443. Novel mechanisms of acute lung injury

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On the effectiveness of steroids in acute lung injury: Experimental separation between inflammation and hypoxemia

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Acute lung injury (ALI) is defined by hypoxemia in the presence of excessive inflammation. Despite of a multitude of clinical trials the role of glucocorticoids in the treatment of ALI is under constant debate. The present study was designed to investigate the effectiveness of dexamethasone in dependence on the type and severity of lung injury and the fraction of inspired oxygen (FiO_2).

C57BL/6 mice were instilled intratracheally with 50 μL HCl at pH 1.5 or 1.8 and were then ventilated with recruitment manoeuvres (RM) and FiO_2=0.3 or 1.0. Another group was ventilated without acid instillation and without RM to induce atelectasis. Dexamethasone [1mg/kg] was injected intravenously at the beginning of ventilation. Lung mechanics were followed by the forced oscillation technique. Cardiovascular parameters, oxygen saturation and body temperature were monitored. Blood gases, cytokines, neutrophil recruitment, microvascular permeability and lung histology were examined.

Dexamethasone attenuated inflammation (neutrophil recruitment, edema formation, cytokine liberation) in all models. Hypoxemia and lung mechanics were improved in the groups instilled with acid pH 1.8 (moderate injury), but not in the groups instilled with acid pH 1.5 (severe injury) or in those with atelectasis. A high FiO_2=1.0 augmented acid-induced lung injury, but did not affect the effectiveness of dexamethasone.

In the present study steroids were highly effective in preventing inflammation under all conditions, whereas they improved the clinical outcome in moderate, but

not in severe lung injury or in cases of derecruitment, suggesting that steroids are effective only in a subclass of patients with ALI.

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LSC 2012 Abstract – Neutrophil trafficking in acute lung injury: A novel human ex vivo model

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Activation and migration of neutrophils into the lung is a central factor in both the onset and progression of acute lung injury (ALI). The assessment of neutrophil biology and trafficking in the lung is fraught with methodological pitfalls.
Human ex vivo ALI models could provide a tractable platform with which to investigate neutrophil trafficking in the lung.
Human lungs from brain dead donors (N=3) were cold preserved and transferred to our institution. Lungs were placed on an ex vivo lung perfusion circuit and following rewarming, E Coli lipopolysaccharide (LPS, 6mg) was injected intrabronchially into the RML. The LLL was used as the control. CT images, tissue biopsies and BALF for cytopsin preparations were taken at T=0 and 4 hours after LPS injury.
CT Images showed ground glass infiltrate in the RML at 4 hours with otherwise normal lung by CT criteria (Figure 1). Compared to control lung (Figure 2A), at 4 hours, LPS injured lung (Figure 2B) demonstrated a neutrophilc alveolitis while BAL cytopsin of LPS injured lung showed a predominance of neutrophils (Figure 2C).

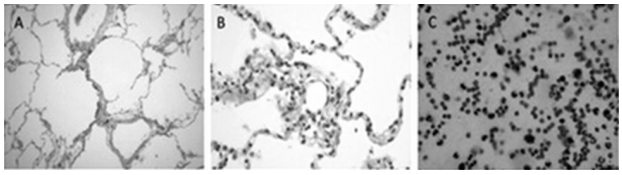

Figure 1. CT image of LPS injured lung at 4 hours.

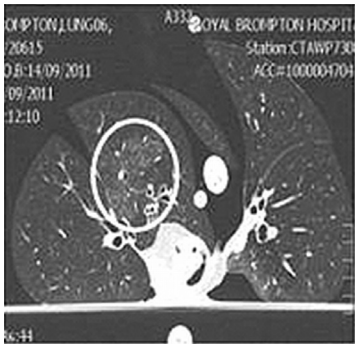

Figure 2

Further characterisation of this novel model allied with established methodology in pulmonary neutrophil trafficking will provide a powerful tool to investigate neutrophil biology in human ALI.

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Sphingosine kinase-1 and sphingosine-1-phosphate promote the development of acute lung injury in pneumoccocal pneumonia

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Pneumonia may evoke acute lung injury. Basal plasma levels of sphingosine-1-phosphate (S1P) contribute to vascular integrity. Pulmonary S1P, mainly synthesized by sphingosine kinase-1 (SphK1), regulates inflammatory mechanisms. We examined SphK1 and S1P in pneumonia.
Serum and post mortem lungs of pneumonia patients were examined. Wildtype-(WT), SphK1$^{-/-}$- and S1P-treated mice were infected with *S. pneumoniae* (*S.pn.*). Murine isolated perfused and ventilated lungs and human endothelial cell (HUVEC) monolayers were exposed to S1P and pneumolysin (PLY). Human lung tissue and human peripheral blood mononuclear cells (PBMCs) were infected with *S.pn.* ex vivo.
S1P was reduced in serum of patients and mice with pneumonia. Pulmonary S1P levels and mRNA expression of SphK1 and S1P receptor 2 (S1PR2) were increased in *S.pn.*–infected WT mice. Lung permeability was decreased in SphK1$^{-/-}$-mice and increased in S1P-treated mice as compared to untreated WT mice in pneumonia. S1P was reduced in ex vivo *S.pn.*-infected human lung tissue. In lungs of pneumonia patients, macrophages carried high amounts of SphK1. *S.pn.*-infected PBMCs showed increased S1P levels and SphK1 expression. S1P (1μM) reduced thrombin-induced but not PLY-induced HUVEC permeability. More S1P (100μM) evoked HUVEC disruption. In isolated lungs, PLY-induced permeability was dose-dependently and synergistically increased by S1P (0.1-1μM). The S1P-induced increase of permeability was abolished by inhibition of S1PR2 or its downstream effector Rho-kinase.
The current data suggest that targeting the SphK1–S1P–S1PR2 axis may provide a therapeutic perspective for prevention of acute lung injury in pneumococcal pneumonia.

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Z antitrypsin polymerization is associated with enhanced pulmonary inflammation

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Severe antitrypsin (AT) deficiency due to polymerization of the Z variant is the commonest genetic cause of emphysema. There is variability in the development and progression of emphysema even in Z-AT homozygotes. We postulated that episodes of pulmonary inflammation could induce polymerization of Z-AT protein thereby further reducing the anti-proteinase protection. 8 Transgenic mice for human Z-AT (Z-mice) and M-AT (M-mice) received 10μg of intrapulmonary LPS. BALF and lung homogenates (LH) were assessed for lung injury and inflammation and AT conformations at day 1, d3 and d7. Z-mice had more lung injury than M-mice; Wet:dry lung ratio was higher in Z- than M-mice, d1, 6.5±0.42 vs. 4.5±0.24, p=0.001; d3, 7.1±0.51 vs. 5.7±0.29, p=0.03. Z-mice had more pulmonary PMNs d1, BALF 71±3x10^{4}cells (mean(SEM)) vs. 52±4, p=0.004; LH intracellular neutrophil elastase (NE), Z- vs. M-mice, median(IQR): d1, 418(490-391)ng/mg vs. 261(285-220), p<0.001. Z-mice had a higher concentration of 8-isoprostane (8-IP) and free NE in BALF compared with M-mice; 8-IP (Z vs. M), d1, 141±29ng/ml vs. 83±19; d3, 211±27 vs. 108±21, p<0.001; BALF, free NE, d1 (Z vs. M), median(IQR) 346(436-316)ng/ml vs. 213(245-113), p=0.001. ELISA and immunoblot revealed that LPS instillation in Z-AT mice led to the development of oxidized-polymers of Z-AT, which further reduces the anti-elastase protection and nullifies the anti-inflammatory effect of AT. This data suggests a molecular mechanism whereby infective exacerbations could further inactivate Z-AT and contribute to the faster decline in lung function in Pi ZZ individuals. This may explain some of the heterogeneity of the lung disease in these individuals.

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Obesity induced by high fat feeding attenuates ventilator-induced lung injury in mice

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Background: Retrospective analysis of Intensive Care data suggests that obesity may confer a survival advantage in Acute Lung Injury (ALI). Development of ventilator-induced lung injury (VILI) is a major determinant of ALI mortality. We have therefore investigated the impact of high fat diet-induced obesity on VILI in mice.
Methods: Male C57BL/6 mice were fed high fat diet for a minimum of 13 weeks, resulting in a mean body weight 30% greater than lean age-matched controls. Animals were anaesthetised and ventilated with high-stretch, standardised as plateau pressure (Pplat) 35-37cmH_2O. Tidal volumes were similar between groups (mean ~1080μl). Mice were ventilated for 180mins or until Pplat increased by 20%. Lung tissue was harvested for wet:dry ratio or processed to a single cell suspension for leukocyte quantification by flow cytometry.
Results: High stretch ventilation induced increases in Pplat and lung wet:dry ratio, and a decrease in p_aO_2 in lean mice, which were all significantly attenuated in obese animals. Leukocyte recruitment (cells/g dry lung mass) also tended to be reduced.

	Pplat increase (cmH_2O)	p_aO_2 decrease (mmHg)	Wet:dry ratio	Neutrophils (×10^7)	Monocytes (×10^7)
Lean	6.0±0.4	111±39	6.5±0.74	12.0±6.4	7.5±4.6
Obese	–1.4±1.7 *	37±29*	4.5±0.27*	5.7±3.0	5.0±1.4

Mean±SD; n=5 per group; *p<0.05 by t-test.

Conclusion: High fat feeding attenuates pulmonary oedema and lung dysfunction

associated with VILI in mice. Numerous metabolic and immunological differences exist between lean and obese subjects. Exploring the mechanisms behind this obesity-mediated protection from VILI may lead to identification of novel pathways and therapeutic targets.
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Ventilator-induced lung injury in severe pneumococcal pneumonia: Protection by adrenomedullin
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Ventilator-induced lung injury (VILI) contributes to mortality in ARDS. Particularly preinjured lungs are susceptible to VILI despite protective ventilation. We previously observed protection against VILI by Adrenomedullin (AM). Here we analyzed impact VILI on lung injury, pulmonary and systemic inflammation, bacterial burden and end-organ injury in established pneumonia. Further AM therapy was investigated.
24h after infection with S. pneumoniae mice were subjected to MV (12ml/kg, 6h) and AM treatment. Lung permeability, oxygenation, lung mechanics, lung and plasma cytokines and leukocytes, bacterial burden in lung, blood spleen, ALT, AST, creatinine and urine output were assessed. Expression of AM and its receptor complex (CRLR; RAMP1-3) were studied
In pneumonia MV aggravated lung injury indicated by increased pulmonary permeability, oxygenation failure and worsening of lung mechanics. MV dramatically increased lung and blood cytokine levels in pneumonia, while lung leukocyte counts in pneumonia were not affected by MV. In pneumonia MV induced leukocytopenia and liver injury. Lung and blood bacterial burden was not affected by MV. MV and pneumonia increased lung AM expression. RAMP1-3 were upregulated in pneumonia but MV reduced its expression. AM protected against MV induced pulmonary hyperpermeability and deterioration of lung mechanics in pneumonia. AM did not alter inflammation but protected against VILI induced liver injury in pneumonia.
MV aggravated lung injury and induced liver injury in pneumonia. MV may pave the way for progression of pneumonia towards sepsis. AM may be a promising adjuvant therapy to limit VILI in pneumonia induced ALI and may protect against end organ damage.

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Proinflammatory distension of the upper airways may contribute to ventilator-induced lung injury
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The pathogenesis of ventilator-induced lung injury (VILI) has been linked to alveolar overdistension, while upper airway stretch has been scarcely considered. We hypothesized that overventilation may contribute to the characteristic features of VILI such as inflammation and hypoventilation by causing upper airway distension. Here, we analyzed the effects of different ventilation strategies on upper airway distension in mice.
Mice were mechanically ventilated at tidal volumes of 6, 10 or 15 ml/kg bw. Spontaneously breathing mice served as controls. Airway distension was imaged by vCT and functional dead space measured by capnography. Release of IL-1α and TNF-α from isolated ventilated murine tracheas was determined by ELISA. Murine and porcine airways were compared by histology and mechanical characteristics were analyzed by the forced oscillation technique.
vCT and capnography both revealed a marked, up to 3-fold increase in upper airway volume during mechanical ventilation. Upper airway distension was dependent on tidal volume and rapidly reversible. In isolated tracheas the release of IL-1β and TNF-α increased significantly with overventilation. Structure and mechanical properties of murine and porcine airways of similar size were essentially similar, indicating that results may be extrapolatable to larger species.
Mechanical ventilation causes rapid, pronounced and reversible distension of upper airways that results in an increased functional dead space and release of inflammatory cytokines in mice. These effects reflect the characteristics of VILI and may thus contribute to the disease not only in mice, but also in larger mammals, including humans.
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Resident alveolar macrophages mediate early alveolar epithelial death signaling and dysfunction
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Acute lung injury (ALI) is characterized by alveolar epithelial dysfunction. We previously showed that early epithelial dysfunction was specifically mediated through tumor necrosis factor (TNF) p55 receptor signaling [1]. This study examined the contribution of resident alveolar macrophages (AM) to this phenomenon following acid aspiration.
C57Bl6 mice were treated intratracheally with liposomes containing either clodronate or PBS. After 48 hours, they underwent intratracheal instillation of hydrochloric acid followed by mechanical ventilation to assess respiratory parameters. Oxygenation, respiratory elastance, alveolar TNF concentration, lung caspase-8 activity and alveolar fluid clearance (AFC) were measured at 90 minutes after acid instillation.
Clodronate liposomes produced an 80% depletion of AMs. AM depletion significantly improved the deterioration in respiratory elastance ($cmH_2O/\mu l$: PBS=0.06±0.008; CLOD=0.05±0.004; $p<0.05$) and PaO_2:FiO_2 (PBS=304±113; CLOD=426±41; $P<0.05$) induced by acid instillation. Additionally, alveolar TNF was significantly reduced (pg/ml: PBS=46.5±25.8; CLOD=15.5±2.7; $P<0.05$), along with attenuated lung caspase-8 activity (arbitrary units: PBS=14763±5466; CLOD=7135±372; $P<0.01$), and improved AFC (%/30min: PBS=3.8±2.6; CLOD=7.1±2.4; $P<0.05$). Caspase-8 activity showed an inverse correlation to AFC (Pearson r=-0.766; $P<0.0001$) implying epithelial death receptor activation.
These data suggests that during ALI induced by acid aspiration, epithelial dysfunction and hypoxemia are a result of epithelial cell death receptor activation by alveolar macrophage-derived TNF.
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Reference:
[1] Patel *et al*. Intensive Care Med. 2011;37(Supplement):S205