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Impaired respiratory function and heightened pulmonary inflammation in episodic binge ethanol intoxication and burn injury

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ABSTRACT

Clinical data indicate that cutaneous burn injuries covering greater than 10% of the total body surface area are associated with significant morbidity and mortality, in which pulmonary complications, including acute respiratory distress syndrome (ARDS), contribute to nearly half of all patient deaths. Approximately 50% of burn patients are intoxicated at the time of hospital admission, which increases days on ventilators by 3-fold, and doubles the length of hospitalization, compared to non-intoxicated burn patients. The most common drinking pattern in the United States is binge drinking, where an individual rapidly consumes alcoholic beverages (4 for women, 5 for men) in 2 h. An estimated 38 million Americans binge drink, often several times per month. Experimental data demonstrate that a single binge-ethanol exposure, prior to scald injury, impairs innate and adaptive immune responses, thereby enhancing infection susceptibility and amplifying pulmonary inflammation, neutrophil infiltration, and edema, and is associated with increased mortality. Since these characteristics are similar to those observed in ARDS burn patients, our study objective was to determine whether ethanol intoxication and burn injury and the subsequent pulmonary congestion affect physiological parameters of lung function, using non-invasive and unrestrained plethysmography in a murine model system. Furthermore, to mirror young adult binge-drinking patterns, and to determine the effect of multiple ethanol exposures on pulmonary inflammation, we utilized an episodic binge-ethanol exposure regimen, where mice were exposed to ethanol for a total of 6 days (3 days ethanol, 4 days rest, 3 days ethanol) prior to burn injury. Our analyses demonstrate mice exposed to episodic binge ethanol and burn injury have higher mortality, increased pulmonary congestion and neutrophil infiltration, elevated neutrophil chemoattractants, and respiratory dysfunction, compared to burn or ethanol intoxication alone. Overall, our study identifies plethysmography as a useful tool for characterizing respiratory function in a murine burn model and for future identification of therapeutic compounds capable of restoring pulmonary functionality.

Introduction

Burn injury is associated with significant morbidity and mortality, with greater than 10% of patients succumbing to their injuries when the burn size exceeds 10% of their total body surface area (ABA, 2014). Clinical data suggest that 42% of the mortality observed in burn patients is due to pulmonary complications (Achauer, Allyn, Furnas, & Bartlett, 1973; Phillips & Cope, 1962). Severe burn, even in the absence of inhalation injury, is a common predisposing factor for the development of Acute Respiratory Distress Syndrome (ARDS) (Liffner, Bak, Reske, & Sjöberg, 2005; Turnage et al., 2002). ARDS is associated with rigid lungs, hypoxemia, and bilateral infiltrates in chest radiographs (Ashbaugh, Bigelow, Petty, & Levine, 1967). Overall, respiratory dysfunction in burn patients is characterized by shallow breathing and fluid accumulation in the lung interstitium, leading to heightened vascular resistance and an increased effort to breathe (Achauer et al., 1973; Turnage et al., 2002). The net result of insufficient gas exchange underlies the large percentage of burn fatalities due to pulmonary complications.

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Binge alcohol drinking is an increasingly prevalent activity, affecting an estimated 38 million adults in the United States (CDC, 2012). It is characterized by either the number of alcoholic drinks one consumes in 2 h (4 for women, 5 for men) or by a blood alcohol concentration of 0.08%. Interestingly, approximately 50% of burn patients are under the influence of alcohol at the time of hospital admission (Grobmyer, Maniscalco, Purdue, & Hunt, 1996; Silver et al., 2008). Intoxicated burn patients have three times as many days on ventilators and an overall twice as long hospital stay compared to burn patients who were not intoxicated (Hadjizacharia et al., 2011; Silver et al., 2008). This leads to an increased risk of pulmonary complications that predisposes burn patients to multiple organ failure, with the lungs preceding all other organs, as well as a higher chance of mortality (Ciesla et al., 2005; Hollingsed, Saffle, Barton, Craft, & Morris, 1993). Notably, the vast majority of intoxicated burn patients are binge drinkers and not chronic dependent drinkers (Howland & Hingson, 1987; Schermer, 2006; Smith & Kraus, 1988). Experimental models of binge alcohol intoxication and burn injury have demonstrated alterations in innate and adaptive immunity that result in marked immune dysfunction, greater susceptibility to infection, and amplified pulmonary inflammation (Bird, Morgan, Ramirez, Yong, & Kovacs, 2010; Bird, Zahs, et al., 2010; Choudhry et al., 2000; Faunce, Gregory, & Kovacs, 1997, 1998; Kawakami, Switzer, Herzog, & Meyer, 1991; Messingham, Faunce, & Kovacs, 2002; Murdoch, Brown, Gamelli, & Kovacs, 2008; Murdoch, Karavitis, Deburghgraeve, Ramirez, & Kovacs, 2011; Patel, Faunce, Gregory, Duffner, & Kovacs, 1999). Previously, our laboratory established that in a mouse model of single-dose binge ethanol exposure and burn injury there is amplified neutrophil infiltration, alveolar wall thickening, and edema in the lungs when alcohol precedes burn injury (Bird, Morgan, et al., 2010; Bird, Zahs, et al., 2010; Chen et al., 2013, 2014; Patel et al., 1999). Since ARDS is associated with similar pulmonary characteristics in humans, including increased neutrophil infiltration, capillary permeability, and pulmonary edema (Dancey et al., 1999; Liffner et al., 2005; Steinvall, Bak, & Sjöberg, 2008), the objective of our study was to determine, using a murine model system, whether intoxication and burn injury and the resulting histological pulmonary congestion affect physiological parameters of lung function.

In these studies, we used non-invasive and unrestrained plethysmography to examine the impact of pulmonary congestion caused by burn injury on breathing patterns and respiratory function (Irvin & Bates, 2003). The Center for Disease Control (CDC) has reported 1 in 6 adults binge drink at least 4 times a month. Additionally, weekend binge drinking is a pattern observed in many cultures (Horvat et al., 2015). To mirror this drinking pattern, our laboratory used a mouse model of episodic binge ethanol intoxication prior to burn injury (adapted from Callaci et al., 2004; Przybycien-Szymanska, Mott, & Pak, 2011; Przybycien-Szymanska, Rao, & Pak, 2010; Qin et al., 2014; Vaagenes et al., 2015) to assess respiratory physiology and gain an understanding of the effect of intoxication on lung function after burn injury.

Overall, our analyses identify plethysmography as a useful tool for characterizing respiratory function in a murine model of ethanol intoxication and burn injury. Our studies demonstrate that episodic binge ethanol intoxication prior to burn injury causes increased pulmonary neutrophil infiltration. The timing of neutrophil accumulation in the lungs parallels the heightened levels of lung neutrophil chemoattractants and is associated with respiratory dysfunction, which likely contributes to diminished survival rates.

Materials and methods

Mice

Male (C57BL/6) mice were purchased from Jackson Laboratories (Bar Harbor, ME) and used at 8–10 weeks old. Mice were housed in sterile micro-isolator cages under specific pathogen-free conditions in the Loyola University Medical Center Comparative Medicine facility. All experiments were conducted in accordance with the Institutional Animal Care and Use Committee. Mice weighing between 22 and 27 g were used in these studies.

Murine model of binge ethanol and burn injury

A murine model of episodic binge ethanol intoxication and burn injury was employed using intraperitoneal injections as described previously (Faunce et al., 1997; Messingham, Fontanilla, Colantoni, Duffner, & Kovacs, 2000; Qin et al., 2014). Animals were given ethanol (1.2 g/kg) or saline vehicle at a dose designed to elevate the blood alcohol concentration (BAC) to 150 mg/dL at 30 min after ethanol exposure (Murdoch et al., 2008). This dose of 150 μL of 20% (v/v) ethanol solution or saline control was given daily for 3 consecutive days, then mice were given 4 days without ethanol or saline, and then given 3 additional daily ethanol or saline doses. Thirty minutes following the final ethanol exposure, when the BAC was 150 mg/dL, the mice were anesthetized (100 mg/kg ketamine and 10 mg/kg xylazine) and their dorsums shaved. The mice were placed into a plastic template exposing 15% of the total body surface area and subjected to a scald injury in a 92–95 °C water bath or a sham injury in room-temperature water (Faunce et al., 1997). The scald injury resulted in an insensate, full-thickness burn (Faunce et al., 1999). The mice were then resuscitated with 1.0 mL saline and allowed to recover on warming pads. All experiments were performed between 8:00 and 9:00 AM to avoid confounding factors related to circadian rhythms. Animals were either euthanized at 24 h or survival was measured out to 7 days post-injury.

Plethysmography

Pulmonary function was assessed at 24h post-injury by using barometric plethysmography (Buxco Research Systems). BAC levels had returned to baseline undetectable levels at this time point (Karavitis, Murdoch, Gomez, Ramirez, & Kovacs, 2008). Mice were placed in an unrestrained whole body barometric plethysmography chamber and allowed to acclimate to the environment before lung function parameters were recorded for 10 min on a breath-by-breath basis. Enhanced pause (Penh), breath frequency (f), tidal volume (TVb), and minute volume (MVb) were analyzed.

Histopathologic examination of the lungs

The upper right lobe of the lung was inflated with 10% formalin and fixed overnight as described previously (Patel et al., 1999), embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). Sections were evaluated using light microscopy (Zeiss AxioVert, Zeiss, Thorndale, CA) and histology photographs were taken at 1000× magnification. To measure pulmonary congestion, photographs were taken in a blinded fashion of 10 high-power fields (400×) per animal and analyzed using the Java-based imaging program ImageJ (National Institutes of Health, Bethesda, MD). The images were converted to binary to differentiate lung tissue from air space and then analyzed for the percent area covered by lung tissue in each field of view as described