Chemico-Biological Interactions 258 (2016) 102–107

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Protective effects of ursolic acid against hepatotoxicity and endothelial dysfunction in mice with chronic high choline diet consumption

Dongyu Li, Daoyuan Ren*, Yiyang Luo, Xingbin Yang**

Shaanxi Engineering Laboratory for Food Green Processing and Safety Control, College of Food Engineering and Nutritional Science, Shaanxi Normal University, Xi'an, 710062, China

ARTICLE INFO

Article history:
Received 28 May 2016
Received in revised form
29 July 2016
Accepted 24 August 2016
Available online 25 August 2016

Keywords:
Choline
Ursolic acid
Preventive effect
Endothelial damage
Liver oxidative injury

ABSTRACT

This study was designed to investigate the preventive effect of ursolic acid (UA), a plant-based pentacyclic triterpenoid carboxyl acid, against vascular endothelial damage and liver oxidative injury in the mice fed with 3% dietary high choline (HC) water. Mice fed 3% HC water for 8 weeks significantly displayed liver oxidative stress and vascular endothelial dysfunction ($p < 0.01$). Furthermore, continuous administration of UA at 400 and 800 mg/kg bw in HC-fed mice could significantly inhibit the HC-induced elevation of serum total cholesterol, total triglyceride, low density lipoprotein-cholesterol, endothelin 1 and thromboxane A_2 levels as well as alanine aminotransferase and aspartate aminotransferase activities, while the HC-induced decline of serum high density lipoprotein-cholesterol, endothelial nitric oxide synthase, nitric oxide and prostaglandin I_2 levels could be markedly elevated following the treatment ($p < 0.05$, $p < 0.01$). UA at 400 and 800 mg/kg bw also increased the hepatic total superoxide dismutase and glutathione peroxidase activities and decreased hepatic malonaldehyde and non-esterified fatty acid levels, relative to HC-treated mice ($p < 0.05$, $p < 0.01$). Moreover, the conventional haematoxylin and eosin staining observation of the liver and vascular tissues suggested that UA exerted a significant protective role against HC diet-induced endothelial damage and liver injury in mice. This is the first report showing high intake of dietary choline can induce liver damage and UA has the potential preventive effect against vascular and liver injury in HC-fed mice.

1. Introduction

Cardiovascular disease (CVD) has become a major public health burden as well as the major cause of mortality and morbidity in the world [1]. The etiology of CVD is complex and multifactorial. There is substantial evidence that oxidative stress and inflammation play an important role in the initiation and progression of CVD [2]. Choline is increasing prominence as a conditionally important nutrient, especially for normal lipid metabolism and membrane function, which may serve many biological functions [3]. Interestingly, the recent study surprisingly reported that choline and its precursor phosphatidylcholine could be metabolized by gut microbiota to produce an intermediate compound known as trimethylamine (TMA), and subsequently TMA was further metabolized by hepatic flavin monooxygenases to form trimethylamine-*N*-oxide (TMAO), which was close associated with the build-up of arterial plaque and CVD [4]. Traditionally, existing researches consider that choline has many biological activities [5], which has inspired people to use choline as a dietary supplement to prevent hepatic damage and increase muscle performance [4]. Several researches have shown that diet with high choline (HC) supplementation of mice can effectively lead to endothelial dysfunction [4]. Therefore, the security of HC-derived diet on the blood vessel and liver deserves more attention and further exploration.

Ursolic acid (UA, 3-hydroxy-12-ursen-28-oic acid) is pentacyclic triterpene acid (Fig. 1A), which is the major component of some traditional medicine herbs or dietary fruits like loquats like loquats. UA is of interest to scientists because of its easy availability and biological activities including glycemic control, antiangiogenic, anti-inflammatory, hepatoprotective, hypolipidemic antiatherosclerotic and antioxidant properties in animals [6,7]. Although the protective effect of UA in plasma and tissues has been evaluated in rats [8], no sufficient work is done to study its preventive effect against cardiovascular damage and liver injury in HC-induced animal model. The purpose of the present study was therefore

* Corresponding author.
** Corresponding author.
E-mail addresses: dyren@snnu.edu.cn (D. Ren), xbyang@snnu.edu.cn (X. Yang).

http://dx.doi.org/10.1016/j.cbi.2016.08.019
0009-2797/

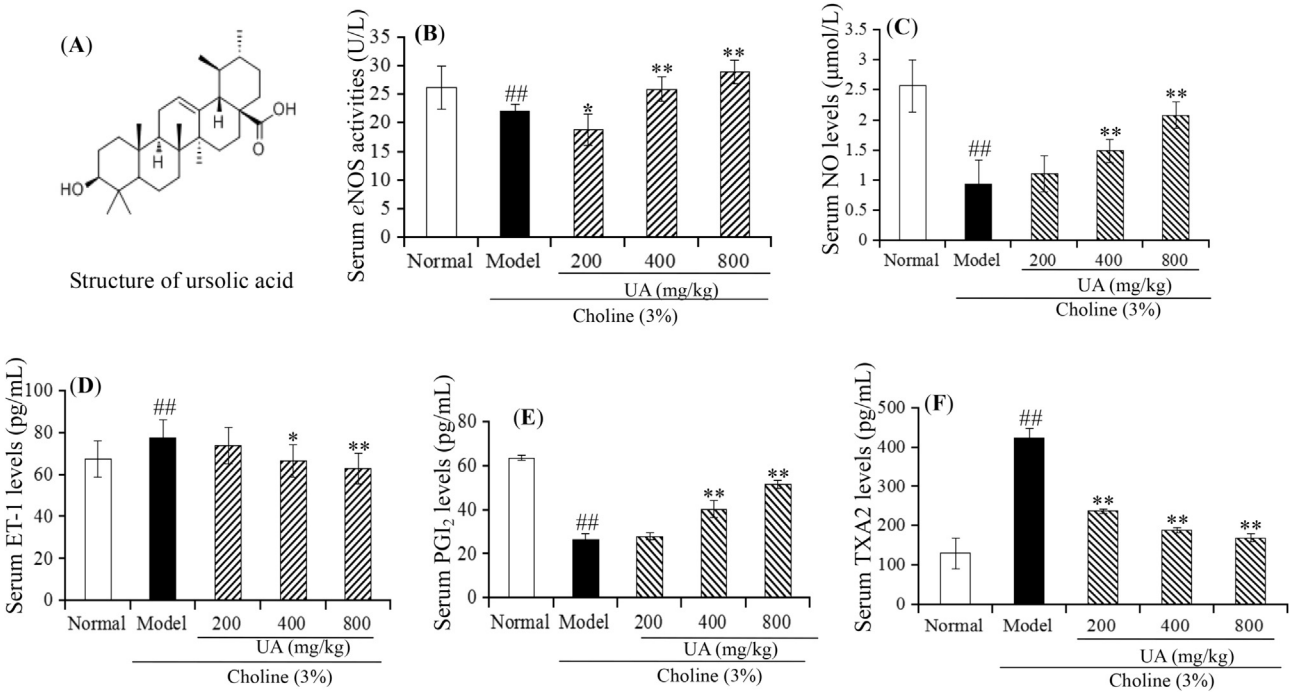

Fig. 1. Structure of ursolic acid (**A**) and its effects on serum levels of *e*NOS (**B**), NO (**C**), ET-1 (**D**), PGI_2 (**E**) and TXA_2 (**F**) in the mice fed 3% dietary choline water for consecutive 8 weeks. Values are presented as means ± SD for 10 mice in each group. $^{\#\#}p < 0.01$, vs the normal group. $^{*}p < 0.05$, $^{**}p < 0.01$, compared to the HC-induced group.

designed to evaluate the potential preventive effects of UA on vascular endothelial damage and liver oxidative stress induced by exposure of mice to 3% dietary choline water for 8 weeks.

2. Materials and methods

2.1. Materials and reagents

Food grade choline chloride was obtained from Senbo Biology Co., Ltd (Xi'an, China). UA was purchased from Waters Co. (Xi'an, China). Haematoxylin and eosin (H&E) were from Shanghai Lanji Technological Development Co. Ltd. (Shanghai, China). Assay kits of serum total cholesterol (TC), total triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were from Changchun Huili Biotechnology Co., Ltd. (Changchun, China). Detection kits for nitric oxide (NO), total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-P*x*), malonaldehyde (MDA), non-esterified fatty acid (NEFA), and ELISA kits of endothelial nitric oxide synthase (*e*NOS), endothelin 1 (ET-1), prostaglandin I_2 (PGI_2), and thromboxane A_2 (TXA_2) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Deionized water was prepared using a Millipore Milli Q-Plus system (Millipore, Bedford, MA, USA). All other regents and chemicals were of analytical grade.

2.2. Animal care and experimental design

Healthy male *Kunming* mice weighting 20–25 g were obtained from the Experimental Animal Center of the Fourth Military Medical University (Xi'an, China). The mice were housed in plastic cages under standard conditions with 12/12 h light-dark cycle at room temperature 22 ± 2 °C and humidity 60 ± 5%, which were provided with tap water and a standard rodent chow with ad libitum feeding (Qianmin Feed Factory). After environmental adaptation for one week, mice were randomly divided into five groups with 10 animals each: normal control group, HC control group (3% dietary choline water), and UA-treated groups (200 mg/kg bw for the low-dose group, 400 mg/kg bw for the middle-dose group, and 800 mg/kg bw for the high-dose group, suspended in 0.5% CMC-Na, 0.4 mL, intragastrically, ig. respectively). The dosage of UA was optimized before the study in mice according to the results of our previous experiments [9,10]. The normal and HC control groups were administered 0.4 mL 0.5% CMC-Na ig. once daily. All the administrations were conducted at between 8:00 to 9:00 a.m. for consecutive 56 days. The 3% dietary choline water was renewed every other day and the body weight of all the mice was measured once a week. The dosage of 3% choline was selected according to the previous report [4], and our pre-experimental results in mice [10]. Food and water intake were monitored weekly, and then the average food and water intake of each mouse in different groups was calculated. Two hours after the last administration, all the mice were fasted strictly, but allowed free access to water as usual for 12 h, and then all the animals were fully anesthetized by the inhalation of isoflurane and sacrificed to obtain blood, livers and blood vessels. The samples of blood were centrifuged at 2000*g* for 20 min, and then gathered the supernate in the tube to obtain the serum stored at 4 °C. The liver tissues were carefully excised and washed with ice-cold normal saline to clear away blood thoroughly, and then frozen at −80 °C immediately for assessment of biochemical parameters. On the basis of the records of the body weight and corresponding liver weight of every mouse, we calculated the hepatosomatic index (HI) according to the following formula: HI = liver weight/body weight × 100%. Part of each liver tissue (cut into several portions) and blood vessels were diverted into 4% paraformaldehyde for histopathological studies. All the experiments were conducted according to the Guidelines of Experimental Animal Administration published by the State Committee of Science and Technology of People's Republic of China.