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Geniposide activates GSH *S*-transferase by the induction of GST M1 and GST M2 subunits involving the transcription and phosphorylation of MEK-1 signaling in rat hepatocytes

Wu-Hsien Kuo[a,b], Fen-Pi Chou[b], Shun-Chieh Young[b], Yun-Ching Chang[b], Chau-Jong Wang[b,*]

[a]*Division of Gastroenterology, Department of Internal Medicine, Armed Forces Taichung General Hospital, ROC, Taiwan*
[b]*Institute of Biochemistry and Biotechnology, College of Medicine, Chung Shan Medical University, No. 110, Sec. 1, Chien-Kuo N. Road, Taichung 402, ROC, Taiwan*

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Abstract

Geniposide, an iridoid glycoside isolated from the fruit of *Gardenia jasminoides* Ellis, has biological capabilities of detoxication, antioxidation, and anticarcinogenesis. We have recently found that geniposide possesses a potential for detoxication by inducing GST activity and the expression of GST M1 and GST M2 subunits. In this study, the signaling pathway of geniposide leading to the activation of GSH *S*-transferase (GST) was investigated. Primary cultured rat hepatocytes were treated with geniposide in the presence or absence of mitogen-activated protein kinase (MAPK) inhibitors and examined for GST activity, expression of GST M1 and M2 subunits, and protein levels of MAPK signaling proteins. Western blotting data demonstrated that geniposide induced increased protein levels of GST M1 and GST M2 (~1.76- and 1.50-fold of control, respectively). The effect of geniposide on the increased protein levels of GST M1 and GST M2 was inhibited by the MEK-1 inhibitor PD98059, but not by other MAPK inhibitors. The GST M1 and GST M2 transcripts as determined by RT-PCR and GST activity were also inhibited concurrently by the MEK-1 inhibitor PD98059. The protein levels of up- and down-stream effectors of the MEK-1, including Ras, Raf, and Erk1/2, and the phosphorylation state of Erk1/2 were found to be induced by geniposide, indicating a two-phase influence of geniposide. The results suggest that geniposide induced GST activity and the expression of GST M1 and GST M2 acting through MEK-1 pathway by activating and increasing expression of Ras/Raf/MEK-1 signaling mediators.

Keywords: Geniposide; GSH *S*-transferase; GST mu class subunits; Hepatocyte; Extracellular-regulated kinase kinase-1

Introduction

Geniposide (Fig. 1), an iridoid glycoside, was first isolated from the fruit of *Gardenia jasminoides* Ellis by Endo and Taguchi (1973) and Inouye and Saito (1969). *G. jasminoides* Ellis frandiflora, makino, a plant in the family of *Rubiaccase*, grows widely in the middle and southern parts of Taiwan. Its fruit, gardenia, has been used as an herbal medicine to treat liver and gall bladder disorders, hepatitis, and acute jaundice (Miyasita, 1976). The crude extract rapidly lowers serum bilirubin and transaminase levels in acute hepatitis (Wu, 1956). The antihyperbilirubinemic effect of *Gardenia* extracts has been reported in animal models (Kong et al., 1977; Miwa, 1954). Lau et al. (1986) proposed that gardenia and geniposide may facilitate the biliary extraction of α-naphthylisothiocyanate and/or its toxic metabolite(s). In our earlier studies, we observed a protective effect of geniposide against the hepatic damage induced by aflatoxin B1 (Wang et al., 1991, 1992b), and the growth of C-6 glioma cells could be inhibited by treatment

Abbreviations: GST, GSH *S*-transferase; MEK-1, extracellular-regulated kinase kinase-1PI 3-K, phosphotidyl inositol 3-kinase; PD, PD98059; SP, SP600125; SB, SB203580; Wt, wortmannin; ARE, antioxidant-responsive element; CDNB, 1-chloro-2, 4-dinitrobenzene; GAPDHglyceraldehydes-3-phosphate dehydrogenase.

* Corresponding author. Fax: +886 4 23248167.
E-mail address: wcj@csmu.edu.tw (C.-J. Wang).

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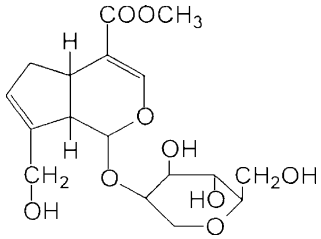

Fig. 1. Structure of geniposide.

with geniposide or its derivative both in vitro (Wang et al., 1992a) and in rats (Wang et al., 1993). Therefore, geniposide possesses the potential as a chemotherapeutic agent against tumor promotion. The mechanism for the anti-aflatoxin B1 genotoxicity action of geniposide is unclear. We recently found that geniposide possesses a potential for detoxication by inducing GSH *S*-transferase (GST) activity via increasing the transcription of GST M1 and GST M2 subunits (Kuo et al., 2004).

GSTs are a family of dimeric enzymes responsible for the metabolism of a broad range of xenobiotics and carcinogens (Mannervik, 1985). According to amino acid sequence similarity and antibody cross-reactivity, the cytosolic GSTs are grouped into seven classes, namely alpha, mu, theta, pi, zeta, chi, and sigma (Nebert and Vasiliou, 2004; Strange et al., 2001). The members of the GST supergene family have diverse structure and catalyze GSH conjugation to some identical but also very different substrates. This concept agrees with the requirement of their multiple functions in the metabolism of a multitude of chemical compounds and other physiologically important processes. It is conventionally thought that the induction of phase II activities is beneficial to detoxication of toxins or carcinogens. Nevertheless, numerous studies showed that the regulation of different GST isoforms was associated with various pathological processes. For example, the induction of GSTP is associated with carcinogenesis (Allan et al., 2001; Batist et al., 1986; Kitahara et al., 1984; Meyer et al., 1985) and drug resistance of tumor tissue (Cheng et al., 1997; Inoue et al., 1995; Yusa et al., 1988). The expression of GSTM and GSTT, on the other hand, is related to cancer susceptibility in human studies (Lear et al., 1996; Rebbeck, 1997). These observations indicate the importance of specifying the effect of chemopreventive agents on the GST system.

Although geniposide was known to increase the activity of GST via inducing the expression of GST M1 and GST M2 subunits (Kuo et al., 2004), a detailed understanding of the mechanism of geniposide's action would still need to be resolved to emphasize its application in cancer chemoprevention. Therefore, we investigated the signal pathway involved in the induction of GST subunits and activity by geniposide in primary cultured rat hepatocytes. The results showed that the pretreatment of MEK inhibitor, but not other MAPK inhibitors, was able to block the geniposide-induce expression of GST M1 and GST M2. Further data demonstrated that geniposide acted via increasing the expression and activity of Ras/Raf/MEK-1/Erk signaling.

Materials and methods

Materials. Geniposide was obtained from Wako Pure Chemical. Tris–HCl, EDTA, SDS, phenylmethylsulfonyl fluoride, bovine serum albumin (BSA), 1-chloro-2, 4-dinitrobenzene (CDNB), PD98059, SP600125, wortmannin, and SB203580 were purchased from Sigma (St. Louis, MO). Protein assay kit was obtained from Bio-Rad Labs (Hercules, CA). William's E medium, calf bovine serum, trypsin–EDTA, and penicillin, streptomycin, and neomycin mixture (PSN) were purchased from Gibco/BRL (Gaithersburg, MD). Polyclonal antibodies against Ras, Raf, ERK, phospho-ERK, and MEK-1 were obtained from Transduction Lab. (KY, USA). Those against GSTM1 and GSTM2 were obtained from Biotrin International (Ireland). Horseradish-peroxidase-conjugated goat anti-rabbit IgG antibody and ECL Western blotting detection kit were purchased from Amersham Biosciences. M-MLV reverse transcriptase and Taq polymerase were purchase from Promega Co.

Preparation of hepatocytes. Male Sprague–Dawley rats (weighing 250–300 g), purchased from the National Taiwan University Hospital Animal Center, were housed three per cage and acclimated under laboratory conditions (18–23 °C, humidity 55–60%, 12:12-h light/dark cycle). The animals were handled according to the guidelines of the Taiwan Society for Laboratory Animal Sciences for the care and use of laboratory animals. The study was approved by the Institute of Animal Care and Use Committee of the Chung Shan Medical University. The hepatocytes were prepared by two-stage collagenase perfusion (Bonney et al., 1974) and were cultured in William's E medium supplemented with 1% PSN antibiotic mixture (penicillin, streptomycin, and neomycin), glutamine (1%), and bovine calf serum (10%), and gassed with O_2/CO_2 (95/5%). The cells were plated at a density of 1×10^5 cells per ml and treated with geniposide, as indicated for the following tests 4 h after attachment. The agents were dissolved in redistilled water. In the experiments testing the effect of the mitogen-activated protein kinase/extracellular-regulated kinase kinase-1(MEK-1) inhibitor PD98059 (PD, 25 μM), JNK inhibitor SP600125 (SP, 20 μM), p38 inhibitor SB203580 (SB, 20 μM), and phosphotidyl inositol 3-kinase (PI 3-K) inhibitor wortmannin (Wt, 5 μM), the cells were preincubated in the presence of inhibitor for 1 h and stimulated with geniposide for the durations indicated.

GST activity assay. The total GST activity was measured according to the method of Habig et al. (1974) using CDNB (1-chloro-2, 4-dinitrobenzene) as substrate. The enzyme activity was expressed as nanomoles GSH conjugate