Potential conflicts of interest

– None to declare

Acknowledgements

– Daniel Melly, Sharon Mumby, Anna Lagan, Lauren Hector, Ruth Bundy, Greg Quinlan
– British Heart Foundation; Garfield Weston Trust
Heme oxygenase (HO): What is it?

• An enzyme that catalyzes the degradation of heme to produce biliverdin, iron and carbon monoxide

Heme oxygenase (HO) 1: What is it?

• HO-1 is an inducible isoform produced in response to oxidative stress, cytokines and heavy metals (eg redox active iron)
• HO-2 is a constitutive isoform expressed under homeostatic conditions
• [HO-3 may work in oxygen sensing]
Sepsis

Anti-oxidant pathway

- Anti-inflammatory transcription factors
- Endogenous anti-oxidants & anti-inflammatory products

Pro-oxidant pathway

- Pro-inflammatory transcription factors
- Chemokines, adhesion molecules

ROS/RNS subtoxic production

Iron

oxidative stress & iron metabolism (including HO-1 activity)

altered redox signalling

Tissue/Organ injury: Evolution or Resolution

oxidative damage
Pathogenesis of the systemic inflammatory syndrome and acute lung injury: role of iron mobilization and decompartmentalization

Anna L. Lagan, Daniel D. Melley, Timothy W. Evans, and Gregory J. Quinlan
Department of Critical Care Medicine, Imperial College School of Medicine, Royal Brompton Hospital, London, United Kingdom
Hemoxygenase, iron and ARDS

Genetic predisposition
- Polymorphism in iron regulatory & HO genes in patients with ARDS

Pathophysiology of SIRS/ALI
- Hemoxygenase and CO production in SIRS

Quantification & significance
- Hemoxygenase in patients with ARDS
- Hemoxygenase and neutrophil apoptosis

Therapeutic Intervention
- Effects of redox modification by albumin in patients with the sepsis syndromes
The prevalence of ferritin light chain gene (FTL) -3381 GG homozygotes was increased in patients with ARDS of extrapulmonary onset (ARDSexp) compared to healthy controls.

- OR: 2.44; 95% C.I, 1.28-4.54; p=0.009

Chest 2008; 133: 1302-1311
Heme oxygenase 2

- A common haplotype of hemeoxygenase 2 (HMOX2) was reduced in patients with ARDS compared to healthy controls, a trend more evident in those with ARDS of pulmonary aetiology

*OR: 0.29; 95% C.I., 0.14-0.60; \( p=0.001 \); **OR: 0.22; 95% C.I., 0.09-0.56; \( p=0.002 \)
Longer (GT)n repeats in the HMOX1 promoter associated with higher HO-1 and reduced ARDS risk.
Hemoxygenase, iron and ARDS

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Increased heme catabolism in critically ill patients: correlation among exhaled carbon monoxide, arterial carboxyhemoglobin, and serum bilirubin IXα concentrations
Effects of HO-1 manifest in CO levels: a possible marker of inflammation

- Is there an optimal range for endogenous CO production?
- Is tolerance for CO lower in patients in critical care?
Carboxyhemoglobin in the critically ill
(n=1935 arterial samples) Mean 1.07
COHb and mortality in ICU

- 1267 patients
- 51,433 measurements of COHb
Summary

• Following surgery necessitating CPB:
  – There was a positive association between COHb and inflammatory markers
  – High maximum, low minimum COHb levels were significantly associated with ICU mortality
Hemoglobinase, iron and ARDS

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Lung hemoxygenase 1 in ARDS
*Critical Care Medicine* 2004; 32: 1130-1135

- Patients with ARDS (AECC criteria)
- Biochemical and immuno histochemistry using BALF and lung tissue from patients and appropriate controls
• HO protein elevated in BAL and lung tissue from patients with ARDS compared to controls
• Correlated negatively with concentrations of redox active iron
• May contribute to changes in iron mobilization, signalling and regulation seen in ARDS
Hemoxygenase, iron and ARDS

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Therapeutic intervention
• Effects of redox modification by albumin in patients with the sepsis syndromes
CPB and the inflammatory response

*Preliminary findings*

- CPB leads to SIRS and:
  - Haemolysis, heme release and depletion of circulating Fe binding proteins
  - Induction of anti apoptotic HO-1 in circulating neutrophils
- Inhibition of HO-1 would:
  - Block haemolysis-induced delay in apoptosis and reduce inflammation
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pre CPB n=19</th>
<th>Post CPB n=19</th>
<th>CPB+16 hrs n=19</th>
<th>CPB+40hrs n=19</th>
<th>* p</th>
</tr>
</thead>
<tbody>
<tr>
<td>WCC, median (IQR)</td>
<td>7.7 (6.0-9.3)</td>
<td>10.3 (8.0-12.3)</td>
<td>9.9 (8.9-11.5)</td>
<td>11.5 (10.1-12.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NC, median (IQR)</td>
<td>4.8 (3.4-6.0)</td>
<td>7.5 (6.3-10.3)</td>
<td>8.2 (7.3-9.6)</td>
<td>9.3 (7.7-10.5)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CRP, median (IQR)</td>
<td>1 (1-4)</td>
<td>2 (1-4)</td>
<td>62 (51-82)</td>
<td>168 (135-218)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PaO2:FiO2</td>
<td>364 (343-410)</td>
<td>354 (257-446)</td>
<td>340 (304-443)</td>
<td>338 (275-421)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Patients with SIRS, n (%)</td>
<td>0</td>
<td>7 (35)</td>
<td>3 (15)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Definition of abbreviations: WCC = White Cell Count; NC = Neutrophil Count; CRP = C-Reactive Protein; SIRS = Systemic Inflammatory Response Syndrome. Percentages expressed relative to the total number of patients. * Analyzed for statistical significance by repeated measures ANOVA with Dunnett's post test comparing all columns with Pre CPB.
IL-8, GM-CSF and MPO significantly elevated postoperatively
Neutrophil apoptosis is delayed by bypass (paired samples)
CPB and the inflammatory response

*First conclusion*

• CPB leads to SIRS and:
  – Elevated inflammatory markers and neutrophil activation
  – [Hemolysis, heme release and depletion of circulating Fe binding proteins]
• HO-1 message & protein are induced in neutrophils post CPB [upper panel]
• HO-1 message and protein are also induced after 4 hr incubation with HO substrate analogue hemin in neutrophils from healthy volunteers [lower]
Hemin [HO-1] inhibits apoptosis: Albumin reverses this effect.
CPB and the inflammatory response

*Second conclusion*

- CPB leads to SIRS and:
  - Elevated inflammatory markers and neutrophil activation
  - Hemolysis, heme release and depletion of circulating Fe binding proteins
  - Induction of anti apoptotic HO-1 in circulating neutrophils
- Albumin abrogated this effect
- A major trial of albumin post CPB is underway
The SAFE Study Investigators

Impact of albumin compared to saline
on organ function and mortality of patients
with severe sepsis

• 1218 patients with severe sepsis at baseline; 603 and 615 randomised to receive albumin and saline respectively

• Two groups similar baseline characteristics

• No difference in renal failure (18.7% albumin group got RRT; 18.2% saline RRT) or SOFA scores

• MVA adjusting for baseline factors in those with complete BL data adjusted odds ratio for death for albumin vs saline 0.71 (95% CI 0.52-0.97; p=0.03)
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Albumin group</th>
<th>Saline group</th>
<th>Odds ratio (95% CI)</th>
<th>Absolute difference (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe sepsis at baseline Status at 28 days: no. (%)</td>
<td>(n = 603)</td>
<td>(n = 615)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead</td>
<td>185 (30.7)</td>
<td>217 (35.3)</td>
<td>0.87 (0.74–1.02)</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Alive in ICU</td>
<td>36 (8.6)</td>
<td>26 (6.5)</td>
<td>1.35 (0.80–2.28)</td>
<td></td>
<td>0.26</td>
</tr>
<tr>
<td>Alive in hospital</td>
<td>135 (35.3)</td>
<td>142 (38.2)</td>
<td>0.88 (0.66–1.19)</td>
<td></td>
<td>0.42</td>
</tr>
<tr>
<td>Duration of stay in ICU (days)</td>
<td>8.2 ± 7.5</td>
<td>7.5 ± 6.7</td>
<td></td>
<td>−0.69 (−1.49 to 0.11)</td>
<td>0.09</td>
</tr>
<tr>
<td>Duration of hospital stay (days)</td>
<td>16.1 ± 9.7</td>
<td>15.6 ± 9.9</td>
<td></td>
<td>−0.47 (−1.58 to 0.63)</td>
<td>0.40</td>
</tr>
<tr>
<td>Duration of mechanical ventilation (days(^a))</td>
<td>6.0 ± 7.2</td>
<td>5.4 ± 6.2</td>
<td></td>
<td>−0.56 (−1.31 to 0.20)</td>
<td>0.15</td>
</tr>
<tr>
<td>Duration of renal replacement therapy (days(^a))</td>
<td>1.2 ± 3.6</td>
<td>1.0 ± 3.1</td>
<td></td>
<td>−0.28 (−0.66 to 0.09)</td>
<td>0.14</td>
</tr>
</tbody>
</table>
Hemoxygenase, iron and ARDS

Genetic predisposition
- SNPs in HMOX 1 gene associated with higher HO-1 and reduced risk of ARDS

Pathophysiology of SIRS/ALI
- ‘Optimal range’ of COHb in patients with SIRS may reflect HO-1 activity

Quantification & significance
- Hemoxygenase elevated in BAL & tissue in ARDS
- CPB delays apoptosis and induces HO-1 in neutrophils

Therapeutic Intervention
- Albumin reverses effect; possibly through ROS scavenging or stabilizing neutrophils

Hemoxygenase: a potential therapeutic target
Is CO pro or anti inflammatory?

Pro

• No effect on cytokine production in experimental endotoxemia in humans

• No benefit in hyperoxia induced lung and tissue injury

• Variable views expressed in literature
  – *Am J Respir Crit Care Med* 2005; 171: 1318
Is CO pro or anti inflammatory?

Anti

- CO suppresses atheroma formation and graft rejection
  - *Nat Med* 2003; 9: 183-190
- Exogenous CO protective in VIALI
  - *Am J Respir Crit Care Med* 2004; 170: 613-620
- Protects against liver failure via NO-induced HO
### Relationship between COHb, bilirubin and markers of inflammation

<table>
<thead>
<tr>
<th>Test</th>
<th>Relationship</th>
<th>p-value</th>
<th>ρ-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WCC/COHb</td>
<td>No relationship</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin/COHb</td>
<td>p&lt;0.0001 ρ=0.155</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WCC/CRP</td>
<td>p&lt;0.0001 ρ=0.298</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP/COHb</td>
<td>p&lt;0.0001 ρ=0.165</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Critical Care Medicine 2007; 35: 1882-7
Cell viability: Hemin inhibits apoptosis [no effect using SnPP]
Effect of Albumin and SnPP on Neutrophil Viability following CPB

Albumin nor SnPP affect neutrophil viability after CPB