Binge Alcohol Exposure Modulates Rodent Expression of Biomarkers of the Immunoinflammatory Response to Orthopaedic Trauma

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Background: Alcohol is a known modulator of the immune system and host-defense response. Alcohol abuse is common in trauma patients, although the influence of alcohol intoxication on the inflammatory response following major orthopaedic injury remains unknown. The aim of this investigation was to examine the influence of binge alcohol exposure on biomarkers of the systemic inflammatory response following bilateral traumatic femoral fracture in a rodent model.

Methods: Ninety-two Sprague-Dawley rats were administered intraperitoneal injections of either saline solution or alcohol for three days. These animals then underwent a sham procedure or bilateral femoral intramedullary pinning and mid-diaphyseal closed fracture via blunt guillotine. The animals were killed at specific time points after the injury. Serum and lung tissue were collected, and twenty-five inflammatory markers were analyzed by immunoassay. Histological sections of lung tissue were evaluated by a board-certified pathologist.

Results: Bilateral femoral fracture significantly ($p < 0.05$) increased multiple serum biomarkers of inflammation. Binge alcohol treatment prior to injury significantly suppressed the increase in serum levels of interleukin (IL)-6, white blood cells, IL-2, IL-10, and C-reactive protein after the fracture. However, alcohol-treated animals were found to have increased pulmonary levels of IL-6, IL-1β, IL-2, and macrophage inflammatory protein-1α following bilateral femoral fracture. In addition, lung tissue harvested following alcohol treatment and injury demonstrated increased pathologic changes, including parenchymal, alveolar, and peribronchial leukocyte infiltration and significantly elevated pulmonary wet-to-dry ratio, indicative of pulmonary edema.

Conclusions: Our results indicate that acute alcohol intake prior to bilateral femoral fracture with fixation in rats modulates the inflammatory response after injury in a tissue-dependent manner. Although serum biomarkers of inflammation were suppressed in alcohol-treated animals following injury, several measures of pulmonary inflammation including cytokine levels, histological changes, and findings of pulmonary edema were significantly increased following fracture with the presence of alcohol.

Clinical Relevance: These findings indicate that alcohol modulates the inflammatory response after a major orthopaedic injury and that analysis of serum markers of inflammation after trauma may not represent the pulmonary inflammatory status in acutely intoxicated patients.

The natural immunoinflammatory response to injury is a complex process important for host recovery[1]. This response typically remains localized at the site of injury, resolving as the patient recovers. However, with serious injury, an exaggerated inflammatory response may overwhelm the threshold of local response, resulting in an imbalance of systemic proinflammatory mediators[2]. This systemic propagation of the inflammatory response has potential to inflict remote organ injury, including acute respiratory distress syndrome or multiple organ failure[1,2].

The prevalence of alcohol abuse has been shown to be elevated in seriously injured trauma patients[3] and has been

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reported in 25% to 40% of trauma patients with associated orthopaedic injuries[4,5]. Alcohol intoxication in trauma patients is associated with longer hospital stays and admission to the intensive care unit after injury, higher injury severity scores, and an increased mortality rate[6,7]. The basis for these findings is multifactorial, but they have been attributed in large part to alcohol-associated modulation of host-defense systems and the immune response. However, the comprehensive effect of alcohol on the immunoinflammatory response to injury is not completely understood. Acute alcohol intoxication is associated with diminishing injury-induced induction of inflammatory markers and suppression of the expression of early lung proinflammatory cytokines in rats[8]. However, acute alcohol ingestion has been shown to increase levels of lung inflammation and neutrophil infiltration following burn injury[9], as well as modulate early proinflammatory responses to hemorrhagic shock[10]. Ultimately, despite the high prevalence of ethanol intoxication in trauma patients, the complete influence of alcohol on the immunoinflammatory response after injury is unknown.

Surgical intervention has been shown to propagate the inflammatory response beyond the site of the initial traumatic injury[2,11]. An ill-timed surgical procedure following injury may exacerbate a hyperinflammatory state with potential to cause systemic disease, including acute respiratory distress syndrome or multiple organ failure. To avoid these complications, increased emphasis has been placed on quantifying the underlying inflammatory status after injury to help guide immediate clinical decisions after injury and base surgical timing[12,13]. Thus, the aim of this investigation was to examine the influence of binge alcohol exposure on biomarkers of the systemic and lung-related inflammatory response following bilateral traumatic femoral fracture in a rodent model. We hypothesized that systemic and local biomarkers of the inflammatory response would be elevated following orthopaedic trauma and that acute alcohol intake prior to injury would modify both the temporal pattern and level of expression of these markers.

Materials and Methods

Experimental Design

The Loyola University Institutional Animal Care and Use Committee approved the animal study used in this investigation. Ninety-two male Sprague-Dawley rats (average weight, 400 g) were used. The rats were randomized into groups consisting of a sham procedure or injury, either with or without the administration of alcohol injections before injury. Animals in sham groups were randomized to receive intraperitoneal injections of either alcohol (eight animals) or saline solution (eight animals) for three days followed by a sham operation. Animals randomized into injury groups were administered either intraperitoneal injections of alcohol or saline solution for three days prior to bilateral femoral intramedullary pinning and mid-diaphyseal closed fracture. Injured rats were killed at four specific time points after injury by CO_2 inhalation and decapitation. At the time of killing, serum and lung tissue were collected and stored. Time points (and sample size) for injured animals administered saline solution injections included six hours (ten animals), twenty-four hours (ten animals), forty-eight hours (eight animals), and seventy-two hours (eight animals) after injury. Time points for animals administered alcohol injections prior to injury included six hours (thirteen animals), twenty-four hours (eleven animals), forty-eight hours (eight animals), and seventy-two hours (eight animals) after injury. The number of animals used in this study was predetermined prior to initiation of the investigation and was estimated on the basis of prior data from our laboratory.

Alcohol-treated animals were administered binge alcohol treatment on three consecutive days prior to injury or sham procedure. A three-day period of alcohol administration before the injury, representing an acute binge, has been previously established in the rodent model[14,15]. Alcohol was administered by a single daily intraperitoneal injection of a 20% (v/v) ethanol-saline solution at a dose of 2 g/kg. This dose was chosen to achieve peak blood alcohol concentrations of approximately 200 mg/dL to simulate intoxicated trauma patients. Alcohol injections were given for two days prior to and then on the day of injury. All control animals were administered a single daily intraperitoneal injection of an equal volume of sterile isotonic saline solution for two days prior to injury and then on the day of injury. Blood alcohol concentration was validated with use of a colorimetric NAD (nicotinamide adenine dinucleotide) reduction assay with use of an alcohol reagent kit (Pointe Scientific, Canton, Michigan). Serum was diluted in reagent (1:200) and incubated ten minutes at 30°C, and absorbance was read at 340 nm.

Fracture Creation and Animal Care

The rats were anesthetized with an intraperitoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg). Before incision, the animals were administered preoperative antibiotics to ensure aseptic surgery (gentamicin, 5 mg/kg) and Buprenex (buprenorphine, 0.15 mg/kg) for postoperative pain in accordance with an established laboratory animal open surgical protocol. After the animal's hind limb was shaved and washed with a povidone-iodine solution, a medial parapatellar incision (approximately 0.5 cm) was created. With the knee extended, the patella was dislocated laterally and the medullary canal was entered through the intercondylar notch. A Dremel Moto-Tool drill (Robert Bosch Tool, Mount Prospect, Illinois) with a 1-mm drill bit was used to penetrate the articular cartilage and subchondral bone in order to enter the medullary canal. The canal was reamed with a 21-gauge needle, and a 1.25-mm Kirschner wire (Synthes, Monument, Colorado) was inserted through the medullary canal into the proximal part of the greater trochanter. The distal portion of the pin was cut flush with the femoral condyles so as not to interfere with knee function. The patella was reduced, and the extensor mechanism and skin were closed with 4-0 Vicryl resorbable sutures (polyglactin; Ethicon, Somerville, New Jersey). After the incision was closed, the diaphyses of both pinned femora were fractured with the use of a blunt guillotine three-point bending apparatus as described previously[16]. Briefly, this device consisted of a 550-g weight dropped 15 cm, driving a blunt guillotine down onto an outstretched rat leg placed across an open platform, creating a three-point bending mechanism. This resulted in a closed femoral fracture with a soft-tissue injury consistent with a blunt force injury (Fig. 1). Pinning fractures at the time of injury was an Institutional Animal Care and Use Committee requirement established to protect animal welfare (ability to eat and drink after injury). In addition, this model has been established as an accepted closed femoral fracture model within the literature[15]. Following injury, animals were resuscitated with an intraperitoneal injection of 5 mL of normal saline solution and were allowed to walk freely. Animals were caged in pairs, and food was placed on the floor of the cage to facilitate normal feeding behavior. Animals were observed to drink freely from their water bottle following injury. Animals surviving for more than twenty-four hours were administered Buprenex (buprenorphine; 0.15 mg/kg) for pain control and saline solution injection (2 mL) for fluid resuscitation every ten to twelve hours.

Serum and Lung Cytokine Assay

Collected blood was immediately placed on ice and allowed to clot for one hour. Blood was then centrifuged (250 × g) for ten minutes. Serum was collected, immediately placed into aliquots, and stored at –80°C until analysis. Serum analysis of twenty-five inflammatory markers (Table I) was determined by ELISA (enzyme-lined immunosorbent assay) performed in-house (BD Bioscience, San Diego, California), which included interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)-α, and C-reactive protein (CRP), or by the

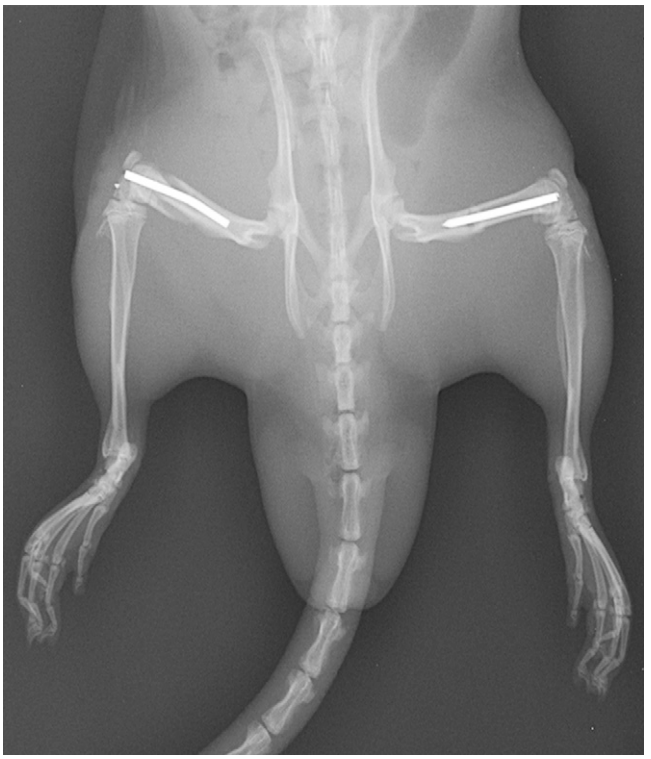

Fig. 1

Radiograph made after injury and surgical stabilization, demonstrating the typical femoral fracture pattern caused by the blunt guillotine and the appropriate placement of intramedullary fixation prior to the fracture.

LINCOplex assay service section of Millipore (St. Charles, Missouri). All samples were run with a dilution of 1:2 in phosphate-buffered saline solution with 2% bovine serum albumin. All samples were run in duplicates, and the average value is reported.

Lungs were collected immediately after the animals were killed. A longitudinal midline thoracotomy incision was made, and the ribs were separated from the sternum. The pulmonary vasculature was isolated and severed. The pulmonary tree was isolated, and lung lobes were separated, snap-frozen in liquid nitrogen, and stored at –80°C until processing. Lungs were prepared for ELISA analysis by adding 1 mL of buffer (EDTA-free protease inhibitor cocktail tablet; Complete Mini; Roche, Mannheim, Germany) to an average of 140 mg of sample. This solution was then subjected to a single freeze-thaw cycle at –80°C and subsequent homogenizing, sonicating, and filtering prior to aliquot and refreeze until analysis at –80°C.

Lung analysis was also conducted by the LINCOplex assay service section of Millipore. These samples were also run in duplicates and analyzed with a BCA (bicinchoninic acid) protein assay kit (Pierce Biotechnology, Rockford, Illinois). Again, the average value of the duplicates is reported.

Histological Analysis of Lung Tissue

Lung tissue was inflated and then harvested from animals immediately after the sham procedure and at six hours and twenty-four hours following bilateral femoral fracture and fixation. Lung tissue was fixed in 10% buffered formalin, processed for histological examination, and embedded in paraffin. Four-micrometer sections were cut, stained with hematoxylin and eosin, and reviewed by two pathologists, blinded to sample type, using a light microscope (Olympus BX41; Leeds, Minneapolis, Minnesota). Leukocyte infiltration was evaluated and graded in a similar fashion to previously published criteria[17]. Lung histological sections were blinded and scored by a pathologist with board certification in anatomic and clinical pathology. Briefly, leukocyte infiltration was assessed for parenchymal-alveolar regions after the entire lung section was examined and the most cellular area identified. The scoring system utilizing the most affected 400× field was as follows: 0 indicated no extravascular leukocytes; 1, less than ten leukocytes; 2, ten to forty-five leukocytes; and 3, less than or equal to forty-five leukocytes. Peribronchial and perivascular leukocyte infiltration was graded similarly, with a score of 0 to 3. Small (≤0.1-mm), intermediate (>0.1 to 0.5-mm), and large (>0.5-mm) caliber bronchial vessels were examined and separately graded. The presence of pulmonary edema was graded as 0 indicating none; 1, focal; 2, multifocal; and 3, diffuse. Degenerative alveolar structures were considered present if distended alveolar spaces with degenerative-reactive pneumocytes were identified. Fluid in alveolar spaces was also evaluated.

Lung Wet-to-Dry Ratio

Lung wet-to-dry ratio measurements were conducted by harvesting the right upper lung lobe and measuring lung weight immediately after tissue harvest. Lungs were incubated in a 70°C oven for seventy-two hours and then were reweighed. The ratio of the weight to dry weight was obtained, and data were analyzed.

Statistical Analyses

Statistical analysis was performed with use of Systat 11 (Systat Software, San Jose, California). One-way analysis of variance (ANOVA) was performed with use of the Tukey post hoc test, and significance was defined as a p value of <0.05.

TABLE I Twenty-five Inflammatory Markers Investigated in Serum and Pulmonary Tissue via Immunoassay

Interleukin (IL)-1α
IL-1β
IL-2
IL-4
IL-5
IL-6
GRO/KC (IL-8 homolog)
IL-9
IL-13
IL-10
IL-12p70
IL-17
IL-18
C-reactive protein
White blood cells
Tumor necrosis factor-α
MIP-1α (macrophage inflammatory protein-1α)
IP-10 (interferon-γ-inducible protein of 10 kDa)
INF-γ (interferon-γ)
Granulocyte-macrophage colony-stimulating factor
Granulocyte colony-stimulating factor
Vascular endothelial growth factor
Leptin
RANTES (protein regulated on activation, normal T expressed and secreted)
Eotaxin

This investigation consisted of statistical tests on twenty-five different biomarkers, which may introduce an aspect of chance associations. Therefore, p values between 0.002 (0.05/25) and 0.05 should be regarded with caution.

Source of Funding

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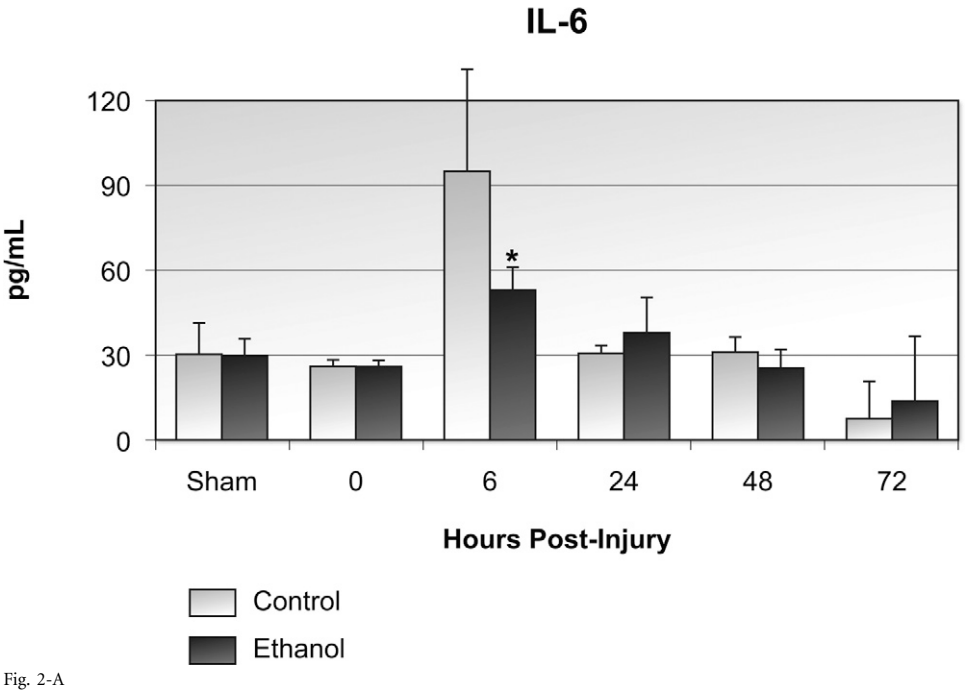

Fig. 2-A

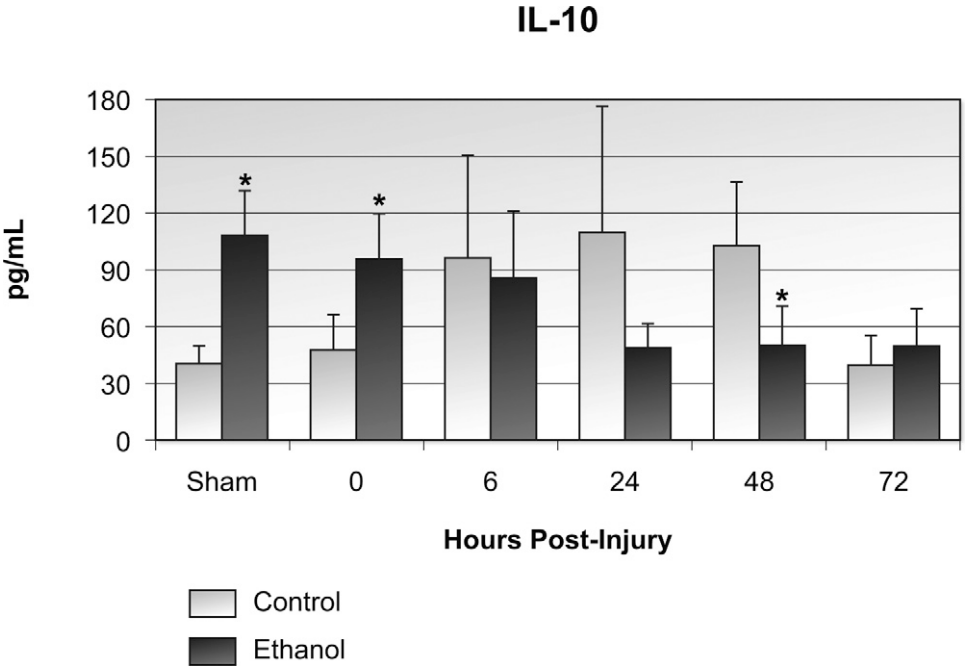

Fig. 2-B

Serum levels of interleukin (IL)-6 (Fig. 2-A), IL-10 (Fig. 2-B), C-reactive protein (CRP) (Fig. 2-C), and white blood cells (WBC) (Fig. 2-D). Data for both alcohol-treated animals and saline-solution treated controls, including the sham surgery group as well as the groups analyzed at zero, six, twenty-four, forty-eight, and seventy-two hours after injury, are given as the mean and the standard deviation. Data for the controls are shown as white bars, and data for the alcohol-treated animals are shown as gray bars. *The difference was significant ($p < 0.05$) compared with the respective control group, according to one-way ANOVA with the Tukey post hoc test.

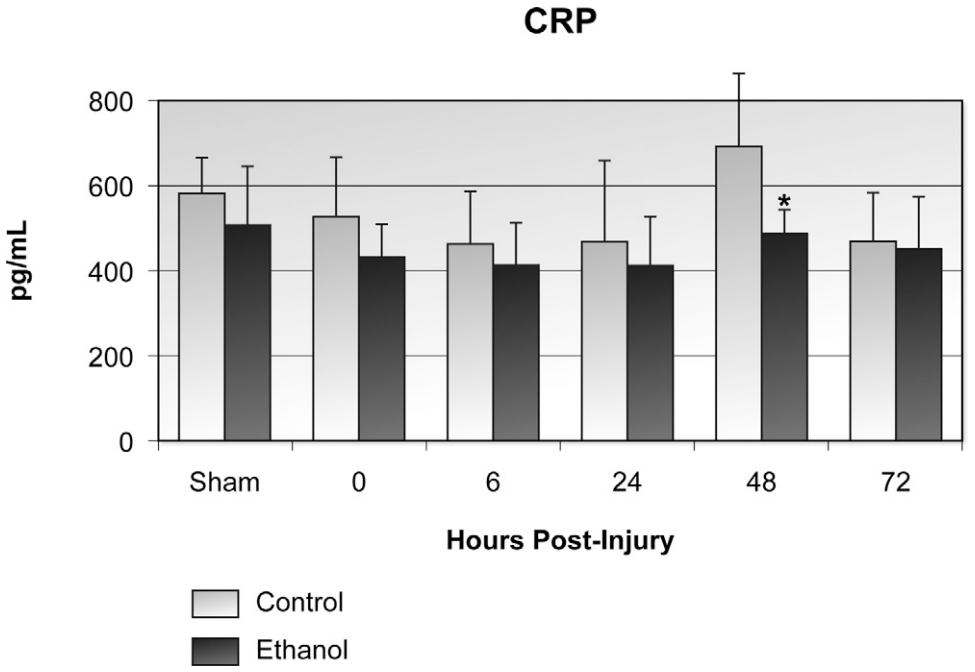

Fig. 2-C

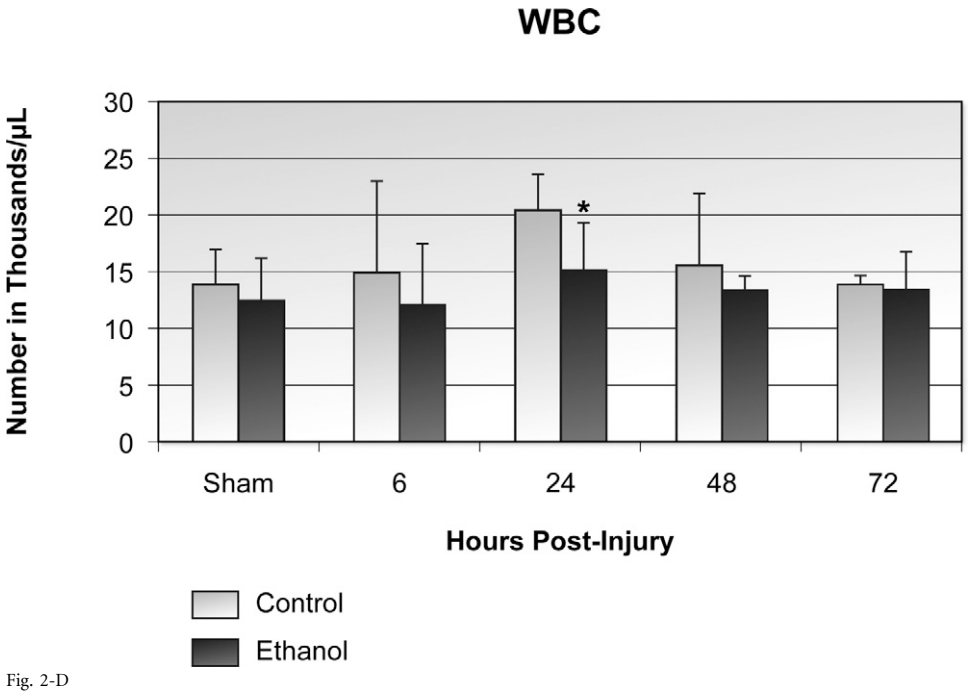

Fig. 2-D

Results

Influence of Injury and Alcohol Administration on Serum Markers of Inflammation

Bilateral femoral fixation and fracture was associated with a significant increase in serum markers of inflammation including IL-2, IL-6, IL-10, granulocyte-macrophage colony-stimulating factor (GM-CSF), growth-related oncogene/keratinocyte-derived chemokine (GRO/KC), monocyte chemotactic protein (MCP)-1, CRP, and white blood cells (WBC). No significant change in serum levels of tumor necrosis factor (TNF)-α were found following fracture. Figures 2-A through 2-D illustrate the effect of binge alcohol treatment before injury on changes in levels of serum markers of inflammation following bilateral femoral fracture with fixation. Compared with animals that did not receive ethanol treatment (the controls), the alcohol-treated animals were found to have significantly ($p < 0.05$ for all) suppressed levels of IL-6 (44% difference; mean and standard deviation, 94.39 ± 29.0 and 52.87 ± 6.2 pg/mL, respectively) at

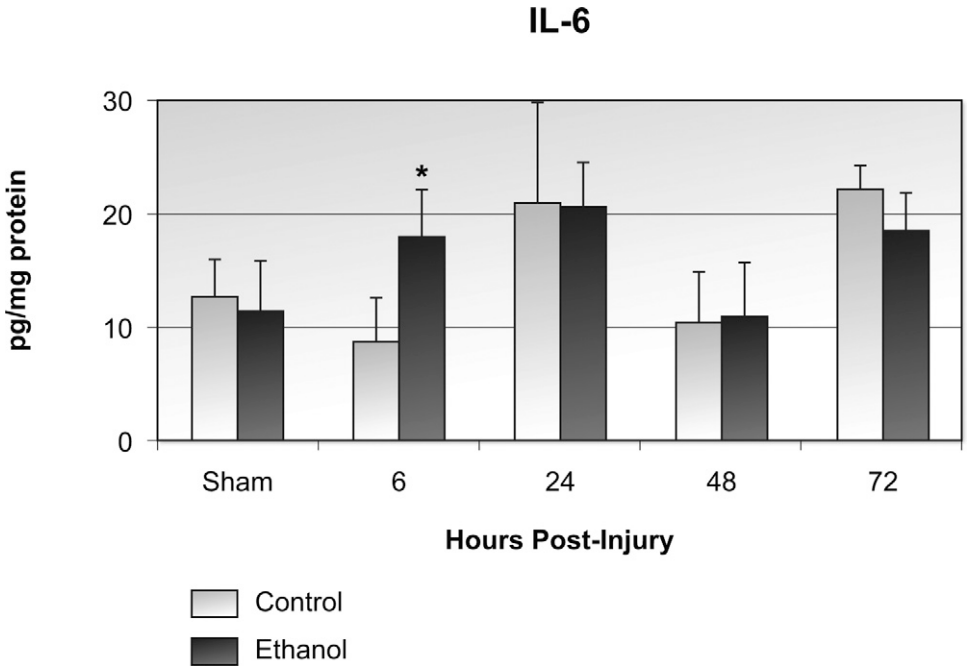

Fig. 3-A

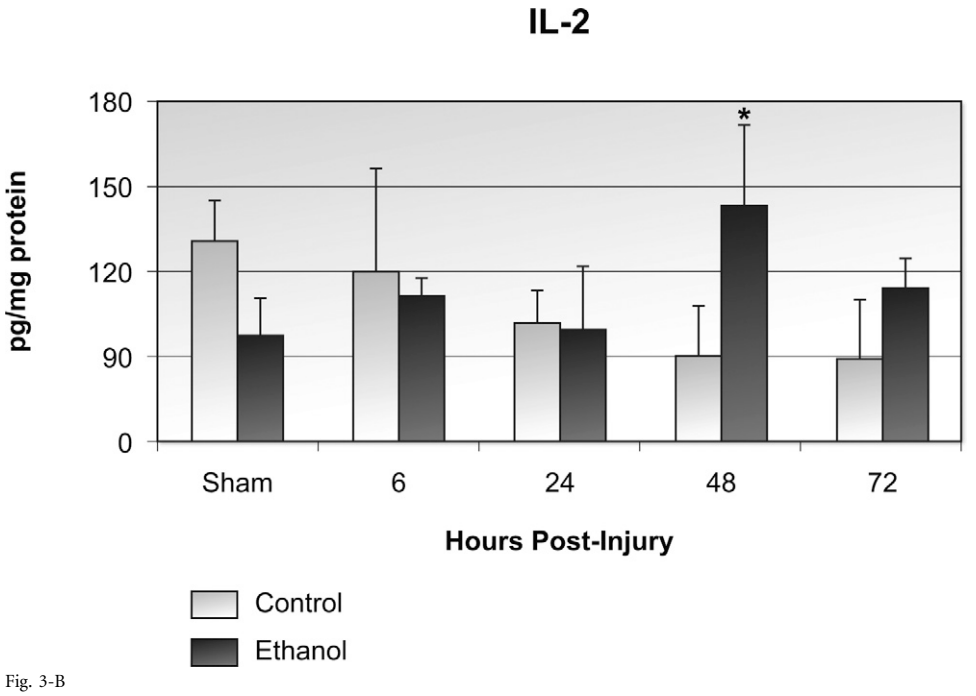

Fig. 3-B

Lung levels of interleukin (IL)-6 (Fig. 3-A) and IL-2 (Fig. 3-B) are given in picograms per milligram of protein. Data for both ethanol-treated animals and saline solution controls, including the sham surgery group as well as the groups analyzed at six, twenty-four, forty-eight, and seventy-two hours after injury, are shown as the mean and the standard deviation. Data for saline solution controls are shown as white bars, and data for ethanol-treated animals are shown as gray bars. *The difference was significant ($p < 0.05$) compared with the respective control group, according to one-way ANOVA with the Tukey post hoc test.

six hours after injury and WBC (11% difference; mean, 15.61 ± 2.5 and 13.86 ± 0.5 × 10^3/μL, respectively) at twenty-four hours after injury. At forty-eight hours after injury, compared with the controls, the alcohol-treated animals had suppressed levels of IL-2 (73.3% difference; mean, 1211.63 ± 162.6 and 323.38 ± 135.06 pg/mL, respectively), IL-10 (51% difference;

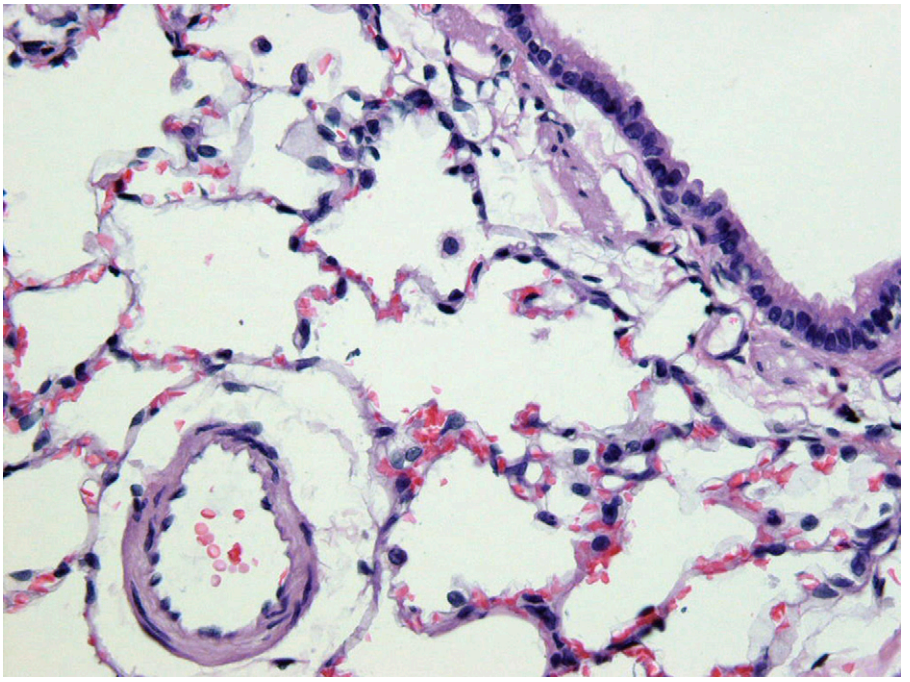

Fig. 4-A

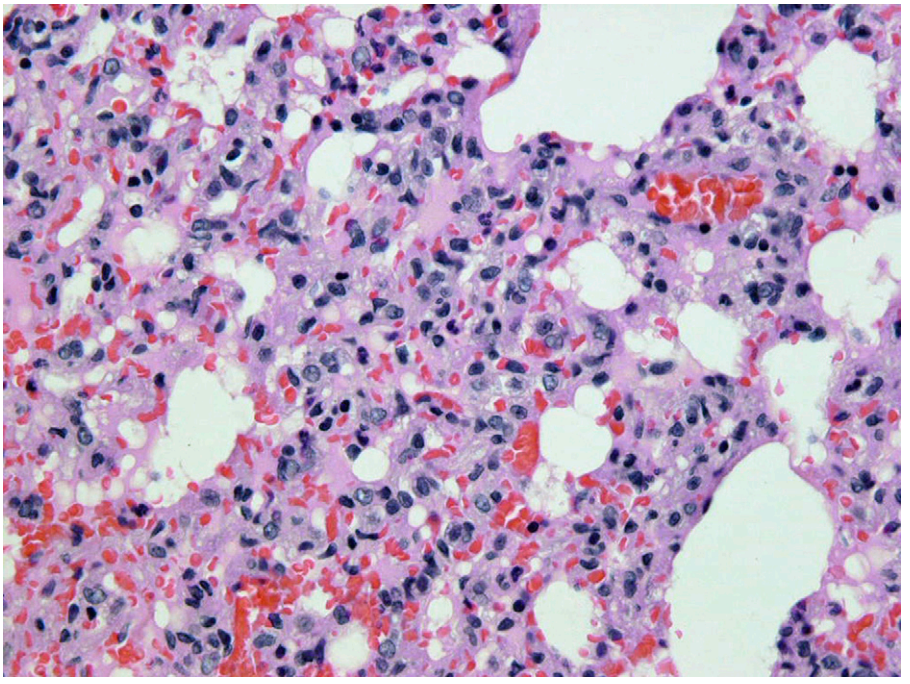

Fig. 4-B

Figs. 4-A and 4-B Photomicrographs of rat pulmonary tissue at 400× magnification with hematoxylin and eosin staining. **Fig. 4-A** Normal lung findings seen in pulmonary tissue obtained from saline solution-treated rats at twenty-four hours following bilateral femoral fracture with fixation, demonstrating normal alveolar parenchyma, bronchioles, and vessels associated with focal minimal to mild interstitial vascular congestion. **Fig. 4-B** Acute injury seen in pulmonary tissue of an alcohol-treated rat obtained at twenty-four hours following bilateral femoral fractures with fixation, demonstrating patchy areas of grade-3 leukocyte infiltration of alveolar interstitium and alveolar spaces associated with pulmonary edema and fibrin. This is consistent with evidence of diffuse alveolar damage associated with acute inflammation.

mean, 101.93 ± 9.36 and 49.82 ± 5.6 pg/mL), and CRP (30% difference; mean, 681.21 ± 44.3 and 480.3 ± 14.0 pg/mL); the differences were significant ($p < 0.05$ for all). Binge alcohol was associated with significantly ($p < 0.05$) elevated serum levels of GM-CSF at six hours after injury (33.5% difference; mean, 356.88 ± 11.5 pg/mL for controls and 476.75 ± 5.1 pg/mL for the ethanol group). Acute alcohol administration also resulted in a significant increase ($p < 0.05$) in preinjury serum levels of the anti-inflammatory cytokine IL-10 (170% increase; mean, 39.67 ± 2.7 pg/mL for controls and 107.41 ± 6.5 pg/mL for the ethanol group). However, by forty-eight hours after injury, these animals were found to have significantly ($p < 0.05$) suppressed IL-10 levels compared with control animals (51% difference; mean, 101.93 ± 9.4 pg/mL for controls and 49.82 ± 5.6 pg/mL for the ethanol group).

Influence of Injury and Alcohol Administration on Pulmonary Markers of Inflammation

Lung levels of GRO/KC, homolog to human IL-8, and IL-1α were both significantly ($p < 0.05$) increased compared with the sham procedure at six hours after injury. Lung levels of IL-6 were associated with a trend toward increased levels at twenty-four and seventy-two hours after injury in saline solution-treated animals. Figures 3-A and 3-B demonstrate the comparison of the pulmonary levels of IL-6 and IL-2 following bilateral femoral fracture in rats both with and without the association of preinjury alcohol binge. Compared with saline solution-treated animals, the animals that had binge alcohol treatment had significantly elevated lung levels of proinflammatory markers ($p < 0.05$ for all) including IL-6 (95% increase; mean, 11.19 ± 1.67 and 21.80 ± 1.58 pg/mL, respectively) at six hours and IL-1β (109% increase; mean, 33.80 ± 2.3 and 70.74 ± 4.1 pg/mL, respectively), IL-2 (177% increase; mean, 20.18 ± 3.9 and 55.88 ± 6.2 pg/mL), and macrophage inflammatory protein (MIP)-1α (103% increase; mean, 4.32 ± 0.47 and 8.77 ± 0.68 pg/mL) at forty-eight hours following the injury.

Influence of Injury and Alcohol Administration on Pulmonary Tissue

Histological analysis of rat lung tissue after the sham procedure confirmed that these animals had minimal pulmonary tissue injury, and no difference between saline solution-treated animals and binge alcohol-treated animals was observed before the injury. After the injury, lung tissue in both saline solution-treated and alcohol-treated animals demonstrated significant histological changes, including parenchymal and alveolar leukocyte infiltration, perivascular leukocyte infiltration, pulmonary edema, and bone marrow elements including cellular hematopoietic cells, fat cells, and, in some cases, bone fragments. We found no measurable difference in pathologic changes between controls and binge alcohol-treated animals at six hours after the injury. However, at twenty-four hours after the injury, rats administered alcohol were found to have

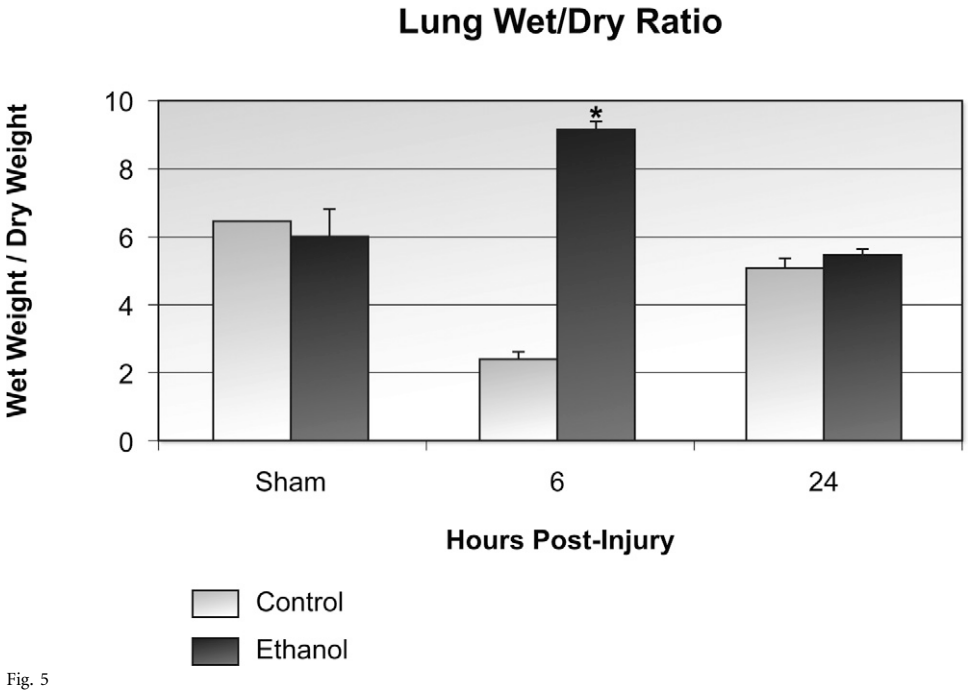

Fig. 5

Wet-to-dry ratio (and standard deviation) of rat lungs pretreated with either saline solution or alcohol and followed by a sham procedure or bilateral femoral fracture with intramedullary fixation. Animals were killed at six hours or twenty-four hours after injury. Data for saline solution-treated animals are shown as white bars, and data for ethanol-treated animals are shown as gray bars. *The difference was significant ($p < 0.05$) compared with the respective control group, according to one-way ANOVA with the Tukey post hoc test.

increased pathologic changes, including parenchymal and alveolar leukocyte infiltration, peribronchial leukocyte infiltration, as well as pulmonary edema and bone marrow emboli, compared with saline solution-treated animals (Figs. 4-A and 4-B).

Figure 5 demonstrates the wet-to-dry relationship between rat lungs treated with saline solution or alcohol before injury. Alcohol-treated animals were found to have a significantly elevated pulmonary wet-to-dry ratio, indicative of increased pulmonary edema, compared with saline solution-treated animals.

Discussion

To our knowledge, the present study represents the first investigation of the influence of binge alcohol treatment on expression of the inflammatory response in a rodent model of traumatic orthopaedic injury. In the current study, the infliction of bilateral femoral fractures in rats resulted in an increase in the levels of serum and pulmonary markers of inflammation. Compared with control animals, animals that had the equivalent of a three-day alcohol binge prior to injury blunted an injury-associated elevation in levels of multiple circulating proinflammatory markers, including IL-6 and CRP. Additionally, alcohol-treated animals were found to have heightened preinjury levels of the anti-inflammatory cytokine IL-10, which was initially elevated but suppressed at twenty-four to forty-eight hours after injury relative to controls. In contrast, associated alcohol administration prior to injury was found to result in significantly increased levels of pulmonary markers of inflammation, including IL-6. In addition, compared with control animals, rats that were administered ethanol developed lung edema and pronounced histological changes following injury including parenchymal, peribronchial, and alveolar leukocyte infiltration.

The natural immunoinflammatory response to injury is a complex process, with the magnitude of the inflammatory response directly proportional to the severity and type of injury[1]. With major injury or a so-called second-hit mechanism, such as an ill-timed surgical procedure, this response can propagate systemically, creating serious host derangement[1,11,12]. Alcohol intoxication, which is present in 25% to 40% of trauma patients with associated orthopaedic injuries[4,5], is a known modulator of multiple components of the immunoinflammatory response including neutrophil function[9,18], chemotaxis of circulating mononuclear cells[19], and pathways of the normal inflammatory cascade[20,21]. The subsequent effect on immune function is dependent on the duration and amount of alcohol consumption[22]. In contrast to chronic alcohol intake, which is associated with a proinflammatory response to injury[10,23], most investigations of short-term acute alcohol exposure with injury demonstrate suppressed levels of circulating proinflammatory cytokines[21,22,24-26]. Mechanisms for this response have been found to be multifactorial, consisting of increased levels of the anti-inflammatory cytokine IL-10[24], inhibition of p38 and nuclear factor-κB activation[21], increased hepatic clearance of circulating cytokines[25], and inhibition of cytokine processing[26]. Subsequently, alcohol-associated downregulation of the normal immune response has been attributed in part to an increased prevalence of postinjury infectious complications contributing to higher morbidity and mortality rates for intoxicated trauma patients[7,27,28].

However, several recent studies have demonstrated a differential immunomodulatory effect of alcohol exposure occurring in pulmonary tissue that appears to vary from the response seen in peripheral circulation[9,14,29,30]. Greiffenstein et al. recently examined the impact of binge alcohol consumption on host responses during trauma and hemorrhage[14]. Those authors demonstrated marked immunosuppression of peripheral proinflammatory blood mononuclear cells, but found that alveolar macrophage responsiveness remained preserved, suggesting the effect from alcohol may be tissue-dependent. Alcohol administration prior to burn injury has been shown to increase lung levels of IL-18, contributing to neutrophil recruitment and activation[9,29]. IL-2 levels are also elevated[30]. In contrast to these studies, Mathis et al. found that acute alcohol intake suppressed hemorrhage-induced proinflammatory cytokine expression in rat lungs; however, spleen tissue response did not change[8]. Ultimately, the effect of acute alcohol intake on the immunoinflammatory response after injury is poorly understood and may vary on the basis of organ type. To date, however, no investigation has examined the influence of alcohol on the inflammatory response following blunt trauma with skeletal injury.

Findings from this study indicate that changes in postinjury serum levels of inflammatory markers in the acutely intoxicated patient may not be representative of the pulmonary inflammatory status. We found IL-6 levels, commonly studied as a readily accessible marker representative of the host inflammatory status[31-33], to be significantly influenced by preinjury acute alcohol administration, resulting in blunting of postinjury serum levels but with associated elevated pulmonary levels. In addition to alterations in serum and pulmonary levels of inflammatory markers, lungs from animals exposed to alcohol and subjected to injury demonstrated an increased wet-to-dry ratio and increased histological pathologic changes, indicative of pulmonary injury, compared with nontreated animals. Clinically, the level of the proinflammatory cytokine IL-6 has been shown to be a reliable index of the magnitude of injury[31], correlated with injury severity and thirty-day mortality following trauma[32], and may be used as a tool to identify seriously injured trauma patients at risk for the subsequent development of multiple organ dysfunction syndrome[33]. IL-6 has also been shown to be a useful index for assessing the risk of a so-called second-hit mechanism produced by the inflammatory burden from subsequent surgical procedures[31]. In a clinical study, Pape et al. found that postinjury IL-6 levels in patients with multiple injuries, including skeletal injury, demonstrated a predictive value for the development of multiple organ failure[33]. The investigators evaluated several parameters of the inflammatory response, reporting that patients with initial IL-6 values of >500 pg/dL (>5 pg/mL) should have an interval delay between primary temporary fracture

stabilization and definitive fracture fixation of more than four days secondary to the potential for development of late multiple organ dysfunction syndromes. The incidence or association of alcohol intoxication in their population was not reported. Thus, our finding of an alcohol-related modulation of serum IL-6 levels after injury may be important when quantifying the postinjury inflammatory response in the intoxicated patient with major orthopaedic injury. Accurate assessment of the magnitude of the inflammatory response is becoming increasingly important in making clinical decisions and guiding postinjury care.

There are several limitations to this study. First, this study is based on a rodent model, and the timing and magnitude of the inflammatory response following injury in rodents is likely different from that occurring in humans. However, understanding of the immunobiology of traumatic injury and influence of associated acute alcohol intake in the rat will provide a platform to design clinically based studies to further understand this important phenomenon in critically injured patients. Second, our rodent injury model required femoral fixation to occur at the same time as fracture, which clearly deviates from the typical clinical timeline of a trauma patient requiring operative fixation. This model was used primarily because pinning of fractures at the time of injury was a requirement established to protect animal welfare. This model is established as an accepted closed femoral fracture model[16], and we believe it simulates the natural inflammatory response similar to that following independent bilateral long-bone fracture because of the blunt mechanism of fracture and associated soft-tissue injury. In addition, both saline solution-treated and alcohol-treated groups underwent injury with the same fixation mechanism and timing. A third limitation is that the rodents used in this study were alcohol-naïve prior to testing. Clinically, trauma patients will not typically be alcohol-naïve and more likely represent a spectrum of repeated alcohol intoxication. Finally, methodology for this investigation included statistical analysis and comparison of twenty-five different biomarkers, which may introduce an aspect of chance associations. Therefore, p values between 0.002 (0.05/25) and 0.05 should be regarded with caution.

In conclusion, this study demonstrates that acute alcohol intake prior to bilateral femoral fracture with intramedullary fixation modulates the postinjury inflammatory response in rats. Elevation in levels of serum markers of inflammation in response to injury was suppressed compared with animals not treated with alcohol; however, several measures of pulmonary inflammation, including cytokine levels, histological analysis, and findings of pulmonary edema, were significantly increased following injury with the presence of alcohol. These findings indicate that analysis of serum markers of inflammation after trauma may not represent the pulmonary inflammatory status in acutely intoxicated patients. In this population, the interpretation of serum markers of inflammation as guides for clinical decisions should be approached with caution.

Appendix

eA Tables showing all data represented by the figures are available with the electronic version of this article on our web site at jbjs.org (go to the article citation and click on "Supporting Data"). ■

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