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Effect of Glycyrrhizae Radix on the Expression of UDP-Glucuronosyltransferase-1A1 (UGT1A1) in Rat Liver

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Abstract – Licorice has been widely used in combination with other herbs or synthetic drugs for various disorders. In an effort to study the effect of licorice roots (Glycyrrhizae Radix, GR) and glycyrrhizin on the hepatic glucuronidation, we have previously found that the pretreatment of GR or glycyrrhizin for 6 days resulted in a marked increase in the enzymatic activity of 3-methylcholanthrene (3-MC)-inducible hepatic UDP-glucuronosyltransferase (UGT) isozyme that has high affinity toward phenolic substrates (*p*-nitrophenol form, UGT1A) in Sprague-Dawley rats. As an approach to elucidate the mechanism for the enzyme activation by licorice in rat liver, we examined the levels of hepatocellular mRNAs for UGT1A upon the treatment of GR or glycyrrhizin. The hepatic mRNAs were extracted from Sprague-Dawley rats and Wistar rats after the treatment of the methanol extract of GR (1 g/kg, *p.o.*), glycyrrhizin (23 mg/kg, *p.o.*) for 6 days, or 3-MC (40 mg/kg, *i.p.*) for 3 days. Using the UGT1A1 cDNA as a probe, we found that the mRNAs for the enzyme were induced by 3-MC treatment while those were influenced neither by GR nor by glycyrrhizin in both strains of rats. These results indicate that the activation of rat liver UGT1A by licorice and glycyrrhizin was not due to the induction of mRNAs for the enzyme.

Keywords □ licorice, glycyrrhizin, UDP-glucuronosyltransferase, mRNA induction

GR has been widely used in combination with other herbs or synthetic drugs for various disorders. The major components of GR 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) 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). Pretreatment of the methanol extract of GR was shown to increase the cumulative biliary and urinary excretions of acetaminophen-glucuronide conjugate after the administration of acetaminophen in male Sprague-Dawley rats (Kim *et al.*, 1993).

Glucuronidation is a major detoxification reaction involved in metabolic conversion of endogenous and exogenous substances to more polar compounds that can be excreted into urine or bile (Kasper and Henton, 1980). Glucuronidation is dependent upon the enzymatic activity of UGT and intracellular concentrations of UDP-glucuronic acid. Several isozymes of UGTs, which are differentially regulated and appear to be specific for different sets of substrates, exist to glucuronidate a large variety of structurally unrelated substances (Bock, 1979; Falany and Tephly, 1983; Mulder, 1992).

According to the recommended nomenclature system (Burchell *et al.*, 1991), the UGT1 family is composed of drug-glucuronidating forms, and the UGT2 family contains steroid-glucuronidating forms. The UGT1A (Emi *et al.*, 1995) accommodates flat, planar substrates such as acetaminoplen and *p*-nitrophenol and is induced by 3-MC. In an effort to study the effect of GR and glycyrrhizin on the hepatic glucuronidation, we have previously found that the treatment of GR or glycyrrhizin caused a marked increase in the enzymatic activity of hepatic *p*-nitrophenol UGT1A (Moon *et al.*, 1995).

The cDNAs of rat liver UGT messengers that were induced by phenobarbital (Mackenzie, 1986) or 3-MC (Iyanagi *et al.*, 1986) have been cloned and characterized.

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Iyanagi *et al.* (1986) have cloned and determined the complete nucleotide sequence of the 1,927-base pairs cDNA encoding mRNA for rat UGT1A. Four exons (A1~A4) have been located in the UGT1A cluster (Emi *et al.*, 1995). Exon A1 (UGT1A1) corresponded to *p*-nitrophenol UGT, exons A2 and A3 encoded distinct isoforms which have not been characterized, while exons A4 was a pseudogene. To determine whether the enzymatic activation of UGT1A by GR was due to the induction of mRNAs for the enzyme in rat liver, we examined the level of hepatocellular mRNA for UGT1A1 upon the treatment of GR or glycyrrhizin using the UGT1A1 cDNA as a probe in the present study.

MATERIALS AND METHODS

Materials

Root of *Glycyrrhizae glabra* was purchased from Kyung-Dong market, Seoul, Korea, and has been deposited in a herbarium at Duksung Women's University. GR was extracted with methanol and freeze-dried. Glycyrrhizin, 3-MC, guanidinium thiocyanate (molecular biology grade), 2-mercaptoethanol (molecular biology grade), phenol (molecular biology grade), MOPS (3-[N-Morpholino] propanesulfonic acid), chloroform, agarose and ethidium bromide (molecular biology grade) were purchased from Sigma Chem. Co. (St. Louis, MO, U.S.A.). The restriction enzymes were purchased from Kosko R & D Center (Kyunggi-Do, Korea) and Promega (Madison, WI, U.S.A.). All other chemicals were reagent grade or molecular biology grade.

Animal treatments

Male Sprague-Dawley and Wistar rats, weighing 220-240 g, were supplied from Toxicology Research Inshitute, Korea FDA. 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.

Rats were pretreated as follows. GR (1 g/kg), glycyrrhizin (23 mg/kg) and water as control were administered daily (*p.o.*) for 6 days. 3-MC (40 mg/kg) in corn oil was administered daily (*i.p.*) for 3 days. The rats were killed by decapitation without the use of any anesthetics 24 hr after pretreatment. The liver was excised, rinsed with saline, blotted and frozen immediately in liquid nitrogen. The liver was stored at -70 ℃ until further experiments.

Preparation of cDNA probes

The UGT1A1 cDNA (Iyanagi *et al.*, 1986) from Wistar rat cloned in pUC18 plasmid was kindly provided by Dr. T. Iyanagi (Himeji Institute of Technology, Hyogo, Japan). The *E. coli* JM83 cells were transformed by the recombinant plasmid containing UGT1A1 cDNA as described in Sambrook *et al.* (1989). The amplified pasmid was subjected to restriction enzyme digestion for identification of the restriction sites for *EcoRI*, *Hind*III, *Pst*I, and *Pvu*II. The 1.2 kb *Eco*RI fragment of cDNA was purified by Geneclean II™ Kit (Bio101 Inc., La Jolla, U.S.A.) and used as a hybridization probe. The cDNA for a housekeeping gene, chicken glutaraldehyde-3-phosphate dehydrogenase (GAPDH) gene (Dugaiczyk *et al.*, 1983) was purified and used as a hybridizing probe. The UGT 1A1 and GAPDH cDNAs were labelled by using ECL direct nucleic acid labelling and detection system™ (Amersham, Buckinghamshire, England).

Northern Blotting

Total RNA was isolated from rat liver by a slight modification of the method of Chomczynski and Sacchi (1987). RNA was fractionated by electrophoresis on 1% agarose gel containing 2% formaldehyde and transferred overnight to nylon filter (Hybond-ECL™ membrane, Amersham). Northern blot analysis was performed as described in Sambrook *et al.* (1989) with a slight modification.

RESULTS AND DISCUSSION

The UGT1A1 cDNA as a 1.2 kb *Eco*RI fragment in pUC18 vector is shown as a heavy bar (Iyanagi *et al.*, 1989; Iyanagi, 1991) in Fig. 1A. The plasmid was amplified in *E. coli* JM83 cells and purified. Restriction enzyme sites for *Eco*RI, *Hind*III, PstI, and *Pvu*II were tested for identification (Fig. 1B, lanes 3~8). The 1.2 kb cDNA insert was isolated from the plasmid and purified (Fig. 1B, lane 9).

Using the isolated 1.2 kb EcoRI fragment of UGT1A1 cDNA as a probe, we carried out Northern blot hybridization analysis of mRNAs from Sprague-Dawley and Wistar rat livers (Fig. 2). It has generally been known that there might not be significant strain difference in the activity of drug metabolizing enzymes. However, we used two strains of rats to ascertain that there is no difference since the UGT1A activity study (Moon *et al.*, 1995) was performed in Sprague-Dawley

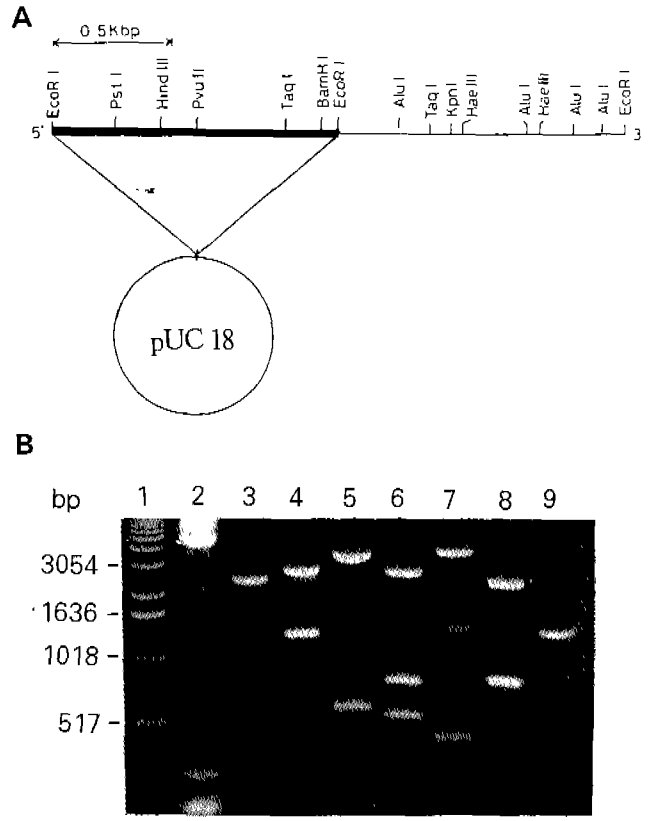

Fig. 1. Restriction map of the UGT1A1 cDNA used as a probe. *A*, Restriction map. Heavy bar represents the 1.2 kb *Eco*RI fragment cloned into the pUC18 plasmid. *B*, Identification of restriction sites and purification of the UGT 1A1 cDNA insert. Restriction enzyme-digested fragments for the pUC18 DNA containing the UGT1A1 cDNA insert were separated in a 1% agarose gel. Each lane represents the following; 1, 1 kb size marker (lane 1); 123 bp size marker (lane 2); uncut plasmid DNA (lane 3); *Eco*RI fragments (lane 4); *Hind*III fragments (lane 5); *Eco*RI and *Hind*III double digested fragments (lane 6); *Pst*I fragments (lane 7); *Pvu*II fragments (lane 8); purified the 1.2 kb *Eco*RI cDNA insert (lane 9).

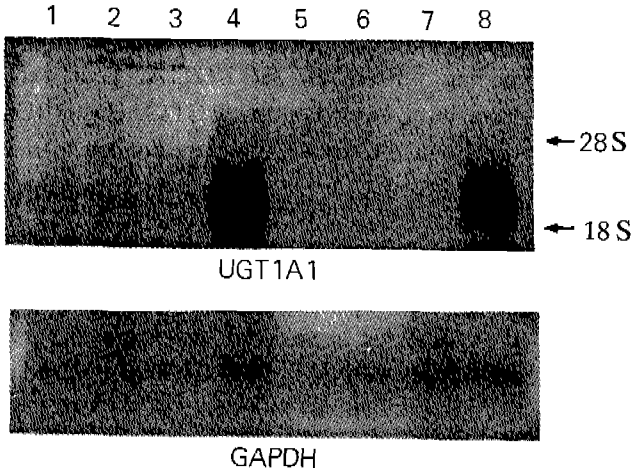

Fig. 2. Effect of GR, glycyrrhizin and 3-MC administration on rat liver UGT1A1 mRNA. The total RNA (30 μg) was detected using a ECL-labelled cDNA probe. Lanes 1~4, Sprague-Dawley rat; lanes 5~8, Wistar rat. Lanes 1 and 5, liver from control rat; lanes 2 and 6, liver from GR-treated rat; lanes 3 and 7, liver from glycyrrhizin-treated rat; lanes 4 and 8, liver from 3-MC-treated rat. Migration of 28S and 18S RNAs is as indicated.

rats and the cDNA probe was obtained from Wistar rat (Iyanagi *et al.*, 1986). A single band of approximately 2.3 kilobases was detected in all lanes of RNAs tested, indicating that the UGT1A1 gene is transcribed in rat livers and the UGT1A1 probe from Wistar rat hybridized well with the corresponding gene in Sprague-Dawley rats. The mRNAs for the enzyme were markedly induced by 3-MC treatment (Fig. 2, lanes 4 and 8). However, those were influenced neither by GR (lanes 2 and 5) nor by glycyrrhizin (lanes 3 and 7) in comparison to control (lanes 1 and 4) in both strains of rats. The data indicate that the activation of rat liver UGT1A by licorice and glycyrrhizin was not due to the induction of mRNAs for the enzyme. The data also showed that the UGT1A1 mRNAs were induced by 3-MC treatment with an equal extent in Sprague-Dawley and Wistar rat livers.

To determine whether the amount of RNAs loaded onto the gel was even, the RNAs were also hybridized to a housekeeping gene, GAPDH gene (Fig. 2). When the blots were stripped of the UGT1A1 probe and probed again with the GAPDH probe, all lanes showed approximately equal concentration of GAPDH mRNA. For labelling and detection of the probes, we used a non-radioisotope ECL system. The hybridized RNA blots showed that the use of ECL replaced ^{32}P successfully.

The activities of the biotransformation enzymes can be enhanced following treatment with chemicals including drugs, pesticides, and natural products. In general, this enhanced activity results from an increase in the rate of synthesis of the biotransformation enzymes, in other words, an enzyme induction. In the present study, we found, however, that the activation of rat liver UGT1A by licorice and glycyrrhizin was not due to the induction of mRNAs for UGT1A1. It would be of interest to find out if the mRNAs for UGT1A2 or UGT1A3 (Emi *et al.*, 1995) were induced by GR treatment.

It is possible that the mechanism for the increase in UGT1A activity by GR involves change in the membrane structure. UGT1A is a membrane-bound enzyme

that is distributed in the endoplasmic reticulum and the nuclear envelope (Roy Chowdhury *et al.*, 1985). The enzymatic activity of UGT1A in the isolated microsomes is markedly stimulated by the treatment of detergents which affects the integrity of the membrane structure (Heirwegh *et al.*, 1972). It is probable that glycyrrhizin induces alteration in membrane fluidity by reacting with the components of membrane or intercalating its moiety into the membrane structure (Nakagawa and Asami, 1981). Another microsomal enzyme, NADPH-cytochrome P-450 reductase is bound to the cytoplasmic surface of the endoplasmic reticulum membrane (Black, 1982). The UGT is known to be involved in drug metabolism in concert with cytochrome P-450, that is, phase I and II reactions take place successively in the endoplasmic reticulum (Iyanagi *et al.*, 1986). It would be interesting to study whether or not activities of the phase I enzymes are also increased upon licorice treatment.

In the previous report (Moon *et al.*, 1995), we found that the intracellular concentration of UDP-glucuronic acid, the required cosubstrate for all glucuronidation reactions in mammals, was increased in rat livers by GR and glycyrrhizin. Thus, it is possible that the increased hepatic concentration of the substrate may be the only cause for the enhanced enzymatic activity of UGT1A. 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 administration of glycyrrhizin *in vivo* may increase the hepatocellular pool of UDP-glucuronic acid. 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 increasing effect of glycyrrhizin on hepatocellular UDP-glucuronic acid concentration does not seem to be due to glucuronic acid which would be produced upon glycyrrhizin administration.

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