The pulmonary endothelial glycocalyx regulates neutrophil adhesion and lung injury during experimental sepsis

Eric P Schmidt1,2, Yimu Yang1, William J Janssen3, Aneta Gandjeva1, Mario J Perez1, Lea Barthel3, Rachel L Zemans3, Joel C Bowman1, Dan E Koyanagi1, Zulma X Yunt3, Lynelle P Smith1, Sara S Cheng4, Katherine H Overdier2, Kathy R Thompson2, Mark W Geraci1, Ivor S Douglas1,2, David B Pearse5 & Rubin M Tuder1

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Background

Acute lung injury (ALI) describes a clinical syndrome of acute respiratory failure with substantial morbidity and mortality.

Between 25-40% of individuals with sepsis and 7% of intensive care patients develop ALI, increasing intensive care unit mortality from 11% to 38% in patients.

Definition of the American-European Consensus Conference Committee: acute onset of diffuse bilateral pulmonary infiltrates by chest radiograph, a PaO2/FiO2 ≤300 for ALI and pulmonary artery wedge pressure (PAWP) ≤18.
Background

Lung infection
Aspiration
Sepsis
Multiple trauma, shock
Other insults

ACUTE LUNG INJURY

Lungs

Resolution of edema; repair of alveolar-capillary membrane
Persistence and progression of injury
  - Multiple organ failure
  - Pulmonary fibrosis
  - Pulmonary vascular destruction

- Alveolar-capillary membrane injury
- Inflammation
- Increased permeability pulmonary edema

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Background

Therapeutic interventions to treat ALI remain limited

Lung-protective ventilation, including low tidal volume and low inspiratory pressure ventilation, has been associated with increased survival rates.

Prone positioning, high-frequency oscillatory ventilation, inhaled nitric oxide and glucocorticoids are also used, but have so far failed to alter mortality rates.

Thus far, no real pathophysiologic-driven therapeutic intervention has become available.

La Presse Médicale; Volume 40, Issue 12, Part 2, December 2011, Pages e585–e594; “Prone positioning in acute respiratory distress syndrome (ARDS): When and how?”
Levels of syndecan-1 and heparan sulfate, both markers for the integrity of the endothelial glycocalix, were markedly higher in the sepsis group and the surgery group compared with the control group.
Background

Mechanistic overview of reactive species-induced degradation of the endothelial glycocalyx during hepatic ischemia/reperfusion injury
Rowan F. van Golena, Thomas M. van Gulika, Michal Hegera, b

Aims

- The mechanisms by which glycocalyx loss occurs during sepsis
- How this loss allows for neutrophil adhesion within the pulmonary circulation
Materials & Methods

Closed-chest pulmonary intravital (in vivo) microscopy
Materials & Methods

Closed-chest pulmonary intravital (in vivo) microscopy

Subpleural microvessels (MV)
Alveolus (A)
Materials & Methods

Animal testing:  
- BL/6 wild-type  
- TNFR1 knockout  
- ICAM-1 knockout

Human lung samples with diffuse alveolar damage (=ALI) and noninjured controls

Immunofluorescence
Flow cytometry
Protein and mRNA expression
In vivo polystyrene microspheres with anti–ICAM-1
Results

LPS degrades the pulmonary ESL via TNF-α

n=5, iv injection
saline
LPS (20 μg per g body weight)
TNF-α (200 ng)
Results

Heparanase mediates LPS-induced ESL degradation

wild-type mice treated with heparinase-III or heat-inactivated heparinase-III (1 U)
n = 4–6 mice per group
Results

Heparanase contributes to septic acute lung injury

heparanase activation (with consequent glycocalyx degradation) is necessary to the development of ALI
Results

Inhibition of heparanase activity with the competitive antagonist heparin completely prevented endotoxemia-induced ESL loss.

Heparin (5 U administered iv)  
*n* = 4–6 mice per group

nonanticoagulant heparanase inhibitor *N*-desulfated/re-*N*-acetylated heparin  
*n* = 4–5 mice per group

Hpse−/− mice  
*n* = 3

However heparin does not interfere with LPS danger signaling.
Results

LPS-induced neutrophil adherence is dependent upon ESL degradation

Adherence of adoptively transferred GFP+ neutrophils within subpleural microvessels

$n = 3$ mice per group
Results

intercellular adhesion molecule 1 (ICAM-1), an endothelial adhesion molecule implicated in endotoxin-induced pulmonary neutrophil adhesion

Neutrophil adhesion in wild-type mice measured via Ly6B immunofluorescence
Results

Visualization of anti-ICAM-1–coated fluorescent microspheres within wild-type mouse subpleural microvessels

LPS (20 μg per g body weight)
LPS (20 μg per g body weight)
heparin (5 U)
heparinase-III (1 U)

These findings provide a teleological rationale for LPS-induced heparanase activation: pathogen-associated molecular patterns prompt endothelial cells to cleave the endothelial glycocalyx, preparing the vascular surface for neutrophil adhesion and subsequent inflammation.
Results

Normal human PMNs were found to express ICAM-1 with 90% positive population, and this expression is augmented by LPS.

The possibility that anti–ICAM-1 microspheres were being captured by neutrophils was excluded, as neutrophil depletion did not prevent microsphere adhesion during

Results

Heparanase is apparent in human sepsis and lung injury

- Heparan sulfate degradation activity measured in plasma, n = 4-7 patients per group
- Heparanase immunofluorescence in normal human lung tissue and in lung biopsies with diffuse alveolar damage

Confocal fluorescent images high heparanase expression (red) endothelial marker CD31 (green)

-> Can heparanase inhibition be lung-protective even if administered after sepsis onset?
Results

Administration of heparin 3 h after intraperitoneal LPS (40 μg per g body weight in 500 μl saline)

Heparin treatment in mice subjected to cecal ligation and puncture (CLP)

Pulmonary heparanase expression (red) after CLP in wild-type mice

Assessment of pulmonary endothelial permeability (Kf)

Pulmonary heparanase expression peaked 48 h after CLP, coincident with an increase in endothelial permeability
Results

To augment CLP-induced neutrophilic alveolitis, CLP was performed the presence of 60% fraction of inspired oxygen (FiO2)

Pulmonary neutrophilic infiltration was apparent 48 h after CLP and was attenuated by delayed heparin therapy. Hpse−/− mice were similarly protected from CLP- and hyperoxia-induced alveolitis and experienced no CLP- and hyperoxia-associated mortality

Heparanase inhibition is protective after sepsis onset
Activated heparanase cleaves heparan sulfate from the pulmonary endothelial glycocalyx

inducing a rapid thinning of the ESL

exposes previously hidden endothelial surface adhesion molecules such as ICAM-1

allowing neutrophil recognition of and adhesion to the endothelial surface
The use of aerosolized unfractionated heparin and N-acetylcystine attenuates lung injury and the progression of acute respiratory distress syndrome in ventilated adult patients with acute lung injury following smoke inhalation.
Thank you for your attention