The Role of Mitochondrial Pyruvate Carrier 1 (MPC1) in Heart Failure and Its Implications for Cardiac Recovery

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THE ROLE OF MITOCHONDRIAL PYRUVATE CARRIER 1 (MPC1) IN HEART FAILURE AND ITS IMPLICATIONS FOR CARDIAC RECOVERY

A Capstone Experience/Thesis Project Presented in Partial Fulfillment
of the Requirements for the Degree Bachelor of Science
with Mahurin Honors College Graduate Distinction
at Western Kentucky University

By
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*****

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ABSTRACT

Heart failure (HF) is a complex syndrome with high mortality rates around the world. HF also has diverse etiology as many things contribute such as hypertension, obesity, coronary artery disease, inflammation, and cardiac arrhythmias. Studies have shown that unloading of a failing heart with a left ventricular assist device (LVAD) can lead to cardiac recovery in a subpopulation of individuals with advanced HF. RNA-sequencing and protein expression analysis of myocardial tissue from HF patients who underwent the LVAD implant and heart transplant indicated that subpopulation of HF patients who responded to LVAD unloading had significantly lower levels of mitochondrial pyruvate carrier 1 (MPC1) in the failing heart, but that is was recoverable following mechanical unloading of the LVAD. The purpose of the present study was to determine the role of MPC1 in the failing heart in an animal model. The hypothesis was that MPC1 deficiency may lead to HF and normalization of MPC1 during LVAD unloading could drive the recovery in adult humans. To test this hypothesis, cardiac-specific deletion of MPC1 in adult mice was generated using locus of x over bacteriophage cre recombinase technology (cre-lopx). Results of the homozygous MPC1 mutant showed the cardiac dysfunction around 11 weeks post tamoxifen induction with an ejection fraction of 15%, increased left ventricular end diastolic diameter (LVEDD) of 7mm, increased left-ventricular mass (LVM) of 150mg, end-systolic volume (ESV) of 110 µl, and a total weight of 24 g. The mice eventually succumbed to HF at around 16
weeks post tamoxifen induction. To further characterize the MPC1 mutant for future experimentation, metabolism, gene expression, mitochondria structure, respiratory function, and myocardium structures in these mice will be examined and compared to the wild type littermate to understand the mechanism and to identify the therapeutic targets for drug development.
This is dedicated to the future Mrs. Kirk and my father. I love you both with all of me.

When my story's told, how will they tell it?  
Will they say I was a giver or remember I was selfish?  
Will they say I was a sinner or pretend I was a saint?  
Will I go down as a winner, what's the picture they gon paint?  
Wouldn't say that I'm a quitter that's one thing I know I ain't  
Will they tarnish, will they taint?  
Glorify me, overthink? say they know me, say I'm great?  
Say I'm phoney, I was fake?  
Say the things about me that they never told me to my face?  
    I was loved I was hated  
    Just a scholar with a dream  
I'm a liar I was honest, I was all of these things  
    When I'm gone let em talk  
    They discussing who I am  
When they bury me just know I was nothing but a man  
    Wasn't nothin' but a man.

-Jermaine Lamarr Cole
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To my brothers, you all have always been my heroes and most likely always will be. Isaac, your creativity is probably the greatest amongst the Kirk kids. Thank you for giving me brotherly advice when I most needed it during my time in college. Israel, your ambition to be the best at whatever you do is motivating. You alone have shown me how to yield significant results without exhausting one's resources. The greatest lesson you ever taught me is that I must sacrifice now for what I want in the future, unless what I want in the future will be sacrificed now. Isaiah, ever since I was a young lad, you always took care of me. The same love and care I see in our mother I also see in you. It is my hope that one day, when I have children, my sons can be molded not in your image, but
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CHAPTER I
INTRODUCTION

Heart failure (HF) is a complex syndrome that is known to have multiple unspecified etiologies (1). Traditionally, this condition is a result of the left ventricle (LV) pumping an insufficient amount of blood to the periphery of the body. Some of the factors that lead to this pumping inefficiency of the LV are diseases and risk factors such as coronary artery disease (CAD), diabetes, and hypertension (2). All of these factors affect the pumping of the left-ventricle in one of two ways, either during systole or diastole. Systole refers to the state of the LV as it is contracting and ejecting blood into the periphery of the body (head, hands, feet, etc.), while diastole refers to the state of the heart when it is in its relaxed state and is being filled with blood (3). In most end stage HF patients, an inability to pump blood to the periphery is far more common; therefore, this project will focus on systolic dysfunction.

In systolic HF, the LV is observed to have a phenotype that is both weak and dilated. In a sample of patients experiencing this form of heart failure, it was noted that they possessed much smaller ejection fractions; they were < 13%, versus a national average healthy heart ejection fraction of 50%< (4). Due to the decrease in blood volume that is pumped to the periphery of the body, the body has a tendency to retain fluid from the blood vessels as a compensatory mechanism. This serves as a temporary solution but quickly results in high fluid volumes that lead to an accumulation of fluid within both the
lungs and the LV, which explains why edema is a hallmark characteristic of a patient with heart failure. This accumulation of fluid is the reason as to why heart failure is termed “congestive heart failure”. Within the LV, high volumes of fluid can lead to other physiological complications such as cardiomegaly and dilated cardiomyopathy (5); both of which can be fatal.

Not only is heart failure a serious health concern, but it is also very common. HF affects approximately 6.5 million Americans with a high concentration of those people being in the Mid-Southeast region of the United States (Figure 1.1).

In 2017, HF contributed one in eight deaths amongst the American population. Mortality rates for individuals with a heart failure diagnosis are high; of those who are diagnosed with end-stage HF, 40%-60% of those patients experience death within five years of diagnosis (6)(7)(20). However, promising therapeutic interventions such as the left ventricular assist device (LVAD) have proven extremely useful in aiding end-stage HF patients as they await transplant, which could extend their lives.

The role of the LVAD is to mechanically pump the blood out of the LV to the periphery of the body (Figure 1.2). A 2001 study at Mount Sinai Hospital sampled end-stage HF patients that were ineligible for transplant to measure the validity and practicality of the device. The study consisted of 129 patients being randomly assigned to one of two groups: LVAD or optical medical management (OMM). Over the course of two years, results showed that patients who underwent LVAD installation outlived their OMM counterparts by as much as two-fold and spent twice as much time outside of the hospital (8).
Figure 1.1 Heart failure mortality rates, 2014-2016
Figure 1.2 Left ventricular assist device unloading diagram illustrates how the mechanical circulatory support device assists the failing heart
In order to better understand the mechanisms that contributed to these improvements, tissue samples were removed both before and after implantation of the LVAD and were analyzed via proteomics. Amongst all the patient tissue samples that were analyzed, a small percentage of the patients (15%) showed a down-regulation before implantation of the LVAD in the gene known as mitochondrial pyruvate carrier 1 (MPC1) (Figure 1.3). MPC1, which is the transporter of the glycolytic end product pyruvate, plays an essential role in shuttling necessary metabolites to the mitochondria to undergo oxidative phosphorylation (21). Following LVAD unloading, a normalization of this gene was seen in this population. Those who had experienced a normalization of the gene also saw significant myocardial improvement, which was measured via echocardiography. The patients who showed improvement and those who did not were termed as responders and non-responders, respectively. Notable changes in cardiac measurements such as left ventricular end-diastolic diameter (LVEDD), fractional shortening (FS), cardiac output (Q), and ejection fraction (EF) all experienced significant improvements, with the most important being EF. Figure 1.4 illustrates the timeline of the installation and unloading of the LVAD, as well as the improvement of the ejection fraction.

The goal of this research was to better understand how this gene is correlated to end-stage HF. We suspect that MPC1 plays an important role in cardiac function. To understand the effects of this gene on the heart, experimental trials in the adult mouse model were conducted to mimic the similar physiological responses seen in adult humans. Due to the similarity in metabolism between the mouse and human model, mice gene behavior is ideal for therapeutic gene targeting/manipulation. Also, due to the
critical ill state of the HF patients and the invasive nature of the study, the mice model was essential to carry out the experiment. Given the data collected on LVAD patients, it was predicted that functional inhibition of the MPC1 gene in mice would induce HF.
Figure 1.3 MPC1 in glucose metabolism pathway
Figure 1.4 Paradigm for understanding myocardial recovery in responders and non-responders
CHAPTER II

METHODOLOGY

LVAD Implantation and Responder Criteria

Patients who expressed a LVEF of 35%> and an LVEDD 60mm< were classified as having reached end-stage HF and were thus eligible to participate in the study. Patients were informed that the LVAD installation procedure required extraction of the core of the apex of the left ventricle. Those who agreed to participate in the study signed a participant agreement form which granted testing and analysis of the surgically extracted core.

Upon extraction of the core, the LVAD was installed into the left ventricle of the patient. Installation of this device required insertion of an inflow tube, which collects the blood from the left ventricle and shuttles it to a pump on the right side of the heart. This pump then propels the blood through an outflow tube, which is inserted into the aorta. The aorta then pumps the received blood from the outflow tube to the periphery of the body.

Following the installation of the LVAD, patients underwent weekly serial echocardiography to assess cardiac functioning. Within the patient population involved in the study, 15% demonstrated myocardial recovery by having an increase in LVEF, and a decrease in LVEDD. Cardiac measures showed that those who demonstrated myocardial recovery possessed a final resulting LVEF of 40%< and an LVEDD of 60mm>. For
patients whose ejection fraction resided 35%>, other cardiac measures were not considered due to an overall lack in pumping efficiency. Those who exhibited myocardial recovery in comparisons to those who did not were termed as responders and non-responders, respectively.

**Protein Analysis of MPC1 in LVAD Patients**

Upon implantation of the LVAD, the left ventricular core was extracted and utilized for testing. Testing showed a down-regulation in the mitochondrial pyruvate carrier 1 gene in the responders. Measurement of the protein concentration of MPC1 was conducted through western blot. In the western blot, ubiquinol-cytochrome c reductase core protein 2 (UQCRC2) was utilized as a house-keeping gene to normalize the levels of MPC1. UQCRC2 is located in complex III of the mitochondrion, which composes a vital portion of the mitochondrial respiratory chain (12). Genes associated with the functioning capacity of the mitochondria are able to be expressed when tested with UQCRC2. In order to better understand the role of the MPC1 gene in the responders, knockout experiments were conducted in mice.

**Mouse Model**

The mice (n=7) used in this study were genetically modified by use of Cre-locus of X-over P1 bacteriophage (loxp) recombinase technology. This technology allows for both spatial and temporal control over gene deletion in mice (9). When fertilization of embryonic stem cells (ES) occurs, insertion of two lox-p sites via a virus during the blastocyst phase of development allows for the lox-p sites to flank a specific gene of interest. In a shortly ensuing step following the insertion of the lox-p sites, the enzyme Cre is, then, able to delete the flanked sequence through recombination. However, in
order to ensure deletions of the MPC1 gene in the myocardial tissue exclusively, α-Myosin Heavy Chain (α-MHC), a known cardiac-specific protein, was used as a promoter (10). In order to ensure that the deletion of the MPC1 gene does not occur until adulthood, the Cre enzyme is formed in conjunction with a fusion protein. This fusion protein, known as Mer, binds Cre to the cytoplasm of the cell, restricting it from translocating to the nucleus to cause deletion (11). Mer, which has two modified estrogen receptor ligand binding domains, can release Cre from the MerCreMer complex when a known estrogen modulator is introduced. The estrogen analog that was used to induce MPC1 deletion in mice was tamoxifen.

Cross breeding of mice containing the α-MHC-MercreMer and mice containing the loxp produced a tamoxifen-inducible adult mouse model. Eight-weeks postnatal mice were injected with tamoxifen to induce MPC1 deletion (Figure 2.1). The tamoxifen-inducible mice that underwent gene excision were termed as knockouts.

Analysis of Cardiac Structure and Function

Serial echocardiography was utilized for both patients and mice to assess cardiac function. The following parameters were measured using echocardiography:

- Left ventricular end-diastolic diameter
- Fractional shortening
- Ejection fraction
- End-diastolic volume
- End-systolic volume
- End-diastolic left-ventricular mass
- Cardiac output
Figure 2.1 Knockout generation of cardiac specific MPC1 knockouts in adult mice
CHAPTER III

RESULTS

Upon LVAD unloading, a small portion of LVAD patients, termed as responders, showed normalization of MPC1 levels in comparison to the donor sample. The criteria for responders was a final resulting LVEF of 35%< and an LVEDD of 60mm>. For non-responders, if ejection fraction resided below 35%, other cardiac measures were not considered due to an overall lack in pumping efficiency. Responders’ MPC1 levels pre-LVAD implantation possessed a ratio value of 0.05. Following LVAD unloading, responders’ MPC1 levels attained a value of 0.10 which is shown in Figure 3.1.

Upon injection of tamoxifen in mice, changes in cardiac structure and function began to be seen eight weeks post-induction. An increase in the left ventricular end-diastolic diameter (LVEDD) of the MPC1 knockout led to cardiomyopathy and is shown in Figures 3.2 and 3.3.

Mice were monitored for a total of 16 weeks post-induction. For the first six weeks, no significant differences were observed between the MPC1 knockouts and their wildtype littermates. Throughout the course of the study, wildtypes maintained an ejection fraction of ≈ 40%, while the MPC1 knockouts LVEF progressively decreased by ≈ 3%/week following Week 7 post-tamoxifen induction. Changes in these ejection fractions are shown in Figure 3.4. Following the changes in ejection fraction, divergence in both LVEDD and end-diastolic left ventricular mass (EDLVM) were seen almost
simultaneously. For the LVEDD and EDLVM, wildtypes dimensions for each were measured to be 5.5 mm and 75 mg at Week 11 post-induction, respectively. For the MPC1 knockouts, LVEDD and EDLVM were measured to be 5.7 mm and 100 mg, respectively. Results for both the LVEDD and EDLVM are illustrated in Figure 3.5.

Echocardiography determined differences between the wildtypes and MPC1 knockouts in regard to fractional shortening (FS), end-systolic volume (ESV), and end-diastolic volume (EDV). Following induction, the MPC1 knockouts experienced dramatic variance in FS of $10\% \leq$ at both Week 9 and Week 16. ESV and EDV for the wildtypes at Week 9 were measured to be 48 $\mu$l and 52 $\mu$l, respectively. The ESV and EDV for the MPC1 knockout were determined to be 48 $\mu$l and 50 $\mu$l, respectively. Results for these measurements are shown in Figure 3.6.

At Week 9, MPC1 knockouts’ total mass experienced a relatively slight decline in mass of $\leq 1$ g/2 weeks, while wildtypes experienced a consistent growth in mass of $\geq 1$ g/2 weeks. Changes in weight loss patterns resemble the survival curve trend, which shows death of MPC1 knockouts at 16 weeks post-induction due to HF (see Figure 3.7).
Figure 3.1 MPC1 was lower in the failing heart of the responders, but not in the non-responders. MPC1 level was normalized following LVAD unloading in responders.
Figure 3.2 Representative image of MPC1 knockout showed dilation of the left ventricle in comparison to the wild type littermate control, 16 weeks post-tamoxifen induction. (n=7)
Figure 3.3 H&E stained sections of hearts from WT and MPC1 KO showing increased LV wall thickness and LV dilation in 16 weeks post-induction MPC1KO hearts (n=3).
Figure 3.4 ECHO analysis revealed significant reduction in EF (A) and FS (B) as early as 9 weeks post-induction in MPC1 knockouts, with respect to littermate wild type.
Figure 3.5 ECHO analysis showed increase in knockouts diastolic diameter (C) and LVM (D) in comparison to wild type littermates.
Figure 3.6 Wildtypes express steady state systolic and diastolic volumes (E)(F), while MPC1 knockouts exhibit varying levels of contraction volume (n=7)
Figure 3.7 Deletion of MPC1 induces heart failure, which leads to gradual loss of body weight (G)(n=7). Kaplan-Meier survival curve of WT and MPC1 KO as a function of weeks post-induction. A total of 3 WT and 3 KO were followed and all 3 KO deceased after 16 weeks of tamoxifen-induced MPC1 deletion (H). Error bars represent SEM, p-value <0.05 was considered significant; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 determined by student’s t-test.
CHAPTER IV
DISCUSSION

The results of this study indicate that end-stage heart failure is strongly correlated with MPC1 deficiency but can be recoverable via therapeutic intervention of LVAD in some heart failure patients. Results of the homozygous MPC1 mutant showed the cardiac dysfunction around 11 weeks post tamoxifen induction with an ejection fraction of 15%, increased left ventricular end diastolic diameter (LVEDD) of 7mm, increased LV mass of 150 mg, end-systolic volume (ESV) of 110 µl, and a weight of 24 g. In regard to weight loss for the MPC1 knockouts, it is primarily understood that this weight loss can be accounted for due to the progression of HF within the mice. The mice eventually succumbed to the failing heart at around 17 weeks post tamoxifen induction.

In a study conducted by Gustafsson et al., patients who underwent implantation of the LVAD experienced an increase in fitness as assessed by 6-min walk test distance, one-year survival of $\geq 80\%$, and, moreover, an increase in quality of life (14). Another study by Burkhoff et al. investigated the biochemical changes associated with gene expression in the failing heart. In this study, changes in the failing heart of the adult modeled a gene sequencing shift that resembled a fetal gene program (15). In the fetal gene program, reliance on the oxidation of glucose as opposed to free fatty acids is identical to what is seen in the failing heart (16).
Our study in particular is of significant importance because it suggests that the level of reverse cardiac remodeling an individual may undergo is tied to their genomic make up. For this study specifically, investigations into MPC1 were the only major changes observed both pre and post LVAD implantation. However, further investigations into other metabolic pathways, such as the electron transport chain, may provide insight into genes affecting HF further down the oxidative phosphorylation pathway.

Investigations into the citric acid cycle have shown that changes in pyruvate carboxylase (PC) may promote antioxidant capacity in the liver (17). If investigations into this same metabolic pathway were investigated in the heart, further insight may be provided as to the underlying cause in MPC1 deficiency.

Moreover, although extensive research has been conducted on the long-term effects of the LVAD in end-stage heart failure patients, little has been done to understand the biochemical modifications that assist in cardiac remodeling. Implantation of the device does indeed warrant an increase in systolic blood flow, but this sudden increase does not necessarily account for myocardial recovery. Studies such as these better aid in providing understanding as to how such remodeling is even feasible. This study in particular challenges the ideology that a person’s genomics play a small role in such a physiological phenomenon when, in fact, it may be a person’s genetic composition which may make such a recovery even possible. Current studies in humans, such as the one conducted by Nakao et al., showed that a person’s contractile velocity is a direct result of proper gene expression (22). In our study, investigations into a single gene, MPC1, showed that inhibition of the gene can indeed cause heart failure in a subset of the HF patients. Further investigations into the regulation of other genes could essentially
provide answers as to if a patient is even able to recover, or if the patient's tissue degradation is far beyond that of cardiac remodeling. Not only would such determinations provide further insight as to how or when a patient will recover, but could help determine future directions of patient treatment.

A potential source of treatment for heart failure patients is through participation in moderate-intense exercise. Physical activity studies within stable HF patients has shown that exercise is both safe and manageable in the failing heart and can reduce the level of hospitalization (23). In conjunction with a reduced amount of hospital care, an increase in health-related quality of life (HRQoL) was reported in most patients who participated in physical activity. Although the above reports elicit promise of change through training of exercise, further investigations into the physiological changes as a result of the activity would be needed to verify its effects. Although exercise has not been found to be statistically effective for those who are already experiencing end-stage HF, it has been shown to act as a form of preventative medicine in patients who were physically active throughout their youth and adulthood. Studies have shown that, although a decline in health is a result of aging, this process is decelerated in individuals who are physically active (24). Exercise has been shown to promote lower blood pressure, lower cholesterol, and reduced incidence of cardiac arrhythmias (25).

Although a vast amount of evidence supports the idea of exercise as a form of preventative medicine, often times the push or admonishment for a patient to become physically active comes long after the point at which exercise can be most optimal. It is simply not enough for health care providers to only recommend physical activity, but physicians should be prescribing it. With individuals who have a known genetic
complication for heart failure, it would be in their (as well as their blood-related family members’) best interest to remain or become physically active to combat the potential detrimental effects of heart failure. One of the largest issues concerning those who have or are currently experiencing heart failure are environmental and behavioral factors surrounding the individual. A common trend that can be seen in patients who have heart failure are things such as poor food access, excessive smoking, moderate-to-high alcohol consumption, and living conditions where a high concentration of pollutants can be found. These much larger issues are topics which need not only further discussion, but further action. Although it will take time for such changes to take place and yield promising results, exercise provides hope not only for better health, but promises those who actively participate in it a better quality of life.
CHAPTER V

CONCLUSION

In summary, this study confirms that deficiency in MPC1 levels leads to heart failure in a subset of the population in a mouse model but can be recoverable following LVAD unloading. These results are similar to what was found in humans. In non-responders, MPC1 levels were not significantly different at either time point (pre- and post-implantation of the LVAD), which could be attributed to a number of different variables. However, we believe that for non-responders, the cause of their heart failure must be due to some other source than MPC1 deficiency.

Due to the loss of the gene in mice, an increase in LVM and LVEDD were seen in the failing heart of the MPC1 knockout. As a result, both enlargement and dilation of the left ventricle were subsequent results which led to a decrease in LVEF. The decrease in LVEF led to a gradual loss of body weight, which in turn led to mortality 16 weeks post tamoxifen induction.

To better understand genetic changes in terms of recovery, usage of single cell sequencing technologies has shown promising results in revealing specific key genes associated with cell types in the heart (i.e. endothelial cells, fibroblast, B-cells etc.) (16). To further characterize the MPC1 mutant, future studies should examine the metabolism, gene expression, mitochondria structure, respiratory function, and myocardium structures in these mice, in comparison to the wild type littermates, to understand the mechanism and identify the therapeutic targets for drug development. Synthetic drug development
would not only help the identified responders in myocardial recovery, but would also increase the statistical odds of non-responders to readily undergo a heart transplant.

Limitations to this study primarily center around the usage of the mouse model. Although their genes are extremely similar to humans, making them suitable for targeted genetic manipulation (13), much about their metabolic pathways still needs to be investigated to better understand specific biochemical responses. Also, therapeutic mechanical interventions for mice have not yet been formulated. This poses issues due to the fact that experimental trials that aim to utilize these interventions are unfeasible, thus limiting procedures and conclusions that focus only on gene inhibition without being able to investigate the mechanisms of recovery and its entirety. Lastly, although usage of the LVAD is extremely beneficial and warrants study, the mere cost of the device and the procedure alone exceed the financial parameters of most patients. In order to combat the financial constraints of this procedure, a device that is more non-invasive, but replicates the same functions as the LVAD, needs to be created. This could give way to not only a larger patient sample size, but also provide more precise echocardiographic data. With HF becoming a growing epidemiological issue, researchers need to investigate the human genome for possible answers as to reversing the process.
BIBLIOGRAPHY


