BBA - Molecular Basis of Disease xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Dysregulation of microRNA biogenesis in the small intestine after ethanol and burn injury

Niya L. Morris[a,b], Adam M. Hammer[a,b], Abigail R. Cannon[a,b], Robin C. Gagnon[a], Xiaoling Li[a], Mashkoor A. Choudhry[a,b,c,*]

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

ARTICLE INFO

Keywords:
Alcohol
miRs
MicroRNA processing
Inflammation
Gut
Trauma

ABSTRACT

Ethanol exposure at the time of burn injury is a major contributor to post-burn pathogenesis. Many of the adverse effects associated with ethanol and burn injury are linked to an impaired intestinal barrier. The combined insult causes intestinal inflammation, resulting in tissue damage, altered tight junction expression, and increased intestinal permeability. MicroRNAs play a critical role in maintaining intestinal homeostasis including intestinal inflammation and barrier function. Specifically, miR-150 regulates inflammatory mediators which can contribute to gut barrier disruption. The present study examined whether ethanol and burn injury alter expression of microRNA processing enzymes (Drosha, Dicer, and Argonaute-2) and miR-150 in the small intestine. Male mice were gavaged with ethanol (~2.9 g/kg) 4 h prior to receiving a ~12.5% total body surface area full thickness burn. One or three days after injury, mice were euthanized and small intestinal epithelial cells (IECs) were isolated and analyzed for expression of microRNA biogenesis components and miR-150. Dicer mRNA and protein levels were not changed following the combined insult. Drosha and Argonaute-2 mRNA and protein levels were significantly reduced in IECs one day after injury; which accompanied reduced miR-150 expression. To further determine the role of miR-150 in intestinal inflammation, young adult mouse colonocytes were transfected with a miR-150 plasmid and stimulated with LPS (100 ng/ml). miR-150 overexpression significantly reduced IL-6 and KC protein levels compared to vector control cells challenged with LPS. These results suggest that altered microRNA biogenesis and associated decrease in miR-150 likely contribute to increased intestinal inflammation following ethanol and burn injury.

1. Introduction

Nearly 500,000 burn injuries are reported each year in the United States resulting in 40,000 hospitalizations [1]. Approximately, 50% of these injuries occur under the influence of alcohol (ethanol) [2–6]. Studies have shown that ethanol exposure at the time of burn injury further confounds the post-burn pathogenesis by delaying wound healing, resulting in longer hospitalization, and increasing susceptibility to infections [4,7,8]. Furthermore, ethanol intoxication at the time of burn injury increases the risk of sepsis and multiple organ failure [8,9]. Additional findings suggest that patients who ingested ethanol prior to burn injury had higher mortality from smaller burn injuries [2,3]. Several lines of evidence suggest a role of gut barrier dysfunction in these co-morbidities associated with ethanol and burn injury [10–16].

Recent findings have demonstrated that the gut barrier disruption following ethanol and burn injury is widely associated with excess inflammation [13,16–18]. These studies suggest that increases in intestinal inflammatory mediators such as IL-18, IL-6 or other chemokines can directly or *via* recruitment of neutrophils cause intestinal tissue damage and alter tight junction protein expression [13,16,17,19–21]. Many studies have shown a role for microRNAs in

Abbreviations: Ago-2, Argonaute-2; IECs, intestinal epithelial cells; LPS, lipopolysaccharide; miR, microRNA; miRISC, miRNA induced silencing complex; PBS, Phosphate-buffered saline; YAMCs, young adult mouse colonocytes

* Corresponding author at: Burn & Shock Trauma Research Institute, CTRE 320, Stritch School of Medicine, Loyola University Chicago Health Sciences Division, 2160 South First Ave, Maywood, IL 60153, USA.

E-mail addresses: nmorris3@luc.edu (N.L. Morris), adhammer@luc.edu (A.M. Hammer), acannon@luc.edu (A.R. Cannon), rgagnon1@luc.edu (R.C. Gagnon), xiali@luc.edu (X. Li), mchoudhry@luc.edu (M.A. Choudhry).

http://dx.doi.org/10.1016/j.bbadis.2017.03.025
Received 9 December 2016; Received in revised form 8 March 2017; Accepted 31 March 2017

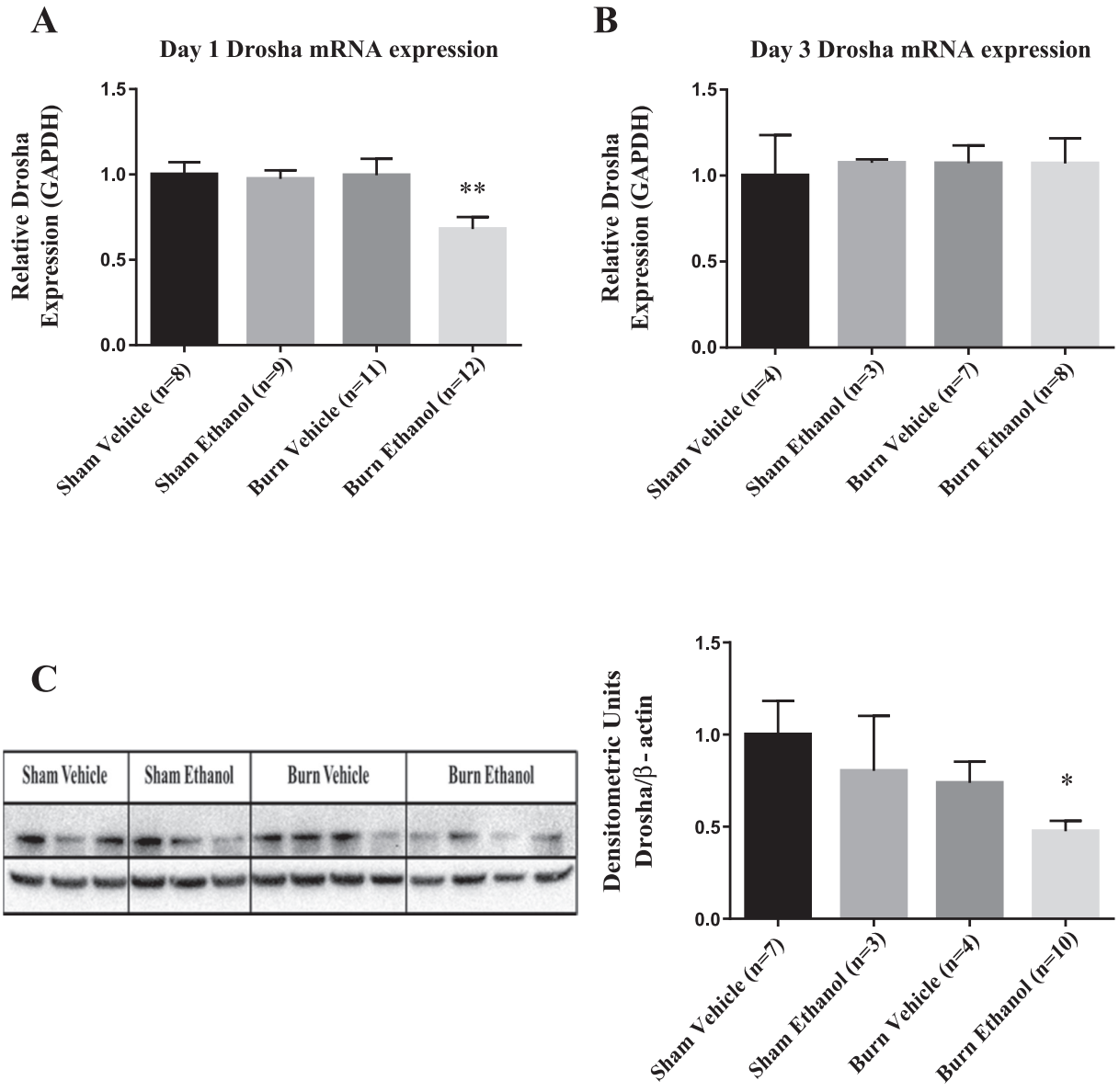

Fig. 1. Drosha mRNA and protein levels are significantly reduced in IECs following ethanol and burn injury.IECs were used to examine Drosha expression day one (A) and day three (B) following ethanol and burn injury. Values were calculated using a ΔΔCT method and normalized to sham vehicle animals. GAPDH was used as an endogenous control. (C) Homogenates from IECs harvested one day after ethanol and burn injury were used to examine Drosha protein levels. Densitometry measurements for Drosha are given as a ratio of Drosha density to β-actin, normalized to sham vehicle animals and the values are shown as means ± standard error of the means of duplicate experiments. $^{*}p < 0.05$, $^{**}p < 0.01$ by One-Way ANOVA compared to sham vehicle.

tissue inflammation following ethanol exposure or tissue injury [22,23]. However, whether microRNAs play a role following ethanol and burn injury remains unknown.

MicroRNAs are small noncoding RNA sequences, which regulate gene expression at the post-transcriptional level [24–27]. Biogenesis of microRNAs occurs in several steps, starting with transcription by RNA polymerase II forming primary microRNA (pri-miRNA), which is then cleaved by Drosha (an RNase III enzyme) resulting in a precursor miRNA (pre-miRNA). The pre-miRNA is exported from the nucleus by exportin-5, where it is cleaved by Dicer [24,25]. Cleavage by Dicer results in a duplex miRNA complex containing both the guide and passenger strand. The guide strand is loaded onto an Argonaute (Ago) protein forming the miRNA induced silencing complex (miRISC); while the passenger stand is usually degraded. The guide miRNA uses partial base pairing to guide miRISC to its target mRNA. Binding of miRISC to the target mRNA allows for miRNA mediated gene regulation [24,25,28].

Numerous studies have illustrated the importance of microRNAs in maintenance of the intestinal barrier [29–31]. Thus, altered expression of microRNAs could negatively affect the intestinal barrier. Changes in microRNAs expression as a result of ethanol and burn injury could potentially alter the levels of pro-inflammatory cytokines, which have been associated with excessive tissue damage and altered tight junction expression [13,16,19–21]. Specifically, miR-150 is predicted to target the inflammatory mediator IL-18 and is down-regulated in sepsis patients and following exposure of cells to bacterial lipopolysaccharide (LPS) *in vitro* [32,33]. Furthermore, Liu et al. demonstrated miR-150 overexpression reduces inflammatory mediators (TNF-α, IL-1β, and IL-6) in monocytes [34].

We examined whether ethanol and burn injury modulates miR-150 expression and microRNA biogenesis in intestinal epithelial cells (IECs). We hypothesized that microRNA expression and biogenesis would be diminished following ethanol and burn injury. Our data suggest that ethanol and burn injury diminishes Drosha and Ago-2 expression.