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REVIEW

Aberrant post-translational protein modifications in the pathogenesis of alcohol-induced liver injury

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Abstract

It is likely that the majority of proteins will undergo post-translational modification, be it enzymatic or non-enzymatic. These modified protein(s) regulate activity, localization and interaction with other cellular molecules thereby maintaining cellular hemostasis. Alcohol exposure significantly alters several of these post-translational modifications leading to impairments of many essential physiological processes. Here, we present new insights into novel modifications following ethanol exposure and their role in the initiation and progression of liver injury. This critical review condenses the proceedings of a symposium at the European Society for the Biomedical Research on Alcoholism Meeting held September 12-15, 2015, in Valencia, Spain.

Key words: Alcohol; Acetylation; Liver; Carbonylation methylation; Dysfunction; Methylation; Glycosylation; Phosphorylation; Ubiquitination; Sumoylation; Betaine; Post-translational protein modification

Core tip: A majority of proteins in our body undergo orchestrated post-translational modifications that influence protein structure and function. Chronic ethanol administration causes aberrant post-translational modification of proteins that play a critical role in the pathogenesis of alcoholic-induced liver damage.

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INTRODUCTION

Ethanol consumption leads to many adverse functional and structural molecular changes in multiple organs and accounts for 2.5 million deaths globally each year[1,2]. Ethanol is mainly metabolized in the liver; this organ is therefore most susceptible to its toxic effects[3,4]. Sustained alcohol misuse produces a wide spectrum of hepatic lesions, the most characteristic being steatosis, hepatitis and fibrosis/cirrhosis[5]. Steatosis, characterized by fat accumulation in hepatocytes, develops in 90% of individuals who drink more than 16 g of alcohol per day[6]. In 30%-40% of individuals reporting chronic alcohol abuse, there is development of hepatitis which is characterized by inflammatory changes in the liver and ballooning degeneration of hepatocytes. In later stages of alcoholic liver disease (ALD), collagen deposition and regenerative nodules can result in the development of fibrosis and cirrhosis, respectively[2]. Better understanding of the mechanisms by which alcohol damages the liver may yield new pharmacologic strategies to blunt, halt, or reverse disease progression, potentially even in inveterate alcoholics.

Hepatic dysfunction due to chronic ethanol consumption is multifactorial involving dysregulation of multiple cellular pathways[7-9]. Significant to the aforementioned dysregulation is abnormal post-translational modification of proteins[10]. About 50%-90% of proteins in our body undergo orchestrated post-translational modifications that could influence protein structure and function. These modifications may be enzymatic or non-enzymatic and play an important role in functions of proteins through the regulation of activity, turnover and localization and/or interactions to maintain cellular hemostasis. These modifications include phosphorylation, acetylation, methylation, glycosylation, ubiquitination, sumoylation and ISGylation. Directed or inadvertent exposure to stressful factors, including chronic ethanol exposure, has been shown to cause aberrant post-translational modifications. Additionally, bioactive products of enzymatic or non-enzymatic lipid oxidation may also cause protein modifications with potential functional damage to protein[11] as well as impact the function of various metabolic pathways. This brief overview will focus on recent advances that have been made using global proteomic approaches and bioinformatics to better understand the impact of these altered post-translational modifications by ethanol.

Alcohol consumption significantly alters several post-translational modifications of proteins and this has been reviewed recently[10]. Here, we review the newly described and pathogenically relevant alterations that play important roles in the progression of ALD. First we discuss the undesired post-translational modifications that may occur *via* electrophilic species including reactive aldehydes (carbonylation), acyl groups (acetylation) and sugar moieties (glycosylation).

CARBONYLATION

A key contributor to the pathogenesis of ALD is enhanced hepatocellular oxidative stress resulting from the production of reactive oxygen species *via* induction of Cyp2E1 as well as xanthine and NADPH oxidases[12-16]. These reactive species, in turn, induce lipid peroxidation of unsaturated fatty acids including linoleic acid forming α/β unsaturated aldehydes[17,18]. The best characterized of these carbonyl-derivatives include 4-hydroxy-2-nonenal (4-HNE), 4-oxo-2-nonenal, malondialdehyde (MDA) and acrolein. Following their formation, these highly reactive lipid electrophiles modify DNA as well as lysine, cysteine and histidine residues on proteins, thereby impairing their structural or catalytic capabilities. Early proteomic approaches to identify carbonylated proteins in ALD used 2-dimensional electrophoresis followed by protein identification. These techniques were not very sensitive and only a handful of proteins were identified[19,20]. A commonality of all these proteins was the fact that all were very highly expressed, which permitted easier identification. Of interest, the majority of identified proteins were involved in either protein folding (heat shock proteins) or hepatocellular oxidative stress responses.

Recent advances in biotin hydrazide chemistry and in the sensitivity of mass spectrometry have allowed for a more in depth proteomic approach to identify less abundant proteins modified by reactive aldehydes in ALD. To date, using global proteomic approaches, over 2000 proteins that undergo carbonylation have been identified in either murine models or in human hepatic tissue isolated from patients with end-stage ALD[21-23]. Using enriched cellular fractions, chronic ethanol consumption led to an increase in carbonylation of microsomal and

cytosolic proteins. Comprehensive pathway analysis of identified proteins revealed that ethanol consumption impacted many different cellular pathways foremost of which are the fatty acid metabolic, tricarboxylic acid cycle and amino acid metabolism. By increasing carbonylation of proteins involved in these pathways, mechanistic links have been proposed for ethanol' s impact on lipid accumulation as well as how acetyl CoA contributes to nutritional imbalances evident in alcoholics. These findings are further supported by an additional study that examined the effects of deletion of glutathione S-transferase A4-4 (GSTA4-4) which functions to remove 4-HNE reducing the effects of reactive aldehydes[24]. Using GSTA4-4 knockout mice and employing proteomics approaches, it was determined that carbonylation was increased in mitochondrial fractions especially in pathways regulating oxidative stress, fatty acid metabolism and amino acid metabolism supporting the contribution of GSTA4-4 in protecting mitochondria from reactive aldehydes (Supplementary Table 1). Concurrently, we have reported that carbonylation is increased in tissue obtained from end-stage alcoholics[23]. Not surprisingly, following mass spectral analysis, increased carbonylation of proteins regulating oxidative stress, metabolic and cytoskeletal processes were increased[24].

GLYCOSYLATION

In cells, glycosylation of proteins contributes to numerous cellular functions including assisting in proper protein folding as well as cell to cell adhesion. Global proteomic approaches and 2-dimensional electrophoresis were performed on microsomal fractions consisting primarily of smooth and rough endoplasmic reticulum, isolated from chronically ethanol fed mice. These studies revealed a significantly decrease in microsomal glycosylation following 8 wk of alcohol consumption. Subsequent bioinformatic pathway analysis revealed significant decreases in glycosylation of proteins regulating protein folding, redox homeostasis and the unfolded protein response among others. These results suggest that decreased glycosylation may contribute to the observed increased in ubiquitinated proteins in murine models of ALD[25].

ACETYLATION

In hepatocytes, acetylation of lysine residues results in regulation of many cellular functions including gene expression and metabolism. As it is metabolized, ethanol is converted to acetaldehyde by alcohol dehydrogenases followed by removal of the aldehyde group by mitochondrial aldehyde dehydrogenases (ALDH2) to produce acetate, which is converted to acetyl CoA[26]. By way of protein acyl transferases, acetyl CoA is then utilized in part as a substrate for protein acetylation. Therefore, it is not surprising that over the last decade, ethanol abuse has been determined to directly affect protein acetylation[27-30]. In murine models of ALD, ethanol decreases expression of the class Ⅲ nicotinamide adenine dinucleotide (NAD+/NADH) dependent protein deacetylases, Sirtuin 1 (SIRT1) and Sirtuin 5 and decreases enzymatic activity of Sirtuin 3[31-35]. All these changes combined decrease the overall cellular deacetylase activity that ultimately results in an increase in protein acetylation. Using traditional Western blotting and immunoprecipitation techniques chronic ethanol induces hyperacetylation of several key metabolic regulators, including PPARγ co-activator 1α, sterol regulatory binding element protein 1 (SREBP-1c) and forkhead transcription factor 1.

In recent experiments, mass spectrometry and proteomic approaches have been applied to identify proteins and pathways that are acetylated following chronic ethanol consumption. Using whole cell extracts and matrix-assisted laser desorption mass spectrometry, Shepard *et al*[30] identified 40 proteins that are acetylated following chronic ethanol administration. Pathway analysis revealed that of these 40, the majority was predominantly mitochondrial proteins and there was a significant preference for proteins regulating lipid metabolism as well as oxidative stress[29,30]. More recently using mitochondrial enriched fractions isolated from ethanol-fed wild-type and SIRT3 knockout mice, we determined that chronic ethanol impacted acetylation of proteins regulating lipid metabolism, oxidative stress, as well as mitochondrial pathways including amino acid biosynthesis and the electron transport chain[32].

SUMOYLATION

A member of the ubiquitin family, SUMO, comprised of four distinct proteins in humans (SUMO-1, -2, -3 and -4), is receiving growing interest since its discovery less than a decade ago[36]. SUMO-4 shows similarity to -2/3 but it is as yet unclear whether it is a pseudogene or merely restricted in its expression pattern[37]. The sumoylation cycle is a multistep process, involving maturation, activation, conjugation and deconjugation, and regulates the function and fate of a large number of proteins involved in many cellular pathways including transcription, intracellular transport, DNA repair, replication, and cell signaling[38,39]. Sumoylation, as an enzymatic cascade, resembles that of ubiquitination, including an ATP dependent step, the E1-activating enzyme Aos1/Uba2 (SAE1/SAE2) forms a thioester bond between its catalytic cysteine (Uba2 C173) and the C-terminal carboxy group of mature SUMO. From there, SUMO is transferred to the catalytic cysteine (C93) of the E2-conjugating enzyme (Ubc9). In the last step of this cascade, an isopeptide bond is formed between SUMO and the 3-amino group of a lysine side chain. Specific isopeptidases, members of the SENP family, ensure reversibility of this modification[40,41].

Sumoylation is often increased under oxidative stress[42]. Recent reports demonstrate that ubiquitin conjugating enzyme 9 (Ubc9), the sole E2 enzyme of sumoylation, is induced in ethanol treated mice[43]. However, the functional significance of this finding remains unknown. However, a number of sumoylated proteins have been identified in the liver after ethanol administration and other injury models. A notable example is the enzyme methionine adenosyltransferase Ⅱ α (MATα2) which has been shown to increase upon ethanol exposure[44] is sumoylated. This modification likely plays a critical role in its stability[45].

Nrf2, a well-characterized transcription factor is known for its role in activating anti-oxidant response element (ARE), forms heterodimers with small Maf (MafG, MafK and MafF) proteins. We recently reported that Nrf2 and MafG are sumoylated and this facilitates their heterodimerization and trans-activation of the ARE in activated hepatic stellate cells[46].

Increased levels of lipopolysaccharide (LPS), a major component of the cell wall of gram-negative bacteria, is frequently found in cirrhotic patients[47] and is observed to lower glutathione (GSH), a potent anti-oxidant. GSH is highly concentrated in the liver and synthesized in the cytosol in a tightly regulated manner. Key determinants of GSH synthesis are the availability of the sulfur amino acid precursor, cysteine, and the activity of the rate-limiting enzyme, glutamate cysteine ligase, which is composed of a catalytic and a modifier (GCLM) subunit. LPS inhibits the sumoylation machinery suppressing the expression of the sole E2 enzyme Ubc9. This results in reduced Nrf2 and MafG sumoylation affecting their heterodimerization and trans-activation of the ARE present in GCLC and GCLM in macrophages and hepatocytes[48].

Although considerable progress has been made in the identification of sumoylated proteins and the characterization of the effects of the modification of these particular substrates, little is known in regards to the global regulation of SUMO conjugation in ALD.

ISOASPARTYL DAMAGE

Ethanol consumption specifically triggers a unique protein post-translational damage as isoaspartate peptide linkages in proteins[49]. This protein isoaspartate damage is due to an inhibition of the protein repair enzyme, protein isoaspartyl methyltransferase (PIMT).

PIMT normally acts to resist the accumulation of isoaspartate damage that arises through protein aging, and as a consequence of oxidative damage to proteins[50]. PIMT is a methyltransferase that utilises *S*-adenosylmethionine (SAM) as a methyl donor. PIMT methylates isoaspartate residues in peptides and proteins, a process that triggers isoaspartate elimination and restoration of protein function. One of the detrimental actions of ethanol consumption is impaired methionine synthase-catalysed remethylation of homocysteine to generate methionine, the metabolic precursor of SAM. A subsequent depletion of SAM availability limits the activity of SAM-dependent methyltransferases, such as PIMT. This inhibition of methylation reactions is further exacerbated by the ethanol-induced increase in the level of *S*-adenosylhomocysteine (SAH), a potent inhibitor of numerous SAM-dependent methyltransferases including PIMT[49,51-54].

Animal studies have demonstrated the benefits of dietary supplementation with betaine, a pro-methylating agent, to counter some of the ethanol-induced changes in the metabolite levels of the methionine metabolic pathway[49,51-54]. Rats fed a control or ethanol liquid diet (36% of calories) for a period of 4 wk with or without dietary supplementation with 1% betaine revealed that the ethanol-induced reduction of the methylation potential (*i.e,.* the lowering of the hepatocellular SAM:SAH ratio to approximately 43% of control level) was rectified in rats fed an ethanol diet supplemented with betaine[49,51-54]. Concomitant with the changes in the SAM:SAH ratio, the ethanol-induced increase of damage to cellular proteins as isoaspartate was also alleviated by betaine supplementation[49,51-54].

A proteomic approach was adopted to investigate the mechanism by which betaine was able to alleviate the ethanol-induced increase of isoaspartate damage. One dimensional and two dimensional protein separations and differential protein staining techniques revealed that betaine supplementation increased the expression of betaine homocysteine methyltransferase-1, methionine adenosyl transferase-1 and glycine N-methyltransferase, and these enzymes act to collectively increase SAM levels and normalise the SAM:SAH ratio[55].

To further investigate the influence of the SAM:SAH ratio on PIMT activity and cellular isoaspartate damage, primary hepatocytes taken from control or ethanol-fed rats were cultured. Cells were incubated *in vitro* with tubercidin or adenosine, agents that elevate cellular SAH levels[56]. These agents produced an additive increase of isoaspartate damage to that detected from ethanol consumption, indicative of an additional lowering of the SAM:SAH ratio and further inhibition of PIMT activity.

To identify liver protein target(s) of PIMT that accrue isoaspartate damage after ethanol consumption, proteins from control and ethanol fed rats were exogenously methylated using PIMT and ^{3}H-SAM methyl donor. Novel, sensitive autoradiographic imaging[57] was used to reveal increased isoaspartate methylation at liver protein bands of 75-80 kDa, 95-100 kDa, and 155-160 kDa. Column chromatography used to enrich isoaspartate-damaged liver proteins indicated that damaged proteins from ethanol-fed rats mirrored those that accumulate in the livers of PIMT knockout mice. The about 160 kDa protein target of PIMT was further purified and

fractionated, and identified as carbamoyl phosphate synthase-1 (CPS-1)[58]. This is a mitochondrial enzyme that catalyses the synthesis of carbamoyl phosphate from ammonia and bicarbonate, and is the first and rate-limiting step of the urea cycle. Resolution of liver proteins by one dimensional polyacrylamide gel electrophoresis and Coomassie blue staining also showed that cytosolic CPS-1 protein levels increased by approximately 20% in rats administered ethanol for 4 wk. A subsequent study of ethanol administration for 8 wk showed that the levels of cytosolic CPS-1 now increased approximately 2-fold over those of control animals; indicating that cytosolic CPS-1 levels correlated with the duration of alcohol consumption. Increased cytosolic CPS-1 was also detected in PIMT knockout mice compared to their control littermates. This release of liver CPS-1 into the cytosol as a response to ethanol consumption or in PIMT knockout mice is presumed to reflect mitochondrial damage and redox stress.

These studies highlight the accumulation of atypical isoaspartate-containing abnormal proteins following chronic ethanol exposure. The animal studies employed, however, are of relatively acute alcohol administration, and it is hypothesised that in alcoholic patients sustained and cumulative isoaspartate protein damage across a broad number of target proteins would ensue and contribute to liver cell damage and pathology.

METHYLATION

Here, we will discuss the role of this post-translational modification in the regulation of innate immunity in hepatitis C virus (HCV) infection combined with ethanol exposure. About 3% of world population is infected with HCV, the most common blood-borne infection in the United States. By 2010, 2.7-3.9 million people were diagnosed with chronic HCV-infection, and there are about 17000 new cases of acute infection per year. Hepatitis C and alcohol are the most widespread causes of liver disease worldwide, and approximately 80% of patients with a history of Hepatitis C and alcohol abuse develop chronic liver injury[59]. Almost one-third of alcoholics with clinical symptoms of liver disease have been infected with HCV, which is four times the rate of HCV infection found in alcoholics who do not have liver disease. Alcohol consumption in HCV-infected patients exacerbates liver disease leading to rapid progression to fibrosis, cirrhosis and even hepatocellular carcinoma[60]. Alcohol-consumption reduces responsiveness of HCV patients to anti-viral treatment; only 7% of heavy drinking HCV patients are responders to interferon therapy[61]. However, despite the direct acting anti-viral agents (DAA) changing the treatment backbone of HCV infection from IFN-ribavirin, the effectiveness of DAA would also depend on the endogenous IFNα-mediated activation of antiviral genes in HCV-infected hepatocytes.

The mechanism by which alcohol consumption exacerbates the course of HCV progression is not clear. Since HCV and alcohol alter innate immunity in hepatocytes, there is a strong possibility that their synergistic effect on innate immunity contributes to HCV spread and progressive liver injury. Activation of an anti-viral innate immune response is based on IFN signaling, which requires activation of IFN-sensitive genes (ISG) *via* the Janus kinase/signal transducers and activators of transcription (JAK-STAT) pathway. Transduction of IFNα signal requires phosphorylation of STAT1 and STAT2. The attachment of STAT1 to DNA becomes possible if STAT1 is methylated on arginine residue(s). It has been shown that HCV subverts the IFNα-mediated JAK-STAT signaling through the reduction of intrahepatic STAT-1 and -2 phosphorylation[62] and reduces STAT-1 methylation leading to suppression of ISGs[63,64].

Ethanol also is known to suppress methylation reactions leading to impaired methylation of multiple proteins and enzymes[49,51-54]. We hypothesize that ethanol potentiates HCV-mediated impairment of methylation-regulated IFN signaling in liver cells, thereby decreasing antiviral protection in liver cells. When HCV-infected Huh7.5-CYP2E1 cells were exposed to an extracellular system that continuously generates the ethanol metabolite, acetaldehyde (Ach) in physiological quantities, STAT1 methylation was suppressed on both arginine and lysine residues[65]. Suppression of STAT1 methylation is regulated by protein arginine N-methyltransferase 1 and lysine methyltransferases, and can be induced by specific methyltransferase inhibitors. The effects of Ach on STAT1 methylation are protein phosphatase 2 dependent. The impaired methylation of STAT1 increases the complex formation between STAT1 and the pathway inhibitor, protein inhibitor of activated STAT-1 (PIAS1), preventing the attachment of IFN-activated STAT1 to DNA followed by antiviral gene activation. This mechanism is schematically presented as Figure 1. Methylation-dependent dysregulations of IFN signaling in hepatocytes were attenuated by supplementation with the pro-methylating agent, betaine[65].

Thus, Ach potentiates the ability of HCV to down-regulate activation of ISGs by interferon and plays a pivotal role in methylation-dependent suppression of innate immunity in hepatocytes, which are primary sites of both HCV replication and ethanol metabolism.

OXIDIZED METABOLITES OF FATTY ACIDS

Alcohol administration results in increased production of enzymatic or non-enzymatic lipid oxidation products, which may also cause protein modifications and potential functional damage to proteins[11] as well as

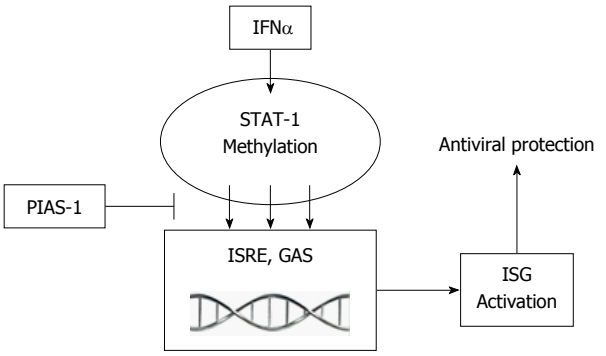

Figure 1 Acetaldehyde suppresses interferon-α signaling in hepatitis C virus -infected liver cells by impairing signal transducers and activators of transcription 1 methylation. The most downstream event in interferon (IFN)α signaling is the attachment of methylated STAT1 to DNA, interferon stimulated response element (ISRE) and gamma-interferon activated site (GAS), for activation of anti-viral interferon-stimulated genes (ISGs). Acetaldehyde suppresses STAT1 methylation, which facilitates increased STAT1 interaction with protein inhibitor of activated STAT 1 (PIAS1, a negative regulator of IFN signaling) preventing STAT1 binding to DNA. This ultimately results in reduced ISG activation and decreased induction of anti-viral proteins.

impact on the function of various metabolic pathways exacerbating alcohol-induced hepatic injury. Many lines of evidence, from animals to humans, have shown that dietary factors, including dietary fat, along with heavy alcohol consumption, play critical roles in the ALD pathogenesis. Indeed, the relative beneficial effects of dietary saturated fat (SF) and damaging effects of dietary unsaturated fat [USF, primarily corn oil/linoleic acid (LA) enriched] on alcohol-induced liver injury have been well documented in experimental animal models of ALD[66-72]. A number of mechanisms have been proposed for the opposing effects of dietary USF *vs* SF in ALD, including (1) induction of lipid peroxidation and oxidative stress[70,73,74]; (2) altered gut microbiota, impaired intestinal barrier integrity, endotoxemia, and associated increase in liver pro-inflammatory cytokine production[66,67,72]; (3) modulation of hepatic lipid metabolism *via* SIRT1-SREBP-1-histone H3 axis[75]; and (4) modulation of hepatocyte nuclear factor-4α expression, a master transcription factor in the regulation of lipid metabolism[76]. A new concept has recently emerged that the bioactive oxidized LA metabolites (OXLAMs), which are formed enzymatically from LA primarily *via* the actions of 12/15-lipoxygenase (12/15-LOX), or non-enzymatically *via* free radical-mediated oxidation in response to oxidative stress, might contribute to ALD pathogenesis. It has been demonstrated that plasma OXLAMs, specifically 9- and 13-hydroxy-octadecadienoic acids (9- and 13-HODEs), were elevated in patients with alcoholic cirrhosis in parallel with the increase in lipoxygenases (15-LOX-1 and 15-LOX-2 mRNA) in the liver samples. The plasma levels of HODEs in patients with ALD were significantly higher than in healthy subjects as well as in NAFLD patients[77]. Further, increased levels of 9- and 13-HODEs were observed in experimental animal models of ALD[78,79] in parallel with the hepatic steatosis, oxidative stress, and inflammation and hepatocyte damage. It has been reported that 9- and 13-HODEs are natural endogenous ligands for the Transient Receptor Potential Vanilloid 1 (TRPV1)[80,81]. Our recent study demonstrated that chronic-binge ethanol-mediated increases in circulating OXLAMs and TRPV1 levels in mice were associated with hepatic steatosis, inflammation and injury[78]. Genetic depletion of TRPV1 did not blunt hepatic steatosis caused by ethanol, but prevented hepatic injury. TRPV1 deficiency protected from hepatocyte death and prevented the increase in pro-inflammatory cytokine and chemokine expression, including TNF-α, interleukin-6, macrophage inflammatory protein-2 and monocyte chemotactic protein-1. Moreover, TRPV1 depletion markedly blunted ethanol-mediated induction of plasminogen activator inhibitor-1, an important mediator of alcohol-induced hepatic inflammation, *via* fibrin accumulation[78]. Exposure of HepG2 cells to 9- and 13-HODEs resulted in activation of TRPV1 signal transduction with the increased intracellular Ca^{2+} levels, suggesting that OXLAM/TRPV1/Ca^{2+} signaling may be a relevant pathway contributing to ALD pathogenesis.

Alcohol consumption increases hepatic oxidative stress with the production of reactive oxygen species. One of the major sources of *in vivo* protein modification during oxidative stress is thought to be oxidative products of polyunsaturated fatty acids (PUFAs). LA is the most abundant PUFA in mammalian tissue. Non-enzymatic oxidative degradation of PUFAs, including LA, generate a variety of lipid peroxidation products (LPOs, *e.g.*, MDA, HNE, acrolein, various epoxyketooctadecenoic acid isomers)[11]. Enhanced amounts of peroxidized phospholipids and their truncation products in the circulation have previously been observed in rats with alcohol-induced liver disease[79]. Some LPOs can modify DNA, peptides and proteins leading to formation of advanced lipoxidation end-products (ALEs). These modifications can potentially cause functional damage to proteins. Numerous LPOs and ALEs exert diverse biologic activities (*e.g.*, damaging and pro-inflammatory effects in some cases) through different as yet not well-defined mechanisms. The role and the significance of oxidized lipids, both dietary and in vivo-produced, as well as possible mechanisms underlying their beneficial or deleterious effects in liver pathology remain to be determined.

CONCLUSION

In summary, chronic ethanol consumption dysregulates post-translational modifications of numerous important proteins that regulate many cellular processes. Not surprisingly, there is considerable overlap in the pathways that are targeted. Understanding how each individual modification affects specific protein function and thereby, alters metabolic pathways will

be of critical importance to deciphering the impact of the aforementioned modifications to alcohol- induced steatosis and hepatocellular damage. It could also provide an insight into disease pathogenesis and progression, and may help to identify additional useful targets of drug action. In addition, the activation of enzymatic and/or non-enzymatic degradation of polyunsaturated fatty acids resulting in the formation of numerous bioactive lipid compounds underlies the deleterious effects of certain dietary fat intake in promoting indices of alcoholic liver damage.

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