Available online at www.sciencedirect.com
SCIENCE DIRECT
Chemico-Biological Interactions 161 (2006) 125–138
Chemico-Biological Interactions
www.elsevier.com/locate/chembioint

Liquiritigenin, an aglycone of liquiritin in *Glycyrrhizae radix*, prevents acute liver injuries in rats induced by acetaminophen with or without buthionine sulfoximine

Young Woo Kim [a,b], Sung Hwan Ki [a], Jong Rok Lee [b], Song Jin Lee [a], Choon Won Kim [c], Sang Chan Kim [b], Sang Geon Kim [a,*]

[a] *College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, Republic of Korea*
[b] *College of Oriental Medicine, Research Center for Biomedical Resources of Oriental Medicine, Daegu Haany University, Daegu, Republic of Korea*
[c] *Department of Diagnostic Medicine Laboratory, Hanyang University Medical School, Seoul, Republic of Korea*

Received 13 January 2006; received in revised form 8 March 2006; accepted 9 March 2006
Available online 27 March 2006

Abstract

Glycyrrhizae radix has been used as one of the oldest and most frequently employed botanicals in both western and oriental countries. Previously, we showed that liquiritigenin (LQ), an aglycone of liquiritin in *G. radix*, exerts cytoprotective effects against heavy metal-induced toxicity in vitro. This study investigated in vivo protective effects of LQ against acute liver injuries induced by acetaminophen (APAP) or APAP plus buthionine sulfoximine (BSO). Liver injuries were assessed by blood biochemistry and histopathology in rats administered with LQ purified from the acid hydrolyates of liquiritin singly (p.o. or i.v., 2–4 days) or in combination with dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxybiphenyl-2,2′-dicarboxylate (DDB), a synthetic derivative of Schisandrin C in *Fructus shizandrae*, and exposed to APAP or APAP + BSO. LQ treatments (oral) effectively decreased liver injuries induced by a single dose of APAP, as evidenced by decreases in hepatic necrosis and inflammation as well as plasma alanine aminotransferase and lactate dehydrogenase activities. LQ, when intravenously applied, enhanced hepatoprotective effect with a greater potency. APAP + BSO led to severe liver injuries, resulting in lethality. LQ pretreatments significantly reduced the potentiated liver necrosis, decreasing mortality. In spite of the improvement in blood biochemistry, DDB failed to protect the liver from injuries induced by APAP or APAP + BSO. Combined treatments of rats with LQ and DDB showed some additive protective effect. The present study demonstrates that LQ efficaciously protects the liver from acute injuries induced by APAP or from APAP-induced severe injuries during GSH deficiency, indicating that LQ is one of the principal cytoprotective components comprised in *G. radix*.

Keywords: Liquiritigenin; Acetaminophen; BSO; DDB; Liver injury; Hepatoprotective effect

Glycyrrhizae radix (*G. radix*, licorice, liquorice) is one of the oldest and most frequently used botanicals in the oriental medicine. *G. radix* extract is recommended for life-enhancing properties and cure of injury or swelling as well as for detoxification [1]. Licorice is also widely used to flavor food and liqueurs. Studies have shown that the extracts of *G. radix* attenuated free radical-induced oxidative damage in the kidney

Abbreviations: ALT, alanine aminotransferase; APAP, acetaminophen; BSO, buthionine sulfoximine; DDB, dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate; GSH, glutathione; HAI, histological activity index; LDH, lactate dehydrogenase; LQ, liquiritigenin

* Corresponding author at: College of Pharmacy, Seoul National University, Sillim-dong, Kwanak-gu, Seoul 151-742, Republic of Korea. Tel.: +82 2 880 7840; fax: +82 2 872 1795.
E-mail address: sgk@snu.ac.kr (S.G. Kim).

0009-2797/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.cbi.2006.03.008

[2], and prevented carcinogenesis induced by toxicants or hormones (e.g., *N*-methyl-*N*-nitrosourea or estradiol) [3].

G. radix comprises flavonoids and pentacyclic triterpene saponin as major constituents, which include liquiritigenin (LQ), liquiritin, isoliquiritigenin, liquiritin apioside, glycyrrhizin and glycyrrhizic acid [4]. Among the components, glycyrrhizin is the major constituent in quantities (4–13%) from the dried roots [1]. Glycyrrhizin induces apoptosis in stomach cancer and promyelotic leukemia cell lines [5], and inhibits HIV replication in monocytes [6]. LQ, an aglycone of liquiritin, is one of the flavonoids comprised in *G. radix*. A study from our laboratories demonstrated that LQ exerted cytoprotective effects against heavy metal-induced toxicity in cultured cells [7]. In spite of the extensive studies on the pharmacological effects of glycyrrhizin and its derivatives, the effects of *G. radix* flavonoids have rarely been studied. In particular, no information but ours is available on the biological effects of LQ.

Acetaminophen (APAP) is one of the most extensively used analgesic and antipyretic agents worldwide. Overdose of APAP or its sporadic reaction causes major morbidity and mortality in its victims. Death from APAP overdose is thought to be secondary to liver failure, which results from massive hepatic necrosis, the hallmark and pathological feature of APAP-induced liver toxicity [8]. The toxicity of APAP is mediated by CYP2E1-mediated oxidative metabolism in the liver and kidney to a highly reactive *N*-acetyl-*p*-benzoquinoneimine (NAPQI). Glutathione (GSH) serves as an oxygen radical scavenger and thus exerts cytoprotective effect. At therapeutic doses, NAPQI is efficiently detoxified by GSH to form an APAP–GSH conjugate.

Although the levels of GSH in cells or tissues are consistently maintained under the normal physiological situations, those decrease in certain pathophysiological states (e.g., APAP intoxication, sulfur amino acids deficiency or protein-calorie malnutrition) [9,10]. A decrease in cellular GSH content promotes oxidative stress and activates cellular proteins (e.g., cell signaling pathways), which may enhance susceptibility of cells to toxic insults. The levels of GSH in the tissues including liver and kidney are decreased by ingestion of a large dose of APAP, NAPQI causing severe tissue injuries [11–13]. Treatment of cells with GSH depleting agents such as buthionine sulfoximine (BSO), diethyl maleate and phorone disrupts a dynamic equilibrium of the GSH pool by inhibiting the essential proteins involved in GSH synthesis or their direct GSH conjugations. Previously, we have shown that a decrease in GSH content enhances the toxicity induced by heavy metals, resulting in cell death [14,15]. A number of studies showed that alterations in the cellular redox-state by disruption of the GSH pool promote liver toxicity elicited by toxicants (e.g., APAP and CCl_4).

In view of the previous observations that LQ protected cells from death induced by heavy metals especially during GSH deficiency and that APAP overdose induced hepatotoxicity while GSH deficiency enhancing susceptibility of cells or animals to APAP, the present study was designed to determine whether LQ protects the liver from APAP intoxication in rats with or without BSO pretreatment. To assess its potential hepatoprotective effects, we used LQ, purified at a relatively large quantity from the acid hydrolyates of liquiritin prepared from *G. radix* extract. The possible synergistic effects of LQ with dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylene dioxybiphenyl-2,2′-dicarboxylate (DDB), a synthetic derivative of Schisandrin C in *Fructus shizandrae*, against APAP-induced acute liver injuries were also determined.

1. Materials and methods

1.1. Materials

DDB was provided from Pharmaking Pharmaceutical Co. (Seoul, Korea). APAP, BSO and methylcellulose (MC) were purchased from Sigma Chemical Co. (St. Louis, MO). Liquiritin (Fig. 1A) standard was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Polyethylene glycol #400 (PEG) was obtained from Yakury Pure Chemical Co. (Kyoto, Japan). Liquiritigenin was suspended in a 40% (v/v) PEG solution, whereas DDB was suspended in a 0.5% (w/v) MC solution.

1.2. Preparation of LQ

Air-dried *G. radix* (3 kg, Wolsung, Daegu, Korea) was successively extracted with 15 L methanol (MeOH) at room temperature for 72 h. The extracts combined were concentrated in vacuo to afford a brown gum (290 g), which was located onto a silica gel column chromatography (CC) (60 cm 230–400 mesh, 1.2 kg) and eluted with 6 L of $CHCl_3$, then with a gradient of $CHCl_3$–MeOH [50:1 (6 L), 30:1 (6 L), and then 15:1 (15 L)]. The fraction of $CHCl_3$–MeOH (15:1) was concentrated to give a dark brown residue (43 g). The obtained residue was chromatographed over silica gel CC (50 cm 230–400 mesh, 350 g) and eluted with $CHCl_3$–acetone (20:1, 5 L), followed by gradient elution of $CHCl_3$–acetone [10:1