Short communication

Investigating ischemia and reperfusion-induced organ damage in severe cardiac arrest: A comprehensive proteomics perspective

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Cardiac arrest (CA) is a life-threatening condition with complex pathophysiology and limited treatment options. To gain deeper insights into the pathological state of vital organs, we employed a proteomics analysis in rodents to assess proteome alterations in the brain, heart, kidney, and liver using a rat model of CA. The brain displayed severe protein alterations in essential cellular pathways, including three major energy-generating pathways after CA, which worsened after resuscitation, resulting in the most significant overall protein changes among the organs. Conversely, the liver, experiencing the most substantial protein alterations post-CA, demonstrated significant recovery, presenting the least protein changes post-resuscitation. This remarkable liver recovery may be attributed to the preserved integrity of its energy generation pathways following CA. Our study provides a comprehensive overview of proteome dynamics in critical organs following CA and resuscitation, offering novel insights into the molecular basis for poor brain recovery. The findings emphasize the importance of the brain's energy production capacity in its functional restoration, highlighting the significance of sustaining adequate energy supply in therapeutic approaches.

Current knowledge of CA pathology is derived from fragmented

data obtained from various models focusing on specific pathways [1], failing to capture the heterogeneity, magnitude, and complexity of CA pathology due to their heterogeneity. To address this limitation, we conducted an untargeted proteomics analysis using high-resolution liquid chromatography tandem mass spectrometry (LC-MS/MS) and tandem mass tag (TMT) labeling [2] to quantify proteins in the brain, heart, kidney, and liver of rats after 20 min CA or 20 min CA followed by 30 min cardiopulmonary bypass (CPB) resuscitation. This model is highly clinically relevant due to its similarity in downtime and outcomes to those of average human CA patients, who typically experience more than 20 min of CA [3]. Moreover, the consistent blood flow maintained by CPB reduces potential variations associated with traditional chest compressions. Detailed procedures for animal surgery, sampling, LC-MS/MS analysis, and data analysis are provided in the supplementary materials and summarized in Fig. 1A.

The sequential steps of mass data profiling to identify and quantify proteins are shown in Fig. S1. Of the identified peptides, 92%—95% were accurate within ±2 ppm. Over 93% had Xcorr values greater than 2, and more than 94% of the proteins were confirmed with multiple unique peptides, validating analytical processes for peptide and protein identification/quantitation. We quantified 3,411, 1,928, 2,731, and 2,879 proteins in the brain, heart, kidney, and liver, respectively, with a total of 4,435 proteins across all four tissues (Figs. 1B and S1D). The steps for statistical analysis are provided in Fig. S2, which includes log2-transformation and normalization. The histograms of all samples show a Gaussian distribution of intensity versus frequency. The multi-scatter plots between two groups for each organ have a Pearson correlation coefficient greater than 0.99. Finally, principal component analysis (PCA) after applying analysis of variance (ANOVA) shows that the proteomes within the same experimental group exhibited low variability, with distinct separation from other groups in all four tissues (Figs. 1C and S2D). These results highlight the significant
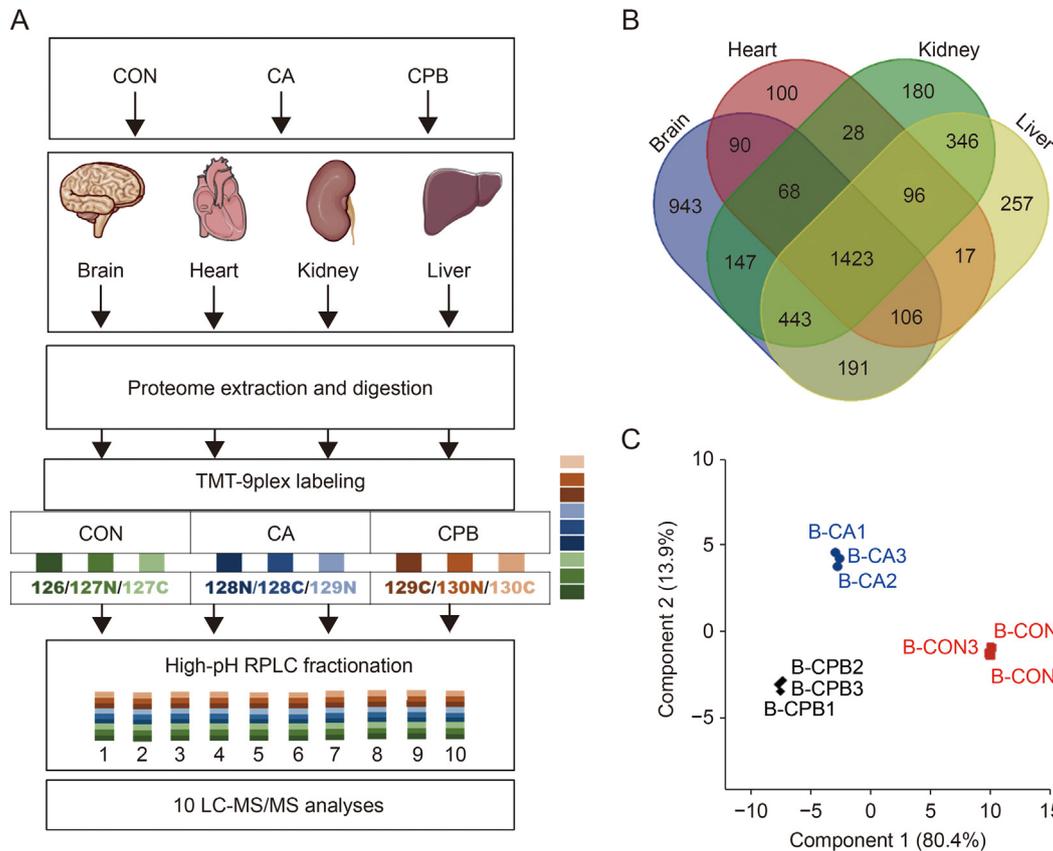
Fig. 1. Experimental workflow and liquid chromatography tandem mass spectrometry (LC-MS/MS) results. (A) Extracted proteome samples of each organ from individual rats were pooled alongside same group and digested with trypsin. Peptide samples were labeled with 3 tandem mass tag (TMT)-labeling reagents for each group, mixed, and then fractionated into 10 fractions for LC-MS/MS analysis. (B) The Venn diagram showing the number of identified proteins in individual organs. (C) Principal component analysis (PCA) after applying analysis of variance (ANOVA) shows that the proteomes within the same group exhibited low variability, with distinct separation from other groups in the brain. Con: Control; CA: cardiac arrest; CPB: Cardiopulmonary bypass.

physiological impact of CA and resuscitation on tissue proteomes and confirm the methodological consistency in the sampling, mass analysis, and data processing steps. The Volcano plot summarizes the overall distribution of differentially expressed proteins following CA and following CPB compared to control (Fig. S3). The list of all peptides and proteins are provided as Supplementary data.

Detailed trend in changes of proteins in individual tissues following CA and resuscitation are shown in heatmaps Fig. 2A. The bar graph summarized changes in the percentage of proteins altered with respect to ischemia alone or resuscitation, as well as proteins that returned to baseline levels in all four organs. In the brain, 60% of proteins were altered after CA, with only 10% normalizing after resuscitation and an additional 15% undergoing further modifications. Consequently, a total of 65% of measured proteins remained altered post-resuscitation, the highest among the four organs. The liver was also severely affected, with 69% of proteins altered post-CA. However, resuscitation normalized a significant portion of proteins, resulting in only 24% remaining altered. The heart and kidney exhibited fewer protein alterations post-CA (45% and 47%, respectively), and resuscitation partially restored protein levels, leaving 33% and 48% altered, respectively. These overall tissue-level changes were consistent with alterations observed in organelles, albeit with some organelle-specific variations (Fig. S4). These findings highlight the striking disparity in the response to resuscitation and recovery between the brain and liver.

We then conducted pathway analysis to evaluate the impact of protein alterations on cellular pathways post-resuscitation. Fig. S5

showcases the top 20 enriched pathways with altered proteins. However, identifying key pathogenic mechanisms was a challenge due to the extensive number of altered pathways, even in the least affected organ, the liver. Thus, we adopted a targeted approach to assess the impact of ischemia and reperfusion on essential cellular pathways (Fig. 2B). Intriguingly, the brain exhibited more significant alterations in these pathways compared to the overall tissue alterations, while the heart and liver displayed an opposite pattern. The kidney demonstrated pathway-to-pathway variations similar to those observed in organelles. Fig. S6 illustrates the interactions of these pathways and their impact on various subcellular organelles, which highlights the pivotal role of mitochondria in their regulation.

Subsequently, we focused on mitochondrial electron transport chain complexes I, III, IV, and V as key components of mitochondria (Fig. 2C, and Tables S1—S4). Since each complex is composed of multiple proteins, not only level changes in whole complexes, but also variations among proteins within each complex represent the degree of alterations that may influence the functionality of mitochondria. CA resulted in significant alterations across all complexes in the four tissues. Remarkably, the brain showed further deterioration after resuscitation, indicating the most pronounced post-resuscitation alteration. Conversely, the heart and liver exhibited substantial recovery of protein alterations. These patterns correlated with changes in mitochondrial respiration activity post-CA [4], validating the functional relevance of our proteomics data.

Our study provides novel proteomics evidence that supports previous concepts regarding brain vulnerability and organ-, organelle-, pathway-specific responses to ischemia-reperfusion.
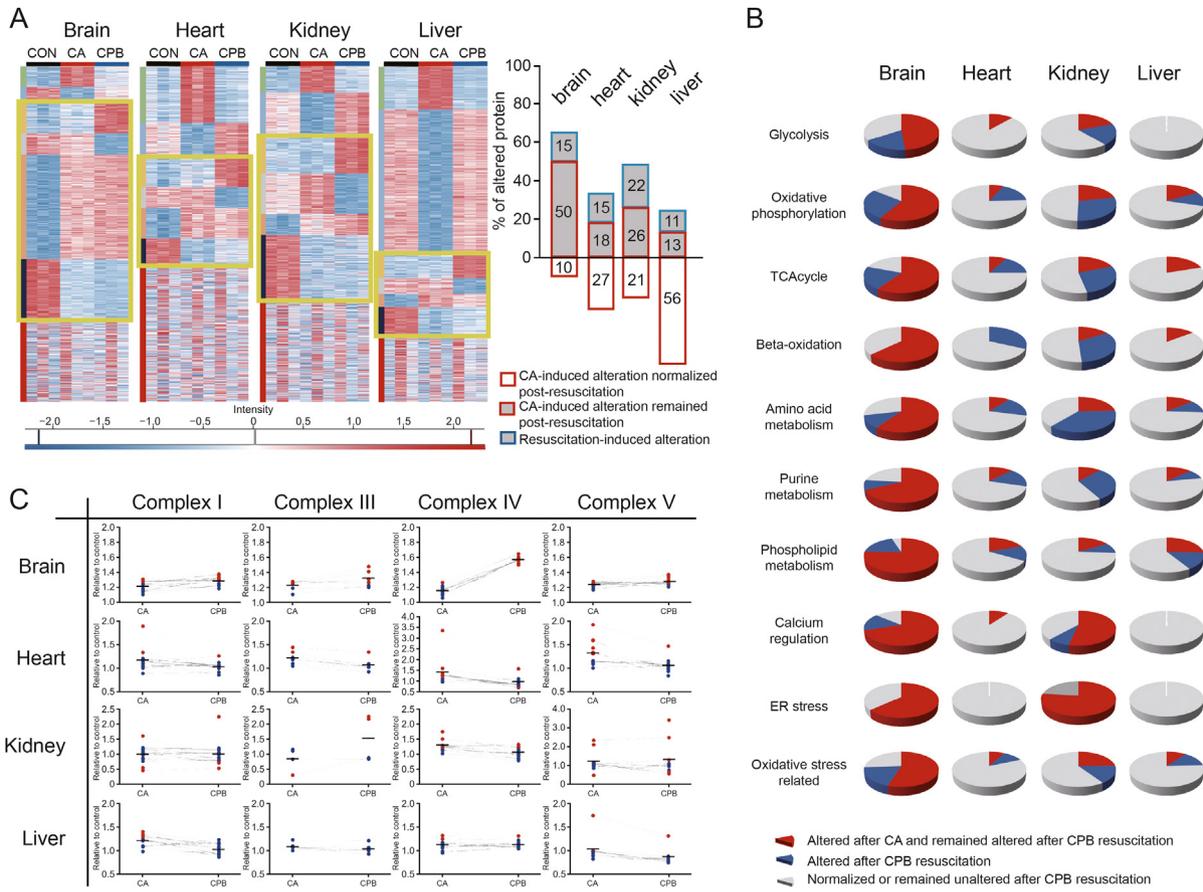
Fig. 2. The visualization of proteomic changes following cardiac arrest (CA) and cardiopulmonary bypass (CPB) resuscitation at the organ, organelle, and pathway levels. (A) Heatmap showed the overall trend in changes of quantified proteins in four tissues. Proteins that remained altered following resuscitation compared to control are highlighted in the yellow rectangle. Bar graphs show % of proteins, which are altered following CA and normalized following CPB (red-white rectangle), altered following CA and remained altered following CPB (red-grey rectangle), and normal following CA and altered following CPB (blue-grey rectangle) in individual organs. (B) The percentage of altered proteins found post-resuscitation in fundamental physiologic pathways that occurred during CA and remained altered despite CPB (red), newly altered after CPB resuscitation (blue), or normalized/remained unaltered after resuscitation (grey). (C) Relative changes in individual proteins of mitochondrial complex I, complex III, complex IV, and complex V following CA and resuscitation normalized to control in the brain, heart, kidney, and liver. Data points outside of ±25% of baseline value (red) and those within (blue). Average levels of individual proteins in each complex are marked with a black bar. Con: Control; TCA: tricarboxylic acid; ER: endoplasmic reticulum.

Importantly, our data quantifies the profound impact of whole-body ischemia, which has previously been hypothesized without supporting evidence. The 65% protein alterations observed in the resuscitated brain after 20 min of CA in our study is greater than ~6% alterations observed after 2 h of middle cerebral artery occlusion [5], demonstrating the magnitude of injury to the brain after whole-body ischemia and reperfusion. Our findings also offer mechanistic insights into the pathological causes of brain damage and the limited recovery observed after CA. Most brain alterations occur during the ischemic phase, particularly affecting essential pathways involved in ATP generation. This limitation in ATP production should hamper the recovery process, even when blood circulation is successfully restored. Consequently, targeted interventions to enhance brain function beyond restoring blood flow are crucial. Furthermore, our study highlights the limitations of current bioinformatics analysis in investigating CA pathology, underscoring the need for dedicated informatics approaches and database schemas specific to CA. Our data and approach will serve as a foundation for future developments in this field.

A limitation in our proteomics analysis is that the use of TMT labeling agents requires sample pooling and high pH reverse-phase liquid chromatography, which may reduce the number of detected peptides and, consequently, the number of identified proteins.

Furthermore, our analysis only encompasses the proteins that were detected. There could be other unidentified proteins that potentially offer further insights into both organ physiology and how alterations can contribute to pathological dysfunction in CA. Overall, our study enhances our understanding of the vulnerability of the brain and the specific responses of organs to ischemia-reperfusion, providing valuable insights for the development of targeted interventions and the advancement of research in the field of CA.

CRediT author statement

Seunguk J. Baek: Data curation, Investigation, Methodology; **Santiago Miyara**: Writing - Reviewing and Editing, Investigation, Methodology; **Lance B. Becker:** Conceptualization, Resources Funding acquisition, Writing - Original draft preparation, Reviewing and Editing; **Junhwan Kim**: Conceptualization, Funding acquisition, Writing - Original draft preparation, Reviewing and Editing.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2023.09.017.

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