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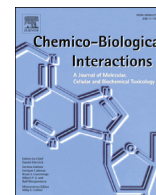

Hepatoprotective effects of allyl isothiocyanate against carbon tetrachloride-induced hepatotoxicity in rat

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ABSTRACT

We evaluated the hepatoprotective activity of allyl isothiocyanate (AITC) against carbon tetrachloride (CCl_4)-induced liver injury in rats. Sprague Dawley rats were orally administered AITC at doses of 5 (AITC 5) and 50 (AITC 50) mg/kg body weight once daily for 3 days, with or without intraperitoneal injection of CCl_4. Serum chemistry was assessed for changes in alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The enzyme activities of superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) were examined in liver tissues, while pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) mRNA expression were analyzed using real-time polymerase chain reaction. And heme oxygenase-1 (HO-1) and ionized calcium binding protein-1 (Iba-1) immunoreactivities were evaluated by Western blot analysis and immunohistochemistry, respectively. In serum chemistry, the oral administration of AITC itself did not affect the serum levels of ALT or AST, furthermore pretreatment with AITC 5 and AITC 50 significantly reduced the ALT and AST activity levels that were elevated in CCl_4-intoxicated rats. In addition, AITC significantly suppressed the reduction of SOD and CAT, and the elevation of MDA, TNF-α mRNA expression, on the other hands, induced the expression of HO-1 compared with those of the vehicle-treated CCl_4 group. The histopathological evaluation and Iba-1 immunoreactivity also supported the hepatoprotective effects of AITC against CCl_4-induced liver injury. These results suggest that AITC ameliorates oxidative liver injury, possibly through reducing lipid peroxidation, enhancing antioxidant enzymes, and suppressing Kupffer cells and macrophages.

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1. Introduction

Many cruciferous vegetables, including radish [1–3], cabbage, kale, horseradish, and wasabi [4], are widely and often consumed by humans [5,6]. Glucosinolates [2,5], in addition to anthocyanins [7], are common ingredients in cruciferous vegetables and may play important roles in various activities, such as anti-oxidation and anti-inflammation [6,8], and commonly divided into aliphatic, aromatic, and indole glucosinolates [5].

Allyl isothiocyanate (AITC) is a hydrolysis product of the glucosinolate sinigrin, which is one kind of aliphatic glicosinolates, as a result of myrosinase activity [9,10], an important component in radish. AITC and other isothiocyanates, including butyl isothiocyanate and phenethyl isothiocyanate, are considered as phytochemical chemopreventive agents [11]. Biologically, it has been known that AITC is metabolized quickly in the liver and AITC metabolites conjugate with glutathione or *N*-acetylcysteine, and are distributed in most organs and tissues, exerting anti-obesity effects

Abbreviations: AITC, allyl isothiocyanate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CAT, catalase; CCl_4, carbon tetrachloride; DAB, diaminobenzidine; HO-1, heme oxygenase-1; Iba-1, ionized calcium binding protein-1; MDA, malondialdehyde; Nrf2, nuclear factor-erythroid-2-related factor 2; PBS, phosphate-buffered saline; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; TNF-α, tumor necrosis factor alpha.

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in rat [12]. Furthermore, it is reported that AITC possesses the antioxidant activity [13], anti-inflammatory [13–15], anti-cancer activities [3,9,16,17], and hypoglycemic effects [18] as well as the amelioration of insulin resistance [19]. Especially, AITC increased the mRNA and protein levels of the cytoprotective nuclear factor-erythroid-2-related factor 2 (Nrf2) and heme oxygenase (HO)-1, which protect cells against radical attacks [13].

Carbon tetrachloride (CCl_4) is a potent hepatotoxin producing centrilobular hepatic necrosis in experimental animals [20–22]. This model has been used widely for the ethnopharmacological evaluation of the beneficial potential of plant extracts [20], as well as studies of mechanical liver injury, which is similar to human liver disease in terms of morphology and the biochemical features of the cellular lesions [23]. CCl_4-induced liver injury is characterized by two subsequent phases: direct oxidative stress, leading to hepatocyte death in the first phase [24], and secondary damage from activated hepatic macrophages, including Kupffer cells, activated by free radicals released by inflammatory mediators, such as tumor necrosis factor-alpha (TNF-α) [25,26] and other pro-inflammatory cytokines [27]. The pathophysiological changes induced by CCl_4 can be ameliorated by medicinal plant extracts [20,28] and vegetables, including the radish and its component sulforaphane [29], which induce antioxidant and cytoprotective effects, possibly through radical scavenging and detoxification.

The aim of the present study was to evaluate whether AITC, an isothiocyanate from glucosinolates sirigin, ameliorates CCl_4-induced liver injury in rats.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals including AITC (377430) and reagents, unless otherwise noted, were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA). The commercial reagent kits for determination of serum chemistry were provided by Beckman Coulter Ireland Inc. (Clare, Ireland). For measurement of superoxide dismutase (SOD) and catalase (CAT) activity, we purchased colorimetric assay kits from abcam (Cambridge, UK).

2.2. Animals

Sprague-Dawley rats of both sexes (8 weeks old), weighing 200–300 g, were used for all experiments (Orient Bio, Gyeonggi-do, Korea). The animals were maintained at a controlled temperature of 25–28 °C under a 12/12-h light/dark cycle and fed a standard diet and water *ad libitum*. All experimental procedures were conducted in accordance with the guidelines for the Care and Use of Laboratory Animals at Jeju National University in Jeju City, Republic of Korea (permission no.: 2014-0026). Every effort was made to minimize the number of animals and their suffering.

2.3. Experimental design

AITC was diluted in phosphate-buffered saline (PBS, pH 7.4) and administered orally. The lethal dose 50 of AITC in rat was 112 mg/kg [30], and we checked the side effect such as gastric hemorrhages by oral administration of 100 mg/kg AITC (data not shown). Thus, doses of 5 (AITC 5) and 50 mg/kg (AITC 50) were used in the present study. To induce liver injury, a 1:1 (v/v) mixture of CCl_4 and sterile olive oil was injected intraperitoneally (1.5 mL/kg), as in previous studies [21,22]. Rats were divided randomly into five groups ($n = 5$); I. Normal control group, II. AITC 50-treated group, III. Vehicle + CCl_4, IV. AITC 5 + CCl_4, V. AITC 50 + CCl_4. AITC was administered 3 consecutive days prior to CCl_4 challenge. Rats were fasted and sacrificed 24 h after CCl_4 injection. Blood samples and liver tissues were collected at the time of sacrifice for serum chemistry and tissue examinations, respectively.

2.4. Histopathological examination

The clinical and laboratory examination described below followed the procedures used in our previous CCl_4-induced liver injury studies [28,31]. The liver tissues were immediately fixed in 10% neutral buffered formalin solution for 2 days and processed routinely for paraffin wax embedding. The liver tissues were cut into 5-μm-thick sections. After deparaffinization, sections were stained with Harris modified hematoxylin and eosin solution (Sigma-Aldrich).

2.5. Measurement of serum biochemical markers

The blood samples (n = 4 animals in each groups) were allowed to coagulate at room temperature and were centrifuged (2000 *g*, 15 min, room temperature), and the serum fraction collected. The serum levels of ALT and AST were detected using commercially available kits (Beckman Coulter Ireland Inc.).

2.6. Determination of lipid peroxidation and antioxidant enzyme levels

The tissue of liver (n = 3 animals in each groups) were frozen immediately and were homogenized in a glass-Teflon homogenizer with 50 mM phosphate buffer (pH 7.4) to obtain a 1:9 (w/v) whole homogenate. Lipid peroxidation was determined by measuring thiobarbituric acid-reactive substances (TBARS) in the homogenate and expressed as the malondialdehyde (MDA) concentration according to our previous study [20]. Briefly, liver was homogenized in ice-cold 1.15% KCl, and 500 μL of the homogenate supernatant were mixed with 3 mL 1% phosphoric acid (Sigma-Aldrich) and 1 mL 0.6% 4,6-dihydroxy-2-mercaptopyrimidine (Alfa Aesar, Ward Hill, MA, USA). The mixture was heated to 100 °C for 45 min. After cooling to room temperature, 3 mL *n*-butanol were added to each sample, followed by shaking. After centrifugation (1500 *g*, 10 min), the supernatant fraction was isolated and the absorbance measured at 540 nm. The amount of TBARS was determined by a standard curve using 1, 1, 3, 3-tetrahydroxypropane (Sigma-Aldrich). Antioxidant enzyme activities, including those of SOD and CAT, were also determined using commercial assay kits (abcam).

2.7. Quantitative real time PCR

DNA was extracted from the livers of normal control, AITC 50, vehicle + CCl_4, AITC 5 + CCl_4, and AITC 50 + CCl_4 groups (n = 4 animals in each groups) using an RNAeasy® Lipid Tissue Mini kit (Qiagen, Venlo, Netherlands), following the manufacturer's protocol. The qPCR was performed by TaqMan Universal Master Mix (Applied Biosystems, Carlsbad, CA, USA) with following primers: TNF-α (forward: 5′-GAC CCT CAC AGT CAG ATC ATC TTC T-3'; reverse: 5′-TGC TAC GAC GTG GGC TAC G-3′), Interleukin-1β (IL-1β) (forward: 5′-CCC TGC AGC TGG AGA GTG TGG-3'; reverse: 5′-TGT GCT CTG CTT GAG AGG TGC T -3′), and GAPDH (forward: 5′-ATG ATT CTA CCC ACG GCA AG -3'; reverse: 5′-CTG GAA GAT GGT GAT GGG TT -3′). All reactions were carried out using the StepOne™ system (Applied Biosystems) with an initial denaturation step (10 min, 95 °C) and 50 cycles of amplification consisting of 95 °C for 15 s and 60 °C for 60 s. Amplification was performed on a Mycycler thermocycler (Bio-Rad, Hercules, CA, USA) with DiastarTM Taq DNA polymerase (SolGent Co. Ltd., Daejeon, Korea) following the manufacturer's protocol.

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