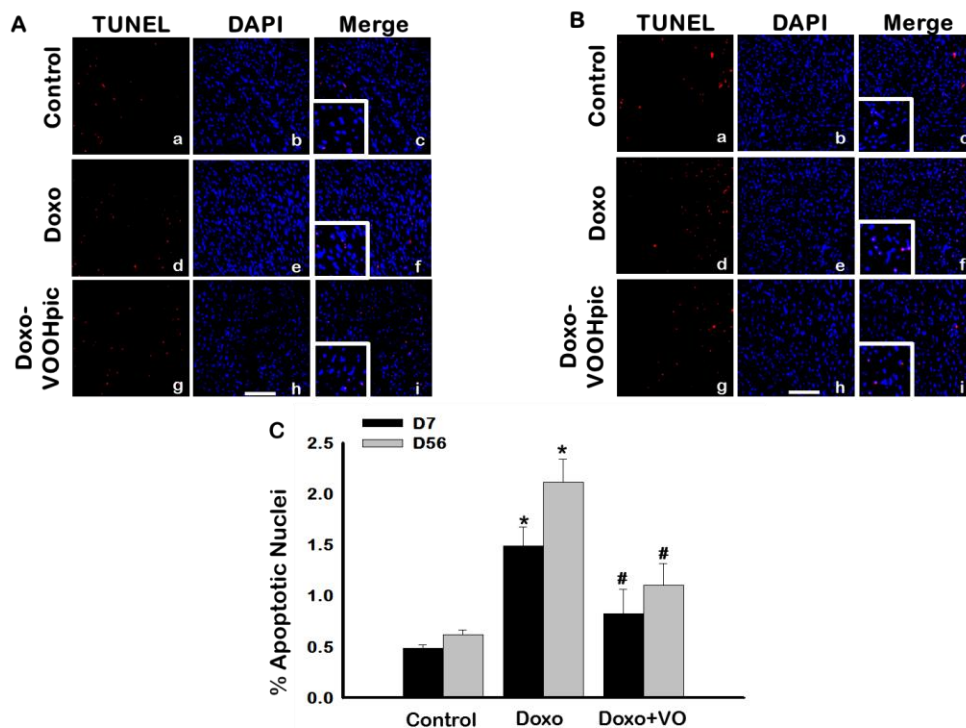
### ***Statistical Analysis***

Statistical analysis was conducted using one-way analysis of variance (ANOVA) and Tukey Test thereafter. Data values are presented as a mean  $\pm$  SEM with p-value  $<0.05$  to show statistical significance between groups.

## CHAPTER 3: RESULTS

### *Effect of VO-OHpic treatment on apoptotic nuclei in the heart*

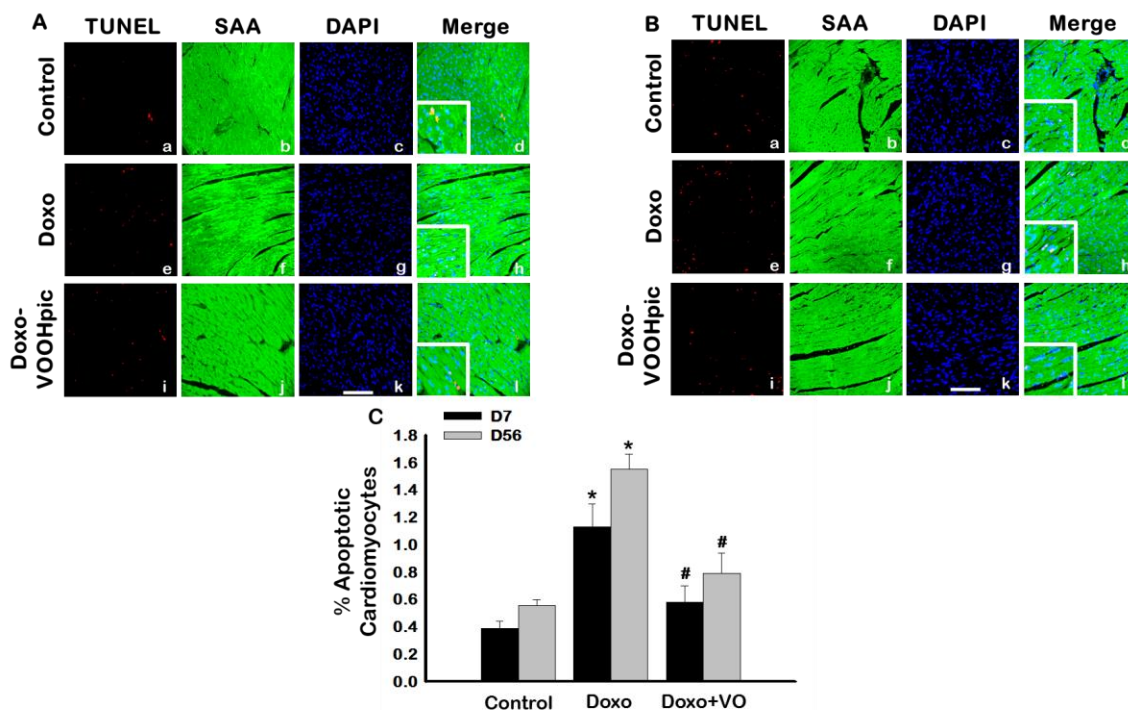
To confirm the effects of VO-OHpic treatment on total apoptotic nuclei, a TUNEL stain was performed. Positive TUNEL cells are quantified as red marker overlaying the blue stained nuclei (DAPI). The number of apoptotic nuclei increased in the Doxo group compared to the control group in both time points (**Figure 1**). However, the Doxo+VO group had a significantly lower percentage of apoptotic nuclei in both time points compared to Doxo group.



**Figure 1:** Percentage of Apoptotic Nuclei following Doxorubicin administration. Panels **A** and **B** display apoptotic nuclei in D7 and D56 mice hearts, respectively. Bar Graph **C** (Middle of image) depicts quantification of the percentage of apoptotic nuclei over total nuclei. \* $p < 0.05$  vs Control, # $p < 0.05$  vs Doxo, Scale Bar = 100 $\mu$ m, N = 5-6.

### ***VO-OHpic treatment reduces percentage of apoptotic cardiomyocytes***

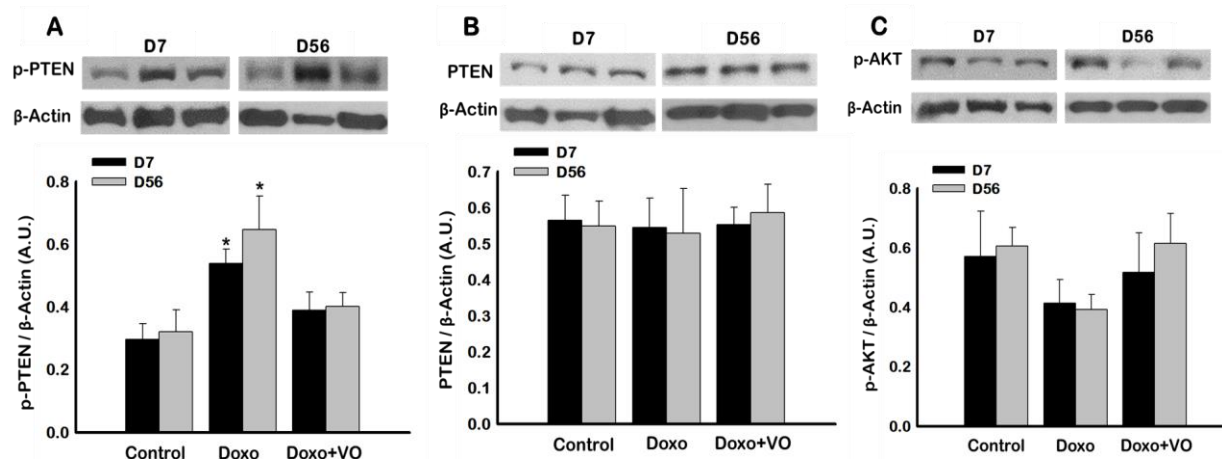
Once it was confirmed that VO-OHpic treatment reduces total apoptotic nuclei, a second TUNEL stain was performed with the addition of the antibody for SAA in order to quantify the percentage of apoptotic cardiomyocytes. TUNEL were stained as described above and SAA in green. The percentage of apoptotic cardiomyocytes increased in animals receiving Doxo treatment (**Figure 2**). In comparison, Doxo+VO animals have a significantly lower amount of apoptotic cardiomyocytes, when compared to Doxo group. Collectively with the first TUNEL stain, it can be said that VO-OHpic administration reduces the number of apoptotic nuclei and cardiomyocytes in a DIC animal heart.



**Figure 2:** Apoptotic Cardiomyocytes following Doxorubicin and VO-OHpic treatment Panels **A** and **B** display apoptotic cardiomyocytes in D7 and D56 mice hearts, respectively. Bar Graph **C** (Middle of image) depicts quantification of the percentage of apoptotic cardiomyocytes over total nuclei. \* $p < 0.05$  vs Control, # $p < 0.05$  vs Doxo, Scale Bar = 100 $\mu$ m, N = 5-6.

### Expression of Cell Survival proteins following VO-OHpic treatment

Western Blotting approaches were combined with staining data to confirm the underlying molecular mechanisms. Western Blotting was performed to measure the levels of proteins p-PTEN, PTEN and p-AKT upon administration of Doxo and the potential therapeutic effects of VO-OHpic (**Figure 3**). The expression of p-PTEN increased upon Doxo administration, whereas the Doxo+VO group exhibited decreased p-PTEN expression. PTEN expression was shown to be similar in all groups, revealing that VO-OHpic administration enacts on PTEN by inhibiting its ability to become phosphorylated. In contrast to p-PTEN results, cell survival protein expression of p-AKT was reduced in the Doxo only group and increased in the Doxo+VO group. This confirms that phosphates available are being uptaken by the PI3K-AKT pathway to stimulate cell survival activity, rather than being uptaken by the cell death protein PTEN.

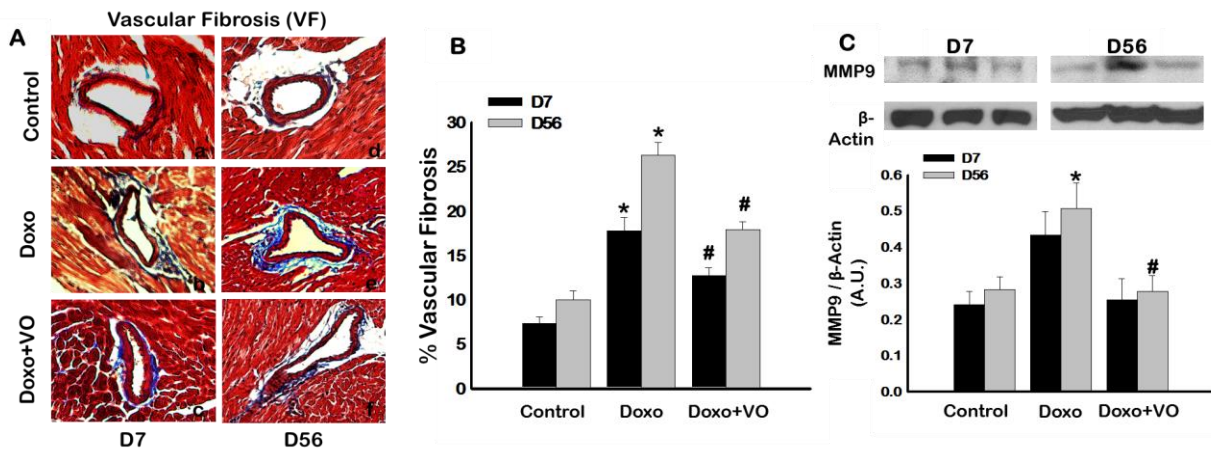


**Figure 3:** Effects of Doxorubicin and VO-OHpic treatments on cell survival protein expression.

Western Blotting of phosphorylated PTEN (**A**), PTEN (**B**) and phosphorylated AKT (**C**). Results quantified in Arbitrary Units (A.U.) based on protein expression. \* $p < 0.05$  vs Control, N = 4-7.

### ***VO-OHpic administration decreases fibrosis in the heart***

To confirm that VO-OHpic is involved in other physiological remodeling procedures, in addition to reduced apoptosis, Masson's Trichrome staining was performed to quantify the presence of collagen as a result of a remodeled heart (**Figure 4A-B**). Upon Doxo administration, the percentage of vascular fibrosis was shown to increase when compared to a control, healthy heart. In contrast to Doxo hearts, Doxo+VO animals demonstrated reduced amounts of vascular fibrosis in both time points. As further confirmation of reduced fibrotic activity, Western Blotting for MMP9, a mediator of extracellular matrix degradation and subsequent fibrosis, was performed (**Figure 4C**). In Doxo group, the expression of MMP9 increased compared to control. In the Doxo+VO group, however, MMP9 expression decreased in both time points. Collectively, these results show that VO-OHpic administration reduces fibrotic activity within the murine DIC heart.

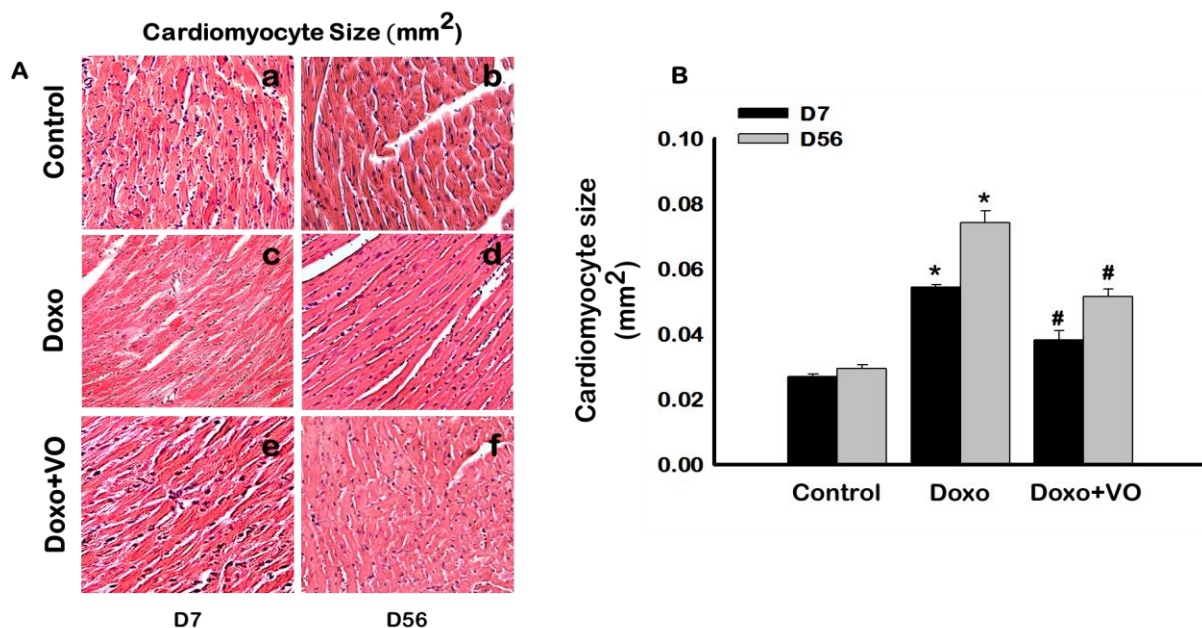


**Figure 4:** Fibrosis within the heart upon Doxorubicin and VO-OHpic treatments. Panel **A** displays Vascular Fibrosis within the murine heart. Bar Graph **B** represents quantitative data of % Vascular Fibrosis over total Vascular Area. MMP9 expression shown to increase in Doxo animals, but decrease with VO-OHpic co-treatment (**C**). \* $p < 0.05$  vs Control, # $P < 0.05$  vs Doxo, 20x Magnification enlarged to show artery, N = 5-6.



### ***Hypertrophy of Cardiomyocytes decreases with VO-OHpic treatment***

Previous studies have shown that cardiomyocyte size expands during cardiac remodeling. To confirm whether VO-OHpic treatment reduces hypertrophy in the DIC heart, Hematoxylin and Eosin (H&E) staining was performed (**Figure 5**). Cardiomyocyte size was shown to increase in animals with Doxo treatment compared to control hearts. In contrast, VO-OHpic administration resulted in a lower overall cardiomyocyte size when compared to Doxo hearts. When paired with previously stated apoptosis and fibrosis data, it can be confirmed that VO-OHpic reduces anatomical structural changes that occur in the heart as a result of Doxorubicin treatment.



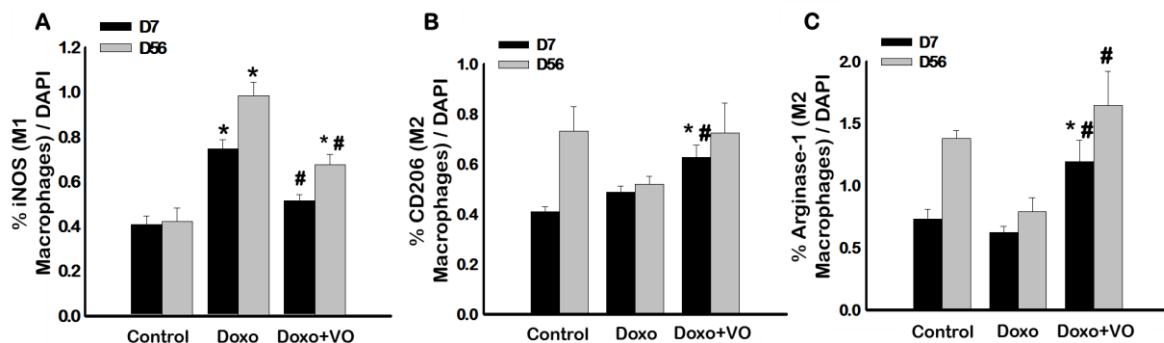
**Figure 5:** Hypertrophy of heart undergoing DIC treatments

Panel **A** represents areas of H&E stained cardiac tissue. Bar Graph **B** reflects average size of cardiomyocyte within the heart. Hypertrophy is reduced in both the D7 and D56 animals. \* $p < 0.05$  vs Control, # $P < 0.05$  vs Doxo, 20x Magnification enlarged areas, N = 5-6.

### ***Doxo+VO reduces M1 Macrophage activity and increases M2 Macrophage activity***

Monocytes have been well documented as cells with the ability to differentiate into M1 pro-inflammatory or M2 anti-inflammatory Macrophage sub populations. To confirm whether VO-OHpic treatment influenced M1 Macrophage populations, immunohistochemistry staining was performed (**Figure 6**). iNOS (inducible Nitric Oxide Synthase) was utilized as a marker of positive M1 Macrophages. In the Doxo animals, the expression of iNOS positive cells/M1 Macrophages increased. In comparison, iNOS levels were reduced in Doxo+VO hearts.

M2 macrophages were evaluated to confirm the presence of anti-inflammatory cell activity in the heart. The markers of CD206 and Arginase-1 were stained for using immunohistochemistry techniques and quantified for the presence of M2 macrophages within the heart (**Figure 6**). The Doxo+VO hearts exhibited an increased number of M2 macrophages compared to Doxo animals. Collectively, it can be concluded that VO-OHpic administration stimulates anti-inflammatory activity within the heart by driving monocytes from M1 macrophage differentiation towards M2 macrophage differentiation.

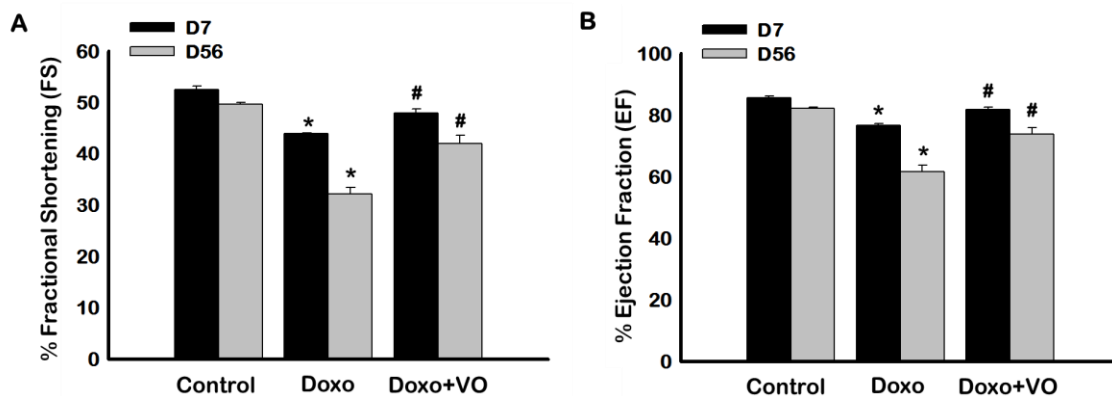


**Figure 6:** Doxo+VO effects on Macrophages

Bar graphs show the presence of iNOS positive M1 pro-inflammatory macrophages (**A**) and M2 Macrophages Arginase-1 (**B**) and CD206 (**C**) in D7 and D56 animals. \*p<0.05 vs Control, #p<0.05 vs Doxo, N = 4-6.

### ***VO-OHpic treatment improves cardiac function***

Prior to sacrifice, animals were subjected to echocardiography to evaluate function of the left ventricle as a result of doxorubicin treatment. In particular, two functional parameters, fractional shortening (FS) (measurement of contractility) and ejection fraction (EF) (measurement of blood ejection into heart) were quantified (**Figure 7**). In Doxo animals, both functional parameters were decreased. Indicative of reduced cardiac function. In contrast, Doxo+VO animals had significantly increased FS and EF compared to Doxo animals, resulting in improved left ventricular function and overall cardiac function.



**Figure 7:** Cardiac function improves with VO-OHpic co-treatment  
Echocardiography results were measured using fractional shortening (**A**) and ejection fraction (**B**). \* $p < 0.05$  vs Control, # $p < 0.05$  vs Doxo, N = 6-7.

## CHAPTER 4: DISCUSSION

Due to PTEN's catalytic pocket and elliptical opening, it makes it unique from other cysteine-based phosphatases, such as tyrosine phosphatases. As a result, VO-OHpic's structure and composition would fit nicely into this active site (Rosivatz et al., 2006). In comparison to other phosphatases, VO-OHpic's structure may be too large for their active sites. It is possible that due to the binding of the VO-OHpic with the PTEN active site, it allows for phosphates to become more accessible to proteins such as AKT and PI3K, thus leading to activation and the progression of survival mechanisms. In addition, VO-OHpic has been shown to be non-competitive in nature (with respect to OMFP substrate, a substrate used in PTEN inhibition studies) (Mak, Vilar, & Woscholski, 2010) as well as a reversible inhibitor (Mak et al., 2010; Spinelli, Lindsay, & Leslie, 2015), making it ideal in therapeutic design in order to optimize treatments for a desired pathology.

Collectively, Immunohistochemistry, Masson's Trichrome and H&E stainings experiments confirmed the progression of cardiac remodeling in doxorubicin animals through apoptotic, fibrotic and hypertrophic mechanisms at both an acute stage (D7) and chronic/long-term stage (D56) post treatment. In comparison, when doxorubicin was combined with VO-OHpic, the amount of cardiac remodeling events within and of the heart decreased. To further validate the physiological events, western blotting was performed to examine cell survival proteins essential to the cardiac remodeling process, in particular phosphorylated/active forms of PTEN and AKT. With co-treatment, p-PTEN expression decreased in Doxo+VO animals and p-AKT expression increased.

In addition to physical changes to the heart, inflammatory cells essential to cardiac remodeling were evaluated. Monocytes and macrophages, important in the phagocytosis

of debris and the secretion of appropriate cytokines and factors, were identified and studied for additional confirmation of VO-OHPic's effects within the heart. VO-OHPic animals demonstrated reduced volumes of positive M1 pro-inflammatory macrophages and increased volumes of M2 anti-inflammatory macrophages. To further confirm the beneficial effects of VO-OHPic administration on inflammatory actions in the heart, additional interleukins and cytokines should be evaluated.

Recently, the inflammatory role of T cells have been a subject of interest in the inflammatory and cardiovascular field as potential mediators of cardiovascular disease development. Naïve T cells can be converted into pro- or anti-inflammatory sub populations and can secrete factors known to play important roles inflammation (i.e. recruiting or stimulating cell activity (Meng et al., 2016). Our preliminary data has shown that VO-OHPic in the DIC heart increases anti-inflammatory T cell activity (data not shown), which may promote cell survival and M1 to M2 macrophage differentiation. Additional studies will be necessary to further elucidate the effects of VO-OHPic on T cell activity.

In conclusion, this study demonstrates that treatment with VO-OHPic reduces doxorubicin induced cardiomyopathy, as evidenced by a reduction in cardiac remodeling events and an increase in cell survival protein activity. In addition, our data suggests a polarization of monocytes from pro-inflammatory M1 macrophages to anti-inflammatory M2 macrophages.

### ***Acknowledgments***

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