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Alcohol potentiates post burn remote organ damage through shifts in fluid compartments mediated by bradykinin

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Abstract

Of the 450,000 burn patients each year, 50% have a positive blood alcohol content and this predisposes them to worsened clinical outcomes. Despite high prevalence and established consequences, the mechanisms responsible for alcohol-mediated complications of post burn remote organ damage are currently unknown. To this end, mice received a single dose of alcohol (1.12 g/kg) or water by oral gavage and were subjected to a 15% total body surface area burn. Animals with a burn alone lost ~5% of their body weight in 24 hours whereas intoxicated and burned mice lost only 1% body weight ($p<0.05$) despite a 17% increase in hematocrit ($p<0.05$) and a 57% increase in serum creatinine ($p<0.05$) over burn injury alone. This retention of water weight despite increased dehydration suggests that intoxication at the time of a burn causes a shift in fluid compartments that may exacerbate end organ ischemia and damage as evidenced by a 3-fold increase in intestinal bacterial translocation ($p<0.05$), a 30% increase ($p<0.05$) in liver weight to body weight ratio, and an increase in alveolar wall thickness over a burn alone. Furthermore, administration of the bradykinin antagonist HOE140 30 minutes after intoxication and burn restored fluid balance and alleviated end organ damage. These findings suggest that alcohol potentiates post burn remote organ damage through shifts in fluid compartments mediated by bradykinin.

Keywords

intoxication; trauma; third spacing; ischemia; ethanol

Introduction

Remote organ damage after burns can be caused by aberrations in the inflammatory response, known as the systemic inflammatory response syndrome (SIRS), though the exact

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mechanisms are currently unknown. The physiologic response to a burn is dependent on the age of the patient, the size and severity of the injury, and patient-specific risk factors such as alcohol use. Alcohol is the most abused substance in the US and the third leading cause of preventable death (1), much of which is associated with unintentional injuries (2). Binge drinking in particular is an increasingly prevalent form of intoxication (3) and is the characteristic pattern of drinking among patients presenting with traumatic injury, including burns (4). Nearly 50% of patients admitted for burns have a positive blood alcohol content (BAC) at the time of admission and this predisposes them to worsened clinical outcomes compared to patients with similar injuries not under the influence of alcohol (5). Specifically, intoxicated patients were found to be twice as likely to acquire an infection, required 60% more surgical procedures, had longer durations of stay in the intensive care unit, and generated more cost than their non-intoxicated counterparts (6, 7). With nearly 450,000 burns requiring medical attention each year in the American healthcare system (8), alcohol greatly contributes to the socioeconomic burden of this destructive injury as both a causative agent and complicating factor in recovery. Despite the high prevalence and established consequences of being intoxicated at the time of a burn injury, there are currently few differences in the treatment and management of burn patients with and without prior alcohol exposure. This may be due in part to the dynamic natural history of burns as well as the complex and duration dependent effects of alcohol. In order to develop much needed targeted therapies, the effects of intoxication on the physiologic response to burn injury need to be studied and manipulated under controlled conditions.

Animal models have provided meaningful insight into the pathophysiology of burn injuries preceded by intoxication. Previous studies in our laboratory and others have demonstrated that relative to either insult alone, the combined injury of intoxication and burn results in elevated levels of neutrophil infiltration and edema in the lung (9), which occurs in an interleukin-6 (IL-6) dependent manner (10). Furthermore, this pulmonary inflammation is accompanied by an increased susceptibility to pulmonary bacterial infections and decreased oxygen saturation compared to non-intoxicated controls (11). This correlates with clinical data linking elevated serum IL-6 in trauma patients to mortality risk (12) as well as findings that burn patients with a positive BAC acquire more pulmonary infections (13). Despite this knowledge, there remains a need to address the currently unknown mechanism by which alcohol intoxication worsens the post burn response and outcome. The need for such understanding is highlighted by the failure of single cytokine therapies in animal and clinical studies of similar injuries leading to SIRS (14). Clinical data demonstrates intoxicated burn patients require more aggressive fluid resuscitation than their non-intoxicated counterparts (6) which may indicate some degree of hypovolemia. This is of significant concern as early ischemia-reperfusion injury may be the inciting event that initiates SIRS, which is linked to pulmonary failure and ARDS (15). One mechanism by which systemic capillary leakiness in this setting may ensue is through the vasoactive mediator, bradykinin. No one to date has investigated the role of bradykinin signaling in the setting of intoxication and burn but alcohol is known to potentiate the action of bradykinin (16) while burn injuries cause systemic bradykinin production (17). To this end, the studies outlined herein examined a possible mechanism by which alcohol exacerbates post burn remote organ damage through potentiating third spacing of fluid and enhancing ischemic damage.

Materials and Methods

Animals

Male C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME) and sacrificed when 8–10 weeks old. Mice were housed in sterile micro-isolator cages under specific pathogen-free conditions in the Loyola University Chicago Comparative Medicine Facility. All experiments were conducted with approval of and strict accordance to the Loyola Institutional Animal Care and Use Committee.

Murine model of intoxication and burn injury

A murine model of a single binge ethanol exposure and burn injury was employed as described previously (18) with minor modifications. Briefly, mice were given a single dose of ethanol (1.12 g/kg) by oral gavage that results in a blood alcohol concentration of approximately 180 mg/dl at 30 minutes (19). The mice were then anesthetized (100 mg/kg ketamine and 10 mg/kg xylazine), their dorsum shaved, weighed, and placed in a plastic template exposing 15% of the total body surface area and subjected to a scald injury in a 92°C water bath or a sham injury in room-temperature water. The scald injury resulted in an insensate, full-thickness burn (20). The mice were then resuscitated with 1.0 ml of 0.9% normal saline and placed on warming pads until recovered from anesthesia. At 30 minutes after burn injury, a separate group of mice was given the selective bradykinin receptor antagonist HOE 140 or saline control. HOE 140 stock solution (0.083 mg/mL) was prepared in saline and administered as a 150 µL intraperitoneal injection to achieve a dose of approximately 0.5 mg/kg. Mice were sacrificed by carbon dioxide narcosis followed by exsanguination 24 hours after injury. All experiments were performed a minimum of three times with one representative data set shown.

Blood and Serum measurements

Blood was collected via cardiac puncture and an aliquot was placed into a microcapillary tube then measured for hematocrit by Hemavet (Drew Scientific, Dallas, TX). The remaining blood was harvested for serum by centrifugation after clotting. Serum aliquots were used to measure blood urea nitrogen and creatinine levels using a DRI-CHEM 7000 (HESKA, Loveland, CO).

Bacterial translocation

Bacterial translocation was assessed as previously described (21, 22). Briefly, 3–5 mesenteric lymph nodes (MLN) per mouse were removed, placed in cold RPMI, and kept on ice. MLN were separated from connective tissue and mechanically homogenized in RPMI using frosted glass slides. Homogenates were plated in triplicate on tryptic soy agar and placed in a 37°C incubator overnight. Colonies were counted the following day, averaged, and divided by the total number of lymph nodes harvested.

Histopathologic examination of the liver and lungs

The whole liver was removed at the time of sacrifice, weighed, and normalized to the total body weight. The upper right lobe of the lung was inflated with 10% formalin and fixed

overnight as described previously (23). The lung was then embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin (H&E). Representative images were taken at 400x magnification.

Statistical analysis

Statistical comparisons (GraphPad Instat) were made between the sham vehicle, sham ethanol, burn vehicle, and burn ethanol treatment groups, resulting in four total groups analyzed. One-way analysis of variance (ANOVA) was used to determine differences between treatment responses, and Tukey's post hoc test once significance was achieved ($p < 0.05$). Statistical comparisons made between the intoxicated burned mice with saline or HOE140 treatment were performed using a Student's t-test and Tukey's post hoc test ($p < 0.05$). Data are reported as mean values $\pm$ the standard error of the mean (SEM).

Results

Intoxication increases post burn dehydration despite fluid retention

Sham injured animals with and without intoxication lost approximately 6% of their total body weight 24 hours after injury, presumably due to effects of anesthesia and stress of physical manipulation (Figure 1A). Similarly, mice receiving a burn alone lost $>5\%$ weight. When intoxication preceded the burn injury, however, mice lost 78% ($p < 0.05$) less body weight than sham injured animals and 73% ($p < 0.05$) less than mice receiving a burn alone (Figure 1A). Despite this apparent retention of water weight, mice undergoing the combined injury of intoxication and burn had an approximate 30% increase ($p < 0.05$) in hematocrit compared to sham injured animals regardless of alcohol exposure and a 17% increase ($p < 0.05$) over a burn alone (Figure 1B). Similarly, intoxication and burn raised serum creatinine levels 3-fold ($p < 0.05$) over sham injury and 1.5-fold ($p < 0.05$) over burn alone (Figure 1CA). A 2.3-fold increase ($p < 0.05$) in serum blood urea nitrogen (BUN) was found after a burn alone compared to sham injury which was raised an additional 1.3-fold when preceded by intoxication (Figure 1D).

Remote organ damage is increased when intoxication precedes burn

Bacterial translocation to the mesenteric lymph nodes was increased >15 -fold ($p < 0.05$) after a burn injury compared to sham injured animals with and without alcohol exposure, and increased an additional 3-fold ($p < 0.05$) when ethanol preceded the burn (Figure 2A). The increase in bacterial translocation with the combined injury was accompanied by a 40% ($p < 0.05$) and 30% ($p < 0.05$) increase in the relative size of the liver compared to sham injury and burn alone, respectively (Figure 2B). Histologic images of the lungs 24 hours after injury demonstrate an increase in alveolar wall thickness and cellularity with a burn alone which is exacerbated with antecedent intoxication (Figure 2C).

Bradykinin antagonism partially restores fluid balance after intoxication and burn

HOE140 treatment 30 minutes after intoxication and burn partially normalized weight loss at 24 hours injury as evidenced by a 10-fold ($p < 0.05$) increase in weight loss compared to mice given saline vehicle (Figure 3A). Bradykinin antagonism with HOE140 also alleviated dehydration after the combined injury as seen by a 12% decrease ($p < 0.05$) in hematocrit

(Figure 3B), 20% reduction ($p<0.05$) in serum creatinine (Figure 3C) and 20% lower ($p<0.05$) BUN (Figure 3D).

Bradykinin antagonism attenuates end organ damage after intoxication and burn

Early treatment with HOE140 after intoxication and burn injury led to a 47% reduction ($p<0.05$) in bacterial translocation to the mesenteric lymph nodes (Figure 4A), a 20% decrease ($p<0.05$) in liver weight to body weight ratio (Figure 4B) and attenuated pulmonary congestion as seen by a diminution of alveolar wall thickness and cellularity (Figure 4C).

Discussion

Burns are a devastating and dynamic injury involving potential complications in every major organ system including cardiac impairment, renal dysfunction, intestinal damage, hepatic derangements and pulmonary failure (17). Of particular note is the interaction between the intestinal microbiome and the liver, known as the gut-liver axis, which helps regulate an immune response with potential systemic consequences. Systemic inflammatory response syndrome (SIRS) is a common complication after a severe burn injury and can lead to substantial sequelae from sterile inflammation, such as acute respiratory distress syndrome (ARDS), or to prolonged immunosuppression with increased susceptibility to infection (24). Alcohol exposure on its own predisposes to ARDS (25) and burn patients who were intoxicated at the time of their injury have greater incidences of infection and require more aggressive antibiotic therapy (7). Animal studies have demonstrated that relative to either insult alone, intoxication prior to burn results in elevated pulmonary inflammation (10) in part caused by hepatic mediators produced in response to intestinal barrier breakdown (19). Furthermore, limiting bacterial translocation after injury attenuates damage in the liver and lungs, highlighting the role of the gut-liver axis in the setting of intoxication and burn (26). Therefore, understanding the mechanism by which alcohol exacerbates intestinal injury after a burn could have significant impact on the management of this at-risk patient population for which there lacks appropriate treatment options.

Full-thickness burns can induce excessive vascular permeability both at the site of injury and in remote organs, leading to detrimental hypodynamic changes in circulation (17). Burn patients who were intoxicated at the time of their injury require greater amounts of fluid resuscitation (6), perhaps indicating a greater degree of fluid extravasation. This is supported by findings contained herein where mice given ethanol and burn injury have increased hematocrit, creatinine, and BUN compared to mice given either ethanol or burn alone (Figure 1B–D). Interestingly, the combination of intoxication and burn led to weight retention 24 hours after injury (Figure 1A). This apparent retention of fluid despite increased dehydration suggests that intoxication at the time of a burn causes a shift in fluid compartments that may exacerbate end organ ischemia and damage in the intestines (Figure 2A). Catecholamine-mediated vasoconstriction of the splanchnic vasculature makes the intestines particularly susceptible to hypovolemia (27), made worse by third spacing of fluid as evidenced by edema in the liver (Figure 2B) and lungs (Figure 2C). One mechanism by which systemic capillary leakiness in this setting may ensue is through the action of the vasoactive agent, bradykinin.

Bradykinin is a potent vasoactive agent produced systemically after a burn with effects of vasodilation and vascular permeability (17). The effects of bradykinin are mediated by two cellular receptors classified as B₁ and B₂. B₁ receptors are present only at low levels in healthy tissues while B₂ receptors are constitutively expressed in most tissues. Acute inflammatory responses such as edema formation are mediated through B₂ receptors whereas chronic inflammation is associated with B₁ activity (28). Accordingly, most experimental and clinical studies examining bradykinin antagonism in an acute inflammatory setting have focused on B₂ receptor blockade. HOE140 is a potent, selective, and long-acting B₂ receptor antagonist and has been used to treat experimental sepsis (29), acute pancreatitis (30), and local inflammation (31) or microvascular changes (32) after thermal injury though with varying degrees of success. B₂ receptor antagonism may be particularly suited to treat the common clinical scenario of intoxication and burn injury because burn injuries cause systemic bradykinin production (17), while its action is known to be potentiated in the presence of alcohol (16), perhaps affording bradykinin undue influence on vascular permeability after this combined injury. In agreement with this, we found that B₂ blockade early after intoxication and burn restores fluid balance and attenuates end organ damage. This highlights the early important role of bradykinin after burn with antecedent alcohol exposure and also suggests third spacing is at least partially responsible for the increased remote organ damage in this setting.

The pathophysiology of both binge intoxication and burn injury converge on bradykinin signaling and could explain numerous observations in animal models and patients as described above. The exacerbated injury in the intestines, liver, and lungs of mice when ethanol precedes burn injury is well documented and agrees with and expands upon the findings of Figure 2. Therapeutic interventions in animals to date have focused on mitigating events downstream of the initial injury, such as manipulating levels of IL-6 (10), IL-18 (33), intracellular adhesion molecule-1 (ICAM-1) (34), and Toll-like receptor 4 (TLR4) (9). While some of these interventions have proven efficacious in providing protection in various organs, as a whole they focus on restoring aberrations that follow the combination of intoxication and burn instead of addressing the underlying cause of the exacerbated injury. The findings of this study suggest that alcohol potentiates post burn third spacing of fluid through the action of bradykinin, perhaps leading to greater intestinal ischemia and initial injury itself. We chose to examine a 24 hour time point because this allows for the greatest differences between treatment groups and avoids any confounding factors related to circadian rhythms. However, we have shown that there is increased intestinal damage as soon as 2 hours after injury when intoxication precedes burn (22). Furthermore, because bradykinin antagonism partially restored fluid balance and attenuated intestinal damage we feel it can be concluded that bradykinin plays an important early role in this scenario, though more direct evidence of bradykinin involvement would be needed to be certain. This does not rule out the significance of other vasoactive agents after burn, such as histamine and serotonin, but given the potentiating effect of ethanol on bradykinin, B₂ antagonism may be particularly suitable therapeutic target in this combined injury.

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Figure 1. Fluid status 24 hours after injury. (A) Total body weight loss; (B) hematocrit, (C) creatinine, and (D) blood urea nitrogen (BUN) levels. * $p < 0.05$ compared to all groups, # $p < 0.05$ compared to sham groups by one-way ANOVA. $N = 4-6$ animals per group.

Figure 2.

Remote organ damage 24 hours after injury. (A) bacterial translocation reported as the number of colony forming units (CFU) per mesenteric lymph node, (B) liver weight to total body weight ratio, (C) Lung histology by H&E staining. Representative images shown at 400x magnification. * $p < 0.05$ compared to all groups by one-way ANOVA. $N = 4-6$ animals per group.

Figure 3.

HOE140 restores fluid balance 24 hours after intoxication and burn. (A) Total body weight loss; (B) hematocrit, (C) creatinine, and (D) blood urea nitrogen (BUN) levels. * $p < 0.05$ by Student's T test. $N = 4-6$ animals per group.

Figure 4.

HOE140 attenuates remote organ damage 24 hours after intoxication and burn. (A) Bacterial translocation reported as the number of colony forming units (CFU) per mesenteric lymph node, (B) liver weight to total body weight ratio, (C) lung histology by H&E staining. Representative images shown at 400x magnification. * $p < 0.05$ by Student's T test. N=4–6 animals per group.