Serum levels of lung-specific proteins as potential biomarkers of pulmonary injury after blast exposure

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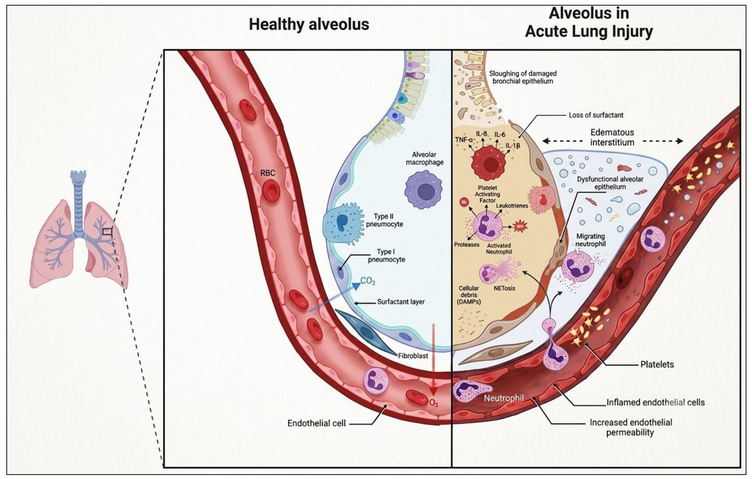
Background

Blast induced lung injury (BLI) caused by explosions has become one of the main causes of death in both military and civilian populations. Overpressure and negative pressure due to blast mainly affects air/gas filled organs such as the lungs. The alveolar-capillary membrane/barrier (ACB) in the lungs (consisting of the surfactant, the alveolar epithelium, the interstitium and the microvascular endothelium) is particularly vulnerable to damage, as the sudden pressure wave disrupts the delicate structure of the capillaries and alveolar walls, leading to hemorrhage, fluid leakage, and impaired gas exchange. This damage triggers the release of cellular proteins, activating resident inflammatory cells, increasing vascular permeability, and promoting inflammatory cell infiltration into the lungs. Circulating proteins from damaged cells offer a means to precisely monitor lung cellular and structural damage by analyzing cell-specific proteins and their messenger RNA levels in biological fluids.

Objective: Only limited studies have evaluated changes in lung-specific proteins in biological fluids such as plasma or serum following blast exposure. Hence, we investigated surfactant A and D [(SFA and SFD), as markers of alveolar injury], club cell protein [(CC16), a marker for epithelial damage] and endothelin-1 (a marker of endothelial injury) in the serum of rats exposed to single and repeated blasts of different intensities at acute and chronic time-points.



Hall S, Berger G, Lehmann C. COVID-19 Lung Injury: Unique and Familiar Aspects of Pathophysiology. *Applied Sciences*. 2024; 14(23):11048.

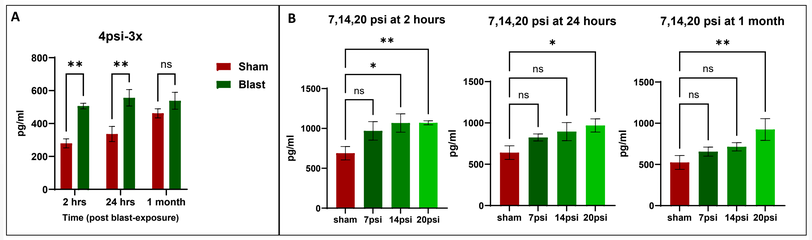
Under normal conditions (left), gas exchange is facilitated by a thin epithelial–endothelial barrier which is maintained by the collective functions of alveolar epithelial cells (pneumocytes), endothelial cells, and alveolar macrophages. During ALI (right), damage to the epithelial–endothelial barrier sets in motion a cascade of inflammatory processes that exacerbate tissue damage, accelerate edema formation, and critically impair gas exchange.

Methods

- Under isoflurane anesthesia, Sprague Dawley rats were exposed to three 4psi blasts (one 4psi blast per day) or two tightly coupled 4 psi and 20 psi or single 7psi, 14psi or 20 psi blast overpressure waves to simulate blast exposure during military training.
- Rats were euthanized under isoflurane anesthesia at 2 hours, 6 hours, 24 hours or 1-month post-blast and serum and lung tissues were collected.
- The protein levels of CC16 and endothelin-1 in the serum and the mRNA expression of CC16 and surfactant proteins (A,B,C, & D) in lung were measured by ELISA and quantitative real-time PCR, respectively.

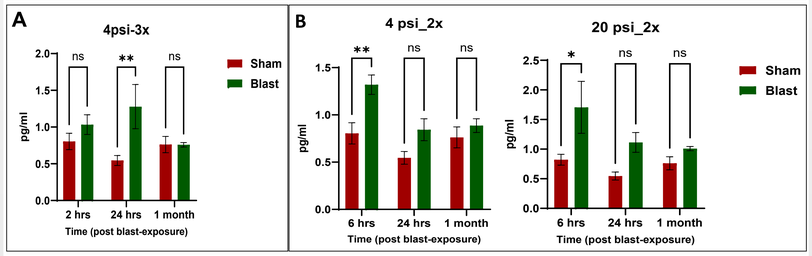
Results

1. Effect of blast exposure on CC16 protein levels in the serum



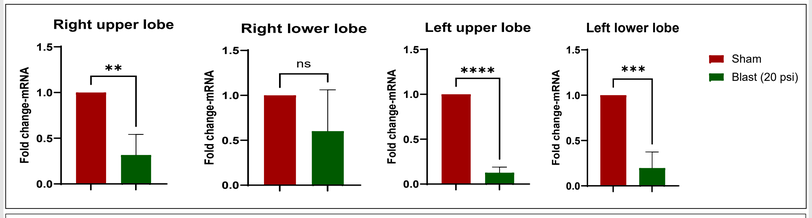
CC16 serum concentrations in rats exposed to either three 4psi blast or single 7psi, 14psi or 20 psi blast and euthanized at 2 hours, 24 hours or 1-month post blast exposure. Statistically significant increase in serum concentrations at 2 hours and 24 hours noted at three 4psi blast exposure (A) and at all time points (2 hours, 24 hours and 1-month) following 20 psi blast (B). Data are presented as mean± SEM between sham and blast group and statistical significance measured by student *t*-test for (A) and ANOVA for (B). Statistically significant differences are shown as *p<0.05, **p<0.01 versus sham group; n=6.

2. Effect of blast exposure on endothelin-1 protein levels in the serum



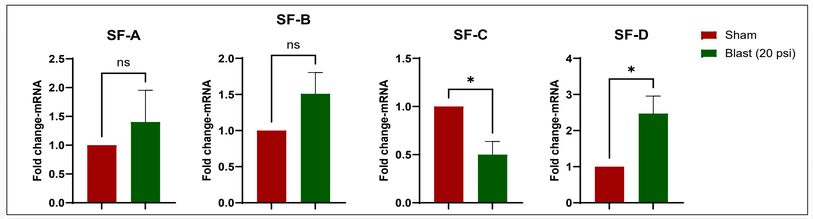
Serum concentrations of endothelin-1 in rats exposed to either three 4psi blast (one per day), or two tightly coupled 4 psi or 20 psi blast and euthanized at 2 hours, 6 hours, 24 hours or 1-month post blast exposure. Statistically significant increase in serum concentrations at 24 hours was noted at three 4psi blast exposure (A) and at 6 hours only following two tightly coupled 4 and 20 psi blast. Data are presented as mean± SEM between sham and blast group and statistical significance measured by student *t*-test. Statistically significant differences are shown as *p<0.05, **p<0.01 versus sham group; n=6.

3. Effect of blast exposure on CC16 mRNA levels in the lungs



Effect of 20 psi blast exposure on CC16 mRNA expression in the different lobes of the lung in rats. Rats were exposed to a single 20-psi blast or sham procedure and euthanized at 24 hours post-exposure. Different lobes of the lung tissues were collected for mRNA expression analysis of CC16 using quantitative real-time PCR. Data are presented as fold change relative to sham controls and represent mean ± SEM (n=6 per group). Statistical significance was determined using student t-test (*p < 0.05,**p < 0.01, ***p < 0.001 and ****p <0.0001).

4. Effect of blast exposure on surfactant protein mRNA levels in the lungs



Effect of 20 psi blast exposure on surfactant protein (SF) mRNA expression in the lung tissues of rats. Rats were exposed to a single 20-psi blast or sham procedure and euthanized at 24 hours post-exposure. Lung tissues were collected for mRNA expression analysis of SF-A, SF-B, SF-C, and SF-D using quantitative real-time PCR. Data are presented as fold change relative to sham controls and represent mean ± SEM (n=4 per group). Statistical significance was determined using student t-test (*p < 0.05).

Conclusions

- Blast exposure causes significant structural damage to the delicate epithelial-endothelial barrier of the lungs. This disruption leads to cellular leakage, and impaired gas exchange.
- Exposure to blasts results in a significant increase in serum concentrations of CC16, an epithelial damage marker showed elevated serum levels at 2 hours and 24 hours, while endothelin-1 (an endothelial injury marker) peaked significantly at 6 to 24 hours.
- A profound downregulation of CC16 mRNA expression across all major lobes of the lung 24 hours following a single 20 psi blast exposure, indicating severe localized transcriptional suppression or cell damage.
- High-intensity blast exposure (20 psi) alters surfactant protein (SF) mRNA expression at 24 hours post-exposure demonstrating a selective disruption of the alveolar surfactant environment.

Future directions

- Analyze the protein expression of CC16, endothelin-1, and surfactant proteins in the lung tissues.
- Validate the utility of CC16, endothelin-1, and specific surfactant proteins as reliable, field-deployable serum biomarkers.
- The chronic effects of blast-induced lung injury (BLI) beyond one month to look for permanent pulmonary fibrosis or delayed-onset respiratory complications.
- Functional assessments of lungs to directly correlate the observed changes in protein/mRNA levels with actual physiological respiratory impairment.

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Disclaimer:

The experiments reported herein were conducted in compliance with the Animal Welfare Act and per the principles set forth in the "Guide for Care and Use of Laboratory Animals," Institute of Laboratory Animals Resources, National Research Council, National Academy Press, 2011.

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