Alternative activation of anti-inflammatory macrophages in the lung by hyperbaric oxygen during *S.aureus* sepsis in mice

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Abstract

BACKGROUND: Hyperbaric oxygen therapy (HBO_2) has been reported to affect inflammatory cytokines such as TNFα and cytoprotective proteins such as HO-1 in sepsis-related acute lung injury (ALI), by complex mechanisms. Here we tested the hypothesis that HBO_2 modulates the pulmonary anti-inflammatory response to sepsis in mice by altering the alveolar macrophage (AM) phenotype. **METHODS**: To study the effect of HBO_2 on lung parenchyma, C57BL/6 mice were divided into four groups: (i) control, (ii) untreated sepsis, (iii) HBO_2-treated sepsis, and (iv) HBO_2 control. Sepsis was induced by intra-peritoneal (IP) implantation of a *S. aureus* ($1x10^8$ cfu)-impregnated fibrin clot. HBO_2 (2.5 ATA) was administered at 6, 24, and 48 hours. Lungs from each group were harvested immediately following each HBO_2 treatment (n = 3 - 4 per time point per group). ALI was quantified by bronchoalveolar lavage (BAL). Western blot and qPCR were performed on whole lung homogenates at each time point. Immunohistochemistry and fluorescence microscopy were used to quantify M1 and M2 macrophage phenotypes. **RESULTS**: Septic mice treated with HBO_2 had decreased BAL fluid cell counts and LDH compared with untreated mice. HBO_2 therapy reduced lung TNFα and IL-1β protein, and increased IL-10 and HO-1 protein expression at 24 and 48 hours post-inoculation. HO-1 and IL-10 localized strongly to macrophages and to bronchial epithelium in all HBO_2-exposed groups. The ratio of alternatively activated anti-inflammatory (M2) to pro-inflammatory (M1) alveolar macrophages was significantly increased at 24 hours in HBO_2-treated sepsis compared to untreated sepsis group (14.2 vs. 2.7 respectively; p<0.001). **CONCLUSIONS**: In mouse lung in sepsis, HBO_2 limits TNFα and IL-1β up-regulation and induces IL-10 and HO-1 expression in alveolar macrophages while increasing the differential expression of macrophages with an M2 phenotype. HBO_2-mediated alternative activation of alveolar macrophages may account for an anti-inflammatory effect of HBO_2 in the lung during sepsis.

Background

Sepsis related ARDS is characterized by a protracted pro-inflammatory state with slow resolution and an inadequate anti-inflammatory response. The reasons for failure of the anti-inflammatory response in ARDS are not well understood. Dysfunction of redox signaling during acute inflammation is thought to play a central role in ARDS development; hence elucidation of the redox-sensitive aspects of pulmonary inflammation may reveal potential therapeutic targets for reducing the incidence of ARDS and improving sepsis survival. Hyperbaric oxygen (HBO_2) has been reported to reduce the severity of acute lung injury in animal models as well as to affect inflammatory molecule expression in lung and other tissues. HBO_2 is bacteriostatic *in vitro*[2,3] and improves survival in an IL-10 dependent-manner[3]. The anti-inflammatory effect of IL-10 is in turn dependent on HO-1 since IL-10 mediated suppression of pro-inflammatory TNF-α and iNOS is lost in *Hmox1* silenced cells. HBO_2 induction of HO-1[6] is in part responsible for attenuation of acute lung injury.[5,6,7] In this study we further investigate key redox sensitive components of the acute inflammatory response to sepsis in mouse lung by examining its effect on inflammatory cytokines and alveolar macrophage phenotype expression. We hypothesize that HBO_2 modulates the acute inflammatory response to sepsis in the lung and improves sepsis induced ALI primarily by recruiting a macrophage dependent anti-inflammatory defense in the lung.

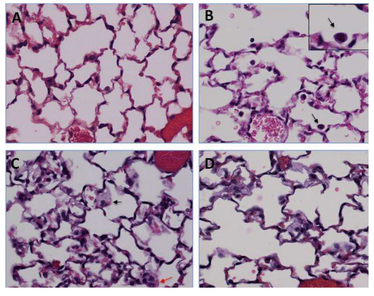
Study Design

	Sepsis	Sepsis- HBO_2	HBO_2
HBO_2 2.5ATA 90mins	-	X	X
S.aureus $1x10^8$ cfu	X	X	-
Vancomycin 6mg/kg	X	X	-
0.9% saline	X	X	-

HBO_2 treatments timed at 6h, 24h, 48h post-inoculation in sepsis-HBO_2 mice and 0h, 24h, 48h in the HBO_2 control group. *n = 12 per group

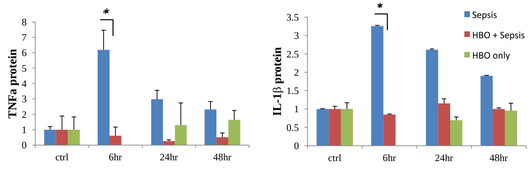
Results

Figure 1. Hyperbaric oxygen attenuates lung inflammation in sepsis at 24hrs



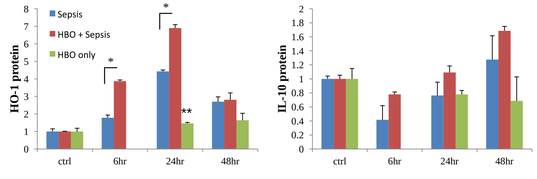
Representative photomicrographs of H&E stained lung tissue: (A) normal control lung. (B) HBO_2 induces mild lung inflammation with recruitment of macrophages into alveolar space (arrow) and mild vascular congestion (C). Septic mice show increased lung inflammation compared to controls with proteinaceous material (black arrow) and occasional neutrophils (red arrow) (D) HBO_2 plus sepsis reduced inflammation compared with sepsis mice with activation of macrophages but absent neutrophils in alveolar space.

Figure 2. HBO_2 suppression of lung pro-inflammatory cytokine induction during sepsis



Densitometry of lung Western analyses for TNF-α and IL-1β protein in sepsis, sepsis-HBO_2 and HBO_2-control mice. TNF-α and IL-1β protein levels are increased by 6hr and are blunted when septic mice are treated with HBO_2 . HBO_2 had no effect on TNF-α and IL-1β protein levels in healthy mice. Bars represent fold change relative to control and are mean ± SEM for n=3 per group and time.

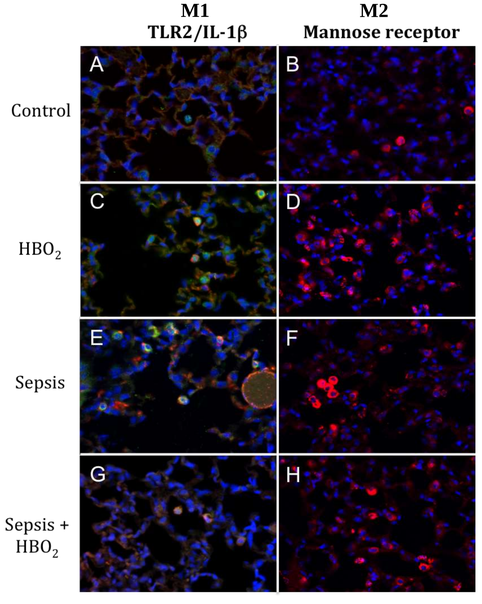
Figure 3. HBO_2 induces anti-inflammatory and anti-oxidant defenses. In lung



Densitometry of lung Western analyses for HO-1 and IL-10 protein in sepsis, sepsis-HBO_2 and HBO_2-control mice. HO-1 and IL-10 protein levels are increased by 24h and 48h respectively in sepsis mice. This increase was further amplified when septic mice received HBO_2 as compared to sepsis mice. In healthy mice, HBO_2 significantly increased HO-1 protein levels at 24h as compared to control, but had no effect on IL-10 expression (*P<0.05 vs. sepsis-control, **P<0.05 vs. control). Bars represent fold change relative to control and are mean ± SEM for n=3 per group and time.

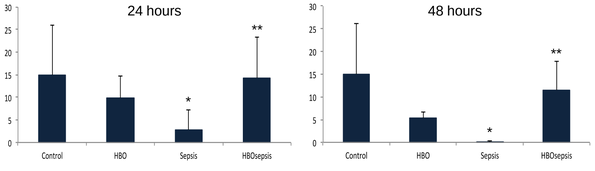
Results

Figure 4. HBO_2 increases expression of the anti-inflammatory alveolar macrophage phenotype at 24hr in lungs of septic mice.



Double-staining of M1 macrophages in mouse lung with TLR-2 (red) and IL-1β (green) and M2 macrophages with mannose receptor (red) reveals increased expression of the M2 phenotype in HBO_2 only (D) when compared to control (B). During sepsis at 24hr M1 phenotype expression is increased as evidenced by increased TLR-2/IL-1β staining (E) and fewer mannose receptor positive macrophages are seen (F). Sepsis plus HBO_2 shows increased mannose positive macrophages (H) compared to TLR-2/IL-1β positive cells (G) (original magnification 100 x).

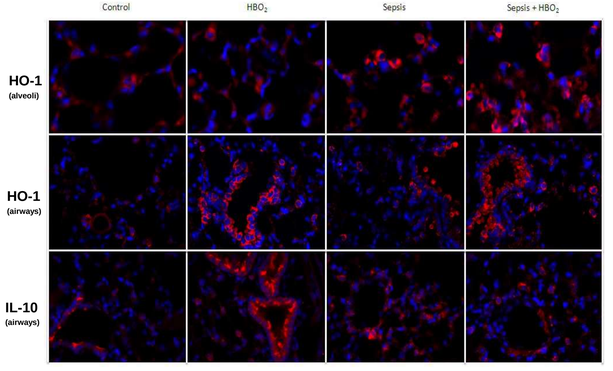
Figure 5. HBO_2 increases M2:M1 phenotype ratio at 24hr and 48hr in sepsis lung



Bars represent ratio of M2:M1 differential cell count/hpf and are mean ± SEM for n=3 per group and time. (*P<0.05 vs control; **P<0.05 vs sepsis-control).

Results

Figure 6. HBO_2 increases HO-1 and IL-10 expression in alveolar macrophages and bronchial epithelium at 24hr.



Mouse lung after inoculation with $1x10^8$ CFU *S.aureus*. Fluorescence immunohistochemistry of HO-1(red) and nuclei (DAPI; blue). Macrophage and bronchial epithelial staining of HO-1 is increased in both HBO_2 only and sepsis plus HBO_2 lung when compared to sepsis only. Bronchial epithelial staining increased in HBO_2 only when compared to all other groups. Sepsis only shows IL-10 staining largely in lung parenchyma and macrophages whereas sepsis plus HBO_2 shows modest staining of both bronchial epithelium and macrophages (original magnification 100 x).

Conclusions

- HBO_2 suppresses early-phase pro-inflammatory response to sepsis in the mouse lung by suppressing induction of TNF-α and IL-1β.
- HBO_2 up-regulates the anti-inflammatory response to acute lung injury (ALI) during sepsis by up-regulation of IL-10 and super-induction of HO-1.
- HBO_2 induces IL-10 and HO-1 expression in alveolar macrophages and bronchial epithelium during sepsis
- HBO_2 increases differential expression of the M2 anti-inflammatory macrophage phenotype in the lung in during sepsis
- HBO_2-mediated alternative activation of alveolar macrophages may account for an anti-inflammatory effect of HBO_2 in the lung during sepsis.

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