Role of Reactive Oxygen Species in Septic Shock

Richard E. Klabunde, Ph.D.
Associate Professor of Physiology
OUCOM
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Outline

- General introduction and definitions
- Pathophysiology of shock
- Pathogenesis of shock
- Current research and potential new therapies
  - Nitric oxide
  - Reactive oxygen species
  - Peroxynitrite
Sepsis

• Systemic inflammatory response to a confirmed infectious process (most commonly caused by bacterial products (e.g., endotoxin)
Severe Sepsis

- Sepsis with either hypotension or systemic manifestations of hypoperfusion
  - Lactic acidosis, oliguria, altered mental status
Septic Shock

• Cardiovascular dysfunction associated with sepsis resulting in hypotension and organ hypoperfusion despite adequate fluid resuscitation
Endotoxemia & Endotoxic Shock

• Endotoxemia
  – Elevated levels of bacterial endotoxins (e.g., lipopolysaccharide, LPS) in the blood.

• Endotoxic Shock
  – Similar to septic shock (hypotension and organ hypoperfusion despite fluids), but with specific involvement of bacterial endotoxins.
Systemic Inflammatory Response Syndrome (SIRS)

- A more general, inclusive term
- Systemic inflammatory response to a variety of severe clinical insults (e.g., infection, burns, trauma, pancreatitis)
Multiple Organ Dysfunction Syndrome (MODS)

- Progressive distant organ failure following severe infectious or noninfectious insults
Morbidity/Mortality of Sepsis and Septic Shock

- Most common cause of death in ICUs
- U.S. cases per year:
  - Sepsis = 400,000
  - Septic Shock = 200,000
  - Death = 100,000

Pathophysiology of Septic Shock

General Clinical Signs

• Flu-like symptoms
  – chills followed by fever
  – general malaise, irritability, lethargy, mental confusion

• Warm skin (early sign)

• Site of infection may or may not be evident
Pathophysiology Cont.

**Cardiovascular**

- Systemic vasodilation and hypotension ($P_{sys} < 90$ mmHg); increased SVR in late stages
- Tachycardia (>100 beats/min)
- Increased cardiac output (hyperdynamic), although contractility is depressed; hypodynamic in late shock
- Ventricular dilation; decreased ejection fraction
- Loss of sympathetic responsiveness
Pathophysiology Cont.

Cardiovascular Cont.

• Hypovolemia due to vascular leakage; central venous pressure may be decreased or increased depending upon fluid resuscitation

• Compromised nutrient blood flow to organs; decreased organ oxygen extraction
<table>
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<th>Hemorrhagic Shock</th>
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<td><strong>Primary CV Origin</strong></td>
<td>Cardiac</td>
<td>Volume</td>
<td>Vascular</td>
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<tr>
<td><strong>Cardiac Output</strong></td>
<td>↓</td>
<td>↓</td>
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<tr>
<td><strong>Vascular Resistance</strong></td>
<td>↑</td>
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<td><strong>Blood Volume</strong></td>
<td>↑</td>
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<td>IV Fluids Antibiotics, Vasopressors, Inotropes</td>
</tr>
</tbody>
</table>
Pathophysiology Cont.

Pulmonary & Renal

- Hyperventilation with respiratory alkalosis
- Pulmonary hypertension and edema
- Hypoxemia (arterial pO₂ < 50 mmHg)
- Reduced pulmonary compliance; increased work
- Respiratory muscle failure
- Renal hypoperfusion and oliguria despite elevated cardiac output
- Acute tubular necrosis and renal failure
Pathophysiology Cont.

Other

- Disseminated intravascular coagulation (DIC)
- Blood dyscrasias
  - leukopenia
  - thrombocytopenia
  - polycythemia
- Central and peripheral nervous dysfunction
- Increased lactate occurs early
Pathogenesis of Septic Shock

- Infectious triggers
  - Cytokine and inflammatory mediator cascade
    - Cardiac dysfunction and microvascular injury
      - Hypotension and shock
Bacterial-Mediated Sepsis (Gram-negative)

Bacteria → LPS + LBP → Macrophage → Mediators of Inflammation

LPS = Lipopolysaccharide
LBP = LPS-Binding Protein

death → CD14
ENDOTOXIN

Vasodilation
Cardiac Depression
Microvascular Leakage
Platelet Aggregation
Leukocyte Adhesion

Tumor Necrosis Factor
Interleukins
Gamma Interferon
Platelet Activating Factor
Leukotrienes
Thromboxanes
Prostaglandins
Histamine
Nitric Oxide
Oxygen Free Radicals
Nitric Oxide Formation

Endotoxin  
Cytokines  

L-arg  
iNOS  
cNOS  

NO  
+  
citrulline  

NO  
+  
GC  

GTP  
cGMP  

Acetylcholine  
Bradykinin  
Substance-P  
Insulin
Actions of Nitric Oxide

• Vasodilation  (direct via cGMP and indirect via inhibition of NE and ET-1 release)
• Inhibits leukocyte-endothelial cell adhesion
• Inhibits platelet adhesion/aggregation
• Modulates vascular permeability
• Scavenges superoxide radicals
• High concentrations are cytotoxic
What role does NO play in septic shock?
NO Formation in Septic Shock

![Graph showing NO production over time post-endotoxemia]

- **NO Production**
- **Time (hours) Post-Endotoxemia**
- **cNOS**
- **iNOS**
Pretreatment with NOS Inhibitors

• Hypothesis:
  – Increased NO production during sepsis causes systemic vasodilation, cardiac depression, increased capillary permeability (leading to decreased blood volume) and hypotension.

• Therefore:
  – Pretreatment with a NOS inhibitor in animal models of sepsis should prevent NO-induced hypotension and edema.
  – Treatment with a NOS inhibitor during sepsis should reverse cardiovascular changes.
Effects of Pretreatment with NOS Inhibitor

6 Hours Post-LPS

NO and Microvascular Leakage

Other Studies

• Pretreatment with NOS inhibitors:
  – Prevents fall in systemic vascular resistance
  – Prevents arteriolar hyporesponsiveness to catecholamines
Therapeutic Efficacy of NOS Inhibitors in Endotoxic Shock

Summary of Role of NO in Septic Shock

• Increased NO production decreases SVR and leads to hypotension.
• Non-specific NOS inhibition restores arterial pressure, but reduces cardiac output and organ perfusion
• NO is involved in microvascular leakage during endotoxemia
• Other studies:
  – NOS inhibition causes pulmonary dysfunction and increases mortality.
  – Selective iNOS inhibition being investigated for therapeutic potential; non-selective inhibitors increase mortality
  – Clinical studies show no benefit on survival
Are nitric oxide inhibitors good or bad in septic shock?

- **Bad** – NOS inhibitors increase
  - Cardiac depression and organ hypoperfusion
  - Thrombosis
  - Leukocyte-endothelial adhesion and inflammation
  - Superoxide anion (NO normally scavenges superoxide)
  - Increase mortality in many studies

- **Good** – NOS inhibitors prevent
  - Excessive vasodilation and hypotension
  - Possible cardiac depression
  - Formation of new, damaging free radical species (e.g., peroxynitrite)
Formation of Reactive Oxygen Species

Cytokines
Endotoxins

\[
\begin{align*}
\cdot & \text{OO}_2^- \\
\rightarrow & \text{H}_2\text{O}_2 \\
\rightarrow & \cdot \text{OH} \\
\rightarrow & \text{HOCl} \\
\rightarrow & \text{H}_2\text{O} \\
\end{align*}
\]

\[
\begin{align*}
\text{Catalase} \\
\text{GSHpx} \\
\text{SOD} \\
\text{MPO} \\
\end{align*}
\]

\[
\text{HOCl} \\
\text{H}_2\text{O}_2 \\
\cdot \text{OH} \\
\cdot \text{O}_2^- \\
\]

Cellular Damage & Dysfunction
Vasodilation
Negative inotropy
Microvascular leakage

\[
\begin{align*}
\text{NAD(P)H oxidases} \\
\text{Cyclooxygenase} \\
\text{Xanthine Oxidase} \\
\text{Nitric Oxide Synthase} \\
\end{align*}
\]

Macrophages
Neutrophils
Endothelial Cells
Myocytes etc.
Free Radicals in Septic Shock

• Reactive oxygen species (ROS) are elevated in sepsis
  – In rat models of acute endotoxemia, superoxide anion increases 3-fold within 60 min and plateaus after about 4 hours (Brovkovych et al, J Physiol Pharmacol 48:633, 1997)
Endogenous antioxidant and scavenging systems can become depleted in severe sepsis, particularly
- Alpha-tocopherol
- Ascorbic acid
- $\beta$-carotene

Survival in septic patients with organ dysfunction is inversely correlated with the reduction in plasma antioxidant potential
(Cowley et al., Crit Care Med 24:1179, 1996)
In animal studies, treatment with ROS scavengers can improve clinical status and decrease mortality.

Animal studies suggest that the following treatments may improve outcome:

- **N-acetyl-L-cysteine** (scavenges hydroxyl radical and hypochlorus acid; replenishes glutathione)
- **Alpha-tocopherol**
- **Ascorbic acid** (treatment during bacteremia in rats prevents hypotension - Armour et al., J Appl Physiol 90:795, 2001)
- **TEMPO** (SOD mimic)
Combined Morbidity* of Endotoxic Rats
(LPS = 15 mg/kg, ip)

*Responses Included:
Uprighting
Locomotory
Toe Pinch

Responses Included:
Uprighting
Locomotory
Toe Pinch

Control
Alpha-tocopherol
Ascorbic acid
N-acetyl-cysteine

(8/16 Survived)
(9/15 Survived)
Effects of ROS Scavenging on LPS-Induced Microvascular Leakage

Figure 2. Inhibition of LPS-induced leakage by pretreatment with tempo. Vertical bars, S.E. (only shown for LPS and Tempo + LPS groups. Number of experiments in parentheses. * p<0.05 compared to all
Unresolved Issues with Antioxidant Therapy

• Is there one antioxidant that is best or should a cocktail be used?
• What is the therapeutic window for antioxidant therapy?
• Do antioxidants increase survival?
• Is there a therapeutic role for inhibitors on ROS production? e.g., xanthine oxididase inhibitors (allopurinol), NAD(P)H oxidase inhibitors
Nitric Oxide and Superoxide Anion Interactions

- Sepsis
- L-Arg → ·NO
- H2O2 → ·OH
- NO2− + ·OH → ONOO− (Peroxynitrite)
- O2 ↔ HOCl
- H2O2 ↔ SOD
- NAD(P)H oxidase, XO, COX, NOS
- Catalase

Reactions involving:
- SOD: Superoxide Dismutase
- MPO: Myeloperoxidase
- NOS: Nitric Oxide Synthase
Superoxide Scavenging of NO and the Formation of Peroxynitrite

• Increased ROS decreases NO, which may contribute to:
  – Leukocyte and platelet adhesion (leading to vascular plugging)
  – Vasoconstriction (leading to impaired perfusion)
• Peroxynitrite formation can cause vasodilation and cardiac depression, as well as cellular damage in general
• Decreasing ROS by antioxidants, for example, may enhance hypotension by increasing NO bioavailability.
  – Therefore, therapy may need to be directed against both ROS and NO.
Nitric Oxide Contributes to ROS-Induced Microvascular Leakage

Peroxynitrite Formation Contributes to PAF-Induced Microvascular Leakage

Summary

• Cardiovascular manifestations of septic shock include:
  – Cardiac depression
  – Systemic vasodilation, hypovolemia and hypotension
  – Reduced organ perfusion

• NO and ROS are implicated in contributing to these cardiovascular changes

• ROS scavengers and selective iNOS inhibitors may provide a new therapeutic approach to improving survival in septic patients