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Geranylgeranylacetone protects against acetaminophen-induced hepatotoxicity by inducing heat shock protein 70

Tadashi Nishida [a], Tatsuya Matsura [a,*], Junya Nakada [a,b], Aki Togawa [a], Masachika Kai [a], Isao Sumioka [c], Yukari Minami [b], Yoshimi Inagaki [b], Yuichi Ishibe [b], Hisao Ito [d], Yoshiji Ohta [e], Kazuo Yamada [a]

[a] *Division of Medical Biochemistry, Department of Pathophysiological and Therapeutic Science, Tottori University Faculty of Medicine, 86 Nishi-cho, Yonago 683-8503, Japan*
[b] *Division of Anesthesiology and Critical Care Medicine, Department of Surgery, Tottori University Faculty of Medicine, Yonago 683-8504, Japan*
[c] *Healthcare Research Institute, Wakunaga Pharmaceutical Co., Ltd., Akitakata 739-1195, Japan*
[d] *Division of Organ Pathology, Department of Microbiology and Pathology, Tottori University Faculty of Medicine, Yonago 683-8503, Japan*
[e] *Department of Chemistry, School of Medicine, Fujita Health University, Toyoake, Aichi 470-1192, Japan*

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Abstract

Geranylgeranylacetone (GGA), an anti-ulcer drug, has been reported to induce heat shock protein (HSP) 70 in several animal organs. The present study was performed to determine whether GGA protects mouse liver against acetaminophen (APAP)-induced injury and whether it has potential as a therapeutic agent for APAP overdose. Hepatic damage was induced by single oral administration of APAP (500 mg/kg). GGA at 400 mg/kg was given orally 4 or 8 h before, or 0.5 h after APAP administration. Treatment of mice with GGA 4 h before or 0.5 h after APAP administration suppressed increases in transaminase activities and ammonia content in blood as well as hepatic necrosis. Such GGA treatment significantly increased hepatic HSP70 accumulation after APAP administration. Furthermore, GGA inhibited increases in hepatic lipid peroxide content and hepatic myeloperoxidase activity after APAP administration. In contrast, GGA neither inhibited hepatic cytochrome P450 2E1 activity nor suppressed hepatic glutathione depletion after APAP administration. The protective effect of GGA treatment 4 h before APAP on hepatotoxicity induced by APAP was completely inhibited with quercetin, known as an HSP inhibitor. In conclusion, GGA has been identified as a new antidote to APAP injury, acting by induction of HSP70. The potential of GGA as a therapeutic tool is strongly supported by its ability to inhibit hepatic injury even when administered after ingestion of APAP.

Keywords: Acetaminophen; Hepatotoxicity; Geranylgeranylacetone; Heat shock protein; Mouse

1. Introduction

Acetaminophen (APAP), the most commonly sold over-the-counter antipyretic analgesic throughout the world, is generally considered harmless at therapeutic doses. However, APAP overdose causes severe and sometimes fatal hepatic damage in humans and

* Corresponding author. Tel.: +81 859 38 6153; fax: +81 859 38 6150.
E-mail address: tmatsura@grape.med.tottori-u.ac.jp (T. Matsura).

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experimental animals (Davidson and Eastham, 1966; Mitchell, 1988). Since the recent reports that use of salicylic acid in combination cold remedies is associated with Reye's syndrome in children with influenza or varicella infection (Belay et al., 1999), it has become more likely that APAP will be used alone. In the United States, the incidence of liver injury due to APAP overdose, either by accident or with suicidal intent, is increasing (Lee, 2004). Thus, it is important to develop a safe and effective antidote against APAP overdose. To date, the mechanisms underlying APAP toxicity are considered to include the following: (1) covalent binding to cellular macromolecules of a reactive intermediate metabolite of APAP produced by cytochrome P450 (CYP), *N*-acetyl-*p*-benzoquinoneimine (NAPQI) (Nelson, 1995), which is normally detoxified by conjugation with reduced glutathione (GSH); (2) to a lesser extent, oxidative stress (Amimoto et al., 1995; Arnaiz et al., 1995). Currently, the agent most widely used in clinical practice for treating APAP-induced liver injury is *N*-acetylcysteine (NAC), a cysteine prodrug (Smilkstein et al., 1988). In addition to NAC, CYP2E1 inhibitors (Li et al., 1977; Sumioka et al., 1998; Sumioka et al., 2001) and antioxidants (Amimoto et al., 1995) may also serve as mechanism-based antidotes for APAP overdose. We have recently reported that treatment of mice with APAP initially induces formation of hepatic heat shock protein 70 (HSP70), followed by HSP25, and that mortality following APAP overdose declines rapidly after the appearance of hepatic HSP25 (Sumioka et al., 2004). These findings suggest that HSP inducers without any side effects might represent a new type of antidote to APAP.

Geranylgeranylacetone (GGA), an acylic polyisoprenoid is an anti-ulcer drug developed in Japan (Hirakawa et al., 1996) and widely used in the clinic (Shirakabe et al., 1995). It has been reported that GGA induces HSP70 in rat gastric mucosa (Hirakawa et al., 1996), heart (Ooie et al., 2001) and liver (Yamagami et al., 2000).

In the present study, we examined whether GGA can protect mouse liver against APAP-induced injury. We are particularly interested in whether GGA is effective when administered after ingestion of APAP, which would indicate that it has potential as a therapeutic agent against APAP overdose.

2. Materials and methods

2.1. Chemicals

APAP was purchased from Wako Pure Chemical Industries (Osaka, Japan). ThioGlo-1^{TM} maleimide reagent was obtained from Calbiochem-Novabiochem Corporation (San Diego, CA). Rabbit anti-HSP70 polyclonal antibody was purchased from Medical & Biological Laboratories Co., Ltd. (Nagoya, Japan). Rabbit anti-HSP25 polyclonal and mouse anti-HSP60 monoclonal antibodies were obtained from Stressgen Biotechnologies Corp. (Victoria, BC, Canada). Quercetin (Q) was purchased from Nakarai Tesque (Kyoto, Japan). GGA was kindly provided by Eisai Co. (Tokyo, Japan). All other chemicals used were of analytical grade.

2.2. Animals

Male ddY mice (five weeks old) were purchased from Japan SLC (Shizuoka, Japan) and housed 5–6 per cage in plastic cages. The animals were maintained on a 12-h light/dark cycle under controlled temperature ($23 \pm 3\,^{\circ}C$) and humidity ($55 \pm 5\%$) for a week before use in experiments. They were allowed free access to standard laboratory food and water, but were fasted overnight just before the onset of the experiment.

All studies were approved by the Institutional Animal Care and Use Committee at Tottori University Faculty of Medicine, and were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Administration of GGA, Q and APAP

APAP (500 mg/kg) was administered orally to mice. GGA (400 mg/kg) was given orally 4 or 8 h before, or 0.5 h after APAP administration. GGA and APAP were suspended in 5% gum arabic and 1% carboxymethyl cellulose sodium salt solution, respectively. The dose volume of each compound and vehicle combined was 10 ml/kg. Mice were anesthetized with diethyl ether and blood samples were taken from the right ventricle with a heparinized syringe 3 or 9 h after APAP administration. After the collection of blood, livers were removed from mice, frozen immediately and stored in liquid nitrogen until assayed. In some experiments, Q was suspended in distilled water and administered orally at a dose of 200 mg/kg 6 h before APAP treatment. The dose volume of Q was 10 ml/kg.

2.4. Measurements of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and blood ammonia

Plasma ALT and AST activities and blood ammonia concentration were determined spectrophotometrically by means of commercially available kits (Wako Pure Chemical Industries, Osaka, Japan).

2.5. Assays for lipid peroxidation and glutathione

The liver was homogenized in 0.05 M phosphate buffer (0.2 M NaH_2PO_4, 0.2 M Na_2HPO_4, 0.2 M Na-EDTA, pH 7.4). Lipid peroxidation in the liver was measured by a colorimetric reaction with thiobarbituric acid as previously described