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Nonalcoholic fatty liver disease induced by 13-week oral administration of 1,3-dichloro-2-propanol in C57BL/6J mice

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ABSTRACT

1,3-Dichloro-2-propanol (1,3-DCP) is a food born chloropropanol contaminant that has been detected during the production process of a wide range of foods. In this study, we investigated the effect of 1,3-DCP on lipid metabolism of mice after 13-week subchronic exposure. The data showed that 1,3-DCP (0.05–0.5 mg/kg/day) could induce nonalcoholic fatty liver disease (NAFLD) in C57BL/6J mice and the NOAEL was 0.01 mg/kg/day. In addition, we studied the signaling pathway to see how 1,3-DCP worked. The data showed that NAFLD induced by 1,3-DCP was due to the dysregulation of AMPK signaling pathway. As far as we are aware, this is the first study to use 13-week subchronic toxicology to investigate the effect of 1,3-DCP on the development of NAFLD in mice. Our study provided evidence for diet contaminants in the development of NAFLD and furthered the safety evaluation of 1,3-DCP through subchronic exposure.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD), which is regarded as the most common liver disease in the developed countries, is a clinic pathological syndrome with a full spectrum that ranges from simple steatosis and nonalcoholic steatohepatitis (NASH) to cirrhosis and hepatocellular carcinoma. It is strongly associated with obesity, hyperlipidemia, type 2 diabetes, metabolic syndrome and cardiovascular disease, occurring in 20–30% of the general population and 9.6% of the children (AlKhater, 2015; Milić and Stimac, 2012). It was reported approximately 30–40% of individuals with hepatic steatosis may progress to NASH, 20–25% of individuals with NASH may progress to advanced stages of hepatic fibrosis and cirrhosis, and 30–40% of NASH patients with cirrhosis may undergo a liver transplant or die of liver-related complications (McCullough, 2004, 2006). As such, NAFLD is a big imminent public health burden. Although the mechanism of NAFLD is not very clear, the two-hit theory in relation to the pathogenesis of NAFLD is widely accepted. In other word, hepatic lipid accumulation occurs first, followed subsequently by the

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development of inflammation and fibrosis (Malaguarnera et al., 2009). The factors from diet, such as dietary fat, fructose and other nutrition factors, exert a main role in the development of dysregulated lipid metabolism and NAFLD (Dumas et al., 2006). Thus, changing of diet and life style is the most popular therapy. However, the benefit from diet and life style change is only modest (Vos, 2014). In addition, diet is complicated, some food contaminants existed in diet also could disturb hepatic lipid metabolism and induce NAFLD, such as glycotoxins, some food additives and food processing contaminants (Jung et al., 2011; Leung et al., 2013; Choi et al., 2013). Thus, it is important to elucidate the relation between some important food contaminants and lipid metabolism.

1,3-Dichloro-2-propanol ($C_3H_6Cl_2O$, 1,3-DCP) is used in high volume as an intermediate in epichlorohydrin production. It is also a member of food borne contaminant, glycerol chlorohydrins, formed from ingestion of food to which hydrochloric acid-hydrolyzed vegetable protein or epichlorohydrin polyamine polyelectrolytes purified drinking water has been added (NIEHS, 2005). 1,3-DCP also can be formed when chloride ions react with glycerol or other lipids in different food stuffs during food processing, cooking and storage (Williams et al., 2010). According to the JECFA evaluation (JECFA, 2007), mean dietary exposure to 1,3-DCP ranges from 0.008 to 0.051 μg/kg/day in the general population and 0.025 to 0.136 μg/kg/day in high consumer (including children). In addition, another research reported higher intakes of 1,3-DCP from 7 to 27 μg/day per capita and 210 μg/day per capita through consuming of soy sauce and dimethylamine-epichlorohydrin copolymer refined corn syrup respectively (NIEHS, 2005). It is reported that 1,3-DCP has hepatotoxicity, nephrotoxicity, neurotoxicity, teratogenicity and mutagenicity (Kim et al., 2007; Williams et al., 2010; Lee et al., 2009). Today, it has gained great attention for its toxic potential as carcinogen and endocrine disruptor in humans (Andres et al., 2013).

In our previous work, we found 1,3-DCP (0.1–1 mg/kg/day) induced hyperlipidemia and hepatic lipid accumulation in mice through 30-day oral administration (Lu et al., 2014). In order to further detect the effect of 1,3-DCP on lipid metabolism in mice after subchronic exposure and find the no observed adverse effect level (NOAEL), we proceeded this 13-week oral administration test and examined the expression of proteins involved in AMPK signaling pathway. Accordingly, we found 1,3-DCP induced NAFLD in mice and identified the NOAEL. Furthermore, the dysregulation of AMPK signaling pathway contributed to this effects. As far as we are aware, this is the first study to use 13-week subchronic toxicology to investigate the effect of 1,3-DCP on the development of NAFLD in mice. A major advantage of this study was that it provided evidence for diet contaminants in the development of NAFLD and furthered the safety evaluation of subchronic administration of 1,3-DCP.

2. Materials and methods

2.1. Animals

Adult C57BL/6J mice weighing 18–22 g were purchased from Jilin University Experimental Animal Center and were acclimatized for 1 week before use in the experimental room (temperature 23 ± 2 °C). Mice were randomly divided into five groups (n = 10/group, half females and half males per group) and were daily gavage-fed water (control) or 1,3-DCP at dose of 0.01, 0.05, 0.1, 0.5 mg/kg/day for 13 weeks. 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- AMP-activated protein kinase (AMPK), anti-phosphorylated-AMPK (p-AMPK) (Thr172), anti-serine-threonine liver kinase B (LKB) 1, anti-glycerol-3-phosphate acyltransferase (GPAT) 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. Clinical observations, body weight and food consumption

All animals were observed daily for clinical signs and mortality. The type, date of occurrence, and severity of signs were recorded individually. Body weight and mean daily food consumption were recorded weekly throughout the study period.

2.4. Serum biochemistry

Blood was collected from all mice, serum was then prepared by centrifugation at 3500 rpm for 15 min and stored at −80 °C until analysis. Serum triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) 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).

2.5. Macroscopic examination and organ weights

Gross lesions were examined from all animals in all groups. The vital organs from each rat, such as brain, heart, liver, spleen, lungs, kidneys, adrenal glands, thymus, testis, epididymides, ovaries and uterus were removed and weighed. Paired organs were weighed together. Organ-to-final-body-weight was calculated.

2.6. Hepatic TC and TG

After removal from the animals, duplicate 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).