Minireview

Pathogenesis of alcoholic liver disease: the role of nuclear receptors

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Abstract

Ethanol consumption causes fatty liver, which can lead to inflammation, fibrosis, cirrhosis and even liver cancer. The molecular mechanisms by which ethanol exerts its damaging effects are extensively studied, but not fully understood. It is now evident that nuclear receptors (NRs), including retinoid x receptor α and peroxisome proliferator-activated receptors, play key roles in the regulation of lipid homeostasis and inflammation during the pathogenesis of alcoholic liver disease (ALD). Given their pivotal roles in physiological processes, NRs represent potential therapeutic targets for the treatment and prevention of numerous metabolic and lipid-related diseases including ALD. This review summarizes the factors that contribute to ALD and the molecular mechanisms of ALD with a focus on the role of NRs.

Keywords: nuclear receptors, alcoholic liver disease, retinoid x receptor alpha, peroxisome proliferator-activated receptors, ethanol, lipogenic transcription factors

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Introduction

Alcoholic liver disease (ALD) is damage to the liver and its function due to alcohol abuse and usually occurs after years of excessive drinking. Changes in the liver include fatty liver, inflammation and cirrhosis.[1–3] Moreover, chronic alcohol consumption is an established risk factor for the development of hepatocellular carcinoma in patients with liver cirrhosis.[4,5] ALD may occur in patients who consume excessive amounts of alcohol. Reports indicate that the risk of developing liver damage depends on the amount of alcohol consumed.[6–8] Evidence suggests that daily ethanol consumption exceeding 40–80 g/d for men and 20–40 g/d for women for 10–12 y may lead to ALD.[1,9,10] Alcohol use remains the most common cause of liver-related mortality and a major cause of death and disability worldwide.[11] Since the 1970s, there has been a gradual decline in ALD mortality in many countries.[9] However, current reports indicate that the incidence of ALD and subsequent deaths is on the rise.[9] In the United States, the mortality rate for ALD in 1993 was 7.9 per 100,000, while in 2000 it had risen to 9.6 per 100,000.[12,13]

Although a clear correlation exists between cumulative alcohol intake and liver disease, only some alcohol drinkers develop signs of ethanol-induced liver damage. It is now clear that individuals differ in their susceptibility to ALD. The susceptibility of individuals to the toxic effects of alcohol consumption may involve complex interactions between genes and the environment. To date, genes encoding the principal alcohol-metabolizing enzymes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), are the only genes that have been firmly linked to vulnerability to alcoholism and possible risk for ALD.[14–18] Other factors that affect susceptibility to ALD include the amount of alcohol ingested over time, ethnicity, female gender, adolescent age, drinking multiple varieties of alcohol, drinking alcohol in-between meals, poor nutrition, obesity, hepatitis C virus infection and polymorphisms in genes (cytochrome P450 2E1 [CYP2E1], glutathione *S* transferases [GSTs] and tumor necrosis factor-α [TNF-α]).[6,9,14,19–26] Epidemiological data indicate that some minority groups suffer more severe adverse effects from alcohol consumption.[27] It has been suggested that some of these consequences may be due to special characteristics of these minority populations, including nutrition, socioeconomic status, hygiene, health-care delivery or other environmental conditions.[28] In addition, reports indicate that polymorphisms in several genes, including ADH and ALDH observed among Asians, African-Americans and Caucasians, may be an important

biological factor contributing to differences in cell and tissue damage, intolerance to alcohol or both observed in these groups.[14,29–31] Among the various ethnic groups, Hispanic and African-American men have the highest risk of alcoholism.[15–17,32]

Ethanol produces a wide spectrum of hepatic injuries in humans and experimental animals, the most characteristic being interference in lipid metabolism. Hepatic lipid homeostasis is maintained by balanced lipid synthesis, catabolism (β-oxidation) and secretion. Alcohol metabolism changes the redox state of the liver, which leads to alterations in hepatic lipid, carbohydrate, protein, lactate and uric acid metabolism. The molecular mechanisms, which account for these alterations, are not completely understood. A large body of evidence implicates the involvement of ligand-activated transcription factors called nuclear receptors (NRs) in alcohol-induced liver injury. These NRs play diverse roles in cellular processes and are receptors for fatty acids, cholesterol, oxysterols and xenobiotics generated after ethanol ingestion. This review, however, will mainly focus on those NRs that are central to the pathogenesis of ALD and their role in orchestrating the complex transcriptional programs that govern lipid and inflammatory homeostasis in ALD.

Ethanol metabolism

Ethanol is metabolized predominantly in the liver via two well-characterized pathways.[18,33,34] The first involves ADH, an NAD^+-dependent enzyme located in the hepatic cytosol, which catalyzes the conversion of ethanol to acetaldehyde, a potent toxicant that accounts for the most toxic effects of ethanol.[33] Acetaldehyde produced from ethanol is further converted to the non-toxic acetate by a mitochondrial ALDH.[34] Both steps are coupled with the reduction of NAD^+ to NADH. The increased NADH/NAD^+ ratio affects the metabolism of carbohydrates and lipids in cytoplasm and mitochondria. This leads to impaired gluconeogenesis and diminished substrate flow through the citric acid cycle, with acetyl CoA diverted to fatty acid synthesis. The NADH-induced inhibition of mitochondrial fatty acid β-oxidation, combined with increased fatty acid synthesis, contributes to the pathogenesis of fatty liver, the initial stage of ALD.[35–38]

Evidence indicates that, rather than being an epiphenomenon of excessive alcohol intake, steatosis could play a direct role in progression to more advanced disease.[39] It is now clear that the severity and pattern of steatosis in ALD predicts the subsequent risk of fibrosis and cirrhosis. The current model of pathogenesis from healthy liver to alcohol-induced liver injury suggests a two-hit progression with steatosis being considered as the 'first hit'. Cellular insults such as oxidative stress, lipid peroxidation, direct lipid toxicity, mitochondrial dysfunction and/or infection causing hepatic inflammation can be the second hit, which lead to alcoholic steatohepatitis.[3] Besides ADH, another enzyme that affects ethanol action is CYP2E1, a microsomal enzyme that metabolizes ethanol at high concentrations and also metabolizes vitamin A, acetaminophen and protease inhibitors.[18,40,41] CYP2E1 activity is induced 2–10-fold after chronic ethanol exposure and has been implicated as the source of oxidative stress.[42]

Oxidative and inflammatory factors influencing ALD

Several pathways or factors, which are known to be involved in the pathogenesis of ALD, are summarized below. Ethanol consumption predominantly results in a decrease in the hepatocyte level of *S*-adenosylmethionine (SAMe) and increases in two toxic metabolites, homocysteine and *S*-adenosylhomocysteine (SAH).[43–50] Furthermore, alcohol causes a shift in the expression of methionine adenosyltransferase (MAT) genes in the liver from MAT1A to MAT2A, which correlates with decreased SAMe levels.[49,50] Chronic ethanol consumption markedly inhibits methionine synthase activity resulting in the depletion of SAMe.[51] Ethanol-induced upregulation of betaine-homocysteine methyltransferase (BHMT) or betaine supplementation can transiently cause elevations in SAMe levels.[51,52] Decreased SAMe levels are associated with decreased antioxidant defense, fibrogenesis, induction of TNF-α and increased DNA strand breaks, all of which lead to liver injury.[50] An association of ethanol-induced hyperhomocysteinemia and endoplasmic reticulum (ER) stress has also been proposed to be important in the observed alcoholic fatty liver, necroinflammation and apoptosis seen after alcohol exposure.[53,54] Recent studies have shown that osteopontin, a matricellular protein, also plays a significant role in ALD.[55] Elevated osteopontin levels correlated with neutrophil infiltration and liver injury.[56] The gender difference in the susceptibility of mice to alcohol-induced liver injury may be due to higher hepatobiliary expression of osteopontin in females than males.[56] Plasminogen activator inhibitor-1 (PAI-1), which inhibits fibrin degradation and mediates inflammatory signaling, is also implicated in alcohol-induced liver injury.[57–59] PAI-1 levels were increased in response to acute and chronic ethanol intake in mice.[59] Additionally, ethanol-induced steatosis and lipid peroxidation is blocked when PAI-1 is absent or low.[59] Reports indicate that PAI-1 mRNA and protein are significantly increased in osteopontin −/− mice, suggesting that osteopontin can suppress PAI-1 expression.[60,61]

The complement pathway, an important component of the innate and adaptive immune response, is involved in the pathogenesis of ALD.[62] The proteins and glycoproteins, which constitute the complement system, are synthesized by the liver hepatocytes, macrophages and other types of cells. The expression of C1, C2, C3, C8 and C9, which are involved in the activation of classical and alternative complement pathways, is induced in alcohol-induced fatty liver.[63–67] Reports indicate that C3 and C5 differentially contribute to the pathogenesis of ethanol-induced liver injury.[67] Ethanol-fed C3-deficient mice did not develop hepatic steatosis, but still had liver injury, as well as increased expression of inflammatory cytokines in the liver.[67] In contrast, ethanol-fed C5-deficient mice developed hepatic steatosis, but were completely protected from

ethanol-induced liver injury and increases in inflammatory cytokines.[67] Interleukins (ILs) have also been shown to play a role in ethanol-induced liver injury. IL-6-deficient mice are more prone to ethanol-induced apoptosis and liver injury.[68] IL-6 exerts its protective effect via an increase in hepatocyte proliferation, induction of antiapoptotic factors, peroxisome proliferator-activated receptors α (PPARα) activity and a reduction in steatosis.[68,69] Reports indicate that nuclear factor eythroid 2-related factor 2 (Nrf2) is induced in ethanol-fed mouse liver, suggesting a protective role of Nrf2 against alcohol-induced liver damage.[70] Nrf2-null mice exhibit impaired detoxification of acetaldehyde and increased sensitivity to alcohol-induced liver injury.[71]

The above pathways involving oxidative stress, antioxidant molecular synthesis, ER stress and inflammation illustrate the complexity of ethanol-induced tissue injury.

Endocrine and metabolic factors influencing ALD

Reports indicate that low levels of adiponectin, an important antisteatotic and anti-inflammatory cytokine produced by adipocytes, may also contribute to steatosis and inflammation in ALD.[72–76] Chronic ethanol administration decreases plasma adiponectin levels as well as hepatic adiponectin receptor-1 (AdipoR1) mRNA levels, leading to steatosis.[75,77] Adiponectin attenuates alcoholic fatty liver by up-regulating PPARα, PPARγ coactivator-1α (PGC-1α) and carnitine palmitoyl transferase-1. In addition, adiponectin down-regulates acetyl-CoA carboxylase (ACC)[19] and fatty acid synthase (FAS), key enzymes involved in fat biosynthesis.[75,78–82] Adiponectin also has anti-inflammatory effects. The administration of adiponectin reduces TNF-α levels in ethanol-fed mice.[75] The action of adiponectin is mediated in part by increase in AMP-activated protein kinase (AMPK) activity.[79,82] It has been proposed that AMPK acts as a metabolic master switch and its activation leads to a concomitant inhibition of energy-consuming biosynthetic pathways, such as FAS.[83] AMPK activation inhibits ACC activity directly by phosphorylation, and inhibits ACC expression indirectly via the suppression of sterol regulatory element-binding protein-1c (SREBP-1c), a key lipogenic transcription factor.[84,85] SREBP-1 activity is regulated by reversible acetylation at specific lysine residues.[86] Findings have demonstrated that sirtuin 1 (SIRT-1), a NAD^+-dependent class III protein deacetylase that regulates lipid metabolism, is involved in ALD.[87] SIRT-1 is known to bind to SREBP-1, resulting in its inactivation via deacetylation. Ethanol exposure reduced the level of SIRT-1 content blocking the SIRT-1-induced deacetylation of SREBP-1.[87] In addition, ethanol-induced transcription of SREBP-1-regulated genes was suppressed by an SIRT-1 agonist, resveratrol.[88] Hepatic SIRT-1 knock down in mice induces the expression of SREBP-1c and its target genes encoding lipid-synthesizing enzymes.[89] Adipose tissues in ethanol-fed rats also express more leptin, a TNF-α-inducible mRNA.[90] Thus, these targeted effects of ethanol on the adipose tissue play important roles in the development of steatosis and inflammation.

Animal models of ALD

Animal models are valuable in elucidating the molecular mechanisms involved in ALD. Two frequently used animal models are the Lieber–DeCarli liquid diet and the Tsukamoto-French intragastric tubing feeding model.[91–94] The Lieber–DeCarli liquid diet model provides an excellent means to reproduce the early stages of ALD, including liver injury, steatosis and oxidative stress as well as studying the relative roles of alcohol, lipids and therapeutic agents. Rodents fed the Lieber–Decarli diet reduce their overall food intake; therefore, pair-fed control animals are mandatory to interpret the experimental results. After determining the caloric intake of the ethanol-treated animal, the exact amount of calories is given to the pair-fed control animal on the next day. Tsukamoto and French developed continuous enteral–ethanol administration via intragastric infusion.[94–96] After laparotomy, a catheter is implanted intragastrically facilitating a continuous infusion of ethanol up to 16.5 g/kg/d. Thus, very high blood-alcohol levels can be achieved. The Tsukamoto–French model produces pathological changes, which resemble human ALD, including microvesicular and macrovesicular fat, megamitochondria, apoptosis, central lobular and pericellular fibrosis, portal fibrosis, bridging fibrosis, central necrosis and infiltrating inflammatory leukocytes as well as lymphocytes.[91,97]

The dietary fat content and composition are principal determinants of the degree of fatty infiltration in animals and humans ingesting alcohol.[98–100] In ethanol-fed rats, robust levels of hepatic triglycerides accumulate when fat content increases above 25% of the calories in the diet.[98] Ethanol with low fat diet (5% of the calories) does not produce fatty liver or evidence of lipid peroxidation unless a high blood-alcohol level is achieved by the intragastric infusion model.[95,101] Studies using the Lieber–DeCarli liquid diet or the intragastric ethanol-fed animal model have demonstrated that diets enriched in saturated fatty acids or medium-chain triglycerides protect against alcohol-induced liver injury. However, diets containing polyunsaturated fatty acids promote liver injury.[82,100,102–105] The administration of saturated fat reversed alcohol-induced liver injury in rats and improved liver pathological changes despite continued ethanol administration.[106,107] Another model that has been used to study the acute ethanol effects on the liver is gastric intubation or intraperitoneal injection in rodents. The binge model originally described by Carson and Pruett was designed to achieve blood-alcohol levels, behavioral effects and physiological changes comparable to those seen in human binge drinking.[108] The dose of alcohol used to mimic human binge drinking in rodents is 4–6 g/kg body weight.[109–111] Binge drinking is the most common pattern of alcohol consumption in school-age youth. The probability of becoming a chronic drinker at an adult age was higher in male and female adolescent binge drinkers than in non-bingers.[112,113] While binge animal models may not completely mimic liver injury in humans, reports

indicate a significant contribution of binge drinking to the incidence of cirrhosis seen in patients with ALD.[114–116]

By taking advantage of genetically manipulated animals, the role of specific genes in the development of ALD has been elucidated. Examples of mouse gene knockouts (KO) include: PPARα, retinoid x receptor (RXRα), SREBP-1c, BHMT-transgenic, CYP2E1, CYP2E1-transgenic, tumor necrosis receptor I (TNF-RI), TNF-RII, NADPH oxidase, cell surface receptor CD14, toll-like receptor 4, intracellular adhesion molecule-1, PAI-1, IL-6, Nrf2 and osteopontin mice.[44,47,71,117–121] The reader is referred to the following references for a discussion of gene-altered mouse models and their impact on the pathophysiology of ALD.[44,46,47,59,117,120–124] This review focuses on the role of NRs on the pathophysiology of ALD.

Nuclear receptors

NRs are transcription factors that regulate diverse processes, including reproduction, embryonic development, cell differentiation and cellular homeostasis.[125,126] NRs are expressed differentially among tissues, and are comprised of at least 49 members, some of which in a circadian manner.[127] Most NRs are composed of four independent but interacting functional modules.[125,128] At the amino-terminus, there is a poorly conserved region called activation function 1, which is responsible for ligand-independent transcriptional activation and is involved in the coordinated interaction of co-activators and co-repressors. Immediately adjacent to activation function 1, there is a highly conserved DNA-binding domain, which contains two zinc-finger motifs, responsible for high-affinity recognition and binding to the canonical DNA hexamer sequences comprising the specific response elements. A small hinge region facilitates the three-dimensional functional organization of the multiple domains. The ligand-binding domain serves the greatest differentiating and identifying function among NRs family members, determining the affinity of receptors for various potential ligands, and is responsible for the species specificity of ligand responsiveness. The C-terminus region, activation function 2, provides ligand-dependent activation, via a complex three-dimensional conformational switch to coordinate interactions with co-regulators.[125,129] However, there are exceptions, for example, a small heterodimer partner lacks a DNA-binding domain and yet heterodimerizes with other NRs often in a ligand-dependent manner.[42,130,131]

The NR family is classified into three groups:[125] steroid hormone receptors, adopted orphan NRs and orphan NRs.[1] NRs that generally bind DNA as homodimers and are activated with high-affinity by steroid hormones are the steroid hormone receptors. Members of this group include the receptor for glucocorticoids, mineralocorticoid, estrogen, androgen and progesterone.[2] The adopted orphan NRs function as heterodimers with RXRs. These orphan receptors are considered adopted, as studies show that they can bind physiological ligands and display physiological effects. Members of this group include the receptors for fatty acids (PPARs), bile acids (farnesoid x receptor [FXR)], oxysterols (liver x receptor [LXR]), xenobiotics (pregnane x receptor [PXR] and constitutive androstane receptor [CAR]) and retinoic acids (retinoic acid receptor).[3] The orphan NRs refer to transcription factors speculated to be NRs based on gene/protein structure, for which no specific ligands have yet been identified, or which appear to lack a functional ligand-binding domain based on structural analysis. Members of this group include nerve growth factor-induced clone B, NR related 1, neuron-derived orphan receptor 1, small heterodimer partner, liver-related homologue 1 and hepatocyte nuclear factor 4α (HNF4α).[125] Different NRs bind to their response elements as homodimers, as heterodimers with RXRs, or as monomers. Binding of ligand to the ligand-binding domain elicits a series of sequential reactions, including conformational changes in the receptors, release of the co-repressor complex and recruitment of co-activators.[128,132] Consequently, the activated NR induces target gene expression. In this way, NRs mediate chemical signals into transcriptional activation of a network of target genes.

NRs and ALD

A number of NRs are important in the pathogenesis of ALD because they act as intracellular sensors of free fatty acids and cholesterol metabolites as well as being involved in inflammatory and xenobiotic signaling. Among them, the role of RXRα and PPARs in ALD has been studied most extensively.

RXR

RXRs (α, β and γ) utilize 9-*cis* retinoic acid as a high-affinity ligand.[133,134] RXRs regulate fundamental biological processes including reproduction, cell differentiation, bone development, hematopoiesis and pattern formation during embryogenesis.[128] Gene KO studies have been conducted on all three RXR genes. Mice missing RXRβ or RXRγ are viable.[135,136] However, RXRα-KO mice are not viable due to defects in cardiac development.[135,137,138] This phenotype was also observed in the embryonic vitamin A deficiency syndrome, suggesting a critical role for RXRα in the vitamin A signaling pathway. Among the RXR isoforms, RXRα is the most highly expressed in the liver.[134,139] To address the role of RXRα in the liver physiology of adult mice, a *cre/lox*-mediated recombination was used to mutate selectively the RXRα gene in adult hepatocytes, by deleting the fourth exon encoding for the majority of the of the RXRα protein.[140] The examined pathways involving class II NRs, such as fatty acid, cholesterol, carbohydrate and xenobiotic metabolic pathways mediated by RXRα are compromised due to hepatocyte RXRα deficiency.[140–143]

RXRα and ethanol metabolism

Ethanol and retinol (vitamin A) share the hydroxyl moiety and are metabolized by common enzymes, ADHs and ALDHs.[144–146] A similar two-step process is involved in the metabolism of both alcohol and retinol, such that the two processes are in competitive inhibition with each

other.[144,147] Both alcohol and retinol are first oxidized to the aldehyde form, and aldehyde is subsequently oxidized to the acid form.[148,149] The hepatic levels of vitamin A, retinoic acid and RXRα are decreased by alcohol administration.[150,151] Reduced serum and hepatic vitamin A concentrations have been found in chronic alcoholics.[152] Thus, reduction in retinoid signaling is implicated in ALD.[144,148,150,153] Although retinoic acid has been shown to be centrally involved in the pathogenesis of ALD, the mechanism and nature of such influences have remained largely unknown.

To understand the role of RXRα in alcohol detoxification and ALD, the effect of hepatocyte RXRα deficiency on the expression of alcohol-metabolizing enzymes was analyzed in hepatocyte RXRα-deficient (hepatocyte RXRα-KO) mice.[123] Hepatocyte RXRα deficiency resulted in a significant increase in hepatic ADH activity and ADH1 protein, but not ADH2 and ADH3 enzyme activities.[123] Moreover, the levels of ADH1, ADH2, ADH3 and ADH4 mRNAs in the livers were not different between the mutant and wild-type mice.[123] In human liver, mitochondrial (low K_m) ALDH2 oxidizes most of the ethanol-derived acetaldehyde; however, in rodents both mitochondrial and cytosolic ALDH isozymes are important in acetaldehyde oxidation.[154] The activities of mitochondrial ALDH2 and cytosolic ALDH are reduced when hepatocyte RXRα is knocked out.[123] Kinetic analysis revealed that the cytosolic high-affinity ALDH1 with high acetaldehyde-oxidizing capacity is more impacted than other ALDH isoforms with RXRα deficiency. Consistent with the decreased cytosolic ALDH1 activity, both ALDH1A1 protein and mRNA levels were significantly reduced in hepatocyte RXRα-KO mouse livers. Accordingly, after a single dose of intragastric administration of ethanol, the blood ethanol levels in the hepatocyte RXRα-KO mice were significantly lower in comparison with that in wild-type mice. Furthermore, hepatic acetaldehyde clearance was slower in hepatocyte RXRα-KO mice than in wild-type mice.[123] These findings provide *in vivo* evidence that the level of *ADH1* expression regulated by RXRα is an important factor in determining the rate of ethanol elimination and influences the risk for alcohol-induced liver injury. The effect of hepatocyte RXRα on ethanol metabolism is summarized in Figure 1.

Both the Tsukamoto–French intragastric tubing alcohol feeding and the Lieber–DeCarli ethanol models were used to study the impact of hepatocyte RXRα on ALD.[44,46] The histological score (fat/necrosis/inflammation/fibrosis) was significantly higher in alcohol-fed hepatocyte RXRα-KO than in wild-type mice. Furthermore, compared with wild-type mice, hepatocyte RXRα-KO mice had significantly lower levels of SAMe and glutathione, which were further reduced after alcohol treatment.[44] Glutamate-cysteine ligase catalytic subunit, glutathione *S*-transferase-μ and glutathione peroxidase (Gpx) 1 gene and protein expression or enzyme activities are down-regulated in the livers of hepatocyte RXRα-KO mice.[155,156]

RXRα and lipid homeostasis

To dissect the role of RXRα in lipid homeostasis during ethanol-induced liver injury, wild-type and hepatocyte RXRα-KO mice were fed ethanol-containing diets or pair-fed control diets for six weeks using the Lieber–DeCarli ethanol model.[46] Liver injury was found in hepatocyte RXRα-KO mice, but not in the wild-type mice.[46] Steatosis induced by ethanol was more pronounced in hepatocyte RXRα-KO mice than in wild-type mice in the intragastric ethanol infusion

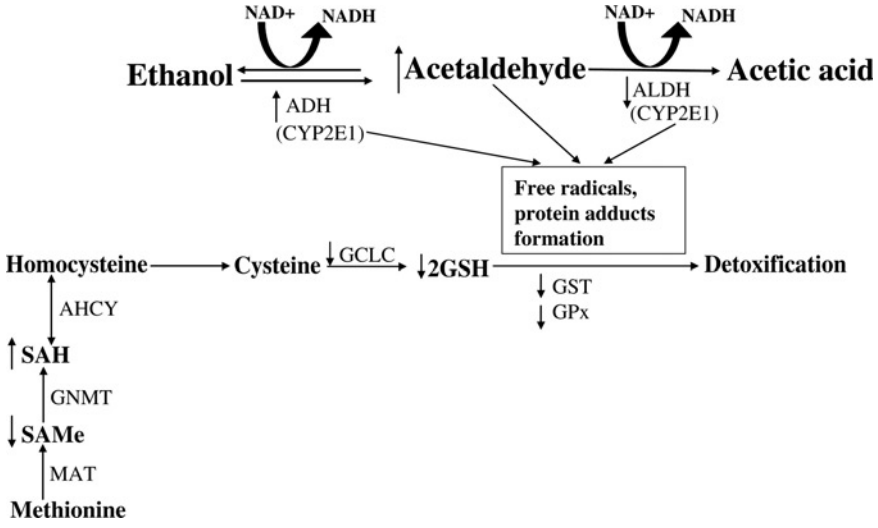

Figure 1 Hepatocyte RXRα modulates ethanol metabolism. ADH catalyzes the conversion of ethanol to acetaldehyde, a potent toxicant that accounts for most of the toxic effects of ethanol. Acetaldehyde produced from ethanol is further converted to the non-toxic acetate by a mitochondrial ALDH2. Both steps are coupled with the reduction of NAD^+ to NADH. Both ethanol and acetaldehyde can be metabolized by CYP2E1. CYP2E1 has been implicated as the source of free radicals generated by ethanol metabolism. Hepatocyte RXRα deficiency results in a significant increase in hepatic ADH activity. In addition, mitochondrial ALDH2 and cytosolic ALDH activity is reduced when hepatocyte RXRα is not expressed. Accordingly, hepatic acetaldehyde clearance is reduced due to the lack of hepatocyte RXRα. Furthermore, SAMe and glutathione levels as well as the expression of GCLC, GST and GPx genes are significantly reduced due to the lack of hepatocyte RXRα. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP2E1, cytochrome P450 2E1; GST, glutathione *S* transferase; GCLC, glutamate–cysteine ligase catalytic subunit; GPx-1, glutathione peroxidase 1; GNMT, glycine *N*-methyl transferase; MAT, methionine adenosyl transferase; AHCY, adenosylhomocysteinase; SAMe, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine

model.[44] Furthermore, ethanol-induced hepatic free fatty acids and cholesterol levels were higher in hepatocyte RXRα-KO than wild-type mice.[46] Basal hepatic liver fatty acid-binding protein (L-FABP) level was reduced with RXRα deficiency.[46,140,157] Ethanol induces L-FABP to provide protection against hepatic free fatty acid toxicity in wild-type mice; however, this protective effect is absent with RXRα deficiency.[46] Furthermore, hepatocyte RXRα-KO mice are more sensitive to the development of steatohepatitis when fed a methionine-choline-deficient (MCD) diet.[157] MCD diet ingestion induces the expression of the SREBP-regulated genes (FAS, ACC-1a and HMG CoA reductase), which in turn increases hepatic lipids and bile acid synthesis.[157] In hepatocyte RXRα-KO mice, the PPARα-mediated fatty acid β-oxidation is compromised, which leads to hepatic fatty acid overload.[157]

RXRα and inflammation

RXRα and other NRs play important roles in the inflammatory process.[158] For example, TNFα and IL-1 can decrease the expression of RXRα, PPARα, PPARγ, LXRα and their co-factors in human hepatoma Hep3B cells.[159] The LPS-induced acute phase response is associated with decreased hepatic proteins involved in lipid metabolism concomitant with the reduction of hepatic RXRα level in mouse and hamster.[160–162] The down-regulation of RXRα in inflamed liver is due to nuclear export and degradation.[160,162] The RXRα-mediated pathways could also be inhibited due to direct interaction between nuclear factor-κB (NF-κB) p65 and the RXRα DNA-binding domain and thus may prevent the binding of RXRα to consensus DNA sequences.[163] These findings clearly demonstrate a down-regulation of RXRα signaling during the inflammatory process. Furthermore, RA inhibits hepatic macrophage TNF-α expression and is known to have anti-inflammatory effects.[151] LG268, which is an RXR-specific ligand, inhibits TNF-α mRNA expression.[151] Although the anti-inflammatory action of RA was found to involve destabilization of the TNF-α mRNA, it also could possibly involve RXRα.[151]

The anti-inflammatory role of RXRα has been demonstrated using ethanol-fed wild-type and hepatocyte RXRα-KO mice.[46] NF-κB regulates the expression of a variety of genes involved in alcohol-induced liver injury.[164–167] Ethanol increases NF-κB p65-binding activity in hepatocyte RXRα-KO mouse liver, but not in wild-type mouse liver.[46] The activation of NF-κB should increase the expression of NF-κB-regulated cytokines and chemokines in hepatocyte RXRα-KO mice.[46] Accordingly, TNF-α, IL-6 and IL-1β mRNA levels are much higher in ethanol-fed hepatocyte RXRα-KO mice than in ethanol-fed wild-type mice.[46]

In response to cytokines or growth factors, the signal transducer and activator of transduction 3 (STAT3) is activated. Activated STAT3 regulates the expression of genes that play key roles in proliferation and inhibition of apoptosis.[168,169] IL-6 is readily detected in patients with ALD, and its concentration correlates positively with the extent of the disease.[170] Furthermore, IL-6-deficient mice are more prone to ethanol-induced apoptosis in the liver.[68] The activation of STAT3 is decreased in ALD patients with cirrhosis.[171] Although IL-6 mRNA and protein levels were induced in ethanol-fed hepatocyte RXRα-KO mice, elevated IL-6 did not result in STAT3 activation.[46] Consistent with this observation, although ethanol induced the expression of anti-apoptotic protein Bcl-xL, a direct STAT3 target gene, in wild-type mice, no increase was found in ethanol-fed hepatocyte RXRα-KO mice.[46] Furthermore, ethanol-mediated reduction of Bcl-2 was greater in hepatocyte RXRα-KO than wild-type mice.[46] Further investigation is needed to explain the different effects of ethanol on Bcl-xL and Bcl-2 expression.[46] Similar to our report, the differential effect of ethanol on Bcl-xL and Bcl-2 expression has also been found.[172] In agreement, apoptotic cells were found in ethanol-fed hepatocyte RXRα-KO mice, but were not noted in ethanol-fed wild-type mice.[46] Similarly, MCD diet-induced proinflammatory gene expressions are higher in hepatocyte RXRα-KO mice than in wild-type mice.[157]

PPARα

PPARα is responsible for peroxisome proliferation. It also regulates lipid metabolism and transport, fatty acid oxidation and glucose homeostasis.[173] PPARα is predominantly expressed in cells or tissues capable of oxidizing fatty acids, such as hepatocytes, heart, muscle, brown adipose tissue and the kidney. PPARα can be activated by natural lipophilic ligands, such as fatty acids and their derivatives, certain leukotriene products and drugs such as non-steroidal anti-inflammatory drugs and fibrates.[174,175] The absence of PPARα expression in KO mice prevents the induction of several hepatic PPARα target genes including acyl-CoA oxidase (ACOX) by peroxisome proliferators.[174,175] There is significant species difference in PPARα expression; human livers express less than 1/10th the level of PPARα mRNA and functional DNA-binding capacity compared with mouse livers.[176] This difference might explain why rodents are more susceptible to the toxic and carcinogenic effects of peroxisome proliferators.[176] PPARα target genes are those that encode fatty acid transporters, proteins involved in export (apolipoprotein [Apo] B), the microsomal triglyceride transfer protein, L-FABP and acyl-CoA dehydrogenase.[173] The activation of these genes results in increased uptake and oxidation of free fatty acids, increased triglyceride hydrolysis and up-regulation of ApoA-I and -II. The net effect is increased fatty acid oxidation, decreased serum triglycerides, a rise in high-density lipoprotein and an increase in cholesterol efflux. The relationship between PPARα and fatty liver became evident when steatosis was observed in aged PPARα KO mice.[177] Starvation activates PPARα.[178,179] PPARα KO mice exhibit steatosis, myocardial lipid accumulation and hypoglycemia during short-term starvation or after high fat diet administration.[178,179]

PPARα is a circadian gene.[180,181] The diurnal variation of lipogenic and cholesterogenic gene expression was attenuated or abolished in PPARα KO mice suggesting the importance of PPARα as a mediator for the circadian regulation of lipid metabolism.[182,183] Both acute and chronic alcohol intake can affect many aspects of circadian rhythms, including physiological, endocrine and behavioral functions. However, the effect of central and peripheral circadian

rhythms involved in alcohol-induced liver injury remains to be addressed.

The role of PPARα in ALD has been demonstrated with the PPARα KO mice.[120] Ethanol-fed PPARα-null mice have hepatocyte damage, which is not found in wild-type mice. Down-regulation of PPARα activity and fatty liver was observed following intragastric ethanol infusion of rats.[184,185] Treatment of ethanol-fed animals with PPARα agonists reduces the toxic effects of ethanol and reverses hepatic fat accumulation.[119,150,184] Fish oil contains *n*–3 fatty acids, which are PPARα activators. Feeding mice fish oil prior to ethanol administration prevents acute ethanol-induced fatty liver.[186] IL-6 treatment ameliorates alcoholic fatty liver, which is in part attributed to the up-regulation of PPARα and increased mitochondrial β oxidation of fatty acids.[69] These results support the importance of PPARα in preventing alcohol-induced fatty liver and injury. However, activation of PPARα using fibrates also causes cholestasis in humans and mice and other undesired effects such as hepatomegaly in rodents.[187–191] Furthermore, ethanol-fed PPARα KO mice have lower levels of GPx, superoxide dismutase and catalase, as well as increased lipid peroxides compared with wild-type controls.[120]

The role of PPARα in alcoholic fatty liver has also been studied in cultured hepatocytes. Ethanol exposure of H4IIEC3 rat hepatoma cells expressing alcohol-metabolizing enzymes causes reduced binding by the RXR/PPARα to a PPARα binding site located on the ACOX promoter.[192] This effect of ethanol is enhanced by the ALDH inhibitor cyanamide and is abolished by the ADH inhibitor, 4-methylpyrazole implicating the involvement of acetaldehyde.[193]

PPARα and inflammation

The role of PPARα in inflammation was first observed using PPARα KO mice.[194] In mice lacking the PPARα gene, the response to topical inflammatory mediator leukotriene B_4 or arachidonic acid, but not phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate, is prolonged compared with the response in wild-type mice.[194] Furthermore, dietary administration of (*n*–3) polyunsaturated fatty acids results in increased survival of the animals when they are challenged with bacteria or endotoxin.[195,196] Fish oil has been found to decrease circulating levels of proinflammatory cytokines (TNF-α, IL-6, IL-1) in patients with endotoxemia.[195] PPARα is able to inhibit inflammatory responses by interfering with NF-κB and activator protein 1 activation through a direct protein–protein interaction with p65 or c-Jun, respectively.[197,198] PPARα is also able to increase inhibitory κB (IκB)-α leading to the prevention of p50- or p65-NF-κB nuclear translocation.[120,199,200] This action leads to the repression of cytokines, cell adhesion molecules and proinflammatory molecules such as inducible nitric oxide synthase (iNOS) and C-reactive protein. The activation of p65-NF-κB was significantly greater in hepatic nuclear fractions from ethanol-fed PPARα KO mice compared with ethanol-fed wild-type mice.[120]

PPARγ

PPARγ is highly expressed in adipocytes, hematopoietic cells and hepatic stellate cells, and to a lesser extent in pancreatic β cells, spleen, skeletal muscle, macrophages and intestinal cells.[201] Hepatocytes express very low levels of PPARγ. PPARγ is involved in adipocyte differentiation, glucose metabolism and lipid storage.[202] Reports indicate that PPARγ ligands can inhibit inflammatory responses by decreasing IL-6, TNF-α, IL-1β secretion and iNOS production in macrophages and Kupffer cells. $PPAR\gamma^{+/-}$ mice have increased susceptibility to experimentally induced arthritis and inflammatory bowel disease.[203] The anti-inflammatory mechanisms mediated by PPARγ involve nuclear export of RelA (p65), thereby preventing NF-κB-induced proinflammatory gene activation.[204,205]

The composition of PPAR isoforms might change in fatty livers. Leptin, which can inhibit appetite, is a peptide hormone produced predominantly by white fat cells. Consequently, mice with leptin deficiency are obese.[206] PPARγ mRNA levels are significantly increased in fatty livers of leptin deficiency mice.[207] The activation of PPARγ causes fatty liver. There are two PPARγ isoforms (γ1 and γ2), and both forms are detectable in hepatocytes. Ethanol increases the mRNA levels of both isoforms and the expression level of downstream target fatty-acid translocase (CD36), a membrane receptor responsible for the uptake of modified forms of low-density lipoproteins and fatty acids from circulation.[186,208] PPARγ is also regulated by chronic alcohol exposure in Kupffer cells and hepatocytes.[209,210] Treatment with the PPARγ agonist pioglitazone prevents the development of alcohol-induced steatosis and inflammation.[211] PGC-1α, a co-activator of PPARγ, is essential for the induction of many ROS-detoxifying enzymes, including GPx1 and SOD2.[212,213] Thus, the role of PGC-1α in ALD warrants investigation.

LXR, PXR, CAR and other lipogenic transcription factors

LXRα and LXRβ might play a role in ALD due to their actions in lipid homeostasis and inflammation.[214–217] The effect of LXR on lipogenesis involves both direct and indirect mechanisms. LXR/RXR heterodimers bind lipogenic gene promoters, such as FAS, or regulate lipogenic gene expression by controlling levels of SREBP-1c, a transcriptional factor known to regulate the expression of a battery of lipogenic enzymes.[218] The activation of SREBP-1c by ethanol feeding was associated with increased expression of lipogenic genes as well as the accumulation of triglyceride in the livers of mice.[219] Transcriptional targets of SREBP-1c include FAS, ACC, stearoyl CoA desaturase (SCD) and fatty acid elongase (FAE).[215,217,220] SREBP-2 is important for the cholesterol biosynthesis pathway; it regulates HMG-CoA synthase, HMG-CoA reductase, farnesyl diphosphate synthase and squalene synthase.[221]

Other NRs that are linked to steatosis are FXR, HNF4α, CAR and PXR. The activation of FXR by bile acids reduces triglyceride levels.[222–225] The triglyceride lowering

effect of bile acids is likely mediated through FXR-induced PPARα activation.[226] FXR represses lipogenesis by interfering with the expression of SREBP-1c through SHP, which inhibits the activity of LXR.[225] As expected, FXR-null mice exhibit massive steatosis when challenged with a high cholesterol/high fat diet.[227] HNF4α functions as a homodimer and is constitutively active through binding of integral fatty acids.[228,229] HNF4α is central to the maintenance of hepatocyte differentiation and is a major *in vivo* regulator of bile acid and lipid homeostasis genes.[230] Hepatocyte HNF4α-deficient mice have hepatic lipid accumulation and reduced serum cholesterol and triglyceride levels.[230] HNF4α is involved in the regulation of gene transcription mediated by PXR, CAR and LXR.[231,232]

Recent studies have also linked CAR to both lipid and glucose metabolism.[233,234] Like PPARα, fasting-induced increases in fatty acids and PPARα agonists induce CAR expression.[233,235,236] The activation of CAR suppresses lipid metabolism and reduces serum triglyceride by decreasing the active form of SREBP-1c.[237] The xenobiotic receptor PXR regulates an SREBP-independent lipogenic pathway by up-regulating the expression of free fatty acid uptake transporter CD36 and several accessory lipogenic enzymes, including SCD-1 and long-chain FAE.[238] PPARγ and CD36 are direct transcriptional targets of PXR.[238] The activation of PXR in mice could induce hepatic triglyceride accumulation in an SREBP-1c-independent manner.[238] Hepatic PXR mRNA levels are significantly increased in mice fed MCD diet.[239] Furthermore, CAR or PXR ligands can increase levels of insulin-induced gene-1, a protein with antilipogenic properties.[240] The role of these receptors in ALD remains to be examined. Figure 2 summarizes the role of NRs in lipid homeostasis.

Conclusions

In summary, the molecular mechanisms leading to ALD are complex. Current research on the role of NRs in ALD has focused mainly on PPARs. Our recent publication shows the hepatoprotective role of PXR in lipopolysaccharide and galactosamine-induced liver injury.[241] Given the pivotal roles of LXR, HNF4α, FXR, CAR and PXR in lipid metabolism, energy homeostasis, bile acid homeostasis and inflammation, attention should now be devoted to these NRs. It is likely that new insights into the pathogenesis of ALD could be achieved via this endeavor. Furthermore, variable NR expression levels may determine target gene expression levels and susceptibility to ALD. Reports indicate that a single-nucleotide polymorphism in the PPARγ gene is associated with the susceptibility to non-alcoholic fatty liver disease in Chinese people.[242] Genetic polymorphism of NRs in contribution to ALD should be studied.

Our published data showed that hepatocyte RXRα, a partner for class II NR superfamily members, plays a significant role in lipid homeostasis, inflammatory process and even alcohol detoxification.[44,46,123,140,142,143,158] Thus, the expression level and functional activity of hepatocyte RXRα should have a significant impact on the disease process. It is important to note that the role of NRs in ALD might be liver cell-type specific. Increased inflammatory responses found in hepatocyte RXRα-KO mice suggests

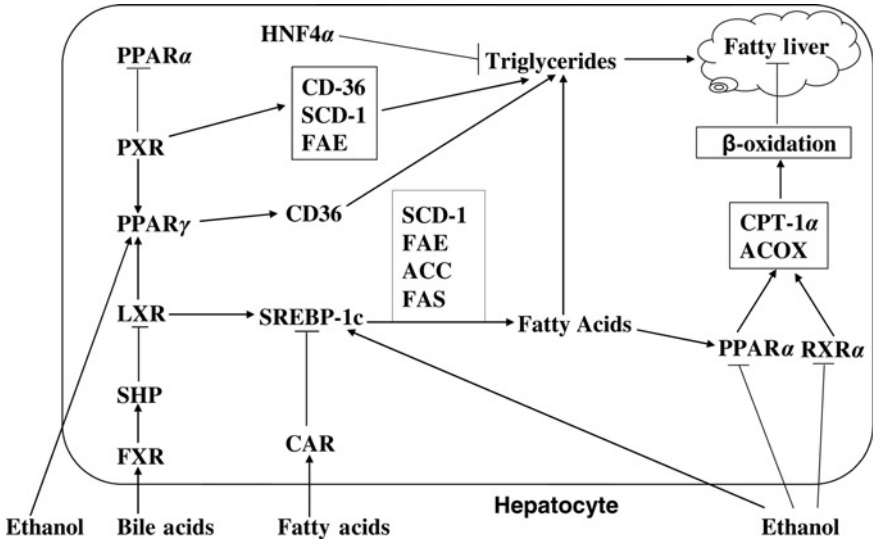

Figure 2 Interaction among nuclear receptors (NRs), alcohol and lipid homeostasis. Research on the role of nuclear receptors in ALD have focused mainly on PPARs. The hepatic RXRα levels are decreased by alcohol administration.[150,151] Down-regulation of PPARα expression and fatty liver are observed following ethanol administration.[184,185] The activation of SREBP-1c by ethanol feeding is associated with increased expression of lipogenic genes as well as the accumulation of triglycerides.[219] The activation of SREBP-1c has been suggested to involve LXR.[218] FXR represses lipogenesis by interfering with the expression of SREBP-1c through SHP, which inhibits the activity of LXR.[225] Ethanol increases the mRNA levels of PPARγ isoforms and the expression level of down-stream target fatty-acid translocase (CD36).[186] Fatty acids can induce CAR expression.[236] The activation of CAR suppresses lipogenesis by decreasing the active form of SREBP-1c.[237] PPARγ, CD36, SCD-1 and FAE are direct transcriptional targets of PXR.[238] HNF4α is central regulator of bile acid and lipid homeostasis genes.[230] These complex interactions among NRs involved in lipid homeostasis determine fat accumulation in the liver. SREBP-1c, sterol regulatory element-binding protein-1c; PPARα, peroxisome proliferator-activated receptors α; CPT-1, carnitine palmitoyl transferase-1; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SCD, stearoyl CoA desaturase; FAE, fatty acid elongase; FXR, farnesoid x receptor, LXR, liver x receptor, PXR, pregnane x receptor; CAR, constitutive androstane receptor; RXR, retinoic x receptor; HNF4α, hepatocyte nuclear factor 4α

the presence of cross-talk between hepatocyte RXRα and the inflammatory response generated from Kupffer cells. Thus, organ- and cell-type-specific KO mice might be necessary to define the role of each NR in ALD.

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