A Unified Theory of Sepsis-Induced Acute Kidney Injury: Inflammation, microcirculatory dysfunction, bioenergetics and the tubular cell adaptation to injury

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Conflicts of Interest


2. Impact of Blood Storage Duration on Physiologic Measures: RECESS Ancillary Study (5RO1 HL101382-03) – Site PI
The classic conceptual model

AKI

Hypovolemia  Heart failure  Major surgery  Sepsis

Shock

Hypoperfusion  Ischemia/hypoxia

“Classic conception”
The classic conceptual model

Exposure to warm ischemia does not necessarily cause AKI

<table>
<thead>
<tr>
<th></th>
<th>ALL</th>
<th>PRCS*</th>
<th>No PRCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIFLE I or F</td>
<td>31.4%</td>
<td>51.7%</td>
<td>6.4%</td>
</tr>
</tbody>
</table>

*PRCS = Post-resuscitation cardiogenic shock

Chua, et al. Resuscitation 2012

Sepsis-induced AKI can occur in the absence of shock

<table>
<thead>
<tr>
<th>CAP</th>
<th>AKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non severe CAP</td>
<td>20.3%</td>
</tr>
<tr>
<td>Non severe sepsis</td>
<td>23.8%</td>
</tr>
<tr>
<td>Not requiring ICU</td>
<td>25%</td>
</tr>
</tbody>
</table>


Exposure to septic plasma causes AKI-like changes in tubular epithelial cells in vitro

Research

_Circulating plasma factors induce tubular and glomerular alterations in septic burns patients_

Filippo Mariano¹, Vincenzo Cantaluppi², Maurizio Stella³, Giuseppe Mauriello Romanazzi², Barbara Assenzi⁴, Monica Cairo³, Luigi Biancone², Giorgio Triolo¹, V Marco Ranieri⁴ and Giovanni Camussi²

Critical Care 2008, 12(R4) (doi:10.1186/cc6848)
Sepsis-induced AKI... is there anything else out there?
Sepsis-induced AKI

Consistent histology findings

1. Microvascular dysfunction

2. Apical tubular epithelial cell vacuolization and loss of brush border

3. Inflammation and oxidative stress

4. Paucity of apoptosis/necrosis

Sepsis-induced AKI (S-AKI) is NOT ATN
Sepsis-induced AKI

Conceptual framework

1. Amplification

- Inflammation (DAMPs, PAMPs)
- Microvascular dysfunction
- Hypoxia
- Immune system

2. TEC response to the amplified alarm signal

AKI phenotype

- Decreased GFR
- Tubular injury
- Paucity of necrosis and/or apoptosis

Wu et al. JASN 2007
Sepsis-induced AKI

Conceptual framework: 1. Amplification of the alarm signal

Reference
3. Singbartl 2011

Hypothesis

Sepsis-induced AKI

Conceptual framework: 2. TEC response to the alarm signal

- Decreased GFR
- Paucity of necrosis and/or apoptosis
- Tubular injury

AKI phenotype

Reference

Hypothesis
- Altered energy balance: ↑AMP:ATP
- Uncoupled respiration
- ROS/RNS ↑
- ψ ↓

Regulation of energy
- Apoptosis
- Protein synthesis

Cell cycle arrest
- S
- G1
- M
- G0

Inflammatory mediators from blood

Mitochondria

S1 Tubular epithelial cell

Tubular epithelial cell S2 segment and beyond

Signal from S1 cells filtered mediators

Sepsis-induced AKI

Preliminary work

Hypothesis

Exogenous stimulation of autophagy improves renal recovery during sepsis.

Stimulation of autophagy:

1. AICAR (5-Aminoimidazole-4-carboxamide 1-b-D-ribofuranoside, Acadesine, N1-(b-D-Ribofuranosyl)-5-aminoimidazole-4-carboxamide)

2. Temsirolimus

Inhibition of autophagy:

3. Compound C
4. VPS34 SiRNA

Sepsis-induced AKI

Model 1A: Cecal ligation and puncture (mice)

Primary outcome: Renal Function (Creatinine, BUN, Cystatin C)

24h

AICAR 100mg/kg
CoC

CLP

8h

Sacrifice and sample collection

Primary outcome
• Creatinine, BUN, Cystatin C

Secondary outcome
• Cytokine expression
• Endothelial adhesion molecule expression
• Leukocyte expression
• Induction of mitophagy (Atg7)


Zuckerbraun Lab
**Sepsis-induced AKI**

Model 1A: Cecal ligation and puncture (mice)

Primary outcome: Renal Function

Activation of AMPK by AICAR protects against cecal ligation and puncture-induced kidney injury

Sepsis-induced AKI
Model 1A: Cecal ligation and puncture (mice)
Secondary outcomes: Cytokine expression

Activation of AMPK by AICAR reverses cecal ligation and puncture-induced increases in serum cytokine levels

Escobar, Gomez, Zuckerbraun. Unpublished data
**Sepsis-induced AKI**

Model 1: Cecal ligation and puncture (mice)

Secondary outcomes: Endothelial activation - ICAM/VCAM

Activation of AMPK by AICAR decreases ICAM expression induced by CLP

![Image of ICAM expression under different conditions](image_url)

- **ICAM 40x**
  - **CONTROL**
  - **AICAR**
  - **Compound C**

Escobar, Gomez, Zuckerbraun. Unpublished data
Sepsis-induced AKI

Model 1: Cecal ligation and puncture (mice)

Secondary outcomes: Leukocyte infiltration – CD45

Activation of AMPK by AICAR reduces leukocyte presence after cecal ligation and puncture

CD45 40x

CONTROL

AICAR

Compound C

Sham

CLP

Escobar, Gomez, Zuckerbraun. Unpublished data
Sepsis-induced AKI

Model 1: Cecal ligation and puncture (mice)
Secondary outcomes: Leukocyte infiltration – CD3

Activation of AMPK by AICAR reduces leukocyte presence after cecal ligation and puncture

CD3
CONTROL
AICAR
Compound C

Sham

CLP

Escobar, Gomez, Zuckerbraun. Unpublished data
Sepsis-induced AKI

Model 1: Cecal ligation and puncture (mice)
Secondary outcomes: Induction of mitophagy (Atg7)

Activation of AMPK by AICAR increases induction of mitophagy beyond the effect of CLP

Escobar, Gomez, Zuckerbraun. Unpublished data
Sepsis-induced AKI
Model 1B: LPS (mice)
Primary outcome: Renal Function (BUN, Cystatin C)

Sepsis-induced AKI
Model 1B: LPS (mice)

Temsirolimus inhibited mTOR and induced autophagy

Temsirolimus facilitated renal recovery (BUN, Cystatin C)

Sepsis-induced AKI
Model 1B: LPS (mice)
Primary outcome: Renal Function (BUN, Cystatin C)

Rosengart Lab

- VPS34 SiRNA or Non target SiRNA
  Tail vein injection (6mg/kg)

- Allocated to LPS vs. Control

- Sacrifice and sample collection

Primary outcome
- BUN, Cystatin C

Secondary outcome
- LC3B (mitophagy induction)

Sepsis-induced AKI
Model 1B: LPS (mice)

VPS34 SiRNA inhibited autophagy and decreased renal recovery at 48 hours after LPS

<table>
<thead>
<tr>
<th>Groups</th>
<th>48 hours after LPS</th>
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<tbody>
<tr>
<td></td>
<td>BUN</td>
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<tr>
<td>Non target</td>
<td>29</td>
</tr>
<tr>
<td>VPS34</td>
<td>133*</td>
</tr>
</tbody>
</table>

Sepsis-induced AKI

Model 2: Cell culture

2A. Macrophages – cytokine expression
2B. Renal endothelial cells – adhesion molecule expression: ICAM/VCAM

Zuckerbraun Lab

Primary outcome
- Macrophages: IL-6, TNF-a, INF-g
- Endothelial cells: ICAM, VCAM

Escobar, Gomez, Zuckerbraun. Unpublished data
Sepsis-induced AKI
Model 2: Macrophage culture
Cytokine expression

Activation of AMPK by AICAR reverses release of IL-6, INF-gamma and TNF-alpha from Macrophages in cell culture

Escobar, Gomez, Zuckerbraun. Unpublished data
Sepsis-induced AKI
Model 2: Renal endothelial cell culture
Expression of ICAM

Activation of AMPK by AICAR decreases ICAM expression induced by LPS in renal endothelial cells

<table>
<thead>
<tr>
<th>ICAM 60x</th>
<th>CONTROL</th>
<th>AICAR</th>
<th>Compound C</th>
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<tbody>
<tr>
<td>Sham</td>
<td><img src="sham_control.png" alt="Image" /></td>
<td><img src="sham_aicar.png" alt="Image" /></td>
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Escobar, Gomez, Zuckerbraun. Unpublished data
Sepsis-induced AKI
Model 2: Renal endothelial cell culture
Expression of VCAM

Activation of AMPK by AICAR decreases ICAM expression induced by LPS in renal endothelial cells

VCAM

60x

CONTROL

AICAR

Compound C

Sham

LPS

Escobar, Gomez, Zuckerbraun. Unpublished data
Sepsis-induced AKI
Summary of findings

1. Over-stimulation of mitophagy via AMPK stimulation or mTOR inhibition, reduces the clinical manifestation of AKI and facilitates recovery.

2. Inhibition of mitophagy caused a decrease in recovery of the renal function after LPS.

3. Stimulation of AMPK decreased the inflammatory response as measured by cytokine release and leukocyte infiltration.

4. Stimulation of AMPK decreased the expression of endothelial adhesion molecules.
Sepsis-induced AKI

Conclusions

1. Mitophagy seems to be an important mechanism through which the kidney responds and recovers from sepsis-induced injury.

2. There was however an important difference on how AICAR and Temsirolimus “protected” the kidney. AICAR decreased the initial injury, whereas Temsirolimus improved its recovery after injury.
   - Different models: LPS vs. CLP
   - Mechanism of action may be different – AICAR decreased inflammatory response.

3. The effect of AMPK may have been exerted through modulation of:
   - Inflammation:
     - Reduces systemic inflammatory mediators
     - Reduces Leukocyte presence in the kidney
   - Microvascular function:
     - Reduces expression of adhesion molecules
   - Mitochondrial quality control processes
     - Over-induction of mitophagy
Sepsis-induced AKI

Future directions

1. Leukocytes
2. TL4-/-

Inflammation

Microvascular dysfunction

Effects of:
1. Leukocyte depletion
2. TLR-4-/-
3. Autophagy stimulation

Tubular epithelial cell response

1. AMPK-/-
2. Acute vs. Chronic stimulation

Acknowledgements
Thank you