ORIGINAL ARTICLE

Reversal of the Effect of Albumin on Gut Barrier Function in Burn by the Inhibition of Inducible Isoform of Nitric Oxide Synthase

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Hypothesis: The use of albumin in the early resuscitation formula after major burn has been forbidden because of its damaging effect on the gut barrier function. We hypothesize that inhibition of the inducible isoform of nitric oxide synthase to stabilize endothelial permeability and to retain albumin in the vascular space will ameliorate the major trauma-induced gut barrier dysfunction.

Design, Interventions, and Main Outcome Measures: In experiment 1, specific pathogen–free rats undergoing 35% total body surface area burn or sham burn were given equal volumes (7.5 mL/kg) of isotonic sodium chloride solution or albumin from femoral veins for fluid resuscitation at 0, 4, or 8 hours after burn. In experiment 2, intraperitoneal *S*-methylisothiourea sulfate (7.5 mg/kg) was given immediately after burn to rats from different groups, as in experiment 1 (SMT groups). At 24 hours after burn, the intestinal mucosa was assayed for myeloperoxidase activity as an index for neutrophil sequestration, the distribution of fluorescein isothiocyanate–dextran across the lumen of small intestine was determined to evaluate the intestinal permeability, and bacterial translocation (BT) to the mesenteric lymph nodes (MLNs) and histological findings in the ileum were also examined.

Results: Compared with sham burn, burn induced significant increases in intestinal mucosa myeloperoxidase activity, intestinal permeability, BT to the MLNs, and villi sloughing in rats. Albumin administration at 0 or 4 hours after burn enhanced the increases in neutrophil sequestration, permeability, and villi sloughing compared with saline injection at the same times. In contrast, injection of albumin in the burn-SMT group did not aggravate these changes in intestinal myeloperoxidase activity, intestinal permeability, BT to the MLNs, and villi edema. Burn-SMT rats with albumin injections at 4 or 8 hours after burn showed significant 35% and 52% decreases, respectively, in intestinal permeability compared with burn-SMT-saline rats. Use of albumin at 8 hours after burn in combination with *S*-methylisothiourea significantly attenuated BT to the MLNs and reduced villi edema.

Conclusions: Early albumin resuscitation aggravated the burn-induced gut damage. Albumin administration and inhibition of the inducible isoform of nitric oxide synthase in combination decreased burn-induced gut barrier dysfunction and reversed the damaging effect of albumin on gut barrier function and decreased BT.

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SEVERE BURN injury produces shock and induces acute gastrointestinal tract derangement that may disrupt gastrointestinal tract mucosa integrity and facilitate bacterial translocation (BT) to the mesenteric lymph nodes (MLNs), liver, and spleen. The magnitude of BT is proportional to the severity of the burn injury.[1] Investigations have shown that the mechanism of BT is due to gut mucosa failure after burn trauma and sepsis.[2] These pathophysiological changes may be the result of mesenteric ischemia and reperfusion injury.[3] Gut ischemia and reperfusion can produce distant organ injury, and the process is partly dependent on neutrophils.[4]

Nitric oxide (NO) was first introduced in 1980 as an endothelium-derived relaxing factor.[5] It is produced from L-arginine by NO synthase (NOS) in all mammalian cells. The 3 forms of NOS in the intestine include a constitutive enzyme normally found in the endothelium lining, a neurally associated constitutive NOS found in neurons of the enteric nervous system, and an inducible enzyme (*i*NOS) that produces large amounts of NO.[6] Basal NO production by constitutive NOS is important in minimizing mucosa and microvascular barrier dysfunction associated with reperfusion of the postischemic intestine.[6] Overproduction of NO may contribute to the intestinal injury after the activation of *i*NOS under the influence of inflamma-

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tory mediators.[7] Studies suggest that inhibition of NO production is beneficial during endotoxemia or sepsis.[8] Although *i*NOS inhibition has been known to ameliorate thermal injury–induced barrier failure,[9] its effect in early albumin treatment of thermal injury has not been investigated.

Albumin plays an important role in the circulation by generating the inward oncotic force that counteracts the outward capillary hydrostatic force. Without albumin, plasma volume cannot be maintained and massive interstitial edema will result.[10] In burn shock that reduces effective circulation, albumin and extracellular fluid are rapidly shifted from plasma and sequestered in interstitial fluid. Hypoalbuminemia is an early and consistent finding after thermal injury and is independently associated with gastrointestinal tract dysfunction and increased rates of infectious morbidity.[11] The use of albumin in the clinical setting continues to generate controversies.[12] The report by the Cochrane Collaboration on the use of albumin in critically ill patients found no evidence showing that albumin administration reduced mortality in patients with hypovolemia, burns, or hypoalbuminemia, but there was a strong suggestion that it might increase mortality.[13] Goodwin et al[14] also found that addition of albumin to crystalloid resuscitation fluids produces no long-lasting benefit on total body blood flow and promotes accumulation of lung water when edema fluid is being reabsorbed from the burn wound in burn patients. However, Carvajal and Parks[15] found that using lactated Ringer solution with albumin did not induce edema in unburned tissues, and it seemed optimal for burn resuscitation by restoring cardiac output and maintaining higher serum albumin levels, colloid osmotic pressure levels, and normal serum osmolality in an ovine burn model.

Besides these disputes and the controversies, many questions remain unanswered. First, will early albumin treatment augment thermal injury–induced gut barrier dysfunction? Second, what is the best fluid resuscitation formula to treat acute major burn injury? Third, if thermal injury–induced systemic hyperpermeability could be ameliorated by *i*NOS inhibitor, can supplemented albumin be retained in the vascular space and reduce the thermal injury–induced gut barrier dysfunction? We hypothesize that inhibition of *i*NOS to stabilize the endothelial permeability and to retain albumin in the vascular space will ameliorate major trauma–induced gut barrier dysfunction. Therefore, by using a model of thermal injury in rats to elucidate the appropriate use of albumin resuscitation, we studied the changes in intestinal myeloperoxidase (MPO) activity, intestinal permeability, and BT to the MLNs after burn when burned rats were treated with albumin alone or in combination with *S*-methylisothiourea sulfate. It is hoped that a better protocol of fluid resuscitation in major trauma and thermal injury could be designed.

METHODS

ANIMALS

Specific pathogen–free Sprague-Dawley rats (weight, 200-275 g) were obtained from the National Laboratory Breeding and Research Center, Taipei, Taiwan. Before the experiments, animals were provided standard rat chow and water ad libitum and housed in a temperature-controlled room with a 12-hour light/dark cycle for at least 1 week. All animal procedures were in compliance with regulations on animals used for experimental and other scientific purposes, approved by the National Sun Yat-Sen University Animal Experiments Committee, Kaohsiung.

EXPERIMENTAL DESIGN

Experiment 1

After the stabilization period, the rats were randomly divided into 1 control group (n=6) and 6 burn groups (n=6 in each) according to the time of saline/albumin administration. The control group was subjected to sham burn, and the burn groups were subjected to a 30% to 35% total body surface area (TBSA) burn injury. All animals received sterile saline for fluid resuscitation right after burn or sham burn. To evaluate the effect of albumin on intestinal neutrophil sequestration and permeability, equal volumes (7.5 mL/kg) of isotonic sodium chloride solution (saline) or human albumin (25% in saline) were given to the burn-saline and burn-albumin groups, respectively, at 0, 4, or 8 hours after burn from the right femoral vein.[16] In the control group, isotonic sodium chloride solution (7.5 mL/kg) was given immediately after the sham burn. All animals were killed at 24 hours after injury, the intestinal mucosa was harvested for MPO assay, MLNs were harvested for BT determination, and midileum tissues were harvested for histological evaluation. The distribution of fluorescein isothiocyanate–dextran (FITC-dextran) across the lumen of small intestine in the rats was measured to assess the intestinal permeability.

Experiment 2

To evaluate the effect of *i*NOS inhibition on intestinal barrier function and BT after injury, the *i*NOS-specific inhibitor *S*-methylisothiourea sulfate dissolved in saline was given to rats intraperitoneally (7.5 mg/kg) immediately after burn (SMT groups). The rats were randomly divided into a sham-SMT group (n=6) and 6 burn-SMT groups (n=6 in each) according to the time of saline or albumin administration (burn-SMT-saline group and burn-SMT-albumin group, respectively) as described in experiment 1. All animals were killed at 24 hours after burn and underwent evaluation for neutrophil sequestration, BT to the MLNs, histological changes, and intestinal permeability.

THERMAL INJURY

The thermal injury procedures were modified from those described by Walker and Mason.[17] The rats were anesthetized with pentobarbital sodium (35 mg/kg) intraperitoneally, and a marked area (mean±SD, 130±15 cm^2) of the shaved dorsal skin was exposed from a wooden template and immersed in 95°C water for 10 seconds. This procedure produced a 30% to 35% TBSA burn of the rats. All animals received intraperitoneal saline (7.5 mL/kg) for fluid resuscitation immediately after the burn or sham treatment. The sham-injured control animals were anesthetized, shaved, and maintained in identical settings except that room temperature water was used for immersion.

TISSUE PREPARATION

The animals were weighed and anesthetized with pentobarbital. The MLNs were removed aseptically and cultured for BT as described by Spaeth et al.[18] The small intestine was dis-

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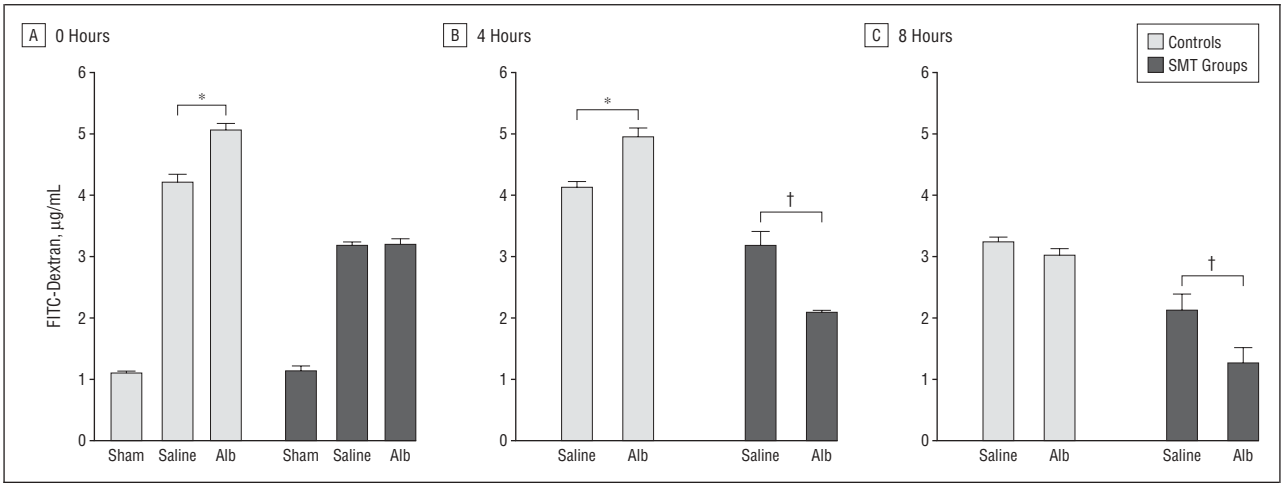

Figure 1. Effect of isotonic sodium chloride solution (saline groups) or albumin (Alb groups) (7.5 mL/kg) supplementation at 0 (A), 4 (B), or 8 (C) hours after thermal injury without or with *S*-methylisothiourea sulfate administration (SMT groups) on the intestinal permeability. Fluorescein isothiocyanate (FITC)–dextran levels in the portal vein 30 minutes after loading into the jejunal loop were measured. Asterisk indicates $P<.05$ vs burn-saline group; dagger, $P<.05$ vs burn-SMT-saline group.

sected out, opened, and cleaned by washing with isotonic sodium chloride solution. The mucosa of the small intestine was taken by scraping with a glass slide, weighed, and homogenized in 3 volumes of ice-cold 50mM Tris hydrochloride buffer (pH, 7.0) containing 300mM sucrose, 1mM dithiothreitol, 100-µg/mL phenylmethanesulfonyl fluoride, 10-µg/mL leupeptin, 10-µg/mL soybean trypsin inhibitor, and 2-µg/mL aprotinin.

DETERMINATION OF INTESTINAL MUCOSA MPO ACTIVITY

The intestinal mucosa was assayed for MPO activity as an index for neutrophil sequestration. Intestinal mucosa was placed in 50mM potassium phosphate buffer (pH, 6.0) with 0.5% hexadecyltrimethylammonium bromide and homogenized. The homogenate was sonicated on ice and centrifuged at 4°C, 3000*g* for 30 minutes. The supernatant (0.1 mL) was added to 2.9 mL of 50mM potassium phosphate buffer (pH, 6.0) containing 0.167-mg/mL *o*-dianisidine and 0.0005% hydrogen peroxide. The rate of change in absorbance at 460 nm was measured for 3 minutes. One unit of MPO activity was defined as the amount of enzyme that reduces 1 mmol of peroxide per minute, and the data were expressed as units per gram of intestinal mucosa.[19,20]

QUANTIFICATION OF INTESTINAL PERMEABILITY

The assay of intestinal permeability was modified from the method described by Otamiri et al.[21] A 20-cm segment of the jejunum was dissected with the beginning at 5 cm distal to the ligament of Treitz with well-protected superior mesenteric vessels. The bilateral end of the isolated jejunum was clamped with rubber bands to prevent the leakage of FITC-dextran. One milliliter of 0.1M phosphate buffer saline (pH, 7.2) containing 25 mg of FITC-dextran (molecular weight, 4400; Sigma-Aldrich Corp, St Louis, Mo) was injected into the lumen. After 30 minutes, a blood sample (100 µL) was taken by a puncture of the portal vein and immediately diluted with 1.9 mL of 50mM Tris (pH, 10.3) containing 150mM sodium chloride. The diluted plasma was centrifuged at 4°C, 3000*g* for 7 minutes, and the supernatant was analyzed for FITC-dextran concentration with a fluorescence spectrophotometer (F-2000; Hitachi Ltd, Tokyo, Japan) at the excitation wavelength of 480 nm and the emission wavelength of 520 nm. Standard curves for calculating the FITC-dextran concentration in the samples were obtained by diluting various amounts of FITC-dextran in a pool of rat plasma, then diluting and centrifuging the standards in the same manner as before measurement.

BT TO THE MLNs

The collected MLNs were weighed and homogenized in 10 volumes of sterile saline. Aliquots (1 mL) of the homogenate from each tissue were plated onto blood agar plates. The plates were examined after aerobic incubation at 37°C for 48 hours. Representative colonies were expressed as colony-forming units (CFU) per gram of organ tissue.

GASTROINTESTINAL TRACT HISTOLOGICAL STUDIES

A longitudinal 1-cm segment of the midileum was harvested from rats in different groups and immersed in 4% formaldehyde solution. The tissue was embedded in paraffin wax, serially sectioned, and stained with hematoxylin-eosin. Intestinal morphological characteristics were evaluated under light microscopy.

STATISTICAL ANALYSIS

All values in the figures are expressed as mean ± SD, and $P<.05$ is considered to be statistically significant. The intestinal mucosa MPO activity, intestinal permeability, and BT between groups were assessed with 1-way analysis of variance, followed by the Scheffé test.

RESULTS

Thermal injury induced significant increases in intestinal mucosa MPO activity, intestinal permeability, and BT to the MLNs in all groups compared with the sham group.

EFFECT OF ALBUMIN, *S*-METHYLISOTHIOUREA, AND *S*-METHYLISOTHIOUREA–ALBUMIN ON INTESTINAL PERMEABILITY

Rats receiving sterile saline at 0, 4, or 8 hours after 30% to 35% TBSA thermal injury showed significant 286%, 278%, and 198% increases ($P<.05$), respectively, in intestinal permeability at 24 hours after thermal injury, as measured by FITC-dextran level (**Figure 1**), compared

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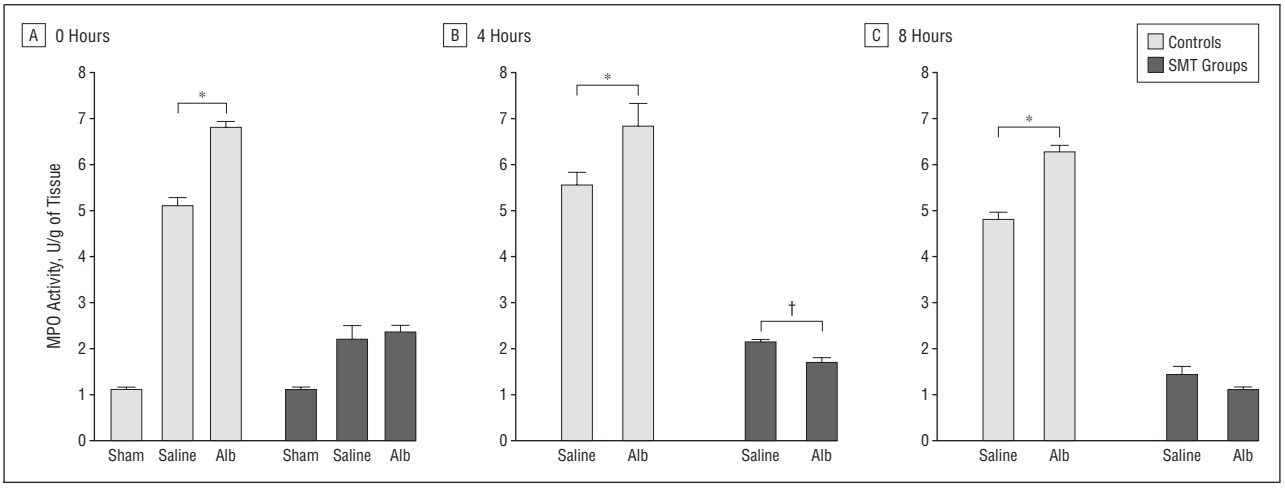

Figure 2. Effect of isotonic sodium chloride solution (saline groups) or albumin (Alb groups) supplementation at 0 (A), 4 (B), or 8 (C) hours after thermal injury without or with *S*-methylisothiourea sulfate administration (SMT groups) on the intestinal myeloperoxidase activity. Asterisk indicates $P<.05$ vs burn-saline group; dagger, $P<.05$ vs burn-SMT-saline group.

with the sham-injured group (4.21±0.13, 4.12±0.1, and 3.25±0.07 µg/mL, respectively, vs 1.09±0.04 µg/mL). As expected, rats receiving albumin at these times after thermal injury also showed significant 364%, 354%, and 178% increases, respectively, in intestinal permeability after thermal injury compared with the sham-injured group (5.06±0.11, 4.95±0.14, and 3.03±0.1 µg/mL, respectively, vs 1.09±0.04 µg/mL). Albumin administration at 0 or 4 hours after burn significantly increased 20% of intestinal permeability compared with administration of saline at the respective times. Sham-SMT animals did not show changes in intestinal permeability. The SMT rats that received saline injection at 0, 4, or 8 hours after injury showed significant decreases of 38%, 34%, and 31% ($P<.05$), respectively, in intestinal permeability compared with rats that received only saline at the same times. The SMT rats undergoing albumin administration at 4 or 8 hours after injury showed further and significant decreases of 35% and 52%, respectively, in intestinal permeability compared with SMT-saline rats. Although SMT rats receiving albumin injection at 0 hours after burn showed no difference in intestinal permeability compared with SMT rats receiving no albumin, there was still a 30% decrease in intestinal permeability of SMT-albumin rats compared with albumin rats.

EFFECT OF ALBUMIN, *S*-METHYLISOTHIOUREA, AND *S*-METHYLISOTHIOUREA–ALBUMIN ON INTESTINAL NEUTROPHIL ACCUMULATION

Rats receiving sterile saline at 0, 4, or 8 hours after 30% to 35% TBSA thermal injury showed significant 364%, 439%, and 326% increases, respectively, in intestinal neutrophil accumulation as assessed by MPO assay at 24 hours after burn compared with sham-injured rats (**Figure 2**). Rats injected with albumin at the same times after burn enhanced the significant increases in intestinal mucosa MPO activity to 577%, 632%, and 510%, respectively. This means that albumin resuscitation at 0, 4, or 8 hours after burn significantly increased intestinal mucosa MPO activity by 46%, 37%, and 43%, respectively, compared with saline resuscitation, suggesting an adverse effect of albumin supplementation on intestinal neutrophil accumulation after burn. Intraperitoneal injection of *S*-methylisothiourea to sham-injured animals did not change the intestinal mucosa MPO activities. The SMT rats that received saline at 0, 4, or 8 hours after thermal injury showed significant 57%, 64%, and 69% decreases, respectively, in intestinal mucosa MPO activity compared with the rats that received sterile saline only at the same times. Although only SMT rats that received albumin at 4 hours after injury showed a significant 20% decrease in intestinal mucosa MPO activity compared with SMT-saline rats, there were significant decreases in MPO activities of SMT-albumin rats compared with albumin rats. This finding suggests that *S*-methylisothiourea administration could eliminate the adverse effect of albumin resuscitation on intestinal neutrophil sequestration after burn.

EFFECT OF ALBUMIN, *S*-METHYLISOTHIOUREA, AND *S*-METHYLISOTHIOUREA–ALBUMIN ON BT TO THE MLNs

Sham-injured rats showed no BT to the MLNs (**Figure 3**), but rats receiving sterile saline at 0, 4, or 8 hours after burn showed BT to the MLNs (837.5±59.1, 785±46.55, and 697.5±17.08 CFU/g of tissue, respectively) at 24 hours after injury. Rats receiving albumin at the same times after injury showed no significant changes in BT when compared with the saline groups. However, rats receiving saline or albumin at 8 hours after burn significantly decreased 17% or 18% of BT, respectively, compared with rats receiving saline or albumin at 0 hours after burn. The SMT rats that received saline at 0, 4, or 8 hours after thermal injury showed significant decreases in BT to the MLNs (18%, 16%, and 26%, respectively) compared with rats receiving saline only at the same times after burn. In contrast, only SMT rats that received albumin injection at 8 hours after injury showed a dramatic effect with an 88% decrease of BT compared with SMT rats that received saline at the same times.

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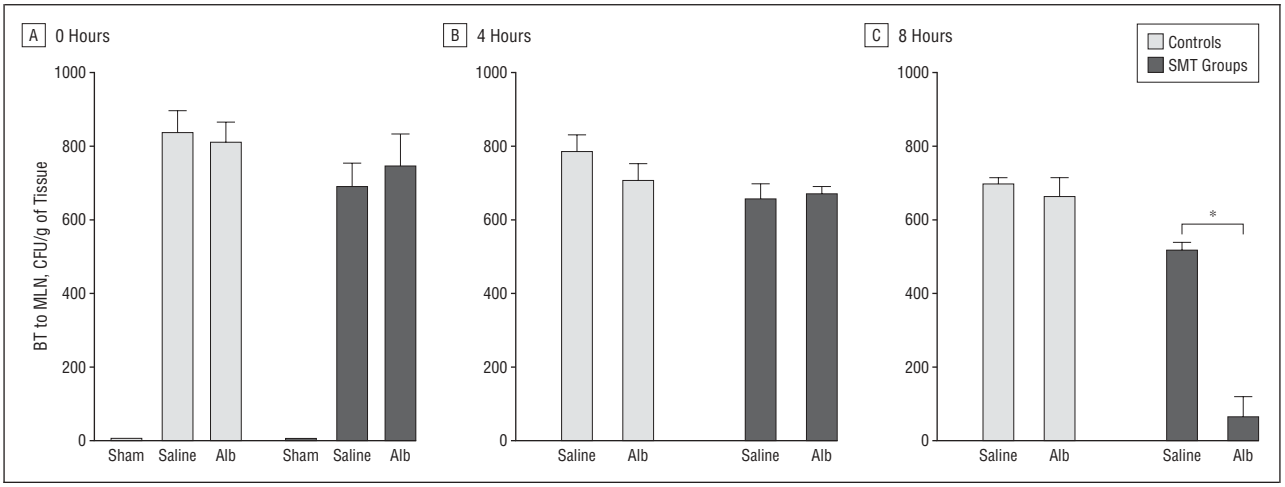

Figure 3. Effect of isotonic sodium chloride solution (saline groups) or albumin (Alb groups) supplementation at 0 (A), 4 (B), or 8 (C) hours after thermal injury without or with *S*-methylisothiourea sulfate administration (SMT groups) on the bacterial translocation (BT) to mesenteric lymph nodes (MLN) (expressed as colony-forming units [CFU] per gram of tissue). Asterisk indicates $P<.05$ vs burn-SMT-saline group.

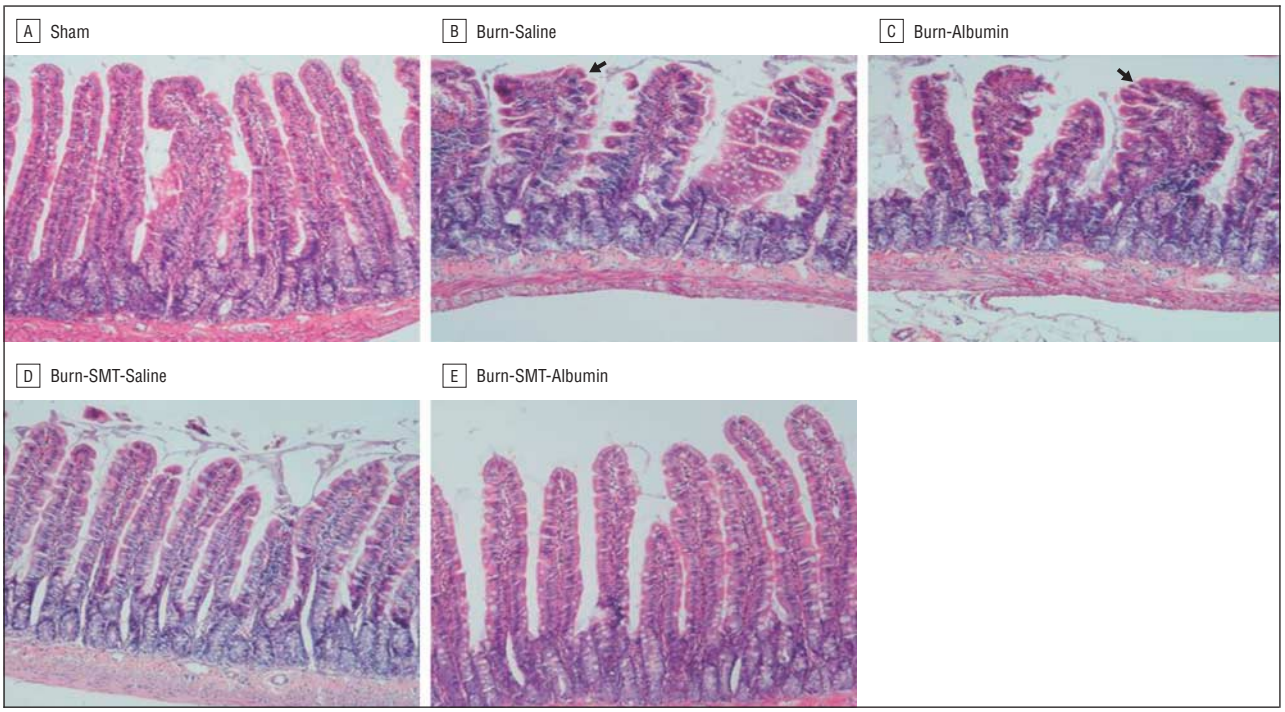

Figure 4. Morphological characterization of ileum from the sham (A), burn–isotonic sodium chloride solution (burn-saline) (B), burn-albumin (C), burn–*S*-methylisothiourea sulfate (SMT)–saline (D), and burn-SMT-albumin (E) groups. Arrows show the atrophy and edema (hematoxylin-eosin, original magnification ×200). The 5 views are each representative of 3 distinct sections prepared from 3 different rats per group and analyzed in a masked fashion.

HISTOLOGICAL ANALYSIS

The midileum of the rats with 30% to 35% TBSA thermal injury that received saline injection (burn-saline group) (**Figure 4**B) showed severe edema and sloughing of the villous tips compared with sham-injured animals (Figure 4A). The rats that received albumin after burn (burn-albumin group) showed a more severe decrease in height and more atrophy and edema of the villous tips compared with the burn-saline group (Figure 4C). The rats that received SMT immediately after burn and saline injection (burn-SMT-saline group) showed less edema and sloughing of the villous tips compared with the burn-saline group (Figure 4D). The rats that received SMT immediately after burn and albumin injection (burn-SMT-albumin group) showed an even more normal appearance of intestinal mucosa compared with the burn-SMT-saline group (Figure 4E).

COMMENT

Burn injury induces immune suppression and increases the susceptibility to infection. The potential cause of sepsis and subsequent multiple-organ failure after thermal injury has been proposed to be the failure of intestinal mucosa to act as a barrier against BT. Neutrophil deposition in tissues (leu-

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kosequestration) after shock may produce local tissue injury from proteases and high-energy oxygen species released from sequestered neutrophils.[16,19] An absence of neutrophils prevents the loss of intestinal vascular barrier properties only in the initial periods after thermal injury.[20] Increased intestinal epithelial permeability is considered to be a quantitative index of injury or dysfunction of the intestinal mucosal barrier. Thermal injury is associated with mesenteric vasoconstriction that leads to damage of the gut mucosa and dysfunction of the gut barrier, resulting in an increased gut permeability and absorption of bacteria and bacterial toxins.[22] Our data demonstrated that 30% to 35% TBSA thermal injury induced neutrophil infiltration in intestinal mucosa, intestinal hyperpermeability, and BT to the MLNs in rats.

Thermal injury leads to a transient capillary leak that recovers exponentially in about 24 hours and peaks between 3 and 6 hours after burn.[23] Although many thousands of burn patients have been saved after the use of fluid resuscitation since early 1950s, the resuscitation formula used has been disputed. The Mount Vernon formula, consisting of colloid-based resuscitation, was mostly used in the burn centers in Great Britain, and the Parkland formula, consisting of crystalloid-based resuscitation, was used in the burn centers in the United States and the rest of the world to treat patients with major burns in the first 24 hours after burn. The effect of albumin treatment on gut neutrophil sequestration and barrier function after burn is still not clear. Sheridan et al[24] found that hypoalbuminemia, defined as a plasma albumin level of 1.0 to 2.5 g/dL, does not have adverse effects on gut function in children with greater than 40% TBSA burns during the initial 4 weeks of care. O'Brien et al[11] studied the effects of human albumin resuscitation on burn-induced immunosuppression, BT, and absorption of gut endotoxin in rats with 20% TBSA scald burn injury and found that animals resuscitated with albumin experienced similar degrees of BT compared with those without albumin supplement. However, burn surgeons consider albumin resuscitation to be unnecessary in the first 24 hours, as albumin will leak from the capillaries of the burned and unburned tissue into the extravascular space and increase the edema. Experiments also showed that restoration and maintenance of plasma protein content is not effective until 8 hours after burn.[25] Our data showed that early albumin supplementation at 0, 4, or 8 hours after 30% to 35% TBSA burn markedly increased neutrophil sequestration in intestinal mucosa. However, only albumin supplementation at 0 or 4 hours after thermal injury aggravated the increase in intestinal permeability. The deteriorating effect of early albumin treatment on gut barrier function was confirmed by histological findings of a more severe decrease in height and of atrophy and edema of the villi in the burn-albumin group compared with the burn-saline group. Therefore, albumin supplementation within the first 8 hours after thermal injury is harmful to the gut barrier function in our model.

It is known that NO produced by *i*NOS plays an important role in the changes in systemic and pulmonary microvascular permeability in combined smoke inhalation and third-degree burn injury.[26] Also, lipopolysaccharide-induced increased vascular permeability is known to be mediated by the NO that is produced by *i*NOS.[27] Arkovitz et al[28] had shown that *S*-methylisothiourea administration could prevent lipopolysaccharide-induced vascular leakage in the lung. In the present study, burned rats injected with a single intraperioneal bolus of *S*-methylisothiourea sulfate (7.5 mg/kg) immediately after injury and supplemented with albumin at 8 hours after burn showed significantly improved intestinal neutrophil sequestration, intestinal hyperpermeability, and BT. These observations suggest that albumin has significant beneficial effects on the improvement of thermal injury–induced intestinal dysfunction in rats as long as *i*NOS activity is suppressed immediately after burn. The beneficial effects of albumin use are less profound if the time interval between albumin resuscitation and *S*-methylisothiourea injection is shortened. Our results show that inhibition of *i*NOS stabilizes the microvascular permeability to keep the albumin in the intravascular space and reverses the effects of albumin on thermal injury–induced gut barrier dysfunction from deterioration to amelioration. Retention of albumin in the vessel by the *i*NOS inhibition generated the inward oncotic force and stabilized the hemodynamic change in gut vasculature; otherwise, massive interstitial edema and neutrophil infiltration would result. In addition, use of albumin in *S*-methylisothiourea–treated burned rats decreased intestinal neutrophil accumulation, intestinal permeability, and BT and prevented thermal injury–induced villi edema and epithelial cell sloughing. Previously, our group showed that injection of intraperitoneal *S*-methylisothiourea sulfate (5 mg/kg every 12 hours for 2 days) could suppress intestinal mucosa *i*NOS activity, thermal injury–induced intestinal permeability increase, and BT.[9] In the present study, by using the synergistic effect of *i*NOS inhibition and albumin, we demonstrated that thermal injury–induced gut barrier dysfunction could be prevented. Clinical burn treatment suggests that albumin supplementation should not begin until 16 hours after burn, because early albumin treatment will increase fluid leakage and edema. From our results, early albumin resuscitation in patients with major burn could be helpful if *i*NOS is inhibited as early as possible after burn. This use of *i*NOS inhibition and albumin in fluid resuscitation might change the fluid resuscitation protocol and decrease the mortality rate in patients with major burn.

Collectively, thermal injury induces intestinal mucosa neutrophil deposition, intestinal permeability, BT, and villi sloughing. Restoration of extracellular fluid in early burn shock with albumin supplementation augments the intestinal damage. Inhibition of *i*NOS before albumin supplementation decreases intestinal mucosa neutrophil infiltration, permeability, and BT and reverses albumin's effects on burn-induced gut barrier dysfunction from damaging to beneficial. Albumin's beneficial effects may be a result of the prevention of permeability increase induced by thermal injury and the consequent retention of albumin in plasma during albumin resuscitation.

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REFERENCES

1. Gianotti L, Alexander JW, Pyles T, James L, Babcock GF. Relationship between extent of burn injury and magnitude of microbial translocation from the intestine. *J Burn Care Rehabil.* 1993;14:336-342.
2. Baron P, Traber LD, Traber DL, et al. Gut failure and translocation following burn and sepsis. *J Surg Res.* 1994;57:197-204.
3. Tokyay R, Zeigler ST, Traber DL, et al. Postburn gastrointestinal vasoconstriction increases bacterial and endotoxin translocation. *J Appl Physiol.* 1993;74:1521-1527.
4. Takeda K, Shimada Y, Okada T, Amano M, Sakai T, Yoshiya I. Lipid peroxidation in experimental septic rats. *Crit Care Med.* 1986;14:719-723.
5. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature.* 1980;288:373-376.
6. Miller MJ, Sadowska-Krowicka H, Chotinaruemol S, Kakkis JL, Clark DA. Amelioration of chronic ileitis by nitric oxide synthase inhibition. *J Pharmacol Exp Ther.* 1993;264:11-16.
7. Goode HF, Howdle PD, Walker BE, Webster NR. Nitric oxide synthase activity is increased in patients with sepsis syndrome. *Clin Sci (Lond).* 1995;88:131-133.
8. Sorrells DL, Friend C, Koltuksuz U, et al. Inhibition of nitric oxide with aminoguanidine reduces bacterial translocation after endotoxin challenge in vivo. *Arch Surg.* 1996;131:1155-1163.
9. Chen LW, Hsu CM, Cha MC, Chen JS, Chen SC. Changes in gut mucosal nitric oxide synthase (NOS) activity after thermal injury and its relation with barrier failure. *Shock.* 1999;11:104-110.
10. Carsin H. Human albumin solutions in the treatment of burned patients: current indications [in French]. *Presse Med.* 1997;26:474-476.
11. O'Brien R, Murdoch J, Kuehn R, Marshall JC. The effect of albumin or crystalloid resuscitation on bacterial translocation and endotoxin absorption following experimental burn injury. *J Surg Res.* 1992;52:161-166.
12. Erstad BL, Gales BJ, Rappaport WD. The use of albumin in clinical practice. *Arch Intern Med.* 1991;151:901-911.
13. Cochrane Injuries Group Albumin Reviewers. Human albumin administration in critically ill patients: systematic review of randomised controlled trials. *BMJ.* 1998;317:235-240.
14. Goodwin CW, Dorethy J, Lam V, Pruitt BA Jr. Randomized trial of efficacy of crystalloid and colloid resuscitation on hemodynamic response and lung water following thermal injury. *Ann Surg.* 1983;197:520-531.
15. Carvajal HF, Parks DH. Optimal composition of burn resuscitation fluids. *Crit Care Med.* 1988;16:695-700.
16. Hansbrough JF, Wikstrom T, Braide M, et al. Effects of E-selectin and P-selectin blockade on neutrophil sequestration in tissues and neutrophil oxidative burst in burned rats. *Crit Care Med.* 1996;24:1366-1372.
17. Walker HL, Mason AD Jr. A standard animal burn. *J Trauma.* 1964;8:1049-1051.
18. Spaeth G, Berg RD, Specian RD, Deitch EA. Food without fiber promotes bacterial translocation from the gut. *Surgery.* 1990;108:240-247.
19. Baskaran H, Yarmush ML, Berthiaume F. Dynamics of tissue neutrophil sequestration after cutaneous burns in rats. *J Surg Res.* 2000;93:88-96.
20. Sir O, Fazal N, Choudhry MA, Goris RJ, Gamelli RL, Sayeed MM. Role of neutrophils in burn-induced microvascular injury in the intestine. *Shock.* 2000;14:113-117.
21. Otamiri T, Sjodahl R, Tagesson C. An experimental model for studying reversible intestinal ischemia. *Acta Chir Scand.* 1987;153:51-56.
22. Chen LW, Hsu CM, Wang JS, Chen JS, Chen SC. Specific inhibition of *i*NOS decreases the intestinal mucosal peroxynitrite level and improves the barrier function after thermal injury. *Burns.* 1998;24:699-705.
23. Judkins K. Burns resuscitation: what place albumin? *Hosp Med.* 2000;61:116-119.
24. Sheridan RL, Prelack K, Cunningham JJ. Physiologic hypoalbuminemia is well tolerated by severely burned children. *J Trauma.* 1997;43:448-452.
25. Demling RH, Kramer G, Harms B. Role of thermal injury–induced hypoproteinemia on fluid flux and protein permeability in burned and nonburned tissue. *Surgery.* 1984;95:136-144.
26. Soejima K, Traber LD, Schmalstieg FC, et al. Role of nitric oxide in vascular permeability after combined burns and smoke inhalation injury. *Am J Respir Crit Care Med.* 2001;163:745-752.
27. Fujii E, Yoshioka T, Ishida H, Irie K, Muraki T. Evaluation of *i*NOS-dependent and independent mechanisms of the microvascular permeability change induced by lipopolysaccharide. *Br J Pharmacol.* 2000;130:90-94.
28. Arkovitz MS, Wispe JR, Garcia VF, Szabo C. Selective inhibition of the inducible isoform of nitric oxide synthase prevents pulmonary transvascular flux during acute endotoxemia. *J Pediatr Surg.* 1996;31:1009-1015.

Correction

Numerical Error. In the Special Article by Cottam et al published in the April issue of the ARCHIVES (2003;138:367-375), the value of the second entry in Table 4 on page 372 under the column titled "Mean Length of Hospital Stay, d" should have been 3.6 rather than 2.6.

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