Crush Syndrome: Identification and Validation of Early Urine Biomarkers in a Combat-Relevant, Large Animal Model

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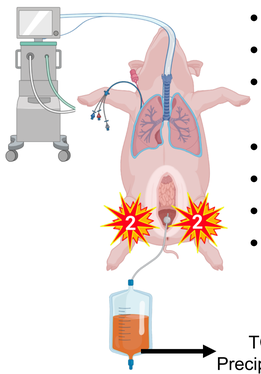
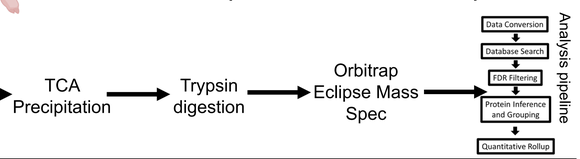
BACKGROUND

- Muscle crush injury and extreme exercise cause acute kidney injury (AKI) through the same muscle protein-driven mechanism.
- AKI due to crush, “crush syndrome” causes hyperkalemia and death and is common in combat-injured warfighters and civilians.[1]
- Exertional rhabdomyolysis limits warfighter effectiveness, requires medical resources, and impairs return-to-duty.[2]
- Our group has repurposed cilastatin sodium for crush syndrome and rhabdomyolysis treatment.[3, 4]
- Clinical trial patient identification, triage, and return-to-duty decisions for novel therapy would be facilitated by a rapid biomarker which identified at-risk patients.
- **Hypothesis**: urine protein interrogation enables lead candidate identification for discrimination between low and high risk of severe crush syndrome and/or hyperkalemia.

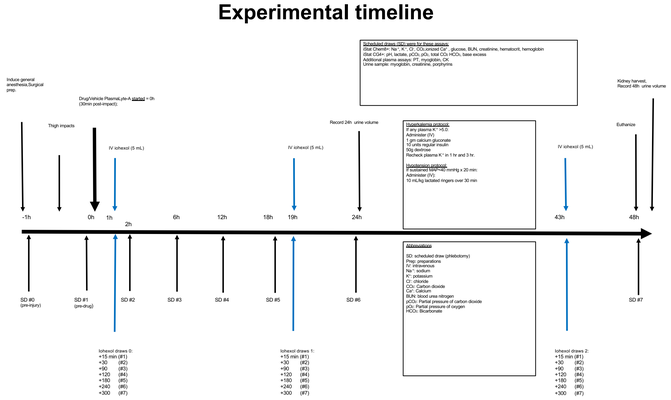
METHODS



- 16 female pigs under general anesthesia
- Urinary catheter & mechanical ventilation.
- 2 explosive-driven impacts to each thigh muscle followed by treatment or vehicle.
- Comparison with no-impact group.
- Treatment published (reference 3).
- 48h protocolized critical care.
- 2.5h after impact urine → mass spec.



Experimental timeline



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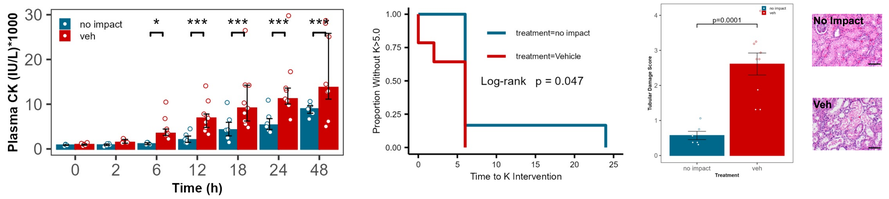
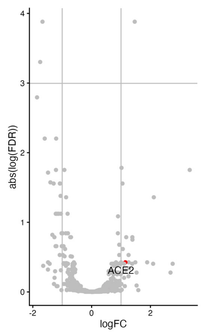
RESULTS

Modeled Crush Syndrome



Crush syndrome model caused elevation of creatine kinase (CK), severe kidney damage at 48h, and life-threatening hyperkalemia (treated by protocol when plasma K>5.0 mEQ/L). From Munhall et al, ref 3.

Biomarker screening and validation



1742 identified proteins

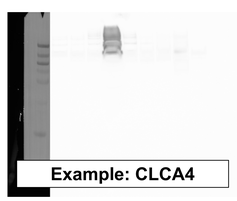
116 differentially expressed proteins

Linear discriminant (LDA) and Scaled Vector (SVM) rankings for K intervention and CK rise slope

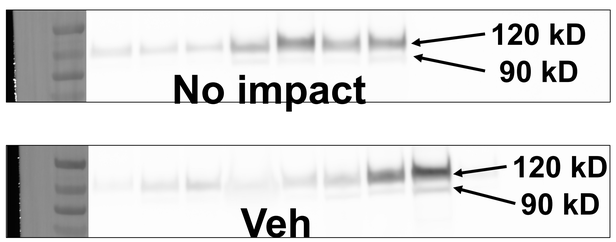
Practicality screen:
1. Human homology
2. Antibody available
3. MW distinct from albumin
4. MW > 50 kD
5. Mass detection compatible with resolution on immunoblot

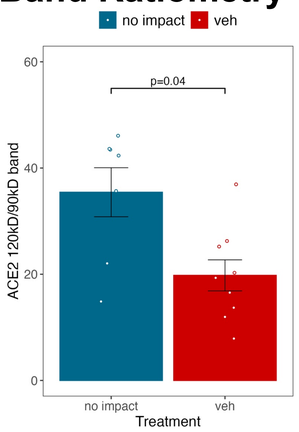
4 candidates:
1. KIF12
2. PDIA4
3. CLCA4
4. ACE2

No immunoblot signal:
1. KIF12
2. PDIA4
3. CLCA4



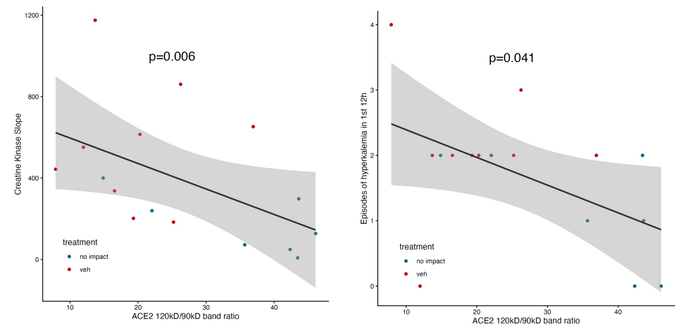
Angiotensin Converting Enzyme 2 Band Ratiometry





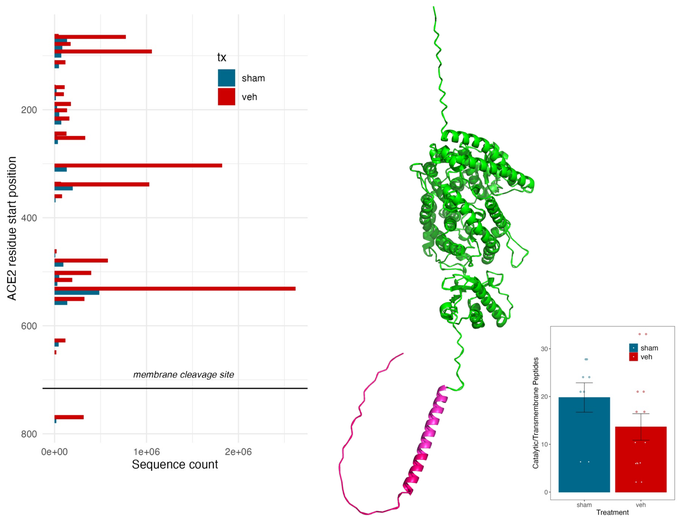
ACE2 displays a dual band. The upper band represents full-length ACE2, while the lower band indicates ACE2 cleaved at the membrane, ”soluble ACE2”. The ratio of 120 kD/90kD bands (Urine Full ACE2 to Soluble, urFACE2) was different across groups.

urFACE2S predicts outcome



Linear regression against (left) progression of crush syndrome (slope of the plasma creatine kinase) and (right) the number of episodes of life-threatening hyperkalemia demonstrated strong relationships. These outcomes are clinically important, representing need for advanced care and lethal disease, respectively.

Revalidation Mass Spectrometry



ACE2 peptides quantifications in the urine mass spectrometry are displayed with their sequence position relative to the site of ACE2 cleavage. Relative ratiometric decrease, similar to that identified on immunoblot, is evident in the peptide/position data, validating the immunoblot observation.

CONCLUSIONS

- We screened 1742 urine proteins identified by mass spectrometry 2.5h after severe muscle injury.
- The ratio of full length to soluble ACE2 in the urine (urFACE2) differentiated injured from non-injured animals.
- urFACE2S strongly predicted progression of crush injury and the lethal complication of crush, hyperkalemia
- urFACE2S may be a practical, early biomarker for crush syndrome requiring advanced treatment.
- The critical next step is identifying human cohorts and performing real-world validation.

ACKNOWLEDGEMENTS

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