UNCLASSIFIED

Mapping the Plasma Proteomic Profiles that Contribute to Endothelial Cell Barrier Integrity Following Trauma and Hemorrhagic Shock

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Introduction

- Trauma with hemorrhagic shock (THS) is a leading cause of death in military casualties.
- In previous studies, we demonstrated a significant temporal elevation in early syndercan-1(SDC1) and thrombomodulin (TM) in the shed blood of two distinct rat models of trauma and hemorrhage, suggesting an early development of trauma or hemorrhagic shock induced-endotheliopathy[1].

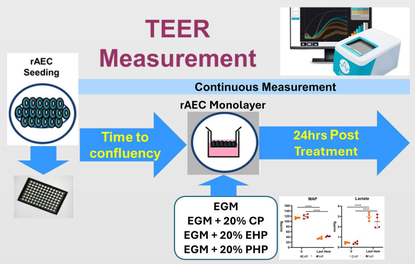
Objectives/Hypothesis

- Demonstrate an ex-vivo model of EoT, characterized by direct damage effect of THS-derived plasma on endothelial tight junction, and the distinct plasma proteomic profiles following trauma and hemorrhagic shock.

Methods

- Plasma was collected from the shed blood of the isoflurane-anesthetized Sprague-Dawley rats that underwent 60% hemorrhage (EHP) or polytrauma (crush injury at liver, intestine, and skeletal muscle and bone fracture) followed by 40% hemorrhage (PHP). Animals were euthanized at the designated end point. Healthy rat plasma was used for control (CP).

Functional Assay



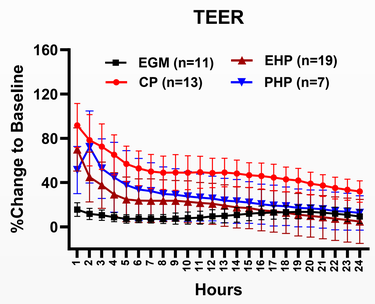
- Rat aortic endothelial cells (rAEC) were cultured in endothelial cell growth medium (EGM) to a monolayer (rAECM) in a CytoView-Z 96-well plate.
- rAECMs were treated with 20% of CP, EHP, or PHP in EGM.
- Trans-Endothelial Electrical Resistance (TEER) was measured by Maestro Edge.

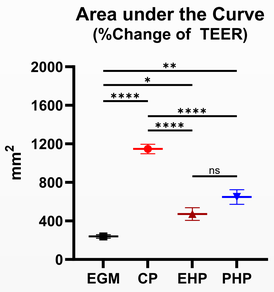
Protein Assay

RayBiotech Rat Proteome profiling array L2 and L3 assays were performed on CP, EHP, or PHP. Signal intensity of EHP and PHP was compared to CP, with a differential 2-fold up- or down-regulation identified. Differential proteins Protein-Protein Interaction (PPI) networks were formed and analyzed through STRING database, with significant PPI network accepted at enrichment p <0.05.

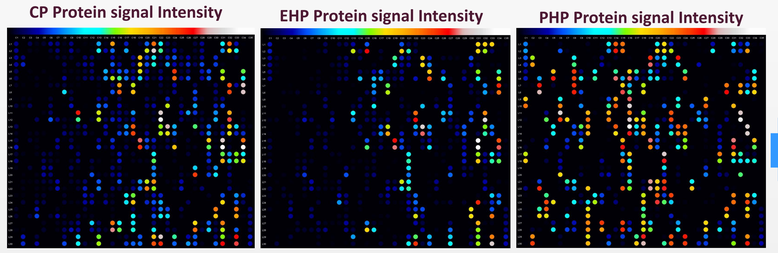
Results

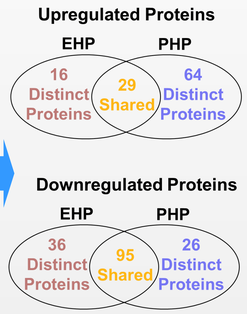
Functional Assay



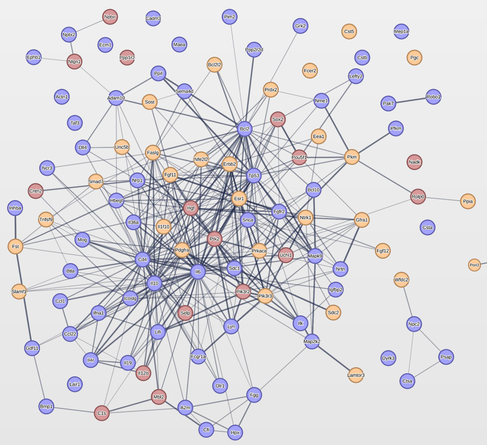


Protein Assay

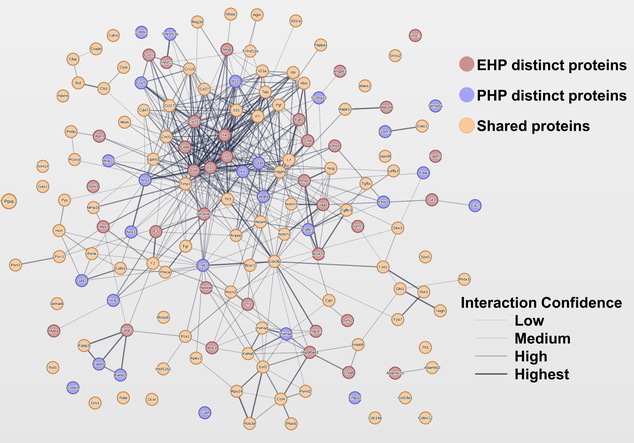




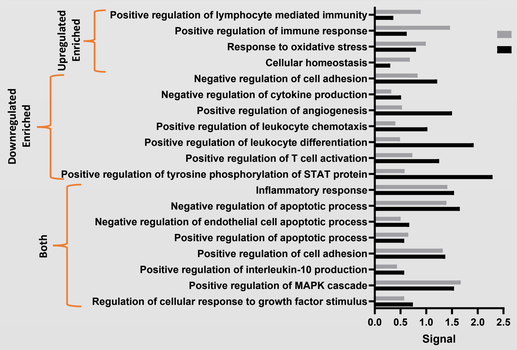
Upregulated PPI Network:



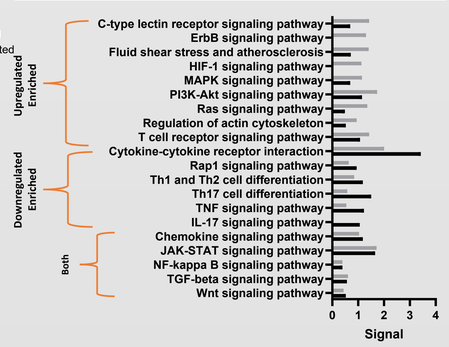
Downregulated PPI Network:



GO Process



KEGG Pathway



Summary

- TEER-AUC was significantly higher in CP compared to EGM, HSP or PTP. No significant difference was found between HSP and PTP.
- EHP was found to have 16 upregulated and 36 down regulated distinct proteins. PHP was found to have 64 upregulated and 26 downregulated distinct proteins. Both profiles shared 29 upregulated and 95 downregulated proteins.
- The Upregulated PPI network was enriched in PI3K-Akt, MAPK, Ras, and HIF-1 pathways, while the downregulated was enriched in TNF, interleukin, Th differentiation, Rap1, and Cytokine receptor pathways. They both shared enrichment in Wnt, TNF-beta, NF-kappa B, and Chemokine pathways.
- The Upregulated PPI network was enriched in oxidative stress, homeostasis, and immunity processes, while the downregulated was enriched in transport Leukocyte activation and chemotaxis processes. They both shared enrichment in adhesion remodeling, and apoptosis or cell death processes.

Conclusions

- This study provides a plasma proteomic profile following trauma and hemorrhagic shock to help elucidate the regulatory mechanisms and biomarkers of acute EoT and identify therapeutic targets.
- This study demonstrates an in vitro model of THS-induced endotheliopathy as characterized by a significant deficit in TEER induced by either EHP or PHP, suggesting potential damage in endothelial tight junctions.

Reference:

1. Chance TC, Meledeo MA, Cap AP, Darlington DN, Bynum JA, Wu X. Syndecan-1 and Thrombomodulin are Early Biomarkers for Development of Endotheliopathy in Trauma and Hemorrhagic Shock. Biology and Medicine. 2023;15(4):8.

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Disclaimer: Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare regulations, and the principles of the Guide for the Care and Use of Laboratory Animals. The facility's Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by the AAALAC International.

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