Experimental and Toxicologic Pathology 67 (2015) 491–498

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Effect of alcohol on diethylnitrosamine-induced hepatic toxicity: Critical role of ROS, lipid accumulation, and mitochondrial dysfunction

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ARTICLE INFO

Article history:
Received 30 May 2015
Accepted 3 July 2015

Keywords:
Alcohol
Diethylnitrosamine
Hepatic injury
ROS
Mitochondrial dysfunction

ABSTRACT

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the seventh in women worldwide. Chronic heavy alcohol consumption is a major risk factor for the development of HCC. However, the mechanism underlying the direct association between alcohol consumption and HCC is far from completely understood. In the present study, we investigated the effect of chronic consumption of alcohol on diethylnitrosamine (DEN)-induced cytotoxicity, which was essential for the malignant transformation. We showed that alcohol deceased survival of mice treated by DEN and promoted DEN-induced toxicity and hepatic injury. In addition, alcohol promoted DEN-induced increase of proinflammatory factors, collagen content and fibrosis-related genes, including collagen1, 3 and 4, TMIP1, TIMP2 and TGFβ1, and compensatory proliferation. Alcohol may increase alcohol dehydrogenase (ADH) and cytochrome P4502E1 (CYP2E1) expression, enhanced reactive oxygen species (ROS) generation, and resulted in a vicious circle between ROS generation, lipid accumulation, and mitochondrial dysfunction, aggravating liver injury and toxicity in DEN-treated mice. These results demonstrated that the combination of alcohol and carcinogens could aggravate carcinogen-induced cytotoxicity in the early phase of rumourigenesis through ADH and CYP2E1-generated ROS and the resultant cytotoxic process. The present study provided direct experimental evidence for alcohol-promoted toxicity and hepatic injury in carcinogen (DEN)-treated mice.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the seventh in women worldwide, and it represents the third most frequent cause of cancer death (Bosetti et al., 2014). The major risk factors of HCC include hepatitis induced by viruses (HBV and HCV), chronic high alcohol consumption, tobacco exposure, aflatoxin, and non-alcoholic steatohepatitis (Purohit et al., 2013). Among them, chronic alcohol consumption is a major risk factor for the development of HCC (Hassan et al., 2002). 10% of those who drink alcohol strongly for a long time may develop HCC (Morgan et al., 2004a). However, it remains difficult to build a direct relationship between alcohol consumption and the development of HCC. It is still tentative whether alcohol consumption contributes to the initiation or the progression of HCC. Thus, it is supposed that high consumption of alcohol causes HCC more easily in patients exposed to well-documented carcinogens (Shibata et al., 1986).

It is well established that diethylnitrosamine (DEN) is a strong hepatocarcinogenic dialkylnitrosoamine, which is frequently used to induce HCC models in rats or mice (Fausto and Campbell, 2010). DEN is a constituent of tobacco smoke, cheddar cheese, curd and fried meals and a number of alcoholic beverages (Bartsch and Montesano, 1984). The characteristic of DEN-induced development of HCC is similar with that occurred in human (Lee et al., 2004). Several weeks of DEN administration could induce DNA damage

Abbreviations: ADH, alcohol dehydrogenase; ALT, alanine transaminase; AST, aspartate transaminase; CYP2E1, cytochrome P4502E1; DEN, diethylnitrosamine; FFA, free fatty acids; HCC, hepatocellular carcinoma; HSCs, hepatic stellate cells; MPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species; TEM, transmission electron microscopy; TG, triglyceride; TNFα, tumor necrosis factor α.

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http://dx.doi.org/10.1016/j.etp.2015.07.003
0940-2993/

and cell death of hepatocytes, resulting in HCC in rodents (Naugler et al., 2007). Cytotoxicity induced by DEN is critical for the malignant transformation (Lauren et al., 1990; Williams et al., 2000, 1996).

In the current study, we aimed to investigate the effect of chronic consumption of alcohol on DEN-induced hepatic injury and to elucidate the possible mechanisms. The results showed that chronic consumption of alcohol significantly increased death rate in DEN-treated mice and liver injury. These effects of alcohol may be attributed to ROS generation, fatty liver and mitochondrial dysfunction, and the subsequent liver fibrosis.

2. Materials and methods

2.1. Materials

β-actin and α-SMA antibodies were purchased from Bioworld Technology. DHE were purchased from Beyotime Institute of Biotechnology. MitoSOX were purchased from Invitrogen. Oil Red O, DEN and most of the chemicals and reagents used in this study were procured from Sigma.

2.2. Animal treatment

All experiments were performed according to the procedures approved by Fourth Military Medical University Animal Care and Use Committee. 80 male 6–8 week C57 mice were purchased from Animal Centre of Fourth Military Medical University. The mice were housed under temperature (23 ± 2 °C) and humidity (55 ± 5%) condition with a standard light (12 h light/dark) cycle. Mice were randomly divided into three groups: Control, DEN, DEN + Alcohol and Alcohol groups. In DEN and DEN + Alcohol groups, mice were intraperitoneally injected with 50 mg/kg DEN for the first time and then given injection of 25 mg/kg DEN every four weeks throughout the experimental periods. Four weeks after the first injection of DEN, mice were orally given alcohol in drinking water throughout the experiment. The procedures of alcohol administration were as follows in order: 2% (v/v) for 3 days, 4% for 3 days, 8% for 10 days, 12 % for 9 days, and finally 16% throughout the experiment. Body weights were recorded every two weeks. Survival rate was recorded every week. The experimental period is 26 weeks. At the end, the mice were anesthetized with sodium pentobarbital, and then livers and blood samples were harvested for the assays.

2.3. ROS determination

ROS was detected by specific probe DHE and MitoSOX as we previously described (Wang et al., 2013). Briefly, frozen sections of livers were stained with 10 μM DHE or 1 μM MitoSOX (kept in the dark, 37 °C) for 30 min. Inflorescence intensity was observed under a laser scanning confocal microscope (Olympus).

2.4. Biochemical analysis

At the end of the animal treatment, blood samples were harvested and serum was separated for estimation of triglyceride (TG), free fatty acids (FFAs), aspartate transaminase (AST) and alanine transaminase (ALT) using commercial kits (Nanjing Jiancheng Company, China). Tumor necrosis factor α (TNFα) and IL6 levels were determined by Elisa kits (CUSABIO, China). Assays were conducted according to the manufacturer's instructions.

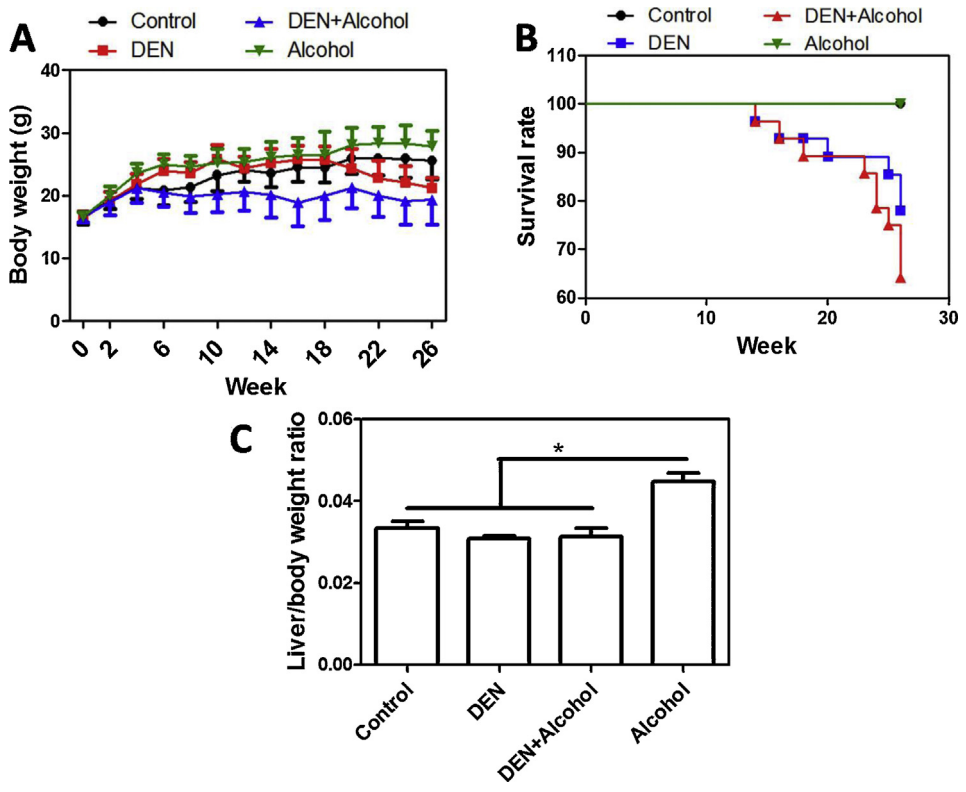

Fig. 1. Effect of alcohol on DEN-induced toxicity.
Mice were injected with DEN every four weeks. For the first time, the dose was 50 mg/kg and mice were injected with 25 mg/kg DEN for the last experimental periods. After the first four weeks, mice were orally given alcohol throughout the experiment. Body weights were recorded every two weeks (A). Survival rate was recorded every week (B). After the experiment, rate of liver/body weight was calculated (C). $^{*}p < 0.05$, between Alcohol and the other groups.