Alcohol 49 (2015) 57–64

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Acute immunomodulatory effects of binge alcohol ingestion

Majid Afshar [a,*], Stephanie Richards [b], Dean Mann [c], Alan Cross [d], Gordon B. Smith [e], Giora Netzer [a,f], Elizabeth Kovacs [g], Jeffrey Hasday [a]

[a] Division of Pulmonary and Critical Care Medicine, University of Maryland School of Medicine, 110 S. Paca Street, Baltimore, MD 21202, USA
[b] School of Medicine, University of Maryland, 22 S. Greene Street, Baltimore, MD 21202, USA
[c] Department of Pathology, University of Maryland School of Medicine, 22 S. Greene Street, Baltimore, MD 21202, USA
[d] Center for Vaccine Development, University of Maryland School of Medicine, 22 S. Greene Street, Baltimore, MD 21202, USA
[e] Shock Trauma and Anesthesiology Research (STAR) Organized Research Center, University of Maryland, 22 S. Greene Street, Baltimore, MD 21202, USA
[f] Department of Epidemiology and Public Health, University of Maryland School of Medicine, 22 S. Greene Street, Baltimore, MD 21202, USA
[g] Alcohol Research Program, Department of Surgery, Loyola University Chicago, Health Sciences Campus, 2160 S. First Avenue, Maywood, IL 60153, USA

ARTICLE INFO

Article history:
Received 19 August 2014
Received in revised form
30 September 2014
Accepted 8 October 2014

Keywords:
Ethanol
Binge drinking
Innate immunity
Cytokines
Antigen-presenting cells
Peripheral blood mononuclear cells

ABSTRACT

Background: Blood alcohol is present in a third of trauma patients and has been associated with organ dysfunction. In both human studies and in animal models, it is clear that alcohol intoxication exerts immunomodulatory effects several hours to days after exposure, when blood alcohol is no longer detectable. The early immunomodulatory effects of alcohol while blood alcohol is still elevated are not well understood.

Methods: Human volunteers achieved binge alcohol intoxication after high-dose alcohol consumption. Blood was collected for analysis prior to alcohol ingestion, and 20 min, 2 h, and 5 h after alcohol ingestion. Flow cytometry was performed on isolated peripheral blood mononuclear cells, and cytokine generation in whole blood was measured by enzyme-linked immunosorbent assay (ELISA) after 24-h stimulation with lipopolysaccharide (LPS) and phytohemagglutinin-M (PHA) stimulation.

Results: An early pro-inflammatory state was evident at 20 min when blood alcohol levels were ~130 mg/dL, which was characterized by an increase in total circulating leukocytes, monocytes, and natural killer cells. During this time, a transient increase in LPS-induced tumor necrosis factor (TNF)-α levels and enhanced LPS sensitivity occurred. At 2 and 5 h post-alcohol binge, an anti-inflammatory state was shown with reduced numbers of circulating monocytes and natural killer cells, attenuated LPS-induced interleukin (IL)-1β levels, and a trend toward increased interleukin (IL)-10 levels.

Conclusions: A single episode of binge alcohol intoxication exerted effects on the immune system that caused an early and transient pro-inflammatory state followed by an anti-inflammatory state.

Introduction

Traumatic injury accounted for 30% of all life years lost in the United States and was the leading cause of death in Americans between 1 and 44 years old (CDC, 2014). Acute alcohol exposure was encountered in more than 30% of trauma patients, and intoxication carried the highest risk for injury (Afshar et al., 2014; Gmel et al., 2006). In preclinical trauma studies, alcohol-induced vasodilation, diuresis, decreased cardiac output, impaired vasoreactivity, and depressed myocardial contractility negatively impacted resuscitation and worsened outcomes (Molina, Sulzer, & Whitaker, 2013). Alcohol-exposed trauma victims who survived their injuries were at increased risk for nosocomial infection and acute respiratory distress syndrome (Afshar et al., 2014; Gmel et al., 2006). Acute alcohol ingestion also decreased resistance to infection-related complications and increased risk for mortality in humans and animals with sepsis (Pruett et al., 2010; Shih et al., 2003). The risks for systemic inflammatory response syndrome and sepsis after alcohol exposure were best described in trauma patients with blood alcohol levels above 100 mg/dL (Griffin, Poe, Cross, Rue, & McGwin, 2009).

Experimental studies have shown acute alcohol exposure resulted in suppressed pro-inflammatory cytokine release in response to an inflammatory challenge, decreased neutrophil recruitment and phagocytic function, and impaired chemotaxis (Molina et al., 2013). Most murine models focused on time points

* Corresponding author. Loyola University Health Systems, 2160 S. First Avenue, Maywood, IL 60153, USA. Tel.: +1 708 216 0461; fax: +1 708 216 6839.
E-mail address: Majid.afshar@luhs.org (M. Afshar).

http://dx.doi.org/10.1016/j.alcohol.2014.10.002
0741-8329/

when blood alcohol was no longer present (Fuance, Gregory, & Kovacs, 1997, 1998), but even in murine models examining very early effects on immune response when blood alcohol was still present, markedly suppressed cellular immunity with inhibition of IL-6 and TNF-α production was found (Goral, Choudhry, & Kovacs, 2004; Goral & Kovacs, 2005). Additional murine *in vivo* and *in vitro* studies showed alcohol suppressed alveolar macrophage phagocytosis of *P. aeruginosa* and inhibited splenic and alveolar macrophage pro-inflammatory cytokine release in response to LPS stimulation as soon as 3 h from alcohol exposure, at physiologically relevant blood alcohol levels (Karavitis, Murdoch, Deburghgraeve, Ramirez, & Kovacs, 2012; Karavitis, Murdoch, Gomez, Ramirez, & Kovacs, 2008). Trauma models with full thickness excisional wound injury and episodic alcohol intoxication exhibited delayed wound closure with dysfunction in several components of the innate immune response, including phagocyte recruitment and chemokine/cytokine production in the alcohol-treated mice (Curtis, Hlavin, Brubaker, Kovacs, & Radek, 2014; Fitzgerald et al., 2007). In trauma patients, acute alcohol exposure was associated with altered immune responsiveness at several days after injury (Szabo, Mandrekar, Verma, Isaac, & Catalano, 1994). The immunomodulatory effects of alcohol in humans have been studied many hours to days after alcohol exposure, when blood alcohol is no longer detectable. Elevated blood alcohol is a frequent occurrence in trauma patients, and understanding the acute effects of alcohol on host response to infection and other inflammatory stimuli has clinical relevance to the care of such patients.

The purpose of this study was to elucidate the early immune effects of binge alcohol ingestion on circulating leukocyte composition and function in healthy human subjects. We used flow cytometry and whole blood cytokine assays to define the temporal changes in circulating leukocyte composition and function in 15 young healthy volunteers during the first 5 h after alcohol ingestion.

Methods

Reagents and antibodies

The following antibodies were purchased from BD Pharmingen, unless otherwise noted: BV421-conjugated anti-CD3 (#562426); BV421-conjugated anti-CD4 (BioLegend, #300532); BV421-conjugated anti-CD56 (BD Horizon, #562751); BV421-conjugated anti-CD86 (#562432); BV421-conjugated anti-HLA-DR (BD Horizon, #562804); BV421-conjugated IgG1, κ Isotype Control (BioLegend, #400158); BV421-conjugated IgG2a, κ Isotype Control (BD Horizon, #562439); BV421-conjugated IgG2b, κ Isotype Control (BioLegend, #400342); FITC-conjugated anti-CD4 (#555346); FITC-conjugated anti-CD14 (#555397); FITC-conjugated anti-CD15 (#562370); FITC-conjugated anti-CD19 (#555412); FITC-conjugated anti-CD25 (#555431); FITC-conjugated anti-CD69 (#555530); FITC-conjugated IgG1, κ Isotype Control (#555748); FITC-conjugated IgG2a, κ Isotype Control (#555573); PE-conjugated anti-CD8 (#555367); PE-conjugated anti-CD11b (BioLegend, #301406); PE-conjugated anti-Cd11c (#555392); PE-conjugated IgG1, κ Isotype Control (#555749); APC-conjugated anti-CD3 (#555335); APC-conjugated anti-CD11a (R&D Systems, #FAB3595A); APC-conjugated anti-HLA-DR (#559866); APC-conjugated IgG1, κ Isotype Control (#555751); and APC-conjugated IgG2a, κ Isotype Control (BioLegend, #400220).

Alcohol dosing protocol

The Institutional Review Board of the University of Maryland, Baltimore approved this study. We recruited 15 healthy human volunteers without a history of an alcohol-use disorder (Hasin, Fenton, Beseler, Park, & Wall, 2012), identified by the Short Michigan Alcohol Survey Tool and the 2010 National Health Survey alcohol questionnaire. All subjects had normal liver function as determined by hepatic function panel laboratory testing, and abstained from alcohol ingestion, smoking, and caffeine for 72 h prior to study enrollment. Alcohol abstinence was confirmed by the urinary biomarkers ethyl glucuronide and ethyl sulfate (Høiseth et al., 2008).

Following screening and enrollment, subjects consumed 100 proof ethanol (50% alcohol by volume) mixed with chilled sugar-free flavored seltzer water in a 1:4 ratio (1 part alcohol spirits to 4 parts seltzer water) over 20 min. The alcohol dose was calculated based on actual body weight and sex (0.89 g/kg body weight for men and 0.81 g/kg for women) to reach a target breath alcohol content (BrAC) >0.1%. After a 20-min absorption period, BrAC level was measured using a BACTrack® S80 Select Breathalyzer (KHN Solutions LLC, San Francisco, CA; sensor accuracy ± 0.005% blood alcohol content [BAC] at 0.050% BAC) and was repeated every 30 min. BAC was measured in heparinized blood prior to alcohol ingestion to confirm an undetectable level. For correlation with BrAC measurements, BAC was also measured at the following times after BrAC level 0.1% was achieved: 20 min, 2 h, and 5 h. When BrAC decreased to ≤0.03% and no significant adverse effects were noted, subjects were discharged from the General Clinical Research Center at the University of Maryland.

Peripheral blood mononuclear cell preparation

Blood was collected for peripheral blood mononuclear cell (PBMC) isolation prior to alcohol ingestion and at 20 min, 2 h, and 5 h after BrAC 0.1% was achieved. Whole blood was collected in sodium heparin tubes and transported to the lab for PBMC isolation. PMBCs were isolated from whole blood within 3 h of collection by density gradient centrifugation with Lymphocyte Separation Medium (Corning, #25-072-CV, density = 1.077–1.080 g/mL), according to the manufacturer's protocol. Cell counts and viability were determined with trypan blue (Corning, #25-900-CI) and manual counting, and PBMCs were cryopreserved in RPMI-1640 Medium (Corning, #15-040-CV) containing 20% heat-inactivated human AB serum and 10% dimethylsulfoxide (American Bioanalytical, #AB00435). Cells were frozen at approximately 2.0×10^6 per vial with an automated step-down freezer (Gordinier Electronics) and stored in the vapor phase of liquid nitrogen at −140 °C for flow cytometric analysis, usually the following day.

On the day of analysis, PBMCs were thawed and suspended in RPMI-1640 Medium with 20% fetal bovine serum. To block non-specific antibody binding to Fc receptors, the cells were incubated in blocking buffer (phosphate-buffered-saline (PBS) 1× with 5% heat-inactivated human AB serum) for 15 min on ice prior to staining with labeled antibodies. Cell counts and viability were again determined with trypan blue, and cells were then stained with antibodies in the dark at 4 °C for 30 min at 1.0 to 2.0×10^5 cells per tube. The cells were subsequently washed with Flow Wash Buffer (PBS with 1% fetal bovine serum) and resuspended in 1% paraformaldehyde for flow cytometry analysis.

Flow cytometry analysis

Sample acquisition was performed using an iCyt Eclipse Flow Cytometer (Sony Biotechnology, Inc.) with a minimum of 2.0×10^4 cells acquired per sample tube. The data were analyzed using iCyt EC800 software, version 1.3.5.