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14. ABSTRACT

Traumatic injury is a leading cause of respiratory failure, where 2-4% of persons with trauma develop acute lung injury/ acute respiratory distress syndrome (ALI/ARDS). Septic shock with acute organ dysfunction is a common complication of severe trauma, with 40% of estimated mortality rates. A key feature in trauma-associated ALI/ARDS and sepsis is the impairment of pulmonary vascular integrity. During sepsis, ALI/ARDS results from activation of innate immune cells and endothelial cells by endotoxins leading to systemic inflammation and oxidative stress, which results in endothelial cell death and loss of integrity of the pulmonary vascular endothelium. However, the mechanisms leading to ALI/ARDS remains elusive.

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1. INTRODUCTION

Traumatic injury is a leading cause of respiratory failure, where 2-4% of persons with trauma develop acute lung injury/ acute respiratory distress syndrome (ALI/ARDS). Septic shock with acute organ dysfunction is a common complication of severe trauma, with 40% of estimated mortality rates. A key feature in trauma-associated ALI/ARDS and sepsis is the impairment of pulmonary vascular integrity. During sepsis, ALI/ARDS results from activation of innate immune cells and endothelial cells by endotoxins leading to systemic inflammation and oxidative stress, which results in endothelial cell death and loss of integrity of the pulmonary vascular endothelium. However, the mechanisms leading to ALI/ARDS remains elusive.

2. KEYWORDS

ACUTE LUNG INJURY, ACUTE RESPIRATORY DISTRESS SYNDROME, SEPSIS, MITOCHONDRIA, MITOCHONDRIAL DYSFUNCTION, METABOBOLOMICS, LIPIDOMICS, BIOMARKERS

3. ACCOMPLISHMENTS

a. What were the major goals of the project?

Goal of the project is to evaluate the multi-omic response to traumatic and septic shock with respect to acute lung injury and define mechanistically, via multi-omics, the therapeutic potential of Orai1 and/or MCU as targets for control of pulmonary vascular inflammation during shock. Both metabolomics and lipidomics analysis was employed to determine the omics profile in the porcine ALI model in this progress report. Metabolomics analysis included several metabolites related to mitochondrial TCA cycle and lipidomics analysis included 10 classes of lipids with a total of 208 lipid species.

To test the hypothesis related to endothelial mitochondrial function, lung endothelial cells (ECs) derived from mice lacking Orai1 and MCU in ECs ($Orai1^{\Delta EC}$ and $MCU^{\Delta EC}$ mice, respectively) with appropriate floxed and cre- controls were studied. N of 6 was used for the control and n of 4-5 for Orai1 and MCU knockout. Mice were challenged with 10mg/kg for 24 hours where urine and

plasma along with lung, kidney, liver and spleen were collected. Therefore, currently we have established ALI mouse model which will allow us to complete major task in Aim1 and Aim 2.

b. What was accomplished under these goals?

Metabolomics and lipidomics analyses were performed in serum from ALI/ARDS traumatic porcine model. Serum was collected from baseline i.e., before the right chest contusion to multiple time-points i.e., 3, 6, and 9 hours post-trauma. Additionally, the omics profile of survivors and non-survivors was also analyzed the non-survivor pigs that died post-injury at 3 hours vs the survivors at 3h.

The analysis demonstrates significant changes from baseline to 3h, 6h, and 9h -post-trauma with several progressive increases in metabolites with time, as shown in the heat map (Figure 1A). Additionally, post-injury changes in the lipidomics analysis are reflected from the lipidomics analysis. An increase in carnitine and acylcarnitine was seen post-injury and a decrease in many membrane lipids such as phosphatidylcholine, as shown in the heatmap, was identified at 9h post-injury (Figure 1B). An increase in carnitine/acylcarnitine may reflect an alteration in the mitochondria function in trauma associated ALI as studies have linked an increase in carnitine/acylcarnitine to the inhibition of beta-oxidation in dysfunctional mitochondria in multiple metabolic disorders[1].

Additionally a decrease in plasma phosphatidylcholine lipid may reflect alveolar dysfunction in the ALI porcine model as 80% of pulmonary surfactant comprises of this lipid[2] which prevents surface tension and movement of fluids into alveoli in normal conditions. And lack of lung surfactant in acute respiratory distress syndrome leads to respiratory failure[3].

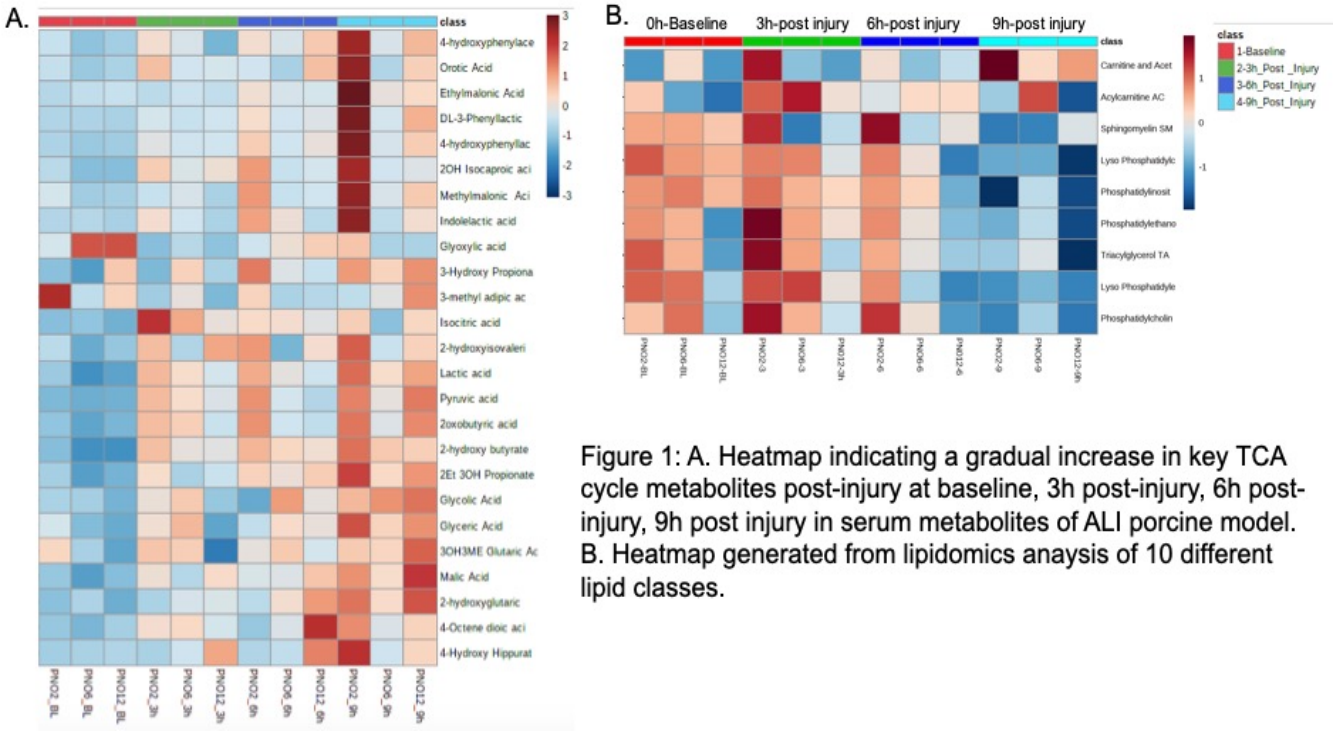

Figure 1: A. Heatmap indicating a gradual increase in key TCA cycle metabolites post-injury at baseline, 3h post-injury, 6h post-injury, 9h post injury in serum metabolites of ALI porcine model. B. Heatmap generated from lipidomics anaysis of 10 different lipid classes.

Furthermore, metabolite differences were stratified by the survivors vs. non-survivors at 3 hours post-trauma as shown by the PCA plot in (Figure 2A). Dramatic differences in metabolite profiles linked to mitochondrial TCA metabolites were observed between the two groups. Additionally, there

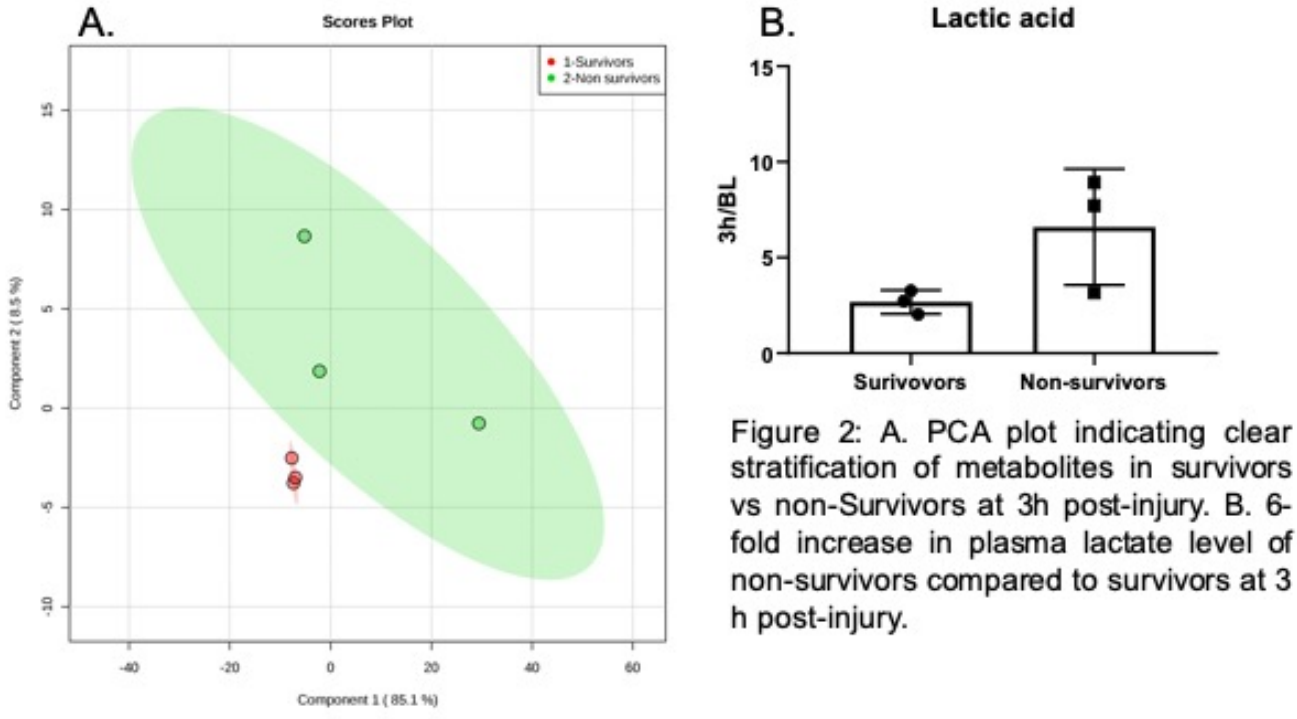

Figure 2: A. PCA plot indicating clear stratification of metabolites in survivors vs non-Survivors at 3h post-injury. B. 6-fold increase in plasma lactate level of non-survivors compared to survivors at 3 h post-injury.

was a 6-fold increase in lactate in the non-survivor group compared to the survivor (Figure 2B). Therefore, suggesting that mitochondrial function was dramatically altered in the porcine model of trauma-induced ALI.

Results from metabolites and lipids from the trauma-induced ALI porcine model suggest that the alteration in the mitochondrial function may underlie the progression of trauma-induced ALI/ARDS, which ultimately leads to impairment of pulmonary vascular integrity. We will further assess these metabolites in a new porcine experimental group to validate the biomarkers that identify those at risk for ALI progression and early mortality.

Therefore, we report that we have accomplished our goal from major task 1 of specific Aim 3 in year 1 of the grant and will be moving forward with the major task 2, which is to test a novel interventional approach in pig trauma-induced ALI model. Metabolomics and lipidomics approach will be employed in blood, urine, and tissue samples from control and a potent cytosolic calcium blocker BTP2 treated model. Additionally, we will determine the MCU oxidation from these samples along with multi-omics analysis.

c. What opportunities for training and professional development has the project provided?

   i. Oral presentation of the project by a graduate student in the MHSRS conference in August 2020.

d. How were the results disseminated to communities of interest?

   i. Yes, results presented at San Antonio Military City Symposium in August of 2020

e. What do you plan to do during the next reporting period to accomplish the goals?

   i. Our next immediate goal is to perform metabolomics and lipidomics on our established murine sepsis-induced ALI model, including control, Orai1, and MCU knockout in the vascular endothelial cells. The goal is to identify intracellular and extracellular signatures of mitochondrial function, EC injury, and barrier dysfunction.

4. IMPACT

a. What was the impact on the development of the principal discipline(s) of the project?

Traumatic injury is the leading cause of ICU admissions, with no current biomarkers available to identify those at risk for common complications. There are direct and indirect causes of traumatic injury in the military setting and civilian life. Respiratory failure from septic shock is a common complication of severe trauma where 2-4% of individuals with trauma develop ALI. A key feature in trauma-associated ALI/ARDS and sepsis is the impairment of pulmonary vascular integrity, where sepsis leads to activation of innate immune cells and endothelial cells by

endotoxins resulting in inflammation and oxidative stress. However, the mechanism leading to sepsis-induced ALI remains elusive.

Oxidative stress has long been implicated in calcium dysregulations, and studies have shown that stressed macrophages can increase cytosolic calcium in the endothelial cells in a paracrine manner[4]. Therefore, an increase in cytosolic calcium has many cascades of effects, especially in the mitochondria, which can be the source for oxidative stress and cell death when overwhelmed with calcium. Keeping this in mind, we hypothesized that modulation of calcium entry from the extracellular space and into the mitochondria will influence the severity of vascular inflammation in ALI.

Our omics data from lipidomics and metabolomics did reflect an alteration in the mitochondrial functions. One of the key points was to able to stratify survivors and non-survivors from the porcine trauma model. This will allow us to identify a molecular signature, a key biomarker that can identify those at risk for progression.

b. What was the impact on the other discipline?

Nothing to Report

c. What was the impact on technology transfer?

Nothing to Report

d. What was the impact on the society beyond science and technology?

Traumatic injury accounts for nearly half of all deaths in civilian life and costs nearly 670 billion in the United States in 2013. Currently, there is no better marker that allows to predict the progression of the injury to acute lung injury or multiple organ injury (MOF). Our research on murine and porcine ALI/ARDS model studies in combination with multi- omics data allows to find new biomarkers and therapeutic targets for trauma associated ALI and MOF.

5. CHANGES/ PROBLEMS

a. Nothing to Report

6. PRODUCTS

a. Oral Presentation

i. Military City USA Trauma Collaborative Research Conference at UT Health San Antonio Trauma and Emergency Surgery. Oral Presentation of abstract submitted to Military Health System Research Symposium (MHSRS) 2020. “ROLE OF MITOCHONDRIA IN ACUTE LUNG INJURY/ ACUTE RESPIRATORY DISTRESS SYNDROME”. August 2020.

7. PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

a. Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

a. Nothing to Report

9. APPENDICES

a. MHSRS 2020 Abstract

ROLE OF MITOCHONDRIA IN ACUTE LUNG INJURY/ACUTE RESPIRATORY DISTRESS SYNDROME

Role of Mitochondria in Acute Lung Injury/Acute Respiratory Distress Syndrome

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Background: Traumatic injury is a leading cause of respiratory failure, where 2-4% of persons with trauma develop acute lung injury/ acute respiratory distress syndrome (ALI/ARDS). Septic shock with acute organ dysfunction is a common complication of severe trauma, with 40% of estimated mortality rates. A key feature in trauma-associated ALI/ARDS and sepsis is the impairment of pulmonary vascular integrity. During sepsis, ALI/ARDS results from activation of innate immune cells and endothelial cells by endotoxins leading to systemic inflammation and oxidative stress, which results in endothelial cell death and loss of integrity of the pulmonary vascular endothelium. However, the mechanisms leading to ALI/ARDS remains elusive.

Method: The development of the ALI/ARDS porcine model is generated by pulmonary contusion in a porcine model. Lipidomics and metabolomics were performed in serum from baseline to multiple time-points post-trauma. Our study also utilizes in vitro as well as in vivo endothelial cell-specific Orai1 and MCU knockout mice to assess the role of SOCE and MCU-dependent Ca^{2+} signaling in triggering vascular inflammation and lung injury in sepsis-associated lung inflammation and ALI/ARDS murine model.

Results: Metabolomics and lipidomics analyses were performed in serum from ALI/ARDS traumatic porcine model. The analysis demonstrates significant changes from baseline to 3h, 6h, and 9h -post-trauma with several increased metabolites with time. Furthermore, metabolite differences were stratified by the survivors vs. non-survivors 24 hours post-trauma. Dramatic differences in metabolite profiles were observed between the

two groups. There was a 6-fold increase in lactate in the non-survivor group compared to the survivor (n=3 each). Mitochondrial TCA metabolites were also differentially altered among the two groups suggesting that mitochondrial function was dramatically altered in this model of trauma-induced ALI. Additional lipidomics data demonstrate alteration of several phospholipids and acylcarnitine levels within 3h of injury, again consistent with altered mitochondrial function. To elucidate the role of mitochondria in lung injury, freshly isolated mouse pulmonary microvascular endothelial cells (MPMVECs) from ORAI1 and MCU knockout showed a marked reduction in proinflammatory ICAM-1 protein expression post LPS challenge. Additionally, altered cytosolic calcium $[Ca^{2+}]_c$ transients and sustained $[Ca^{2+}]_m$ uptake was seen in control; however; this phenotype was not observed in Orai1 or MCU knockout. Strikingly, 60% of the MCU knockout mice challenged with gram-negative bacteria *K. pneumoniae* survived up to 6 days, whereas control mice survived only 3 days post-infection. Mice protected against the sepsis challenge has a reduction in lung inflammation, leukocyte infiltration, and bronchoalveolar lavage (BAL) protein and lung wet/dry ratio.

Conclusion: Analysis from metabolomics and lipidomics data from porcine serum established that mitochondrial function is closely involved with lung injury and potentially with mortality. Similarly, in vivo and in vitro studies on Orai1 and MCU knockout in the endothelial cells subjected to sepsis challenge suggests a contribution of MCU to endothelial cell activation during injury. Collectively, our murine and porcine ALI/ARDS model studies in combination with multi-omics data reveal that changes in mitochondrial function are associated with endothelial cell activation, proinflammatory response, and barrier dysfunction. Mitochondrial protectants could be a new therapeutic target for acute lung injury.

Learning objective 1: Describe an important role of mitochondria as a new therapeutic target for acute lung injury.

Learning objective 2: Discuss therapeutics to reduce the incidence and/or severity of ALI/ARDS and/or other lung injury secondary to trauma, transfusion, infection, burns, hemorrhagic shock, inhalation, and/or oxygen exposure.

Learning objective 3: Research on the etiology and prevention of ALI/ARDS caused by the host's responses to trauma, transfusion, burns, infection, hemorrhagic shock, inhalation, and/or oxygen exposure.

10. REFERENCES

1. Bjørndal B, Alterås EK, Lindquist C, Svardal A, Skorve J, Berge RK. Associations between fatty acid oxidation, hepatic mitochondrial function, and plasma acylcarnitine levels in mice. *Nutrition & Metabolism*. 2018;15(1):10. doi:10.1186/s12986-018-0241-7

2. Bernhard W, Hoffmann S, Dombrowsky H, et al. Phosphatidylcholine Molecular Species in Lung Surfactant. 2001;25:7.

3. Whitsett JA, Wert SE, Weaver TE. Alveolar Surfactant Homeostasis and the Pathogenesis of Pulmonary Disease. *Annu Rev Med*. 2010;61:105-119. doi:10.1146/annurev.med.60.041807.123500

4. Madesh M, Hawkins BJ, Milovanova T, et al. Selective role for superoxide in InsP3 receptor-mediated mitochondrial dysfunction and endothelial apoptosis. *J Cell Biol*. 2005;170(7):1079-1090. doi:10.1083/jcb.200505022