Hypertonic Saline Infusion for Pulmonary Injury Due to Ischemia-Reperfusion

Conor J. Shields, MB, AFRCSI; Desmond C. Winter, MD, FRCSI; Brian J. Manning, MB, AFRCSI; Jiang Huai Wang, PhD; William O. Kirwan, MCh, FRCSI; H. Paul Redmond, BSc, MCh, FRCSI

Hypothesis: Inhibition of neutrophil-endothelial cell interactions by hypertonic saline (HTS) may confer protection against organ injury in states of immunologic disarray. This study tested the hypothesis that infusion of HTS modulates the development of end-organ injury in a model of lower-torso ischemia-reperfusion injury.

Design: Ischemia-reperfusion injury was induced in 30 male Sprague-Dawley rats by infrarenal aortic cross clamp for 30 minutes, followed by reperfusion for 2 hours. At 0 and 60 minutes of reperfusion, intravenous HTS (7.5% sodium chloride, 4 mL/kg) was administered to 6 rats each, and another 12 received either 4 or 30 mL/kg of isotonic sodium chloride solution. Six rats received HTS, 4 mL/kg, before ischemia. At 2 hours, we assessed liver function, pulmonary injury, neutrophil infiltration (myeloperoxidase activity), endothelial permeability (bronchoalveolar lavage and wet-dry weight ratios), and proinflammatory cytokine levels (tumor necrosis factor \( \alpha \) and interleukin 6).

Results: Infusion with HTS before or after ischemia significantly reduced end-organ injury. Histopathologic pulmonary injury scores were markedly attenuated in the HTS group (5.82±1.3) and the HTS pretreated group (4.91±1.6) compared with the isotonic sodium chloride solution groups (8.54±1.1) (\( P = .04 \)). Pulmonary neutrophil sequestration (2.07±0.23) and increased endothelial permeability (4.68±0.44) were manifest in animals resuscitated with isotonic sodium chloride solution compared with HTS treatment (1.54±0.19 [\( P = .04 \)] and 2.06±0.26 [\( P = .02 \)]) and pretreatment (1.18±0.12 [\( P = .04 \)] and 1.25±0.07 [\( P = .002 \)]). In addition, a significant reduction in serum tumor necrosis factor \( \alpha \) (\( P = .04 \)) and interleukin 6 (\( P = .048 \)) levels was observed, whereas HTS resuscitation attenuated the upsurge in aspartate transaminase (\( P = .03 \)) and alanine transaminase levels (\( P = .047 \)).

Conclusions: Resuscitation with HTS attenuates the pulmonary edema and tissue injury due to lower-torso ischemia-reperfusion and maintains a more benign immunologic profile.

Arch Surg. 2003;138:9-14

An episode of acute lower-torso ischemia followed by restitution of perfusion may engender a diffuse and potentially life-threatening systemic inflammatory response,\(^1\) of which lung injury is the most pertinent manifestation.\(^2\) The propensity of an extrapulmonary pathologic condition to induce an acute response in the lung is predicated on the complex interplay of proinflammatory and anti-inflammatory factors.\(^3\) Although the mechanisms that initiate progression to end-organ injury remain ill defined,\(^4 \) excessive neutrophil cytotoxicity is compellingly implicated in microvascular hyperpermeability, with spillage of protein-rich transudate into the alveolar spaces (hallmarks of the acute respiratory distress syndrome).\(^5,6\) The pivotal importance of neutrophil accumulation in the instigation of pulmonary injury is substantiated by the findings of studies using agents to abrogate neutrophil cytotoxicity.\(^11\) Teleologically, the cytotoxic potential of neutrophils equips the host to combat septic challenge. However, in states of immunologic disarray, as prevails in systemic inflammatory response syndrome, the unleashing of their destructive faculties results in tissue damage.\(^11\)

The mediation of neutrophil entrapment in the lung is a consequence of complement activation, cytokine production, and stimulation of alveolar macrophages and adhesion molecule expression.\(^11\) Secretion of chemoattractant substances, including tumor necrosis factor \( \alpha \) (TNF-\( \alpha \)), interleukin 1\( \beta \) (IL-1\( \beta \)), and complement factor C5a, results in enticement of neutrophils to the pulmonary microvasculature.\(^13,14\) Pulmonary infiltration of activated neutrophils and their ultimate degranulation of proteolytic en-
zymes and reactive oxygen species results in the stig-
matas of lung injury. This manifests clinically as pro-
gressive hypoxemia with radiologic evidence of diffuse
infiltrations, and it has been described in patients after
aortic aneurysm repair.

The immunomodulatory effects of hypertonic sa-
line (HTS) infusion provide potential strategies for at-
tenuating inappropriate neutrophil activation. Previous
studies by Shields et al have demonstrated significant
attenuation of end-organ injury in an animal model of
pancreatitis with HTS resuscitation. The benefits of tran-
sient hyperosmolar resuscitation extend to the attenua-
tion of receptor-mediated polymorphonuclear leuko-
cyte functions, including the down-regulation of
neutrophil oxidative burst activity and adhesion mole-
cule expression and the suppression of polymorpho-
nuclear leukocyte activation. The inhibition of neu-
rophil-endothelial cell interactions by HTS infusion may
confer protection against organ injury in inappropriate
inflammatory states.

This study tests the hypothesis that HTS infusion
modulates the immunologic profile and development of
downstream pulmonary neutrophil infiltration as de-
scribed previously. Myeloperoxidase activity was used as a
marker of pulmonary neutrophil infiltration as described else-
where. Protein concentrations and myeloperoxidase activity
were measured spectrophotometrically.

END-ORGAN INJURY PARAMETERS

Immediately before euthanasia, blood samples were collected
via cardiac puncture for biochemical determination of liver
function. Plasma levels of aspartate transaminase and alanine
transaminase were assessed, and serum levels of the cytokines
TNF-α and IL-6 were determined using an enzyme-linked
immunosorbent assay kit (R & D Systems, Minneapolis, Minn) and
were estimated spectrophotometrically (MRX Microplate
Reader; Dynatech Technologies Inc, Chantilly, France). At 2
hours of reperfusion, a midline sternotomy was performed.
The right upper lobe of the lung was excised, fixed in 10%
formalin, and stained with hematoxylin-eosin. Samples were
simultaneously assessed by 2 observers (C.J.S., D.C.W.) who
were masked to the experimental manipulation, using a stan-
dardized scoring system (Table 1). Pulmonary endothelial
leak and edema were assessed by measuring lung wet-dry
weight ratios and bronchoalveolar lavage protein content as
described previously. Myeloperoxidase activity was used as a
marker of pulmonary neutrophil infiltration as described else-
where. Protein concentrations and myeloperoxidase activity
were measured spectrophotometrically.

SURVIVAL STUDY

A survival study was performed to validate the efficacy of HTS
therapy. Lower-torso IR injury was induced in 30 animals as
described in the “Model of IR-Induced Lung Injury” subsec-
tion. Animals were randomized to receive either isotonic so-
odium chloride solution (0.9% sodium chloride, 30
mL/kg) or HTS (4 mL/kg, n = 10) or 30 mL/kg, n = 10)
ment, another group received large-volume isotonic sodium
chloride resuscitation (0.9% sodium chloride, 30
mL/kg). Samples from control animals were used to provide
baseline biochemical and histopathologic variables. All ani-
imals were humanely killed at the conclusion of 2 hours of reper-
fusion. This experiment was conducted after receiving ap-
proval by the Biological Services Unit of the National University
of Ireland and the Department of Health and Children in Ire-
land (Dublin).

| Table 1. Histologic Injury Scoring System |
|---------------------------|-----------------------------|
| Lung Injury               |                             |
| 0 – Absent                | Congestion                  |
| 1 – Mild                  | Inflammatory infiltrate     |
| 2 – Moderate              | Hemorrhage                  |
| 3 – Severe                | Intra-alveolar macrophages  |
| 0 – Absent                |                             |
| 1 – Present               |                             |

RESULTS

Restoration of perfusion resulted in a significant derange-
ment of liver function, represented by marked eleva-
tions in plasma levels of the liver enzymes aspartate trans-
aminase and alanine transaminase in all experimental
animals compared with controls. The potent immuno-
modulatory effects of HTS were evident in the attenua-
tion of this acute response in animals resuscitated with
HTS (Figure 1).

Significant elevations in the concentrations of the
potent proinflammatory cytokines responsible for ini-

©2003 American Medical Association. All rights reserved.
ating the acute phase response to IR injury, TNF-α and IL-6, were observed in all animals subjected to lower-torso IR injury; however, this upsurge was attenuated in the HTS pretreatment and treatment groups (Figure 2 and Figure 3). The degree of pulmonary injury sustained as a consequence of IR injury differed markedly between the experimental groups. Tissue samples from the large- and small-volume isotonic sodium chloride solution groups exhibited significant interstitial congestion and hemorrhage, with pronounced derangement of the alveolar architecture. Animals treated and pretreated with HTS evinced significantly lower mean pulmonary injury scores than their isotonic sodium chloride solution counterparts (Table 2).

**SURVIVAL STUDY**

A significant increase in survival was noted in the HTS therapy groups. At 12 hours, survival in the small- and large-volume isotonic sodium chloride solution groups was only 40% and 30%, respectively, whereas animals resuscitated with HTS exhibited 80% survival (Figure 5), a significant increase in survival.

**COMMENT**

This study demonstrates that HTS resuscitation precludes progression to end-organ injury in states of immunologic disarray arising from IR injury. To our knowledge, no effective intervention strategies currently exist to modulate clinically relevant reperfusion injury and its sequelae. Ventilation is the principal therapeutic maneuver for acute respiratory distress syndrome, although recent interest has focused on suppressing the overexuberant inflammatory response. Attempts to affect the interplay between proinflammatory and anti-inflammatory cytokines may offer the best prospect of diminishing pulmonary involvement, but there are few accepted therapeutic endeavors in this regard. Regulators of cellular signal transduction pathways such as p38 mitogen-activated protein kinase and nuclear factor κB inhibitors provide potential pharmacologic targets for suppressing unchecked and unrestrained inflammation, although the pharmacodynamics or toxicity of these agents may limit their value.
in critically ill patients with multiple organ dysfunction syndrome.

Hypertonic saline infusion has been shown to be an effectual means of restoring circulating volume in hemorrhagic shock, exerting favorable effects on cardiac contractility, blood pressure, and peripheral tissue perfusion.28 However, the recognition that HTS infusion can also serve to dampen an overexuberant inflammatory response, and may modulate polymorphonuclear leukocyte cytotoxicity, has revived interest in it as a resuscitative agent. Plasma sodium chloride concentration is tightly regulated, being a major determinant of plasma osmolality. Previous studies have demonstrated that infusion of 7.5% sodium chloride results in a rapid, al-

Table 2. Pulmonary Injury, Neutrophil Infiltration, and Endothelial Permeability*

<table>
<thead>
<tr>
<th></th>
<th>Lung Injury Score†</th>
<th>MPO Activity, µMPO/g</th>
<th>BAL Protein Content, µg/mL</th>
<th>Lung Wet-Dry Weight Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.03 ± 0.01</td>
<td>1.02 ± 0.11</td>
<td>112 ± 13</td>
<td>1.09 ± 0.21</td>
</tr>
<tr>
<td>Isotonic sodium chloride solution</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small volume</td>
<td>8.54 ± 1.1</td>
<td>2.07 ± 0.23</td>
<td>632 ± 67</td>
<td>4.68 ± 0.44</td>
</tr>
<tr>
<td>Large volume</td>
<td>8.12 ± 1.0</td>
<td>1.92 ± 0.15</td>
<td>815 ± 48</td>
<td>4.89 ± 0.32</td>
</tr>
<tr>
<td>Hypertonic saline (pretreatment)</td>
<td>4.91 ± 1.6</td>
<td>1.18 ± 0.12</td>
<td>203 ± 14</td>
<td>1.25 ± 0.07</td>
</tr>
<tr>
<td>Hypertonic saline</td>
<td>5.82 ± 1.3</td>
<td>1.54 ± 0.19</td>
<td>224 ± 23</td>
<td>2.06 ± 0.26</td>
</tr>
<tr>
<td>P-value‡</td>
<td>.04</td>
<td>.04</td>
<td>.04</td>
<td>.05</td>
</tr>
</tbody>
</table>

Abbreviations: BAL, bronchoalveolar lavage; MPO, myeloperoxidase.
*Data are given as mean ± SEM.
†See Table 1 for scoring system.
‡Kruskal-Wallis 1-way analysis of variance on ranks test, hypertonic saline (pretreatment) and hypertonic saline vs the isotonic sodium chloride solution groups.
though transient, rise in plasma sodium levels to 40M above isotonic levels,29 which remain elevated for up to 4 hours.30

The failure of isotonic resuscitation, irrespective of volume, to attenuate the pulmonary inflammatory response subsequent to IR injury further confirms the profound effects of osmotic shock on neutrophil function. Although HTS infusion significantly facilitates microvascular circulation, reducing endothelial cell swelling by establishing a potent osmotic gradient,31 the tendency for a hypertonic environment to disrupt neutrophil-endothelial cell interaction and to induce neutrophil apoptosis32 may account for the amelioration of lung injury discerned in this study. As dysfunction of the regulatory mechanism of apoptosis of entrapped neutrophils is a pivotal component in the propagation of the massive inflammatory response evident in systemic inflammatory response syndrome,33–36 administration of a proapoptotic hypertonic solution may induce neutrophils to enter the programmed cell death sequence, thereby curbing the tendency toward further alveolar epithelial destruction. This hypothesis is sustained by the finding of markedly diminished myeloperoxidase activity in the lungs of HTS-treated animals. Furthermore, the diminished pulmonary injury observed with osmotic perturbation correlates with decreased endothelial permeability, signifying that HTS exposure also results in a moderate discharge of transudate into the alveolar spaces.

The derangement of liver function observed in the isotonic sodium chloride solution group was not replicated in the HTS groups, a finding in agreement with previous studies.35 This may have a significance that transcends that of hepatic protection, as hepatic amplification of pulmonary inflammation via the immunologic potency of the Kupffer cell has been implicated in the exacerbation of lung injury.14 The correlation with increased survival further emphasizes the importance of diminution of lung involvement in efficacious intervention.

The present study demonstrates a significant inhibition of the cytokine-driven inflammatory response, with exposure to hypertonicity resulting in a marked diminution of serum levels of TNF-α and IL-6. The signaling molecule TNF-α exerts a considerable amplifying effect on the acute inflammatory response, triggering up-regulation of neutrophil β2 integrin expression, and that of the corresponding endothelial ligand, intercellular adhesion molecule-1. This facilitates neutrophil-endothelial interaction and ultimately engenders endothelial hyperpermeability. The predominant role of inflammatory cytokines in promoting neutrophil recruitment, adherence, and extravasation is further emphasized by the observation of a positive correlation between extent of reperfusion injury and serum concentration of IL-6.3 The attenuation of IL-6 expression exhibited by HTS-treated animals further emphasizes the suppression of the inflammatory response provoked by hypertonic resuscitation.

The results of this study affirm the potent immunomodulatory effects of HTS infusion. Hypertonic therapy attenuates neutrophil-mediated end-organ insult by guarding against the deleterious effects of unrestrained neutrophil sequestration and activation and encourages a more benign immunologic profile. A clinical trial of hypertonic resuscitation in hyperinflammatory states is warranted.

Accepted for publication July 27, 2002.

This study was presented at the annual meeting of the Surgical Infection Society (Europe), Madrid, Spain, May 3, 2002.

Corresponding author and reprints: H. Paul Redmond, BSc, MCh, FRCSI, Department of Academic Surgery, Cork University Hospital, Wilton, Cork, Ireland (e-mail: redmondhp@shb.ie).

REFERENCES
