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

Niya L. Morris [a,b], Xiaoling Li [a], Zachary M. Earley [a,b], Mashkoor A. Choudhry [a,b,c,*]

[a] Alcohol Research Program, Burn and Shock Trauma Research Institute, Department of Surgery, Loyola University Chicago Health Sciences Division, Maywood, IL 60153, USA
[b] Integrative Cell Biology Program, Loyola University Chicago Health Sciences Division, Maywood, IL 60153, USA
[c] Department of Microbiology and Immunology, Loyola University Chicago Health Sciences Division, Maywood, IL 60153, USA

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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.

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 results 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

* Corresponding author. Burn & Shock Trauma Research Institute, Bldg. 110/EMS; Room 4236, Stritch School of Medicine, Loyola University Chicago Health Sciences Division, 2160 South First Ave., Maywood, IL 60153, USA. Tel.: +1 708 327 2463; fax: +1 708 327 2813.
E-mail address: mchoudhry@luc.edu (M.A. Choudhry).

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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 s. Mice in the sham group were immersed in lukewarm water for 7–8 s. 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 9000 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 (Fig. 1).

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 (Fig. 2).

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