Alcohol ingestion before burn injury decreases splanchnic blood flow and oxygen delivery

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Alcohol ingestion before burn injury decreases splanchnic blood flow and oxygen delivery. Am J Physiol Heart Circ Physiol 288: H716–H721, 2005. First published September 23, 2004; doi:10.1152/ajpheart.00797.2004.—Recent studies from our laboratory have shown that alcohol and burn injury impair intestinal barrier and immune functions. Although multiple factors can contribute to impaired intestinal barrier function, such an alteration could result from a decrease in intestinal blood flow (BF) and oxygen delivery (DO2). Therefore, in this study, we tested the hypothesis that alcohol ingestion before burn injury reduces splanchnic blood flow and oxygen delivery. Rats (250 g) were gavaged with alcohol to achieve a blood ethanol level in the range of 100 mg/dl before burn or sham injury (25% total body surface area). Day 1 after injury, animals were anesthetized with methoxyflurane. Blood pressure, cardiac output (CO), ±dP/dt, organ BF (in ml·min⁻¹·100 g⁻¹), and DO2 (in mg·ml⁻¹·100 g⁻¹) were determined. CO and organ BF were determined using a radioactive microsphere technique. Our results indicate that blood pressure, CO, and ±dP/dt were decreased in rats receiving a combined insult of alcohol and burn injury compared with rats receiving either burn injury or alcohol alone. This is accompanied by a decrease in BF and DO2 to the liver and intestine. No significant change in BF to the coronary arteries (heart), brain, lung, skin, and muscles was observed after alcohol and burn injury. In conclusion, the results presented here suggest that alcohol ingestion before burn injury reduces splanchnic BF and DO2. Such decreases in BF and DO2 may cause hypoxic insult to the intestine and liver. Although a hypoxic insult to the liver would result in a release of proinflammatory mediators, a similar insult to the intestine will likely perturb both intestinal immune cell and barrier functions, as observed in our previous study.

hemodynamic; liver; intestine; shock; trauma; ethanol; cardiovascular response; thermal injury

Nearly 50% of burn and a similar percentage of trauma patients are found positive for alcohol at the time of hospital admission (6, 21, 25). Previous studies have suggested that the severity of injury is increased in patients who have consumed alcohol before injury compared with those who have not (for reviews, see Refs. 6, 21, and 25). These studies have shown that intoxicated patients require frequent intubations, experience delayed wound healing, and unnecessary longer hospital stays (6, 7, 11, 18, 21, 23–25). As a consequence, enormous resources are consumed to treat trauma patients who are positive for blood alcohol compared with those who sustained injuries in the absence of alcohol. Furthermore, intoxicated patients are more susceptible to infection and are more likely to die than injured patients who are not intoxicated at the time of injury (6, 18, 21, 23–25). In contrast, some studies suggest that alcohol does not influence the severity of injury (7, 11), whereas others have suggested that alcohol is protective (for a review, see Ref. 25). Although a definitive reason for the disparity in the existing clinical data is not known, factors such as age, sample size, triage criteria, and severity of injury are potential contributors to the observed differences in previous studies. On the other hand, results from experimental studies clearly support that alcohol intoxication further complicates postinjury pathogenesis (for reviews, see Refs. 6, 21, and 25).

The findings from these studies suggest that alcohol ingestion before burn injury exacerabtes the suppression of immune defense and increases the susceptibility to infection (4, 6, 17, 25, 27). Regardless of the initial injury, infection and the subsequent development of multiple organ dysfunction (MOD) remain the major cause of morbidity and mortality in these patients (6, 25, 26). The onset of multiple organ failure is presumably induced by a series of events including an uncontrolled production of proinflammatory mediators and suppression of immune cell functions.

Recent studies from our laboratory have shown that alcohol ingestion before burn injury impairs intestinal immune and barrier functions (4). This results in increased bacterial translocation. Alterations in intestinal immune/barrier function and subsequent bacterial translocation have been implicated in MOD in critically injured or ill patients as well as in animal models of acute injuries (5, 6, 8, 19, 26). Although several factors are likely to contribute to alterations in intestinal immune and barrier function in alcohol and burn injury, such alterations could also result from a decrease in blood flow and oxygen delivery. As a consequence, oxygen availability decreases below the level that is needed to meet regional metabolic demands. This results in tissue hypoperfusion, leading to hypoxic conditions. Studies have shown that hepatocellular, intestinal, and renal dysfunctions occur early after burn, trauma, and hemorrhagic shock and that these dysfunctions are associated with tissue hypoperfusion (1, 16, 20, 30, 33, 34). Similarly, alcohol ingestion has also been shown to influence hemodynamic responses (9, 11, 12, 22, 28). However, no study to date has evaluated the hemodynamic response to a combined insult of alcohol and burn injury. Therefore, this study tested the hypothesis that alcohol ingestion before burn injury reduces splanchnic blood flow and oxygen delivery (DO2).

MATERIALS AND METHODS

Rat model of acute alcohol and burn injury. The experiments described herein were carried out in adherence to the National Insti-
tutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Alabama (Birmingham, AL). As described previously (4, 17), rats were randomly divided into four experimental groups: 1) saline + sham, 2) alcohol + sham, 3) saline + burn, and 4) alcohol + burn. Blood alcohol levels equivalent to 90–100 mg/dl were achieved by oral gavage with 5 ml of 20% alcohol in saline. Four hours after being gavaged, rats were anesthe-
tized with pentobarbital sodium (60 mg/kg; Abbot Laboratory). Hairs were shaved from their dorsal surface body area. For the burn procedure, rats were transferred into a template designed to expose 25% of the total body surface area (TBSA). Rats were then immersed in a hot water bath (95–97°C) for 10 s. Sham burn rats were subjected to identical anesthesia and other treatments except that they were immersed in lukewarm water. The animals were dried immediately and given fluid resuscitation (~10 ml physiological saline). Rats were allowed to recover from anesthesia and then returned to their cages, after which they were allowed food and water ad libitum. Rats were killed on day 1 after injury and the following measurements were performed:

**Measurement of heart performance, cardiac output, and blood flow.** Rats were anesthetized with intravenous injections pentobarbital (30 mg/kg). Heart performance was measured as described previously (15) with minor modifications. A polyethylene-50 catheter was inserted into the left ventricle via the right carotid artery. The position of the catheter was confirmed by recording the characteristic left ventricular pressure curve. With the use of a heart performance analyzer (Micro-Med; Louisville, KY), variables of heart performance such as the maximal rate of pressure increase (+dP/dt max) and decrease (−dP/dt max) were determined and documented as mmHg per second.

After heart performance was measured, cardiac output and organ blood flow were determined using a radioactive microsphere technique (2). Briefly, strontium-85-labeled microspheres (DuPont/NEN; Boston, MA) were suspended in 10% dextran containing 0.01% Tween 80 to prevent aggregation. A 0.2- to 0.25-ml suspension of microspheres with an activity of 4.4105 cpm was injected manually into the left ventricle of each rat via the left ventricular catheter for 20 s at a constant rate. The reference blood sample was withdrawn from the femoral arterial catheter into a 3-ml syringe beginning 20 s before microsphere infusion and was continued for an additional 60 s at a rate of 0.7 ml/min using a pump (Harvard Apparatus; South Natick, MA). Normal saline (1.4 ml) was infused manually immediately after the microsphere infusion to replace the volume of blood lost. Rats were then killed by an overdose of methoxyflurane inhalation; various organs were harvested, weighed, and placed in one or more test tubes; and organ radioactivity was counted using a Wallac automatic gamma-counter (1470 Wizard, Wallac; Gaithersburg, MD). The reference blood sample was transferred from a syringe into a test tube for radioactivity measurement. The remaining microspheres, which were left in the syringe after injection, were also counted. Cardiac output (in ml·min⁻¹·100 g⁻¹) and organ blood flow (ml·min⁻¹·100 g⁻¹) were calculated as previously described (2).

**Determination of DO₂, oxygen consumption, and oxygen extraction ratio.** As described previously (2), a 3.5-Fr umbilical vessel catheter (Sherwood; St. Louis, MO) was placed in the hepatic vein through the jugular vein for hepatic venous blood sampling. The exact position of the hepatic venous catheter tip was confirmed at autopsy. A 1-ml heparinized syringe with a 22-gauge needle was inserted into the portal vein, secured with superglue to prevent blood leakage, and used for portal venous blood sampling. Blood samples (0.15 ml each) were collected immediately after microsphere infusion from the femoral artery, femoral vein, hepatic vein, portal vein, and renal vein. Oxygen saturation and oxygen content were determined using an OSM hemoximeter (Radiometer; Copenhagen, Denmark). DO₂ of the whole body and intestine was calculated by multiplying arterial oxygen content with cardiac output or blood flow, respectively. Hepatic arterial DO₂ was determined by multiplying arterial oxygen content with hepatic arterial blood flow, and portal DO₂ was determined by multiplying portal oxygen content with portal blood flow. Total hepatic DO₂ was calculated as the sum of hepatic arterial DO₂ and portal DO₂. Oxygen consumption (VO₂) was calculated by multiplying the oxygen content difference between the arterial and venous blood with cardiac output or organ blood flow. Hepatic VO₂ was determined by calculating the difference in oxygen content between the hepatic artery and hepatic vein multiplied by hepatic arterial blood flow plus the difference in oxygen content between the portal vein and hepatic vein multiplied by portal blood flow. The oxygen extraction ratio (in %) was calculated by VO₂/DO₂ × 100.

**Statistical analysis.** All data are presented as means ± SE. The differences between groups were analyzed using ANOVA (GB-STAT School Pack software, Dynamic Microsystems; Silver Spring, MD). A difference of P < 0.05 was considered significant.

**RESULTS**

**Hemoglobin and blood gases.** There was no difference in total hemoglobin and hematocrit between sham and burned injured rats regardless whether or not they received alcohol. Similarly, blood 0₂ and CO₂ contents were not found to be different in the various experimental groups (Table 1).

**Heart performance.** As shown in Fig. 1A, there was no difference in blood pressure in rats receiving sham injury with and without alcohol ingestion. Similarly, burn injury alone did not affect blood pressure. A significant decrease in blood pressure was noted in rats receiving the combined insult of alcohol and burn injury compared with sham injured rats regardless of their alcohol ingestion and rats receiving burn injury alone without prior alcohol ingestion. Similarly, cardiac output was also decreased in rats gavaged with alcohol before burn injury compared with rats receiving either burn injury alone or sham injured rats (Fig. 1B). Although there was a significant increase in +dP/dt in burn injured rats compared with sham injured rats, it was significantly decreased in rats receiving the combined insult of alcohol and burn injury compared with the rats receiving either insult alone (Fig. 2A). −dP/dt was also suppressed in rats gavaged with alcohol compared with the group gavaged with saline. A similar trend of a decrease in −dP/dt was observed in burn injured rats gavaged with alcohol, but this trend was not found to be significantly different from other experimental groups (Fig. 2B).

**Organ blood flow.** There was an increase in intestinal (Fig. 3A) and hepatic (Fig. 3B) blood flow in sham injured rats

<table>
<thead>
<tr>
<th>pH</th>
<th>Saline</th>
<th>EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.35 ± 0.1</td>
<td>7.36 ± 0.1</td>
<td>7.38 ± 0.2</td>
</tr>
<tr>
<td>PO₂, mmHg</td>
<td>97.4 ± 3.4</td>
<td>101 ± 5.2</td>
</tr>
<tr>
<td>PCO₂, mmHg</td>
<td>49.6 ± 2.0</td>
<td>45.3 ± 2.5</td>
</tr>
<tr>
<td>Hemoglobin, g/l</td>
<td>13.5 ± 0.4</td>
<td>13.7 ± 0.8</td>
</tr>
<tr>
<td>SO₂, %</td>
<td>91.1 ± 0.45</td>
<td>92.7 ± 0.98</td>
</tr>
<tr>
<td>O₂ content, %</td>
<td>17 ± 0.7</td>
<td>17.9 ± 1.3</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>41.5 ± 1.3</td>
<td>42.1 ± 2.6</td>
</tr>
</tbody>
</table>

Table 1. Blood gases and other parameters on day 1 after alcohol and burn injury

- Values are means ± SE; n = 5 animals/group. EtOH, ethanol; SO₂, oxygen saturation.
gavaged with alcohol compared with rats gavaged with saline. Burn injury alone did not influence intestinal and hepatic blood flow compared with sham injured rats. In contrast, intestinal and hepatic blood flow was significantly suppressed in rats receiving a combined insult of alcohol and burn injury compared with rats receiving either injury alone. Furthermore, our data as presented in Table 2 suggest that lower hepatic blood flow is likely due to the decrease in portal flow rather than hepatic arterial flow. Hepatic arterial blood flow is increased in sham rats gavaged with alcohol and in burn injured rats gavaged with or without alcohol compared with the sham injured rats gavaged with saline. In addition to the intestine and liver, blood flow to the kidney was also decreased after the combined insult of alcohol and burn injury compared with rats receiving either burn injury or alcohol alone. No change in blood flow in the coronary arteries (heart), brain, lung, skin, and muscles was observed after alcohol and burn injury (Table 2).

**Systemic DO2, VO2, and oxygen extraction.** A significant increase in systemic (Fig. 4) as well as intestinal (Fig. 5A) and hepatic (Fig. 5B) DO2 was observed in rats gavaged with alcohol compared with sham injured rats gavaged with saline. No difference in DO2 was noted in burn injured rats gavaged with saline compared with sham injured rats. In contrast, a significant suppression in DO2 in the circulation as well as in the intestine and liver was observed in rats receiving combined alcohol and burn injury compared with burn injured rats gavaged with saline or sham injured rats irrespective of their treatment.

**DISCUSSION**

Previous studies from our laboratory have shown that alcohol ingestion before burn injury exacerbates intestinal immune suppression and impairs barrier functions (4). Multiple factors such as neutrophil infiltration and the production of cytokines have been proposed to contribute to the loss of mucosal integrity in burn, hemorrhagic shock, and sepsis (6, 8, 15, 32). However, an alteration in intestinal immune and barrier function could also result from changes in intestinal blood flow. Therefore, in this study, we attempted to determine the effect of alcohol ingestion before burn injury on heart performance and organ blood flow. Previous studies have suggested that the cardiovascular response to thermal injury has two separate phases (20, 29). The first, or initial, phase, which immediately follows the injury, is characterized by decreased blood flow to the intestine and liver, followed by a second phase, which is characterized by increased blood flow to the heart and brain (20, 29). The results presented in Fig. 6A further suggested that oxygen extraction was not affected in rats gavaged with alcohol. VO2 (Fig. 6B), on the other hand, was found to be nonsignificantly increased in rats gavaged with alcohol compared with sham injured rats gavaged with saline. In contrast, burn injury alone did not influence oxygen extraction and consumption. However, the combined insult of alcohol and burn injury resulted in a significant increase in both oxygen extraction and VO2 compared with rats receiving either burn injury alone in the absence of prior alcohol ingestion or in sham injured rats gavaged with saline.
tissues and organs and is thought to be caused by hypovolemia after injury. This initial phase is followed by a hypermetabolic phase characterized by increased blood flow to the tissues and organs and increased internal core temperature. Lorente et al. (20) found that burn patients have a hemodynamic profile similar to that of other trauma patients. They observed that burn injury results in marked systemic and pulmonary vasoconstriction and low DO2 and VO2. The findings from these studies further suggested that compared with survivors, non-survivors showed more systemic acidosis, lower cardiac index, more systemic hypotension and pulmonary hypertension, higher right and left filling pressures, lower DO2 and VO2, higher oxygen extraction, and higher pulmonary and systemic vascular resistance index. Similarly, in a sheep model of combined burn (40% TBSA) and smoke inhalation, Sakurai et al. (29) showed a biphasic hemodynamic response.

In addition to the biphasic response, many studies have shown a decrease in blood flow to the ileum, colon, pancreas, and spleen after burn injury (3, 10, 12–14, 29). These findings further indicated that the recovery from the initial low perfu-

**Table 2. Organ blood flow on day 1 after alcohol and burn injury**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Sham</th>
<th>EtOH</th>
<th>Burn</th>
<th>EtOH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>EtOH</td>
<td>Saline</td>
<td>EtOH</td>
</tr>
<tr>
<td>Hepatic portal</td>
<td>105.1±5.2</td>
<td>132.9±9.0*</td>
<td>121.3±18.5</td>
<td>65.9±2.7†</td>
</tr>
<tr>
<td>Hepatic artery</td>
<td>6.5±0.4</td>
<td>11.5±1.5*</td>
<td>9.5±2.3</td>
<td>10.2±2.9*</td>
</tr>
<tr>
<td>Kidney</td>
<td>478.8±4.6</td>
<td>500±59.5</td>
<td>477±87</td>
<td>318±30.2†</td>
</tr>
<tr>
<td>Skin</td>
<td>12.0±3.9</td>
<td>9.14±1.6</td>
<td>6.2±0.46*</td>
<td>9.1±1.6</td>
</tr>
<tr>
<td>Muscle</td>
<td>8.5±1.7</td>
<td>7.8±1.9</td>
<td>10.6±1.84</td>
<td>13.8±4.48</td>
</tr>
<tr>
<td>Coronary</td>
<td>534.5±38.4</td>
<td>463±37.8</td>
<td>486±51.9</td>
<td>477±83.3</td>
</tr>
<tr>
<td>Brain</td>
<td>47.7±5.8</td>
<td>51.6±2.4</td>
<td>45.4±6.0</td>
<td>47.3±8.2</td>
</tr>
<tr>
<td>Lung</td>
<td>64.7±13.8</td>
<td>65.9±18.3</td>
<td>62.2±4.3</td>
<td>61.5±6.7</td>
</tr>
</tbody>
</table>

Values are means ± SE (in ml·min⁻¹·100 g⁻¹); 5 animals/group. *P < 0.05 vs. sham gavaged with saline; †P < 0.05 vs. sham and burn-injured rats gavaged with saline.
In this study, we used a rat model of alcohol and burn injury in which rats were gavaged with alcohol 4 h before they received 25% TBSA burn injury. In this model, we did not observe a significant effect of burn injury on hemodynamic responses. In contrast, hemodynamic responses were significantly altered when burn injury was combined with prior alcohol intoxication. These findings corroborate further the suggestion that mild burn injury as has been described here and as used in a study by Carter et al. (3) did not produce hemodynamic alterations. However, if the mild injury is superimposed with additional stress factors, it produces alterations in cardiac functions and organ blood flow similar to those observed in experimental models of severe injury (31). McDonough et al. (22) have shown that in conscious guinea pigs, intraperitoneal administration of a moderate dose of alcohol before trauma potentiates cardiac dysfunction and decreases the mean time to death. Phelan et al. (28) showed that alcohol intoxication blunted counterregulatory responses to blood loss. The decrease in mean arterial blood pressure was achieved after lesser blood loss in the group subjected to a combined insult of ethanol and hemorrhage. However, the findings reported by Horton (12) suggest that intrajejunal administration of alcohol in dogs did not influence further posthemorrhagic shock cardiac dysfunction despite significantly greater acidosis in the intoxicated group. We observed that cardiac output and blood flow in the liver and small intestine were significantly lower in alcohol and burn injured rats compared with sham injured rats and rats subjected to burn injury in the absence of prior alcohol ingestion. Furthermore, in our studies, the decrease in total hepatic blood flow is likely due to a decrease in portal blood flow after alcohol and burn injury because no change or even an increase in hepatic arterial blood flow was observed 24 h after alcohol and burn injury. Similar findings have been reported previously in an experimental model of hemorrhagic shock (2). Similar to cardiac output and blood flow, DO2 was significantly lower in the liver and small intestine after alcohol and burn injury compared with rats receiving either insult alone. In contrast, oxygen extraction and VO2 were significantly increased in both organs. In this study, we did not find changes in oxygen extraction and consumption in heart, brain, lung, skin, and muscles. Although the mechanism of such differential responses remains unknown, previous studies have shown that the splanchnic hemodynamic response to shock is characterized by a selective vasoconstriction of the vessels in the mesentery (2, 16). Although this vasoconstriction may provide an advantage by preserving perfusion of vital organs after alcohol and burn injury, the decreased blood flow and DO2 to the small intestine and liver can lead to hypoxemia. The deficit in DO2 in the face of increased oxygen extraction and VO2 are likely to add to hypoxic insult to the liver and intestine. Several studies have shown that the postischemic diet creates an inflammatory environment that provokes multiple organ failure (8, 13, 15, 30). Wang et al. (34) have shown that depressed hemodynamics and blood flow result in the development of the immunosuppressive responses observed after trauma hemorrhage. Furthermore, the intestinal hypoperfusion after burn injury may lead to increased permeability and bacterial translocation (13, 30, 34). Although a definitive cause for impaired intestinal immune and barrier function after alcohol and burn injury remains to be established, reduced oxygen transport is likely to play a role in producing intestinal dysfunction after alcohol and burn injury.

Fig. 6. Oxygen extraction (A) and consumption (B) on day 1 after alcohol and burn injury. Values represent means ± SE from 5 animals/group. *P < 0.05 vs. sham regardless of alcohol ingestion and burn gavaged with saline; #P < 0.05 vs. sham gavaged with saline and burn gavaged with saline.

With regard to alcohol intoxication, studies have shown that both acute and chronic alcohol ingestion result in altered hemodynamic responses (9, 11, 12, 22, 28). We observed that 24 h after the ingestion of alcohol, there was an increase in blood flow and DO2 to the intestine and liver in the absence of injury. Although many of the previous studies have shown that acute alcohol ingestion results in impaired heart functions, some have suggested that acute alcohol ingestion improves cardiac function (9, 11, 12, 22). The contradictory reports of acute alcohol-induced cardiovascular changes are likely due to the differences in animal models, routes of alcohol administration (intraperitoneal vs. intragastric), the amount of alcohol consumed, and the time at which the studies were performed after alcohol intoxication.

In this study, we used a rat model of alcohol and burn injury in which rats were gavaged with alcohol 4 h before they consumed, and the time at which the studies were performed with regard to alcohol intoxication, studies have shown that acute alcohol intoxication improves cardiac function (9, 11, 12, 22). With regard to alcohol intoxication, studies have shown that acute alcohol intoxication improves cardiac function (9, 11, 12, 22). The contradictory reports of acute alcohol-induced cardiovascular changes are likely due to the differences in animal models, routes of alcohol administration (intraperitoneal vs. intragastric), the amount of alcohol consumed, and the time at which the studies were performed after alcohol intoxication.
In conclusion, our results indicate that a combined insult of alcohol and burn injury causes a decrease in blood flow and DO₂ to the liver and intestine. Such decreases in blood flow and DO₂ may cause hypoxic insult to the liver and intestine. Whereas a hypoxic insult to the liver would result in the release of proinflammatory mediators, a similar insult to the intestine will perturb both intestinal immune cell and barrier functions. Thus results from these studies suggest that reduced splanchnic blood flow and DO₂ may contribute to impaired intestinal immune and barrier function after alcohol and burn injury.

ACKNOWLEDGMENTS
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