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1,3-Dichloro-2-propanol induced hyperlipidemia in C57BL/6J mice via AMPK signaling pathway

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ABSTRACT

1,3-Dichloro-2-propanol (1,3-DCP) is a well-known contaminant that has been detected in a wide range of foods. Dietary intake represents the greatest source of exposure to 1,3-DCP. In the study, we first found 1,3-DCP could induce hyperlipidemia in C57BL/6J mice below 1 mg/kg/day. We investigated serum lipid profile, liver total cholesterol (TC) and triglyceride (TG), histopathology of Liver and adipose tissue. The results showed 1,3-DCP dose dependently increased serum TG, TC and low-density lipoprotein cholesterol (LDL-C), decreased serum high-density lipoprotein cholesterol (HDL-C), increased relative liver weight, liver TG and TC, relative adipose tissue weight and enlarged the size of adipose cells. Because AMPK signal pathway is important in the process of lipid metabolism, we further investigated the effects of 1,3-DCP on AMPK signaling pathway in murine models. The results showed that 1,3-DCP (0.1–1 mg/kg/day) decreased p-AMPK/tAMPK ratio, p-ACC/tACC ratio, PPARα expression, but increased FAT, SREBP1, HMGCR and FAS expression. These observations indicated that 1,3-DCP induced hyperlipidemia in C57BL/6J mice at least partially through regulating AMPK signaling pathway.

1. Introduction

Hyperlipidemia is characterized by abnormally elevated levels of one or more lipids and/or lipoproteins in the blood and pathological lipid qualities (Chien et al., 2013). It is considered as one of the most important risk factor of cardiovascular disease (Vijayakrishnan et al., 2013), cancer (Borena et al., 2011), diabetes (Hwang et al., 2009), diabetic neuropathy (Smith and Singleton, 2013), primary aldosteronism (Sang et al., 2013) and chronic kidney disease (Ryu et al., 2009). Despite improvements in lipid-lowering therapy during the last decades, hyperlipidemia still remains a great hazard to people health. In United States approximately one sixth of the adult population suffers from high total cholesterol (TC) (Vijayakrishnan et al., 2013). Hyperlipidemia results from complex interactions between genetic, dietary and environmental factors. Among them, dysregulation of lipid metabolism along with diet is one of the major causes of hyperlipidemia. Excessive absorption of lipids from diet or aberrant lipid metabolism in the body will lead to hyperlipidemia (Kumar et al., 2013).

1,3-Dichloro-2-propanol (1,3-DCP, $C_3H_6Cl_2O$) is a member of a group of chemicals known as chloropropanols, which are foodborne contaminants that can be formed when chloride ions react with glycerol and other lipids in different foodstuffs during food processing, cooking and storage. (Collier et al., 1991; Williams et al., 2010). Routes of exposure to 1,3-DCP include water, air, soil and food (Crews et al., 2003). The potential risk of 1,3-DCP to humans has recently increased due to its increased production and widespread use. Today, it has gained great attention for its toxic potential as carcinogen via genotoxic mechanism, and acts as an endocrine disruptor in humans and animals (Andres et al., 2013). Besides, 1,3-DCP has hepatotoxicity, nephrotoxicity, neurotoxicity, teratogenicity and mutagenicity (Kim et al., 2007; Williams et al., 2010; Eder et al., 2006; Lee et al., 2009). The toxic mechanism includes lipid peroxidation, DNA oxidation, GSH depletion, oxidative stress, mitochondrial membrane potential breakdown and intracellular $[Ca^{2+}]i$ increase (Katoh et al., 1998; Park et al., 2010; Hammond and Fry, 1999). However, there are no research concerned about the lipid metabolism exposure to 1,3-DCP. Thus, the present study was to investigate the effects of 1,3-DCP on lipid metabolism in mice and further to study the AMP-activated protein kinase (AMPK) signaling pathway to determine how 1,3-DCP affects by Western-blot. To our knowledge, no research exists systematically addressing the effects and mechanism of 1,3-DCP induced hyperlipidemia.

Abbreviations: 1,3-DCP, 1,3-dichloro-2-propanol; ACC, acetyl-CoA carboxylase; AMPK, adenosine 5′-monophosphate-activated protein kinase; FAS, fatty acid synthase; FAT, fatty acid translocase; FBS, fetal bovine serum; HMGCR, 3-hydroxy-3-methylglutaryl CoA reductase; p-ACC, phosphorylated acetyl CoA carboxylase; p-AMPK, phosphorylated AMP-activated protein kinase; PPAR, peroxisome proliferator activated receptor; SREBP, Sterol regulatory element-binding protein.

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In addition, we chose dose of 1,3-DCP from 0.1 to 1 mg/kg (lower than estimated no observed adverse effect level 1.4 mg/kg). The results will provide evidence for understanding the toxic mechanism of 1,3-DCP at low dose, thereby, will lay foundations for searching more sensitive biomarkers, which could be early detected of the toxicity exposure to 1,3-DCP.

2. Materials and methods

2.1. Animal studies

Adult C57BL/6J mice weighing 18–22 g were purchased from Jilin University Experimental Animal Center and acclimatized for 1 week before use in the experimental room (temperature 23 ± 2 °C). Mice were randomly divided into eight groups (n = 10/group, four groups are female and four groups are male) and were daily gavage-fed water (control) or 1,3-DCP in water at dose of 0.1, 0.5, 1 mg/kg/day for 4 weeks. During the experiment, the body weight and food intake were recorded. At the end of the experimental period, whole blood, liver and adipose tissue were collected from mice that had fasted for 12–14 h before sacrifice. All of the procedures were in strict accordance with the guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.2. Reagents

1,3-DCP was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Anti-acetyl-CoA carboxylase (ACC), anti-phosphorylated ACC (p-ACC) (Ser79), anti-AMPK, anti-phosphorylated-AMPK (p-AMPK) (Thr172), anti-Sterol regulatory element binding protein (SREBP)-1, anti-fatty acid synthase (FAS), anti-peroxisome proliferator activated receptor (PPAR) α, anti-fatty acid translocase (FAT), anti-3-hydroxy-3-methylglutaryl CoA reductase (HMGCR) and β-actin were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, USA).

2.3. Serum lipid profile analysis

Blood was collected from all mice and held at 4 °C for 10 h, serum was then prepared by centrifugation at 3500 rpm for 15 min and stored at −80 °C until analysis. Serum triglyceride (TG), TC, low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were enzymatically analyzed with a commercial kit (Zhejiang Elikan Biological Technology, Inc. China) and quantified by BECKMAN CX5 PRO automatic blood analyzer (Beckman Coulter, Inc. US).

2.4. Determination of TC and TG in liver

After removal from the animals, duplicate 1 g portions of the fresh liver were homogenized and extracted with chloroform–methanol mixture (2:1, v/v) as described by Folch et al. (1957). The concentration of TC and TG in liver was measured by enzymatic colorimetric methods using commercial kits (Applygen Technology, Inc. China).

2.5. Histopathology of liver and adipose tissue

The fresh liver and abdominal adipose tissue from the abdominal cavity were collected and were weighed to calculate the relative weight (relative liver or adipose tissues weight (g) = liver weight (or adipose tissues weight)/body weight × 100 g). Then liver and adipose tissue were obtained from all mice fixed in 10% buffered formalin and embedded in paraffin. After fixation, sections (3 μm) were routinely stained with hematoxylin and eosin stain (HE) for studying lipid accumulation and adipocyte size with an Olympus BX41 Microscope (magnification, ×400; Olympus, Tokyo, Japan). Adipose cell size distribution was determined in fixed cells as described previously (Takahashi et al., 2002).

2.6. Western blot analysis

The protein from liver was harvested in a cold RIPA buffer (50 mM Tris pH7.4, 150 mM NaCl, 1% TritonX-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, and 1 mM PMSF) containing sodium orthovanadate leupeptin and sodium fluoride. The mixtures were homogenized on ice for 30 min, then, were centrifuged at 12,000 rpm at 4 °C for 10 min. The protein content of the supernatants was determined with BCA Protein Assay Kit (Beyotime Institute of Biotechnology, China) using bovine serum albumin as a standard.

Equal amounts of protein samples (80 μg) were loaded into each lane of SDS–PAGE (sodium dodecyl sulfate–polyacrylamide gels) and electrophoresed under denaturing conditions. Subsequently, proteins were electro-transferred onto a polyvinylidene difluoride (PVDF) membrane. After blocking with 5% nonfat milk for 2 h, blots were incubated with primary antibodies at 4 °C overnight, followed by incubation with peroxidase-conjugated secondary anti-mouse antibody. The bound antibodies visualization was detected by ECL plus (GE Healthcare Buckinghamshire, UK). Protein quantity was determined by densitometry using Quantity One version 4.62, software (Bio-Rad Inc., USA).

2.7. Statistical Analysis

All data were expressed as the means ± S.D. and were analyzed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA), using one-way analysis of variance (ANOVA) and Student's t-test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effect of 1,3-DCP on body weight and food intake in C57BL/6J mice

As shown in Fig. 1, during the 4-week administration of 1,3-DCP (0.1–1 mg/kg/day), there was no significant difference in food intake and body weight, regardless of sex. And no death or clinical signs were observed among rats treated with 1,3-DCP.

3.2. Effect of 1,3-DCP on serum lipid profile in C57BL/6J mice

As shown in Fig. 2, 1,3-DCP (0.1–1 mg/kg/day) significantly and dose-dependently increased the serum TC, LDL-C, and TG compared with control. However, serum HDL-C was significantly decreased, indicating that 1,3-DCP induced hyperlipidemia in C57BL/6J mice.

3.3. Effect of 1,3-DCP on hepatic lipid accumulation in C57BL/6J mice

As shown in Fig. 3, the relative liver weight, hepatic TG and TC content of mice treated with 1,3-DCP (0.1–1 mg/kg/day) was significantly increased compared to control in dose-dependence manner at all sexes. From the gross appearance of liver we could see that the liver has a pale yellow appearance and a greasy consistency dose dependently. The HE staining of liver section also showed more lipid droplet appeared in liver treated with 1,3-DCP, indicating that 1,3-DCP induced lipid accumulation in liver.

3.4. Effect of 1,3-DCP on adipose tissue in C57BL/6J mice

As shown in Fig. 4, the relative adipose tissue weight was increased compared to control. The adipocyte size was also greatly increased by 1,3-DCP (0.1–1 mg/kg/day) dose dependently, indicative of cell hypertrophy. Also there was no sex difference.

3.5. Effects of 1,3-DCP on lipid metabolism-related signaling pathway of liver in C57BL/6J mice

As shown in Fig. 5, the ratio of p-AMPK/tAMPK, p-ACC/tACC and the expression of PPARα were both significantly decreased with the administration of 1,3-DCP (0.1–1 mg/kg/day). Conversely, the expression of FAT, SREBP1, HMGCR and FAS were significantly increased.

4. Discussion

In our study, we have demonstrated that 1,3-DCP (0.1–1 mg/kg/day) didn't affect the food intake and body weight of C57BL/6J mice in both sex. It seems no significant clinic toxicity to mice. While in fact, 1,3-DCP (0.1–1 mg/kg/day) dose dependently increased serum TG, TC, LDL-C and significantly decreased serum HDL-C, which are the typical syndrome of hyperlipidemia. Meantime, 1,3-DCP (0.1–1 mg/kg/day) significantly increased relative liver weight, relative adipose tissue weight, liver TG and TC, induced lipid accumulation in liver and greatly enlarged the size of adipose cells of C57BL/6J mice. Interestingly, the body weight didn't change, though relative adipose tissue weight was increased. It might indicate that the lean