Environmental Toxicology and Pharmacology 56 (2017) 15–20

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

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

Research Paper

Formaldehyde alters triglyceride synthesis and very low-density lipoprotein secretion in a time-dependent manner

Jianying Bai[a,*], Pan Wang[a], Yanfei Liu[a], Yan Zhang[a], Yaofu Li[a], Zhen He[a], Lifang Hou[a,b], Ruifeng Liang[a]

[a] Department of Environmental Health, School of Public Health, Shanxi Medical University, Taiyuan 030001, PR China
[b] Center for Population Epigenetics, Robert H. Lurie Comprehensive Cancer Center and Department of Preventive Medicine, Northwestern University Feinberg School of Medicine, Chicago, IL 60611, USA

ARTICLE INFO

Keywords:
Formaldehyde
HepG2 cells
Triglyceride metabolism
VLDL secretion

ABSTRACT

Formaldehyde is a common indoor air pollutant that is toxic to the liver. This study aimed to investigate the effects of formaldehyde on triglyceride metabolism in human hepatocellular carcinoma cells (HepG2). Cell viability was detected using a MTT (3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide) assay. Following treatment with different concentrations of formaldehyde for 24 and 48 h, the intra and extra-hepatocellular triglyceride (TG) content was determined using a chemical-enzymatic method; Western blotting was used to detect the levels of fatty acid synthesis and VLDL-related proteins. Our results showed that cell viability significantly decreased after formaldehyde treatment (0.5–12.5 mM, 24/48 h). Extracellular TG levels in the hepatocytes increased after formaldehyde treatment at 0.004 mM–0.1 mM for 24 h. SREBP-1c, ACC, FASN, and MTP, CES3 and DGAT1 proteins increased significantly after 24 h of formaldehyde treatment. Intracellular TG levels decreased for 48 h treatment of formaldehyde. AMPKα increased significantly in all tested groups and p-AMPK increased significantly after 0.1 mM formaldehyde treatment for 48 h. Our results indicated that short–term formaldehyde exposure balances triglyceride metabolism by promoting hepatocellular TG synthesis and VLDL secretion; Long-term formaldehyde disturbs the TG metabolism balance in the hepatocytes.

1. Introduction

Formaldehyde (FA) is a saturated aliphatic aldehyde that is soluble in water, ethanol, and other organic solvents. As an important chemical material, it has been used in the manufacture of resin, rubber, plastic, and other materials. FA is also a common indoor air pollutant and has many effects on human health. In recent years, the occupational hazards of indoor FA pollution have received increasing attention and studies have shown the various toxic effects of FA (Tong et al., 2006; Yu and He, 2004; Feng and Zhai, 2008; Zhang et al., 2003). Especially studies have focused its effect on lipid peroxidation and pathological changes of the liver (Xue et al., 2007). Animal studies suggested that FA could cause irregular changes of the rough endoplasmic reticulum, smooth endoplasmic reticulum expansion, and cytoplasm loss in rat liver cells (Cikmaz et al., 2010).

The liver plays a unique role in regulating lipid metabolism. Many chemicals can cause fatty liver degeneration and hepatic steatosis (Torres et al., 2016). Hepatic steatosis ultimately occurs because of an imbalance in TG synthesis and breakdown (Douglas, 2013; Nguyen et al., 2008) and affects one-third of adults in developed countries (Starley et al., 2010; Wang et al., 2015; Fan and Farrell, 2009; Cohen et al., 2011). Whether FA could cause fat-related metabolic disorders in the human liver and the underlying mechanisms are still unclear. Therefore, in this study, we focused on TG metabolism in human hepatocellular carcinoma cells (HepG2) to investigate the changes of fat metabolism after exposure to FA.

2. Materials and methods

2.1. Cell culture and treatment

HepG2 cells (Shanghai Institute of Cell Biology, China) were first cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) (Gibco, Life Technologies Corporation, Grand Island, NY, USA),

Abbreviations: FA, formaldehyde; TG, triglyceride; ELISA, enzyme-linked immunosorbent assay; AMPK, AMP-activated protein kinase; p-AMPKα, phospho-AMP-activated protein kinase α; SREBP-1c, sterol regulatory element binding protein-1c; ACC, acetyl-CoA carboxylase; p-ACC, phospho-acetyl-CoA carboxylase; FASN, fatty acid synthase; MTP, microsomal triglyceride transfer protein; DGAT1, sn-1,2-diacylglycerolacyl transferase1; CES3, carboxylesterase3; VLDL, very low-density lipoprotein; DNL, de novo lipogenesis

* Corresponding author.
E-mail address: jybai66@sxmu.edu.cn (J. Bai).

http://dx.doi.org/10.1016/j.etap.2017.08.023
Received 15 May 2017; Accepted 26 August 2017
Available online 30 August 2017
1382-6689/

containing 10% calf serum (Sijiqing, Hangzhou, China) in an incubator (5% CO_2, 37 °C). The medium was replaced every 2–3 days. Cells were then digested with 0.25% tryptase and passaged after they achieved 80% adhesion. Cells in the logarithmic phase were selected for further experiments In which six treatment groups were created: one negative control group (complete medium), two positive control groups (1 mM oleic acid group and 2.5 μg/ml Brefeldin A group), and three FA treatment groups (Sigma). The FA concentrations in the FA treatment groups were 0.004, 0.02, and 0.1 mM separately according to the results of preliminary 3-(4,5-dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide (MTT) (Wuhan Boster Biological Technology Co., Ltd, China) assays.

2.2. Detection of cell viability

Cell viability was detected using the MTT assay. The cells were seeded routinely at a density of 10,000 cells/cm^2 into a 96-well culture plate with 100 μL/well FA or control solution. After 24 and 48 h of treatment, the supernatants were removed and the cells were washed with 100 μL of PBS for three times. The cells were then added into 100 μL of medium with 20 μL MTT reagent and incubated for 4 h. Formazan detergent reagent (100 μL) was then added until the purple precipitate was dissolved. Absorbance was determined at 570 nm using a microplate reader.

2.3. Detection of intra-hepatocellular and extra-hepatocellular TG

Detection of TG in hepatocytes was performed using an organic extraction method: the glycerophosphate oxidase-peroxidase (GPO-POD) method (Applygen Technologies Inc., China) with minor adjustments (Schwartz and Wolins, 2007; Bai et al., 2016). The cells were seeded routinely into a 6-well culture plate. After treatment, the supernatants were removed and the cells were washed by 2 mL phosphate buffered saline (PBS). They were then dispersed with 400 μL of PBS/10 mM EDTA at pH 7.4. Cells (200 μL) were centrifuged and lysed for protein measurement. Cells (200 μL) were added to glass tubes containing 2 mL of isopropanol-hexane-water (IHW) for organic extraction, and then vortexed and incubated for 30 min. Hexane-diethyl ether (500 μL, 1:1) was added to each tube. Samples were mixed by vortaxing, and then incubated for 10 min at room temperature. Water (1 mL) was added to separate the phases by vortaxing. The tubes were then covered with aluminum foil and incubated at 20–25 °C until the phases separated. Then 400 μL organic phase was pipetted into a new glass tube and evaporated with nitrogen at 50 °C using a 96-well format manifold. Thereafter, 10 μL water and 190 μL TG reagent were added to each tube according to the TG detection kit instructions. The tubes were vortexed, covered with parafilm, and incubated for 30 min at 37 °C with shaking at 100 rpm. Then, 150 μL of TG reagent plus sample from each tube was transferred to a flat-bottom 96-well plastic microplate and the absorbance was determined at 550 nm using a microplate reader. To determine the extra-hepatocellular TG, the cells were seeded routinely into 6-well culture plates. The supernatants were collected after exposure to FA for 24 and 48 h, and TG was extracted using an organic extraction method similar to that used for intracellular TG detection and evaporated using a 96-pin microplate format gas manifold (Schwartz and Wolins, 2007). Then, 1 mL supernatant was used for extraction instead of 200 μL of cell suspension and 800 μL of organic phase was evaporated with nitrogen. The TG level was determined as before.

2.4. Detection of protein levels

The intracellular proteins were collected using loading buffer (50 mM Tris-HCl at pH = 6.8, 5% glycerol, 5% β-mercaptoethanol, 5% SDS, and 0.25% bromophenol blue) after treatment for 24 and 48 h. Cell pellets were obtained by centrifugation (10,000 rpm for 10 min at 4 °C) and their protein concentrations were quantified. For detection of p-ACC,ACC, AMPKα and p-AMPKα, the proteins were collected using extraction buffer containing 50 mM Tris-HCl, pH7.4, 0.5% SDS and protease inhibitor cocktail P8340 (Sigma).Using 5% (for ACC and p-ACC only) or 10% separating gel and a 5% spacer gel, 20 μg of protein sample was collected for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the separated proteins were transferred electronically to a nitrocellulose membrane, blocked with 5% skim milk at room temperature for 1 h, and then incubated with the corresponding primary antibodies (Table 1) overnight in a 4 °C shaker. Subsequently, the membranes were shaken and rinsed three times using PBS with Tween 20 (PBST) at room temperature, and the membranes were incubated with the secondary antibody coupled with horse radish peroxidase (Table 2) for 1 h with shaking, rinsed with PBST three times at room temperature, and the immunoreactive proteins were detected using enhanced chemiluminescence (ECL, Thermo Scientific). β-Actin was detected as a loading control for same amount of total protein. After the films were developed, the optical density of the immunoreactive protein bands was analyzed semiquantitatively using Image-Pro Plus 6.0 software.

Table 1
Primary antibodies used for western blotting.

Primary antibody	Company	Catalogue #	Dilution
Rabbit anti-human AMPKα	Cell Signal Technology	#2532	1:1000
Rabbit anti-human p-AMPKα (Thr172)	Cell Signal Technology	#2531	1:1000
Rabbit anti-human SREBP1c	Protein Tech, China	14088-1-AP	1:1000
Rabbit anti-human ACC1	Protein Tech, China	21923-1-AP	1:1000
Rabbit anti-human p-ACC1(Ser79)	Cell Signal Technology	#3661	1:1000
Rabbit anti-human FASN	Protein Tech, China	10624-2-AP	1:1000
Rabbit anti-human MTP	abcam	Ab63467	1:1000
Mouse anti-human DGAT1	Santa Cruz Biotechnology	Sc-271934	1:500
Rabbit anti-human CES3	Protein Tech, China	14587-1-AP	1:2000
Mouse anti-human β-actin	Wuhan Boster Biological Technology Co., Ltd., China	BM0005	1:1000

2.5. Statistical analysis

One-way analysis of variance was used to compare the mean values of various groups. Multiple comparisons between groups were performed using Dunnett's *t*-test. A difference with a P-value < 0.05 was considered statistically significant. All the statistical analyses were performed using SPSS (Version 22.0. Armonk, NY: IBM Corp.)

Table 2
Secondary antibodies used for western blotting.

Secondary antibody	Company	Catalogue #	Dilution
Goat anti-rabbit IgG	Wuhan Boster Biological Technology Co., Ltd., China	BA1050	1:5000
Goat anti-mouse IgG	Wuhan Boster Biological Technology Co., Ltd., China	BA1055	1:5000