Blunt Chest Trauma in Mice after Cigarette Smoke-Exposure: Effects of Mechanical Ventilation with 100 % O₂

Katja Wagner1,2, Michael Gröger1, Oscar McCook1, Angelika Scheuerle3, Pierre Asfar4, Bettina Stahl1, Markus Huber-Lang5, Anita Ignatius6, Birgit Jung7, Matthias Duechs7, Peter Möller3, Michael Georgieff2, Enrico Calzia1, Peter Radermacher1, Florian Wagner1,2

1 Institut für Anästhesiologische Pathophysiologie und Verfahrensentwicklung, Ulm, Germany, 2 Klinik für Anästhesiologie, Universitätsklinikum, Ulm, Germany, 3 Institut für Pathologie, Universitätsklinikum, Ulm, Germany, 4 Laboratoire HIFIH, UPRES EA 3859, PRES l’UNAM, IFR 132, CNRS UMR 6214, INSERM U1083, Université Angers, Département de Réanimation Médicale et de Médecine Hyperbare, Centre Hospitalier Universitaire, Angers, France, 5 Klinik für Unfall-, Hand-, Plastische und Wiederherstellungschirurgie, Universitätsklinikum, Ulm, Germany, 6 Institut für Unfallchirurgische Forschung und Biomechanik, Universitätsklinikum, Ulm, Germany, 7 Abteilung Respiratory Diseases Research, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach/Riss, Germany

* peter.radermacher@uni-ulm.de

Abstract

Cigarette smoking (CS) aggravates post-traumatic acute lung injury and increases ventilator-induced lung injury due to more severe tissue inflammation and apoptosis. Hyper-inflammation after chest trauma is due to the physical damage, the drop in alveolar PO₂, and the consecutive hypoxemia and tissue hypoxia. Therefore, we tested the hypotheses that 1) CS exposure prior to blunt chest trauma causes more severe post-traumatic inflammation and thereby aggravates lung injury, and that 2) hyperoxia may attenuate this effect. Immediately after blast wave-induced blunt chest trauma, mice (n=32) with or without 3-4 weeks of CS exposure underwent 4 hours of pressure-controlled, thoraco-pulmonary compliance-titrated, lung-protective mechanical ventilation with air or 100 % O₂. Hemodynamics, lung mechanics, gas exchange, and acid-base status were measured together with blood and tissue cytokine and chemokine concentrations, heme oxygenase-1 (HO-1), activated caspase-3, and hypoxia-inducible factor 1-α (HIF-1α) expression, nuclear factor-κB (NF-κB) activation, nitrotyrosine formation, purinergic receptor 2X4 (P2XR4) and 2X7 (P2XR7) expression, and histological scoring. CS exposure prior to chest trauma lead to higher pulmonary compliance and lower PaO₂ and Horovitz-index, associated with increased tissue IL-18 and blood MCP-1 concentrations, a 2-4-fold higher inflammatory cell infiltration, and more pronounced alveolar membrane thickening. This effect coincided with increased activated caspase-3, nitrotyrosine, P2XR₄, and P2XR₇ expression, NF-κB activation, and reduced HIF-1α expression. Hyperoxia did not further affect lung mechanics, gas exchange, pulmonary and systemic cytokine and chemokine concentrations, or histological scoring, except for some patchy alveolar edema in CS exposed mice. However, hyperoxia attenuated tissue HIF-1α, nitrotyrosine, P2XR₇, and P2XR₄ expression, while it increased HO-1 formation in CS exposed mice. Overall, CS exposure aggravated post-traumatic
inflammation, nitrosative stress and thereby organ dysfunction and injury; short-term, lung-
protective, hyperoxic mechanical ventilation have no major beneficial effect despite attenua-
tion of nitrosative stress, possibly due to compensation of by regional alveolar hypoxia
and/or consecutive hypoxemia, resulting in down-regulation of HIF-1α expression.

Introduction
Blunt chest trauma is frequently associated with poly-trauma, and independently contributes
to mortality if acute lung injury (ALI) develops [1]. Epidemiological data demonstrate that
active or passive cigarette smoking (CS) is associated with the development of ALI after blunt
trauma [2], and that active cigarette smoking increases the susceptibility to develop Acute
Respiratory Distress Syndrome (ARDS) despite younger age and better overall general health
status [3]. Scarce data, however, are only available in experimental animals, and the results are
conflicting: in mechanically ventilated rats, pre-challenge CS exposure aggravated tissue
inflammation and apoptosis, but had only marginal effects in spontaneously breathing animals
[4]. Data on lung mechanics, gas exchange or histological changes were not reported in that
study. Moreover, CS exposure even suppressed the pro-inflammatory responses of alveolar
macrophages during halothane and isoflurane anaesthesia [5].

Lung contusion due to blunt chest trauma induced both pulmonary and systemic hyper-
inflammation, oxidative stress, and enhanced apoptosis [6–8]. The pulmonary and systemic
hyper-inflammatory response is due to the physical damage per se, the trauma-related drop in
alveolar O₂ tension [6], and the consecutive hypoxemia and tissue hypoxia [9]. Chronic
obstructive pulmonary disease (COPD) is also associated with pulmonary and systemic inflam-
mation, oxidative and nitrosative stress, and apoptosis [10,11], at least in part as a result of
alveolar hypoxia and hypoxemia [12–15]. Finally, in mice, CS exposure-induced COPD
[16,17] lead to a similar degree of pulmonary inflammation [18] as that induced by blunt chest
trauma in otherwise healthy littermates [8].

It is well-established that long-term hyperoxia causes ALI characterised by oxidative stress
and enhanced cell death [19]. Scarce data, however, are only available on the interaction
between CS exposure and hyperoxia: pre-natal exposure to the CS component benzo[a]pyrene
potentiates immediate post-natal hyperoxic lung injury [20], and long-term (over five days)
post-natal exposure to hyperoxia had an additive effect on the histological lung damage and
organ dysfunction in CS-induced COPD during adulthood [21]. However, in various animal
models resulting from haemorrhage [22–25], ischemia/reperfusion injury [26,27] and poly-
microbial sepsis [28–30] short-term ventilation with 100% O₂ resulted in attenuated inflamma-
tion and reduced apoptosis [26,27,29–32], and ultimately improved organ function and sur-
vival. Therefore, in anesthetized, resuscitated, and mechanically ventilated mice we tested the
hypotheses that 1) CS exposure prior to blunt chest trauma aggravates the post-traumatic pul-
monary and systemic inflammatory response and thereby organ dysfunction and injury, and
that 2) short-term hyperoxia may attenuate this effect.

Materials and Methods
The study protocol was approved by the University Animal Care Committee and the federal
authorities for animal research of the Regierungspräsidium Tübingen, Baden- Württemberg,
Germany (protocol no. 1046). The experiments were performed in accordance with the
National Institutes of Health Guidelines on the Use of Laboratory Animals. A total of n = 36
C57BL/6J mice of either gender at an age of 10–16 weeks and weighing 23 ± 2 g were obtained from Charles River (Kisslegg, Germany), housed in isolated, ventilated cages under a 12-hours light-dark cycle, and received food and water ad libitum. Four of these mice did not undergo CS exposure, anesthesia, chest trauma, and surgery, and served as controls for immunoblotting and electrophoretic mobility shifts (EMSA).

Cigarette smoke-induced pulmonary inflammation

In order to address the 1st hypothesis, 16 mice were exposed to cigarette smoke ("CS") over 3–4 weeks for 5 days per week in an exposure box as described previously [18]. Thereafter, 1 week was allowed as a recovery period prior to the blast experiment. This approach was chosen to avoid any acute stress effect induced by the CS exposure procedure per se. Mice received 4 cigarettes (Roth-Händle without filters, tar 10 mg, nicotine 1.0 mg, carbon monoxide 6 mg, Badische Tabakmanufaktur Roth-Händle®, Lahr, Germany) on day 1, 6 ones day 2, and 8 ones for the following days of the exposure period lasting for 15 min for each cigarette, which was followed by 8-minutes with fresh air (15 L.min⁻¹), and an additional 24-minutes break after each second cigarette. A semi-automatic cigarette lighter and smoke generator with an electronic timer was used to control the exposure (Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany). Particle concentration was monitored by a real time ambient particle monitor (MicroDust Pro, Casella, Amherst, NH, USA). In pilot experiments, this CS-exposure procedure had not caused any effect on the behavior, body weight, or respiratory pattern. Control animals ("Non-CS", n = 16) were exposed to room air.

Anaesthesia, blast wave, surgery, and experimental protocol

Mice were anesthetized with a mixture of 2.5% sevoflurane (Sevorane, Abbott, Wiesbaden, Germany) in 50% O₂ and N₂ and buprenorphine i.p. (1 μg·g⁻¹). Blunt chest trauma was induced by a single blast wave centred on the thorax as described previously [8]. Briefly, compressed air rapidly ruptures a Mylar polyester film (Du Pont de Nemur, Bad Homburg, Germany), which releases a reproducible single blast wave toward the animal’s mid-sternal chest, and thus induces a reproducible contusion of the lungs without remote organ damage. Immediately after trauma, animals received i.p. ketamine (85 μg·g⁻¹), midazolam (0.9 μg·g⁻¹), and fentanyl (0.18 μg·g⁻¹), and were placed on a procedure bench equipped with a closed-loop system to control body temperature [8,33,34]. An incision was made in the anterior neck to expose trachea, right internal jugular vein, and right carotid artery. The trachea was intubated, and the lungs were mechanically ventilated with a pressure-controlled, lung-protective ventilation strategy using a specially designed small animal ventilator (FlexiVent, Scireq, Montreal, Canada). After a lung recruitment manoeuver consisting of an inspiratory hold at 18 cm H₂O over 5 seconds, the initial respirator settings were: tidal volume 8 μL·g⁻¹, respiratory rate 150 breaths·min⁻¹, inspiratory/expiratory time ratio 1:2, PEEP 5 cm H₂O. Recruitment manoeuvres were repeated hourly, because this approach allowed maintaining thoraco-pulmonary compliance in the physiological range by preventing atelectasis, and reduced pulmonary inflammation [35]. In order to address the 2nd hypothesis n = 8 animals in each group were randomly assigned to mechanical ventilation with 100% O₂ (FiO₂ 1.0), whereas the other mice were ventilated with air (FiO₂ 0.21). Catheters were inserted into the jugular vein, the carotid artery, and the bladder. Anaesthesia was maintained with continuous i.v. ketamine (100–150 μg·g⁻¹·h⁻¹), midazolam (0.2–0.3 μg·g⁻¹·h⁻¹), and fentanyl (1–1.5 μg·g⁻¹·h⁻¹). Anaesthetic drugs were titrated to reach deep sedation and analgesia as documented by complete tolerance against noxious stimuli. To maintain mean arterial pressure > 50 mmHg animals received 12 μL·g⁻¹.
of hydroxyethyl starch in a balanced electrolyte solution (Tetraspan 6%, 130, 0.42, Braun Medical, Melsungen, Germany).

**Measurements**

All animals were studied over four hours of mechanical ventilation. Systemic haemodynamics (heart rate, mean blood pressure) and body temperature were recorded hourly. The static thoraco-pulmonary compliance was measured hourly by incrementally increasing the airway pressure up to a maximum inspiratory pressure of 20 cm H₂O and using the indwelling software of the respirator that allows automatic recording of the inspiratory and expiratory pressure-volume loop. Arterial blood samples were taken hourly for blood gases and pH. The respiratory rate was titrated to maintain arterial PCO₂ at 30–40 mmHg, the PEEP level was titrated according to the arterial PO₂: if the PaO₂/FiO₂ ratio was ≥ 300 mmHg PEEP was decreased to 3 cmH₂O, and if 100 mmHg < PaO₂/FiO₂ < 200 mmHg, it was increased to 8 cm H₂O. At the end of the observation period, animals were killed through blood withdrawal via the carotid artery. Immediately thereafter the respiratory tubes were clamped at end-expiration, i.e. PEEP level, the thorax was opened, and the lungs were removed.

**Blood and lung tissue preparation**

Whole blood was immediately spun, and plasma was stored at -80°C until analysis. The right lung was sampled, immediately frozen in liquid nitrogen and stored at -80°C for cytokine measurements, immunoblotting and EMSA. The left lung was fixed in formalin and paraffin-embedded for histology and immunohistochemistry.

**Cytokine concentrations**

Plasma and lung tissue levels of the cytokines and chemokines tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-10, IL-18, keratinocyte chemoattractant (KC), and monocyte chemotactic protein-1 (MCP-1) were measured by a mouse multiplex cytokine kit (Bio-Plex Pro Cytokine Assay, Bio-Rad, Hercules, CA) in accordance to the manufacturer’s instructions [8,33,34]. In brief, the appropriate standards and samples were added to a filter plate. The samples were incubated with antibodies chemically attached to fluorescent-labelled micro beads. Thereafter, premixed detection antibodies were added to each well, and streptavidin-phycocerythrin was added. Beads were then re-suspended, and the cytokines reaction mixture was quantified using the Bio-Plex protein array reader. Data were automatically processed and analysed by Bio-Plex Manager Software 4.1 using the standard curve produced from recombinant cytokine standards. Levels below the detection limit of the assays were set to zero for statistical purposes. Due to technical problems, plasma cytokine concentrations are from n = 7 animals in the “CS” groups, while lung tissue levels are from n = 6 and n = 7 of the air-ventilated “non-CS” and “CS” mice, respectively.

**Cell extracts, EMSA, and immune-blots**

Lung tissue was homogenized and lysed in lysing buffer. For cell extract preparation, cells were re-suspended, lysed on ice and centrifuged. The supernatant (protein extract) was stored at -80°C. For the assessment of the expression of haeme oxygenase-1 (HO-1), cleaved caspase-3 and HIF-1α, protein concentrations were determined, and equal total protein aliquots (20–60 μg) were separated by SDS-PAGE and transferred by Western blotting [8,33]. After blocking, the membranes were incubated with commercially available primary antibodies (anti-HO-1, Abcam, Cambridge, NY; anti-cleaved caspase 3, Cell Signaling, Danvers, MA; anti-
HIF-1α, Thermo Fisher Scientific, Waltham, MA). The primary antibodies were detected using horseradish peroxidase-conjugated secondary antibodies (Cell Signaling, Danvers, MA or Santa Cruz, Dallas, TX). The membranes were subjected to chemo-luminescence using SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific). Exposed films were scanned, and intensity of immune-reactivity was measured using NIH Image J software (http://rsb.info.nih.gov/nih-image). Actin and vinculin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) served as the loading controls. All immune-blots were repeated twice.

Activation of the nuclear transcription factor κB (NF-κB) was determined using electrophoretic mobility shift assay (EMSA) as described recently [8,33,34]: cell extracts were incubated with Poly-doxy-inosinic-deoxy-cytidylic acid (poly-dI-dC) and 32P-labeled double stranded oligonucleotide containing the NF-κB (HIVκB-site) (5′-AGT TGA GGG GAC TTT CCC AGG C-3′, Biomers, Ulm). Complexes were separated in polyacrylamide gels and exposed to X-ray films. A phosphor-imager and image analysis software (AIDA Image Analyzer, Raytest, Straubenhardt) allowed quantifying the radioactively labeled NF-κB.

For comparison between individual gels, the intensity of each band was related to that of two loaded control animals which had not undergone surgical instrumentation and trauma. Therefore, the immune-blot and EMSA data are expressed as fold increase over the mean of the two control values using the mean value of the three individual gels for each experimental animal.

**Histology and immunohistochemistry**

The left lung was formalin fixed and embedded in paraffin for histological and immunohistochemistry analysis. Adapting recently published scoring systems [36] the haematoxylin eosin-stained lung sections were analysed by two experienced pathologist (A.S., P.M.) blinded for the group assignment for alveolar collapse (i.e. dystelectasis or atelectasis), emphysematous over-distension, inflammatory cell (i.e. neutrophils, macrophages, and lymphocytes, respectively) infiltration, thickening of the alveolar membranes, protein debris in the airspaces, and alveolar oedema. These parameters were scored on a scale ranging from 0 (normal lung histology without pathological findings), 0.5 (minor histological injury), 1 (moderate and patchy histological injury), 1.5 (major histological injury < 25% of the lung involved), and 2 (major histological injury 25–50% of the lung involved).

Immunohistochemistry detection of nitrotyrosine formation and expression of the purinergic receptors 2X7 and 2X4 (P2XR7, P2XR4) was performed as follows: the paraffin sections were deparaffinized in xylene and graded ethanols, boiled twice in sodium citrate buffer for heat-induced epitope retrieval before being exposed to the primary antibody (anti-nitrotyrosine, Millipore, Schwalbach, Germany; anti-P2X7-Receptor and -P2X4-Receptor, Alomone Labs, Jerusalem, Israel,). Primary antibody detection was performed by a secondary antibody and visualized with a red chromogen (Alkaline Phosphatase-conjugated Goat-Anti-Rabbit IgG, Jackson Immuno Research, West Grove, USA). Slides were visualized using a Zeiss Axio Imager A1 microscope using a 10x objective (EC Plan-NEOFLUAR). Four distinct random 800 μm² square regions were quantified for intensity of signal using the image analysis AxioVision 4.8 software (Zeiss, Jena, Germany). Therefore, results are presented as densitometric sum red [8,34].

**Statistical analysis**

All data are presented as median (quartiles) unless otherwise stated. The sample sizes were based on our previous experience [8], for which a statistical power analysis using the Horovitz-index (PaO2/FiO2 ratio), thoraco-pulmonary compliance and lung tissue NF-κB activation as
main criteria and based on two-sided testing, $\alpha = 0.05$, power 80% and non-parametric analysis of variance had yielded a minimum of $n = 8$–10 for eight experimental groups. The scope of the present study was to assess the effects of CS-exposure prior to and hyperoxia immediately after blunt chest trauma. Therefore, in an attempt to reduce the number of animals, we did not study sham-operated mice and used $n = 8$ in each of the four experimental groups. After exclusion of normal distribution using the Kolmogorov-Smirnov-test, intergroup differences were analysed with a Kruskal-Wallis one way ANOVA on ranks, and a subsequent Dunn’s test for multiple comparisons using a two-tailed hypothesis testing. Differences were considered statistically significant when $p < 0.05$. All quantitative graphical presentations and statistical analyses were performed using the GraphPad Prism 5, version 5.04, software (GraphPad Software Inc., La Jolla, CA).

**Results**

**Effects of cigarette smoke-exposure**

Table 1 demonstrates that systemic haemodynamics, metabolism, and acid-base status did not differ between the four experimental groups. However, CS exposure prior to chest trauma was associated with a higher static thoraco-pulmonary compliance post trauma and lower $\text{PaO}_2$ and Horovitz-index.

Table 2 shows that CS exposure prior to trauma increased post-traumatic lung tissue IL-18 and blood MCP-1 concentrations, the latter, however, did not reach statistical significance ($p < 0.1$ each during air and $\text{O}_2$ ventilation).

Table 3 demonstrates that the CS exposure-induced alterations in pulmonary gas exchange and lung cytokine concentrations coincided with a two- to four-fold higher tissue infiltration of neutrophils, smokers’ macrophages, and lymphocytes, as well as with more pronounced alveolar wall thickening.

| Table 1. Parameters of systemic hemodynamics, lung mechanics, pulmonary gas exchange, metabolism, and acid-base. |  |
| Non-CS $\text{FiO}_2$ 0.21 | CS $\text{FiO}_2$ 0.21 | Non-CS $\text{FiO}_2$ 1.0 | CS $\text{FiO}_2$ 1.0 | p-value K-W ANOVA |
| Heart rate [min$^{-1}$] | 340 (340;348) | 355 (320;375) | 395 (364;443) | 322 (318;350) | 0.089 |
| Mean arterial pressure [mmHg] | 54 (54;58) | 56 (52;57) | 57 (56;60) | 58 (55;60) | 0.326 |
| Glucose [mmol L$^{-1}$] | 5.7 (5.4;6.6) | 4.5 (3.7;4.8) $\uparrow$ | 5.0 (4.4;6.4) | 5.2 (4.8;5.8) | 0.024 |
| Lactate [mmol L$^{-1}$] | 1.8 (1.1;2.0) | 1.1 (0.9;1.1) | 1.2 (0.8;1.3) | 0.9(0.8;1.5) | 0.199 |
| Minute ventilation [L min$^{-1}$] | 750 (745;810) | 810 (768;828) | 800 (760;830) | 750 (748;758) | 0.227 |
| Compliance [L cm H$_2$O$^{-1}$] | 79 (75;93) | 97 $\uparrow$ (82;106) | 75 (65;85) | 90 $\uparrow$ (87;96) | 0.028 |
| $\text{PaO}_2$ [mmHg] | 102 (97;106) | 78 $\uparrow$ (75;82) | 379 $\uparrow$ (318;395) | 370 $\uparrow$ (329;424) | < 0.001 |
| PEEP [cm H$_2$O] | 3.0 (3.0;3.0) | 3.0 (3.0;3.5) | 3.0 (3.0;3.0) | 3.0 (3.0;3.5) | 0.272 |
| $\text{PaO}_2/\text{FiO}_2$ ratio [mmHg] | 491 (460;505) | 368 $\uparrow$ (358;391) | 379 $\uparrow$ (318;395) | 370 (329;424) | 0.007 |
| $\text{PaCO}_2$ [mmHg] | 35 (32;38) | 32 (32;35) | 37 (29;37) | 37 (34;43) | 0.460 |
| Arterial pH | 7.32 (7.30;7.34) | 7.30 (7.23;7.33) | 7.32 (7.31;7.35) | 7.30 (7.28;7.34) | 0.565 |
| Arterial base excess [mmol L$^{-1}$] | -7.4 (-8.5;-6.9) | -8.1 (-9.7;-6.8) | -6.8 (-8.0;-6.5) | -7.0 (-10.0;-5.4) | 0.617 |

$\uparrow$ depicts $p < 0.05$ vs. the corresponding Non-CS group,

$\downarrow$ depicts $p < 0.05$ vs. the corresponding air group (K-W ANOVA Kruskall-Wallis analysis of variance on ranks with post-hoc Dunn’s test for multiple comparisons).

doi:10.1371/journal.pone.0132810.t001
Typical examples of the histological items are shown in Fig 1. Whereas CS exposure did not affect post-traumatic lung tissue HO-1 expression (Fig 2), it markedly increased activated caspase-3 (Fig 3) expression and NF-κB activation (Fig 4), and reduced HIF-1α formation (Fig 5). CS exposure was also associated with increased tissue nitrotyrosine formation (Fig 6), P2XR7 and P2XR4 expression (Figs 7 and 8).

Table 2. Plasma and lung tissue cytokine and chemokine concentrations. Plasma (in pg mL⁻¹) and lung tissue (in pg mg protein⁻¹) cytokine and chemokine concentrations at the end of the four-hours observation period (n = 8 in each group) obtained from mice without (Non-CS) and with (CS) cigarette smoke exposure over 3–4 weeks prior to blunt chest trauma and mechanical ventilation with air (FiO₂ 0.21) or 100% O₂ (FiO₂ 1.0). All data are median (quartiles).

|                  | Non-CS FiO₂ 0.21 | CS FiO₂ 0.21 | Non-CS FiO₂ 1.0 | CS FiO₂ 1.0 | p-value K-W ANOVA |
|------------------|------------------|--------------|-----------------|------------|------------------|
| IL-1β [pg mL⁻¹] | 32 (25;36)       | 37 (22;49)   | 23 (13;29)      | 13 (9;19)  | 0.215            |
| IL-1β [pg mg protein⁻¹] | 309 (209;457) | 531 (204;631) | 223 (118;332) | 285 (245;325) | 0.413            |
| IL-6 [pg mL⁻¹]  | 212 (71;475)     | 511 (310;863)| 123 (93;275)   | 177 (122;434)| 0.304            |
| IL-6 [pg mg protein⁻¹] | 5 (5;20)       | 9 (8;11)     | 11 (8;18)      | 5 (4;7)    | 0.116            |
| IL-10 [pg mL⁻¹] | 99 (72;171)      | 153 (92;255) | 85 (53;126)    | 95 (80;177) | 0.467            |
| IL-10 [pg mg protein⁻¹] | 47 (43;53)    | 38 (34;40)   | 42 (39;45)     | 48 (45;51) | 0.153            |
| KC [pg mL⁻¹]    | 275 (146;421)    | 265 (156;644)| 115 (97;136)   | 223 (212;324)| 0.369            |
| KC [pg mg protein⁻¹] | 570 (313;833) | 606 (469;671) | 320 (224;497) | 450 (391;586) | 0.204            |
| MCP-1 [pg mL⁻¹] | 772 (528;2517)   | 1395 (889;7011)| 265 (208;779)  | 3060 (1859;3389) | 0.099            |
| MCP-1 [pg mg protein⁻¹] | 141 (135;156) | 137 (123;166) | 136 (114;150) | 129 (121;135) | 0.622            |
| TNF-α [pg mL⁻¹] | 90 (84;104)      | 111 (82;137) | 86 (61;93)     | 93 (86;108)  | 0.456            |
| TNF-α [pg mg protein⁻¹] | 113 (75;133)  | 112 (102;121)| 122 (111;145) | 113 (88;144) | 0.707            |
| IL-18 [pg mL⁻¹] | 234 (165;464)    | 189 (169;217)| 179 (147;220)  | 148 (105;208) | 0.429            |
| IL-18 [pg mg protein⁻¹] | 603 (533;673) | 973 (795;1045) | 563 (440;600) | 882 (629;1088) | 0.019            |

§ depicts p < 0.05 vs. the corresponding Non-CS group,
$ depicts p < 0.05 vs. the corresponding air group (K-W ANOVA Kruskall-Wallis analysis of variance on ranks with post-hoc Dunn’s test for multiple comparisons).

doi:10.1371/journal.pone.0132810.t002

Table 3. Parameters of lung histopathology. Parameters of lung histopathology at the end of the four-hours observation period (n = 8 in each group) obtained from mice without (Non-CS) and with (CS) cigarette smoke exposure over 3–4 weeks prior to blunt chest trauma and mechanical ventilation with air (FiO₂ 0.21) or 100% O₂ (FiO₂ 1.0) (n = 8 in each group). Parameters were scored as 0 (normal lung histology without pathological findings), 0.5 (minor histological injury), 1 (moderate and patchy histological injury), 1.5 (major histological injury < 25% of the lung involved), and 2 (major histological injury 25–50% of the lung involved). All data are median (quartiles).

|                  | Non-CS FiO₂ 0.21 | CS FiO₂ 0.21 | Non-CS FiO₂ 1.0 | CS FiO₂ 1.0 | p-value K-W ANOVA |
|------------------|------------------|--------------|-----------------|------------|------------------|
| Dystelectasis/atelectasis | 1.0 (0.5;1.0) | 0.5 (0.5;1.0) | 1.0 (0.5;1.0) | 0.5 (0.5;0.6) | 0.683            |
| Emphysema        | 1.5 (1.5;2.0)    | 1.5 (1.0;1.5) | 1.3 (1.0;1.5)  | 1.1 § (1.0;1.3) | 0.011            |
| Alveolar membrane thickening | 1.0 (1.0;1.0) | 1.5 § (1.5;1.8) | 1.0 (1.0;1.5) | 1.5 § (1.4;1.6) | 0.047            |
| Lymphocytes      | 1.0 (1.0;1.0)    | 2.0 § (1.8;2.3) | 1.5 (1.5;2.0)  | 2.0 § (1.9;2.5) | < 0.001          |
| Neutrophils      | 0.5 (0.5;0.8)    | 2.0 § (2.0;2.5) | 0.8 (0.5;1.0)  | 1.5 § (1.5;2.0) | < 0.001          |
| Macrophages      | 3.5 (3.3;3.8)    | 12.0 § (7.0;13.5) | 3.5 (3.0;5.6) | 14.0 § (11.5;15.8) | < 0.001          |
| Protein debris in the airspaces | 1.0 (0.5;1.0) | 0.5 (0.5;0.8) | 1.0 (1.0;1.0) | 0.8 (0.5;1.0) | 0.074            |
| Alveolar edema   | 0.5 (0.3;0.5)    | 0.5 (0.5;1.0) | 1.0 (0.9;1.1)  | 1.5 § (0.9;2.0) | 0.022            |

§ depicts p < 0.05 vs. the corresponding Non-CS group,
$ depicts p < 0.05 vs. the corresponding air group (K-W ANOVA Kruskall-Wallis analysis of variance on ranks with post-hoc Dunn’s test for multiple comparisons).

doi:10.1371/journal.pone.0132810.t003
Effects of hyperoxia

Tables 1 and 2 demonstrate that lung-protective mechanical ventilation at FiO₂ 1.0 did not further affect lung mechanics, gas exchange or pulmonary and systemic cytokine and chemokine concentrations. Table 3 shows that ventilation with 100% O₂ did not affect lung histology except for some patchy alveolar edema in CS exposed mice, however, without any deleterious effect on organ function (see Table 1). While ventilation with 100% O₂ markedly increased lung tissue HO-1 expression (Fig 2) in CS exposed mice, it did not further affect activated caspase-3 (Fig 3) and HIF-1α expression (Fig 4), or NF-κB activation (Fig 5), and even attenuated nitrotyrosine formation (Fig 6), and P2XR₇, and P2XR₄ expression (Figs 7 and 8).

Fig 1. Typical examples of the histopathological items scored. Upper panel: Lung region with little histopathological abnormalities, i.e. no dystelectasis/atelectasis, normal thickness of alveolar membranes, and little lymphocyte immigration. Dotted arrows show some degree of protein debris in the airspaces. Lower panel: Lung region with major histological injury (alveolar membrane thickening and lymphocyte immigration grade 1.5 each). Solid arrows show alveolar smokers' macrophages within the airways.

doi:10.1371/journal.pone.0132810.g001
Discussion

This study investigated the effect of short-term hyperoxia on post-traumatic pulmonary and systemic inflammation and lung injury in mice that had undergone CS exposure prior to blunt chest trauma. The major findings were that i) CS exposure prior to trauma aggravated trauma-induced lung dysfunction and organ injury due to more pronounced pulmonary inflammation and nitrosative stress, ultimately resulting in enhanced apoptosis and tissue damage, and that ii) lung-protective mechanical ventilation with 100% O2 partially attenuated this effect.

Effects of cigarette smoke-exposure on the acute trauma response

Smoking is directly related to the development of ALI after severe blunt trauma [2]. Our findings well agree with this observation: mice that had undergone CS exposure prior to trauma showed impaired pulmonary gas exchange and more pronounced lung histological damage. Despite the comparable histological evidence of emphysematous lung over-distension, the higher static thoraco-pulmonary compliance in the CS exposed mice further supports this notion: CS abuse is the most important etiological factor for COPD [37], which in turn, is
associated with a hyperinflation-induced increase of the static compliance [38], even when total lung capacity is still normal [39].

Murine models of CS-induced COPD are characterised by pulmonary inflammation and mediator release: after four days of CS-exposure, animals presented with a several-fold increase of neutrophil accumulation and pro-inflammatory cytokine and chemokine concentrations in the bronchoalveolar lavage (BAL) fluid [18,40]. In addition, CS exposure caused a two- to three-fold increase of markers of oxidative and nitrosative stress in the BAL fluid [41]. This effect resulted in enhanced apoptosis as documented by increased expression of the activated caspase-3 [41,42]. Finally, CS exposure increased ventilator-induced lung injury due to aggravated tissue inflammation and apoptosis in alveolar type II cells [4]. Our findings well agree with these observations: despite the 1-week recovery period aiming to avoid any procedure-related stress, CS-exposed mice presented with higher NF-κB activation, nitrotyrosine formation, and tissue IL-18 concentrations. The latter finding is of particular interest in the context of the CS exposure-related higher expression of the P2XR7 and P2XR4: inflammatory-regulated production of IL-1β and IL-18 has been referred to as a key mechanism of injurious ventilation- [43] and hyperoxia-induced [44] ALI via P2XR7 and subsequent inflammasome activation and IL-1β secretion: Genetic P2XR7 deletion reduced the endotoxin-induced release of IL-1β and thereby attenuated the impairment of lung mechanics and the
histological organ damage in vivo [45], and P2X7 activation aggravated the endotoxin-related vascular hypo-reactivity in vitro [46]. Co-expression of the P2X4 enhanced the P2X7-related inflammatory response [47], and up-regulation of P2X4 was referred to compensate for P2X7 depletion [48]. Finally, in addition to their role for the development of ALI, P2X7 and P2X4 activation was shown to assume major importance for CS-related lung injury: CS exposure caused up-regulation of both the P2X4 and P2X7 [49], and either pharmacological blockade or genetic deletion of the P2X7 attenuated the pulmonary IL-1β and IL-18 accumulation after CS exposure [50]. Consequently, our experiments support P2X4 and P2X7 as potential therapeutic targets both in ALI [51] and CS-induced COPD [52,53].

Pre-traumatic CS exposure reduced lung tissue HIF-1α expression after chest trauma. At first glance, this finding is in contrast to data from both CS exposed animals [54] and patients with COPD [55,56]. The increased nitrosative stress in CS exposed mice may have assumed importance in this context: under normoxic conditions, hyper-inflammation-induced nitric oxide (NO) release impairs HIF-1α degradation due to inhibition of prolyl-hydroxylases (PHD) [57]. However, excess NO formation under hypoxic conditions may also reduce HIF-1α accumulation because of an NO-mediated feedback with expression of PHD and/or O2-redistribution to PHD resulting from NO-induced inhibition of mitochondrial respiration [58].
Effects of hyperoxia on the acute trauma response

In mice without pre-traumatic CS exposure, pure O2 ventilation was associated with a significantly lower PaO2/FiO2 ratio than in the corresponding air-ventilated animals. This effect was most likely due to O2 breathing-induced instability of lung regions with low ventilation/perfusion-ratios [59] rather than due to O2 toxicity: O2 ventilation i) did not affect gas exchange or lung mechanics in mice after CS exposure, and ii) was not associated with any biochemical or morphological sign of aggravated pulmonary inflammation. Equivocal data are available whether short-term mechanical ventilation with 100% O2 induces a pulmonary and systemic inflammatory response: in rats, 120 minutes of pure O2 ventilation caused a marked increase in extravascular lung water and the BAL fluid neutrophil, chemokine and cytokine content, but this effect required the use of injurious tidal volumes (20 mL·kg⁻¹). In contrast, tidal volumes similar to our study (7 mL·kg⁻¹) had no effect [60]. In rabbits, four hours of mechanical ventilation with 100% O2 significantly increased BAL polymorphonuclear leukocytes and MCP-1 concentrations, associated with increased alveolar-capillary permeability. Again, this effect was only present with high (25 mL·kg⁻¹) tidal volumes, while clinically more relevant (10 mL·kg⁻¹) tidal volumes had no effects [61]. Finally, in mice incrementally increasing the FiO2 during 120 minutes of mechanical ventilation (tidal volume 7–8 mL·kg⁻¹) did not alter airway resistance, tissue...
elastance nor BAL fluid concentrations of chemokines and cytokines [62]. Scarce data are only available on the effects of non-injurious mechanical ventilation with O2 in the presence of sepsis- or trauma-induced ALI: low-tidal volume (7 mL \text{kg}^{-1}) mechanical ventilation with 100\% O2 caused more pronounced pulmonary and systemic inflammation than spontaneous air breathing, but the effects of hyperoxia per se remain open, since mechanically ventilation with lower FiO2 values was not studied [63]. In swine with fecal peritonitis-induced septic shock, lung-protective, pure O2 mechanical ventilation up to 24 hours did not cause any aggravation of pulmonary or systemic inflammation or histological damage [29,30].

Hyperoxia may have attenuated pulmonary and systemic inflammation by compensating regional pulmonary hypoxia as well as tissue hypoxia resulting from chest trauma-induced hypoxemia: hypoxic hypoxia (FiO2 0.1) of incremental duration caused a time-dependent increase of the BAL fluid neutrophil count and albumin content reflecting alveolar-capillary leakage [64], and further aggravated endotoxin-induced ALI [65]. MCP-1 is a crucial mediator of this inflammatory response [66], and in our experiments, blood MCP-1 levels were three

---

**Fig 6. Results of the immunohistochemistry for nitrotyrosine.** Typical examples (upper panel) and quantitative analysis (lower panel) of immunohistochemistry for lung tissue nitrotyrosine formation from mice without (dotted boxplots) and with (hatched boxplots) cigarette smoke exposure prior to blunt chest trauma and mechanically ventilated with air (white boxplots) and 100\% O2 (grey boxplots) (n = 8 in each group). All data median (quartiles, range), $ p < 0.05$ vs. corresponding cigarette smoke exposure group, $ p < 0.05$ vs. corresponding air ventilation group (Kruskall-Wallis analysis of variance on ranks with post-hoc Dunn’s test for multiple comparisons).

doi:10.1371/journal.pone.0132810.g006
times lower in pure O$_2$-ventilated mice without CS exposure prior to trauma (772 (528;2517) vs. 265 (208;779) pg·mL$^{-1}$, $p = 0.08$). Pure O$_2$ ventilation was also associated with reduced lung tissue nitrotyrosine formation, no matter whether mice had been exposed to CS or not. This finding well agrees with previous findings on the effects of short-term exposure to hyperoxia on oxidative and nitrosative stress: two or three hours of intermittent O$_2$ breathing after zymosan injection increased the activity of tissue antioxidant enzymes [31], and long-term exposure to FiO$_2$ 0.8 attenuated lung nitrotyrosine formation with intra-tracheal carrageenan-induced pneumonitis [67]. Hence, our findings suggest that under acute stress, short-term hyperoxia can counteract the nitrosative stress associated with CS exposure-induced COPD. Hyperoxia attenuated the P2XR$_7$ expression both in mice with and without CS exposure prior to chest trauma, whereas it reduced P2XR$_4$ expression only in animals with CS exposure. Hence, short-term hyperoxia may be of particular interest in acute stress under conditions of chronic hypoxia (aem): hypoxic hypoxaemia induced by exposure to simulated altitude not only increased lung tissue P2XR$_4$ mRNA, but also in the right ventricle, which went alongside with pulmonary artery hypertension and consecutive right ventricular hypertrophy [68].

Fig 7. Results of the immunohistochemistry for P2X$_7$. Typical examples (upper panel) and quantitative analysis (lower panel) of immunohistochemistry for lung tissue expression of the purinergic receptor P2X$_7$ from mice without (dotted boxplots) and with (hatched boxplots) cigarette smoke exposure prior to blunt chest trauma and mechanically ventilated with air (white boxplots) and 100% O$_2$ (grey boxplots) ($n = 8$ in each group). All data median (quartiles, range). § $p < 0.05$ vs. corresponding cigarette smoke exposure group. $\$ p $< 0.05$ vs. corresponding air ventilation group (Kruskall-Wallis analysis of variance on ranks with post-hoc Dunn’s test for multiple comparisons).

doi:10.1371/journal.pone.0132810.g007
Albeit we lack a direct proof, it is tempting to speculate that the hyperoxia-related attenuation of pulmonary and systemic inflammation was due to down-regulation of HIF-1α expression: Both genetic deletion [69,70] and pharmacological blockade [71] of HIF-1α attenuated the local and systemic inflammatory response and thereby reduced the severity of ALI after remote [69,70] or direct [71] organ damage. Finally, the neuro-protective effect of exposure to both hyper- and normobaric O2 was associated with a two to three-fold reduction of HIF-1α expression [72].

Limitations of the study

It could be argued that despite the marked differences in the inflammatory response, the CS-related effects on lung mechanics, gas exchange, and histology lack physiological significance: overall they were moderate, and the Horovitz-index always remained above 300 mmHg, i.e. above the definition threshold of ALI. Of note, we [8,51] and others [73] found a similar dissociation between significantly increased levels of inflammatory biomarkers in the lung and

Fig 8. Results of the immunohistochemistry for P2X₄. Typical examples (upper panel) and quantitative analysis (lower panel) of immunohistochemistry for lung tissue expression of the purinergic receptor P2X₄ from mice without (dotted boxplots) and with (hatched boxplots) cigarette smoke exposure prior to blunt chest trauma and mechanically ventilated with air (white boxplots) and 100% O₂ (grey boxplots) (n = 8 in each group). All data median (quantiles, range). § p < 0.05 vs. corresponding cigarette smoke exposure group. $ $ p < 0.05 vs. corresponding air ventilation group (Kruskall-Wallis analysis of variance on ranks with post-hoc Dunn’s test for multiple comparisons).
lacking effects on lung mechanics and gas exchange in mice after chest trauma [8,51] and polymicrobial sepsis [73]. Only few studies reported Horovitz-indices compatible with the definition of ALI (< 300 mmHg) in mechanically ventilated mice: animals were either ventilated with injurious tidal volumes (12–40 mL·kg⁻¹) [74–78], or 24 hours after injection of endotoxin, i.e. in the presence of prolonged, severe ALI [79]. Moreover, we used pressure-controlled, lung-protective ventilation that also comprised an initial lung recruitment manoeuvre. Consequently, any further damage beyond the effect of lung contusion per se or due to injurious mechanical ventilation was avoided. In fact, neither pulmonary mechanics nor gas exchange deteriorated during the 4-hour observation period. Our approach is in contrast to previous experiments, which explicitly studied the effect of CS on tissue inflammation and apoptosis related to ventilator-induced lung injury [4]. However, in that study, data on lung mechanics, gas exchange or histological changes were not reported at all.

Clearly, the short duration of the mechanical ventilation precludes any conclusion on the long-term effects. However, a recent review article showed that only two studies described mechanical ventilation in mice of up to eight hours, while in the other reports only four to six hours of mechanical ventilation were used [80]. Finally, we cannot exclude that correcting trauma-related hypoxaemia using an FiO₂ of 0.3–0.4 rather than air ventilation in the control group may have produced similar or even better results when compared to the hyperoxia group.

Conclusion

In a murine model of COPD induced by CS exposure, pulmonary and systemic inflammation as well as nitrosative stress were aggravated after blunt chest trauma, which ultimately resulted in increased severity of post-traumatic organ dysfunction and injury as documented by impaired gas exchange and more pronounced histological damage. Overall, short-term, lung-protective mechanical ventilation with 100% O₂ did not have a major therapeutic effect despite attenuation of nitrosative stress. The latter was possibly due to correction of regional alveolar hypoxia and/or consecutive hypoxemia, resulting in HIF-1α down-regulation.

Acknowledgments

Very special thanks are dedicated to Rosemarie Mayer and Rosa Maria Engelhardt for their skillful technical assistance.

Author Contributions

Conceived and designed the experiments: KW PA MH AI M. Georgieff EC FW PR. Performed the experiments: KW M. Gröger FW. Analyzed the data: KW OM AS M. Gröger BS FW. Contributed reagents/materials/analysis tools: AS OM MH BS MD. Wrote the paper: KW PA AI PM M. Georgieff BJ FW PR. Cigarette smoke exposure: BJ MD.

References

1. Shah CV, Locallo AR, Lanken PN, Kahn JM, Bellamy S, Gallop R, et al. The impact of development of acute lung injury on hospital mortality in critically ill trauma patients. Crit Care Med 2008; 36:2309–2315 doi:10.1097/CCM.0b013e3181804c78 PMID: 18664786
2. Calfee CS, Matthay MA, Eisner MD, Benowitz N, Mariah C, Pittet JF, et al. Active and passive cigarette smoking and acute lung injury after severe blunt trauma. Am J Respir Crit Care Med 2011; 183:160–165
3. Hsieh SJ, Zhuo H, Benowitz NL, Thompson BT, Liu KD, Matthay MA, et al. Prevalence and impact of active and passive cigarette smoking in acute respiratory distress syndrome. Crit Care Med 2014; 42:2058–2068 doi: 10.1097/CCM.0000000000000418 PMID: 24942512
4. Hirsch J, Chalkley RJ, Bentley T, Burlingame AL, Frank JA. Double impact of cigarette smoke and mechanical ventilation on the alveolar epithelial type II cell. Crit Care 2014; 18:R50 doi: 10.1186/cc13795 PMID: 2466941

5. Kotani N, Hashimoto H, Sessler DI, Yatsu Y, Muraoka M, Matsuki A. Exposure to cigarette smoke impairs alveolar macrophage functions during halothane and isoflurane anesthesia in rats. Anesthesiology 1999; 91:1823–1833 PMID: 10598627

6. Knöferl MW, Liner UC, Seitz DH, Perl M, Brückner UB, Kinzl L, et al. Cardiopulmonary, histological, and inflammatory alterations after lung contusion in a novel mouse model of blunt chest trauma. Shock 2003; 19:519–525 PMID: 12785006

7. Hoth JJ, Weiss JD, Brownlee NA, Hillbould EM, Meredith JW, McCall CE, et al. Toll-like receptor 4-dependent responses to lung injury in a murine model of pulmonary contusion. Shock 2009; 31:376–381 doi: 10.1097/SHK.0b013e3181862279 PMID: 18665044

8. Wagner F, Scheuerle A, Weber S, Stahl B, McCook O, Knöferl MW, et al. Cardiopulmonary, histologic, and inflammatory effects of intravenous Na2S after blunt chest trauma-induced lung contusion in mice. J Trauma 2011; 71:1569–1567 doi: 10.1097/TA.0b013e118238842e PMID: 21857260

9. Eltzschig HK, Carmeliet P. Hypoxia and inflammation. N Engl J Med 2011; 364:656–665 doi: 10.1056/NEJMra0910283 PMID: 21323543

10. Tudor RM, Petrace I. Pathogenesis of chronic obstructive pulmonary disease. J Clin Invest 2012; 122:2749–2755

11. Schmidt EP, Tuder RM. Role of apoptosis in amplifying inflammatory responses in lung diseases. J Cell Death 2010; 2010:41–53 PMID: 22081757

12. Seimetz M, Parajuli N, Pichl A, Veit F, Kwapiszewska G, Weisel FC, et al. Inducible NOS inhibition reverses tobacco-smoke-induced emphysema and alveolar hypoplasia in mice. Cell 2011; 147:293–305 doi: 10.1016/j.cell.2011.08.035 PMID: 22000010

13. Chao J, Wood JG, Gonzalez NC. Alveolar hypoxia, alveolar macrophages, and systemic inflammation. Respir Res 2009; 10:54 doi: 10.1186/1465-9921-10-54 PMID: 19545431

14. Gonzalez NC, Wood JG. Alveolar hypoxia-induced systemic inflammation: what low PO2 does and does not do. Adv Exp Med Biol 2010; 662:27–32 doi: 10.1007/978-1-4419-1241-1_3 PMID: 20204767

15. Fröhlich S, Boylan J, McLoughlin P. Hypoxia-induced inflammation in the lung? A potential therapeutical target in acute lung injury? Am J Respir Cell Mol Biol 2013; 48:271–279 doi: 10.1186/rcmb.2012-0137TR PMID: 23087053

16. Vlahos R, Bozinovski S, Gualano RC, Ernst M, Anderson GP. Modelling COPD in mice. Pulm Pharmacol Ther 2006; 19:12–17 PMID: 16286233

17. Gould NS, Min E, Gauthier S, Chu HW, Martin R, Day BJ. Aging adversely affects the cigarette smoke-induced glutathione adaptive response in the lung. Am J Respir Crit Care Med 2010; 182:1114–1122 doi: 10.1164/rcrm.201003-0442OC PMID: 20922927

18. Wollin L, Pieper M. Tiotropium bromide exerts anti-inflammatory activity in a cigarette smoke mouse model of COPD. Pulm Pharmacol Ther 2010; 23:345–354 doi: 10.1016/j.pupt.2010.03.008 PMID: 20362889

19. Kallett RH, Matthay MA. Hypoxic acute lung injury. Respir Care 2013; 58:123–141 doi: 10.4187/respcare.01963 PMID: 23271823

20. Thakur VS, Liang YW, Lingappan K, Jiang W, Wang L, Barrios R, et al. Increased susceptibility to hyperoxic lung injury and alveolar simplification in newborn rats by prenatal administration of benz[a]pyrene. Toxicol Lett 2014; 230:322–332 PMID: 24657529

21. McGrath-Morrow SA, Lauer T, Collaco JM, Yee M, O’Reilly M, Mitzner W, et al. Neonatal hyperoxia contributes additively to cigarette smoke-induced chronic obstructive pulmonary disease changes in adult mice. Am J Respir Cell Mol Biol 2011; 45:610–616 doi: 10.1165/rcmb.2010-0259OC PMID: 21239606

22. Bitterman H, Brod V, Weisz G, Kushnir D, Bitterman N. Effects of oxygen on regional hemodynamics in hemorrhagic shock. Am J Physiol Heart Circ Physiol 1986; 271:H203–H211

23. Efrati S, Berman S, Aharon GB, Siman-Tov Y, Averbukh Z, Weissgarten J. Application of normobaric hyperoxia therapy for amelioration of haemorrhagic shock-induced acute renal failure. Nephrol Dial Transplant 2008; 23:2215–2222 doi: 10.1093/ndt/gfn095 PMID: 18400820

24. Meier J, Kemming GI, Kisch-Wedel H, Blum J, Pape A, Habler OP. Hyperoxic ventilation reduces six-hour mortality after partial fluid resuscitation from hemorrhagic shock. Shock 2004; 22:240–247 PMID: 15316394

25. Blasiole B, Bayir H, Vagni VA, Janesko-Feldman K, Cheikhia A, Wisniewski SR, et al. Effect of hyperoxia on resuscitation of experimental combined traumatic brain injury and hemorrhagic shock in mice. Anesthesiology 2013; 118:649–663 doi: 10.1097/ALN.0b013e318280a42d PMID: 23299361
26. Waisman D, Brod V, Wolff R, Sanop E, Chemin M, Weinraub Z, et al. Effects of hyperoxia on local and remote microcirculatory inflammatory response after splanchnic ischemia and reperfusion. Am J Physiol Heart Circ Physiol 2003; 285:H643–H652 PMID: 12714329

27. Sukhotnik I, Brod V, Lurie M, Rahat MA, Shnizer S, Lahat N, et al. The effect of 100% oxygen on intestinal preservation and recovery following ischemia-reperfusion injury in rats. Crit Care Med 2009; 37:1054–1061 doi: 10.1097/CMM.0b013e31819d095c PMID: 19237917

28. Buras JA, Holt D, Orlow D, Bellkoff B, Pavlides S, Reenstra WR. Hyperbaric oxygen protects from sepsis mortality via an interleukin-10-dependent mechanism. Crit Care Med 2006; 34:2624–2629 PMID: 16932333

29. Barth E, Bassi G, Maybauer DM, Simon F, Gröger M, Öter S, et al. Effects of ventilation with 100% oxygen during early hyperdynamic porcine fecal peritonitis. Crit Care Med 2008; 36:495–503 PMID: 18091553

30. Hauser B, Barth E, Bassi G, Simon F, Gröger M, Öter S, et al. Hemodynamic, metabolic and organ function effects of pure O2 ventilation during established fecal peritonitis-induced septic shock. Crit Care Med 2009; 37:2465–2469 doi: 10.1097/CCM.0b013e31819d095c PMID: 1953193

31. Hou L, Xie K, Li N, Qin M, Lu Y, Ma S, et al. 100% oxygen inhalation protects against zymosan-induced sterile sepsis in mice: the roles of inflammatory cytokines and antioxidant enzymes. Shock 2009; 32:451–461 doi: 10.1097/SHK.0b013e31819c391a PMID: 19174736

32. Waisman D, Brod V, Rahat MA, Amit-Cohen BC, Lahat N, Rimar D, et al. Dose-related effects of hyperoxia on the lung inflammatory response in septic rats. Shock 2012; 37:95–102 doi: 10.1097/SHK.0b013e3182356fc3 PMID: 21921827

33. Wagner F, Wagner K, Weber S, Stahl B, Knöferl MW, Huber-Lang M, et al. Inflammatory effects of hypothermia and inhaled H2S during resuscitated, hyperdynamic murine septic shock. Shock 2011; 35:396–402 doi: 10.1097/SHK.0b013e3181ff1fe0e PMID: 20938376

34. Wagen K, Wachter U, Vogt JA, Scheuerle A, McCook O, Weber S, et al. Adrenomedullin binding improves catecholamine responsiveness and kidney function in resuscitated murine septic shock. Intensive Care Med Exp 2013; 1:2

35. Reiss LK, Kowallik A, Uhlig S. Recurrent recruitment manoeuvres improve lung mechanics and minimize lung injury during mechanical ventilation of healthy mice. PLoS ONE 2011; 6:e24527 doi: 10.1371/journal.pone.0024527 PMID: 21935418

36. Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, et al. An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. Am J Respir Crit Care Med 2011; 44:725–738

37. Geldmacher H, Biller H, Herbst A, Urbanski K, Allison M, Buist AS, et al. The prevalence of chronic obstructive pulmonary disease (COPD) in Germany. Results of the BOLD study. Dtsch Med Wochenschr 2008; 133:2609–2614 PMID: 19052996

38. Papandrinopoulou D, Tzouda V, Tsoukalas G. Lung compliance and chronic obstructive pulmonary disease. Pulm Med 2012; 2012:542769 doi: 10.1155/2012/542769 PMID: 23150821

39. O'Donnell DE, Lavenezia P. Physiology and consequences of lung hyperinflation in COPD. Eur Respir Rev 2006; 15:61–67

40. Geraghty P, Wyman AE, Garcia-Arcos I, Dabo AJ, Gadhvi S, Foronjy R. STAT3 modulates cigarette smoke-induced inflammation and protease expression. Front Physiol 2013; 4:267 doi: 10.3389/fphys.2013.00267 PMID: 24101903

41. Guan SP, Tee W, Ng DSW, Chan TK, Peh HY, Ho WE, et al. Andrographolide protects against cigarette smoke-induced oxidative lung injury via augmentation of Nrf2 activity. Br J Pharmacol 2013; 168:1707–1718 doi: 10.1111/bph.12054 PMID: 23146110

42. Park JW, Ryter SW, Kyung SY, Lee SP, Jeong SH. The phosphodiesterase 4 inhibitor rolipram protects against cigarette smoke extract-induced apoptosis in human lung fibroblasts. Eur J Pharmacol 2013; 706:76–83 doi: 10.1016/j.ejphar.2013.02.049 PMID: 23499692

43. Dolinay T, Kim YS, Howrylak J, Hunninghake GM, An CH. Inflammasome-regulated cytokines are critical mediators of acute lung injury. Am J Respir Crit Care Med 2012; 185:1225–1234 doi: 10.1164/rccm.201201-0003OC PMID: 22461369

44. Kolliputi N, Shaik RS, Waxman AB. The inflammasome mediates hyperoxia-induced alveolar cell permeability. J Immunol 2010; 184:5819–5826 doi: 10.4049/jimmunol.0902766 PMID: 20375306

45. Monçô-Ribeiro L, Cagido VR, Lima-Murad G, Teixeira-Santana P, Riva DR, Borojecic R, et al. Lipopolysaccharide-induced lung injury: Role of P2X7 receptor. Respir Physiol Neurobiol 2011; 179:314–325 PMID: 21982752
46. Chiao CW, Tostes RC, Webb RC. P2X7 receptor activation amplifies lipopolysaccharide-induced vascular hyporeactivity via interleukin-1β release. J Pharmacol Exp Ther 2008; 326:864–870 doi: 10.1124/jpet.107.135350 PMID: 18599654

47. Kawano A, Tsukimoto M, Mori D, Noguchi T, Harada H, Takenouchi T, et al. Regulation of P2X7-dependent inflammatory functions by P2X4 receptor in mouse macrophages. Biochem Biophys Res Commun 2012; 420:102–107 doi: 10.1016/j.bbrc.2012.02.122 PMID: 22405772

48. Weinhold K, Krause-Buchholz U, Rödel G, Kasper M, Barth K. Interaction and interrelation of P2X7 and P2X4 receptor complexes in mouse lung epithelial cells. Cell Mol Life Sci 2010; 67:2631–2642 doi: 10.1007/s00018-010-0355-1 PMID: 20405163

49. Cicko S, Lucattelli M, Müller T, Lommatzsch M, De Cunto G, Cardini S, et al. Purinergic receptor inhibition prevents the development of smoke-induced lung injury and emphysema. J Immunol 2010; 185:688–697 doi: 10.4049/jimmunol.0904042 PMID: 20519655

50. Eltom S, Stevenson CS, Rastrick J, Dale N, Raemdonck K, Wong S, et al. P2X7 receptor and caspase 1 activation are central to airway inflammation observed after exposure to tobacco smoke. PLoS ONE 2011; 6:e24097 doi: 10.1371/journal.pone.0024097 PMID: 21915284

51. Hafner S, Wagner K, Wepler M, Matallo J, Gröger M, McCook O, et al. Physiological and immune-biological characterization of a long-term murine model of blunt chest trauma. Shock 2015; 43:140–147 doi: 10.1097/SHK.0000000000000277 PMID: 25526372

52. Lucattelli M, Cicko S, Müller T, Lommatzsch M, De Cunto G, Cardini S, et al. P2X7 receptor signaling in the pathogenesis of smoke-induced lung inflammation and emphysema. Am J Respir Cell Mol Biol 2011; 44:423–429 doi: 10.1165/rcmb.2010-0038OC PMID: 20508069

53. Müller T, Viera RP, Grimm M, Dürk T, Cicko S, Zeiser R, et al. A potential role for P2X7R in allergic airway inflammation in mice and humans. Am J Respir Cell Mol Biol 2011; 44:456–464 doi: 10.1165/rcmb.2010-019OC PMID: 20508067

54. Jiang H, Zhu Y, Xu H, Sun Y, Li Q. Activation of hypoxia-inducible factor-1α via nuclear factor-κB in rats with chronic obstructive pulmonary disease. Acta Biochem Biophys Sin 2010; 42:483–488

55. Yasuo M, Mizuno S, Kraskauskas D, Bogaard HJ, Natarajan R, Cool CD, et al. Hypoxia inducible factor-1α in human emphysema lung tissue. Eur Respir J 2011; 37:775–783 doi: 10.1183/09031936.0002910 PMID: 20562128

56. Lee SH, Lee SH, Kim CH, Yang KS, Lee EJ, Min KH, et al. Increased expression of vascular endothelial growth factor and hypoxia inducible factor-1α in lung tissue of patients with chronic bronchitis. Clin Biochem 2014; 47:552–559 PMID: 24463065

57. Metzen E, Zhou J, Jeekelmann W, Fandrey J, Brüne B. Nitric oxide impairs normoxic degradation of HIF-1α by inhibition of prolyl hydroxylases. Mol Biol Cell 2003; 14:3470–3481 PMID: 12925778

58. Berchner-Pfannenschmidt U, Tug S, Kirsch M, Fandrey J. Oxygen-sensing under the influence of nitric oxide. Cell Signal 2010; 22:349–356 doi: 10.1016/j.cellsig.2009.10.004 PMID: 19861159

59. Dantzker DR, Wagner PD, West JB. Instability of lung units with low V/Q ratios during O2 breathing. J Appl Physiol 1975; 38:886–895

60. Quinn DA, Moufarrej RK, Volokhov A, Hales CA. Interactions of lung stretch, hyperoxia, and MIP-2 production in ventilator-induced lung injury. J Appl Physiol 2002; 93:517–525

61. Sinclair SE, Altemeier WA, Matute-Bello G, Chi EY. Augmented lung injury due to interaction between hyperoxia and mechanical ventilation. Crit Care Med 2004; 32:2496–2501 PMID: 15599157

62. Cannizzaro V, Berry LJ, Zosky GR, Turner DJ, Hantos Z, Sly PD. Impact of supplemental oxygen in mechanically ventilated adult and infant mice. Respir Physiol Neurobiol 2009; 165:61 doi: 10.1016/j.resp.2010.0032810 July 30, 2015 19 / 20

63. Müller HC, Witzenrath M, Tscherning T, Gutbier B, Hippenstiel S, Santel A, et al. Adrenomedullin attenuates ventilator-induced lung injury. Thorax 2010; 65:1077–1084 doi: 10.1136/thx.2010.135996 PMID: 20971983

64. Majdipour C, Jewell UR, Kneller S, Ziegler U, Schwendener R, Booy C, et al. Decreased alveolar oxygen induces lung inflammation. Am J Physiol Lung Cell Mol Physiol 2002; 284:L360–L367. PMID: 12383872

65. Vuichard D, Gantert MT, Schimmer RC, Suter D, Booy C, Reyes L, et al. Hypoxia aggravates lipopolysaccharide-induced lung injury. Clin Exp Immunol 2005; 141:248–260 PMID: 15996189

66. Chao J, Donham P, van Rooljen N, Wood JG, Gonzalez NC. Monocyte chemotactrant protein-1 released from alveolar macrophages mediates the systemic inflammation of acute alveolar hypoxia. Am J Respir Cell Mol Biol 2011; 45:53–61 doi: 10.1165/rcmb.2010-0264OC PMID: 20813992

67. Fišáková B, Vytášek R, Miková D, Vízek M. Hypoxia attenuated nitrotyrosine concentration in the lung tissue of rats with experimental pneumonia. Physiol Res 2004; 53:487–492 PMID: 15479126
68. Ohata Y, Ogata S, Nakanishi K, Kanazawa F, Uenoyma M, Tominaga S, et al. Expression of P2X4R mRNA and protein in rats with hypobaric hypoxia-induced pulmonary hypertension. Circ J 2011; 75:945–954 PMID: 21378451

69. Feinman R, Deitch EA, Watkins AC, Abungu B, Colorado I, Kannan KB, et al. HIF-1 mediates pathogenic inflammatory responses to intestinal ischemia-reperfusion injury. Am J Physiol Gastrointest Liver Physiol 2010; 299:G833–G843 doi: 10.1152/ajpgi.00065.2010 PMID: 20689059

70. Suresh MV, Ramakrishnan SK, Thomas B, Machado-Arnada D, Bi Y, Talarico N, et al. Activation of hypoxia-inducible factor-1α in type 2 alveolar epithelial cell is a major driver of acute inflammation following lung contusion. Crit Care Med 2014; 42:e642–e653 doi: 10.1097/CCM.0000000000000488 PMID: 25014067

71. Jiang H, Huang Y, Xu H, Li QF. Inhibition of hypoxia inducible factor-1α ameliorates lung injury induced by trauma and hemorrhagic shock in rats. Acta Pharmacol Sin 2012; 33:635–643 doi: 10.1038/aps.2012.5 PMID: 22465950

72. Calvert JW, Cahill J, Yamaguchi-Okada M, Zhang JH. Oxygen treatment after experimental hypoxia-ischemia in neonatal rats alters the expression of HIF-1α and its downstream target genes. J Appl Physiol 2006; 101:853–865 PMID: 16728520

73. Uematsu S, Engelberts D, Peltekova V, Otu hakowski G, Post M, Kavanagh BP. Dissociation of inflammatory mediators and function: experimental lung injury in nonpulmonary sepsis. Crit Care Med 2013, 41:151–158 doi: 10.1097/CCM.0b013e318267606f PMID: 23128385

74. Albeceita G, Fernandez A, Parra D, Gonzalo JA, García-Prieto E, Taboada F. Mechanical ventilation causes monocyte deactivation in intact and endotoxin-treated mice. J Trauma 2008; 64:470–476 doi: 10.1097/TA.0b013e31814931ac PMID: 18301217

75. Wilson MR, Patel BV, Takata M. Ventilation with ‘clinically relevant’ high tidal volumes does not promote stretch-induced lung injury in the lungs of healthy mice. Crit Care Med 2012; 40:2850–2857 PMID: 22890257

76. Wolthuis EK, Vlaar APJ, Choi G, Roelofs JJTH, Juffermans NP, Schultz MJ. Mechanical ventilation using non-injurious ventilation settings does not promote lung injury in the absence of pre-existing lung injury in healthy mice. Crit Care 2009; 13:R1 doi: 10.1186/cc8008 PMID: 19152704

77. Vlaar APJ, Wolthuis EK, Hofstra JJ, Roelofs JJTH, Boon L, Schulz MJ, et al. Mechanical ventilation aggravates transfusion-related acute lung injury induced by MHC-1 class antibodies. Intensive Care Med 2010; 36:879–887 doi: 10.1007/s00134-010-1802-z PMID: 20221752

78. Müller-Redetzky HC, Will D, Hellwig K, Kummer W, Tschernig T, Pfell U, et al. Mechanical ventilation drives pneumococcal pneumonia into lung injury and sepsis in mice: protection by adrenomedullin. Crit Care 2014; 18:R73 doi: 10.1186/cc13830 PMID: 24731244

79. Rossi JL, Velentza AV, Steinhorn DM, Watterson DM, Wainwright MS. MLCK210 gene knockout or kinase inhibition preserves lung function following endotoxin-induced lung injury in mice. Am J Physiol Lung Cell Mol Physiol 2007; 292:L1327–L1334 PMID: 17307813

80. Reiss LK, Kowallik A, Uhlig S. Recurrent recruitment manoeuvres improve lung mechanics and minimize lung injury during mechanical ventilation of healthy mice. PLoS ONE 2011; 6:e24527 doi: 10.1371/journal.pone.0024527 PMID: 21935418