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Effect of Glycyrrhizae Radix on the Glucuronidation in Rat Liver

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Pretreatment of Glycyrrhizae Radix (GR) to male Sprague-Dawley rats was demonstrated to increase excretion of acetaminophen-glucuronide conjugate when bile and urine were assayed after administration of acetaminophen. In order to study the effect of GR on the glucuronidation in rats, we examined enzymatic activities of hepatic UDP-glucuronosyltransferases (UDP-GT1 and UDP-GT2) and intracellular concentrations of hepatic UDP-glucuronic acid (UDP-GA), upon the administration of GR (1 g/kg body weight, *p.o.*) or glycyrrhizin (23 mg/kg body weight, *p.o.*), a major component of GR, for 6 days. GR and glycyrrhizin caused increases in specific activities of UDP-GT2 111% and 96%, respectively. Specific activity of UDP-GT1 was increased 25% by GR treatment whereas it was not significantly increased by glycyrrhizin. Concentrations of UDP-GA were increased 257% by GR and 484% by glycyrrhizin. These data indicate that GR activated glucuronidation and thus suggest the possibility that GR may influence detoxification of xenobiotics in rat liver.

Key words : Glycyrrhizae Radix (GR), Glycyrrhizin, Glucuronidation, Uridine diphospho-glucuronosyltransferase (UDP-GT), Uridine 5'-diphophoglucuronic acid (UDP-GA)

INTRODUCTION

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.

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.

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). Glucuronidation is dependent upon the enzymatic activity of UDP-glucuronosyltransferase (UDP-GT) and intracellular concentration of UDP-glucuronic acid (UDP-GA). Several isozymes of UDP-GTs, which are differentially regulated and appear to be specific for different sets of substrates, exist to glucuronidate a large variety of structurally unrelated substances. UDP-GT1 and GT2 forms were identified (Mulder, 1992; Falany and Tephly, 1983; Bock *et al.*, 1979). UDP-GT2 accommodates flat, planar substrates and is induced by 3-methylcholanthrene (3-MC), whereas UDP-GT1 accepts bulky ones and its inducer is phenobarbital. In the present study, effects of Glycyrrhizae Radix (GR) and its major component, glycyrrhizin, on the ac-

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tivities of UDP-GT1 and UDP-GT2 as well as on the concentration of UDP-GA were determined in order to study if GR affects glucuronidation in rat liver.

MATERIALS AND METHODS

Materials

Roots of *Glycyrrhizae Glabra* were purchased from Kyung-Dong market. GR was extracted with methanol and freeze-dried. Glycyrrhizin, 3-methylcholanthrene (3-MC), phenobarbital, UDP-GA, p-nitrophenol, diethylstilbestrol (DES), sucrose, bovine serum albumin (BSA), triton-X 100, corn oil, magnesium chloride, sodium phosphate monobasic, sodium phosphate dibasic, Trizma base, Brij 58, saccharic acid-1,4-lactone and CHAPS were purchased from Sigma Chemical Co. (St. Louis, MO, USA). [Monoethyl-^{3}H] diethylstilbestrol (2.92 TBq/mmol) was purchased from Amersham (Buckinghamshire, England).

Animals

Male Sprague-Dawley rats, weighing 220-240 g, were supplied from National Institute of Safely Research. They were provided tap water and lab chow (Shinchon Co., Korea) *ad libitum* and were housed at 23°C, 55±10 % humidity, in a 12-hr light/12-hr dark cycle.

Determination of glycyrrhizin content in GR by HPLC

Dried methanol extract of GR (111 mg) was diluted 5-fold with methanol. Glycyrrhizin content in GR was determined by HPLC analysis using μ-Bondapak C_{18} column (Waters Associate Inc., USA). GR and glycyrrhizin were eluted with acetonitrile/water/acetic acid (40 : 59 : 1) at a flow rate of 1.0 ml/min. Elution of the components in GR was detected at 254 nm.

Effect of GR and glycyrrhizin on the glucuronidation in rats

Rats were pretreated as follows: GR (1 g/kg), glycyrrhizin (23 mg/kg) and water as controls were administered orally daily for 6 days. 3-MC (20 mg/kg) in corn oil and phenobarbital (75 mg/kg) in saline were administered intraperitoneally (*i.p.*) daily for 4 days. The experiments were performed 24 hr later. After pretreatments, each rat was decapitated without the use of any anesthetic, the liver was excised, rinsed with saline, blotted and frozen immediately in liquid nitrogen. The liver was stored at -70°C until further analysis.

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-GT 2 activity was determined by the procedure of Reinke *et al.* (1986), with a slight modification as follows: Incubations were performed in 50-ml erlenmeyer flask with 2 ml of 0.05 M phosphate buffer, pH 7.0, containing 3 mM UDP-GA, 1 mM $MgCl_2$, 0.02% bovine serum albumin, microsomes (1mg/ml), 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 ml 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 ml of the supernatant fraction with 2.0 ml of 1.6 M glycine buffer, pH 10.3, and reading absorbance at 436 nm.

Microsomal UDP-GT1 activity was determined with DES as aglycone substrates (Hjelle *et al.*, 1985) as follows: Prior to assay for UDP-GT1 activity, microsomal pellets were resuspended in 0.25 M sucrose containing 8 mM 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), a nondenaturing zwitterionic detergent. The reaction mixture (0.5 ml) contained 0.2 M Tris · HCl, pH 7.4, 10 mM $MgCl_2$, 0.2 mM ^{3}H-DES (1.5 Ci/ml), 2.2 mM saccharic acid 1,4-lactone and microsomes (1 mg/ml) was incubated at 37°C. Reaction was started by the addition of 2 mol UDP-GA and terminated after 10 min by the addition of 0.5 ml of ethanol. Chloroform, 5 ml, was added to extract the unreacted DES from the reaction mixtures by shaking vigorously for 10 min. After extraction, radioactivity in an aliquot (200 μl) of the aqueous phase was determined by liquid scintillation counting (Tri-Carb 2000 series, Packard Instrument Co., USA). Hepatic UDP-GA was determined by the methods of Watkins *et al.* (1982). Protein contents in microsomes were determined by the method of Bradford (1976) using Bio-Rad Protein Assay Kit (Bio-Rad, California, USA).

RESULTS

The content of glycyrrhizin in GR was determined as 2.3% by HPLC analysis (Fig. 1). Effects of GR (1 g/kg, *p.o.*, 6 days) and glycyrrhizin (23 mg/kg, *p.o.*, 6 days) on the enzymatic activity of UDP-GT2 were examined (Fig. 2). GR and glycyrrhizin increased the specific activity of UDP-GT2 in similar degrees, 111% and 96%, respectively, when 3-MC (20 mg/kg in corn oil, *i.p.*, 4 days) which is an inducer of the enzyme increased the activity 416%. As shown in Fig. 3, the specific activity of UDP-GT1 was increased 25% by GR treatment. Glycyrrhizin treatment, however, did not alter the activity of UDP-GT1. Phenobarbital (75 mg/kg, *i.p.*, 4 days), a known inducer of UDP-GT1,

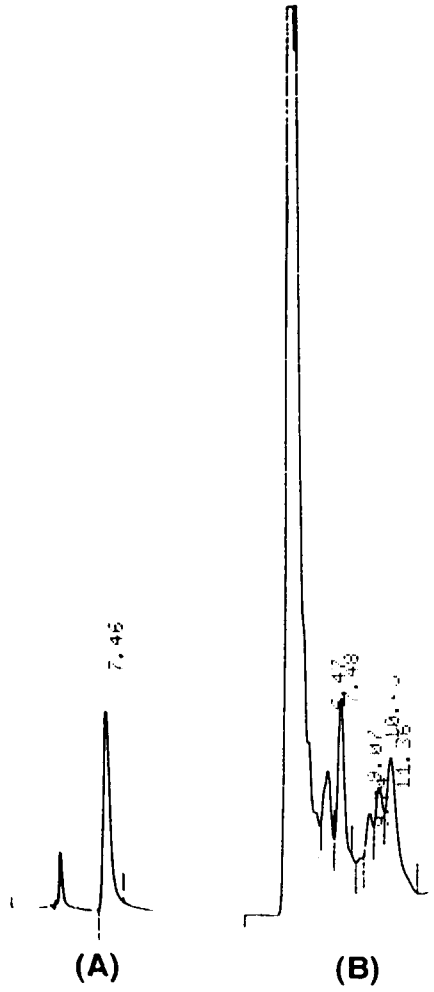

Fig. 1. Quantitative analysis for glycyrrhizin in GR by HPLC using μ-Bondapak C_{18} column. GR and glycyrrhizin were eluted with acetonitrile/water/acetic acid (40 : 59 : 1) at a flow rate of 1.0 ml/min. Elution of the components in GR was detected at 254 nm. (A) Glycyrrhizin standard. (B) Methanol extract of GR

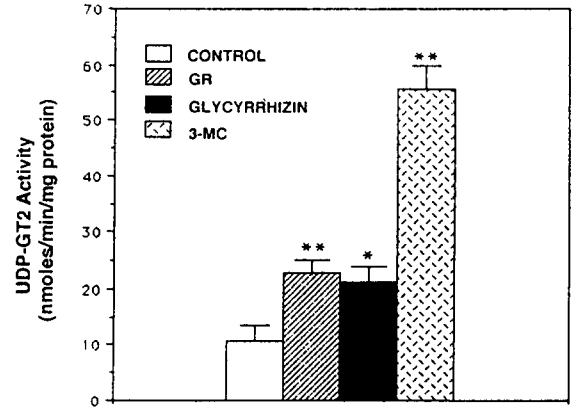

Fig. 2. Effect of GR, glycyrrhizin and 3-MC on hepatic UDP-GT2 activity. The UDP-GT2 activities were determined in livers of rats treated with GR (1 g/kg, *p.o.*, 6 days), glycyrrhizin (23 mg/kg, *p.o.*, 6 days), 3-MC (20 mg/kg in corn oil, *i.p.*, 4 days) or water (*p.o.*, 6 days) as control. Bars represent the means+S.E. of five rats. *Significantly different ($p<0.05$) from control. **Significantly different ($p<0.01$) from control

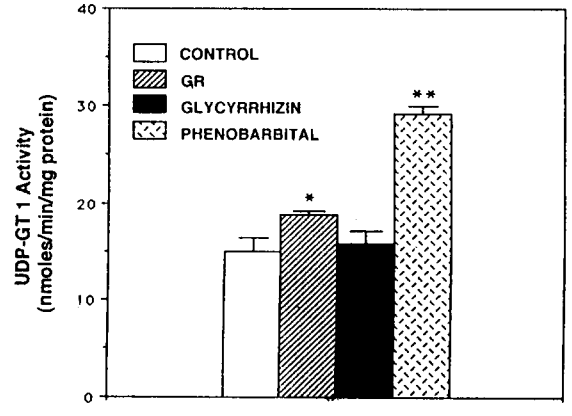

Fig. 3. Effect of GR, glycyrrhizin and phenobarbital on hepatic UDP-GT1 activity. The UDP-GT1 activities were determined in liver of rats treated with GR (1 g/kg, *p.o.*, 6 days), glycyrrhizin (23 mg/kg, *p.o.*, 6 days), phenobarbital (75 mg/kg, *i.p.*, 4 days) or water (*p.o.*, 6 days) as control. Bars represent the means+S.E. of five rats. *Significantly different ($p<0.05$) from control. **Significantly different ($p<0.01$) from control

was used as a positive control and caused 95% increase in the enzymatic activity. Hepatic concentrations of UDP-GA were determined in GR- and glycyrrhizin-treated rats (Fig. 4). GR and glycyrrhizin treatment increased the UDP-GA concentrations 257% and 484%, respectively. These findings indicate that licorice and its major component glycyrrhizin activated hepatic glucuronidation in Sprague-Dawley rats.

DISCUSSION

The present study clearly demonstrated that GR increased the activity of hepatic UDP-GT2 (Fig. 2) and UDP-GT1 (Fig. 3) activities as well as the hepatic concentration of UDP-GA (Fig. 4) in rats. The results presented here indicate that increasing effect of GR on the excreted amount of acetaminophen-glucuronide conjugate, which was demonstrated previously (Kim *et al.*, 1983) may have resulted from stimulation of the hepatic glucuronidation pathway by GR pretreatment in rats. This result, along with the reported protective effect of glycyrrhizin on enzyme leakage from injured rat hepatocytes (Nakamura *et al.*, 1985), suggested a possible application of GR and/or glycyrrhizin for detoxification of xenobiotics.

As shown in Fig. 2, GR and glycyrrhizin increased the UDP-GT2 activity in similar degrees. These data suggest that the inducing effect of GR on hepatic UDP-GT2 activity was due to glycyrrhizin contained in GR. However, the possibility that there may be

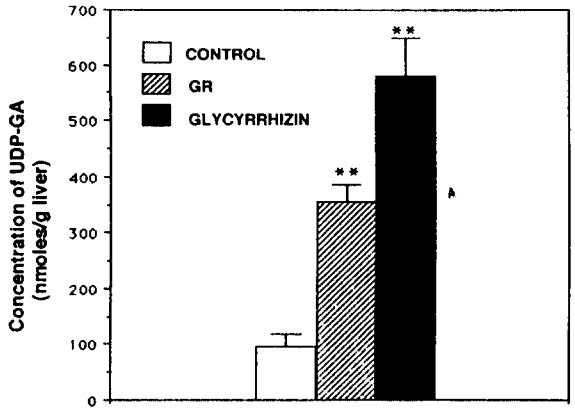

Fig. 4. Effect of GR and glycyrrhizin on hepatic UDP-GA concentration. The UDP-GA concentrations were determined in livers of rats treated with GR (1 g/kg, *p.o.*, 6 days), glycyrrhizin (23 mg/kg, *p.o.*, 6 days) or water (*p.o.*, 6 days) as control. Bars represent the means+S.E. of five rats. **Significantly different ($p<0.01$) from control

another UDP-GT2 inducer and inhibitor present in the mixture of GR still remains. The data on the UDP-GT1 activity (Fig. 3) indicate that GR caused an increase in UDP-GT1 activity in a lesser degree compared to the increasing effect on UDP-GT2 activity and that the effect might be due to other component (s) than glycyrrhizin in GR. It is noteworthy that the increased substrate availability (Fig. 4) did not enhance the enzymatic activity of UDP-GT1 upon glycyrrhizin treatment.

It has been reported that the activity of UDP-GT2 was increased in livers of rats treated with 3-MC (Mulder, 1992). Iyanagi *et al.* (1986) have cloned and determined the complete nucleotide sequence of the 1,927-base pairs cDNA encoding rat mRNA for UDP-GT2. They demonstrated that the level of the UDP-GT2 mRNA was increased in livers of rats treated with 3-MC (4 mg/100g body weight) daily for 3 days. It would be worthwhile to study if UDP-GT2 mRNA is induced by the treatment of GR in rat liver using a fragment of the cDNA as a probe. The cytochromes P-450c and d, located in the endoplasmic reticulum, are also induced in rat livers by the treatment of 3-MC (Sogawa *et al.*, 1985). Therefore, the two species of cytochrome P-450 and UDP-GT2 which catalyze the two successive reactions in drug metabolism, are coinduced transcriptionally in livers of 3-MC-treated rats. It would also be interesting to study whether or not the phase I enzymes such as cytochrome P-450 are also induced in response to GR treatment.

The hepatic concentrations of UDP-GA were also increased by GR (Fig. 4). The result which shows that glycyrrhizin caused an increase about twice as much as GR did suggests a possibility that strong inducing effect of glycyrrhizin on the concentration of hepatic UDP-GA might be interfered by other component(s) in GR. Based on the fact that glycyrrhizin is hydrolyzed to an aglycone, glycyrrhetic acid, and two molecules of glucuronic acid, it can be suggested that the inducing effect of glycyrrhizin on the concentration of hepatic UDP-GA may be due to the increased level of glucuronic acid upon the administration of glycyrrhizin *in vivo*. However, it has been demonstrated that the rate-determining step in UDP-glucuronic acid synthesis is UDP-glucose dehydrogenase (Hjelle, 1986), indicating that manipulation of glucuronidation rate by carbohydrate supplementation is probably of limited usefulness. Therefore, the possibility that the UDP-GA-inducing effect of glycyrrhizin was due to the glucuronic acid which would be produced upon glycyrrhizin administration is rare.

As mentioned in Introduction, glucuronidation is dependent on the enzymatic activity of UDP-GT and intracellular concentration of UDP-GA. The data obtained in this study demonstrate that GR and/or glycyrrhizin enhanced both the enzyme activity and the substrate availability. These results suggest that GR and glycyrrhizin have a potential to activate glucuronidation pathways in rat liver and thus may contribute to detoxification of xenobiotics in rats.

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