Systemic Inflammatory Biomarkers of Blast Induced Lung Injury: Studies in a Sheep Experimental Model

Jayasree Nath and Adolph Januszkiewicz

Walter Reed Army Institute of Research, Silver Spring, MD.

Abstract

Our studies in human bronchial epithelial cells (HBEC) have documented early pro-inflammatory responses following brief exposure to acute oxidative stress, as exemplified by marked increases in levels of pro-inflammatory cytokines like TNFα, IL-1β, IL-6, IL-8 and IFNγ. These studies also demonstrated significant increase in NO/nitrite levels, 3-nitrotyrosine formation, expression of NOS and ICAM-1, and an increased adhesion of inflammatory neutrophils to HBEC and cell death via both apoptosis and necrosis (J. Inhal. Toxicol., 2004, 2007). Consistent with our *in vitro* data, related studies in a blast overpressure (BOP) shock wave-mediated rat lung injury model have also confirmed oxidative stress-mediated increase in levels of pro-inflammatory cytokines/chemokines, cell adhesion molecules and infiltration of inflammatory neutrophils into the injured lung within 1-2 hours post-injury (Crit. Care Med., 2004). In an attempt to further delineate early systemic biomarkers of blast - induced lung injury, which may have real-life translational potential in the detection and/or treatment of multi-organ injury associated with battlefield - related acute trauma/stress, we extended our studies in a sheep experimental model of blast-induced lung injury. A sensitive Proteome array-based Multiplex ELISA was used to monitor early changes in blood levels of multiple pro-inflammatory cytokines/chemokines and some neurotrophic factors. Heparinized venous blood was collected from control and experimental sheep at 5, 30 and 60 min. post-blast and the centrifuged plasma samples were stored at -80 degree, until further analyses. Changes in blood levels of several pro-inflammatory mediators were monitored, to identify potential systemic inflammatory 'biomarkers' of blast-induced trauma/injury, and to correlate those changes with the severity and extend of blast-induced injury and its patho-physiological outcome. While the concentrations of various tested analytes ranged between 5-200 pgm/ml, our studies demonstrated a significant decrease in levels of a number of pro-inflammatory cytokines/chemokines like IL-1β, TNF-α, MIP-1α, MCP-1, GRO-α, C-reactive protein (CRP), and also that of brain-derived nerve growth factor (BDNF) and β-nerve growth factor (β-NGF), within 30-60 minutes post-blast. Of particular relevance, this was most apparent in blood samples collected from the sheep in which the severity and extend of injury was most extensive (n=5). Although the data is preliminary, the scientific rationale and experimental approach of this study have real-life translational potential in early detection and/or treatment of blast-induced multi-organ injury/trauma including closed or mild traumatic brain injury, which currently poses a major medical challenge to U.S. Military Medicine and Combat Casualty Care.

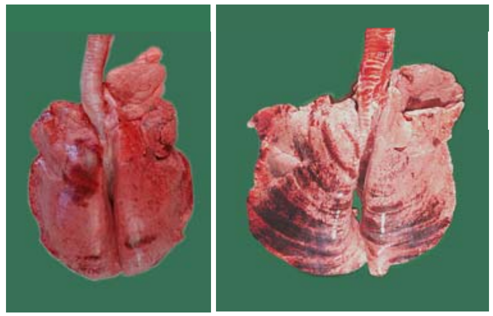
Introduction

Impact of blast overpressure (BOP) shockwave - mediated blunt trauma (BT) can have adverse effects on the systemic vasculature of the lung and other major organ systems including the brain which can contribute toward the overall outcome of an inflicted blast-induced BT. Early systemic inflammation and acute oxidative stress-mediated cell/tissue injury are major secondary causative agents of multi-organ failure and resultant death following exposure to BOP-mediated shockwaves. The lung is one of the primary targets of blast-induced BT and the primary features of such injury consist of pulmonary contusion, rupture of capillaries and release of free hemoglobin and resultant oxidative stress, changes in cell-cell interaction, hemorrhagic lesions, acute systemic inflammation, edema, and circulatory depression. Similar changes may also occur during blast-induced closed or mild traumatic brain injury, which is of major medical concern for today's U.S. Military Medicine and/or Combat Casualty Care. These internal injuries and its associated patho-physiological changes often have no external signs, are difficult to diagnose, and therefore, are frequently underestimated or not detected in a timely fashion. Therefore, delineation of early systemic inflammatory biomarkers of blast-induced trauma and/or injury, can provide valuable diagnostic tools for both pre-and post-deployed health assessment of active-duty U.S. solders The latter may have real-life translational potential in timely detection of BOP-mediated internal injuries in a battlefield scenario.

Objective

The primary objective of this study in a large animal model was to identify early inflammatory 'biomarkers' in the peripheral blood within 0 - 2 hour post-exposure to a defined BOP-mediated BT, which can act as a reliable determinant for both the severity of an inflicted 'blunt trauma' and its patho-physiological consequence on major organ systems, which may lead to multi-organ dysfunction/failure and resultant impairment or death.

BOP-Induced Sheep Lung Injury



Low Impact High Impact

Experimental Approach

- A sensitive Multiplex sandwich ELISA that uses plate-based Proteome Arrays and can simultaneously detect up to 16-20 analytes at 1-5 pgm/ml, was used for monitoring changes in blood levels of various pro-inflammatory biomarkers.
- Heparinized venous blood from unexposed and BOP-exposed animals were collected at 0 -60 min. post BOP and the plasma sample were stored at -80 degree until further analyses.
- Lung pathology was monitored/compared in pre- and post - BOP exposed animals.
- Levels of various cytokines/chemokines and neurotrophic factors in sheep plasma samples were measured using SearchLight® Proteomic Arrays (Pierce Biotechnology, Woburn, MA).

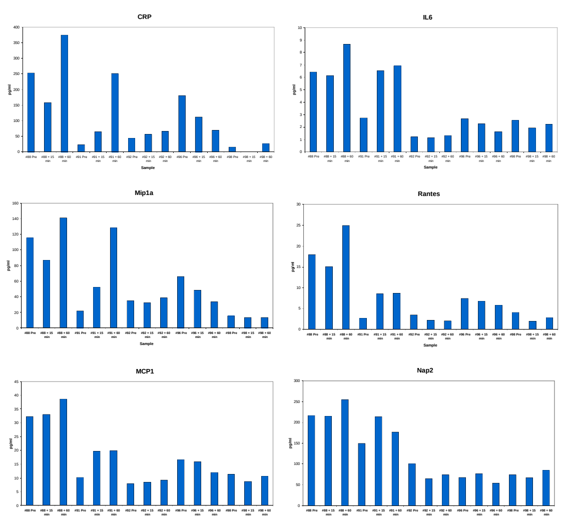
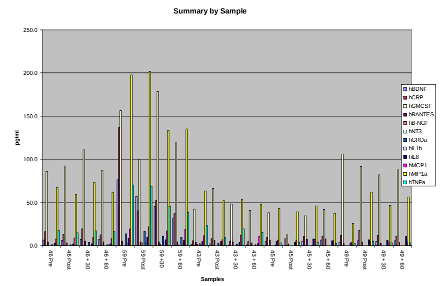


Figure 1.



Target Number	Survival Time (max 1 hour)	Est. Surface Lung Injury (%)	Pathological Observations	Right Lung Wt. (grams)	Left Lung Wt. (grams)
9943	1 hour	28	unremarkable	280	248
9946	5 minutes	30	Fractured ribs Multifocal contusions (gut, diaphragm, liver, pancreas) Tracheal bloody froth	227	219
9949	1 hour	45	Focal Ecchymoses diaphragm Multifocal contusions gut Liver hematoma Tracheal bloody froth	238	229
9945	1 hour	45	Fractured ribs Multifocal contusions left heart Multifocal contusions (gut, liver, pancreas) Tracheal bloody froth	304	280
9959	45 minutes	61	Hemoperitoneum Multifocal contusions (gut, liver, spleen, pancreas) Spleen laceration Tracheal bloody froth	292	319

Table 1: Based on observable lung injury, target 43 was the least injured, and 59 the worst. Total lung weight is not always indicative of lung damage, since lung weight vary considerably among animals. 59 is the only target that sustained a laceration in the gut to cause hemoperitoneum. 59, 49, and 46 are the only targets to emit bloody froth from the trachea.

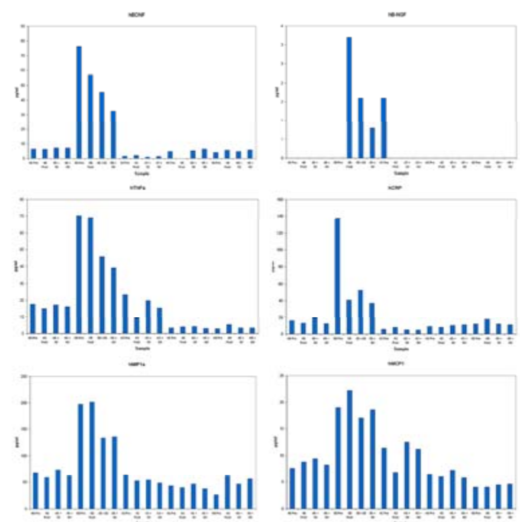


Figure 2.

Results

- Our studies in a sheep experimental lung-injury model clearly demonstrate an early systemic inflammatory response and significant increases in levels of a number of pro-inflammatory cytokines/chemokines in the circulating blood levels (within 0-1 hour), when the extend and severity of blast-induced injury was relatively less (or mild).
- Of particular relevance, our overall data obtained from these preliminary studies suggest an inverse correlation between the injured host's ability to elicit an early systemic inflammatory response and the magnitude and severity of blast-induced trauma/injury.
- Because timely diagnosis and treatment of blast-induced mild traumatic brain injury and its associated cognitive dysfunctions have recently emerged as a major medical concern of today's military medicine, we also monitored pre- and post - blast levels of some of the neurotrophic factors.
- As shown in Fig. 2, a significant post-blast decrease in blood levels of BDNF and β-NGF were noted, where the severity and extend of injury were more extensive and serious.
- As shown in Fig. 1, a marked increase (5-fold) in blood levels of C-reactive protein (CRP), a clinical biomarker of cardiovascular inflammation, is particularly noteworthy in the context of early systemic inflammation following acute lung injury
- Application of a sensitive SearchLight™ Proteomic Array-based Multiplex ELISA was very effective in detection and quantification of up to 16 analytes (at 1-5pgm/ml) in small volumes of blood, which may find useful application in a real-life clinical scenario.

Summary

The information gathered from our present study in a sheep experimental model of BOP-induced lung injury provides valuable insights for designing effective strategies/tools for early detection of BOP-induced lung and other major organ injuries including the brain. Early systemic inflammatory biomarkers detectable in small volume of peripheral venous blood, offer potential therapeutic targets for effective monitoring of solder performance in a battlefield scenario, in a relatively non-invasive manner. The results of our preliminary study suggest that the ability of a biological system to successfully respond to a given trauma has a 'systemic threshold', beyond which it fails to mount an inflammatory response to defend the host against an inflicted trauma or 'insult'. C-reactive protein, a well-known inflammatory biomarker of cardiovascular disease, appears to be an effective 'biomarker' for assessment of BOP-mediated acute lung injury in a sheep experimental model. The experimental approach may find application in the assessment of other major organ injuries, including the brain. Further studies are necessary, to establish functional correlates between early systemic inflammatory biomarkers and BOP-mediated multi-organ injury and its patho-physiological consequences.

Conclusion

The information gathered from this study may find real-life application in prediction and/or early assessment of both the severity of an inflicted trauma and the final outcome/extend of a given BOP/BT-mediated impact and its patho-physiological outcome. Our present data combined with the results of our previous studies suggest that there is a systemic threshold of a given 'biological system' to respond to a given trauma, which may ultimately determine the fate of the injured host.

Acknowledgements

The authors would like to thank all other WRAIR colleagues and collaborative team members, who made this study possible.

References

1. Ayyagari, V., Januszkiewicz, A., and Nath, J. Toxicology, 197, 148 -163, 2004.
2. Ayyagari, V., Januszkiewicz, A., and Nath, J. Inhal. Toxicol. , 19, 181 -194, 2007.
3. Gorbounov, N., McFaul, S., Van Albert, S., Morrisette, C., Zaucha, G., Nath, J. Crit. Care Med. 32, 1028 -1034, 2004.
4. Nath, J., Lavigne, L., Burns, C., Januszkiewicz, A. Free Rad. Biol. Med., 37 (suppl. 1), S53, 2005.
5. Nath, J. and Januszkiewicz. Proc. of Army Sci. Conf., 2008.

Disclaimer: The opinions and assertions contained herein are the private views of the authors and are not to be construed as official, nor do they reflect the views of the Department of the Army or the Department of Defense. This study was supported by the Department of Army.