The Role of Ceramide in the Pathogenesis of Alcoholic Liver Disease

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Abstract

Aims: Ceramide is an important second messenger in the sphingomyelin signaling pathway. In this review, we will focus on the potential role of ceramide in the pathogenesis of alcoholic liver disease (ALD).

Methods: We have summarized the relevant studies and reviews about the role of ceramide in ALD. In addition, we have discussed the role of acid sphingomyelinase and protein phosphatase 2A in ALD, which are associated with ceramide and hepatic steatosis.

Results: Recent studies have proved that the immunoreactivity and content of ceramide were increased, both in experimental models of chronic alcohol-induced steatohepatitis and human livers with severe chronic alcohol-related liver disease. Consistent with that, the levels of protein phosphatase 2A and acid sphingomyelinase were increased. Of relevance, the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) was inhibited, which could block the fatty acid oxidation and promote its synthesis.

Conclusions: It was hypothesized that ethanol promoted ceramide accumulation and increased PP2A activity by activating ASMase, which may be an important mechanism in the inhibitory effect on AMPK phosphorylation and then contributed to the progression of steatosis. ASMase, a specific mechanism of ceramide generation, was proved to be a regulator of steatosis, fibrosis, lipotoxicity and endoplasmic reticulum stress.

INTRODUCTION

Chronic alcohol abuse is one of the leading causes of liver-associated morbidity and mortality (McCallough et al., 2011). Alcoholic liver disease (ALD) is a chronic disease with a histological spectrum ranging from hepatic steatosis, steatohepatitis, fibrosis, and ultimately to cirrhosis. Due to the incomplete understanding of pathogenesis, there is little progress has been made in ALD treatment. In chronic ALD, liver function is compromised due to the generation of toxic lipids, i.e. ceramide. Ceramide represents a family of lipids generated from fatty acid and sphingosine. Growing evidence suggests that ceramide may play a key role in the pathogenesis and progression of ALD. The immunoreactivity and abundance of ceramide increased, which has been proven in both experimental models of chronic alcohol-induced steatohepatitis and human livers with severe chronic ALD (Longato et al., 2012; Ramirez et al., 2013). According to previous studies, it is likely that the accumulation and altered profiles of hepatic ceramide are closely associated with the pathogenesis of ALD. Acid sphingomyelinase (ASMase), a specific mechanism of ceramide generation, was proved to be regulating steatosis, fibrosis, lipotoxicity and endoplasmic reticulum stress (ER stress). ASMase may also modulate alterations of the methionine cycle and phosphatidylcholine homeostasis (Garcia-Ruiz et al., 2015). Adenosine monophosphate-activated protein kinase (AMPK) is central to the pathogenesis of alcoholic-induced hepatic steatosis (You et al., 2004). The group of Liangpunsakul found that the inhibitory effect of ethanol on AMPK phosphorylation was mediated partly through increasing the levels of ceramide and activation of protein phosphatase 2A (PP2A) (Liangpunsakul et al., 2012). So we attempt to discuss the effect of ceramide on AMPK and its role in the pathogenesis of ALD.
CERAMIDE AND ASMase

It showed that abnormal shifts in membrane lipid composition led to the disrupted cellular signaling (Anderson and Borlak, 2008; Inokuchi, 2010), with hepatic steatosis involved in. The accumulation of toxic lipids, such as free fatty acids, free cholesterol, and ceramides can all have lipotoxic effects in hepatocytes (Malhi and Gore, 2008; Neuschwander-Tetri, 2010). The role of ceramide has been proved in the pathogenesis of steatohepatitis in experimental models of chronic ethanol (Setschedi et al., 2011). Ceramide can be generated from sphingomyelin hydrolysis by ASMase. Recently, numerous studies have proved that ethanol increases the mRNA levels of ASMase and ceramide content in the livers of ethanol-fed mice.

Sphingolipids are ubiquitous constituents of eukaryotic membranes, which play an important role in the regulation of signal transduction pathways (Novgorodov and Guzd, 2009). Over the past decades, it has become clear that ceramide, as a second messenger in the sphingomyelin signaling pathway, regulates diverse cellular responses, including cell apoptosis, differentiation, proliferation and in the pathogenesis of insulin resistance and lipotoxicity. The Ceramide family contains about 50 distinct molecular species, which are characterized by different acyl chains, their desaturation and hydroxylation (Novgorodov and Guzd, 2009). Ceramide is distributed in cell membranes, and its generating is based on several mechanisms: (a) the de novo synthesis from 1- and 2-acylcerin and palmitoyl-CoA catalyzed by the action of serine palmitoyl transferase (SPT) and ceramide synthases (CERS); (b) the hydrolysis of membrane sphingomyelin by the action of sphingomyelinases (SMases), of which the ASMase and neutral SMase are of major correlation; (c) Ceramide is metabolized by ceramidase to sphingosine, which can be recycled after acetylation of sphingosine, utilizing the ‘salvage pathway’ (limuro et al., 1997; Liangpunsakul et al., 2010, 2012; Longato et al., 2012). Ceramide affects cellular functions and causes apoptosis through modulating the phosphorylation states of various kinds of protein, activating enzymes which promote apoptosis, and inhibiting Akt phosphorylation (Arboleda et al., 2007). Clugston observed that there was an almost 2-fold increase of total ceramide levels in the liver of alcohol-fed mice. However, it was equivalent in alcohol-fed and control mice in plasma (Clugston et al., 2011). The study also found that alcohol exposure was associated with a significant decrease in serum levels of sphingosine and sphinganine, while in the liver, they both increased in alcohol-fed mice. Given that sphingosine and sphinganine are both precursors for the generation of ceramide, the increased abundance in the liver correlates well with the increased ceramide levels (Clugston et al., 2011). Myriocin is an inhibitor of ceramide synthesis via inhibiting serine palmitoyl transferase which is needed in de novo pathway. Chronic alcohol-induced hepatic steatosis was associated with increasing ceramide levels, and importantly, the steatosis was significantly resolved after myriocin treatment. In chronic ethanol-fed rats livers, there were increased densities of lipid vacuoles and pronounced variability in shape and size of mitochondria. But it proved that myriocin reduced the abundance and size of lipid droplets, rendered mitochondria more uniform and enhanced the endoplasmic reticulum profiles (Tong et al., 2014). Furthermore, myriocin reduced the alcohol-associated sinusoidal fibrosis, which was the beginning stage of fibrogenesis.

Ceramides can be synthesized by different ceramide synthase. CERS1 has a high specificity for generating C18:0 ceramide. CERS2 and CERS4 mainly synthesize C20:0, C22:0, C24:1, C24:0 and C26:0 ceramides. Shorter chain ceramides like C14:0 and C16:0 ceramides are mainly generated by CerS5 and CerS6 (Levy and Futerman, 2010; Longato et al., 2012; Ramirez et al., 2013). Ceramide can be recycled after acetylation of sphingosine by the ‘salvage pathway’. Hanamatsu demonstrated that high levels of serum sphingosine C18:0, C20:0, C22:0 and C24:0 were significantly correlated with the parameters of obesity, insulin resistance, and lipid metabolism (Hanamatsu et al., 2014). And then ceramide may be related to these parameters. In the livers of chronic ethanol-fed rats, ceramide profiles differed significantly from control which was analyzed by liquid chromatography tandem-mass spectrometry (LC-MS/MS): higher levels of C14:0 and C18:0 ceramide, and relatively lower levels of C16 and C20 ceramide. Increasing levels of C16:0 ceramide could promote cell death (Osawa et al., 2005; Seumois et al., 2007), while C18:0 ceramide may inhibit cell growth (Koybasi et al., 2004). The levels of C18, C18:1, C22:1 and C24:1 ceramide species were increased in mice fed with the Lieber-DeCarli liquid ethanol (EtOH) diet, while other species (C14, C16, C20, C22, C24 and C26 ceramide) were not affected. Analogously, it was reported that C18 ceramide was increased in a metabolomic study of EtOH feeding (Zhao et al., 2011). Liangpunsakul observed that the levels of C16, C18 and C24 ceramide were higher in ethanol-fed mice than control, when administered imipramine, an inhibitor of ASMase, C16 and C24 ceramide were significantly decreased, associated with the improvement of hepatic steatosis (Liangpunsakul et al., 2010, 2012). Hence, it indicated that the magnitude and specificity on individual ceramide species differed in response to inhibition of ceramide synthesis. It proved that ceramide immunoactivity was augmented and the expression of ceramide-related genes were increased in the livers of chronic ethanol-fed rats. The expression of CERS4 and SPTL2 (long-chain subunit of serine palmitoyl transferase) contributed to the accumulation of hepatic ceramide via biosynthetic mechanisms. In addition, the increasing of SMPD1 (sphingomyelin phosphodiesterases) expression may promote the generation of ceramide through catabolic mechanisms. What is more, similar results have been showed in other models of chronic steatohepatitis (de la Monte et al., 2009; Lyn-Cook et al., 2009; Setschedi et al., 2011).

ASMase could relocate from intracellular compartments to the plasma membrane, contributing to the catabolism of sphingomyelin and ceramide formation in lysosomes (Tani et al., 2005, 2007). It plays an important role in sphingosine hydrolysis and ceramide generation within lipid rafts (Bollinger et al., 2005; Novgorodov and Guzd, 2009), and then exerts an influence on several biological processes. Fernandez (Fernandez et al., 2013) found that alcohol increased the activity of ASMase and the level of total ceramide in ASMase−/− mice, without activation of NSMase. Furthermore, it showed that there was a significant increase in C16:0 and C18:0 ceramide species and a relatively minor increase in C24:0 in ASMase−/− mice, while these species were decreased in alcohol-fed ASMase+/− mice. Tumor necrosis factor-α (TNF-α) is a stimulus for the generation of ceramide by activation of ASMase (Lawler et al., 1998). It has been proved that ASMase knockout animals were resistant to the steatosis and apoptotic effects of TNF-α on the liver (Fernandez-Checa et al., 2005). Previously study demonstrated that imipramine reduced the level of ceramide in cultured hepatoma cells incubated with ethanol (Liangpunsakul et al., 2010). Consistent with that, when administered imipramine in ethanol-fed mice, the hepatic ceramide content was decreased, and the activity of ASMase and PP2A was also inhibited (Liangpunsakul et al., 2012). However, Liangpunsakul considered that the increased activity of ASMase was not because of TNF-α in the liver. There might be alternative pathways that were responsible for ASMase activation, including oxidative stress and ER stress. One study has reported that when administered amitriptyline, another
ASMase inhibitor, the formation of ceramide in the liver of LEC rats (a genetic model for Wilson disease) was limited, and the hepatic steatosis, fibrosis and nuclear pyknosis were reduced, which finally improved their survival (Lang et al., 2007). In ASMase−/− mice, amitriptyline treatment could also decrease alcohol-induced macrosteatosis and accumulation of triglyceride and free fatty acid, without causing sphingomyelin accumulation.

Another mechanism by which ethanol could raise the level of ceramide is through the induction of stearoyl-CoA desaturase-1 (Scd-1) (You et al., 2004). The level of ceramide and the expression of SPT mRNA were decreased in Scd-1 knockout group, which was related to the increased rate of fatty acid oxidation, reduced fatty acyl-CoA level, and the activation of AMPK (You et al., 2004). Ethanol feeding could induce the expression of Scd-1 in mouse liver (You et al., 2002; Fernandez et al., 2008) through its effect on sterol regulatory element binding protein 1 (SREBP-1) and ceramide (Supakul and Liangpunsakul, 2011).

CERAMIDE AND PP2A

Ethanol can activate PP2A (Mathias et al., 1998). PP2A is a major protein serine/threonine phosphatase that regulates many cellular functions and signaling pathways, including apoptosis, insulin signaling, and the Wnt/β-catenin pathways (Janssens and Goris, 2001; Supakul and Liangpunsakul, 2011). The holoenzyme complex of PP2A consists of three subunit isoforms. The A subunit is a structural subunit, playing a role in stabilizing the C subunit. The C subunit is catalytic subunit and its structure may be the most conserved. The regulatory B subunits are diversity, which influence the specificity of the target protein and its cellular location. PP2A can be activated by ceramide, which was reported to bind to the B subunit. In this regard, PP2A was identified as a ‘ceramide-activated protein phosphatase (CAPP)’ (Mathias et al., 1998; Mukhopadhyay et al., 2009). An inhibitor of protein phosphatase 2A (I1PP2A) may be related to the mechanism of the activation of PP2A by ceramide. I1PP2A can bind to ceramide directly, which may decrease its association with PP2A, so that the activity of PP2A was increased (Deacuc et al., 2000). It was proved that ethanol treatment of rat hepatoma cells (H4IEC3) has significantly increased cellular ceramide content and PP2A activity (Fernandez-Checa et al., 2005; Liangpunsakul et al., 2008). When the hepatoma cells were treated with ceramide analogue, the level of AMPK phosphorylation was reduced (Liangpunsakul et al., 2010). So we considered that the effect of ethanol on AMPK appears to be mediated partly through increasing the levels of ceramide and activation of PP2A.

Ceramides impair the Akt/PKB signaling through activation of PP2A which dephosphorylates Akt (Tereul et al., 2001; Ruvolo, 2003), and through the inhibition of protein-kinase-C-ζ (PKC-ζ) dependent translocation of Akt to the plasma membrane, which is required for its function (Powell et al., 2003). Ramirez found that chronic ethanol feeding significantly reduced hepatic total level of Akt, as well as the phosphorylated and relative phosphorylated levels of Akt in Long Evans rat model (Ramirez et al., 2013). Although these results were not observed in ALD patients, Longato hypothesized that the Akt pathway may be inhibited in the earlier and less severe stages of ALD (Longato et al., 2012).

PP2A AND AMPK

It was hypothesized that the effect of ethanol on AMPK appears to be mediated partly through increasing the levels of ceramide and activation of PP2A. It has been shown that the basal activity of AMPK was inhibited and the amount of AMPK protein was reduced, no matter in ethanol treatment of cultured cells or in ethanol-fed mice (You et al., 2004). The group of Liangpunsakul reported that ethanol inhibited AMPK was through the inhibition of protein kinase Cζ, which phosphorylated LKB1 at Ser-428, and LKB1, the upstream kinases for AMPK. They found in H4IEC3 cells, ethanol markedly reduced the level of p-PKC-ζ and p-LKB1 (Liangpunsakul et al., 2008). Our previous work showed that binge drinking in the rat induced hepatic steatosis which was related to the reduced expression of AMPK (Zhou et al., 2015). Then we demonstrated that the impaired Adiponectin-sirtuin 1 (SIRT1)-AMPK signaling pathway may be an important pathogenic mechanism in ethanol-induced hepatic steatosis (Jang et al., 2015). Correnti found that adiponectin knockout mice had increased alcohol-induced hepatosteatosis and hepatomegaly. While restoring circulating adiponectin levels using recombinant adiponectin, the hepatic steatosis ameliorated (Correnti et al., 2014). They observed equivalent reductions of AMPK protein in alcohol-fed wild-type and adiponectin knockout animals compared with control; however, the level of ceramide was increased in adiponectin knockout mice. Moreover, pharmacological inhibition of ceramide synthesis in adiponectin knockout mice abrogated alcohol-mediated steatosis, which suggest that adiponectin reduces alcohol-induced steatosis through regulation of ceramides and AMPK. Here we discuss another potential mechanism involved PP2A for the inhibitory effect of ethanol on AMPK.

AMPK is known to act as a key metabolic regulator through phosphorylating target enzymes involved in lipid metabolism, such as acetylCoA carboxylase (Se moois et al., 2007) and carnitine palmitoyl transferase I (CPT I) (Winder and Hardie, 1999). Studies have demonstrated that AMPK is the major protein kinase for the inactivation of ACC and the activation of malonyl CoA decarboxylase. When ACC is inhibited, the cellular concentration of malonyl-CoA is decreased, and then the inhibition of CPT I is relieved, ultimately leading to the fatty acid oxidation increased (You et al., 2004). CPT I is the rate-limiting enzyme in the transport of long-chain fatty acids into the mitochondrial matrix, while ACC is regarded as the rate-limiting enzyme in fatty acid biosynthesis in the liver (Abu-Elheiga et al., 2001). Malonyl CoA is the product of ACC, and it is both a precursor for the biosynthesis of fatty acids and a potent inhibitor of mitochondrial fatty acid oxidation at the CPT I step. The phosphorylation of ACC leads to the inhibition of ACC activity, and when ACC is inhibited, the inhibition of CPT I relieves, and fatty acid oxidation increases (Ruderman et al., 1999). Conversely, when ACC phosphorylation is inhibited by ethanol through the inhibition of AMPK, ACC activity and malonyl CoA concentrations are increased. Above that, it finally leads to increased lipogenesis and reduced fatty acid oxidation, which contributes to the development of fatty liver.

The activity of AMPK is regulated by AMP and its phosphorylation. It has been described that the tumor suppressor kinase LKB1 can phosphorylate AMPKα at threonine 172 in vitro. However, both PP2A and protein phosphatases 2C (PP2C) are able to regulate mammalian AMPK, since that they can dephosphorylate and inactivate AMPK (Moore et al., 1991). By using the yeast saccharomyces cerevisiae as a model, one study reported that PP2A regulatory subunit A could coimmunoprecipitate with AMPK-α2 catalytic subunit (Gimeno-Alcaniz and Sanz, 2003). Wu (Wu et al., 2007) found that palmitate suppressed AMPK at Thr172 in LKB1-deficient bovine aortic endothelial cells (BAECs), implying that its inhibitory effects on AMPK might be independent of LKB1. In addition, palmitate increased the activity of PP2A. When PP2A was suppressed, the
inhibition on AMPK-Thr172 phosphorylation was abolished, no matter by okadaic acid, a potent inhibitor of protein phosphatases, or PP2A-specific siRNA. Further, C2-ceramide, a cell-permeable analog of ceramide, can mimic the effects of palmitate above. So we conclude that palmitate inhibits the phosphorylation of AMPK through the activation of ceramide-dependent PP2A.

Liangpunsakul proved that okadaic acid increased the phosphorylation of AMPK in H4IEC3 cells treated with ethanol, and significantly reversed the inhibitory effect of ethanol on H2O2-induced AMPK phosphorylation (Liangpunsakul et al., 2008). It was also observed that PP2A was coimmunoprecipitated with AMPK-α subunit, and ethanol increased PP2A activity by 30% in immunoprecipitates of cell lysates. By using PP2A siRNA, the suppression of PP2A could make AMPK and ACC phosphorylation increased, but it had no effect on LKB1 phosphorylation. Additionally, when the hepatoma cells were transfected with PP2A siRNA, the inhibitory effect of ethanol on AMPK and ACC phosphorylation was attenuated. Due to the studies above, it is hypothesized that the inhibitory effect of ethanol on AMPK involved PP2A. In conclusion, PP2A and the pathways regulating its activity should be considered as therapeutic targets to prevent alcoholic fatty liver disease (Liangpunsakul et al., 2008).

The inhibitory effect of ethanol on AMPK can be ameliorated by okadaic acid or silencing PP2A (Izuka et al., 2004); however, ethanol may also reduce AMPK activity through additional actions not involving PP2A. Carbohydrate response element binding protein (ChREBP) is a transcription factor. It was found to directly promote the transcription of the lipogenic enzymes genes, such as ACC and fatty acid synthase (FAS) (Ishii et al., 2004; Liangpunsakul et al., 2013). AMPK may alter the ChREBP chromatin and its acetylation. ChREBP is also under the control of PP2A, which dephosphorylates several phosphorylation sites on ChREBP, such as Ser196, Thr666 and Ser568 (Uyeda and Repa, 2006). According to the effects of ethanol on AMPK and PP2A, it is likely that ethanol might enhance the fatty acid synthesis by increasing the activity of ChREBP. In addition, exogenous ceramide could make ChREBP activity increased, however, the function of ceramide on ChREBP might be not as great as that of ethanol (Liangpunsakul et al., 2013). It was proved that treatment with physiologically relevant concentrations of ethanol could strongly activate ChREBP in hepatoma cells, leading to an increase of the expression of ACC and FAS, which was known to be regulated by ChREBP. It could significantly increase the hepatic levels of mRNA and protein expression of ChREBP when given alcohol intragastrically to rats for 4 weeks, compared to pair-fed controls (Li et al., 2008). Studies also showed that if activating AMPK by using 5-aminomimidazole-4-carboxamide ribonucleotide (AICAR) (Kawaguchi et al., 2002) or transfection with a constitutively active AMPK expression plasmid, the effect of ethanol on ChREBP activity could be blocked. Since ethanol inhibits AMPK activity even in the presence of okadaic acid, there may be additional effects on ChREBP due to reducing the phosphorylation at critical regulatory sites (Liangpunsakul et al., 2013). The results of current studies indicate that the activation of ChREBP may be a potential mechanism for the development of alcoholic fatty liver.

CERAMIDE, ASMASE AND ER STRESS IN ALD

Ceramide can be synthesized in endoplasmic reticulum by de novo synthesis way. Ceramide could inhibit AMPK (Liangpunsakul et al., 2010) and promote local hepatocellular injury (Anderson and Borlak, 2008). One possible mechanism was that AMPK may reduce the endoplasmic reticulum (ER) stress and apoptosis (Kuznetsov et al., 2011). Ethanol metabolism leads to oxidative stress in the endoplasmic reticulum. Recent studies have manifested that ethanol exposure could lead to increasing activation of ER stress responses in the livers of experimental animals (Esfandiari et al., 2005; Dara et al., 2011). ER stress promotes oxidative injury and inflammation, which may contribute to the progression of ALD. Ceramide can incorporate into the structure called lipid rafts, which may accelerate oxidative stress and pro-apoptosis signaling. Increasing oxidative stress results in reactive oxygen species (ROS) formation, lipid peroxidation and cell death. Reactive oxygen species could stimulate ER stress and increase ceramide generation (Himuro et al., 1997). ER structure was disrupted in ethanol-exposed livers, accompanying with increased mRNA levels of ER stress genes (Longato et al., 2012; Ramirez et al., 2013).

ER stress may act as a critical role in ALD (Fernandez et al., 2013), since it regulates hepatic steatosis and liver injury (Ji and Kaplowitz, 2003, 2006). It was hypothesized that ASMase could induce ER stress. Exogenous ASMase can upregulate the expression of ER stress markers in both HepG2 cells and primary hepatocytes. When HepG2 cells was cultured with exogenous ASMase or NSMase, both enzymes could increase ceramide levels by 2–3-fold. However, after exposure to exogenous ASMase, the levels of ER stress markers were increased. Consistent with that, another study indicated that exogenous ASMase could increase the expression of ER stress markers, while it was failed to induce those changes despite similar ceramide generation when incubated with NSMase (Garcia-Ruiz et al., 2000). According to above results, ASMase may cause ER stress specifically. Furthermore, it prevented alcohol-induced ER stress when ASMase was inhibited by amitriptyline in wild-type mice. It was also proved that ASMase−/− mice were refractory to alcohol-induced ER stress, moreover, the inhibition of ASMase could protect against alcohol-mediated sensitization to lipopolysaccharide (LPS), which could lead to liver injury (Fernandez et al., 2013).

Since endoplasmic reticulum Ca2+ storage could regulate unfolded protein response (UPR) and ER stress, previous studies reported that ceramide made Ca2+ release from the ER to the cytosol in HeLa cells (Pintos et al., 2001). Ca2+ plays a key role to maintain the ER homeostasis, because Ca2+ loss could result in ER stress (Feng et al., 2003). Thapsigargin is a specific inhibitor of the ER Ca2+ pump sarco/endo-plasmic reticulum calcium ATPase (SERCA). In HepG2 cells, ASMase decreased thapsigargin-inhibited ER Ca2+ release, indicating lower ER Ca2+ storage. These findings fit with the concept that aberrant lipid composition in the ER regulates SERCA and hence ER Ca2+ homeostasis (Fu et al., 2011). Furthermore, the data exhibited a 2–3-fold increase in the expression of ASMase as well as ER stress markers in liver biopsies from patients with acute hepatitis. It suggested that alcohol induced ASMase activation and ER stress, at least in part, by disrupting ER Ca2+ homeostasis, supporting the potential role of ASMase in human ALD.

ASMase is expressed by nearly any cell type and generally located in endosomal/lysosomal compartment. Moreover, it has been found in lipid rafts, a specific microdomain of the plasma membrane, serving as a signaling platform via cell surface receptors. Previous studies combined ASMase-derived ceramide with cell death, even if the direct roles are still poorly defined. There may be a functional relationship between ASMase and cathepsins, because cathepsin D (CtsD) was participated as a target of ceramide in lysosomal compartments (Heinrich et al., 1999). It was observed that cathepsin B (CtsB)-deficient hepatocytes were resistant to TNF-dependent cell death (Guicciardi et al., 2000). Moles (Moles et al., 2009) reported a new function of CtsB
Fig. 1. The role of ceramide in alcoholic liver disease. Ethanol could activate ASMase, then hydrolyzing sphingomyelin to promote ceramide accumulation, further increasing PP2A activity, and then inhibiting the effect of ethanol on AMPK phosphorylation. When AMPK phosphorylation is blocked, it regulates downstream target enzymes involved in lipid metabolism, leading to fatty acid synthesis increased and its oxidation decreased, which promotes the progression of steatosis. It is hypothesized that ASMase could induce ER stress by disrupting ER Ca²⁺ homeostasis and prohibiting the proliferation of hepatic stellate cells, which contributes to hepatic steatosis and liver fibrogenesis. ASMase, acid sphingomyelinase; SM, sphingomyelin; ER stress, endoplasmic reticulum stress; PP2A, protein phosphatase 2A; AMPK, adenosine monophosphate-activated protein kinase.

and CtsD in mediating the activation of hepatic stellate cells (HSCs) and liver fibrosis. Hepatic fibrosis is a wound-healing response to chronic injury (Iredale, 2007; Moles et al., 2010). HSCs mediate the fibrotic component in the liver. Myofibroblasts are likely derived from a deal of cellular sources including HSCs activation (Friedman, 2008). Moles showed that selective stimulation of ASMase occurred during the transdifferentiation/activation of primary mouse HSCs into myofibroblast-like cells, accompanying with CtsB and CtsD processing. When inhibiting ASMase or blunting CtsB/D processing, the activation and proliferation of mouse and human hepatic stellate cells were prohibited (Moles et al., 2010). Above all, ASMase may play a role in the mechanism of liver fibrogenesis.

CONCLUSIONS

The steatosis of liver is regarded as reversible, but persistent injury and inflammation can further develop to chronic steatohepatitis. Dysregulated lipid metabolism promotes ceramide accumulation, resulting in lipotoxic states and activating oxidative stress and ER stress pathways. Taken together, we conclude that ethanol may promote ceramide accumulation by activating ASMase, and further activating PP2A. PP2A can dephosphorylate and inactivate AMPK, so that it may be an important mechanism in the inhibitory effect of ethanol on AMPK phosphorylation. When the phosphorylation of AMPK is blocked, the fatty acid synthesis is increasing while its oxidation is decreased, which leads to the progression of steatosis (Fig. 1). Thus, activation of AMPK or inhibition of ceramide generation may be beneficial to control the alcoholic fatty liver. Myricin reduced several histopathologic-al and ultrastructural abnormalities in experimental ALD, which demonstrates that reducing hepatic ceramide levels can help to restore the structural and functional integrity of the liver (Tong et al., 2014). Ceramide is involved in several pathways of ethanol-induced liver injury. Although monotherapy with myricin was sufficient to restore liver structure and function in chronic ALD, future studies should still be investigated to explore pharmacologic therapy of blocking ceramide synthesis or accelerating its rate of degradation. In addition, the role of PP2A inhibitors and compounds interfering with ceramide synthesis may deserve further study, AMPK activators seem to be attractive compounds for the treatment of ALD. It is said that small-molecule inhibitors of ASMase are available for use in humans (Liangpunsakul et al., 2012), which may provide a novel therapeutic strategy. In summary, the treatment of chronic alcohol-related liver disease requires multi-pronged strategies to reverse the effects of dysregulated lipid metabolism and other possible mechanisms.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES


