Toxicology and Applied Pharmacology 311 (2016) 34–41

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Role of dietary fatty acids in liver injury caused by vinyl chloride metabolites in mice

Lisanne C Anders [a,b], Heegook Yeo [a], Brenna R Kaelin [a], Anna L Lang [a], Adrienne M Bushau [a], Amanda N Douglas [a], Matt Cave [a,b,c,d,e], Gavin E Arteel [a,c], Craig J McClain [a,b,c,d,e], Juliane I Beier [a,c,*]

[a] Department of Pharmacology and Toxicology, University of Louisville Health Sciences Center, Louisville, KY 40292, USA
[b] Department of Medicine, University of Louisville Health Sciences Center, Louisville, KY 40292, USA
[c] Hepatobiology and Toxicology Program, University of Louisville Health Sciences Center, Louisville, KY 40292, USA
[d] Diabetes and Obesity Center, University of Louisville Health Sciences Center, Louisville, KY 40292, USA
[e] Robley Rex Louisville VAMC, Louisville, KY 40206, USA

ARTICLE INFO

Article history:
Received 26 August 2016
Revised 24 September 2016
Accepted 27 September 2016
Available online 28 September 2016

Keywords:
PVC
TASH
Plastic
Hepatotoxicity
Unsaturated fat
Saturated fat

ABSTRACT

Background: Vinyl chloride (VC) causes toxicant-associated steatohepatitis at high exposure levels. Recent work by this group suggests that underlying liver disease may predispose the liver to VC hepatotoxicity at lower exposure levels. The most common form of underlying liver disease in the developed world is non-alcoholic fatty liver disease (NAFLD). It is well-known that the type of dietary fat can play an important role in the pathogenesis of NAFLD. However, whether the combination of dietary fat and VC/metabolites promotes liver injury has not been studied.

Methods: Mice were administered chloroethanol (CE - a VC metabolite) or vehicle once, 10 weeks after being fed diets rich in saturated fatty acids (HSFA), rich in poly-unsaturated fatty acids (HPUFA), or the respective low-fat control diets (LSFA; LPUFA).

Results: In control mice, chloroethanol caused no detectable liver injury, as determined by plasma transaminases and histologic indices of damage. In HSFA-fed mice, chloroethanol increased HSFA-induced liver damage, steatosis, infiltrating inflammatory cells, hepatic expression of proinflammatory cytokines, and markers of endoplasmic reticulum (ER) stress. Moreover, markers of inflammasome activation were increased, while markers of inflammasome inhibition were downregulated. In mice fed HPUFA all of these effects were significantly attenuated.

Conclusions: Chloroethanol promotes inflammatory liver injury caused by dietary fatty acids. This effect is far more exacerbated with saturated fat, versus poly-unsaturated fat; and strongly correlates with a robust activation of the NLRP3 inflammasome in the saturated fed animals only. Taken together these data support the hypothesis that environmental toxicant exposure can exacerbate the severity of NAFLD/NASH.

1. Introduction

Vinyl chloride is an organochloride used in industry to create the polymer, polyvinyl chloride (PVC), and its production was recently estimated at 27 million metric tons annually (Sass et al., 2005). VC has been identified as a solvent degradation product at many Superfund sites. It is present in landfill leachate and in the groundwater near military installations where it exists as a microbial metabolite of trichloroethylene (Kielhorn et al., 2000). Another significant source of exposure is in the chemical industry, especially in the United States, Europe and China (Kielhorn et al., 2000; WHO, 2004). VC is also found in significant concentrations in the ambient air surrounding manufacturing complexes. However, the main environmental exposure risk stems from contaminated groundwater in areas surrounding production sites. Because VC is a gas, in homes located above contaminated groundwater, VC can migrate through soil and foundations to enter basements or living spaces. Owing to its widespread presence in EPA superfund sites and its known potential human risk, VC is ranked #4 on the ATSDR Hazardous Substance Priority List (U.S.Department of Health and Human Services PHS, 2006).

VC is a known human hepatotoxicant that has been demonstrated to cause several benign and malignant diseases, including hepatocellular carcinoma (HCC), hemangiosarcoma and toxicant associated steatohepatitis (Wahlang et al., 2013). To date, however, these direct effects of VC exposure are hypothesized to be caused only by high occupational exposures and have limited relevance with existing VC safety regulations. The effect of low environmental VC exposure in contrast to high occupational VC exposures has not been studied. Importantly,

* Corresponding author at: Department of Pharmacology and Toxicology; University of Louisville; Louisville, KY, 40202, USA.
E-mail address: juliane.beier@louisville.edu (J.I. Beier).

http://dx.doi.org/10.1016/j.taap.2016.09.026
0041-008X/

the impact of low exposure needs to consider other factors that may increase hepatotoxicity. In this context, the effect of underlying disorders or other insults that may modify risk is critical.

In the liver, the concept of multiple factors contributing to disease is well-known. Indeed, numerous studies have now established that modifications to the liver that are pathologically negligible can become hepatotoxic in response to another factor. This multiple 'hit' paradigm has been described in numerous models of liver injury such as alcoholic (Beier et al., 2011; Beier et al., 2009) and non-alcoholic fatty liver diseases (Yang et al., 1997; Day and James, 1998). Although lifestyle and diet are major factors in the etiology of this class of diseases, current research suggests that environmental factors also affect their development and progression. The similarity of VC metabolism to that of other hepatotoxicants known to sensitize the liver, such as ethanol (Bolt, 2005), suggests that VC may also cause fatty liver. Indeed, recently our laboratory has demonstrated that the VC metabolite chloroethanol renders livers more sensitive to hepatotoxicity caused by the injection of bacterial lipopolysaccharide (LPS); this exacerbation of liver damage is characterized by a more robust inflammatory response and enhanced cell death (Anders et al., 2016).

It has become clear that the type of dietary fat consumed plays an important role in the pathogenesis of liver disease, independent of caloric load (Juarez-Hernandez et al., 2016). For example, in non-alcoholic fatty liver disease (NAFLD), saturated fatty acids (SFA) but not poly-unsaturated fatty acids (PUFA) have been shown to cause inflammation and cell death (Csak et al., 2011; Miura et al., 2013). However, it has also been shown that PUFAs can exacerbate liver injury and markers of inflammation in a murine NAFLD model (Provenzano et al., 2014). In contrast, in models of alcoholic liver disease (ALD), PUFA (such as linoleic acid) but not SFA, exacerbated alcohol-related liver injury, possibly via pro-inflammatory bioactive oxidized linoleic acid metabolites (OXLAMs) (Nanji et al., 1995; Kirpich et al., 2013; Martinez-Clemente et al., 2010; Uderhardt and Kronke, 2012; Vangaveti et al., 2010). Overall, the role of dietary fatty acids and their effects on liver injury and inflammation is still controversial and many aspects remain unclear (Juarez-Hernandez et al., 2016). Importantly, the potential interaction between dietary fat type and VC/metabolites to promote liver injury has not been studied.

2. Methods

2.1. Animals and treatments

Eighty male C57BL/6J mice, 8 weeks old, from Jackson Laboratory (Bar Harbor, ME) were housed in a pathogen-free barrier facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, and procedures were approved by the local Institutional Animal Care and Use Committee. Mice were fed low fat control or high fat diets (see below and Fig. 1a and b) for 10 weeks. At the end of the 10 weeks 40 animals were administered chloroethanol (CE; 50 mg/kg i.g.) and 40 animals were administered vehicle (water) 24 h prior to sacrifice. The concentration of CE was determined by others not to directly cause liver damage (Kaphalia and Ansari, 1989) and was validated in a previously published study by our group (Anders et al., 2016). Based on the fraction of VC that is estimated to be metabolized to CE and its apparent volume of distribution in rodents, this concentration equates to ~100 ppm inhalation exposure of VC over a short period of time. At sacrifice, animals were anesthetized with ketamine/xylazine (100/15 mg/kg, i.m.). Blood was collected from the vena cava just prior to sacrifice (exsanguination), and citrated plasma was stored at −80 °C for further analysis. Portions of liver tissue were snap-frozen in liquid nitrogen, embedded in frozen specimen medium (Sakura Finetek, Torrance, CA), or were fixed in 10% neutral buffered formalin.

2.1.1. Low saturated fat diet (LSFA). 13% calories as fat; Casein 195.0 g/kg, DL-Methionine 3.0 g/kg, Sucrose 120.0 g/kg, Corn Starch 432.89 g/kg, Maltodextrin 100.0 g/kg, Anhydrous Milkfat 37.2 g/kg, Soybean Oil 12.8 g/kg, Cellulose 50.0 g/kg, Mineral Mix, AIN-76 (170915) 35.0 g/kg, Calcium Carbonate 4.0 g/kg, Vitamin Mix, Teklad (40060) 10.0 g/kg, Ethoxyquin, antioxidant 0.01 g/kg; (Harlan Laboratories, Madison, WI).

2.1.2. High saturated fat diet (HSFA). 42% calories as fat; Casein 195.0 g/kg, DL-Methionine 3.0 g/kg, Sucrose 341.31 g/kg, Corn Starch 75.0 g/kg, Maltodextrin 75.0 g/kg, Anhydrous Milkfat 210.0 g/kg, Cholesterol 1.5 g/kg, Cellulose 50.0 g/kg, Mineral Mix, AIN-76 (170915) 35.0 g/kg, Calcium Carbonate 4.0 g/kg, Vitamin Mix, Teklad (40060) 10.0 g/kg, Ethoxyquin, antioxidant 0.04 g/kg; (Harlan Laboratories, Madison, WI).

2.1.3. Low polyunsaturated fat diet (LPUFA). 13% calories as fat; Casein 195.0 g/kg, DL-Methionine 3.0 g/kg, Sucrose 120.0 g/kg, Corn Starch 432.79 g/kg, Maltodextrin 100.0 g/kg, Corn Oil 50.0 g/kg, Cholesterol 0.1 g/kg, Cellulose 50.0 g/kg, Mineral Mix, AIN-76 (170915) 35.0 g/kg, Calcium Carbonate 4.0 g/kg, Vitamin Mix, Teklad (40060) 10.0 g/kg, Ethoxyquin, antioxidant 0.01 g/kg; (Harlan Laboratories, Madison, WI).

2.1.4. High polyunsaturated fat diet (HPUFA). 42% calories as fat; Casein 195.0 g/kg, DL-Methionine 3.0 g/kg, Sucrose 341.36 g/kg, Corn Starch 49.5 g/kg, Maltodextrin 100.0 g/kg, Corn Oil 210.0 g/kg, Cholesterol 2.0 g/kg, Cellulose 50.0 g/kg, Mineral Mix, AIN-76 (170915) 35.0 g/kg, Calcium Carbonate 4.0 g/kg, Vitamin Mix, Teklad (40060) 10.0 g/kg, Ethoxyquin, antioxidant 0.04 g/kg; (Harlan Laboratories, Madison, WI).

2.2. Biochemical analyses, histology and immunohistochemistry

Oral glucose tolerance was evaluated at 4 and 8 weeks during the feeding protocol (Fig. 1a for timeline). Mice were fasted for 6 h, then blood was sampled via tail cut immediately after fasting to determine baseline. Following oral administration of 2 mg/kg D−/(+)-glucose (Sigma, St. Louis, MO) in 4 ml/kg of sterile saline solution, blood was sampled and glucose concentrations measured at 15, 30, 60 and 90 min. Glucose concentrations were determined using an Accu-Chek Aviva Plus glucometer and test strips (Roche Diagnostics Corp., Indianapolis, IN). Plasma transaminases (ALT and AST) were determined using standard kits (Thermo Fisher Scientific, Middletown, VA). Paraffin embedded liver sections were stained with hematoxylin & eosin (H&E) and neutrophil accumulation was assessed by chloroacetate esterase stain (CAE; Sigma, St. Louis MO). CAE-positive cells were counted using Metamorph Image Analysis Software (Molecular Devices, Sunnyvale, CA) and are expressed as positive cells per 1000 hepatocytes. Hepatic lipids were extracted from snap-frozen liver samples as described previously (Bligh and Dyer, 1959; Kaiser et al., 2009). Hepatic and plasma lipids were determined using standard clinical chemistry reagents for cholesterol, and triglycerides (Infinity, Thermo Fisher Scientific, Middletown, VA). Liver sections were stained with Oil Red O (ORO) for visualization of neutral lipids, as described previously (Beier et al., 2009).

2.3. Immunoblots

Liver samples were homogenized in RIPA buffer (Beier et al., 2006) containing protease and phosphatase inhibitor cocktails (Sigma, St. Louis, MO). Samples were loaded onto SDS-polyacrylamide gels (Invitrogen, Thermo Fisher Scientific, Grand Island, NY), followed by electrophoresis and Western blotting onto PVDF membranes (Hybond P, GE Healthcare Bio-Sciences, Pittsburgh, PA). Primary polyclonal antibodies for mouse ATF3, CHOP, HMGB1, Caspase 1, and NLRP3 were used and compared to GAPDH (Cell Signaling Technology; Beverly, MA). Densitometric analysis was performed using UN-SCAN-IT gel (Silk Scientific Inc., Orem, UT) software.