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ANIMAL MODELS

Transient Receptor Potential Vanilloid 1 Gene Deficiency Ameliorates Hepatic Injury in a Mouse Model of Chronic Binge Alcohol-Induced Alcoholic Liver Disease

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Experimental alcohol-induced liver injury is exacerbated by a high polyunsaturated fat diet rich in linoleic acid. We postulated that bioactive oxidized linoleic acid metabolites (OXLAMs) play a critical role in the development/progression of alcohol-mediated hepatic inflammation and injury. OXLAMs are endogenous ligands for transient receptor potential vanilloid 1 (TRPV1). Herein, we evaluated the role of signaling through TRPV1 in an experimental animal model of alcoholic liver disease (ALD). Chronic binge alcohol administration increased plasma OXLAM levels, specifically 9- and 13-hydroxy-octadecadienoic acids. This effect was associated with up-regulation of hepatic TRPV1. Exposure of hepatocytes to these OXLAMs *in vitro* resulted in activation of TRPV1 signal transduction with increased intracellular Ca^{2+} levels. 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 proinflammatory cytokine and chemokine expression, including tumor necrosis factor-α, IL-6, macrophage inflammatory protein-2, and monocyte chemotactic protein 1. *TRPV1* depletion markedly blunted ethanol-mediated induction of plasminogen activator inhibitor-1, an important alcohol-induced hepatic inflammation mediator, via fibrin accumulation. This study indicates, for the first time, that TRPV1 receptor pathway may be involved in hepatic inflammatory response in an experimental animal model of ALD. TRPV1-OXLAM interactions appear to play a significant role in hepatic inflammation/injury, further supporting an important role for dietary lipids in ALD. *(Am J Pathol 2015, 185: 43—54; http://dx.doi.org/10.1016/j.ajpath.2014.09.007)*

Alcohol consumption remains one of the most common and important causes of liver disease in the United States and worldwide. Alcoholic liver disease (ALD) ranges from steatosis and steatohepatitis to advanced injury, such as fibrosis, cirrhosis, and hepatocellular carcinoma. It has been estimated that 15% to 30% of heavy drinkers develop advanced ALD.[1—3] Despite the significant progress made on ALD pathogenesis, the specific mechanism(s) responsible for ALD development and progression remain poorly understood. Due, in part, to this incomplete understanding of the mechanisms by which alcohol damages the liver, there is still no Food and Drug Administration—approved therapy for this common and often devastating disease. Understanding the molecular mechanisms involved in the pathogenesis of alcohol-induced liver injury may, therefore, lead to the development of new therapeutic options and/or preventive interventions.

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Dietary fat is an important determinant of ALD development and progression.[4–7] Recent publications have shown that experimental and clinical alcohol-induced liver steatosis and injury were associated with elevated oxidized linoleic acid metabolites (OXLAMs), specifically 9- and 13-hydroxy-octadecadienoic acids (9- and 13-HODEs).[8,9] It has been reported that 9- and 13-HODEs are natural endogenous ligands for the transient receptor potential vanilloid 1 (TRPV1).[10,11] The TRPV1 receptor is a ligand-gated nonselective cation channel with high permeability for Ca^{2+},[12] which is expressed in many cells and tissues, including liver.[13–22] The TRPV1 is a polymodal molecular detector of multiple stimuli responding to a large variety of physical (eg, noxious heat), and chemical (eg, H^+ ions) stimuli. In addition to HODEs, several exogenous and endogenous TRPV1 agonists have been identified, including capsaicin,[12] cannabinoids,[23] retinoids,[24] and metabolites of arachidonic acid.[25]

Accumulating evidence suggests an important role of TRPV1 in several diseases and pathological conditions, including chronic pain,[26] neurogenic inflammation,[27] diabetes,[28,29] metabolic syndrome and obesity,[19,30] and liver diseases.[13,31,32] To the best of our knowledge, there are no data assessing the role of TRPV1 in ALD. The present study evaluates the role of TRPV1 in the development of ethanol-induced liver steatosis, inflammation, and injury using an experimental animal model of ALD. Our findings collectively indicate that the genetic deficiency of *TRPV1* protects against alcohol-induced liver inflammation and injury but not steatosis. Our data point toward a role for TRPV1-OXLAM receptor-ligand interactions as a potentially relevant pathway contributing to alcohol-mediated steatohepatitis.

Materials and Methods

Animal Model of ALD

Animals were housed in a pathogen-free barrier facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and the study protocol was approved by the University of Louisville (Louisville, KY) Institutional Animal Care and Use Committee. Eight-week-old male *TRPV1* knockout mice (B6.129X1-TRPV1^{tm1Jul}/J, 11th backcross generation) and their genetically unaltered wild-type (WT; C57Bl6/J) counterparts were obtained from the Jackson Laboratory (Bar Harbor, ME). Animals were fed Lieber-DeCarli control (isocaloric maltose-dextrin) or ethanol (5% w/v) liquid diets ad libitum for 10 days plus a single binge ethanol administration (5 g/kg, body weight, 20% ethanol) by gavage, whereas mice in control groups were gavaged with isocaloric dextrin maltose.[33] Both diets were prepared fresh daily. In the control group diet, the levels of protein, carbohydrate, and fat were held constant at 17%, 43%, and 40% of total energy, respectively. In the alcohol diet, ethanol (35% of total calories) was substituted for carbohydrate energy. The diet was enriched in corn oil containing a high amount of polyunsaturated linoleic fatty acid, and purchased from Research Diet (New Brunswick, NJ). At the conclusion of the experiment, the mice were anesthetized; and blood and tissue samples were obtained. Plasma was stored at −80°C. Portions of liver tissue were frozen immediately in liquid nitrogen, whereas others were fixed in 10% neutral-buffered formalin or embedded in frozen specimen medium (Tissue-Tek OCT compound; Sakura Finetek, Torrance, CA).

Blood and Liver Biochemical Analysis

Plasma alanine transaminase (ALT) and aspartate transaminase (AST) activity, cholesterol, triglycerides (TGs), glucose, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very LDL (VLDL) were determined by Lipid Panel Plus using the Piccolo Xpress chemistry analyzer (Abaxis, Union City, CA). Blood alcohol levels were measured using nicotinamide adenine dinucleotide-alcohol dehydrogenase (NAD-ADH) Reagent Multiple Test (Sigma, St. Louis, MO), according to the manufacturer's instructions. Plasma endotoxin levels were measured with the Limulus Amoebocyte Lysate kit (Lonza, Walkersville, MD). For the determination of hepatic lipid levels, hepatic lipids were extracted with an aqueous extract from chloroform and methanol. Hepatic TGs were measured, as previously described,[34] using TG reagent (Thermo Fisher Scientific Inc., Middletown, VA). Liver cholesterol was assayed using reagents from Sigma.

Liver Histological Examination and Staining

For histological analysis, liver sections were fixed in 10% buffered formalin and embedded in paraffin. Tissue sections (5 μm thick) were prepared and stained with hematoxylin and eosin. Oil-Red-O staining was performed to evaluate hepatic fat accumulation. Apoptotic cells were identified by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay using the ApopTag Peroxidase *In Situ* Apoptosis Detection kit (Millipore, Billerica, MA), according to the manufacturer's instructions. Neutrophil accumulation in the livers was assessed by chloroacetate esterase (CAE) staining using a commercially available kit (Sigma), according to the manufacturer's instructions. Immunofluorescence detection of hepatic fibrin deposition was performed in frozen tissue, as previously described.[35]

Hepatic Caspase-3 Activity Assessment

Caspase-3 activity was determined using 200 μg whole liver protein with the caspase-3 colorimetric kit (Abcam, Cambridge, MA), according to the manufacturer's instructions.

RNA Isolation and Real-Time RT-PCR Assay

Total liver RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. Reverse transcription was performed with qScript cDNA Supermix (Quanta Biosciences, Gaithersburg, MD) and