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Effects of Glycyrrhizae Radix on Acetaminophen-induced Hepatotoxicity in Mice

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Abstract—In order to study if Glycyrrhizae Radix (GR) has protective effects on hepatotoxicity of acetaminophen in mouse, one of the species which are sensitive to acetaminophen-induced hepatotoxicity, effects of GR on liver weight to body weight ratio, serum alanine and aspartate transaminase (ALT and AST) activities, hepatic UDP-GT2 activity, and histopathologic changes were determined in acetaminophen-treated mice. Liver weight to body weight ratio and UDP-GT2 activity in mouse liver were not altered by GR. However, GR pretreatment lowered serum ALT and AST activities by 77% and 90%, respectively, and diminished the degree of centrilobular necrosis caused by acetaminophen in liver as determined by histopathologic observation. These results suggest a possible protective effect of GR against the acetaminophen-induced hepatotoxicity in mice.

Keywords □ Glycyrrhizae Radix (GR), glycyrrhizin, acetaminophen, hepatotoxicity.

Licorice has been widely used in combination with other herbs or synthetic drugs for various disorders. The major components of licorice are glycyrrhizin and glycyrrhetinic acid. Glycyrrhizin has been reported to have various biological activities including anti-inflammatory (Finney *et al.*, 1958; Yamamoto *et al.*, 1963; Ohuchi *et al.*, 1981), anti-allergic (Sotomatsu *et al.*, 1959; Kuroyanagi *et al.*, 1966; Inoue *et al.*, 1987), anti-gastric ulcer (Doll *et al.*, 1962), and anti-viral (Baba *et al.*, 1987; Pompei *et al.*, 1979; Hino *et al.*, 1981; Ito *et al.*, 1987) activities. It has also been demonstrated that glycyrrhizin attenuated liver damage caused by carbon tetrachloride, allyl formate, and hepatotoxin in rats (Nakamura *et al.*, 1985). The exact mechanism of action, however, has not been reported yet.

Acetaminophen is a widely used analgesic and antipyretic drug which produces hepatotoxicity in both humans and laboratory animals at excessive dosages (Black, 1984). The drug is mainly metabolized in the liver by glucuronidation and sulfation (Jollow *et al.*, 1974). A few percent of a dose is metabolized by cytochrome P-450 to an electrophilic arylating intermediate, N-acetyl-*p*-benzoquinoneimine, that produces liver injury unless it is trapped by conjugation with endogenous glutathione (Dahlin *et al.*, 1984), forming the acetaminophen-glutathione. Considerable species variation exists in acetaminophen-induced hepatotoxicity (Gregus *et al.*, 1988). Species such as mouse and hamster have been shown to be highly susceptible to toxic effects of acetaminophen whereas rat is quite resistant (Moldeus, 1978).

As an approach to elucidate the possible *in vivo* interaction of synthetic drugs and herbs which are frequently used in combination in Asia, the effect of licorice on the metabolism of acetaminophen was examined in male Sprague-Dawley rats (Kim *et al.*, 1993). The pretreatment of the methanol extract of licorice roots (*Glycyrrhizae glabra*, 1 g/kg, *p.o.*) for 6 days significantly increased the cumulative biliary and urinary excretions of acetaminophen-glucuronide conjugate after the administration of acetaminophen (150 mg/kg, *i.v.*) without affecting thioether and sulfate conjugates. The results suggested that GR may enhance detoxification of acetaminophen since glucuronidation is a major detoxification reaction involved in metabolic conversion of endogenous and exogenous substances to more aqueous soluble compounds that can be excreted into urine or bile (Kasper and Henton, 1980). The effect of GR pretreatment on acetaminophen-induced hepatotoxicity was studied in mice which are reported

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to be sensitive to toxic effects of acetaminophen.

Materials and Methods

Materials

Roots of *Glycyrrhizae glabra* was purchased from Kyung-Dong market. GR was extracted with methanol and freeze-dried. Glycyrrhizin, 3-methylcholanthrene (3-MC), phenobarbital, uridine-5'-diphosphoglucuronic acid (UDP-GA), acetaminophen, *p*-nitrophenol, sucrose, bovine serum albumin (BSA), triton X-100, corn oil, magnesium chloride, sodium phosphate monobasic and sodium phosphate dibasic were purchased from Sigma Chemical Co.(St. Louis, MO, USA). Alanine transaminase (ALT) and aspartate transaminase (AST) reagents were purchased from Technicon Chemicals Co., Belgium.

Animals

Male ICR mice, weighing 30~35 g, were supplied from National Institute of Safety Research. They were provided tap water and lab chow (Shinchon Co., Korea) *ad libitum* and were housed at 23℃, 55± 10% humidity, in a 12-hr light/12-hr dark cycle.

Effects of GR on the acetaminophen-induced hepatotoxicity

Mice were pretreated with methanol extract of GR, 1 g/kg, and water as control orally for 6 days. One day later, acetaminophen, 400 mg/kg, was administered intraperitoneally and fasted. After 24 hr, mice were anesthetized with diethylether and blood was collected in heparinized syringe from abdominal vein. Liver was excised, rinsed with saline, blotted, weighed and stored at −70℃. Plasma was prepared by centrifugation of blood at 3,500 g for 10 min. Plasma ALT and AST activities were determined with kits purchased from Technicon Chem. Co., Belgium. Liver weights per 100 g mouse were calculated as an estimate of liver swelling. UDP-GT2 activity was measured in hepatic microsomes that had been isolated by differential centrifugation. The microsomes were washed once and resuspended in 0.25 M sucrose. Microsomal UDP-GT2 activity was determined by the procedure of Reinke *et al.* (1986), with a slight modification as follows : Incubations were performed in 50 m*l* Erlenmeyer flask with 2 m*l* of 0.05 M phosphate buffer, pH 7.0, containing 3 mM UDP-GA, 1 mM $MgCl_2$, 0.02% bovine serum albumin, microsomes (1 mg/m*l*), 0.05 % triton X-100 and 0.5 mM p-nitrophenol. After 2-min preincubation without UDP-GA, incubations were initiated by the addition of UDP-GA and terminated after 5 min by the addition of 0.5 m*l* of 5% trichloroacetic acid. The precipitated proteins were removed by centrifugation at 1,000 g for 5 min. The remaining *p*-nitrophenol was determined by diluting 0.5 m*l* of the supernatant fraction with 2.0 m*l* of 1.6 M glycine buffer, pH 10.3, and reading absorbance at 436 nm. Liver slices were also analyzed by routine light microscopy after staining paraffin-embedded samples with hematoxylin and eosin.

Statistics

The data were analyzed by Student's *t*-test. The ALT and AST activities were analyzed by Satterthwaite's modified *t*-test for unequal variances.

Results

In order to determine the effect of GR on acetaminophen-induced hepatotoxicity in ICR mice, plasma ALT, AST and AP activities, liver weight to body weight ratio, and hepatic UDP-GT2 activity were examined in addition to histopathologic observation upon pretreatment of GR (1 g/kg, *p.o.*, 6 days) in acetaminophen (400 mg/kg, *i.p.*)-treated mice. Shown in Table I is the effect of GR on ALT and AST activities in plasma collected 24 hours after the administration of acetaminophen. The increase of ALT and AST activities in acetaminophen-treated group were 80 folds and 20 folds, respectively, compared to the control group. Treatment of GR before the injection of acetaminophen, however, markedly alleviated the transaminase activities compared to acetaminophen-treated group. The ALT activity was decreased by 77% and the AST activity was reduced by 90%. The administration of GR by itself had no effect on the transaminase activities. The AP activity was not changed by acetaminophen treatment (data not shown). GR did not alter the liver weight to body weight ratio and hepatic UDP-

Table I. Effect of Glycyrrhizae Radix (GR) on the plasma alanine and aspartate transaminase (ALT and AST) activities in acetaminophen-treated mice.

Treatment	Activity (U/m*l* of plasma)	
	ALT	AST
Control	34± 6	63± 9
Acetaminophen	2725± 1045**	1279± 541**
GR	28± 3	49± 5
GR+Acetaminophen	633± 347** [a]	126± 42* [a]

Animals were sacrificed 1 day after administration of acetaminophen (400 mg/kg, *i.p.*). Values are means± S.E. of five to fourteen mice. *Significantly different ($p<0.05$) from control. **Significantly different ($p<0.01$) from control. [a] Significantly different ($p<0.1$) from acetaminophen-treated group according to Satterthwaite's modified *t*-test for unequal variances.

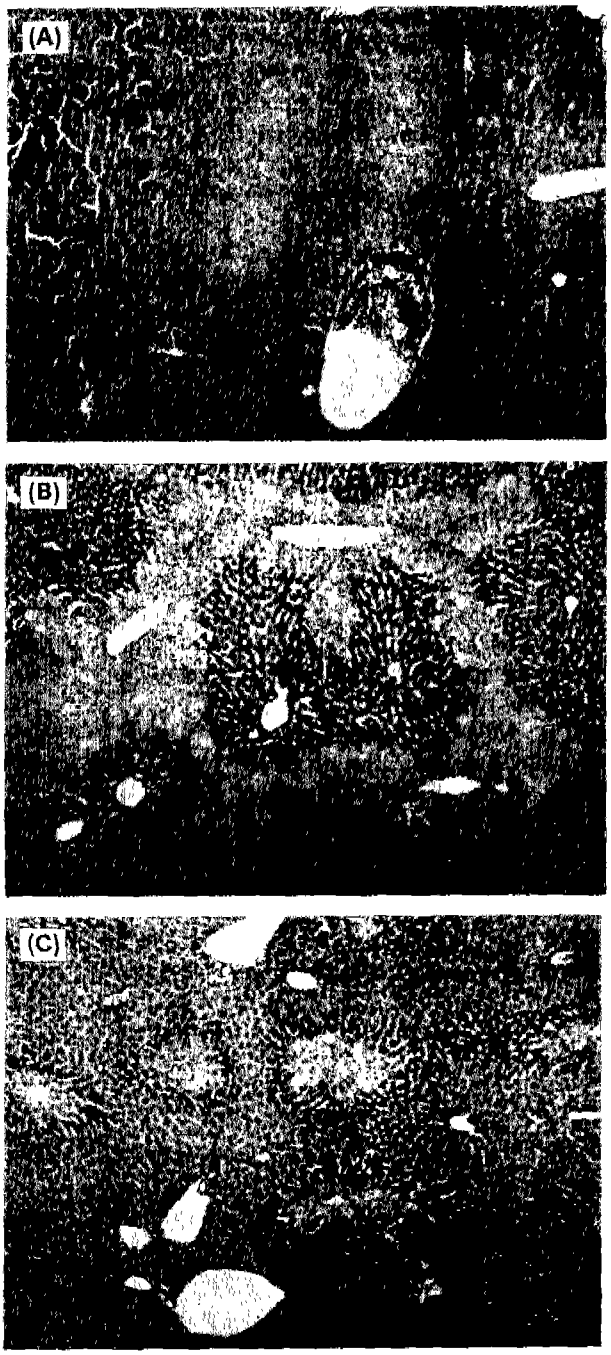

Fig. 1. Histopathologic analysis of the effect of GR pretreatment on acetaminophen-treated mouse livers. Male ICR mice were pretreated with GR (1 g/kg) or water orally for 6 days. Fasted mice were sacrificed 24 hours after the administration of acetaminophen (400 mg/kg, *i.p.*). Liver sections were then prepared, stained with hematoxylin and eosin, and observed in a light microscope with a magnification of 40X. (A) The liver of control mouse having normal hepatocytes and lobular architecture. (B) The liver of acetaminophen-treated mouse, illustrating severe necrosis of hepatocytes in the centrilobular area expanded into the midzonal area. (C) The liver of acetaminophen-treated mouse which was pretreated with GR. The degree and the area of centrilobular necrosis caused by acetaminophen were considerably diminished.

GT2 activity in acetaminophen-treated mice significantly (data not shown).

Effect of GR on acetaminophen-induced hepatic necrosis was studied by histopathological observation and is shown in Fig. 1. The liver of control mice showed normal hepatocytes and lobular architecture (Fig. 1A). Mice treated with 400 mg/kg of acetaminophen developed acute hepatic necrosis which was readily observable histologically (Fig. 1B). Acetaminophen induced a typical severe necrosis in the centrilobular area expanded into the midzonal area. Pretreatment of GR considerably diminished the degree and the area of centrilobular necrosis caused by acetaminophen in liver (Fig. 1C).

Discussion

The results of the present experiments to study the effect of GR pretreatment on acetaminophen-induced hepatotoxicity showed that GR pretreatment lowered plasma ALT and AST activities 77% and 90%, respectively (Table I), and diminished the degree of centrilobular necrosis caused by acetaminophen in liver as determined by histopathologic observation (Fig. 1). These results suggest a possible protective effect of GR against the acetaminophen-induced hepatotoxicity in mice.

Pathological increase in enzyme activities in the serum could be due either to rupture of the cells or impaired permeability of their surface membranes. It is known that serum enzyme activities increase after exposure to acetaminophen. It has been demonstrated that glycyrrhizin prevented releases of lactose dehydrogenase and AST caused by CCl_4 from cultured rat hepatocytes (Nakamura *et al.*, 1985). Glycyrrhizin has also been reported to have a protective effect against hemolysis induced by other saponins or a cationic surfactant (Segal *et al.*, 1977), indicating that glycyrrhizin may induce alteration in membrane fluidity by reacting with the components of membrane or intercalating its moiety into the membrane structure. Therefore, the present results on ALT and AST suggest a possibility that glycyrrhizin in GR may have protective effect against leakage of transaminases due to acetaminophen overdose *in vivo*.

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