WORKING WITH THE SYSTEMIC INFLAMMATORY RESPONSE: REMEMBERING THE COMPLEXITIES

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Disclosures

Barbados Diabetes Foundation
Bayer Healthcare
British Heart Foundation
Cave Shepherd Ltd.
Cellplex
Destiny Group of Companies
Edmund Cohen Foundation
Medicor International
Point Care Technologies
What is the systemic “inflammatory” response?
CYTOKINES:

proinflammatory

The cytokine response to CPB has two phases:

- a proinflammatory phase dictated by contact with the foreign surface
- an antiinflammatory phase dictated by the body (i.e. a homeostatic response)
Contact of blood with the foreign surface of the bypass circuit activates:

- Cytokines and white cells
- Coagulation System
- Fibrinolytic System
- Platelets
- Complement System
- Hemolysis

Need for a more holistic view of the "systemic inflammatory response":

“… activation of complement, coagulation, fibrinolytic, and kallikrein cascades, activation of neutrophils with degranulation and protease enzyme release, oxygen radical production, and the synthesis of various cytokines from mononuclear cells”

**Intervention:** pharmacologic/circuit modification approaches

**Intervention:** clinical management changes
**TITLE:** An evidence-based review of pharmaceutical interventions to limit the systemic inflammatory response in cardiac surgery  
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**AFFILIATION:** Edmund Cohen Laboratory for Vascular Research, University of the West Indies, Barbados; *The Dartmouth Institute for Health Policy and Clinical Practice, Lebanon, NH, USA; †London Health Sciences Center, London, Ontario, Canada; §Flinders Medical Center, Adelaide, Australia.  
**INTRODUCTION:** We report here the first evidence-based review of pharmaceutical strategies to limit the systemic inflammatory response in adult coronary artery bypass grafting surgery.

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Heart Surgery Forum (2008), in press
What are some plausible targets for attenuating the systemic inflammatory response?
intrinsic coagulation

XII

Kallikrein → Bradykinin

Thrombin

Platelet activation
Leukocyte activation
Endothelial activation
Controlled thrombin generation

Fibrinogen

Fibrin

Hemostasis
Controlled thrombin generation

PAR1

Fibrinogen

Thrombin burst

Fibrin

new drug target?

pl rich occlusive thrombus
Proteolytic activation of PAR1

Cleavage by thrombin (or other serine proteases)

= tethered ligand (SFLLRN)

? Platelets - aggregation
? Endothelial cells - inflammation
? Neural cells - apoptosis

activating signals
TRA-PCI: Phase II what happened?

American Coll Cardiol Apr 2007

Schering Plow announces 30,000 patient Phase III trial April 2007

??
fibrinolysis  intrinsic  coagulation

tPA

Plasmin

Kallikrein → Bradykinin

XII

Thrombin

Platelet activation  Leukocyte activation  Endothelial activation
Bradykinin

XII

fibrinolysis
intrinsic
coagulation

Plasmin

Platelet activation

tPA

XII

Thrombin

PAR1

Neurodegeneration

Apoptosis

Neuron

PAR1

Glial Cell

(+) tPA

(+) tPA

(+)
Question: can plasmin activate neural cells via PAR1?

- A172 glial cells grown *in vitro*

- Plasmin
- Aprotinin
- TXA
- EACA

X
activation of PAR1 cell changes (5 min) (2 hours)

Pls Apr P + A med

Plasmin (5 µM)
Aprotinin (200 KIU/ml)

control
plasmin

Greenidge et al - unpublished
Complement is activated

**Classical pathway**

XIIa

C1 → C2 → C4 → C4bC2a

C3 → C4b.C2a.C3b → C3a

**Terminal pathway**

C3b

C3

C5b

C5b-9

Cell Lysis

**Alternate pathway**

C3b

C3

Heparin:protamine + IgG
fibrinolysis  intrinsic coagulation  classical complement

tPA  tPA

| Platelet activation | Leukocyte activation | Endothelial activation |

| Plasmin  | Kallikrein  | Bradykinin  | C3  |

| Thrombin  | IgM/IgG  | C5  |
fibrinolysis

intrinsic coagulation

classical complement

white cell adhesion

tPA

Plasmin

Kallikrein

Bradykinin

IgM/IgG

C3

C5

Platelet activation

Leukocyte activation

Endothelial activation

Thrombin
Adhesion to plastic has been described as “frustrated phagocytosis” and engages inflammatory and ROS signaling pathways.

Snerbulent splench-sucker that captures prey by sticking to their faces and suffocating them - its suckers are impossible to prize off. Unfortunately, it can’t catch anything as it can’t move from the rock it was born on.

— “Flanimals” by Ricky Gervase
fibrinolysis

intrinsic coagulation

classical complement

white cell adhesion

hemolysis/
RBC transf

tPA → Plasmin

XII → Kallikrein → Bradykinin

IgM/IgG → C3 → C5

Platelet activation

Leukocyte activation

Endothelial activation

Oxidative stress

RBC transf

Platelet activation

Leukocyte activation

Endothelial activation

Oxidative stress
Red Blood Cell Changes

Figure 21-1. Scanner electron micrograph showing red blood cells taken from the patient preoperatively.

Figure 21-5. Homologous red blood cells stored for 15 days.

Slide courtesy of: Bruce Spiess
Hemolysis

Weight: 80 kg
TBV: 5.3 L
Damaged blood: 7.3 mL

Free plasma Hb [mg/dL]

Slide courtesy of: Filip de Somer
Extracorpuscular free hemoglobin:

**THE FENTON REACTION:**

\[ \text{Hb-Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Hb-Fe}^{3+} + \text{OH}^- + \cdot\text{OH} \]

**NITRIC OXIDE CONSUMPTION:**

\[ \text{Hb-Fe}^{2+} + \text{NO} \rightarrow \text{Hb-Fe}^{3+} + \text{NO}_3 \]

- Renotoxic
- Atherogenic
- Vasoconstrictive
- \( \downarrow \) NO leads to \( \uparrow \) activation of leukocytes and platelets

Figure 2. Number of transfused PRBC used vs incidence of ARF.

Odds Ratio for renal failure = 1.23 per Unit PRBC
Hemoglobin scavenging macrophages

- ROS
- cytokines
- proteases
local trigger

Ischemia/Reperfusion

Midline cerebral artery

Ritter LS et al. Stroke 2000;31:1153
Measuring leukocyte extravasation by cantharidin-induced skin blisters
Extravasation of leukocytes into skin blisters (x10^5 cells)

<table>
<thead>
<tr>
<th></th>
<th>Pre-op</th>
<th>Peri-op</th>
<th>t-test P-value</th>
<th>Wilcoxon P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>4.84 (2.14 – 10.92)</td>
<td>24.48 (10.30 – 58.16)</td>
<td>0.013</td>
<td>0.043</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.79 (1.21 – 6.44)</td>
<td>14.37 (5.64 – 36.65)</td>
<td>0.014</td>
<td>0.028</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.16 (0.51 – 2.67)</td>
<td>6.37 (2.80 – 14.49)</td>
<td>0.014</td>
<td>0.028</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.40 (0.18 – 0.86)</td>
<td>1.73 (0.77 – 3.92)</td>
<td>0.009</td>
<td>0.018</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.40 (0.16 – 1.01)</td>
<td>1.02 (0.41 – 2.53)</td>
<td>0.093</td>
<td>0.063</td>
</tr>
</tbody>
</table>

Summary: leukocytes in organ injury

- Inflammatory leukocytes extravasate into tissues and organs post CPB
- Ischemia/reperfusion injury is a potent trigger for leukocyte extravasation
- Extent of injury depends on leukocyte- and endothelial- activation
ABNORMAL BLOOD FLOW

HYPERCOAGULABILITY

ENDOTHELIAL INJURY


local trigger

vessel trauma/injury
Intact internal elastic lamina

Graft intima

Elastin stain 200X

Prior to pressure distention

After pressure distention

Internal elastic lamina disrupted

Slide courtesy of: Rob Poston
Intraoperative Conduit Imaging

Slide courtesy of: Rob Poston
OCT detection of RA spasm during harvest

<table>
<thead>
<tr>
<th></th>
<th>Proximal</th>
<th>Middle</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>LA = 4.25</td>
<td>LA = 4.17</td>
<td>LA = 3.04</td>
</tr>
<tr>
<td>harvest</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Spasm</td>
<td>LA = 1.74</td>
<td>LA = 2.04</td>
<td>LA = 1.01</td>
</tr>
<tr>
<td>after</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>harvest</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Percent Spasm, %

Electrocautery

$p = 0.032$

Harmonic Scalpel

Slide courtesy of: Rob Poston
aortic x-clamp:
- *multiple*
- *excessive force*

Preventing local triggers:

- Whole team approach
  - *gentle* handling & clamping of vessels
  - minimize ischemia duration
  - optimize perfusion parameters
  - cell saver/leukofiltration
  - temperature/emboli monitoring
systemic factors + local trigger = organ injury

**Intervention:** pharmacologic/circuit modification approaches

**Intervention:** clinical management changes
Consensus Statement: Defining Minimal Criteria for Reporting the Systemic Inflammatory Response to Cardiopulmonary Bypass

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ABSTRACT

The causal factors of the systemic inflammatory response to cardiopulmonary bypass (CPB) have not been well described. The systemic inflammatory response is broadly defined as an acute, nonspecific, immune response characterized by the release of proinflammatory cytokines, leukocyte activation, and vascular endothelial activation. The current consensus statement is designed to provide a framework for the reporting of the systemic inflammatory response to CPB.

Definition of the Systemic inflammatory Response

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Consensus Statement writing group
Systemic factors

- Fibrinolysis
- Intrinsic coagulation
- Classical complement
- White cell adhesion
- Hemolysis/RBC transf.

Local trigger

- Vessel trauma
- Endothelial denudation
- Ischemic injury
- Endothelial activation

Systemic/local

- Bleeding
- Humoral, white cell & vascular activation
- Oxidative stress

Systemic factors

- Systemic factors
- Local

Heart
Brain
Kidney
Gut
Lung
Fluid shifts in the brain

“Fixing the heart: must the brain pay the price?”

Mark Newman, Circulation 2004
Whole blood PAR1 function assay

- Blood samples taken before, during and after CPB
- Platelet binding to leukocytes used as a surrogate for platelet activation

1. Gate on leukocyte subpopulation
2. Determine % of platelets in the leukocyte gate
3. Assess ability of platelets to be activated via PAR1 by adding PAR1 agonist TRAP-6
Aprotinin has a weak $K_i$ for thrombin

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>$K_i$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>trypsin</td>
<td>$6 \times 10^{-11}$</td>
</tr>
<tr>
<td>plasmin</td>
<td>$9 \times 10^{-11}$</td>
</tr>
<tr>
<td>plasma kallikrein</td>
<td>$3 \times 10^{-8}$</td>
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<tr>
<td>tissue kallikrein</td>
<td>$3 \times 10^{-10}$</td>
</tr>
<tr>
<td>Factor XIIa</td>
<td>$&gt; 1 \times 10^{-6}$</td>
</tr>
<tr>
<td>Factor XIa</td>
<td>$1 \times 10^{-7}$</td>
</tr>
<tr>
<td>elastase</td>
<td>$&gt; 1 \times 10^{-6}$</td>
</tr>
<tr>
<td>thrombin</td>
<td>$&gt; 1 \times 10^{-6}$</td>
</tr>
</tbody>
</table>
Conclusions on mechanism of action

Aprotinin inhibits binding of thrombin to the hirudin-like binding site on PAR1.

Leads to a reduced rate of PAR1 hydrolysis by thrombin.

Fits the inhibitory profile of aprotinin.