Published in final edited form as:

Alcohol Clin Exp Res. 2013 April ; 37(4): 550–557. doi:10.1111/acer.12011.

Binge Ethanol and Liver: New Molecular Developments

Shivendra D. Shukla, Stephen B. Pruett, Gyongyi Szabo, and Gavin E. Arteel

Department of Medical Pharmacology and Physiology (SDS), University of Missouri School of Medicine, Columbia, Missouri; Department of Basic Sciences (SBP), Mississippi State University, Mississippi State, Mississippi; Department of Internal Medicine (GS), University of Massachusetts Medical School, Worcester, Massachusetts; Department of Pharmacology and Toxicology (GEA), University of Louisville Health Sciences Center, Louisville, Kentucky

Abstract

Binge consumption of alcohol is an alarming global health problem. Binge (acute) ethanol (EtOH) is implicated in the pathophysiology of alcoholic liver disease (ALD). New studies from experimental animals and from humans indicate that binge EtOH has profound effects on immunological, signaling, and epigenetic parameters of the liver. This is in addition to the known metabolic effects of acute EtOH. Binge EtOH alters the levels of several cellular components and dramatically amplifies liver injury in chronically EtOH exposed liver. These studies highlight the importance of molecular investigations into binge effects of EtOH for a better understanding of ALD and also to develop therapeutic strategies to control it. This review summarizes these recent developments.

Keywords

Acute Ethanol; Alcoholic Liver Disease; Binge Ethanol; Liver Injury

Alcoholic liver disease (ALD) is characterized by fatty liver, hepatitis, fibrosis, and cirrhosis. The frequency, dose, and duration of ethanol (EtOH) consumption are important in the manifestation of the damaging effects of EtOH (Zakhari and Li, 2007). However, in most cases, livers of individuals who have consumed alcohol for many years appear normal, and only about 15% of chronic alcoholics develop the liver disease. These data indicate that other factors (genetic and environmental), besides daily alcohol consumption, contribute to overall risk for developing ALD.

Binge consumption of alcohol is on the rise at an alarming rate worldwide. A binge is defined by the National Institute on Alcohol Abuse and Alcoholism as consumption of 5 and 4 drinks for men and women, respectively, in 2 hours to produce a blood EtOH level of more than 0.08% (80 mg/dl). Binge drinking is remarkably common in the United States. About 43% of college students reported at least 1 binge episode during the previous month (Alcohol and Health, 2000). About 38 million U.S. adults binge drink according to a 2010 survey by Center for Disease Control. The overall prevalence of binge drinking among

adults in the 48 states and the District of Columbia was 17.1% with an estimated annual economic cost of 168 billion dollars (CDC Report, 2012). Although the definition of chronic alcohol abuse may be based on temporal, physiological, and psychological end points, a span of 10 years of chronic drinking is indicative of dependence. The epidemiological evidence demonstrates that binge drinking in chronic alcoholics augments liver injury (Alcohol and Health, 2000; Reuben, 2007). Binge EtOH appears to be a major trigger for the progression of steatosis to steatohepatitis.

The description of acute, binge, and chronic EtOH treatments in the in vitro or culture systems remains a source of confusion and ambiguity. This also applies to in vivo studies to an extent. For example, most of the cell culture models where EtOH is used for a few hours to a few days are essentially representative of binge or acute effects of EtOH. These treatments are not chronic in the context of human situation. In most cases, the genotypic and phenotypic properties of cultured cells also change with longer time of culturing. In the case of in vivo examination in animal models, the time of treatment with EtOH can be theoretically compared with humans. For the purpose of comparison let us consider that the life span of well-fed mice and rats is 2 and 4 years, respectively. This can be compared to about 80 years of life span of a healthy human. On this basis, chronic alcohol abuse in human for 10 years will relate approximately to 14 weeks EtOH treatment in mice or 28 weeks in rats. But, most of the mice or rat studies, in vivo, use 4 to 12 weeks (equivalent to 1.5 to 4.5 years in humans). Thus, shorter EtOH treatments in vivo may be more reflective of binge/acute effects than chronic EtOH effects. This issue remains to be appreciated and addressed in a systematic manner. It should also be mentioned that EtOH sensitivity in human, rat, mice, and other animal models (e.g., drosophila, zebrafish) can also vary due to differences in populations, species, and strains.

Relatively little is known with regard to the effects of binge drinking on the liver, as compared to the current knowledge of effects of chronic EtOH exposure. In animal models, several approaches have been considered to examine the effects of binge EtOH. This includes single binge, intermittent repeat binge, and chronic EtOH exposure followed by episodes of binge. Evidences from such animal studies are providing mechanistic information on binge EtOH effect relevant to ALD. It is increasingly being recognized that the cellular effects of EtOH are due to modulation of immunological, metabolic, signaling, and epigenetic pathways (Beier and McClain, 2010; Gao et al., 2011; Shukla et al., 2008). Binge has profound effect on these mechanisms (Fig. 1). A mechanistic distinction between binge (acute) and chronic effects of EtOH has the potential to identify novel targets that amplify liver injury in binge abusers. Although emergence of this problem has been recognized, it is only recently that the molecular insight into the binge effect is beginning to be revealed. This review highlights these new developments.

ACUTE ETOH EXPOSURE AND MODULATION OF INFLAMMATION DURING INTOXICATION

The liver is exposed to the highest concentrations of EtOH and it is also the major site of EtOH metabolism. There is evidence that acute EtOH exposure inhibits hepatic

mitochondrial DNA synthesis (Demeilliers et al., 2002). Acute EtOH also impairs mitochondrial metabolism and dynamics (Gao and Bataller, 2011; Hoek et al., 2002). Mitochondrial dysfunction has been implicated in the pathogenesis of ALD. It is also relevant to note that elimination kinetics of EtOH, mediated by ADH1, ADH3, and CYP2E1, can alter with dose of EtOH (Haseba et al., 2012) and may also play role in the binge effects.

The effects of acute exposure on immunological parameters in the liver are largely unknown. Acute EtOH consumption has decreased resistance to infection-related complications and increased risk of mortality in humans and rodents with sepsis (Griffin et al., 2009; Pruett et al., 2010). Growing evidence indicates EtOH functionally suppresses cells of the innate immune system and decreases clearance of bacteria. In mice and rats, EtOH is most often administered through gastric intubation or intraperitoneal injection. EtOH at concentrations up to 32% (v/v) in water administered by gavage does not cause histologically obvious necrosis or hemorrhage in the gastrointestinal tract as do larger concentrations (Carson and Pruett, 1996). In this model, EtOH dosages of 4 to 6 g/kg yield a peak blood EtOH concentration of 43.4 to 86.8 mM (200 to 400 mg/dl). The lower end of this range is commonly achieved in human binge drinkers, and the upper end is near the maximum concentration found in humans (Jones and Holmgren, 2009). It is important to point out that EtOH clearance is more rapid in mice than in humans, so equivalent biological effects, which are often best predicted by area under the concentration versus time curve (AUC) of the drug in question, may require higher peak blood levels in mice than typically observed in humans. For example, the alcohol clearance rate in humans is approximately 20 mg/dl/h in contrast to approximately 40 mg/dl/h in mice (Carson and Pruett, 1996); therefore, the same peak concentration of alcohol will be cleared much more rapidly in mice, making a much smaller AUC. In general, animals with small body weight metabolize EtOH at faster rates (sometimes 5 times or higher) than larger animals.

Findings from several laboratories are consistent with the idea that EtOH intoxication inhibits inflammatory response, at least in part, by inhibiting signaling through Toll-like receptors (TLRs) when a potent external TLR stimulus is provided during EtOH intoxication (Dai and Pruett, 2006). Results indicate acute EtOH redistributes TLR4 and reorganizes the actin cytoskeleton, thereby impairing receptor clustering. This effect blunts full activation of TLR4-mediated downstream signaling, such as NF- κ B and MAPK pathways that are involved in the production of cytokines and other inflammatory mediators. Activation of NF- κ B by injection of lipopolysaccharide (LPS) is particularly inhibited by acute EtOH intoxication in the general anatomical region occupied by the liver (Dai and Pruett, 2006; Pruett and Fan, 2009). This suggests innate immunity in the liver may be compromised to a greater extent than in other anatomical locations, possibly due to higher EtOH concentrations in the liver than in other locations. Signaling or responses through all TLRs tested to date (TLR2/6, TLR3, TLR5, TLR7, and TLR9) are inhibited by acute exposure to EtOH (Goral and Kovacs, 2005), but the mechanism by which this occurs has only been examined in detail for TLR4 as described earlier.

Results can be entirely different when the effect of acute EtOH administration is examined distal from intoxication. Specifically, acute EtOH administered in vivo is reported to activate

various cellular signaling pathways and to enhance subsequent inflammatory effects in the liver induced by LPS (Aroor et al., 2010; Zhong et al., 1999), when EtOH was administered 24 hours before LPS (Beier et al., 2009). Although it may initially seem that findings of anti-inflammatory and pro-inflammatory effects of EtOH in different experimental systems are contradictory, gut permeability and timing of the inflammatory stimulus relative to EtOH exposure seem to be important. For example, EtOH at 6 g/kg, but not 3 g/kg induces a robust acute phase response 24 hours after administration, even though both of these dosages inhibit the induction of IL-6 by LPS when measured 3 hours after dosing with LPS (3.25 hours after EtOH). In contrast, EtOH at 6 g/kg did not induce an acute phase response in mice with a mutated, hyporesponsive TLR4 gene, suggesting that the acute phase response in mice treated with EtOH alone is also dependent on LPS, presumably from the gastrointestinal tract.

INTERACTIONS AMONG ACUTE ETOH EXPOSURE, RESISTANCE TO INFECTION, AND HEPATOTOXICITY

Although EtOH abuse is associated with an increase in a wide range of infections, evidence for increased risk of sepsis or posttraumatic infection is one of the best documented in the case of binge drinking (Griffin et al., 2009). A substantial portion of individuals who had EtOH in their blood at the time of injury or illness were binge drinkers, not chronic heavy drinkers (Lin et al., 2009). Several studies (Goral and Kovacs, 2005; Pruett et al., 2010) have demonstrated that acute EtOH intoxication interferes with innate immune responses. The liver has a central role in the regulation of host innate immune responses as it has one of the largest populations of resident macrophages (Kupffer cells) and natural killer cells, which are key components of the innate immune response. It is also the major site for removal of bacteria and endotoxins from systemic circulation, particularly those arising from the gastrointestinal tract. Studies in both humans and animal models indicate that acute EtOH consumption significantly increases mortality in sepsis (Rehm et al., 2010).

Cytokines play a central role in the manifestation of sepsis. The concentrations of many cytokines that are normally low in plasma (in the pg/ml range) increase to ng/ml or even $\mu\text{g/ml}$ levels during sepsis, ultimately leading to an exaggerated pro-inflammatory response. Several studies (Goral and Kovacs, 2005; Pruett et al., 2010) have indicated that acute EtOH intoxication decreases the concentration of pro-inflammatory cytokines but is still associated with a poor outcome in sepsis. A probable explanation for this effect is that while a hyper pro-inflammatory response is detrimental, the host still needs protection from the invading microorganisms, which requires the initiation of an innate immune response. This was demonstrated in a recent study using wild-type and TLR4 hyporesponsive mice. The TLR4 hyporesponsive group had better survival in a sepsis both in absence and in presence of acute EtOH than the wild-type group (Pruett et al., 2010). An evaluation of the cytokine response in the serum and peritoneal fluid (Bhatty et al., 2011) showed that hyporesponsive TLR4 decreased the concentration of the pro-inflammatory cytokines thereby preventing an exaggerated pro-inflammatory response, while cytokines induced through receptors other than TLR4 were apparently sufficient to initiate an innate immune response leading to bacterial clearance. EtOH significantly decreases the production of most pro-inflammatory

cytokines induced by TLR4 (Bhatty et al., 2011) leading to decreased bacterial clearance by neutrophils and macrophages and ultimately decreased survival (Pruett et al., 2010).

Acute EtOH is associated with increased gut permeability (Enomoto et al., 2001), which can cause the normal bacterial flora and the toxins produced by them to enter circulation and thereby the hepatic circulation. In a mouse model of peritonitis leading to sepsis, acute EtOH increased bacterial load (*Escherichia coli*) in the peritoneal cavity and also inhibited clearance mechanisms (Pruett et al., 2010). Studies in animal models have shown that an acute administration of EtOH enhances the hepatotoxicity of endotoxin, but this effect is observed only with low doses of endotoxin (Shibayama et al., 1991). In contrast, the decreased hepatocellular function which is known to occur early after the onset of sepsis has been suggested to be associated with the release of pro-inflammatory cytokines such as TNF α and IL-6; the levels of both of these cytokines are suppressed by acute EtOH exposure in humans (Norkina et al., 2008) and animal models (Pruett et al., 2010).

Binge EtOH exposure (6 g/kg) increases the concentration of serum amyloid A and serum amyloid P, the major acute phase proteins in mouse. IL-6 is considered to be the major inducer of acute phase protein. Other cytokines that are implicated to play a role in an acute phase response include TNF α , IL-1, TGF- β , and IFN- γ . It is intriguing that acute EtOH exposure leads to an anti-inflammatory response but is associated with an increased acute phase response. In the mouse, low doses of EtOH alone do not induce an acute phase response while an exacerbated response was observed with 6 g/kg of EtOH which was maximal 24 hours after dosing and was TLR4 dependent. Both low and high doses of alcohol suppress the acute phase response to LPS (Pruett and Pruett, 2006).

ROLE OF CYTOKINES, IRAK, AND MICRORNA IN BINGE ETOH

As alluded above, one of the distinguishing features of acute and chronic alcohol is the differences in the inflammatory cascade. Acute intoxication and chronic alcohol consumption mediate opposite effects on inflammation involving different molecular pathways. Cells of the innate immune system such as monocytes and macrophages are equipped with broad range of pattern recognition receptors that sense danger signals from pathogens and injury. Increasing evidence suggests that pattern expression and activation of pattern recognition receptors occur in the liver not only in the immune cells but also in parenchymal cells (Mandrekar and Szabo, 2009). Alcohol binge in humans induces molecular signatures of TLR tolerance in monocytes/macrophages. Acute alcohol intoxication in vivo results in attenuated production of TNF α and IL-1 β in response to ex vivo stimulation with LPS (a TLR4 ligand) in human blood monocytes (Mandrekar et al., 2006). TLR4-induced TNF α production involves tightly regulated signaling events that include sequential recruitment and activation of the MyD88, the common TLR adaptor, IRAK1/4 kinase activation, that triggers activation of the IKK complex that phosphorylates the inhibitory NF- κ B molecules (IKKs) to allow nuclear translocation of the p65/p50 stimulatory NF- κ B dimers (Mandrekar and Szabo, 2009). This event results in the activation of the inflammatory cascade as most pro-inflammatory cytokine genes, including TNF α , have 1 or multiple NF- κ B-binding sites in their promoter regions. Observation of decreased TNF α in human monocytes correlated with decreased nuclear translocation and DNA

binding of the NF- κ B transcription factor after acute alcohol binge and these in vivo observations were also reproducible in vitro. TLR4-induced phosphorylation of IKK α and IKK β was also inhibited by acute alcohol exposure in vitro (Mandrekar et al., 2009).

A single exposure of innate immune cells to LPS results in robust induction of the intracellular signaling pathway to induce TNF α and other pro-inflammatory cytokines (Mandrekar and Szabo, 2009). However, repeated LPS exposure yields an attenuated response, a phenomenon called “TLR tolerance.” TLR tolerance is mediated by up-regulation of negative regulators of TLR signaling and is characterized by a molecular signature including up-regulation of NF- κ B p50 homodimers, Bcl3, and IRAK-M. Interestingly, acute alcohol in human monocytes as well as in RAW 264.7 macrophages results in a preferential induction of p50/p50 NF- κ B homodimers (Mandrekar and Szabo, 2009; Mandrekar et al., 2009). In the later phase of LPS stimulation, p65/p50 nuclear levels decrease and the p50/p50 homodimers become the predominant forms of NF- κ B. Because p50 has only nuclear localization and DNA-binding domain but lacks a transactivation domain, p50/p50 homodimers act as inhibitors of target gene induction and contribute to TLR tolerance. Induction of p50/p50 homodimers by acute alcohol contributes to the attenuated LPS response and TNF α induction in monocytes and thus, contributes to TLR4 tolerance (Oak et al., 2006).

Previous studies indicated that in addition to the TLR4 “tolerance” phenomenon, acute alcohol exposure in monocytes/macrophages interferes with early signaling events in lipid rafts. Acute alcohol interferes with recruitment of TLR4 into the lipid rafts that are cholesterol-rich membrane micro-domains that facilitate signaling events. It has been shown that acute alcohol displaces TLR4 from lipid rafts and thereby prevents optimal LPS-induced downstream signaling (Dai et al., 2005). Downstream from lipid rafts, decreased phosphorylation of IRAK and decreased kinase activity of IKK α and IKK β were reported that correlated with decreased NF- κ B activation and attenuated TNF α production (Mandrekar and Szabo, 2009). These observations suggest that acute alcohol has some direct effects on signaling but it also can modulate inflammatory processes at various levels of the signaling cascade.

ETOH SENSITIZES MACROPHAGES/KUPFFER CELLS VIA UP-REGULATION OF MIR-155 TO TLR4-INDUCED INFLAMMATORY CYTOKINE OVERPRODUCTION

The role of the TLR4 and its ligand, LPS, has been extensively studied in ALD. Recent investigations have dissected the involvement of the downstream signaling pathways of TLR4 in alcohol-induced liver disease and found that mice deficient in the TLR adaptor, MyD88, were not protected from alcohol-induced liver damage and steatosis. Furthermore, the protective effect of both TLR4 and IRF3 deficiency in alcohol-induced liver damage, inflammation, and liver steatosis was demonstrated (Petrasek et al., 2011). One of the mechanisms by which prolonged alcohol exposure leads to liver disease is via its effects on Kupffer cells. It has long been shown that Kupffer cells from livers of alcohol-fed mice show an increased susceptibility to TLR4/LPS-induced signaling resulting in overproduction of

TNF α (Thurman et al., 1998). However, the mechanism by which alcohol sensitizes Kupffer cells to increased TNF α production is still not fully understood.

Recently, small noncoding RNAs termed “microRNA (miR)” are emerging as fine tuners of numerous biological processes. For example, miR-155, miR-146a, and miR-125b have been linked to inflammation, cancer, and cardiovascular abnormalities (Bala et al., 2011). In particular, miR-155 is a key player in inflammatory diseases via its ability to regulate genes involved in immunity. Unlike most other miRNAs that inhibit expression of the target gene product, miR-155 has an augmenting effect on TNF α production. Induction of miR-155 was reported in acute alcohol-treated macrophages (Bala et al., 2011).

BINGE ETOH EFFECTS ON EPIGENETIC HISTONE MODIFICATIONS AND MAP KINASE SIGNALING

Binge EtOH also influences epigenetic parameters. In a recent study, binge EtOH was administered intraperitoneally in rats and EtOH dose- (1 to 5 g/kg body weight) and time- (1 to 4 hours) dependent alterations in various parameters were monitored. Steatosis and necrosis (serum ALT) of the liver increased in 4 hours suggesting modest liver injury after acute EtOH treatment. It was observed that acute EtOH caused increase in the phosphorylation of ERK1/2 MAP kinase and a modest increase in the phosphorylation of p38 MAPK and JNK. Although these 3 MAP kinases were rapidly activated by binge EtOH at 1 hour, the necrosis occurred at 4 hours and correlated to the activation of ERK1/2 (Aroor et al., 2010). A single oral dose of EtOH has been shown to cause more than 2-fold changes in 28 genes by microarray analysis. These included genes involved in fatty acid synthesis, for example, fatty acid synthase, stearyl-CoA desaturase, and also sterol regulatory element binding protein 1 (Yin et al., 2007).

The role of histone H3 phosphorylation at serine-10 (P-H3-S10) and serine 28 (P-H3-S28) in binge EtOH liver injury in vivo has also been studied and was found to have different sensitivities to EtOH dose and time exposure. Increases in dually modified-phosphoacetylated histone H3 at K9/S10 were also observed. The changes in histone H3 phosphorylation and phosphoacetylation were also accompanied with expression of early response genes (c-Fos, c-Jun, MKP-1). Chromatin immunoprecipitation assays of liver samples after acute EtOH indicated that increased histone H3 phosphorylation at serine 10 and 28 were associated with the promoters of c-Jun. On the other hand, increased P-H3-S28 was associated with the promoter of the plasminogen activator inhibitor-1 (PAI-1). It was demonstrated that these modifications are differentially involved in the mRNA expression of genes after acute EtOH exposure in vivo (James et al., 2012).

Results on MAP kinase signaling in chronic followed by single or 3 binge EtOH administration are quite intriguing. Chronic EtOH treatment resulted in mild steatosis and necrosis, whereas chronic EtOH followed by binge EtOH exhibited marked steatosis and significant increase in necrosis. Chronic followed by binge EtOH treatment also showed significant increase (compared to chronic EtOH alone) in the phosphorylation of ERK1 & ERK2, and RSK. Interestingly, the phosphorylation of JNK and p38 MAPK did not increase by the binge. There was also increase in mRNA for egr-1 and PAI-1, but not TNF α in

chronic EtOH followed by binge EtOH treatment (Aroor et al., 2011; Park et al., 2005). Among other alterations, the activated levels of ERK1, and more so ERK2, were remarkably amplified by binge suggesting a role of these isotypes in the binge amplification of the liver injury. Thus, 1 or 3 episodes of binge EtOH administration augment liver injury after chronic EtOH intake in the rat model. It appears likely that accumulation of fat by chronic EtOH or acute EtOH alone may be due to mechanisms independent of ERK1/2 activation, whereas increased accumulation of fat by binge in chronically treated animals is predominantly due to ERK2 activation (Aroor et al., 2011; Park et al., 2005).

A common situation in the progression of liver damage and vascular injury in human is result of heavy EtOH binge superimposed on chronic EtOH intake. New evidence from an experimental animal model, where binge EtOH was administered after chronic EtOH treatment, indicated that binge caused decreased mRNA of low-density lipoprotein-receptor (LDL-R) and increased mRNA levels of the angiotensinogen gene in liver (Aroor and Shukla, 2011). It is relevant to note here that increases in plasma LDL cholesterol and angiotensin are cardiovascular risk factors in human alcoholics. It implies that binge EtOH-induced alterations in liver have consequences on the cardiovascular system. Binge EtOH thus affects inter-organ cross-talk. This is further supported by increases in the PAI as discussed in the next section. Such animal models are experimentally close to clinical conditions, as heavy binge drinking episode in patients chronically consuming alcohol is the most common trigger for admission of patients with steatohepatitis (Crosse and Anania, 2002). A study of a large cohort of drinkers with consecutive biopsies suggested the concept of multiple hits of alcoholic hepatitis occurring in the same patients as the prime determinant in the progression of alcoholic liver injury (Mathurin et al., 2007). Animal data demonstrate that binge EtOH intake is an important factor in augmenting liver damage during chronic EtOH intake in rat (Aroor et al., 2011) and mice (Ki et al., 2010). In a recent proteomic analysis, it was observed that binge EtOH in vivo altered the levels of a number of proteins that include carbonic anhydrase 3, Cyp2E1, and protein disulfide isomerase associated protein 3 (Aroor et al., 2012b). Furthermore, the nuclear translocation of proteins like CREB, ERK1/2, and PPAR α were also disrupted by binge EtOH (Aroor et al., 2012a). This highlights changes in cellular, nuclear proteins, and malfunction of nuclear transport of proteins caused by binge EtOH treatment in vivo.

ACUTE ETOH EXPOSURE IN RELATION TO FIBRIN ACCUMULATION

One of the earliest hepatic changes caused by alcohol consumption is steatosis. While originally thought to be a pathologically inert histological change, more recent work indicates that steatosis may play a critical role not only in the initiation, but also in the progression of ALD. Indeed, previous work by Thurman and colleagues (1998) in the rat has shown that treatments that blocked experimental ALD caused by chronic enteral feeding also protected against the effects of acute bolus EtOH on the liver (e.g., steatosis). It is therefore possible that protection against acute alcohol-induced steatosis can be used as a predictive tool to identify new therapies to test in the chronic model. In addition to direct changes caused by EtOH administration, acute models of EtOH exposure are also useful for studying the alteration by EtOH of the response of liver to other xenobiotics. For example, acute EtOH exposure is well known to exacerbate liver pathology induced by acetaminophen

or LPS. One such model employs EtOH (4 to 6 g/kg) as the first “hit,” followed by a subsequent exposure to LPS approximately 24 hours later. Under these conditions, liver damage caused by endotoxin is greatly exacerbated by EtOH administration. This effect of EtOH is in contrast to the blunting of the LPS response caused by acute EtOH during the intoxication phase. Indeed, such a priming effect of EtOH to LPS-induced liver damage is not observed if the LPS is injected sooner after alcohol exposure (e.g., 2 hours), indicating that the effect of EtOH is not likely EtOH per se, but indirect effects of the drug on the liver. Employing the acute EtOH + LPS, it was shown that impaired fibrinolysis, leading to fibrin extracellular matrix (ECM) accumulation, contributes to the enhanced inflammatory injury caused by EtOH preexposure under these conditions. These results have implications not only for mechanistic insight into acute alcohol-induced liver damage, but also into chronic ALD, which also appears to be mediated, at least in part, by altered fibrin ECM accumulation.

It also appears that mechanisms of acute and chronic alcohol-induced liver injury overlap to some extent (Arteel et al., 1997; Thurman et al., 1998). For example, Enomoto and colleagues (2000) showed that compounds previously shown to protect against chronic alcohol-induced liver damage (Adachi et al., 1994, 1995; Iimuro et al., 1996) also protected rat liver against acute EtOH-induced steatosis (Table 1). Therefore, acute exposure can in principle be used to mimic very early changes caused by EtOH (Arteel et al., 1996).

ACUTE ETOH, PAI-1, AND HEPATIC RESPONSE

PAI-1 is an acute phase protein that is usually only expressed in adipocytes and endothelial cells, but it can be highly expressed by most cells in response to stress. The classical role of PAI-1 is to inhibit the plasminogen activators, tPA and uPA. Via attenuating the activation of plasminogen to plasmin, PAI-1 plays a major role in fibrin metabolism by blocking fibrinolysis. The role of PAI-1 in fibrin accumulation in vascular disease is well understood to contribute to endothelial dysfunction and inflammation. Although the liver can produce large amounts of PAI-1 in response to stress, the role of PAI-1 in liver diseases is not well understood. EtOH exposure rapidly and robustly induces PAI-1 expression in the mouse liver. Furthermore, alcohol-induced steatosis was prevented by genetic or pharmacologic inhibition of PAI-1 expression (Bergheim et al., 2006). Similar protection against EtOH-induced steatosis was observed in TNFR1^{-/-} mice in that study, a strain in which the increase in PAI-1 expression caused by EtOH was also completely blunted. Thus, PAI-1 plays a critical role in alcohol-induced steatosis.

Steatosis and inflammation are linked in the progression of ALD. Preventing steatosis is often sufficient to block inflammation in experimental ALD. Indeed, the enhanced LPS-induced inflammatory liver injury caused by acute EtOH preexposure was shown to be mediated, at least in part, by fibrin accumulation in livers, mediated by an inhibition of fibrinolysis by PAI-1 (Beier et al., 2009). Although PAI-1 is well known to be induced during inflammation, how PAI-1 may actually mediate inflammation is less understood. First, the “classical” role of PAI-1 in impairing fibrinolysis may also contribute to inflammation. For example, fibrin matrices have been shown to be permissive to chemotaxis and activation of monocytes and leukocytes. Furthermore, fibrin clots disrupt the flow of

blood within the hepatic parenchyma (i.e., hemostasis), the subsequent microregional hypoxia and hepatocellular death may directly (e.g., via HIF1 α) and indirectly alter inflammatory cell signaling (Ganey et al., 2004). Thus, acute EtOH is a useful exposure regimen for modeling hepatic responses to alcohol abuse. This exposure regimen may not only model alcohol binges in humans, but it may also be useful as a screening tool for later stages of chronic alcohol exposure.

CONCLUSIONS AND FUTURE INVESTIGATIONS

The binge EtOH, itself or after chronic EtOH, is increasingly being demonstrated to have profound effects on the liver. Epidemiological data also support it. The definition of binge and its comparison with chronic, especially as it relates to human scenario, has to be assessed carefully. In vitro and in vivo studies in animals and humans show that a number of molecular components that represent metabolic, immunologic, and epigenetic entities are affected by binge/acute EtOH. These include changes in TLR4 receptor, cytokines, histone modifications, microRNAs, and components involved in inflammation. It also appears that chronic EtOH exposure renders liver more susceptible to binge/acute EtOH-induced alterations leading to an increased injury. These developments highlight the important involvement of binge EtOH in liver injury. Identification of molecular targets affected by binge has the potential for developing newer therapeutic strategies. The fact there is an alarming rise in binge drinking makes this issue even more compelling to address.

There are several issues ripe for investigation regarding binge (acute) EtOH-induced increase in liver injury. These include (i) A better understanding of various experimental animal models of binge EtOH exposure. (ii) Identification of specific molecular targets (genes and proteins) that are distinct from chronic EtOH effects and that may play role(s) in enhancing the susceptibility of the liver to injury. (iii) Translational approaches to utilize information from binge studies for the development of new therapeutic strategies to treat ALD. There is good basis to state that the examination of binge (acute) effects of EtOH (in combination with chronic effects) may be more helpful than studies of chronic EtOH effects alone, in identifying molecular mechanisms of ALD and avenues to control it.

References

- Adachi Y, Bradford BU, Gao W, Bojes HK, Thurman RG. Inactivation of Kupffer cells prevents early alcohol-induced liver injury. *Hepatology*. 1994; 20:453–460. [PubMed: 8045507]
- Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. *Gastroenterology*. 1995; 108:218–224. [PubMed: 7806045]
- Alcohol and Health. Tenth Special Report to the US Congress on Alcohol and Health. US Department of Health and Human Services PHS-NIAAA; 2000. NIH Publication, U.S. Department of Health & Human Services
- Aroor AR, Jackson DE, Shukla SD. Elevated activation of ERK1 and ERK2 accompany enhanced liver injury following alcohol binge in chronically ethanol-fed rats. *Alcohol Clin Exp Res*. 2011; 35:2128–2138. [PubMed: 21790671]
- Aroor AR, Jackson DE, Shukla SD. Dysregulated phosphorylation and nuclear translocation of cyclic AMP response element binding protein (CREB) in rat liver after chronic ethanol binge. *Eur J Pharmacol*. 2012a; 679:101–108. [PubMed: 22269225]

- Aroor AR, James TT, Jackson DE, Shukla SD. Differential changes in MAP kinases, histone modifications, and liver injury in rats acutely treated with ethanol. *Alcohol Clin Exp Res*. 2010; 34:1543–1551. [PubMed: 20586759]
- Aroor AR, Lowery JR, Restrepo RJ, Mooney BP, Shukla SD. A proteomic analysis of liver after ethanol binge in chronically ethanol treated rats. *Proteome Sci*. 2012b; 10:29. [PubMed: 22545783]
- Aroor AR, Shukla SD. Binge ethanol intake in chronically exposed rat liver decreased LDL receptor and increased angiotensinogen gene expression. *World J Hepatol*. 2011; 3:250–255. [PubMed: 21969878]
- Arteel GE, Iimuro Y, Yin M, Raleigh JA, Thurman RG. Chronic enteral ethanol treatment causes hypoxia in rat liver tissue *in vivo*. *Hepatology*. 1997; 25:920–926. [PubMed: 9096598]
- Arteel GE, Raleigh JA, Bradford BU, Thurman RG. Acute alcohol produces hypoxia directly in rat liver tissue *in vivo*: role of Kupffer cells. *Am J Physiol*. 1996; 271:G494–G500. [PubMed: 8843775]
- Bala S, Marcos M, Kodys K, Csak T, Catalano D, Mandrekar P, Szabo G. Up-regulation of microRNA-155 in macrophages contributes to increased tumor necrosis factor alpha (TNF α) production via increased mRNA half-life in alcoholic liver disease. *J Biol Chem*. 2011; 2:1436–1444. [PubMed: 21062749]
- Beier JI, Luyendyk JP, Guo L, von Montfort C, Staunton DE, Arteel GE. Fibrin accumulation plays a critical role in the sensitization to lipopolysaccharide-induced liver injury caused by ethanol in mice. *Hepatology*. 2009; 49:1545–1553. [PubMed: 19291788]
- Beier JI, McClain CJ. Mechanisms and cell signaling in alcoholic liver disease. *Biol Chem*. 2010; 391:1249–1264. [PubMed: 20868231]
- Bergheim I, Guo L, Davis MA, Lambert JC, Beier JI, Duveau I, Luyendyk JP, Roth RA, Arteel GE. Metformin prevents alcohol-induced liver injury in the mouse: critical role of plasminogen activator inhibitor-1. *Gastroenterology*. 2006; 130:2099–2112. [PubMed: 16762632]
- Bhatty M, Jan B, Tan W, Pruett SB, Nanduri B. Role of acute ethanol exposure and TLR4 in early events of sepsis in a mouse model. *Alcohol*. 2011; 45:795–803. [PubMed: 21872420]
- Carson EJ, Pruett SB. Development and characterization of a binge drinking model in mice for evaluation of the immunological effects of ethanol. *Alcohol Clin Exp Res*. 1996; 20:132–138. [PubMed: 8651442]
- CDC Report. Binge drinking prevalence, frequency, and intensity among adults-United States, 2010. *MMWR Morb Mortal Wkly Rep*. 2012; 61:14–19. [PubMed: 22237031]
- Crosse KI, Anania FK. Alcoholic hepatitis. *Curr Treat Options Gastroenterol*. 2002; 5:417–423. [PubMed: 12408778]
- Dai Q, Pruett SB. Ethanol suppresses LPS-induced Toll-like receptor 4 clustering, reorganization of the actin cytoskeleton, and associated TNF- α production. *Alcohol Clin Exp Res*. 2006; 30:1436–1444. [PubMed: 16899048]
- Dai Q, Zhang J, Pruett SB. Ethanol alters cellular activation and CD14 partitioning in lipid rafts. *Biochem Biophys Res Commun*. 2005; 1:37–42. [PubMed: 15896296]
- Demeilliers C, Maisonneuve C, Grodet A, Mansouri A, Nguyen R, Tinel M, Letteron P, Degott C, Feldmann G, Pessayre D, Fromenty B. Impaired adaptive resynthesis and prolonged depletion of hepatic mitochondrial DNA after repeated alcohol binges in mice. *Gastroenterology*. 2002; 123:1278–1290. [PubMed: 12360488]
- Deng X, Luyendyk JP, Zou W, Lu J, Malle E, Ganey PE, Roth RA. Neutrophil interaction with the hemostatic system contributes to liver injury in rats cotreated with lipopolysaccharide and ranitidine. *J Pharmacol Exp Ther*. 2007; 322:852–861. [PubMed: 17505017]
- Enomoto N, Ikejima K, Yamashina S, Enomoto A, Nishiura T, Nishimura T, Brenner DA, Schemmer P, Bradford BU, Rivera CA, Zhong Z, Thurman RG. Kupffer cell-derived prostaglandin E(2) is involved in alcohol-induced fat accumulation in rat liver. *Am J Physiol Gastrointest Liver Physiol*. 2000; 279:G100–G106. [PubMed: 10898751]
- Enomoto N, Ikejima K, Yamashina S, Hirose M, Shimizu H, Kitamura T, Takei Y, Sato N, Thurman RG. Kupffer cell sensitization by alcohol involves increased permeability to gut-derived endotoxin. *Alcohol Clin Exp Res*. 2001; 25:51S–54S. [PubMed: 11410742]

- Ganey PE, Luyendyk JP, Maddox JF, Roth RA. Adverse hepatic drug reactions: inflammatory episodes as consequence and contributor. *Chem Biol Interact.* 2004; 150:35–51. [PubMed: 15522260]
- Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology.* 2011; 141:1572–1585. [PubMed: 21920463]
- Gao B, Seki E, Brenner DA, Friedman S, Cohen JI, Nagy L, Szabo G, Zakhari S. Innate immunity in alcoholic liver disease. *Am J Physiol Gastrointest Liver Physiol.* 2011; 300:G516–G525. [PubMed: 21252049]
- Goral J, Kovacs EJ. In vivo ethanol exposure down-regulates TLR2-, TLR4-, and TLR9-mediated macrophage inflammatory response by limiting p38 and ERK1/2 activation. *J Immunol.* 2005; 174:456–463. [PubMed: 15611271]
- Griffin R, Poe AM, Cross JM, Rue LW 3rd, McGwin G Jr. The association between blood alcohol level and infectious complications among burn patients. *J Burn Care Res.* 2009; 30:395–399. [PubMed: 19349899]
- Haseba T, Kameyama K, Mashimo K, Ohno Y. Dose-dependent change in elimination kinetics of ethanol due to shift of dominant metabolizing enzyme from ADH 1 (class I) to ADH 3 (class III) in mouse. *Int J Hepatol.* 2012; 2012:1–8.
- Hoek JB, Cahill A, Pastorino JG. Alcohol and mitochondria: a dysfunctional relationship. *Gastroenterology.* 2002; 122:2049–2063. [PubMed: 12055609]
- Iimuro Y, Ikejima K, Rose ML, Bradford BU, Thurman RG. Nimodipine, a dihydropyridine-type calcium channel blocker, prevents alcoholic hepatitis due to chronic intragastric ethanol exposure in the rat. *Hepatology.* 1996; 24:391–397. [PubMed: 8690410]
- James T, Aroor AR, Lim RW, Shukla SD. Histone H3 phosphorylation (S10, S28) and phosphoacetylation (K9/S10) are differentially associated with gene expression in liver of rats treated in vivo with acute ethanol. *J Pharmacol Exp Ther.* 2012; 340:237–247.
- Jones AW, Holmgren A. Age and gender differences in blood-alcohol concentration in apprehended drivers in relation to the amounts of alcohol consumed. *Forensic Sci Int.* 2009; 188:40–45. [PubMed: 19394172]
- Ki SH, Park O, Zheng M, Morales-Ibanez O, Kolls JK, Bataller R, Gao B. Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: role of signal transducer and activator of transcription 3. *Hepatology.* 2010; 52:1291–1300. [PubMed: 20842630]
- Lin JN, Tsai YS, Lai CH, Chen YH, Tsai SS, Lin HL, Huang CK, Lin HH. Risk factors for mortality of bacteremic patients in the emergency department. *Acad Emerg Med.* 2009; 16:749–755. [PubMed: 19594458]
- Mandrekar P, Bala S, Catalano D, Kodys K, Szabo G. The opposite effects of acute and chronic alcohol on lipopolysaccharide-induced inflammation are linked to IRAK-M in human monocytes. *J Immunol.* 2009; 2:1320–1327. [PubMed: 19561104]
- Mandrekar P, Catalano D, White B, Szabo G. Moderate alcohol intake in humans attenuates monocyte inflammatory responses: inhibition of nuclear regulatory factor kappa B and induction of interleukin 10. *Alcohol Clin Exp Res.* 2006; 30:135–139. [PubMed: 16433741]
- Mandrekar P, Szabo G. Signalling pathways in alcohol-induced liver inflammation. *J Hepatol.* 2009; 6:1258–1266. [PubMed: 19398236]
- Mathurin P, Beuzin F, Louvet A, Carrié-Ganne Balian A, Trinchet J, Dalsoglio D, Prevot S, Naveau S. Fibrosis progression occurs in a subgroup of heavy drinkers with typical histological features. *Aliment Pharmacol Ther.* 2007; 25:1047–1054. [PubMed: 17439505]
- Norkina O, Dolganiuc A, Catalano D, Kodys K, Mandrekar P, Syed A, Efros M, Szabo G. Acute alcohol intake induces SOCS1 and SOCS3 and inhibits cytokine-induced STAT1 and STAT3 signaling in human monocytes. *Alcohol Clin Exp Res.* 2008; 32:1565–1573. [PubMed: 18616672]
- Oak S, Mandrekar P, Catalano D, Kodys K, Szabo G. TLR2- and TLR4-mediated signals determine attenuation or augmentation of inflammation by acute alcohol in monocytes. *J Immunol.* 2006; 12:7628–7635. [PubMed: 16751410]
- Park P, Lim RW, Shukla SD. Involvement of histone acetyltransferase (HAT) in ethanol induced acetylation of histone H3 in hepatocytes: potential mechanism for gene expression. *Am J Physiol.* 2005; 289:G1124–G1136.

- Petrasek J, Dolganiuc A, Csak T, Nath B, Hritz I, Kodys K, Catalano D, Kurt-Jones E, Mandrekar P, Szabo G. Interferon regulatory factor 3 and type I interferons are protective in alcoholic liver injury in mice by way of crosstalk of parenchymal and myeloid cells. *Hepatology*. 2011; 2:649–660. [PubMed: 21274885]
- Pruett BS, Pruett SB. An explanation for the paradoxical induction and suppression of an acute phase response by ethanol. *Alcohol*. 2006; 39:105–110. [PubMed: 17134663]
- Pruett SB, Fan R. Ethanol inhibits LPS-induced signaling and modulates cytokine production in peritoneal macrophages in vivo in a model for binge drinking. *BMC Immunol*. 2009; 10:49. [PubMed: 19765273]
- Pruett SB, Fan R, Cheng B, Glover M, Tan W, Deng X. Innate immunity and inflammation in sepsis: mechanisms of suppressed host resistance in mice treated with ethanol in a binge-drinking model. *Toxicol Sci*. 2010; 117:314–324. [PubMed: 20624996]
- Rehm J, Baliunas D, Borges GL, Graham K, Irving H, Kehoe T, Parry CD, Patra J, Popova S, Poznyak V, Roerecke M, Room R, Samokhvalov AV, Taylor B. The relation between different dimensions of alcohol consumption and burden of disease: an overview. *Addiction*. 2010; 105:817–843. [PubMed: 20331573]
- Reuben A. Alcohol and the liver. *Curr Opin Gastroenterol*. 2007; 23:283–291. [PubMed: 17414844]
- Shibayama Y, Asaka S, Nakata K. Endotoxin hepatotoxicity augmented by ethanol. *Exp Mol Pathol*. 1991; 55:196–202. [PubMed: 1936215]
- Shukla SD, Velazquez J, French SW, Lu S, Ticku MK, Zakhari S. Emerging role of epigenetics in the actions of alcohol. *Alcohol Clin Exp Res*. 2008; 32:1525–1534. [PubMed: 18616668]
- Thurman RG, Bradford BU, Iimuro Y, Knecht KT, Arteel GE, Yin M, Connor HD, Wall C, Raleigh JA, Frankenburg MV, Adachi Y, Forman DT, Brenner D, Kadiiska M, Mason RP. The role of gut-derived bacterial toxins and free radicals in alcohol-induced liver injury. *J Gastroenterol Hepatol*. 1998; 13:S39–S50. [PubMed: 9792033]
- Yin H, Kim M, Kim J, Kong G, Kang K, Kim H, Yoon B, Lee M, Lee B. Differential gene expression and lipid metabolism in fatty liver induced by acute ethanol treatment in mice. *Toxicol Appl Pharmacol*. 2007; 223:225–233. [PubMed: 17655900]
- Zakhari S, Li TK. Determinants of alcohol use and abuse: impact of quantity and frequency patterns on liver disease. *Hepatology*. 2007; 46:2032–2039. [PubMed: 18046720]
- Zhong Z, Arteel GE, Connor HD, Schemmer P, Chou SC, Raleigh JA, Mason RP, Lemasters JJ, Thurman RG. Binge drinking disturbs hepatic microcirculation after transplantation: prevention with free radical scavengers. *J Pharmacol Exp Ther*. 1999; 290:611–620. [PubMed: 10411569]

Fig. 1.
Binge ethanol has multiple effects on the liver.

Table 1

Mechanistic Overlap Between Acute and Chronic Liver Damage Caused by Alcohol: Summary of Work in Which Pharmacologic or Genetic Interventions Protected Against Both Acute (Steatosis) and Chronic (Steatohepatitis) Experimental Alcohol-Induced Liver Injury in Rats and/or Mice

	Protected against hepatic changes caused by	
	Acute EtOH	Chronic EtOH
GdCl ₃	+ (Enomoto et al., 2000)	+ (Adachi et al., 1994)
Antibiotics	+ (Enomoto et al., 2000)	+ (Adachi et al., 1995)
Nimodipine	+ (Enomoto et al., 2000)	+ (Iimuro et al., 1996)
TNFR1 ^{-/-} mice	+ (Bergheim et al., 2006)	+ (Deng et al., 2007)
PAI-1 ^{-/-} mice	+ (Bergheim et al., 2006)	+ (Bergheim et al., 2006)

EtOH, ethanol; PAI-1, plasminogen activator inhibitor-1.