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EXPLORING THE EFFECTS OF ZINC ON THE HEART DURING ISHEMIA/REPERFUSION INJURY

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EXPLORING THE EFFECTS OF ZINC ON THE HEART DURING ISHEMIA/REPERFUSION INJURY

by

DAVID NGUYEN

Presented to the Faculty of the Honors College of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

HONORS BACHELOR OF SCIENCE IN BIOLOGY

THE UNIVERSITY OF TEXAS AT ARLINGTON

May 2020

ACKNOWLEDGMENTS

I would like to start out with thanking Dr. Zui Pan for giving me the opportunity to work in her lab and agreeing to be my mentor for my honors project. Dr. Pan has shown me on what it means to become a real scientist, that it is a time consuming and sometimes frustrating process but, a rewarding one once you get your data. She was always willing to help in any way possible and to set time aside for me even though she has a very busy schedule. She is awesome in that she actively encourages undergraduate research, as she wants to offer the opportunity to contribute to science that will improve the lives of people and help fight disease. Not only has she helped me with my research project, she has truly made me a better critical thinker in that to not be afraid to ask questions as well as a better presenter.

I would also like to thank Dr. Sangyong Choi who is a post doctor under Dr. Pan, he invested countless of his hours in me and got me up to speed on proper lab techniques. Although I may have messed up a couple experiments, he has never once gotten mad and offers amazing constructive criticism and advice! He is truly a great teacher and I very much look up to him. I would also like to give a shoutout to Dr. Yan Chan and soon to be doctors Xian Liu and Lam Tran who also work under Dr. Pan. They made going to research lab very enjoyable and were always a pleasure to be around and happy to help me out.

Finally, I would like to thank my past research professor Dr. Wei Chen. I originally started my undergraduate research with him in the field of physics. He is what sparked my interest in research in the first place, and with Dr. Lalit Chudal, Dr. Sunil Sahi, and Nil

Padley who supported me along the way. I eventually wanted to branch off into more biology related research. So, I am very thankful he introduced me to Dr. Pan!

Finally, one final shout out to Jonathan Phan, without him mentoring me I would not have made it as far as I have now. Always giving me encouragement even when things looked dim.

I try to live by my dear friend Moses Eboh's quote "Just try your hardest, it's not all about being the best, rather it's knowing you worked your hardest to get to where you are and that is what matters". I am very proud of what I have accomplished at my time in the University of Texas at Arlington and having said that MAV UP 🤟.

May 05, 2020

ABSTRACT

EXPLORING THE EFFECTS OF ZINC

ON THE HEART DURING

ISHEMIA/REPERFUSION

INJURY

David Nguyen, B.S. Biology

The University of Texas at Arlington, 2020

Faculty Mentor: Zui Pan

Zinc, one of the most abundant minerals on earth may be the answer to protecting the heart from damage and malfunction. It has been found that supplementing the heart with zinc-ionophore during Ischemia/Reperfusion(I/R) injury can prevent the heart from damage. As it was found that during a heart attack, intracellular zinc levels were low as compared to the control. Thus, to deliver zinc more effectively to the heart we had two questions: Which zinc channel(s) or transporter(s) is involved in the reduction of intracellular zinc? Can enhancing such an intrinsic protein, instead of using side effects-prone zinc ionophore, protect the heart from I/R injury? Thus, it is necessary to identify the mechanistic factors regulating cardiac zinc homeostasis.

A real-time PCR experiment was done to detect the mRNA SLC5A3 to see if there was any zinc homeostasis expressed in Zebrafish if they can be a good candidate for other experiments. There was indeed a relationship between the channel and zinc homeostasis. An MTT assay test was also performed to study if the addition of zinc had any effect during ischemia reperfusion injury conditions. Finally, a western blot was done to detect SLC5A3 protein.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

CHAPTER 1

INTRODUCTION

1.1 Introduction

Each year there are approximately 1.5 million heart attacks that happen every year in the United States. Each time a cardiac arrest happens there is a condition called I/R Injury that happens. Simply, during a heart attack (ischemia) there is no oxygen flow at this stage there is no damage to the heart yet. Only when the heart is beating again, and oxygen is introduced (reperfusion) is when there is inflammation and oxidative damage to the heart. We want to reduce or prevent I/R injury from happening in the first place which is where supplementation of Zinc comes in. It has been found that low levels of zinc are found intracellular during a heart attack. The hypothesis is that if we selectively target the SLC5A3 gene it can be a good candidate for being a zinc transporter.

Solute Carrier Family 5 Member 3 (SLC5A3), This gene encodes SMIT1, a sodium myo-inositol transporter and had never been linked to zinc metabolism. To test this hypothesis, it is necessary to know whether SLC5A3 is indeed present in cardiomyocytes and if it is a good transporter for zinc. Thus, the first part of my project is to examine the expression profile of SLC5A3 in various cardiomyocytes, including HL-1 cells, a mouse atrial cardiomyocytes cell line, isolated adult mouse ventricular and atrial cardiomyocytes.

HL-1 Cells is a cardiac muscular cell line from an adult female mouse. The reason we use this is to do preliminary studies in testing ischemia-reperfusion conditions as well as looking at Slc5A3 by PCR.

Polymerase chain reaction (PCR), an experiment which allows DNA to be amplified “replicated” to be large enough to be studied. This is important as we can see the expression levels of SLC5A3 at various time points. Typically, a housekeeping gene or control gene is used to during this experiment. MTT Assay Test, a test which allows for assessing cell metabolic activity under various environments.

CHAPTER 2

METHODOLOGY

2.1 Cell Lines and Cell Culture

HL-1 cell lines were cultured in Claycomb Medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) (VWR, USA) and Norepinephrine 0.1mM and L-Glutamine 2mM and 1% penicillin/streptomycin (Corning™ Antibiotic-Antimycotic Solution) at 37°C in a 5% CO2 humidified incubator. Zinc Nitrate Hexahydrate were purchased from Sigma-Aldrich (USA).

2.2 Mitochondrial Activity Assay

The mitochondrial activity was detected using MTT assay, a test which allows for assessing cell metabolic activity under various environments. On a 96 well plate, HL-1 cells were incubated with 10,000 cells per well then exposed to various environments such as ischemia conditions with the addition of 10 uM of zinc for various time periods. After that, cells were returned to normal conditions for 13 hours, then they were incubated in 100mL per well 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution (0.5 mg/mL) for 2 h at 37 °C. Incubate for 3-4 hours until cells are stained black/purple, then remove medium and add 150uL DMSO to ALL wells including background while minimizing the amount of bubbles. Then with a UV-visible spectrophotometer the plates were read at 540nm.

2.3 Quantitative Real-Time PCR

To start we exposed Zebra fish the to various environments such as Hypoxia and Normoxia conditions for certain amount of time periods. Next lysed the heart cell then extract the RNA via an RNA filtrate kit which uses DNase to degrade DNA leaving only RNA. Then by using specific housekeeping genes and certain targeting genes we will able to see if there was any zinc homeostasis via that certain channel we are testing. Finally, SYBR green 1 is added to use as a fluorescence for the PCR machine to detect SLC5A3.

2.4 Western-Blotting

To start we will grow HL-1 cells and expose them to various environments such as Hypoxia and Normoxia conditions. Western blot utilizes gel electrophoresis, which detects abundance of a specific protein in a sample. We start off by lysed cells which then you add a protease inhibitor along with Radioimmunoprecipitation assay buffer (RIPA buffer). We then activate it via heat, then load onto an 8% gel containing 7.6 mL diH20,4mL acrylamide, 4mL 1.5 M Tris pH 8.8, 160 uL 10% SDS, 160uL 10% APS, 16 uL TEMED. The stacking gel was 6%. Then our solution was added, the gel is then attached to an electrophoresis machine which applies current, as proteins are negatively charged the proteins will get pulled from the top of the gel to the bottom via the positive charge. The higher molecular weight proteins will be near the top of the gel and the lower molecular weights at the bottom of the gel as they can travel more effectively though the gel. After this is done, we will not be able to see anything on the gel, only the markers which is our standards. We will have to transfer this to a special paper membrane 2x transblot filter paper which will be able to hold onto the proteins well. We then use a process called electro-transfer which allows the proteins from the gel to be transferred onto the paper.

Confirming that the standard marker has transferred, we then want to block unreacted sites on the membrane to reduce amount of nonspecific binding proteins. This is done by 5% TBST, the solution created was 1:1000 SLC5A3 antibody to milk. The SLC5A3 antibodies will make your specific protein of interest essentially luminance under UV light. We then load it into the machine, and it will finally show us the results of our test.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Real-Time PCR Zebrafish Heart

Zebra fish is a useful model for cardiac functional genomics research equipped with 1) a simple genetic manipulation tool, 2) many cardiac genes conserved in human, 3) similar heart rate to human compared to mice, and 4) transparent body enabling optical fluorescent imaging. Zebra fish has two SLC5A3 isoforms, slc5a3a and slc5a3b, both of which are orthologous to human SLC5A3 (77% and 79%, respectively). According to RNA-seq data (GSE94617, GSE120236) (Lai et al., 2017; Van Steenbergen et al., 2017), slc5a3b is a major isoform abundantly expressed in the heart. The hypothesis that SLC5A3 mediates zinc in zebra fish heart as well.

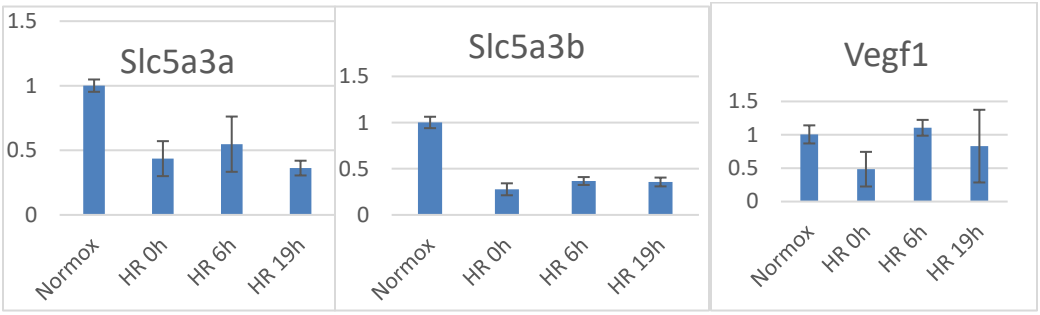

Figure 3.1: PCR Data of Slc5a3a / SLC5A3B / Housekeeping Hif1aa

Figure 3.1 shows Realtime PCR Data of Zebrafish heart cells exposed to hypoxia conditions (A). This tests for expression levels for SLC5A3A(B), SLC5A3B levels (C), Vegf1 Housekeeping gene expression level. As we can see, slc5a3a and slc5a3b is

expressed in zebra fish heart. Based on the results slc5a3a isoform is more abundant compared to slc5a3b.

3.2 Western Blot Data of HL-1 Cells in Hypoxia

To examine the expression profile of SLC5A3 in cardiomyocyte cells it was necessary to know whether SLC5A3 is indeed present in HL-1 Cells. So, by using western blot we can confirm is there is an expression level at all.

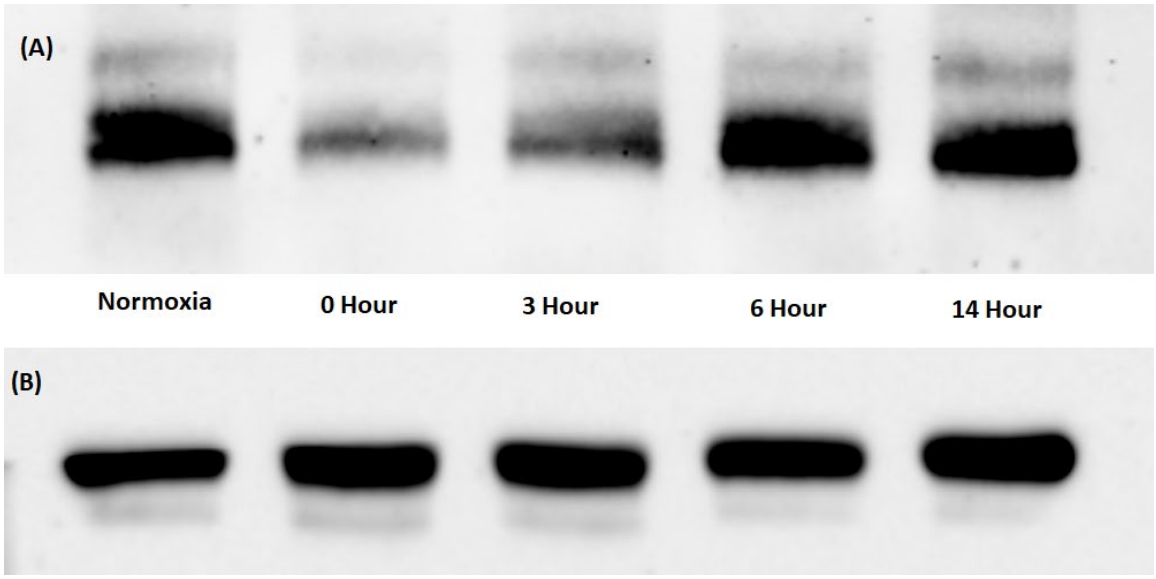

Figure 3.2: Expression of SMIT1 levels in HL-1 cells when exposed to hypoxia conditions measured by western-blotting method. (A) SMIT1 in HL-1 Hypoxia. (B) Alpha-tubulin in HL-1 Hypoxia which was used as a loading control.

There is clearly a relationship between SMIT1 expression levels as time went on after being exposed to hypoxia conditions for 12 hours. At 0 Hr. there seems to be little SMIT1 level, but as time went on when exposed normal O2 levels, the expression level rose. Ideally, I should have data of western blot with the addition of zinc but due to COVID19 I was unable to perform this experiment.

3.3 Mitochondrial Activity Assay of HL-1 Cells with Zinc

To test whether zinc has any effects when supplementing the heart with zinc-ionophore during I/R injury if it can prevent the heart from damage, this test was made. We replicated a I/R injury by growing all HL-1 cells at 37°C in a 5% CO2 humidified incubator for 12 hours. Then media was replaced, and 10uM zinc was added, and the control was allowed to sit in the 5% CO2 incubator while the hypoxia cells were placed into a incubator with 5% oxygen and the rest nitrogen for a total of 12 hours. After that the media was replaced once more in all and allowed to incubate in the Normoxia conditions for 6 hours. Then MTT procedure was employed.

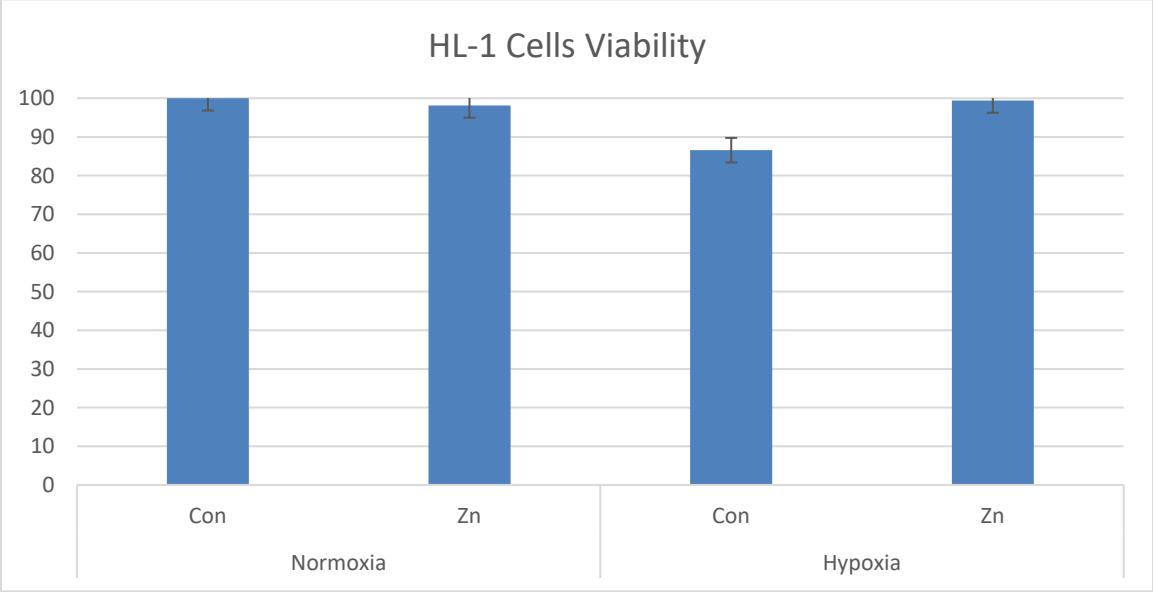

Figure 3.3: The Addition of Zinc reduces cell death of HL-1 cells in Hypoxia conditions. Cell viability measurement. HL-1 cells were treated Zinc in Normoxia and Hypoxia conditions.

According to our results, in the Normoxia conditions the addition of zinc seems to be lower than our control, but this could be due to a margin of error as indicated by the error bars. However, in the Hypoxia conditions we see a big difference in cell viability in

that compared to our control group, the addition of zinc seems to increase HL-1 Cells viability rate.

CHAPTER 4

CONCLUSION

In this paper we try to examine SMIT1A is a possible candidate for being a zinc channel(s) or transporter(s) is involved in the reduction of intracellular zinc. Also, with the addition of zinc, does it really protect the heart from I/R injury. So, we did a real time PCR to see if Zebrafish is a good candidate to do future testing on to see whether they express SLC5A3, which they do. This means that zebrafish is a good live test subject to do more studies on SLC5A3 being a possible zinc transporter. A western blot was also performed to see SMIT1 ‘s expression level during a stimulated I/R attack. I would have also done a western blot with the addition of zinc to see how it changes the expression level. But due to covid-19 and the lab shutting down this was not possible. Finally, an MTT assay was performed if zinc had any effect during an I/R injury, there does seem to be a possible correlation with zinc protecting the cells from hypoxia conditions. Overall, there is still much exploration on SMIT1 being a zinc transporter and I hope to continue this project to release more data soon.

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BIOGRAPHICAL INFORMATION

David Nguyen is a senior earning a Bachelor of Science in Biology at the University of Texas at Arlington. He entered The University of Texas at Arlington in Summer of 2017 and will graduate Spring 2020. Starting out research he meets a physics professor Dr. Wei Chen, and this is where he started to research on nanoparticles specifically Sodium Bismuthate and how it is a possible it may destroy cancer cells while not harming normal tissue as it is pH sensitive. He soon got published in "Detection of Hexavalent Chromium by Copper Sulfide Nanocomposites". Wanting to get into more biology-based research, he was introduced to Dr. Zui Pan, and this is where he started his research on the effects of zinc on the heart. David is planning to apply to medical school this fall.