



Mechanisms of alcohol induced liver injury in rats and treatments

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Abstract

Alcohol induced liver injury i.e. Alcoholic Liver Disease (ALD) or alcoholic hepatitis is the most severe form of all the alcohol-induced liver lesions. Animal models of ALD mainly involve mild liver damage (that is steatosis and moderate inflammation) whereas severe alcoholic hepatitis in humans occurs in the setting of cirrhosis and is associated with severe liver failure. It is a complex process that includes a wide spectrum of hepatic lesions, from steatosis to cirrhosis. Cell injury, inflammation, oxidative stress, regeneration and bacterial translocation are key drivers of alcohol-induced liver injury. The consequences of alcohol consumption on health vary according to the drinking pattern (excessive or not, acute or chronic); environmental and individual factors are also key drivers. Alcohol-induced liver injury includes fatty liver, fibrosis and alcoholic hepatitis; these basic lesions can occur separately, simultaneously or sequentially in the same patient. This Review discusses the main pathways associated with the progression of liver disease, as well as potential therapeutic strategies targeting these pathways.

Keywords: ALD, Animal models, liver injury. Hepatitis.

Introduction

Excess alcohol drinking is the major cause of liver-related diseases. It is important to bear in mind that ALD is often diagnosed at very advanced stages of the disease. It is an intricate process, which appears to involve a metabolic product of ethanol oxidation (5, 10), cytochrome P450 induction (9, 35), enhanced oxidative stress (60, 107), depletion of antioxidant defenses (48), lipid peroxidation (55), generation of aldehydic products, the effects of mitogenic and fibrogenic cytokines (29, 70) and complex interactions between liver parenchymal and non parenchymal cells with the hepatic stellate cells. It is hepatic lipocyte or fat-storing cell now recognized as the primary source of extracellular matrix (29). The extent of alcoholic liver fibrosis correlates significantly with hepatic levels of products of lipid peroxidation such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) (55), aldehydes that directly stimulate collagen synthesis and/or gene expression by fibroblasts and hepatic stellate cells. As previously shown, the

concentrations of liver malondialdehyde (MDA) liver 4-hydroxynonenal (4-HNE), and serum aminotransferases (ALT, AST) were significantly elevated by ethanol infusion alone. ((15,120). This Review discusses the relevance of certain pathways according to the type of liver lesion, and of potential molecules targeting these pathways in relation to the pattern and the severity of liver injury.

Cell injury and ethanol metabolism

Molecules of ethanol diffuse easily across cell membranes. Many factors influence ethanol absorption and metabolism, including gender, age, ethnicity and body weight, and <10% of ethanol is directly eliminated by the lungs, kidneys and sweat in its unchanged form (88). The main metabolic pathway involved in the biotransformation of ethanol is oxidation into acetaldehyde (Figure 2). This process uses NAD⁺ and is primarily achieved by based on

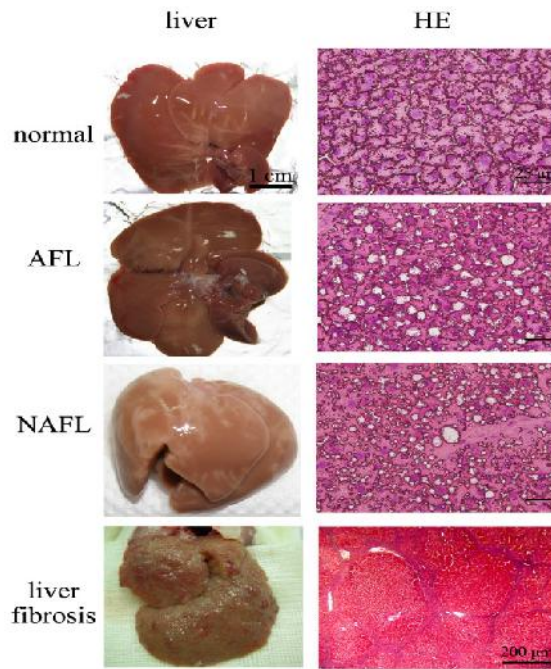


Figure 1: Histological analysis and comparisons among four groups of rats. Images of livers from normal control, rats with alcoholic fatty liver (AFL), non-alcoholic fatty liver (NAFL, fed 60% fructose) and liver fibrosis (left column) were analyzed by hematoxylin and eosin (H&E) staining (right column). In AFL and NAFL rats, livers were filled with prominent fatty change. The scale bar represents 25 µm for normal, AFL and NAFL; but 100 µm for liver fibrosis.

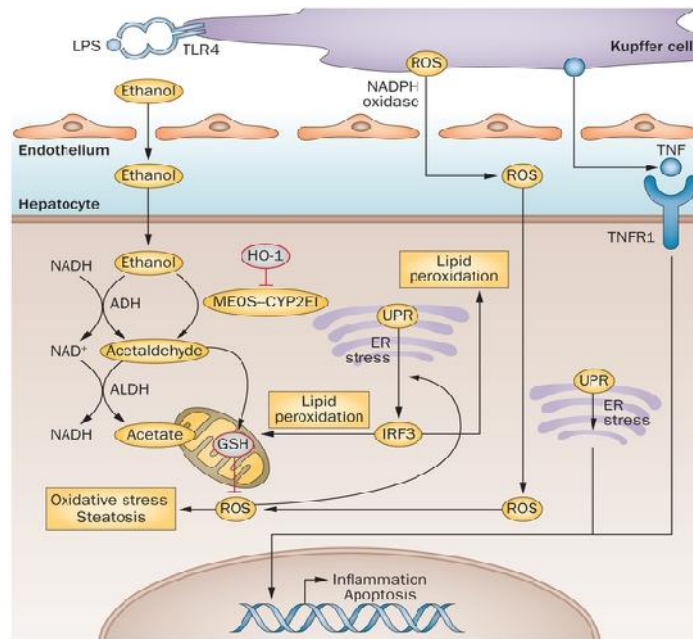


Figure 2: Metabolism of ethanol and related cell injury. Ethanol is mainly metabolized by alcohol dehydrogenase and MEOS into acetaldehyde, which is responsible for the generation of ROS. ROS cause oxidative stress, ER stress and steatosis. ROS are also generated through activation of NADPH oxidase in Kupffer cells. All these changes in hepatocyte metabolism lead to inflammation and apoptosis.

Abbreviations: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP2E1, cytochrome P450 2E1; ER, endoplasmic reticulum; GSH, reduced glutathione; HO-1, heme oxygenase 1; IRF3, interferon regulatory factor 3; LPS, lipopolysaccharide; MEOS, microsomal ethanol oxidation system; NAD, nicotinamide adenine dinucleotide; ROS, reactive oxygen species; TNFR1, tumor necrosis factor receptor 1; TLR-4, Toll-like Receptor 4; UPR, unfolded protein response

cytochromes P450 and in particular CYP2E1 (12, 62). Under normal conditions, CYP2E1 accounts for 10% of ethanol oxidation (68) but this system is inducible by its substrates, in particular ethanol itself. CYP2E1 is mainly expressed in the perivenous zone (Rappaport zone) which might partially explain why alcohol-related liver injury is more frequent in this part of the hepatic lobule (11, 63). Regardless of the involved pathway, after oxidation most acetaldehyde is converted into acetate by aldehyde dehydrogenase, which is expressed in the cytoplasm and in mitochondria. This reaction is catalyzed by NAD⁺/NADH and increases the amounts of NADH in the liver (62). As alcohol dehydrogenase, CYP2E1 and aldehyde dehydrogenase are mainly expressed in hepatocytes, most of the direct cellular toxicity of ethanol affects these cells. Ethanol metabolism leads to accumulation of reactive oxygen species (ROS), mainly hydrogen peroxide (H₂O₂) and superoxide anion O₂⁻ (12) which is exacerbated by hypoxia, bacterial translocation and the release of pro inflammatory cytokines. Given their short half-life and high reactivity, these radicals quickly bind to ethanol or iron atoms to form reactive metabolites such as hydroxyl radical (OH), ferrous oxide (FeO) or hydroxyethyl radical (CH₃CHOH), which are all responsible for lipid peroxidation of cell membranes. Mitochondria (through their respiratory chain), the endoplasmic reticulum (through CYP2E1) and Kupffer cells (through NADPH oxidase) are the main sources of ROS. During alcohol-induced liver injury (128), iron is also involved in oxidative stress and promotes fibrosis by catalyzing the formation of ROS (101, 103). The cytotoxic effects of ethanol metabolism and ROS lead to cell death; evidence exists of apoptosis and necrosis in ALD (20). DAMPs (damage-associated molecular patterns) are released after cell death, mainly necrosis, and trigger macrophage and neutrophil activation, fibrogenesis and hepatic regeneration (69). Senescence via natural killer (NK) cells and autophagy are regulators of liver inflammation after cell death and the release of DAMPs (6,109). Although it is tempting to hypothesize that these molecules (for example, HMGB1 and miR-122) have a key role in alcohol-induced cell death, few data are available in ALD compared with other liver diseases such as NAFLD or paracetamol-induced liver failure. Chronic exposure to ethanol induces glutathione depletion, which makes hepatocytes more sensitive to oxidative stress as reduced (not oxidized) glutathione protects cells against ROS (30, 31, 32, 41, 50,129). Heme oxygenase-1 (HO-1) is also a protective factor against oxidative stress in ALD because it blocks CYP2E1

activity, reducing generation of ROS. The endoplasmic reticulum is a central organelle for the maturation of proteins, in particular their folding process. In response to various stimuli, incomplete maturation produces an accumulation of unfolded proteins in the lumen of the endoplasmic reticulum that activate several pathways leading to endoplasmic reticulum stress (50). In ALD enhanced endoplasmic reticulum stress, due in part to ROS and hyperhomocysteinemia, participates in inflammation through the activation of NF- κ B and JNK, and in apoptosis, in particular through disruption of cellular calcium homeostasis and the activation of the CHOP-GADD153 pathway (52). IRF3 links endoplasmic reticulum stress to mitochondria and subsequent hepatocyte apoptosis by activation of caspases, but also to liver inflammation in hepatocytes and liver mononuclear cells by stimulating the interferon and the NF- κ B pathways (53). Endoplasmic reticulum stress, amplified by elevated homocysteine levels, contributes to alcohol tissue damage and is counterbalanced by transcription genes involved in the unfolded protein response, such as GRP78, GRP94 or Herp (53, 54). Another consequence of endoplasmic reticulum stress is the activation of the steatogenic pathways in hepatocytes, in particular via SREBPs (sterol regulatory element-binding proteins) (20, 54). Ethanol also generates endoplasmic reticulum stress in stellate cells through inhibition of a protective autophagy phenomenon, leading to their activation in fibrogenic cells (38).

Mechanisms of alcohol-induced steatosis:

Chronic alcohol consumption promotes steatosis by disrupting hepatic lipid metabolism via SREBPs and PPAR- α (peroxisome proliferator-activating receptor), which are directly influenced by AMPK (5' adenosine monophosphate-activated protein kinase) (Figure 3). SREBP1c exerts its deleterious role by increasing fatty acid biosynthesis through fatty acid synthase and enzymes responsible for fatty acid desaturation such as stearoyl-CoA desaturase (137). SREBP expression is increased by acetaldehyde and TNF (27) which also stimulate its maturation in hepatocytes (61). SREBP might be down regulated by betaine (also known as glycine betaine) administration (54) Conversely; PPAR- α prevents ethanol-induced steatosis (84). Its expression is reduced in hepatocytes by acetaldehyde (38, 61) and during chronic alcohol intake by down regulation of RXR- α , which prevents its heterodimerization to PPAR- α and subsequently alters PPAR- α signalling. As in metabolic steatosis, AMPK downregulation is central to alcohol-induced

lipid accumulation by stimulating SREBP1c and inhibiting PPAR- expression (10, 80,141). AMPK expression is decreased by ethanol *in vitro* and in experimental models of alcoholic steatosis (39, 138) AMPK down regulation also causes a decrease in fatty acid oxidation mediated by acetyl-coA carboxylase and carnitin palmitoyltransferase (27). Lipid metabolism disturbances are also worsened by endoplasmic reticulum stress and ROS. The metabolic pathways sirtuin-1 (136, 139) cannabinoid receptors (51, 67) complement fractions (16–105) and PPAR- 56 might also participate in alcohol-induced steatosis. All of these mechanisms increase lipogenesis and decrease fatty acid oxidation and export during chronic alcohol consumption. Insulin resistance has a key role in alcohol-induced steatosis, as shown by the faster disease progression in patients who are

overweight and drink to excess compared with normal weight individuals who drink to excess (87–108). However, data also suggest that modest alcohol intake might result in a decrease in inflammatory lesions in patients with NAFLD (26). In animal models, ethanol feeding induces insulin resistance, macrophage infiltration and the production of proinflammatory cytokines such as TNF, IL-6 and CC-chemokine ligand 2 (CCL2, also known as MCP-1) in adipose tissue (56). Adiponectin is down regulated in those models leading to a decrease in fatty acid oxidation, an increase in fatty acid synthesis and inhibition of AMPK and PPAR- (131, 133,140). Decreased production of adiponectin has also been observed in adipose tissue due to endoplasmic reticulum stress through an accumulation of homocysteine in adipocytes (111).

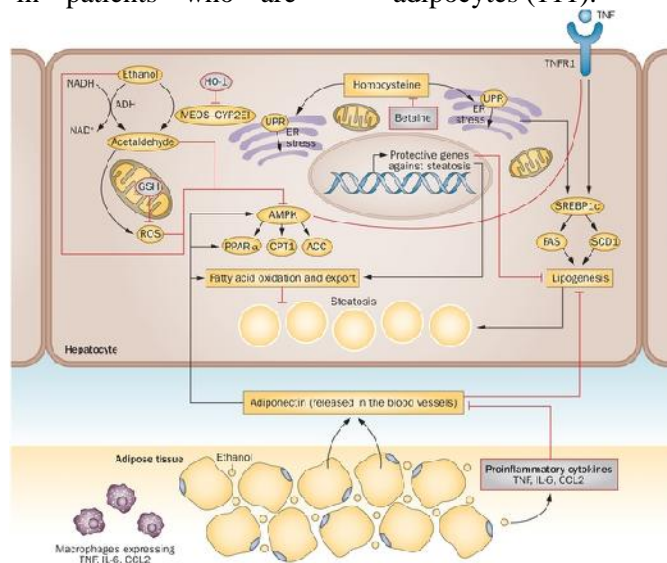


Figure 3: Mechanisms of alcohol-related steatosis. Chronic alcohol consumption leads to steatosis via generation of acetaldehyde, ROS and ER stress. The consequences are blockade of PPAR and of AMPK, which is responsible for fatty acid oxidation and export via ACC and CPT-1. In addition, chronic alcohol consumption induces SREBP1c activation, which is responsible for fatty acid synthesis through FAS and fatty acid desaturation through SCD1. TNF also leads to steatosis by activating SREBP1c. Betaine reduces homocysteine levels, which thus enhances ER stress. Chronic alcohol consumption also induces adipose tissue inflammation, which decreases the release of the protective adiponectin, thus favoring steatosis. All these mechanisms lead to disruption of hepatic lipid metabolism by increasing lipogenesis and decreasing fatty acid oxidation and export. Abbreviations: ACC, acetyl CoA carboxylase; AMPK, adenosine monophosphate-activated protein kinase; CCL2, CC-chemokine ligand 2; CPT1, carnitin palmitoyltransferase 1; ER, endoplasmic reticulum; FAS, fatty acid synthetase; PPAR- , peroxisome proliferator-activated receptor ; ROS, reactive oxygen species; SCD1, stearoyl-CoA desaturase 1; SREBP1c, sterol regulatory element-binding protein 1c; TNFR1, tumour necrosis factor receptor 1.

Treatments

Generally speaking, whatever the severity of disease, recovery from alcohol-induced liver injury is dependent upon cessation of alcohol consumption. Indeed, long-term survival (>5 years) is better in

patients with cirrhosis who remain abstinent than in those who begin drinking again (102,123).

1. Immunity, dysbiosis and ALD
 2. Regeneration and ALD
 3. Targeted treatment of ALD
- Corticosteroids

Immunity, dysbiosis and ALD

Chronic alcohol exposure in humans and animal models increases circulating concentrations of lipopolysaccharide (LPS) compared with controls (66–68) and the severity of hepatic injury is correlated to serum levels of LPS (45, 75, 100, 117). Exogenous administration of LPS induces necroinflammatory lesions in the liver (4, 75) whereas administration of antibiotics or probiotics reduces the severity of hepatic lesions in ethanol-fed rats (1, 85). After translocation from the gut lumen to the liver, LPS and other pathogen-associated molecular patterns (PAMPs) are recognized by pathogen-recognition receptors (PRRs), including TLRs (Toll-like receptors, mainly TLR4 which is responsible for LPS recognition) and NOD1 and NOD2 (nucleotide oligomerization domain) receptors. As well as LPS–TLR4, liver inflammation is also mediated by other bacterial compounds, which explains why the injection of other bacterial motifs also leads to recruitment of inflammatory cells (43). More specifically, the activation of TLR2 and TLR6 (which are both responsible for recognition of bacterial lipopeptides) and TLR9 (which recognizes bacterial DNA-containing unmethylated CpG motifs) leads to an increase in the proinflammatory cascade in ALD. After binding to TLR4 via LBP (lipopolysaccharide binding protein), LPS activates Kupffer cells via a signalling cascade that includes CD14, MD-2 (also known as lymphocyte antigen (7) and finally results in the activation of mitogen-activated protein kinases (such as ERK1, ERK2, JNK and p38), NF- κ B and AP-1 (42, 127). When these pathways are activated, Kupffer cells release ROS, adhesion molecules such as ICAM-1 (intracellular adhesion molecule 1) and VCAM-1 (vascular cell adhesion protein 1), chemokines (IL-8 and CCL2) and proinflammatory cytokines (TNF, IL-1 and IL-6) (10, 142). Targeting NADPH oxidase in Kupffer cells might be an interesting therapeutic target because of its role in the defense against bacteria, in inflammatory responses, generation of ROS and in the control of hepatic fibrosis (12, 21, 122). Finally, the intracellular labile iron fraction regulates activation of NF- κ B in Kupffer cells and the cascade of inflammation pathways associated with the early stages of alcohol-induced liver injury (65, 110, 121 and 130). Leukocyte recruitment amplifies the inflammatory response to LPS. In humans, alcoholic hepatitis is characterized by the presence of large amounts of cytokines that are highly sensitive to PAMPs, such as RANTES (also known as C-C chemokine receptor type 1) IL-8, Gro- α and CCL2 (2, 18, 76, and 116). leading to neutrophil recruitment in the liver. Besides their role in neutrophil infiltration, proinflammatory changes in

Kupffer cells also seem to enhance steatosis (3, 78) The role of Kupffer cells is not just deleterious in ALD—these cells can also adopt an anti-inflammatory phenotype and release IL-10 (8, 125).

Although innate immunity has a central role in the pathogenesis of ALD, adaptive immunity might also contribute to the progression of alcohol-induced liver injury. Indeed, chronic alcohol consumption leads to the development of antibodies directed against lipid peroxidation products, which activate an adaptive immune response (10). More specifically, alcohol consumption stimulates splenic T cells and natural killer T (NKT) cells in the liver, leading to the development of a cytotoxic response against hepatocytes, which might be related, at least in part, to Fas and TNF receptor 1 signalling (115) Other cells, such as stellate cells, might also participate in the release of inflammatory mediators, such as CCL2, IL-8, TNF and IL-1 (7, 99, 118). When considering the effects of bacterial translocation on the pathogenesis of ALD, the question of modifications in gut microbiota (that is, dysbiosis) in patients with excess alcohol consumption has become an important issue. Bacterial overgrowth and dysbiosis have been observed in animal models¹ with subsequent intestinal oxidative stress, leaky gut and intestinal inflammation (14, 82, 109, 134). In dysbiosis, ethanol targets gut microbes by decreasing their ability to synthesize saturated fatty acids; by contrast, supplementation of saturated long-chain fatty acids restores eubiosis, reduces gut leakiness and ALD (13). Compared with nondrinkers, some patients who drink to excess have been shown to have colonic dysbiosis, including a lower percentage of *Bacteroidetes* and a higher percentage of *Proteobacteria*, which are associated with higher levels of endotoxins. This finding suggests that PAMP-induced liver inflammation might be enhanced in these individuals (83). Quantitative metagenomics has also shown that patients with cirrhosis can be identified according to clusters of cognate bacterial species compared with healthy individuals, suggesting that gut microorganisms have a role in the severity of liver disease (106). Thus probiotics might be able to help limit the progression of ALD. Indeed, preliminary studies have shown that the administration of probiotics (for example, *Lactobacillus sp.*) restored gut microbiota, reduced hepatic inflammation in animals (37) restored the phagocytic capacities of neutrophils in patients with alcoholic cirrhosis (114) and improved liver enzyme levels in heavy drinkers. Prebiotics have also been shown to be protective in NAFLD and can be viewed as a promising strategy in ALD, although no data support their use at present (22, 59 and 77).

Regeneration and ALD

Liver regeneration is a key target for the development of new treatment molecules in ALD, in particular alcoholic hepatitis. Regeneration is necessary to restore functional liver tissue after insults such as partial hepatectomy or toxic injury. Hepatocytes proliferate in the absence of chronic liver disease, but in ALD this mechanism is not as effective when liver progenitor cells might act as facultative cells for regeneration (discussed later) (23, 79). Models of partial hepatectomy have identified TNF, IL-6, STAT-3 and NF- κ B as the key drivers of liver regeneration (19, 34, 124, 126, and 132). In alcohol-fed mice, steatosis is associated with increased production of ROS and inhibition of mature hepatocyte proliferation. Chronic ethanol exposure also impairs liver regeneration by limiting DNA synthesis by mature hepatocytes and impairing normal miRNA signalling during the regeneration process (119). At a cellular level, chronic alcohol consumption induces senescent replication of hepatocytes and proliferation of liver progenitors. An important missing piece of the research puzzle is the lack of an experimental model with altered liver regeneration.

Targeted treatment of ALD

Corticosteroids were proposed in the AASLD (American Association for the Study of Liver Diseases) (91) and EASL (European Association for the Study of the Liver) guidelines to treat severe forms of alcoholic hepatitis (defined by a Maddrey's discriminant function ≥ 32) on the basis of meta-analyses showing a reduction in short-term mortality (71, 72). Nevertheless, despite the positive effects of this treatment on short-term survival, mortality 6 months after the onset of disease is still ~30–40% (5, 35). Because some patients do not respond to existing corticosteroids, it is crucial to identify nonresponders rapidly. Scoring systems for alcoholic hepatitis include the Glasgow Alcoholic Hepatitis Score, the ABIC (age–bilirubin–INR–creatinine) score and the Lille model (25, 36 and 66). The formula of the Lille model includes the effect of corticosteroids on bilirubin levels after 7 days of treatment, which is highly predictive of outcome (73, 74). The difference in bilirubin level is combined with age, the presence of renal insufficiency, albumin level, and prothrombin time and bilirubin level at the beginning of treatment to generate a score that ranges from 0 to 1. The ideal cut-off of 0.45 can be used to define responders to corticosteroids (Lille score <0.45) and nonresponders (Lille score ≥ 0.45). The therapeutic response can be

refined further by defining three populations: complete responders (Lille score <0.16); partial responders (Lille score $0.16–0.56$); and null responders (Lille score ≥ 0.56). Corticosteroids can be withdrawn after 7 days in null responders, because the outcome of these patients is not different from that of patients treated with placebo (72). In theory, corticosteroids help treat severe alcoholic hepatitis because of their anti-inflammatory properties; however, the underlying pathways of this treatment have still not been clarified. In animal models, alcohol-induced liver injury was prevented by the administration of antibodies against TNF in wild-type mice and was not observed in mice lacking TNF receptor (49, 135). Because of this evidence from animal models, the first studies focused on TNF pathways. Levels of TNF its p55 soluble receptors and IL-8 (a strong chemo attractant responsible for neutrophil recruitment) are elevated in patients with severe alcoholic hepatitis (86, 116). Compared with those with alcoholic cirrhosis or healthy livers. On the other hand, IL-10 levels are unaffected by the presence of alcoholic hepatitis, suggesting an imbalance between proinflammatory and anti-inflammatory cytokines. Although preliminary studies showed a correlation between baseline serum TNF levels and poor outcome, this relationship has not been confirmed in further studies (112). After corticosteroid treatment, IL-8 and TNFRp55 (soluble TNF receptor p55) plasma levels decrease, particularly TNFRp55 in survivors, whereas IL-10 levels increase. Conversely, serum TNF levels are unchanged after corticosteroid therapy, although *ex vivo* experiments have shown that this treatment limits the ability of neutrophils and monocytes to produce TNF in the presence of LPS. (Figure 4).

Patients with alcoholic hepatitis have decreased sensitivity to corticosteroids—as shown by defective inhibition of lymphocyte proliferation—and so another strategy to improve patient outcome could be to increase the effect of this treatment in this group. Interestingly, patients who respond to corticosteroids were shown to have better *ex vivo* sensitivity to this drug than no responders (24, 57). The results of this trial were confirmed in an independent cohort and a suggested threshold of 60% maximum intensity (of lymphocyte proliferation) was proposed to define resistance to corticosteroids. Sensitivity to corticosteroids is improved as liver dysfunction recovers and might be restored by theophylline (a xanthine), potentially via enhanced recruitment of histone deacetylases, which silence the expression of proinflammatory genes. Basiliximab (a monoclonal antibody triggering the IL-2 receptor [anti-CD25])

might be another therapeutic option to compensate for defective sensitivity to corticosteroids (24). Another option to target signalling pathways involved in histone acetylation might be decreasing acetate levels via inhibition of acetyl-coA synthetase, leading to downregulation of proinflammatory genes (IL-6, IL-8 or TNF) in macrophages exposed to ethanol (58).

Downregulation of proinflammatory genes in macrophages and monocytes can also be obtained by stimulation of GILZ (glucocorticoid-induced leucine zipper), whose expression is stimulated by corticosteroids (44). New molecules that increase sensitivity to corticosteroids could be potential targets to improve patient management.

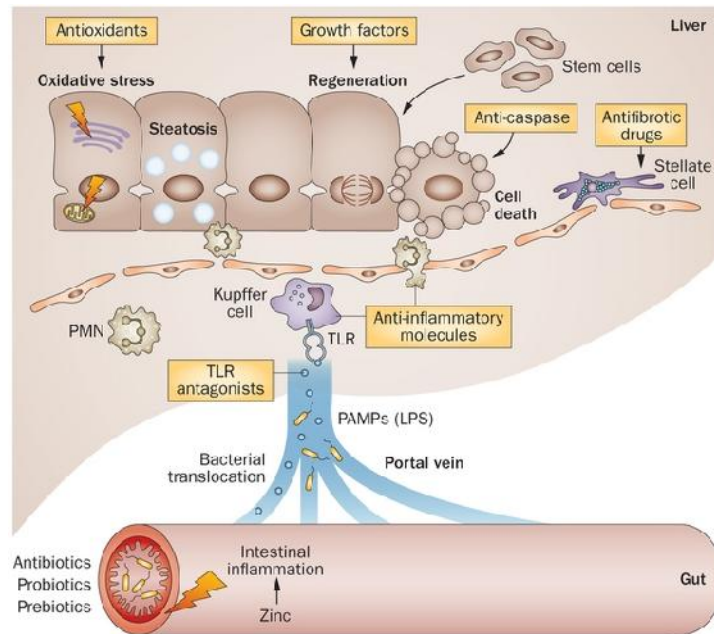


Figure 4: Potential targeted therapies in ALD. There are many potential targets to treat ALD, including the gut microbiota, liver inflammation, regeneration, cell death and oxidative stress. Several of them are being studied
 Abbreviations: ALD, alcoholic liver disease; LPS, lipopolysaccharide; PAMPs, pathogen-associated molecular patterns; PMN, polymorph nuclear neutrophil; TLR, Toll-like receptor.

Other treatments

Oxidative stress is one of the key mechanisms that drives early ALD, in particular steatosis. As the precursors of glutathione have been shown to improve steatosis in rodents (10, 35, 64) it is tempting to evaluate these molecules in humans. S-adenosylmethionine could be an interesting therapeutic option for ALD because this molecule restores mitochondria levels of glutathione, whereas N-acetylcysteine does not (17, 40) Betaine supplementation reduces steatosis in ethanol-fed mice by decreasing endoplasmic reticulum stress by conversion of homocysteine to methionine (54) However, no data yet support the use of these two molecules in humans in the early stages of ALD. Antioxidant molecules alone are not effective in severe forms of alcoholic hepatitis (104). Although experimental studies suggested that N-acetylcysteine was the best candidate, this drug did not improve liver injury or patient outcome in randomized studies (81, 113). These molecules could be considered preventive

treatment to reduce the risk of disease progression, although prospective data are needed. Conversely, the combination of N-acetylcysteine with prednisolone was associated with a greater early improvement in liver function and a lower incidence of hepatorenal syndrome than prednisolone alone, associated with a trend towards improved short-term survival (89). The mechanisms underlying this synergistic effect have not yet been elucidated. Further studies investigating this combination are needed.

Conclusion

New drugs should be tested on the basis of the main mechanisms involved in the type of liver injury, according to novel findings from translational research and animal models. For example, in patients with alcoholic hepatitis, an interesting potential area for investigation, the most promising options include molecules targeting inflammation such as TLR4

antagonists or IL-1 receptor antagonists (such as anakinra), or those targeting bacterial translocation, apoptosis (for example, emricasan) and/or liver regeneration, as well as antibiotics. In patients in the early stages of ALD, probiotics, prebiotics, molecules limiting oxidative stress and alleviating steatosis and/or reducing the progression of fibrosis should be developed, in close association with the management of alcohol dependence. Scientific studies have made major progress in understanding the pathogenesis of and the effect of treatment in ALD. This Review discusses the need to build a bridge from the bench to the bedside as well as to adapt the design of future studies to the histological lesions of the disease and to mortality. For example, the sample size in studies evaluating molecules to reduce disease progression in patients with alcoholic steatosis must be extremely large with an end point that has been accepted by health agencies, such as the risk of developing cirrhosis. The study populations in these trials will have to be as large as those in cardiovascular studies and will have to use noninvasive markers to assess progression to cirrhosis. On the other hand, studies on alcoholic hepatitis must focus on short-term outcome and the development of surrogate markers to identify and validate new therapeutic pathways.

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