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Regional variation in expression of pro-inflammatory mediators in the intestine following a combined insult of alcohol and burn injury

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Abstract

The intestine is segmented into functionally discrete compartments (duodenum, jejunum, ileum, and colon). The present study examined whether alcohol combined with burn injury differently influences cytokine levels in different parts of the intestine. Male mice were gavaged with alcohol (~2.9 g/kg) 4 h prior to receiving a ~12.5% total body surface area full thickness burn. Mice were sacrificed 1, 3, and 7 days after injury. The intestine segments (duodenum, jejunum, ileum, and colon) were harvested, homogenized, and analyzed for inflammatory mediators (IL-6, IL-18, and KC) using their respective ELISAs. KC levels were significantly increased in the jejunum, ileum, and colon following alcohol and burn injury as compared to shams. The increase in KC was ~28-fold higher in the colon as compared to the levels observed in duodenum following alcohol and burn injury. Both IL-6 and IL-18 levels were significantly elevated in both the ileum and colon following the combined insult. There was a ~7-fold increase in IL-6 levels in the colon as compared with the duodenum after the combined insult. Levels of IL-18 were increased by ~1.5-fold in the colon as compared to the ileum following alcohol and burn injury. The data suggest that pro-inflammatory mediators are differentially expressed in the intestine following alcohol and burn injury.

Keywords

inflammation; cytokines; chemokines; ethanol; gut

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Introduction

Approximately 450,000 burn injuries are reported each year in the United States (“Burn incidence and treatment in the US: 2013 fact sheet”, 2013). Nearly half of the reported burn injuries occur under the influence of alcohol (Choudhry et al., 2004; Haum et al., 1995; Jones, Barber, Engrav, & Heimbach, 1991). Multiple studies demonstrate that the combined insult results in delayed wound healing, longer hospitalization, and increased susceptibility to infection. Additionally, intoxicated patients also have significantly higher mortality rates and die from smaller burns (Choudhry et al., 2004; Haum et al., 1995; Jones et al., 1991). Experimental models of alcohol and burn injury corroborate clinical data illustrating that the combined insult results in adverse effects, including an increased innate response, particularly resulting in excess inflammation (Bird & Kovacs, 2008; Li, Akhtar, Kovacs, Gamelli, & Choudhry, 2011). Alternatively, alcohol and burn injury result in depression of the adaptive immune response with a decreased T cell response (Choudhry, Fazal, Goto, Gamelli, & Sayeed, 2002; Choudhry et al., 2004). Experimental data have also established that the combined insult further exacerbates production of inflammatory mediators (Li et al., 2011; Zahs, Bird, Ramirez, Choudhry, & Kovacs, 2013). Such an increase in inflammatory mediators following combined alcohol and burn injury is likely to contribute to multiple organ dysfunction/failure (Choudhry et al., 2004).

The intestine is the second largest immunological organ consisting of functionally discrete compartments – duodenum, jejunum, ileum, and colon. Additionally, the intestine is the largest bacterial reservoir within the body. Collectively, the intestine is responsible for nutrition absorption and maintaining an interface to prevent gut bacterial translocation (Mowat & Agace, 2014). Regions of the intestine (duodenum, jejunum, ileum, and colon) are functionally distinct and contain regional variations in antigen-presenting cells and bacterial content (Denning et al., 2011; Hao & Lee, 2004; Mowat & Agace, 2014). It is well established that the intestinal bacterial content increases progressively from the duodenum to the colon, where the colon contains the largest bacterial population (Hakansson & Molin, 2011; O’Hara & Shanahan, 2006). Both alcohol and burn injury alone perturb intestinal structural and functional integrity (Costantini et al., 2009; Gosain & Gamelli, 2005; Magnotti & Deitch, 2005). Similarly, alcohol combined with burn injury has been demonstrated to cause increased intestinal permeability and bacterial translocation (Choudhry et al., 2004; Li, Akhtar, & Choudhry, 2012; Rendon, Li, Akhtar, & Choudhry, 2013; Zahs et al., 2013). Previously, our lab also showed that acute alcohol exposure prior to burn injury results in significantly increased levels in inflammatory mediators in the terminal ileum (Li, Schwacha, Chaudry, & Choudhry, 2008; Li et al., 2011). However, regional variations of cytokine production following alcohol and burn injury have not been examined. The present study examined whether alcohol combined with burn injury differently influences the expression of cytokines in various parts of the intestine.

Materials and Methods

Animals

Adult 8–10-week-old male C57BL/6 mice (22–25 g) were obtained from Harlan Laboratories (Indianapolis, IN, USA). Mice were housed and acclimated for 2 weeks prior

to experimentation. All animal procedures were conducted in accordance with the Animal Care and Use Committee at Loyola University Chicago Health Sciences Division.

Mouse model of acute alcohol intoxication and burn injury

Mice were randomly divided into four groups: sham vehicle ($n = 11-12$), sham alcohol ($n = 10-12$), burn vehicle ($n = 5-8$), and burn alcohol ($n = 6-8$). As described previously, alcohol- or water-treated mice were gavaged with 0.4 mL of 25% alcohol in water (~2.9 g/kg) or water, respectively (Li et al., 2011). Four hours after the gavage, mice were anesthetized by intraperitoneal injection with a cocktail of ketamine and xylazine (80 mg/kg and 1.25 mg/kg, respectively). The dorsal surface was shaved and mice were transferred to a template, which is fabricated to expose ~12.5% of the total body surface area (TBSA). Mice in the burn group were immersed in 85–90 °C water for 7–8 sec. Mice in the sham group were immersed in lukewarm water for 7–8 sec. Mice were then dried and resuscitated with an intraperitoneal injection of 1.0 mL physiological saline (Li et al., 2011). Mice were returned to their cages and given water and food *ad libitum*.

Tissue harvesting

Mice were euthanized 1, 3, and 7 days after injury. The duodenum, jejunum, ileum, and colon were removed and immediately transferred into liquid nitrogen (Li et al., 2011).

Preparation of tissue homogenates

For the measurement of inflammatory mediators, tissue from the various groups was sonicated in phosphate-buffered saline (PBS) containing a protease inhibitor cocktail (Sigma Chemical Co., St. Louis, MO). Homogenates were cleared by centrifuging at 9,000 rpm at 4 °C for 30 min and stored at –80 °C.

Measurement of cytokines in tissue homogenates

IL-6, IL-18, and KC levels in tissue homogenates were determined by enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Mouse IL-6 and KC ELISA kits were purchased from R&D Systems (Minneapolis, MN), and mouse IL-18 ELISA kit was purchased from eBioscience (Santa Clara, CA).

Statistical analysis

All statistical analysis was performed using ANOVA with Tukey-Kramer's *post hoc* test (GraphPad InStat Software, San Diego, CA). A $p < 0.05$ was considered statistically significant.

Results

IL-6 levels significantly elevated in the ileum and colon following alcohol and burn injury

No change was observed in IL-6 levels in duodenum and jejunum following alcohol and burn injury compared to shams. IL-6 was significantly elevated in the ileum 1 day following burn injury alone compared to sham animals. A similar increase in IL-6 levels was noted in ileum and colon 1 day after a combined insult of alcohol and burn injury compared to sham.

While the increases in IL-6 levels were similar (2.5-fold) in both ileum and colon after alcohol and burn injury compared to shams, the net IL-6 amount was much higher in colon compared to small intestine (e.g., duodenum, jejunum, and ileum). An analysis of the data revealed a ~7-fold increase in IL-6 levels in the colon following alcohol and burn injury as compared to duodenum and ~3-fold compared to ileum. IL-6 levels were normalized to shams by days 3 and 7 after alcohol and burn injury.

IL-18 significantly elevated in ileum and colon following alcohol and burn injury

IL-18 is a pro-inflammatory cytokine and is associated with intestinal tissue damage (Akhtar, Li, Chaudry, & Choudhry, 2009; Akhtar, Li, Kovacs, Gamelli, & Choudhry, 2011). There was no change in IL-18 levels in jejunum after alcohol and burn injury compared to shams. Although IL-18 tended to increase in duodenum 1 day following the combined insult of alcohol and burn injury, this was not found to be significant compared to shams. IL-18 levels were only significantly elevated in the ileum and colon following alcohol and burn injury compared to shams. Furthermore, in contrast to IL-6, the net elevation in IL-18 levels was only 1.5-fold higher in the colon as compared with the ileum following alcohol and burn injury. Similar to IL-6, there was no change in IL-18 levels in the intestine on days 3 and 7 after alcohol and burn injury compared to shams.

KC levels increase proximal to the colon after combined alcohol and burn insult

To establish whether chemokine levels are also differentially affected in different parts of the gut following alcohol and burn injury (similar to cytokines), we analyzed KC levels in different parts of the intestine on days 1, 3, and 7 after alcohol and burn injury. KC levels were not significantly elevated in duodenum in any of the days following alcohol and burn injury. We observed a significant increase in KC levels following burn injury alone in the ileum and colon. Furthermore, KC levels were significantly elevated in the jejunum, ileum, and colon 1 day following alcohol and burn injury as compared to sham animals. KC levels progressively increased following alcohol and burn injury, the more proximal to the colon. KC levels increased ~28-fold in the colon following alcohol and burn injury as compared with duodenum and ~4-fold compared to ileum. The elevation in KC, however, was not observed in the jejunum, ileum, and colon on days 3 and 7 after alcohol and burn injury compared to shams.

Discussion

The intestine facilitates water and nutrient absorption. The intestine also functions in the maintenance of an immunological and physical barrier which prevents the gut bacteria from entering into extra-intestinal sites. The intestine is compartmentalized into four anatomically and functionally distinct segments (Mowat & Agace, 2014). These compartments of the intestine have different bacterial content, with the bacterial number increasing progressively from the duodenum to the colon. The duodenum and jejunum contain 10^2 – 10^4 colony forming units (cfu)/g, whereas the ileum's bacterial content is 10^{10} cfu/g. The colon's bacterial content is 10^{10} – 10^{12} cfu/g (Hakansson & Molin, 2011; O'Hara & Shanahan, 2006). Alcohol and burn injury have been associated with increased bacterial translocation (Choudhry & Chaudry, 2006). Alcohol exposure has also been demonstrated to increase

epithelial cell susceptibility to infection (Wood et al., 2013). Due to the regional variations in bacterial content and increased bacterial translocation because of alcohol and burn injury, it is important to determine if there are any differences in the inflammatory response in different parts of the intestine.

In our study, although we observed an increase in the pro-inflammatory cytokine levels in the jejunum, ileum, and colon, levels were several-fold higher in the colon compared to jejunum or ileum as a consequence of alcohol and burn injury. While the actual cause for differential expression of these cytokines/chemokines in various parts of the intestine remains to be established, the progressive increase in bacterial density from the duodenum to the colon is likely to contribute to an excess inflammatory response in the distal part (i.e., colon) compared to jejunum or ileum. In addition to bacteria, bacterial products such as endotoxins may also vary in different parts of the GI tract, and such differences in endotoxins are likely to contribute to regional differences in the inflammatory response in the GI tract. These findings implicate a role of bacterial overgrowth and endotoxins in intestinal inflammation.

The neutrophil chemokine, KC, increases more proximal to the colon following alcohol and burn injury. Circulating neutrophil sequestration to the site of injury following the combined insult is believed to be a major contributing factor to multiple organ failure. Neutrophils are the immediate responders following injury. An earlier study from our laboratory has shown an increase in neutrophil infiltration into the tissue following a combined insult of alcohol and burn injury (Akhtar et al., 2009). We observed that combined alcohol and burn insult significantly elevated KC levels in the jejunum, ileum, and colon compared to shams within 24 h after injury. Such an increase in KC is likely to cause neutrophil recruitment to the intestine, as observed in our previous studies. Similarly, the increase in IL-6 levels following alcohol exposure has also been associated with increased intestinal permeability (Al-Sadi et al., 2014). Additionally, findings from both patients and animal models indicate a relationship between the elevated levels of IL-6 and the development of septic complications (Hack et al., 1989; Remick, Bolgos, Copeland, & Siddiqui, 2005).

IL-18 has also been demonstrated to have a role in increased gut leakiness following combined alcohol and burn injury. Results from that study showed a role of IL-18 in decreased claudin-1 phosphorylation and occludin expression and phosphorylation (Li et al., 2012). Similarly, occludin dephosphorylation has been demonstrated to increase intestinal permeability (Dunagan, Chaudhry, Samak, & Rao, 2012). IL-18 levels were elevated in the duodenum, ileum, and colon following alcohol and burn injury. The elevation in duodenum, however, was not found to be significantly different from shams. We have previously demonstrated a role of IL-18 in increased neutrophil O_2^- production and their recruitment to the intestinal tissue. IL-18 has also been shown to delay neutrophil apoptosis (Akhtar et al., 2011). Such an increase in IL-18-dependent neutrophil infiltration into the intestinal bed may contribute to intestinal tissue damage and intestinal barrier disruption, leading to gut bacterial translocation following alcohol and burn injury (Rendon et al., 2013). This increase in translocation of bacteria or endotoxin is likely to contribute to sepsis and organ dysfunction under those conditions.

Together, the present study provides evidence that alcohol combined with burn injury causes inflammation throughout the intestinal tract but the colon or large intestine exhibits a more severe inflammatory response after combined alcohol and burn injury. Further studies are necessary to examine how regional changes in bacterial content in the gut lead to induction of the inflammatory response following alcohol and burn injury.

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Highlights

- Alcohol combined with injury results in increased inflammation in the intestine.
- The results further add that inflammatory mediators are differentially expressed in various parts of the intestine following alcohol and burn injury.
- The levels of inflammatory mediators were several fold higher in the colon compared to jejunum or ileum as a consequence of alcohol and burn injury.

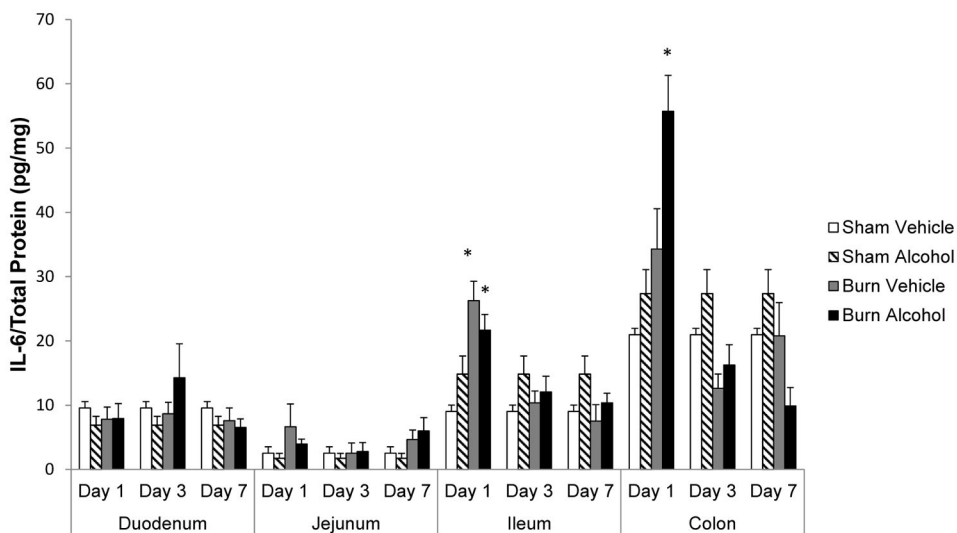


Figure 1. Effects of combined alcohol and burn injury on IL-6 production in intestinal tissue 1, 3, and 7 days post-injury, $*p < 0.05$ by ANOVA compared to respective sham vehicle. The data shown are mean \pm SEM of $n = 5-12$ animals per group. All sham vehicle and sham alcohol animals from all the days tested were pooled together.

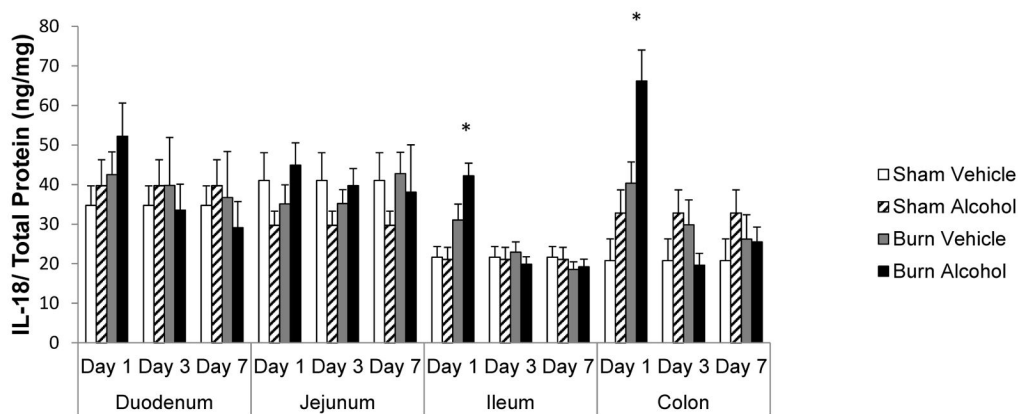


Figure 2. Effects of combined alcohol and burn injury on IL-18 production in intestinal tissue 1, 3, and 7 days post-injury, * $p < 0.05$ by ANOVA compared with respective sham vehicle. The data shown are mean \pm SEM of $n = 5-12$ animals per group. All sham vehicle and sham alcohol animals from all the days tested were pooled together.

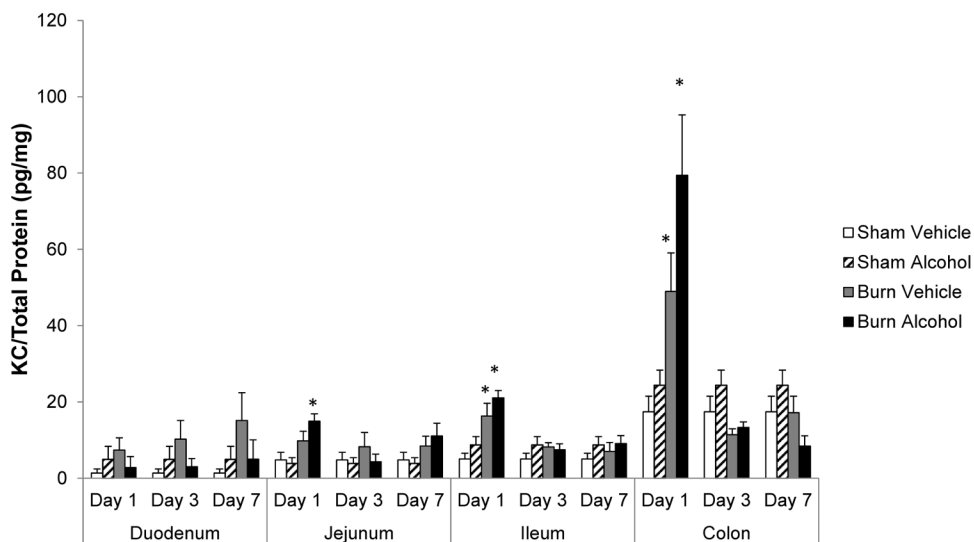


Figure 3. Effects of combined alcohol and burn injury on KC production in intestinal tissue 1, 3, and 7 days post-injury, * $p < 0.05$ by ANOVA compared with respective sham vehicle. The data shown are mean \pm SEM of $n = 5-12$ animals per group. All sham vehicle and sham alcohol animals from all the days tested were pooled together.