Food and Chemical Toxicology 48 (2010) 3137–3143

Contents lists available at ScienceDirect

Food and Chemical Toxicology

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

Anti-fibrotic effects of the anthocyanins isolated from the purple-fleshed sweet potato on hepatic fibrosis induced by dimethylnitrosamine administration in rats

Jae Ho Choi [a,b], Yong Pil Hwang [a], Chul Yung Choi [c], Young Chul Chung [d,**], Hye Gwang Jeong [a,*]

[a] Department of Toxicology, College of Pharmacy, Chungnam National University, 220 Gung-dong, Daejeon 305-764, Republic of Korea
[b] College of Pharmacy, Chosun University, 375 Seosuk-dong, Gwangju 501-759, Republic of Korea
[c] Department of Pharmaceutical Engineering, College of Public Health and Natural Science, Korea International University, Jinju 660-759, Republic of Korea
[d] Department of Food Science, College of Public Health and Natural Science, Korea International University, Jinju 660-759, Republic of Korea

ARTICLE INFO

Article history:
Received 1 June 2010
Accepted 15 August 2010

Keywords:
Purple sweet potato
Anthocyanins
Dimethylnitrosamine
Hepatic fibrosis
α-SMA
Collagen

ABSTRACT

In current study, we investigated the protective effects of the anthocyanin fraction (AF) obtained from the purple-fleshed sweet potato on hepatic fibrosis induced by dimethylnitrosamine (DMN) administration in rats. Treatment with DMN for 4 weeks produced marked liver fibrosis as assessed by increased serum alanine aminotransferase and aspartate aminotransferase activity and hepatic collagen content. These increases were inhibited by treatment with AF prior to the administration of DMN. In addition, AF inhibited DMN-induced reductions in rat body and liver weights in a dose-dependent manner. Histopathological evaluation of the rat livers revealed that AF reduced the incidence of hepatic fibrosis lesions and inhibited DMN-induced increases in α-smooth muscle actin (α-SMA) and collagen type I and III expression levels. AF also decreased DMN-induced expression levels platelet-derived growth factor receptors-beta, tumor necrosis factor-alpha and transforming growth factor-beta. This study demonstrates that AF administration can effectively improve liver fibrosis caused by DMN, and may be used as a therapeutic option and preventive measure against hepatic fibrosis.

1. Introduction

Hepatic fibrosis is caused by a variety of pathological factors, including alcohol consumption, drug abuse, autoimmune disease, metabolic disease, constant cholestasis, and hepatic viruses (Friedman, 2000). Hepatic fibrosis, a response to chronic liver injury, results from the excessive deposition of extracellular matrix (ECM) proteins and can ultimately lead to cirrhosis of the liver (Hsiang et al., 2005). Hepatic stellate cells (HSCs) are pericytes found in the perisinusoidal space of the liver (a small area between the sinusoids and hepatocytes). In response to liver injury HSCs undergo rapid activation, which leads to functional and morphological changes. Activated HSCs are proliferative and fibrogenic and cause the accumulation of ECM proteins, including transforming growth factor-beta (TGF-β), alpha-smooth muscle actin (α-SMA) and collagen (Friedman, 2008; Kisseleva and Brenner, 2008; Henderson and Iredale, 2007; Wallace et al., 2008).

During hepatic fibrogenesis, HSCs are activated by reactive oxygen species (ROS), growth factors, and profibrogenic cytokines released from damaged hepatocytes and Kupffer cells (Kisseleva and Brenner, 2007), and their cognate receptors are associated with this transition. Among these factors, autocrine and paracrine signaling via platelet-derived growth factor (PDGF), a potent ligand for PDGF receptors (PDGFRs), stimulates HSC growth and proliferation (Lotersztajn et al., 2005; Pinzani and Marra, 2001). Also, it has been reported that hepatocellular injury mediates inflammation response by tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) and enhances the proliferation of cultured stellate cells and their collagen synthesis (Casini et al., 1994; Hierholzer et al., 1998; Ramadori and Armbrust, 2001).

Dimethylnitrosamine (DMN) leads liver fibrogenesis and subsequent cirrhosis. DMN exhibits a potent hepatotoxicant through metabolic activation by cytochrome P450 2E1 (CYP2E1) in animal experimental models (George et al., 2001). DMN-induced hepatic fibrosis model to reproduce most of the features observed during human liver fibrosis, such as ascites, nodular regeneration, overproduction of extracellular matrix including collagen and histopathological changes (Bataller and Brenner, 2005). It is a valuable animal model for studying mechanisms of hepatic fibrosis, and may provide a model for the rapid screening of anti-fibrotic agents.

Anthocyanins, a class of naturally presenting polyphenol compounds, are widely distributed in fruits, beans, cereals, and vegetables. In animal models, the biological activity of anthocyanins includes powerful antioxidant effects (Shih et al., 2007), anti-inflammatory effects (Karlsen et al., 2007), and anti-tumor

* Corresponding author. Tel.: +82 42 821 5936.
** Corresponding author. Tel.: +82 55 751 8316.
E-mail addresses: fnjung@hanmail.net (Y.C. Chung), hgjeong@cnu.ac.kr (H.G. Jeong).

0278-6915/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2010.08.009

properties through the stalling of the growth of pre-malignant cells (Shih et al., 2005). Anthocyanins also help to prevent obesity, hyperglycemia (Tsuda et al., 2003), and asthma (Park et al., 2007). The component part of vegetables and fruits containing abundant plant polyphenols is associated with a low risk of life-style-related diseases, such as cardiovascular disease (Hertog et al., 1993; Keli et al., 1996; Aviram and Fuhrman, 1998; Arts and Hollman, 2005). Recently, purple sweet potato (*Ipomoea batatas*) has aroused extensive attention because of its unique color, nutrition and its role in health care (Lila, 2004). There is a high content of anthocyanin pigments in the tuber of some purple sweet potato cultivars. The anthocyanins from purple sweet potato are more stable than the pigments of strawberry, red cabbage, perilla and other plants. So purple sweet potatoes have been regarded as a good source of stable anthocyanins as a food colorant and purple sweet potato color could be recognized as a physiologically functional food factor. Anthocyanins of purple sweet potato possess biological functions such as scavenging free radicals, anti-mutagenicity, anti-carcinogen activity and anti-hypertensive effects (Ahmed et al., 2010). We previously reported that the anthocyanin fraction (AF) obtained from the purple-fleshed sweet potato has a potent hepatoprotective effect in acetaminophen (APAP)-induced hepatic damage mouse model (Choi et al., 2009). AF upregulated the activities of antioxidants such as glutathione and glutathione S-transferase, and acts as a free radical scavenger. Also, AF inhibited the APAP-induced hepatotoxicity through blocking of CYP2E1-mediated APAP bioactivation. However, the anti-fibrotic effects of AF have not reported. In current study, we investigated the anti-fibrotic effects of AF on hepatic fibrosis induced by DMN administration in rats.

2. Materials and methods

2.1. Preparation of AF

AF was purified from the aqueous extract of whole bodies of purple-fleshed sweet potato supplied by the Ji San Food Co. (Hamyang, Korea). AF was prepared as described elsewhere (Lee et al., 2000) and their compositions were previously published. The compositions of the purple-fleshed sweet potato of anthocyanin were cyanidin-3-O-glucoside chloride, malvidin-3-O-glucoside chloride, pelargonidin-3-O-glucoside chloride, and peonidine-3-O-glucoside chloride (Goda et al., 1997). Briefly, uniformly sized tubers without defects were washed, peeled, diced into 0.5-cm cubes, and freeze-dried. Freeze-dried tuber samples (0.5 g) were homogenized in 15 mL ethanol/water (85:15 vol/vol) using an Ultra Turrax (Divtech Equipment Co., Cincinnati, OH), Tissumizer (30,000 rpm) and stored for 12 h at −20 °C. Supernatants obtained by centrifugation were concentrated, diluted to 5 mL using 0.01% aqueous HCl (whole extract), and passed through C-18 Sep-Pak cartridges (Waters, Milford, MA) preconditioned with 0.01% acidified methanol to absorb anthocyanins (Lee et al., 1997). Anthocyanins were obtained by eluting columns with 0.01% methanolic HCl, concentrating eluates (under a nitrogen flow), and reconstituting with either alcohol or dimethyl sulfoxide. The yield of dried residue corresponded to 2.5% of the original dry whole body weight. This was powdered in a grinder, passed through a 40-mesh sieve, and stored at −20 °C until use.

2.2. Animals and treatment

Five-week-old male Sprague–Dawley (SD) rats were obtained from Daehan Biolink (Chungbuk, Korea). The animals were allowed free access to Purina rodent chow (Seoul, Korea) and tap water and were maintained under specific pathogen-free conditions. Animals were acclimatized to the temperature (22 ± 2 °C) and humidity (55 ± 5%) of controlled rooms with a 12-h light/dark cycle for at least 1 week prior to experimentation. All animal experiments were performed according to the rules and regulations of the Animal Ethics Committee, Chosun University.

The rats were divided into six groups. To induce hepatic fibrosis, we administered DMN (Sigma Chemical Co., St. Louis, MO) dissolved in sterile saline (10 mg/kg body weight) as an intraperitoneal injection three times per week for 4 weeks. AF was dissolved in saline. Rats were intragastrically administered 50, 100, and 200 mg/kg of AF per day six times per week for 4 weeks. The control and DMN-treated groups were administered saline (intragastrically) without drug administration. The animals were sacrificed on day 29 (Fig. 1). Each group consisted of five rats. Livers were excised, were weighed, and underwent histopathological examination and determination of collagen content using the Sircol collagen assay kit (Biocolor, Belfast, Northern Ireland).

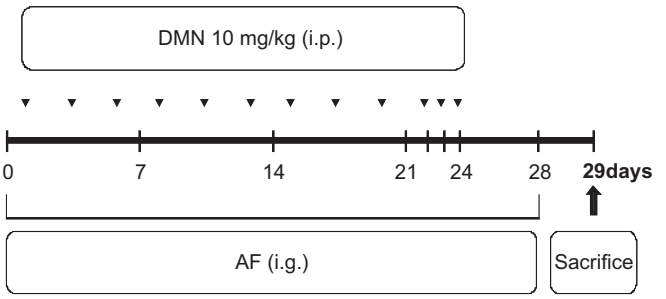

Fig. 1. Schematic diagram of the experimental procedure. Rats were divided into six groups. To induce hepatic fibrosis, we administered DMN dissolved in sterile saline (10 mg/kg body weight) by intraperitoneal (i.p.) injection three times per week for 4 weeks. AF was dissolved in saline. Rats were intragastrically (i.g.) administered 50, 100, and 200 mg/kg/day of AF six times per week for 4 weeks. The control and DMN-treated groups were administered saline alone (i.g.) without drug. The animals were sacrificed on day 29. Each group consisted of five rats.

2.3. Hepatotoxicity studies

To assess hepatotoxicity, we measured the serum activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using spectrophotometric diagnostic kits (Sigma Chemical Co., St. Louis, MO, USA).

2.4. Histological examinations

The left lateral lobe of the liver was sliced, and tissue slices were fixed in 10% buffered-neutral formalin for 24 h. The fixed liver tissue slices were embedded in paraffin, sectioned, deparaffinized, and rehydrated using standard techniques. Sections 5 μm in thickness were subjected to hematoxylin and eosin and Masson's trichrome staining prior to examination (Vyberg et al., 1987). An arbitrary scope was given to each microscopic field viewed at a magnification of 100×. A minimum of 10 fields were scored per liver slice. The extent of fibrosis was graded as 0, no increase; 1, slight increase; 2, moderate increase; 3, distinct increase; or 4, severe increase. The extent of periportal bridging, intralobular degeneration, portal inflammation, and fibrosis was also graded according to Knodell's scoring method (Moragas et al., 1998).

2.5. Collagen content

The right lobe of the liver (0.2 g) was homogenated with 0.5 M acetic acid containing 1 mg pepsin (at a concentration of 10 mg tissue/10 ml acetic acid solution). The resulting mixture was then incubated for 24 h at 4 °C with stirring. Liver collagen content was determined by assaying total soluble collagen using the Sircol collagen assay kit (Biocolor, Belfast, Northern Ireland) according to the manufacturer's instructions. Acid soluble type I collagen supplied with the kit was used to generate a standard curve.

2.6. Semi-quantitative RT-PCR

Total RNA was extracted from frozen liver samples with RNAiso reagent (Takara, Kyoto, Japan) according to manufacturer's protocol and stored at −80 °C until use. Then 0.5 μg RNA was used for reverse transcription and amplified by polymerase chain reaction (PCR) using the access RT-PCR system Takara thermal cycler (TaKaRa, Seoul, Korea). The PCR amplification protocol was 30 cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 45 s. The termination cycle included a prolonged extension at 72 °C for 7 min. Amplified products were resolved by 2% agarose gel electrophoresis, stained with ethidium bromide, and photographed under ultraviolet light. The coding sequences of the genes are presented in Table 1.

2.7. Western blotting

To analyze protein expression, we homogenized liver tissues in a Potter–Elvehjem homogenizer with four volumes (w/v) of 10 mM Tris–HCl (pH 7.4) containing 0.15 M KCl, 0.1 mM EDTA, 1 mM dithiothreitol, and 0.01 mM phenylmethoxysulfonyl fluoride. Then 50–150 μg protein was routinely resolved by SDS–PAGE, transferred to a PVDF membrane (Amersham Pharmacia Biotech, Piscataway, NJ, USA), and probed with the appropriate primary and secondary antibodies. PDGFR-β, COL1A1, COL3A1, and β-actin (C4) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The secondary antibody was a horseradish peroxidase-coupled anti-rabbit or mouse IgG (Beverly, MA, USA). Anti-α-SMA antibody was purchased from Dako (Glostrup, Denmark). Membranes were probed with an ECL western blot detection system according to the manufacturer's instructions.