International Immunopharmacology 47 (2017) 95–105

Contents lists available at ScienceDirect

International Immunopharmacology

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

Multi-targeted protection of acetaminophen-induced hepatotoxicity in mice by tannic acid

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ARTICLE INFO

Article history:
Received 23 October 2016
Received in revised form 23 March 2017
Accepted 28 March 2017
Available online 1 April 2017

Keywords:
Tannic acid
Acetaminophen
Anti-oxidation
Anti-inflammation
Anti-apoptosis

ABSTRACT

Tannic acid (TA) is the polyphenol that has beneficial health effects against oxidative stress. However, the hepatoprotective effects of TA are still relatively unknown. In the present study, we evaluated the effects of TA on an acetaminophen (APAP)-induced hepatotoxicity model, which was established by administration of 400 mg/kg of APAP. The levels of alanine transferase (ALT), aspartate transferase (AST), dendothelin-1 (ET-1), nitric oxide (NO) and malondialdehyde (MDA) in the APAP-induced hepatotoxicity mice were significantly increased (up to ~200%), while their levels were reduced by pretreatment with TA (25 and 50 mg/kg) ($P < 0.05$). The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in the APAP-induced hepatotoxicity mice were significantly reduced (lower to ~65%), while their activities were increased by pretreatment with TA (25 and 50 mg/kg) ($P < 0.05$). In addition, pretreatment with oral TA (25 and 50 mg/kg) for 3 days before the APAP administration dose-dependently ameliorated changes in hepatic histopathology, suppressed overexpression of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), c-fos, c-jun, NF-κB (p65) and caspase-3 (all $P < 0.05$), downregulated bax and upregulated bcl-2, nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) (all $P < 0.05$) in the liver. These results indicate that TA exhibits significant hepatoprotective effects against APAP-induced hepatotoxicity and suggest that the hepatoprotective mechanisms of TA may be related to anti-oxidation, anti-inflammation and anti-apoptosis.

1. Introduction

Acetaminophen (APAP), which is a commonly recommended drug to relieve fever and pain, is safe in therapeutic doses, but fatal hepatotoxicity can result from excessive doses due to hepatic centrilobular necrosis [1]. Under normal conditions, the free radical induced by APAP metabolism can be eliminated by the antioxidant defense system. Once the balance is disrupted, these radicals induce oxidative stress and lead to hepatocellular injury [2]. Lipid peroxidation, mitochondrial damage and ATP depletion in proteins are responsible for oxidative stress in APAP-induced hepatotoxicity [3]. Thus, the agent(s) with anti-oxidant properties may have protective effects against the hepatocellular injury.

Tannic acid (TA) (Fig. 1) is a natural compound that is found in high levels in fruits and vegetables, such as strawberries, beans, grapes, persimmons, cocoa and tea. People consume, on average, 1 g/day of TA in the United States, and TA is also considered to be a safe food additive [4]. Furthermore, laboratory tests have shown that drinking red wine raises high-density lipoprotein-cholesterol and plasma antioxidant levels more than compared to drinking white wine [5]. Incubating white wine with grape skins, which contain a significant fraction of TA, endows white wine with a similar ability to inhibit low-density lipoprotein-oxidation [6]. This effect is simulated by anti-oxidants contained in red wine, and TA plays an important role against liver injury induced by significant alcohol consumption [7]. TA has also been reported to limit the mutagenicity of polycyclic aromatic hydrocarbons in salmonella typhimurium and Chinese hamster V79 cells and suppress the tumorigenicity of polycyclic aromatic hydrocarbons and *N*-methyl-N-nitrosourea in mouse skin, lungs and forestomachs [8]. The anti-carcinogenic and anti-mutagenic abilities of TA potentially derive from its anti-oxidant properties, which reduce the production of reactive metabolites and their downstream deleterious effects [8,9]. TA was reported to possess multiple beneficial pharmacological activity including cardioprotective effects on isoproterenol-induced myocardial damage

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http://dx.doi.org/10.1016/j.intimp.2017.03.027
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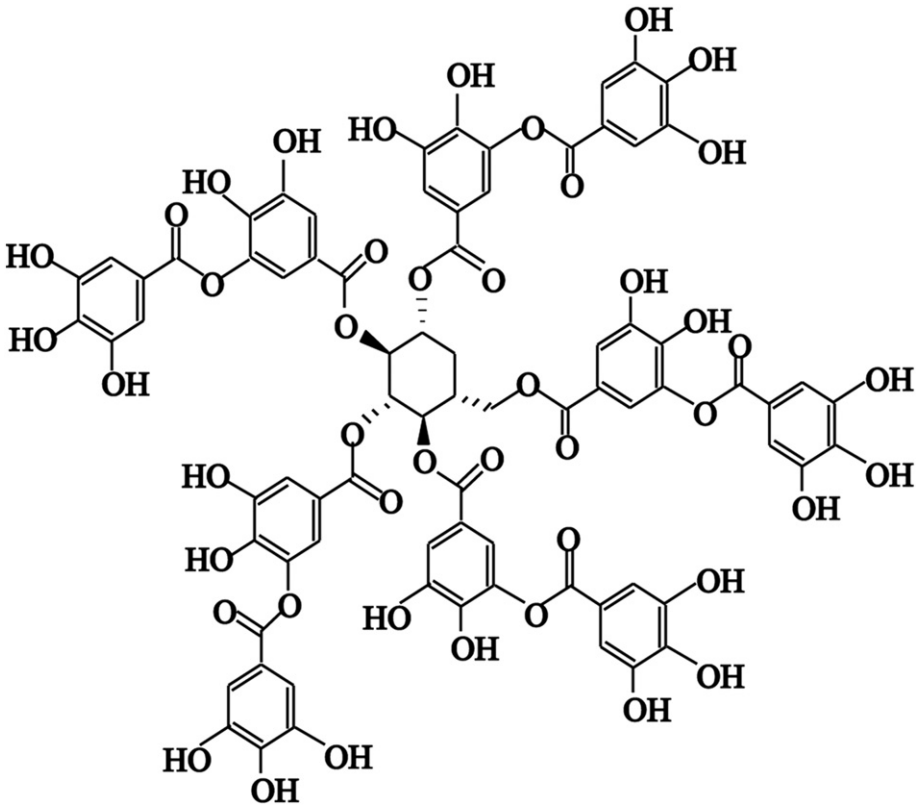

Fig. 1. Chemical structure of tannic acid (TA).

[10], vasodilative effects on rat mesenteric arteries through activation of Kv7.4 and Kv7.3/7.5 K^+ channels [11], inhibitory effects on L-type Ca^{2+} channels, Ca^{2+} transient and contractility in rat myocardium [12] and ameliorative effects on liver fibrosis [13].

Based on the anti-oxidant properties of TA, we hypothesize that it may protect the liver from injury of reactive oxygen species induced by overdoses of APAP. We accordingly investigated the hepatoprotective effects of TA by assessing the morphological and biochemical changes in an APAP-induced hepatotoxicity model. Also, we explored the underlying mechanisms of TA on hepatotoxicity by measuring hepatotoxicity-related factors.

2. Materials and methods

2.1. Drugs and reagents

APAP and TA were acquired from Sigma Chemicals (St. Louis, MO, USA). Silymarin was obtained from Xi'an Yihe Bioengineering Institute (Xi'an, China). Unless otherwise specified, the rest of the chemicals were acquired from Sigma Chemical Co. (St. Louis, MO, USA), which are of maximum purity.

2.2. Animals

The Experimental Animal Center of Hebei Medical University is responsible for the provision of Kunming mice (20–22 g) under standard conditions (22 ± 2 °C, 50–60% relative humidity, 12-h light-dark cycle). All procedures were carried out under the Guidelines of Animal Experiments from the Committee of Medical Ethics, Ministry of Health of China, and were approved by the Ethics Committee for Animal Experiments of Hebei Medical University (approval number: HEBMU-2014-10; approval date: October 25, 2014).

2.3. Influence of TA on normal mouse liver

A test was firstly conducted on mice to evaluate the safety of TA. 20 mice were randomly assigned to 2 groups: control and H-TA groups. Each group received oral administration of either normal saline solution for the control group or TA (50 mg/kg) alone for the H-TA group one time every day for 3 days. The physical status, behavior, signs of morbidity and mortality of mice were monitored every day. 12 h after the last administration of TA, the blood and tissue samples of mice in each group were collected for further analyses. No adverse effects on mice behavior and mortality were observed during the entire observation period.

2.4. Hepatotoxicity model and treatments

The 50 mice were allocated into five groups after acclimation ($n = 10$): Control, APAP, L-TA + APAP, H-TA + APAP and Sily + APAP groups. The control group was given normal saline solution orally. The L-TA + APAP, H-TA + APAP and Sily + APAP groups were given TA (25 and 50 mg/kg) or silymarin (100 mg/kg) orally for 3 consecutive days [2,13]. 2 h after the last TA or silymarin administration, mice were given APAP (400 mg/kg) to establish an APAP-induced hepatotoxicity model [14,15]. The blood and tissue samples were collected 12 h after APAP administration for further analyses.

2.5. Biochemical analysis

The collected blood samples were centrifuged at 3000 rpm for 15 min at 4 °C, and the levels of ALT and AST in the serum were assayed using the Reitman and Frankel method [16] with a commercial detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), which is based on the colorimetric method.