



Published in final edited form as:

Alcohol. 2012 December ; 46(8): 783–787. doi:10.1016/j.alcohol.2012.05.005.

Alcohol and epigenetic changes: Summary of the 2011 Alcohol and Immunology Research Interest Group (AIRIG) meeting

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Abstract

On November 18, 2011, the 16th annual Alcohol and Immunology Research Interest Group (AIRIG) meeting was held at Loyola University Medical Center in Maywood, Illinois. The focus of this year's meeting was alcohol's effect on epigenetic changes and possible outcomes induced by these changes. Two sessions, which consisted of talks from invited speakers as well as presentations of selected abstracts, were held in addition to a poster session. Participants presented information on alcohol-induced alterations in histone modifications and gene expression along with immunologic responses to alcohol. Speakers shared new research specifically on histone deacetylase enzyme expression and modifications due to alcohol and the downstream effect of these modifications may have on gene expression and tissue damage. Additional studies suggested that alcohol exacerbates inflammation when combined with other insults such as infection, trauma, inhalation injury, and disease.

Keywords

Alcohol; Epigenetics; Histone; Immune response; Inflammation

Introduction

The tissue and cell-type specific modulation of gene expression profiles by alcohol consumption is well documented, though the identification of epigenetic factors as key mediators of this effect is an exciting, recent advancement in the field. An epigenetic trait was recently re-defined as “a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence” (Berger, Kouzarides, Shiekhatar, & Shilatifard, 2009), hence gene expression patterns in different tissues from the same organism are unique, even though every cell carries the identical genetic code. Epigenetic regulation can involve DNA methylation, covalent modification (methylation, acetylation, phosphorylation, ubiquitinylation, ADP-ribosylation, and sumoylation) of select histone amino acids, and non-coding RNAs. Age, environment, drugs, and toxins, including alcohol, directly influence epigenetics. Epigenetic memory may be a contributing factor in the

“second-hit hypothesis” which postulates that alcohol exposure (the first hit) exaggerates physiological responses to toxins, injury, or infection (the second hit).

The effect of alcohol exposure on histone tail modification appears to be both dose-dependent and tissue-specific. For example, researchers utilized a rat binge ethanol model to compare the modification status of one specific histone lysine residue, H3K9, across 11 different tissues and found that global H3K9 acetylation was robustly increased in liver, lung, spleen, and testes, while no changes were observed in blood vessels, pancreas, colorectum, stomach, heart, brain or kidney, and no global change in H3K9 methylation was observed in any tissue (Kim & Shukla, 2005). The activity and protein expression of several enzymes mediating histone acetylation, including the histone acetyltransferases (HATs), are also up-regulated by chronic alcohol in the liver, as is overall DNA methylation (Bardag-Gorce et al., 2007). In the brain, ethanol withdrawal-induced seizures are associated with NMDA receptor hyperexcitability and excitotoxicity and likely involve changes in DNA methylation in the *NR2B receptor* subunit promoter (Marutha Ravindran & Ticku, 2004, 2005), further demonstrating the multi-factorial, tissue-specific epigenetic consequences of alcohol consumption.

Alcohol can also mediate highly specific epigenetic changes. Cultured primary rat hepatocytes treated with ethanol demonstrate distinct, gene-specific histone methylation changes, including altered methylated H3K4 and H3K9 (Pal-Bhadra et al., 2007). Since methylation of H3K4 and H3K9 have opposite functional consequences, resulting in either transcriptional repression or activation, understanding how ethanol modulates these effects will undoubtedly identify potential therapeutic targets. Interestingly, tumor necrosis factor- α (TNF α), a predominant cytokine in inflammation, is regulated by H3K9 methylation (Gazzar, Yoza, Hu, Cousart, & McCall, 2007), suggesting that the prolonged inflammatory state associated with alcoholism may be partially mediated by epigenetic dysregulation of TNF α expression. Hence, epigenetic alterations may play an important role in the aberrant immune response observed following alcohol exposure combined with other challenges such as disease, trauma, infection, or injury.

To further address the relationship between epigenetics and alcohol consumption, the 2011 Alcohol and Immunology Research Interest Group (AIRIG) meeting was held on November 18, 2011, at Loyola University Stritch School of Medicine in Maywood, Illinois. The meeting was sponsored by the Alcohol Research Program and Department of Surgery at Loyola University Stritch School of Medicine in Maywood, Illinois, the Society for Leukocyte Biology, and the National Institute on Alcohol Abuse and Alcoholism. Meeting organizers were Drs. Melanie D. Bird, Mashkoor A. Choudhry, and Elizabeth J. Kovacs (Loyola University Stritch School of Medicine), Thomas J. Waldschmidt (University of Iowa), and Lou Ann Brown and Theresa Gauthier (Emory University). Talks from invited speakers and presentations of selected abstracts were given throughout two plenary sessions. During a separate session, twenty-five posters were also available for viewing and continued discussion of alcohol's effect on epigenetic changes, histone modifications, and inflammation.

Epigenetics

Dr. Samir Zakhari opened the meeting with an overview of the current literature examining alcohol and epigenetic changes. This was followed by the first plenary session, chaired by Drs. Melanie Bird (Loyola University Stritch School of Medicine) and Ping Zhang (Michigan State University), in which presenters described new studies on alcohol, specific epigenetic modifications, and outcomes of these alterations. Dr. Craig McClain, University of Louisville, shared new data to help define how binge alcohol consumption results in

epigenetic histone modifications leading to changes in gene expression, which contributes to liver injury (Kim & Shukla, 2006). Hepatic histone deacetylase (HDAC) mRNA expression, HDAC activity, and histone acetylation were evaluated in a mouse (C57BL/6) model of oral ethanol gavage. Liver steatosis and injury were also assessed in this model by measuring hepatic triglycerides accumulation and plasma ALT activity. Specifically, Dr. McClain indicated that binge alcohol exposure resulted in alterations of hepatic HDAC mRNA expression including down-regulation of HDAC 1, 7, 9, 10, 11, and up-regulation of HDAC 3. Furthermore, a decrease in total liver HDAC activity and histone hyperacetylation was observed. These events were associated with microvesicular hepatic fat accumulation and elevated ALT levels. It was proposed that binge alcohol-induced microvesicular hepatic steatosis and liver injury likely occur due to the deregulation of hepatic HDAC mRNA expression. The molecular mechanisms underpinning the HDAC alterations and their individual contribution to the development of hepatic steatosis and injury are still a source of active investigation.

Dr. Asha Jacob, North Shore University Hospital-Long Island Jewish Medical Center, provided new insight into a possible role for cold inducible RNA binding protein (CIRP) in alcohol-induced brain injury. Excessive alcohol ingestion has been associated with cerebral dysfunction (Victor & Dreyfus, 1961), and interestingly, brain glucose metabolism is decreased in alcoholics (Gilman et al., 1990). The molecular mediator(s) responsible for such decrease in neuronal activity have not been elucidated; however, cortex levels of CIRP mRNA were elevated in a rat model of cerebral ischemia (Liu, Zhang, & Xue, 2010). To determine if CIRP also had a role in alcohol-induced brain injury, Dr. Jacob used ethanol-exposed wild type and CIRP knockout mice and analyzed brain glucose metabolism using fludeoxyglucose (^{18}F FDG) in a microPET scanner. Alcohol-exposed CIRP knockout mice had elevated brain activity as compared to that of wild-type mice suggesting that CIRP may be responsible for the observed decrease reported in alcoholics. A different set of experiments indicated that rats exposed to ethanol for 15 h had a significant increase in CIRP protein levels in the hippocampus and CIRP protein was also present in the cerebrospinal fluid. Interestingly, CIRP mRNA and protein were increased in BV2 cells (a murine microglia cell line) after 48 h of ethanol exposure. Dr. Jacob also examined the expression of peroxisome proliferator activated receptor-gamma (PPAR- γ), a known mediator of neuroprotection. Significant decreases in both PPAR- γ mRNA and protein were found in BV2 cells following ethanol exposure. These data suggest that CIRP may act as a novel mediator of alcohol-induced brain dysfunction.

Dr. Shivendra Shukla from the University of Missouri presented data further delineating how alcohol-induced histone modifications may promote tissue damage. Both *in vitro* and *in vivo* studies have demonstrated that alcohol exposure causes an increase in histone H3 acetylation at lysine 9 (H3K9), histone H3 methylation at lysines 4 and 9, and histone phosphorylation at serine 10 and serine 28. Alcohol-induced acetylation and methylation of H3K9 have opposing effects on gene expression (Rice et al., 2007). It is noteworthy that acute and binge alcohol exposure affects these modifications differently than those observed after chronic alcohol exposure. In a rat model, acute alcohol caused H3K9 acetylation in the liver, but 4 weeks of chronic alcohol treatment did not show global H3K9 acetylation. Surprisingly, even in the absence of global acetylation, an association of a specific gene (e.g. alcohol dehydrogenase 1) promoter with acetylated histones was increased. There was also a degree of gene selectivity in their association behavior. When chronically-treated rats were additionally given a second hit of binge alcohol an elevation in global histone H3 acetylation was observed. These studies indicate a role for histone modifications in binge ethanol-induced liver damage. Taken together, these investigations highlight importance of multiple modifications on histone H3 by ethanol.

Dr. Kristina Bailey, University of Nebraska Medical Center, presented data examining alcohol-induced histone modifications in airway epithelial cells and the possible effects of these alterations on TLR2 expression. Alcohol is known to perturb the innate immunity of the airway epithelium. Toll-like receptors (TLR) constitute a significant element of this system. Interestingly, brief alcohol exposure causes an increase in TLR2 expression in the airway epithelium (Bailey, Wyatt, Romberger, & Sisson, 2009); however, preliminary experiments suggest that in this epithelium prolonged alcohol exposure leads to a decrease in TLR2 expression. As little is known about the mechanism of this decrease, Dr. Bailey examined whether prolonged alcohol exposure modulates HDAC activity ultimately leading to decreased TLR2 expression. Using normal human bronchial epithelial (NHBE) cells treated with the HDAC inhibitor, Trichostatin A, it was found that TSA-treated cells had reduced TLR2 expression compared to cells treated only with media alone. This suggests that HDAC inhibition may lead to decreased TLR2 expression. Further experiments examined whether alcohol modulates any members of the HDAC family. Alcohol-exposed NHBE cells had decreased HDAC 3, 4 and 10 suggesting that alcohol has a specific effect on these HDACs as compared to a global effect on all HDACs. A transformed airway epithelial cell line, 16HBE14o-, was used to study the effects of alcohol on HDAC activity; however, no change in HDAC activity was observed between ethanol-treated cells and cells exposed to media alone. This work demonstrates that alcohol decreases HDAC 3, 4 and 10 expression in the airway epithelium which potentially leads to the decrease in TLR2 expression after prolonged alcohol exposure.

The last presentation in this session was given by Dr. Ali Keshavarzian of Rush University, who shared some of his laboratory's work on alcohol's effect on circadian gene expression and the role of these genes on susceptibility to intestinal tissue damage. It is known that some alcoholics develop intestinal permeability ultimately leading to endotoxemia and tissue damage. Previous studies have demonstrated that gut-derived endotoxin is necessary to activate the inflammatory response, which can promote tissue injury (Bode, Kugler, & Bode, 1987). Recent data indicate that disruption of circadian rhythms increases the susceptibility of damage in the colon (Preuss et al., 2008); however, the effect of alcohol on circadian gene (*Clock* and *Per2*, specifically) expression and the role of CYP2E1 in altering this expression is unknown. Using a Caco-2 cell monolayer system, Dr. Keshavarzian found that alcohol induced an elevation in monolayer permeability and the CLOCK and PER2 proteins. These alcohol-induced changes were CYP2E1 dependent and inhibition of *Clock* or *Per2* via siRNA reduced alcohol-induced permeability of the monolayer. Interestingly, CYP2E1 siRNA blocked the alcohol-induced elevation in *Clock* gene expression. Further experiments in a rodent model demonstrated that rats, which already had increased total gut permeability due to alcohol exposure, also had augmented PER2 protein levels in their duodenum and proximal colon as compared to control rats. This work suggests a new mechanism for alcohol-induced intestinal permeability and tissue damage.

Alcohol and inflammation

The second plenary session, chaired by Dr. Katherine Radek (Loyola University Stritch School of Medicine) and Corey Parlet (University of Iowa) was comprised of speakers addressing new insights into the role of alcohol on the inflammatory response. Dr. Joanne Weinberg of the University of British Columbia presented studies examining prenatal alcohol exposure's impact on adjuvant-induced arthritis. Development and function of the immune system is negatively affected by prenatal alcohol exposure, which causes elevated hypothalamic-pituitary-adrenal axis activity (Zhang, Sliwowska, & Weinberg, 2005). In this model of prenatal alcohol exposure, pregnant dams received an alcohol-containing liquid diet or control diets. Female offspring were exposed to chronic mild stress during adolescence followed by injection of complete Freund's adjuvant (to induce arthritis) in

adulthood. Dr. Weinberg found that stress exposure increased disease incidence and severity when compared to rats not exposed to stress. Furthermore, animals exposed to prenatal alcohol displayed greater inflammation irrespective of stress exposure. All non-stressed, control-fed animals had resolution of disease by day 60 post injection; however, 60% of non-stressed, prenatal alcohol-exposed rats still did not have resolution of the disease. Further analysis suggested that corticosterone (CORT) may be protective against the development of adjuvant-induced arthritis as non-stressed animals had higher levels of CORT.

Dr. Liza Makowski of the University of North Carolina at Chapel Hill continued the second session with a discussion of adipose inflammation following alcohol exposure and burn injury. Alcohol is a known modulator of the inflammatory response, and can aggravate this response following trauma (Bird & Kovacs, 2008). The contribution of adipose tissue to this heightened inflammation is unknown; therefore, Dr. Makowski's laboratory measured adipose levels of the pro-inflammatory cytokine, IL-6, in a mouse model of acute alcohol exposure and burn injury. Alcohol alone did not induce adipose tissue inflammation as compared to saline treatment. Burn injury stimulated elevations in both IL-6 mRNA and protein in adipose tissue. The combined insult of alcohol exposure and burn injury led to a significant rise in IL-6 at the protein and mRNA level when compared to either exposure alone. The glucose transporter GLUT1 and glucose metabolism have previously been shown as vital elements for the pro-inflammatory macrophage response (Gamelli, Liu, He, & Hofmann, 1996). Alcohol exposure did not affect GLUT1 mRNA levels in adipose tissue; however, burn injury induced an increase in GLUT1 mRNA, and exposure to alcohol and burn trauma synergistically elevated GLUT1 expression further than either insult alone. Interestingly, these data suggest a role for adipose tissue in the pro-inflammatory response induced by the combined insult of acute alcohol exposure and burn injury.

Dr. Ilhem Messaoudi, Oregon National Primate Research Center, spoke next and focused on the impact of alcohol self-administration on immune homeostasis in a nonhuman primate model. Chronic ethanol results in alterations in both endocrine and immune function and increased susceptibility to infection (Rivier, 1996; Szabo & Mandrekar, 2009). Several mechanisms underlying alcohol-mediated immune suppressive outcomes have been identified, and these range from structural changes in mucosal barriers to functional alterations in immune cell function (Fernandez, Koval, Fan, & Guidot, 2007; Goral, Karavitis, & Kovacs, 2008; Zhong, Zhao, McClain, Kang, & Zhou, 2010). As the impact of alcohol abuse on immune function predominantly comes from cross-section studies and rodent/NHP models, Dr. Messaoudi took advantage of a nonhuman primate model where rhesus or cynomolgus macaques (*Maccaca mulatta* and *Maccaca fascicularis*) are first trained to consume alcohol in a schedule-induced polydipsia followed by 22 h/day access to both water and alcohol. The use of this model allows for complex longitudinal designs that span the critical periods of induction as well as correlate the individual differences in consumption with outcomes. Using this model, changes in plasma protein and hormone were measured in adult male cynomolgus monkeys at baseline, induction of water and ethanol self-administration, and after 4 months and 12 months of 22-hr daily access to ethanol and water. Forty-five proteins were affected after alcohol exposure with adrenocortico-tropic hormone (ACTH) and cortisol changing significantly, and 28 proteins suppressed during ethanol self-administration. Eight proteins were elevated across the course of the experiment while nine showed biphasic changes, and 10 were stable across the experiment. Cortisol and ACTH levels were highest during induction. Furthermore, the lifetime quantity of ethanol consumed after 12 months strongly correlated with CD40, CD40 ligand (CD154) and CCL22 levels. This model presents a new opportunity for understanding how alcohol self-administration affects the immune system.

The rest of the second session was comprised of short talks selected from submitted abstracts. Sun-Mi Choi of Louisiana State University Health Sciences Center detailed her work examining alcohol's effect on signaling pathways during MRSA pneumonia infection. Methicillin-resistant *Staphylococcus aureus* (MRSA) pneumonia incidence rates have increased in the past 5 years. MRSA infections are an important public health concern as infections are often associated with bronchiectasis, compromised lung function, and high mortality rates (Tacconelli & De Angelis, 2009). Interestingly, recent data indicate that patients diagnosed with MRSA are more likely to have abused alcohol in the past and these patients are more likely to die from sepsis (Kaech et al., 2006). To investigate whether acute alcohol exacerbates MRSA infection, a mouse model of alcohol exposure and MRSA (USA300) infection was employed. Elevated bacterial loads, as well as, increases in interleukin (IL)-1 β , IL-6, and tumor necrosis factor- α (TNF α) were found in alcohol-exposed mice as compared to mice not receiving alcohol. Alcohol exposure also led to decreased expression of Reg3 γ , an antimicrobial c-type lectin. Induced by signal transducer and activator of transcription 3 (STAT3) signaling, Reg3 γ is expressed in paneth cells and as revealed in this presentation, Reg3 γ is also expressed in lung epithelium. In an *in vitro* model using mouse lung epithelial (MLE12) cells, alcohol was found to inhibit IL-6-induced Reg3 γ expression. When alcohol-exposed mice were given recombinant Reg3 γ after MRSA infection there was an elevated clearance of USA300. Sun-Mi's work indicates that acute alcohol exposure causes decreased MRSA clearance from the lungs via inhibition of STAT3-induced production of the antimicrobial protein Reg3 γ .

The next short talk was given by Jacqueline Bouchard from Boston University School of Medicine. Her research focused on whether alcohol could trigger or exacerbate asthma. Asthma and binge drinking represent two significant public health issues that are both on the rise. Several clinical studies suggest that ethanol intoxication can worsen asthma symptoms within 15 min of drinking (Geppert & Boushey, 1978; Gong, Tashkin, & Calanese, 1981); however, no controlled animal laboratory study has investigated the interactions between acute ethanol exposure and allergic allergen-sensitized mice. Employing a mouse model where animals are first sensitized to cockroach allergen (CRA) then given alcohol one week later, it was found that within minutes of a single alcohol exposure a myriad of asthmatic parameters, including hallmarks of asthma such as eosinophil infiltration, mucus production, and airway obstruction were triggered in allergen-sensitized mice. Alcohol also induced rapid mast cell degranulation in the absence of a concurrent allergen exposure, suggesting a non-IgE mechanism of degranulation. Naïve, non-sensitized mice subjected to acute alcohol intoxication failed to demonstrate any of these alcohol-induced inflammatory responses. These studies suggest that alcohol can trigger asthma in allergen-sensitized, atopic mice, but will not trigger the same inflammation in normal, non-atopic mice.

Dr. Paul Thevenot from Louisiana State University Health Sciences Center gave the next presentation on his work examining airway remodeling following alcohol exposure and combustion-generated particulate matter (CGPM). Inhalation of particulate matter is known to worsen respiratory diseases such as asthma (Gent & Bell, 2010). Effects of alcohol on CGPM-induced asthma attacks were explored in a mouse model in which animals were fed a chronic alcohol diet and then exposed to laboratory-generated CGPM. Mice exposed to both alcohol and CGPM had elevated airway resistance and hyperresponsiveness as compared to non-exposed mice suggesting even greater pulmonary structural changes than observed in mice exposed to CGPM alone. Further experiments examined alveolar macrophage viability following alcohol and CGPM exposure as these macrophages are critical for clearance of inhaled particulate matter. While more macrophages were found in the bronchoalveolar lavage fluid of dually-exposed mice, a decrease in interstitial alveolar macrophages was also detected. Reduced interstitial macrophages may negatively impact stimulation of epithelial repair as well as the pulmonary immune response to alcohol and

CGPM exposure. To support this, Dr. Thevenot also presented data indicating that mice exposed to both insults have less cytotoxic T cells as compared to control mice. These data indicate that alcohol exposure increases pulmonary dysfunction when combined with particulate matter inhalation and may also prevent the necessary immune response to repair any damage incurred by this inhalation injury.

The final presentation of the day was given by Dr. Christopher Davis of Loyola University Chicago who described the implications of alcohol intoxication at the time burn injury and smoke inhalation. Each year nearly 80,000 deaths in the U.S. are linked to excessive alcohol use (CDC, 2004). Moreover, binge drinking is associated with violence and unintentional injuries, and its impact on those injured by burns is striking; however, the effect of binge drinking on burn patients with inhalation injuries (INI) is not well described. In a prospective study, bronchoscopy was performed on burn patients when INI was suspected. These patients were grouped as Blood Alcohol Content (BAC) negative, BAC 1–79 mg/dL, and BAC \geq 80 mg/dL. Compared to BAC negative patients, binge drinkers with an INI had much higher carboxyhemoglobin levels (% COHb), though the groups did not differ in terms of INI grades, hospital charges, antibiotic days, days on the ventilator, ICU days, hospital length of stay, incidence of sepsis, pneumonia, and transfusions. Despite similarities between groups, binge drinkers had considerably smaller skin burns than did their non-drinking counterparts and significantly lower revised Baux scores (an index used to correlate mortality with burn size, age, and inhalation injury). Though no binge drinking patient succumbed to their injuries as compared to 32% of non-drinkers, the difference in survival was not significant after adjusting for age and % TBSA. Of the burn patients, binge drinkers with an INI had smaller dermal burns than non-drinkers, though both their outcomes and consumption of health care resources were similar to their non-drinking patients. Data presented by Dr. Davis indicate that patients who are intoxicated at the time of burn and inhalation injury have altered carboxyhemoglobin levels and require just as much health care intervention as non-intoxicated patients despite a smaller burn size.

Summary

This year's meeting focused on alcohol's effects on epigenetic changes, gene expression, and how these may affect other immunologic parameters. Whether it be acute, binge, chronic, or prenatal, alcohol exposure has been shown to cause tissue damage, alterations in both neuroendocrine and immune systems, and susceptibility to infection (Bird & Kovacs, 2008; Goral et al., 2008; Szabo & Mandrekar, 2009; Zhang et al., 2005). These outcomes are often associated with changes in gene expression. Studies presented here suggest that alcohol-induced epigenetic modifications may be one mechanism responsible for aberrant gene expression thus promoting the elevated inflammatory state observed in alcohol models. Furthermore, laboratory and clinical data presented indicate that when combined with other insults, ethanol exposure can produce elevated symptom severity and tissue injury in rodent models of disease, infection, injury, and inhalation. Overall, the findings presented here suggest a new connection between alcohol exposure, gene expression, and downstream detrimental outcomes. Alcohol-induced modulation of epigenetic marks also represents a new and fertile area to analyze in an attempt to better understand the role of alcohol in inflammation and tissue damage.

Acknowledgments

The authors and participants would like to thank the NIAAA (AA016751), the Loyola University Chicago Burn and Shock Trauma Institute, and the Alcohol Research Program for financial support of the meeting. Support was also provided by NIH R13 AA017084, R01 AA012034 (EJK), R13 AA 020768 (EJK), F31 AA019913 (AZ) and T32 AA013527 (EJK). The authors would also like to thank Letta Kochalis for technical and logistical support.

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