Hypertonic Saline and Pentoxifylline Reduces Hemorrhagic Shock Resuscitation-Induced Pulmonary Inflammation Through Attenuation of Neutrophil Degranulation and Proinflammatory Mediator Synthesis

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Background: **Ringer's lactate (RL), the current standard resuscitation fluid, potentiates neutrophil activation and is associated with pulmonary inflammation. Resuscitation with hypertonic saline and pentoxifylline (HSPTX) has been shown to attenuate hemorrhagic shock–induced injury when compared with RL. Because the neutrophil plays a major role in post-shock inflammation, we hypothesized that HSPTX reduces pulmonary inflammation after resuscitation in comparison to RL.**

Methods: **Sprague-Dawley rats underwent controlled shock and were resuscitated with RL (32 mL/kg) or HSPTX (4 mL/kg 7.5% NaCl + pentoxifylline 25 mg/kg). Animals who did not undergo shock or resuscitation served as controls. After 24 hours, bronchoalveolar lavage fluid (BALF) and lung tissue were collected. Cytokine induced neutrophil chemoattractant (CINC) was measured in BALF by enzyme-linked immunosorbent assay. Matrix metalloproteinases (MMP)-2 and -9 were measured by zymography. Hemeoxygenase-1 (HO-1) was assessed by Western blot and immunohistochemistry.**

Results: **HSPTX resuscitation led to a 62% decrease in CINC levels compared with RL ($p < 0.01$). BALF MMP-2 expression was attenuated by 11% with HSPTX ($p = 0.09$). Lung MMP-2 and MMP-9 expression was reduced by 89% ($p < 0.01$) and 76%, respectively ($p < 0.05$). Lung HO-1 expression declined by 34% with HSPTX in comparison to RL ($p < 0.01$), indicating less oxidative injury. Lung immunohistochemistry localized HO-1 to neutrophils, macrophages, and airway epithelial cells.**

Conclusion: **Collectively, the attenuation of pulmonary inflammation with HSPTX after shock when compared with RL is associated with downregulation of neutrophil activation, oxidative stress, and proinflammatory mediator production.**

Key Words: **Pentoxifylline, Hypertonic saline, Hemorrhagic shock, Acute lung injury, Matrix metalloproteinases, Heme oxygenase-1, Interleukin-8, Inflammation.**

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Hemorrhagic shock results in peripheral tissue hypoxia and generalized ischemia. Although fluids are capable of restoring adequate tissue perfusion, they are implicated in the pathogenesis of ischemia-reperfusion injury, which results in systemic neutrophil activation and proinflammatory mediator production.[1,2] If inflammation continues unabated, it can result in end organ injury involving the liver, intestine, and, most notably, the lung.

The development of acute lung injury (ALI) and its most severe form, acute respiratory distress syndrome (ARDS), is associated with considerable morbidity and mortality in trauma patients.[3] This condition is characterized by increased alveolar-capillary membrane permeability, edema, and hypoxia secondary to injury at the level of the endothelial basement membrane.[4,5]

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Although the exact mechanism responsible for the development of inflammatory injuries remains unknown, the neutrophil, through the release of proteases and synthesis of reactive oxygen species (ROS), has been shown to play a central role.[6–8] One of the earliest mediators implicated in the development of inflammation and ALI, interleukin (IL)-8, functions as a chemoattractant and mediates neutrophil migration into the lung parenchyma. IL-8 is also involved in neutrophil adhesion molecule receptor expression and degranulation.[9] Significantly increased levels of IL-8 have also been demonstrated in the bronchoalveolar lavage fluid (BALF) of trauma patients who go on to develop ARDS.[10]

Matrix metalloproteinases (MMP)-2 and -9 are released from neutrophil granules and are capable of digesting type IV collagen present in the basement membrane.[11] Significantly higher levels of MMP-2 and MMP-9 have been demonstrated in the bronchoalveolar lavage fluid BALF of ARDS patient compared with healthy subjects, implicating their role in lung injury.[12]

ROS not only contribute to the pathogenesis of inflammation, they also induce the expression of hemeoxygenase-1 (HO-1), an enzyme which protects tissues against oxidative insults through its role in heme degradation.[13,14] Upregulation of HO-1 expression has been demonstrated with ALI and

resuscitation after hypoxia and serves as a reliable marker of the degree of oxidative stress sustained within tissues.[15]

Racemic Ringer's lactate (RL), the current standard fluid utilized in hemorrhagic shock, has been implicated as a contributor to ischemia-reperfusion injury because of its effects on neutrophil function. RL-induced neutrophil activation and endothelial dysfunction have been observed in animal studies.[16,17] In vitro experiments have demonstrated a dose-dependent increase in neutrophil oxidative burst and β2-integrin expression with the administration of RL.[18] These shortcomings have fueled the search for a superior resuscitation fluid.

The administration of hypertonic saline (HS) as a small-volume resuscitative fluid has been investigated as a potential alternative to RL. Although both fluids are capable of conferring hemodynamic stability, HS attenuates the increases in neutrophil activation and vascular permeability seen with RL.[19,20] HS has also been shown in animal models of hemorrhagic shock to reduce lung injury and bacterial translocation when compared with RL.[21,22]

Pentoxifylline (PTX), a methylxanthine derivative and nonspecific phosphodiesterase inhibitor, has recently gained attention as an immune modulator in sepsis and hemorrhage. It has the capacity to attenuate neutrophil activation and lung injury in endotoxemia and to prevent endothelial damage, neutrophil adhesion, and mortality in animal models of ischemia-reperfusion.[23–26] PTX alone is not capable of volume expansion and must be administered with fluid in the treatment of shock.

Because several similarities exist between the immunomodulatory effects of HS and PTX, their combination (HSPTX) would result in a small volume resuscitative fluid with anti-inflammatory properties. This novel fluid strategy has been shown to attenuate in vitro neutrophil activation and tumor necrosis factor (TNF)-α production more efficiently than HS infusion alone.[27] Our laboratory has recently demonstrated that HSPTX infusion can produce hemodynamic stability in an animal model of hemorrhagic shock without the subsequent development of histologic RL-induced reperfusion injury to the lung and intestine.[28] In this series of experiments, we hypothesized that postresuscitative pulmonary inflammation would be downregulated by HSPTX when compared with RL. To study this, we examined the effects of the resuscitative fluids on oxidative stress, neutrophil activation, and proinflammatory mediator synthesis in an in vivo model of hemorrhagic shock.

MATERIALS AND METHODS

The experiment was approved by the University of California Animal Subjects Committee and in accordance with guidelines established by the National Institutes of Health.

Experimental Model

Male Sprague-Dawley rats (300–400 g) were purchased from Harlan Sprague-Dawley (San Diego, CA). A 12-hour light and dark cycle was instituted, and food and water were provided ad libitum. Animals were anesthetized with ketamine and xylazine by intraperitoneal injection. A right inguinal incision was performed, and the femoral artery and vein were cannulated with polyethylene catheters (PE50). The venous catheter was utilized for injection of resuscitative fluids, and the arterial catheter was used to withdraw blood and monitor the mean arterial pressure (MAP). Blood was withdrawn during a period of 10 minutes until a MAP of 35 mm Hg was obtained. Controlled hypotension was then maintained at 35 $\pm$ 5 mm Hg for 1 hour by withdrawal or reinfusion of blood as necessary.

The animals were randomly divided into three groups according to the treatment received. Sham animals (n = 5) underwent cannulation without shock or resuscitation and served as negative controls. RL-resuscitated animals (n = 7) received 32 mL/kg of RL. HSPTX-treated animals (n = 7) received 4 mL/kg of 7.5% NaCl + 25 mg/kg of PTX (Sigma, St. Louis, MO). PTX was dissolved in HS without the infusion of any additional fluid. The dose of PTX was chosen based on studies from our laboratory demonstrating its safety and lack of hypotension.[28] The volume of RL infusion was calculated to animals three times their shed blood volume as done in clinical practice and to give equivalent sodium loads to RL and HSPTX treated animals. After resuscitation with the respective fluid, shed blood was reinfused. As seen in our prior study, there was no significant difference between preshock, shock, and resuscitation MAP or the shed blood volume between the groups.[28] The body temperature of the animals was maintained at 37°C throughout the experiment.

Bronchoalveolar Lavage Collection

At the end of volume resuscitation, the catheters were removed, the incision was closed, and the animals were returned to their cages. The animals were killed via cardiac puncture 24 hours after the completion of shock and resuscitation. Immediately after death, the trachea of each animal was accessed through a tracheotomy. The lungs were instilled with 10 mL of sterile normal saline under direct vision to avoid overdistension. The BALF was obtained and centrifuged at 250 g for 10 minutes. The supernatant was subsequently collected and stored at –70°C.

Lung Procurement and Tissue Extraction

After the bronchoalveolar lavage was completed, the right lung of each animal was excised and frozen in liquid nitrogen. Lung tissue was homogenized in 1 mL of ice-cold T-PER Tissue Protein Extraction Reagent and 1% protease inhibitor cocktail (Pierce Biotechnology, Rockford, IL). Homogenates were centrifuged at 10,000 g for 5 minutes and the supernatant was stored at –70°C. The total protein concentration of each extract was determined using the bicinchoninic acid protein assay (Pierce Biotechnology).

The left lung of each animal was stored in 10% phosphate-buffered saline (PBS) formalin, embedded in par-

affin using an automated processing unit (Autotechnicon), cut into 5-μm cross sections, and transferred onto glass slides for immunohistochemical analysis.

BALF CINC Content

The concentration of CINC in the BALF was measured quantitatively using enzyme-linked immunosorbent assay (n = 5 per group). CINC is a member of the IL-8 family present in rats and functions as a neutrophil chemoattractant similar to IL-8 in humans. The wells of a 96-well immunoplate (NUNC Brand, Rochester, NY) were coated with the capture antibody, goat anti-CINC antibody diluted 1:396 in coating buffer, and incubated overnight at 4°C (Peptide Institute, Osaka, Japan). Nonspecific binding sites were blocked with a buffer comprised of 5% nonfat dry milk (Sigma) in sterile PBS (Irvine Scientific, Santa Ana, CA). The wells of the plate were washed with 0.05% Tween 20 (Fischer Scientific, Pittsburgh, PA) in PBS. Undiluted BALF was incubated on the plate at room temperature for 2 hours. After washing, the secondary antibody (rabbit anti-CINC) was diluted 1:2,000 in blocking buffer and dispensed onto the plate for incubation at room temperature. The conjugate, goat antirabbit horseradish peroxidase (HRP; Cell Signaling, Beverly, MA) was instilled on the plate and incubated at room temperature for 30 minutes. An Immunopure TMB substrate kit (Pierce Biotechnology) was used for detection. The reaction was stopped with 2 mol/L H_2SO_4 after 15 minutes and read at 450 nm with a microplate reader (Molecular Devices, Sunnyvale, CA). Data are presented as nanogram per milliliter.

Lung MMP-2 and MMP-9 Activity

MMP-2 and MMP-9 activity were measured in lung samples (n = 4 per group) by zymography under nonreducing conditions. MMP identity was verified by running human standards (Chemicon International, Temecula, CA) diluted 1:1000 in 2× Tris-Glycine Sample Buffer (Invitrogen, Carlsbad, CA). Two micrograms of each sample was diluted in sample buffer and loaded onto a Novex 10% Zymogram Gel copolymerized with gelatin (Invitrogen). Gels were run in Tris-Glycine SDS Running Buffer at 130 V for 90 minutes. After electrophoresis, gels were incubated in Renaturing Buffer (Invitrogen) for 30 minutes at room temperature. Gels were then incubated with Developing Buffer (Invitrogen) for 30 minutes at room temperature, followed by an overnight incubation with fresh developing buffer at 37°C. Gels were stained with Coomassie brilliant blue R-250 and rinsed frequently with destaining solution (50% methanol, 40% water, and 10% acetic acid). MMP activity appeared as clear bands against a blue background. Gels were standardized and scanned in black and white for analysis.

BALF MMP-2 Activity

Using the methods as outlined for the lung above, MMP-2 activity was obtained in the BALF using 2.5 μg of each sample diluted in 2× Tris-Glycine Sample Buffer.

Lung Heme Oxygenase-1 Protein Content

Then 20 μg of each lung sample (n = 4 per treatment group) was boiled and loaded onto an 8% to 16% Tris-Glycine polyacrylamide gradient gel (Invitrogen) and run at 130 V for 90 minutes. The gel was then transferred to a nitrocellulose membrane (Invitrogen) at 27 V for 90 minutes. The membrane was blocked with 5% milk in Tris buffered saline with Tween 20 (TBS/Tween 20 Sigma) for 1 hour and incubated at 4°C overnight with rabbit anti-HO-1 polyclonal antibody diluted 1:1,000 (StressGen Biotechnologies, Victoria, BC, Canada). The membranes were then washed with TBS/Tween 20 and incubated for 1 hour at room temperature in blocking buffer with the secondary antibody, HRP-linked antirabbit immunoglobulin (Ig) G diluted 1:2,000 and HRP-conjugated antibiotin antibody diluted 1:4,000 (Cell Signaling, Danvers, MA). The Supersignal West Pico Chemiluminescent Kit (Pierce Biotechnology) was applied to the membrane for 5 minutes, and the membrane was exposed to radiographic film for detection of antibody complexes.

Lung Heme Oxygenase-1 Immunostaining

After deparaffinization, slides (n = 5 per group) were incubated in Target Retrieval Solution (DAKO, Carpinteria, CA) at 95°C for 20 minutes and cooled. All subsequent steps were conducted in a humid chamber at room temperature. Endogenous peroxidase activity was quenched with 1.5% H_2O_2 for 5 minutes. Sections were blocked with 5% nonfat milk and 1% bovine serum albumin (BSA; Sigma) for 20 minutes, and then incubated in rabbit polyclonal HO-1 antibody diluted 1:500 in blocking serum for 1 hour. Sections were washed with PBS and incubated with biotinylated rabbit secondary antibody diluted 1:500 in PBS. Specific labeling was detected using the Elite ABC peroxidase kit and DAB substrate (Vector laboratories, Burlingame, CA). Slides then underwent counterstaining with Hematoxylin (Richard-Allan Scientific, Kalamazoo, MI).

Statistical Analysis

All values are expressed as the mean ± SEM of n observations where n represents the number of animals. MMP activity and HO-1 content was quantified with UNSCAN-IT Gel Digitizing software (Silk Scientific, Orem, UT) and is presented as the mean of the pixel total for each band. Each assay was performed in duplicate or triplicate where appropriate. Statistical significance of differences among groups was determined by analysis of variance (ANOVA) followed by Bonferroni correction. A p value <0.05 was considered significant.

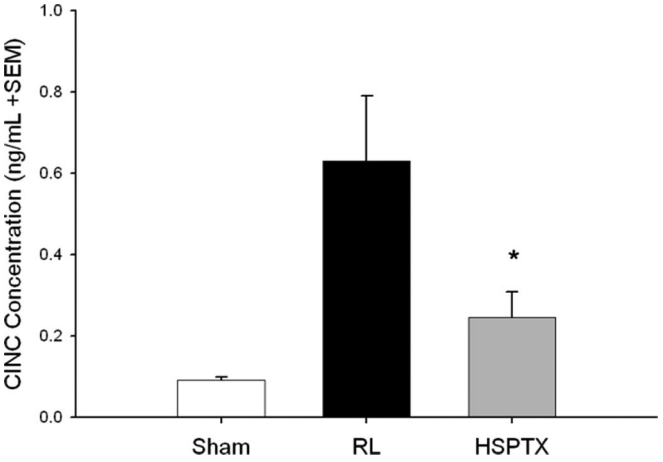

Fig. 1. *Resuscitation-induced CINC Levels. CINC levels demonstrated significant attenuation in animals resuscitated with HSPTX. Each column corresponds to the mean CINC concentration (ng/mL) + SEM of five independent experiments.* *p $<$ *0.01 versus RL.*

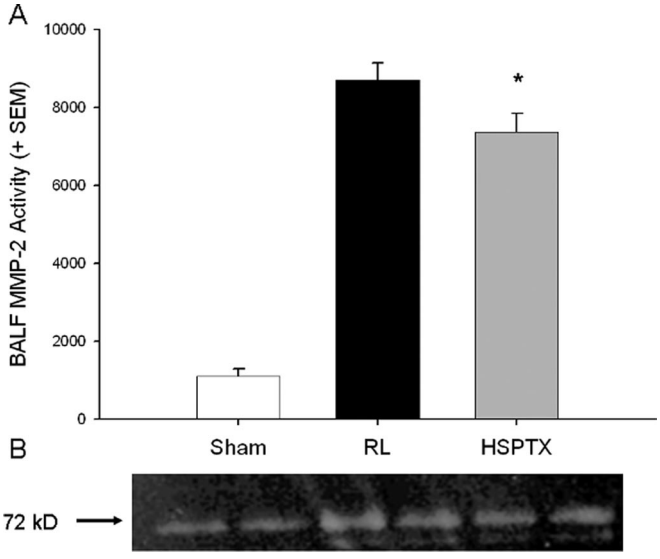

Fig. 2. *BALF MMP-2 activity.* (A) *MMP-2 activity in the BALF was reduced by 23% when HSPTX was used for resuscitation when compared with Ringer's lactate. Each column corresponds to the mean band pixel total + SEM of four independent experiments.* *p = *0.09 versus RL.* (B) *Representative zymogram of MMP-2 activity.*

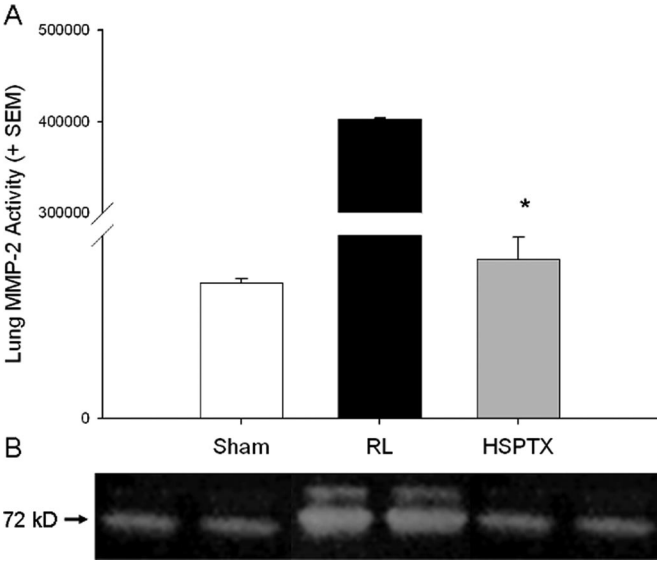

Fig. 3. *Lung MMP-2 activity.* (A) *MMP-2 activity in lung tissue was reduced by 89% when HSPTX was used for resuscitation when compared with Ringer's lactate. Data corresponds to the mean band pixel total + SEM of four independent experiments.* *p $<$ *0.01 versus RL.* (B) *Representative zymogram of MMP-2 activity.*

RESULTS

BALF CINC Content

CINC, as a member of the IL-8 family, regulates chemotaxis and directional neutrophil migration during ischemia-reperfusion injury in the rat lung. At 24 hours after resuscitation, both the RL and HSPTX animals had higher levels of CINC than the Sham group ($p > 0.01$; Fig. 1). HSPTX resuscitation led to a 62% decrease in CINC levels when compared with their RL counterparts (0.63 ± 0.15 ng/mL versus 0.24 ± 0.06 ng/mL; $p < 0.01$).

MMP-2 Activity

Matrix metalloproteinases are released upon neutrophil degranulation and assist neutrophil sequestration in the lung. In the BALF, MMP-2 activity was amplified 67% and 92% for HSPTX and RL-treated animals in comparison to sham animals (7,372 ± 469 and 8,710 ± 436 versus 1,100 ± 194; $p < 0.05$), although activity seen with HSPTX was less than RL ($p = 0.09$; Fig. 2). Similar findings were observed in the lung tissue itself. Resuscitation with RL resulted in a dramatic increase in lung MMP-2 activity (Fig. 3). HSPTX infusion reduced MMP-2 activity by 89%, when compared with RL-treated counterparts (43,476 ± 6,062 versus 402,500 ± 1,586; $p < 0.01$). In addition, the activity of MMP-2 with HSPTX administration was similar to that seen in sham animals (43,476 ± 6,062 versus 36,883 ± 1,242, respectively).

Lung MMP-9 Activity

Lung MMP-9 activity was significantly increased by 64% with RL resuscitation when compared with sham animals ($p < 0.01$; Fig. 4). HSPTX animals demonstrated a marked 76% reduction in activity when compared with those receiving RL (114,473 ± 11,031 versus 491,150 ± 7,692; $p < 0.05$).

Lung Heme Oxygenase-1 Expression

HO-1 serves as a marker of oxidative stress and its activity is induced by the presence of reactive oxygen species. After the insult of ischemia and reperfusion with RL, a sharp increase in HO-1 expression was seen in the lung (Fig. 5). HSPTX was able to attenuate this response by 33%

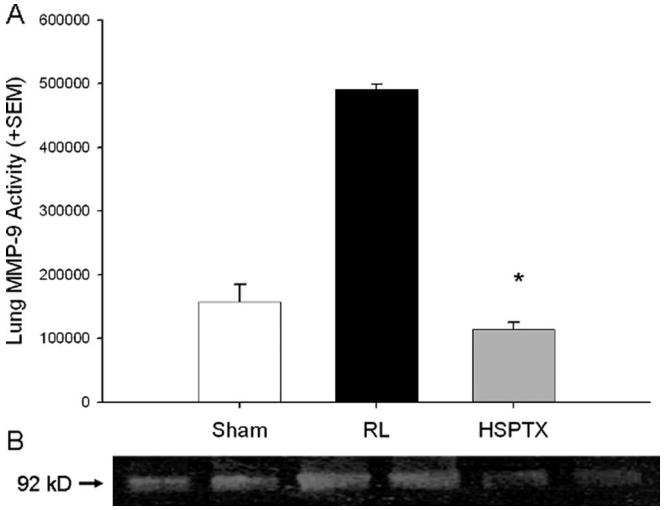

Fig. 4. *Lung MMP-9 activity. (*A*) Lung MMP-9 activity declined by 75% when HSPTX resuscitation was compared with RL. Data corresponds to the mean band pixel total + SEM of four independent experiments. *p < 0.05 versus RL. (*B*) Representative zymogram of MMP-9 activity.*

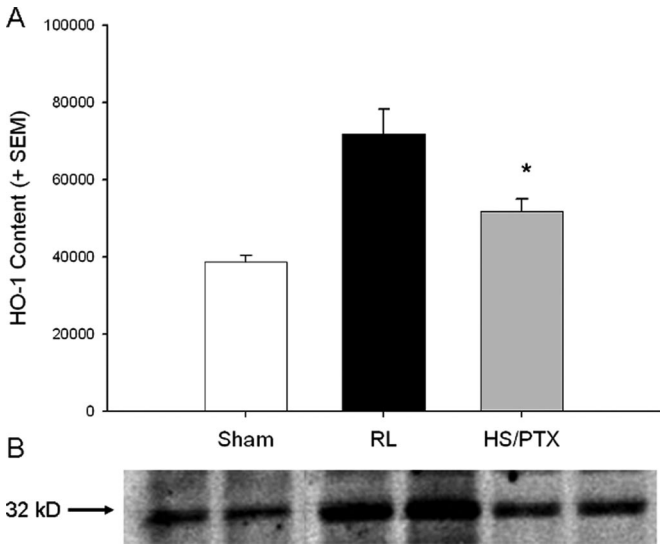

Fig. 5. *Lung hemeoxygenase-1 expression. (*A*) Histogram illustrating the relative decline in HO-1 expression seen with HSPTX. Data represents the mean band pixel total + SEM of four independent experiments. *p < 0.01 versus RL. (*B*) Representative Western blot of lung HO-1 protein after hemorrhagic shock and resuscitation.*

(71,725 ± 6,450 versus 51,842 ± 3,264; $p < 0.01$). In addition, HO-1 levels were similar between the Sham and HSPTX groups.

Lung Heme Oxygenase-1 Immunohistochemistry

Cellular localization for HO-1 was performed to identify the cell populations responsible for its expression in a semi-quantitative manner. Intense staining was seen in those specimens who underwent resuscitation with RL (Fig. 6), which parallels the results seen with the HO-1 western blot. Similar levels of staining were seen in sham and HSPTX sections. HO-1 expression was mainly localized to alveolar macrophages and neutrophils (Fig. 5). However, airway epithelial cells also exhibited apical staining that mirrored the activity seen in the inflammatory cells (Fig. 7). These findings demonstrate the activity of multiple cell populations in response to ALI.

DISCUSSION

Hemorrhagic shock is a major cause of death in the setting of trauma. In contrast to patients who die early as a result of uncontrolled bleeding, those who survive the initial period of shock and resuscitation may go on to develop an uncontrolled systemic inflammatory response that culminates with end organ injury. ROS produced during the reperfusion period are responsible for neutrophil activation which, if unregulated, may result in the development of ALI and ARDS.[4,5]

Recently, conventional fluid resuscitation with RL has been challenged by numerous studies documenting its effects on neutrophil activity and endothelial dysfunction.[16–18] The D- and L-isoforms of lactate are both present in racemic RL classically administered to patients in shock. D-lactate has been implicated as the component responsible for the potentiation of neutrophil oxidative burst seen with RL infusion.[29] These findings have sparked the search for alternative resuscitation strategies utilizing various fluids and pharmacological adjuncts. For these proposed fluid to become clinically applicable, however, they must first be tested against the racemic RL, the current standard resuscitation fluid.

Our laboratory has proposed HSPTX as a small volume resuscitative fluid with anti-inflammatory properties for the treatment of hemorrhagic shock. In animal studies, both racemic RL and HSPTX have the ability to restore blood pressure and perfusion effectively. The severity of histologic lung and intestinal damage, however, was much greater when RL was administered.[28] Therefore, we proposed that HSPTX attenuates resuscitation-induced pulmonary inflammation is associated with the downregulation of oxidative stress, neutrophil function, and proinflammatory mediator synthesis in comparison to RL.

When ischemia-reperfusion occurs, the presence of ROS induces protective mechanisms in the lung, such as the release of HO-1. HO-1, the rate-limiting enzyme in heme degradation, converts heme into biliverdin while producing carbon monoxide.[30] Its role in cytoprotection is mediated through carbon monoxide induced-inhibition of proinflammatory cytokines as well as its antioxidant properties.[11] HO-1 activity is induced through oxidative injury, and its degree of expression correlates with the intensity of the oxidative stress.[15] In our study, HO-1 expression was highest in RL-treated animals, indicating a higher degree of oxidative stress and presence of ROS after reperfusion in that spe-

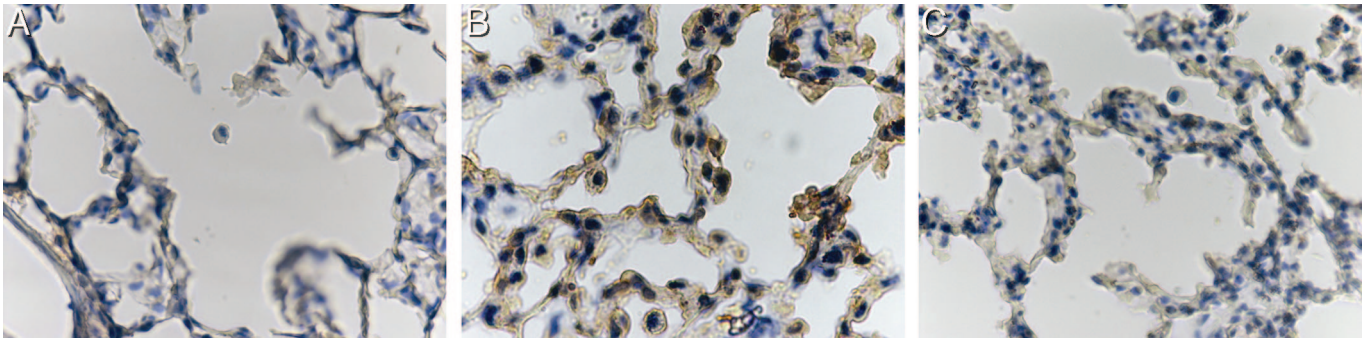

Fig. 6. *Localization of hemeoxygenase-1 in lung tissue. Figures demonstrate representative micrographs (600× magnification) of lung sections with immunohistochemical localization of HO-1 in sham animals (*A*), those treated with RL (*B*), and those resuscitated with HSPTX (*C*). Expression is localized to macrophages and neutrophils and is most intense with RL resuscitation.*

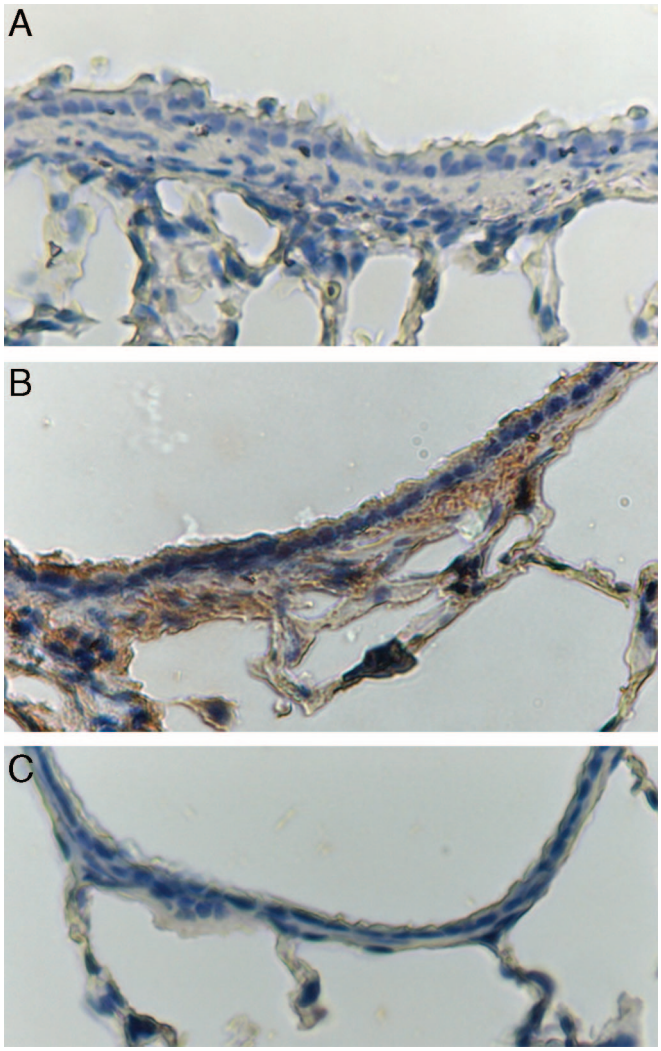

Fig. 7. *Localization of hemeoxygenase-1 in lung tissue. Figure demonstrates representative micrographs (600× magnification) of lung airway epithelial cells located at the top of each panel and the relative expression of HO-1 in sham animals (*A*), those treated with RL (*B*), and those resuscitated with HSPTX (*C*).*

cific treatment arm when compared with either HSPTX or sham animals.

Because elevated levels IL-8 have been demonstrated in trauma patients who go on to develop ARDS and multisystem organ failure, we examined the relative levels of CINC, a marker for a series of chemoattractants found in the rat, in the BALF.[10,31] Although the exact relationship between CINC in rats and IL-8 in humans is unknown, they serve similar roles as signals for the directional migration of neutrophils. Although best known for its role as a stimulant of pulmonary neutrophil sequestration, IL-8 also induces neutrophil lysosomal enzyme release and adhesion molecule expression.[9] In previous studies, inhibition of IL-8 through specific antibodies resulted in attenuation of ARDS in a rabbit endotoxemia model.[32] In this study, HSPTX infusion resulted in a significant reduction in CINC levels when compared with RL. Therefore, the decline in pulmonary reperfusion injury demonstrated with HSPTX is associated with the downregulation of CINC.

The pathogenesis of inflammation requires not only the migration and but also the activation and degranulation of neutrophils. Under normal circumstances, degranulation allows for the targeted delivery of potentially toxic enzymes to the appropriate stimulus while limiting host organ damage. MMP-2 and MMP-9, members of the zinc-dependent endopeptidase family, reside in tertiary granules and function as facilitators of neutrophil sequestration by enzymatically digesting type IV collagen of the extracellular matrix and pulmonary endothelial basement membrane. In the case of ALI, the uncontrolled release of MMPs results in tissue damage and propagation of inflammation, and their relative level of expression associated with the severity of ARDS.[12,14] Metalloproteinase inhibition has been shown to reduce lung injury and improve survival in septic animal models.[33,34] We observed a marked reduction in MMP-2 and MMP-9 activity in the lung in those animals resuscitated with HSPTX. These results indicate that the attenuation of neutrophil degranulation and the downregulatory effects of HSPTX on pulmonary inflammation may be interrelated. Although a decrease in MMP-2 was also evident in the BALF, it was not statistically significant. This observation may be a result of, in part, the

fact that the BALF is a less sensitive marker than actual tissue when measuring cytokine and enzyme levels.

A positive feedback loop between IL-8 and MMP-9 has been described. IL-8 has been shown to induce MMP-9 release through interaction with CXC chemokine receptor 1 and 2 on the surface of neutrophils.[35] In turn, MMP-9 can enzymatically cleave the amino terminal of IL-8, resulting in a tenfold increase in IL-8 activity.[36] Therefore, one may also postulate that the effects of HSPTX on metalloproteinases may potentially be a result of attenuation of IL-8. Further studies are needed to determine a causal relationship.

The mechanism by which HSPTX reduces reperfusion injury is dependent on the action of its two constituents. Both HS and PTX have been shown to improve microcirculatory blood flow in hemorrhagic shock.[37,38] PTX is well known for its hemorheologic properties and has been shown to restore cardiac indices in an in vivo model of shock when infused as an adjunct to RL.[39] More recently, PTX, through nonspecific phosphodiesterase inhibition and attenuation of TNF-α, has been investigated for its broad anti-inflammatory potential in septic and hemorrhagic shock secondary to its effect on neutrophil degranulation, adhesion, and oxidative burst.[1,40] The use of PTX as an adjunct to standard RL resuscitation after shock has been studied by our laboratory and has resulted in similar finding observed with HSPTX in this study (unpublished data). The disadvantage of large-volume resuscitation resulting from the simple combination of PTX and RL still remains. Therefore, intravascular volume expansion with a small volume of HS with PTX is a more attractive alternative.

In addition to its superior effects on volume expansion, HS has the capacity to attenuate neutrophil function. The suppression of neutrophil adhesion and respiratory burst seen with HS in animal hemorrhagic shock models has been attributed to the resultant hypertonicity produced by infusion.[20,41,42] In addition, the transient hyperchloremic acidosis seen with HS infusion improves the acid-base status of the patient more effectively than RL.[43] Thus the combination of HS and PTX combines the advantage of low volume infusions while attenuating the inflammatory cascade involved in reperfusion injury.

Strategies aimed at prevention of gut mucosal injury have been proven effective in the reduction of pulmonary inflammation.[44,45] Intestinal mucosal ischemia leads to the generation of toxic oxygen radicals and proinflammatory mediators that travel through the mesenteric lymph toward the lung where they initiate the activation of macrophages and neutrophils.[46] HS and PTX have each been shown to reduce bacterial translocation in a rat model of hemorrhage,[47] and our laboratory has documented attenuation of intestinal edema, necrosis, and villi disruption when HSPTX is used instead of RL for resuscitation in hemorrhagic shock.[28] Most recently, Cruz et al. have demonstrated significant reductions if the gastric mucosal-arterial P_{CO_2} gradient in an animal model of hemorrhagic shock when HSPTX was used for resuscitation over both RL and HS alone.[48] Further investigations examining the mechanisms by which HSPTX reduces intestinal injury and resultant end-organ damage are currently ongoing in our laboratory.

In conclusion, the reduction in pulmonary inflammation demonstrated with HSPTX is associated with attenuation of oxidative stress, diminished neutrophil degranulation, and chemoattractant production when compared with RL, the current standard resuscitation fluid. This novel resuscitation strategy may have therapeutic potential in the attenuation of ischemia-reperfusion injury seen after severe hemorrhagic shock.

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