

Oxidative stress and endoplasmic reticulum stress induced hepatic steatosis

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ABSTRACT

The hepatic steatosis is strongly associated with obesity, hyperlipidemia, and type 2 diabetes mellitus. Experimental and clinical studies increasingly show that the oxidative stress and endoplasmic reticulum stress (ER stress) contribute in many ways to the pathogenesis of hepatic steatosis. Although there is a plethora of studies on ER stress and associated diseases, the core pathophysiological mechanism of ER stress-associated ROS has not yet been fully clarified. In this review, we will discuss the correlation of ER stress with oxidative stress and lipid disorders, and the understanding of ER stress and its cofactors in pathological processes may provide new perspective on disease development and control.

Keywords: Oxidative stress, endoplasmic reticulum stress, hepatic steatosis.

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INTRODUCTION

Hepatic steatosis, the excessive accumulation of triglycerides in the liver, is one of the hallmarks of obesity-related pathologies (Cohen et al., 2011). Two processes that are becoming increasingly recognized as inducers of hepatic steatosis are endoplasmic reticulum (ER) and oxidative stress. Many of the diseases that feature ER and oxidative stress are associated with lipids disorder, including diabetes, atherosclerosis, renal disease, and neurodegenerative disease, and are becoming epidemic among humans in modern society. Nonetheless, progress in translating this understanding into useful therapeutic strategies has been disappointing. An important approach to this problem is to gain a more in-depth understanding about molecular and cellular mechanisms and links between ER stress and oxidative stress, particularly in the process of hepatic steatosis and in settings relevant to disease processes. Recent studies indicate that ER stress and oxidative stress both are intertwined with the lipids disorder. However, the mechanisms that link ER stress and oxidative stress are very poorly characterized. In this review, we attempt to summarize the relation between ER stress and oxidative stress, the role of oxidative stress in lipids disorder, and finally the mechanisms of ER stress underlying hepatic steatosis.

OXIDATIVE STRESS AND HEPATIC STEATOSIS

The role of oxidative stress in liver steatosis production has been discussed in relation to alterations in metabolic transcription factors expression. Hepatic steatosis is one multifactorial disease and oxidative stress has been implicated in its pathogenesis, although a causal relationship or a pathogenic link between hepatic steatosis and oxidative stress has not been established (Anderson and Borlak, 2008; Videla et al., 2006; Gawrieh et al., 2004). However, many human and animal studies have observed the association between disease state of NAFLD and biomarkers of oxidative stress or lipid oxidation (Koek et al., 2011; Raszeja-Wyszomirska et al., 2012; Rolo et al., 2012; Zein et al., 2012). Increased levels of ROS and lipid oxidation products and decreased levels of antioxidant enzymes such as superoxide dismutase (SOD) and catalase and antioxidant compounds such as glutathione have been observed in patients of NAFLD compared with those observed in the healthy subjects (Videla et al., 2004).

Lipid oxidation products are useful biomarkers for oxidative stress *in vivo*, since the mechanisms of lipid oxidation is now well understood and their physiological levels in human fluids and tissues are high enough to be

identified and quantified with gas chromatography-mass spectrometry (GC/MS) or high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Sparvero et al., 2010; Yin et al., 2011). Several studies have measured systemic markers of oxidative stress status in NAFLD and an increase in markers such as HODE, HETE, 8-isoP, TBARS, MDA, oxidized LDL, hydroxydeoxyguanosine (8-OHdG), and thioredoxin (TRX) has been observed in NASH patients. Several groups found higher levels of lipid peroxidation products in NASH patients compared with NAFL or healthy people (Zein et al., 2012; Sparvero et al., 2010). Hepatic level of 8-OHdG, a reliable marker of oxidative DNA damage, was increased in NAFLD patients, especially in NASH patients (Yin et al., 2011). The expression of hemoxygenase-1 (HO-1), which may protect the cells against oxidative stress, is induced in NASH patients as an adapting response (Malaguarnera et al., 2005). It was reported that the levels of α -tocopherol, lutein, zeaxanthin, lycopene, and β -carotene were significantly decreased in NASH patients compared with those of controls (Erhardt et al., 2011). Cytokines, which are related to oxidative stress with inflammatory and destructive properties, have a predominant role in the regulation of liver lipid accumulation leading to hepatic steatosis (Kuppan et al., 2012; Zhao et al., 2011). Malondialdehyde (MDA) and superoxide dismutase (SOD) were studied as markers of oxidative stress. The lipid peroxidation product MDA is a presumptive marker for oxidative stress and an indicator of free radical production. SOD constitutes a primary defense against oxidative stress (Poli, 2000; Zhang et al., 2008). Oxidative stress is believed to play a major role in the mechanism underlying the hepatic lipid accumulation in hepatic steatosis. Besides improving lipid peroxidation, previous reports have suggested a link between increased hepatic oxidative stress and lipid accumulation (Alkhoury et al., 2009). Lipid overaccumulation has been proposed to result in steatosis and oxidative stress of hepatocytes (Sozio et al., 2010).

The increased evidence showing the involvement of oxidative stress in the pathogenesis of hepatic steatosis has stimulated studies on the effects of antioxidants. Notably, recent studies suggesting the beneficial effect of vitamin E against hepatic steatosis have attracted much attention (Cheng et al., 2012; Sanyal et al., 2010; Pacana and Sanyal, 2012). It is noteworthy that vitamin E acts as an efficient peroxyl radical-scavenging antioxidant *in vivo* to prevent lipid peroxidation, but not against the oxidation mediated by enzymes and non-radical oxidants such as hypochlorite (Pattison et al., 2009; Niki, 2013). The protective effects of vitamin E against liver injury have been confirmed in many animal studies (Chung et al., 2010; Yachi et al., 2013). Oxidative stress has been reported to be one major cause of liver steatosis by alcohol. Antioxidants such as N-acetylcysteine attenuate acute alcohol-induced steatosis (Lu et al., 2012; Yang et

al., 2012). It was found that a JNK inhibitor partially blocked acute ethanol-induced steatosis (Yang et al., 2012:44). CBD is a nonpsychotic cannabinoid, which has been shown to have anti-inflammatory and antioxidant properties. CBD has also been reported to function as an antioxidant in preventing glutamate toxicity and preventing neurotoxicity by acute alcohol (Hamelink et al., 2005). Increasing autophagy can protect cells from injury by various stimuli, as inhibition of autophagy increases toxicity to cells. Genetically enhancing autophagy by overexpressing Atg7 could alleviate hepatic steatosis induced by a high-fat diet (Yang et al., 2010). Carbamazepine, an FDA-approved antiepileptic drug, can alleviate fatty liver by inducing autophagy (Puls et al., 2013). In contrast, loss of autophagy *in vitro* or *in vivo* increases lipid accumulation in cells and in liver (Yang et al., 2012; Wu et al., 2012). The recent finding indicated the cannabidiol protects mouse liver from acute alcohol-induced steatosis through multiple mechanisms including attenuation of alcohol-mediated oxidative stress, prevention of JNK MAPK activation, and increasing autophagy (Yang et al., 2014).

ER STRESS AND LIPID DISORDERS

Recently, it has been suggested that ER stress is involved in hepatic lipid metabolism (Malhi and Kaufman, 2011). A possible link between ER stress and hepatic steatosis was first suggested by Ozcan et al, who observed an upregulation of several ER stress markers in the liver of ob/ob or high-fat diet-fed mice, both characterized by the presence of an hepatic steatosis (Malhi and Kaufman, 2011). Since, several studies have confirmed the activation of the ER stress pathway in the steatotic liver of various animal models (Kammoun et al., 2009; Mu et al., 2009) or in humans (Gregor et al., 2009; Puri et al., 2008). The hepatic X-box binding protein 1 (XBP1) efficiency exhibits decreased expression of lipogenic genes (Lee et al., 2008). Furthermore, liver-specific IRE1a and whole-body activating transcription factor 6 (ATF6) knockout mice exacerbate hepatic steatosis upon pharmacological ER stress (Yamamoto et al., 2010; Zhang et al., 2011). Thus, the hepatic activation of ER stress suggests a role of this cellular pathway in the onset of hepatic steatosis.

Despite these findings, it is not completely understood how elevated ER stress in the liver contributes to hepatic steatosis. The unfolded protein response (UPR) appears to be activated in several liver diseases, which are associated with steatosis, raising the possibility that ER stress-dependent alteration in lipid homeostasis is a mechanism that underlies the steatosis (Malhi and Kaufman, 2011). Indeed, several distinct enzymatic lipogenic pathways, including the fatty acid elongation machinery, cholesterol biosynthesis and complex lipid biosynthesis, are compartmentalized in the ER. In

addition, the ER stress response is correlated with elevated transcripts of lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). Recent studies using nonalcoholic fatty liver disease models have demonstrated a key interconnectedness between hepatic steatosis and ER stress (Lake et al., 2014). All three arms of the UPR and their downstream signaling molecules are involved in the regulation of lipid metabolism (Lee et al., 2008; Rutkowski et al., 2008). IRE1 β -null mice fed a high-fat and high-cholesterol diet develop hyperlipidemia, indicating its important role in lipid metabolism (Iqbal et al., 2008). Conversely, lipids can induce ER stress. Excess cholesterol induces acute ER stress by perturbing sarco-endoplasmic reticulum Ca²⁺-ATPase (SERCA) structure and SERCA-mediated Ca²⁺ homeostasis in the ER (Feng et al., 2003). In addition, increased lipid synthesis in the obese liver results in an increased ratio of phosphatidylcholine to phosphatidylethanolamine, which inhibits SERCA activity. Impaired SERCA activity leads to ER Ca²⁺ depletion and ER stress (Fu et al., 2011). SERCA plays a critical role in maintaining ER Ca²⁺ homeostasis and normal ER function (Mei et al., 2013). Moreover, the recent identification of Hsp70, Hsp90 and protein disulfide isomerase (PDI), which are involved in protein folding, as *in vivo* targets for modification by the lipid peroxidation product 4-HNE, suggests that impairment of their activity could contribute to the initiating events of ER stress and subsequent hepatic lipid accumulation (Smathers et al., 2011).

ER stress and lipogenesis, VLDL assembly and secretion

Although it is now established that hepatic steatosis is associated with the appearance of endoplasmic reticulum stress, the mechanisms and the factors involved in this process are currently unknown. The ER is the major site for lipid synthesis and VLDL assembly. Disturbed ER homeostasis can stimulate lipogenesis (Kammoun et al., 2009) and inhibit hepatic VLDL secretion (Caviglia et al., 2011; Ota et al., 2008; Qiu et al., 2006), which led to overt hepatic steatosis. Recent studies have pointed out a link between activation of endoplasmic reticulum stress and lipogenesis. It has been reported that ER stress suppresses the expression of lipogenic genes through repression of SREBP1, a key lipogenic transcription factor (Rutkowski et al., 2008). In contrast to these results, it has been reported that ER stress potently activates SREBP1c, which subsequently leads to elevated hepatic lipogenesis (Kammoun et al., 2009; Lee et al., 2012). Indeed, the hepatic deletion of XBP1 leads to a decrease in the de-novo lipid synthesis (Lee et al., 2008). Another study showed that sustained dephosphorylation of eIF2 α by overexpression of the phosphatase GADD34 is associated with a decrease in

hepatic lipogenesis and the appearance of steatosis in high-fat diet-fed mice (Oyadomari et al., 2008). PERK-dependent signaling contributes to lipogenic differentiation by maintaining sustained expression of lipogenic enzymes (Bobrovnikova-Marjon et al., 2008). ATF4 is also required for lipogenic gene expression in white adipose tissue, as evidenced by ATF4 knockout mice having less white adipose tissue (Yoshizawa et al., 2009). Conversely, the overexpression of a constitutively active form of ATF6 stimulated fatty acid synthesis in NIH-3T3 cells (Bommiasamy et al., 2009). Recently, it has been reported that CREB-H was processed in response to tunicamycin-induced ER stress (Zhang et al., 2006), and that defective CREB-H alleles in humans associate with extreme hypertriglyceridemia, supporting the physiological significance of CREB-H in lipid homeostasis (Lee et al., 2011). Thus, further studies are required to explore how ER stresses mediate hepatic lipogenesis.

ER stress and β -oxidation

Very few studies have analyzed whether activation of endoplasmic reticulum stress has consequences on mitochondrial β -oxidation. However, as lipogenesis is activated by endoplasmic reticulum stress, it is tempting to speculate that in these conditions, the increase in malonyl CoA concentration should induce an inhibition of carnitine palmitoyltransferase-1 (CPT1) activity and, thus, a decrease in mitochondrial β -oxidation. On the other hand, given the fact that mitochondria and endoplasmic reticulum are closely related by a physical and functional manner (Giorgi et al., 2009), it is relevant to infer that endoplasmic reticulum stress could influence directly mitochondrial function and conversely. A recent study pointed out that a mitochondrial dysfunction obtained by oligomycin treatment leads to endoplasmic reticulum stress via modifications of calcium signaling in a human liver cell line (Lim et al., 2009). Another mechanism that could participate in the onset of hepatic steatosis is a decrease in VLDL synthesis and secretion (Sreekumar et al., 2003). Apolipoprotein B100 and microsomal transfer protein have well-known roles in hepatic VLDL production (Choi and Ginsberg, 2011). The levels of microsomal transfer protein mRNA and protein or secretion of apolipoprotein B100 were greatly mitigated by ER stress inducer, tunicamycin (Yamamoto et al., 2010; Choi and Ginsberg, 2011; Fuchs et al., 2012; Qiu et al., 2011). It was demonstrated that endoplasmic reticulum stress leads to a decrease in apoB100 secretion by inducing its co-translational proteasomal degradation but without affecting its mRNA (Ota et al., 2008). This result was confirmed by another study, which showed that endoplasmic reticulum stress decreases apoB100 content by two different ways: degradation of apoB100 and attenuation of apoB translation via the PERK branch

of UPR (Qiu et al., 2009). Finally, a recent study has demonstrated that apoB100 accumulation in the endoplasmic reticulum in response to increased lipid delivery to hepatic cells leads to the initiation of endoplasmic reticulum stress (Su et al., 2009), which could, in return, induce a decrease in apoB100 content in the liver (Ota et al., 2008; Qiu et al., 2009). It was reported that ER stress in metabolic disorders reduces HDL biogenesis due to impaired hepatic ABCA1 function (Röhrli et al., 2014). The up-regulation of hepatic VLDLR is one of the potential links between ER stress and hepatic steatosis. Based on its expression profile, the hepatic VLDLR may not actively deliver lipids from lipoproteins under normal conditions. However, increased hepatic VLDLR levels during severe or prolonged ER stress might result in the transport of lipids into the liver to prepare for extreme conditions (Jo et al., 2013).

CONCLUSIONS

Cells have basal level of reactive oxygen species (ROS) for signaling and normal function. In contrast, ROS levels increase upon exposure to toxic agents such as irradiation and environmental pollutants or during enzymatic reactions (Malhotra and Kaufman, 2007; Perjes et al., 2012). The oxidative folding process is a major folding mechanism in ER physiology. ER stress-associated oxidative environment may also be correlated with ER stress-associated ROS. ER protein oxidation and mitochondrial oxidative phosphorylation are well-described ROS sources during ER stress. Meanwhile, the role of ER stress-associated NADPH oxidase(s) as a potential ROS source is not yet fully clarified. It is of note, however, that NADPH oxidase 4 (Nox4), one of the NADPH oxidase isoforms, has recently been implicated as a possible ROS source during strokes (Radermacher et al., 2013). Additionally, Nox4-associated ROS alter UPR signaling and promote Ras activation (eventually activating RohA) at the cytosolic face of ER. The main functional implication of signaling is autophagy such that when either Nox4 or autophagy protein 5 (Atg5) is disabled, cells undergo apoptosis (Wu et al., 2010).

In conclusion, the hepatic steatosis has evolved a complex and intertwined set of signaling pathways. Although these pathways are not yet fully characterized, it is becoming clear that ER stress and oxidative stress are intimately involved in the pathology of hepatic steatosis.

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