45 Endothelial Damage Occurs Early After Inhalation Injury as Measured by Increased syndecan-1 Levels

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Introduction: Inhalation injury is a significant cause of morbidity and mortality in the burn patient population. However, the pathogenesis of inhalation injury and its potential involvement in burn shock is not well understood. Pre-clinical studies have shown endothelial injury, as measured by syndecan-1 levels, to be involved in the increased vascular permeability seen in shock states. Furthermore, the lung has been identified as a site of significant syndecan-1 shedding. Here we aim to characterize the contribution of endotheliopathy caused by inhalation alone in a swine model.
Methods: Eight female Yorkshire pigs were used in this experiment. A custom-made smoke box was employed to deliver smoke via endotracheal tube directly into the swine lungs. Carboxyhemoglobin levels were then titrated to a level of 50-75%. Blood was collected at induction of anesthesia, pre-injury, 30 minutes, and at hours 1, 2, 4, 6, and 12 post-injury and was stored in EDTA tubes from which plasma was separated and stored for future analysis. Pigs were necropsied immediately after completion of the experiment and lung samples were placed in all-protect and flash frozen. Histology was performed on lung sections and a validated, published scoring system composed of 5 parameters (neutrophils in the alveolar space, neutrophils in the interstitial space, hyaline membrane formation, protein detritus in the alveolar space and septum thickening) was used to assess lung injury severity (between 0 and 1).Plasma Syndecan-1 (SDC-1) was quantified by ELISA. All data was compared to Syndecan-1 levels measured at induction. Conditions were analyzed with one-way ANOVA with multiple comparisons and Dunnett's correction for multiple comparisons.
Results: Syndecan-1 levels at induction were 13.74 ± 2.03 ng/ml. Pre-injury and 30 minutes post-injury levels remained similar. Syndecan-1 levels at hour 2 post-injury increased 37% from induction (18.36 ± 1.28 ng/ml, p=0.0057). This trend continued with a 47% percent increase from induction at hour 4 post-injury (19.62 ± 2.15 ng/ml, p=0.0033) and a 49% increase from induction at hour 6 post-injury (20.42 ± 2.43 ng/ml, p=0.0011). Histological sections showed higher lung injury severity compared to control pigs (0.1-0.3 vs. 0.5-0.74, p< .05).
Conclusions: Significant increases in syndecan-1 levels in this animal model provide evidence for a connection between smoke inhalation injury and endothelial injury. Furthermore, the endotheliopathy that leads to burn shock could be exacerbated by inhalation injury, leading to the poor clinical outcomes that are often seen in patients with combined burn and inhalation injuries. Future research should focus on the mechanisms underlying inhalation injury and its contribution to shock physiology.

46 Bardoxolone-methyl Microparticles Ameliorate Immune Dysfunction Following Burn and Inhalation Injury Through the NRF2/KEAP1 Axis

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Introduction: Severe burn injury leads to many systemic stresses that can seriously impact multiple systems throughout the body. These stresses are further exacerbated if there is a combined burn and inhalation injury, which leads to increased morbidity and mortality for many patients. Combined burn and inhalation injury causes an intense systemic inflammatory response and activation of the innate immune system which can lead to inflammatory complications, such as systemic inflammatory response syndrome and multiple organ failure. Nuclear Factor-Erythroid-2-Related Factor (NRF2) is a transcription factor that acts to downregulate overt damaging pro-inflammatory and oxidative responses and maintain immune homeostasis. This transcription factor remains bound to Kelch-like ECH-associated protein 1 (KEAP1) in the cytoplasm. Under oxidative stress, NRF2 dissociates from KEAP1 and translocates to the nucleus where it facilitates the transcription of anti-inflammatory and antioxidant genes. We hypothesized that NRF2 is a key regulator after burn and inhalation injury, and activation of NRF2 can limit the severity of burn and inhalation injury.
Methods: To test this, we have developed a mouse model of combined cutaneous burn and woodsmoke inhalation injury. After burn and inhalation injury, we found that $NRF2^{-/-}$ knockout mice have higher mortality compared to wild-type (WT) mice and suffer from increased vascular permeability and lung edema, suggesting that this transcription factor is important for controlling morbidity and mortality. In WT mice, NRF2 is activated following burn and inhalation injury, however, based on immunohistochemical staining, it is not sufficiently induced following this insult since it remains in the cytoplasm and is unable to transcribe anti-inflammatory genes. Therefore, we treated WT mice with an intraperitoneal injection of bardoxolone-methyl microparticles, a NRF2 activator that separates KEAP1 from NRF2 in the cytoplasm, immediately after burn and inhalation injury in WT mice
Results: We observed significant decreases in mortality as well as reduced concentrations of certain pro-inflammatory cytokines in the blood and bronchoalveolar-lavage fluid of these mice. In the lungs of these mice, there was an upregulation in numerous pathways involved in the management of inflammation and immune response compared to mice that did not receive bardoxolone-methyl microparticles.
Conclusions: In conclusion, treatment with bardoxolone-methyl microparticles immediately after burn and inhalation injury might be effective for reducing the severity of the inflammatory response and limiting inflammatory complications.