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Protective effect and mechanism of action of diallyl disulfide against acetaminophen-induced acute hepatotoxicity

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ABSTRACT

The aim of this study was to investigate the potential protective effects of diallyl disulfide (DADS) against acetaminophen (AAP)-induced acute hepatotoxicity and elucidate the molecular mechanisms underlying these protective effects in rats. Treatment with AAP caused acute hepatotoxicity manifested by elevated levels of aspartate aminotransferase and alanine aminotransferase with corresponding histopathological changes and high levels of oxidative stress in the livers. AAP treatment also caused hepatocellular apoptosis with phosphorylation of c-Jun-N-terminal protein kinase (JNK). In addition, AAP caused activation of nuclear factor kappaB (NF-κB) concurrent with induction of inflammatory mediators. In contrast, pretreatment with DADS effectively attenuated acute liver injury and oxidative stress caused by AAP. DADS pretreatment suppressed cytochrome P450 2E1 (CYP2E1) levels in a dose-dependent manner and inhibited elevation of CYP2E1 activity induced by AAP. DADS pretreatment suppressed the phosphorylation of JNK and attenuated hepatocellular apoptotic changes. In addition, DADS inhibited the nuclear translocation of NF-κB and subsequent induction of inflammatory mediators. Overall, these results indicate that DADS confers a protective effect against oxidative stress-mediated JNK activation and apoptotic changes caused by AAP in the rat livers. This may be due to its ability to inhibit CYP2E1, enhance antioxidant enzymes activities, and suppress NF-κB activation.

1. Introduction

Acetaminophen (AAP) is a widely used, effective analgesic and antipyretic drug, which is safe when prescribed at the therapeutic level (Bessems and Vermeulen, 2001). At therapeutic doses, approximately 80% of AAP is conjugated to glucuronic acid or sulfate in hepatocytes and excreted through the bile or urine (Mitchell and Jollows, 1975). The remaining 5–10% of AAP is metabolized by cytochrome P450 (CYP), a superfamily of hemecontaining monoxygenases that metabolize a large number of compounds (Nelson, 1990). Among the CYPs, there are 57 genes that have been found to be functionally significant, of which CYP2E1 is known to play an important and predominant roles in metabolism and subsequent toxicity of AAP (Gonzalez, 2005; Zanger and Schwab, 2013). By CYP2E1, AAP metabolized to a highly reactive intermediate N-acetyl-*p*-benzoquinone imine (NAPQI) (Nelson, 1990). NAPQI is rapidly detoxified via conjugation with reduced glutathione (GSH). However, AAP overdose can cause excessive production of NAPQI, which in turn depletes of GSH levels in hepatocytes and leads to subsequent hepatocellular death (Mitchell et al., 1973). Recent studies have demonstrated that production of reactive oxygen species induced by AAP causes mitochondrial damage and early activation of mitogen-activated protein kinases, especially c-Jun-N-terminal protein kinase (JNK) (Latchoumycandane et al., 2007; Hanawa et al., 2008).

Oxidative stress activates signal transduction pathways involving transcription factors, such as nuclear factor kappa B (NF-κB) (Dambach et al., 2006). NF-κB is known to regulate the gene expression controlling inflammatory mediators, including

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inducible nitric oxide (iNOS), cyclooxygenase-2 (Cox-2), and tumor necrosis factor-α (TNF-α). It is an important regulator of numerous inflammatory mediators, several of which have been implicated in AAP-induced hepatotoxicity (Laskin and Gardner, 2003). Many studies have shown that antioxidant and anti-inflammatory agents effectively protect against acute hepatotoxicity induced by AAP overdose (Girish et al., 2009; Nagi et al., 2010; Oz and Chen, 2008). In this regard, inhibition of AAP metabolic activation achieved by blocking CYP2E1, and/or inhibition of NAPQI-mediated oxidative stress achieved by increasing GSH levels and activating the antioxidant defense system, can confer protection from AAP-induced hepatotoxicity (Jaeschke et al., 2011).

Garlic (*Allium sativum* L.) possesses diverse biological activities, including anticarcinogenic, antidiabetic, antioxidant, and anti-inflammatory properties (Agarwal, 1996; Wang et al., 1996). Garlic oil contains more than 20 organosulfur compounds (OSCs) that are believed to play a major role in the reported biological activities of garlic (Wu et al., 2002). Among various OSCs, diallyl disulfide (DADS) is beneficial to human health, particularly because of its protective effects against carcinogenesis and chemically induced toxicity (Siess et al., 1997; Sheen et al., 2001; Shin et al., 2016). Many of the pharmacological effects of DADS may result from modulation of phase I and II metabolizing enzymes (Reicks and Crankshaw, 1996; Guyonett et al., 1999). DADS not only inhibits CYP2E1-related metabolism but also enhances antioxidant properties (Dwivedi et al., 1998; Ko et al., 2017). However, the mechanism by which DADS elicits hepatoprotective and antioxidant effects against AAP-induced hepatic damage is still unclear. Therefore, the aim of the present study was to evaluate the protective effects of DADS against AAP-induced oxidative hepatic injury and to elucidate the mechanisms underlying these protective effects in rats.

2. Materials and methods

2.1. Animals and environmental conditions

Twenty-four male Sprague–Dawley rats aged 9 weeks were obtained from a specific pathogen-free colony at Samtako Co. (Osan, Republic of Korea) and used after 1 week of quarantine and acclimation. Two animals per cage were housed in a room maintained at a temperature of 23 ± 3 °C and a relative humidity of 50 ± 10% with 12 h light/dark cycles and 12–18 air changes/h. Commercial rodent chow (Samyang Feed, Wonju, Republic of Korea) sterilized by radiation and sterilized tap water were provided *ad libitum*. The Institutional Animal Care and Use Committee of Chonnam National University approved the protocols for the animal study, and the animals were cared for in accordance with the Guidelines for Animal Experiments of Chonnam National University.

2.2. Test chemicals and treatment

AAP (CAS No. 103-90-2) was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). DADS was purchased from Tokyo Kasei Chemical Co. (Tokyo, Japan). All other chemicals were of the highest grade commercially available. DADS was dissolved in corn oil, and AAP was dissolved in a saline solution that was kept in a boiling water bath. The volumes of AAP (20 mL/kg body weight) and DADS (10 mL/kg body weight) administered daily were calculated based on the most recently recorded body weight of the individual animal. DADS was administered to rats by oral gavage once daily for 5 days at 100 mg/kg/day. The rats were given a single oral dose of AAP (4 g/kg) to induce liver injury 3 h after the final DADS treatment (Sharifudin et al., 2013; Verma et al., 2013). All animals were sacrificed 24 h after AAP administration.

2.3. Experimental groups and dose selection

Twenty four healthy male rats were randomly assigned to four experimental groups: 1) vehicle control, 2) DADS, 3) AAP, and 4) DADS + AAP ($n = 6$ per group). The effective dose of DADS was based on an earlier study and our previous study (Wu et al., 2002; Lee et al., 2014).

2.4. Necropsy and serum biochemical analysis

All treated animals were anesthetized via carbon dioxide inhalation for blood sample collection 24 h after administration of AAP. Blood samples were drawn from the posterior vena cava and all rats were euthanized via exsanguination after bleeding. The serum samples were collected via centrifugation at 800*g* for 10 min within 1 h after collection and stored in the −80 °C freezer before analysis. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using the Fuji Dri-chem 4000i automatic analyzer (Fujifilm Co., Tokyo, Japan).

2.5. Histopathological examination

After bleeding, a portion of the livers was dissected and fixed in 10% neutral buffered formalin solution for 2 weeks. The remaining livers were snap-frozen in dry ice and stored at −80 °C for subsequent biochemical analyses. The fixed tissues were processed routinely, and were embedded in paraffin, sectioned to 4-μm thickness, deparaffinized, and rehydrated using standard techniques. The sections were stained with Harris' hematoxylin and eosin stain for microscopic examination (Leica DM LB2; Leica, Wetzlar, Germany). All observations were performed manually with a light microscope with ×10 and ×20 objective lenses and a ×100 oil immersion lens in a totally blinded manner.

2.6. Determination of lipid peroxidation and GSH levels, and antioxidant enzymes activities

Oxidative stress in the livers was assessed by evaluating the levels of malondialdehyde (MDA, a marker of lipid peroxidation) and GSH, as well as glutathione reductase (GR), glutathione *S*-transferase (GST), superoxide dismutase (SOD), and catalase activities. The concentration of MDA was assayed by monitoring thiobarbituric acid reactive substance formation using the method described by Berton et al. (1998). GSH content was measured using the method described by Moron et al. (1979). The activities of antioxidant enzymes including GR, GST, SOD, and catalase were determined using commercial assay kits (Cayman Chemical, Ann Arbor, MI, USA). Total protein contents were determined using the method by Lowry et al. (1951), using bovine serum albumin as a standard.

2.7. Preparation of hepatic cytosolic/nuclear fraction

A frozen liver samples were cut into small pieces and washed with ice-cold (10 mM Tris-HCl, pH 7.4). Samples were homogenized in a glass-Teflon homogenizer with a suitable hypotonic lysis buffer (10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, and 0.1 mM EGTA) containing protease inhibitor cocktail and dithiothreitol (DTT) as a reducing agent to lyse the cell membranes. The lysate was incubated on ice for 15 min and NP-40 was added to a final concentration of 0.5%, and then centrifuged at 250 *g* for 15 min. The supernatant (cytosolic fraction) was collected and stored at −80 °C for subsequent analyses. The pellet containing the nuclear fraction was resuspended in extraction buffer (20 mM HEPES, pH 7.9, 500 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, and protease