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Cell death mechanism in an isolated wood smoke inhalation induced-ARDS large animal model

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Cell death mechanism in an isolated wood smoke inhalation induced-ARDS large animal model

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Abstract

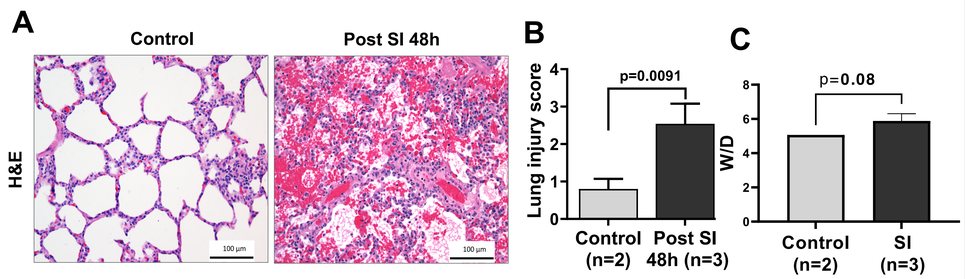
Acute respiratory distress syndrome (ARDS) is a lethal disease condition in critically ill patients with a reported mortality rate reaching 45%. The current treatment modalities available for severe ARDS are invasive and carry significant risk for patients. Most published studies involving smoke inhalation utilize another simultaneous injury (such as cutaneous burn) to increase pathology burden of their animal models. This introduces confounding variables to investigations which aim to concentrate on inhalation injury. In this study, we evaluated the potential molecular targets associated with isolated smoke inhalation-induced ARDS.

We observed an increase in lung injury score and wet/dry ratio 48h post smoke inhalation together with upregulation of inflammatory markers, IL-1β and IL-6 levels. Furthermore, there was a decrease in phosphorylation of cell survival marker Akt and an increase in pro-apoptotic protein BAX at 48h post smoke inhalation. These results indicate that smoke inhalation induced inflammatory processes resulting in increased apoptosis and decreased cell survival in lung parenchymal cells. Use of this unique model may be of benefit in studying the pathophysiology of inhalation injury and for the development of novel therapeutic strategies.

Materials and Methods

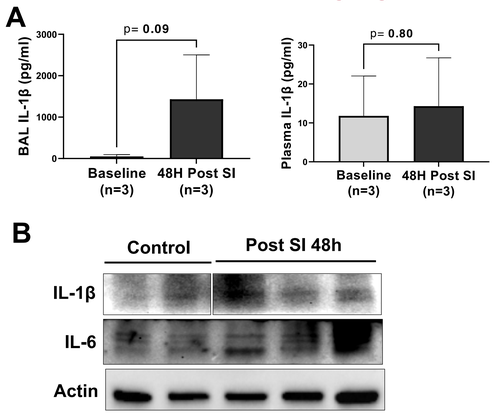
Smoke inhalation: Duroc pigs (50±5 kg) were exposed to oak wood smoke for 2 hours while intubated. Continuous monitoring and serial blood collection was performed via catheters placed in the carotid artery, internal jugular vein, pulmonary artery, and femoral artery. Pigs were euthanized at 48h post smoke inhalation after final blood sampling, and lung tissue was collected for analysis.
H&E: Analysis was performed in Tissue Science Facility, UNMC to determine lung injury.
Wet/Dry ratio: Lung tissues were dried in an incubator at 60ºC for 5 days and weighed again (dry weight).
Cytokine analysis: ELISA was performed using Quantikine® ELISA kit from R&D systems.
Western Blots: Analysis was performed using actin as loading control.

Fig 1: Wood smoke inhalation induces injury to lung parenchyma



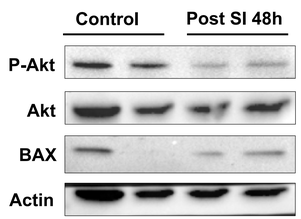
(A&B) Smoke inhalation leads to an increase in total lung injury score together with increase in wet/dry ratio (C) in SI animals compared to control.

Fig 2: Wood smoke inhalation increases inflammatory cytokines



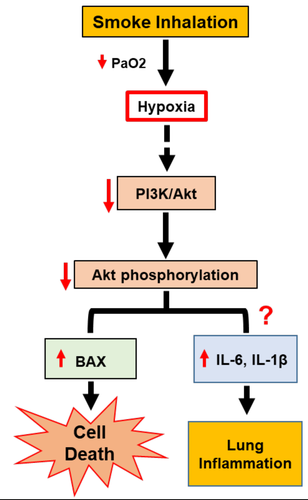
(A&B) Inflammatory cytokines IL-1β and IL-6 were upregulated 48h post smoke inhalation contributing to in acute lung inflammation.

Fig 3: Wood smoke inhalation induces cell death



Smoke inhalation downregulates phosphorylation of cell survival marker Akt and upregulates pro-apoptotic marker BAX leading to lung parenchymal cell death.

Fig 3: Proposed pathway



Conclusion and Future Directions

We report that isolated wood smoke inhalation-induced inflammatory processes and downregulates Akt phosphorylation resulting in increased apoptosis and decreased cell survival in lung parenchymal cells. Use of this unique model of injury from wood smoke exposure may be of benefit in studying the pathophysiology of inhalation injury and for the development of novel therapeutic strategies.

Future studies will assess the apoptotic and cell survival mechanism involved in isolated wood smoke inhalation-induced ARDS.

References

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