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The Effect of Glycyrrhizae Radix on the Metabolism of Acetaminophen

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Abstract—The effects of Glycyrrhizae Radix (GR) on the metabolism of acetaminophen (AA) were examined in male Sprague-Dawley rats. The methanol extract of GR (500 mg/kg) was administered orally to rats for 6 days. AA and its metabolites excreted in bile, urine and blood within 120 min after dosing of AA (150 mg/kg, i.v.) were assayed by HPLC. Treatment of rats with the methanol extract of GR significantly increased the cumulative biliary excretion of AA-glucuronide (156% of the control) and decreased that of AA-sulfate (63% of the control). The cumulative urinary excretion of AA-glucuronide was also significantly increased to 132% of the control. GR treatment significantly increased total (biliary plus urinary) excretion of AA-glucuronide (172% of the control) without influencing thioether and sulfate conjugates of AA. The results clearly show that GR enhances UDP-glucuronosyl transferase-mediated detoxication of AA, but may not influence sulfotransferase-mediated and cytochrome P-450-mediated metabolites formation.

Keywords □ *Glycyrrhizae Radix*, acetaminophen metabolites.

Glycyrrhizin, extracted from the root of licorice (*Glycyrrhiza glabra*), and its aglycone, glycyrrhetinic acid, have been demonstrated to have various biological activities such as anti-inflammatory (Finney *et al.*, 1958; Hino *et al.*, 1981; Yamamoto *et al.*, 1963), anti-allergic (Sotomatsu *et al.*, 1959; Kuroyanagi and Saito, 1966; Kuroyanagi *et al.*, 1962; Inoue *et al.*, 1987), anti-gastric ulcer (Takagi *et al.*, 1969; Doll *et al.*, 1962) and anti-viral (Baba and Shigeta, 1987; Pompei *et al.*, 1979) activities. Glycyrrhizin has also been reported to have inhibitory effects on the chronic hepatitis (Abe *et al.*, 1982) and the *in vitro* infectivity and cytopathic activity of human immunodeficiency virus (Ito *et al.*, 1987). Glycyrrhizin and glycyrrhetinic acid have also been found to be inhibitory on corticosteroid 5α-, 5β-reductase and 11β-dehydrogenase activities in rat liver and kidney *in vivo* and *in vitro* (Monder *et al.*, 1989). It has been demonstrated that glycyrrhizin attenuated the liver damage caused by carbon tetrachloride, allyl formate, and other hepatotoxins in rats (Nakamura *et al.*, 1985). The exact mechanism of action, however, has not been elucidated yet.

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AA 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 AA-glutathione. Studies using bile duct-cannulated hamsters, mice, rats, rabbits and guinea pigs have shown that AA-glutathione and AA-glutathione-derived thioether conjugates (AA-cysteinylglycine, AA-cysteine and AA-mercapturate) are excreted into bile and only a small fraction of AA-thioether conjugates appears in urine as AA-mercapturate (Gregus *et al.*, 1988). Madhu *et al.* (1989) suggested that the biliary excretion of AA-glutathione reflects the formation of reactive AA metabolite *in vivo*, and thus, concluded that measuring biliary excretion of AA-glutathione appeared to be a useful method for studying the effect of endogenous and exogenous influences on toxication of AA *in vivo*.

In the present work, we have studied the effect of GR on the overall biotransformation of AA by simulta-

neous examination of urinary and biliary excretions of AA metabolites.

Materials and Methods

Animals and Chemicals

Male Sprague-Dawley rats, weighing 250-300g, were supplied from National Institute of Safety Reasearch, Seoul, Korea. They were provided tap water and lab chow (Shinchon Diet Co., Korea) *ad libitum* and were housed at 23℃, 55± 10% humidity, in a 12-hr light/12-hr dark cycle. AA was purchased from Sigma Chemical Co, U.S.A.. *Glycyrrhiza glabra* was purchased from Kyung-Dong market and extracted with methanol. The methanol extract of GR was lyophilized and stored at refrigerator until sample preparation was made. Pentobarbital was purchased from TCI Chemical Co., Japan.

Treatments

Rats were pretreated with methanol extract of GR dissolved in distilled water (500 mg/kg, p.o., daily), in a volume of 10 ml/kg, for 6 days. Control rats were received tap water for the same period of time.

Surgical Procedures

Rats were anesthetized with pentobarbital (50 mg/kg, i.p.). To maintain patent airways, a tracheotomy was performed on each animal. The right carotid artery and the common bile duct were cannulated using polyethylene tubing(PE-50) and a 23-gauge needle attached to PE tubing for collection of blood and bile samples, respectively. The urinary bladder was exteriorized through a middle incision of the abdominal wall. It was emptied by gentle compression before AA administraton. Anesthetic-induced hypothermia and the associated change in bile flow were prevented by using a heat lamp.

AA Administration and Sample Collection

AA was administered into the saphenous vein at dosage of 150 mg/kg dissolved in saline containing 10% mannitol. Bile and urine samples were collected for 2 hr after dosing in 15 min collection periods. Arterial blood samples (120~150 μl) were collected in heparinized tubes at 2, 5, 10, 20, 40, 60, 90, and 120 min after the AA injection. The bile, urine and blood samples were used immediately or stored at −70℃ until analysis could be performed. In order to maintain a constant urine flow, 1 ml/kg of saline containing 5% mannitol was injected through the carotid cannula every 15 min after AA administration. The volumes of bile and urine collected at each period were measured gravimetrically assuming unity for the specific gravities. Plasma was prepared by centifugation of blood at 3500×g for 10 min.

Analysis of AA and AA Metabolites

Biliary and urinary excretion rates of AA and its metabolites were calculated as the products of the flow rates and concentration in bile and urine. The concentrations of AA and its metabolites in bile, urine and blood samples were determined by HPLC as described by Howie *et al.* (1977). Blood, bile and urine were diluted 5-, 100- and 100-fold, respectively, with methanol and centrifuged before analysis. AA and its metabolites were separated using a C_{18}-μ Bondapak reverse phase column (3.9×300 mm; Waters Associates, Inc., Boston, MA) preceded by a precolumn (3.9×25 mm) packed with μCorasil (Waters Associates Inc.). AA and its metabolites were eluted with water/methanol/acetic acid (900 : 80 : 8) at a flow rate of 1.5 ml/min. The elution of the metabolites was monitored by absorbance at 254 nm. Chromatographic analysis of control bile, urine and blood samples from the rats did not contain any interfering peaks. Quantitation of AA and its metabolites was based on integrated peak areas. The concentrations of AA metabolites were determined using an AA standard curve because their molar extinction coefficients are reported to be the same as AA (Howie *et al.*, 1977).

Statistical Analysis

Data were analyzed by one-way analysis of variance. Haseken's test was used to compare the means. The significance level was set at $p<0.05$.

Results

Quantitative analysis for the excretions of AA and its metabolites was performed by using HPLC. The biliary excretion rates of AA and its metabolites in control and GR-treated rats are depicted in Fig. 1. The excretion rate of AA-glucuronide, the most abundant metabolite of AA in bile, was the highest at the second 15 min collection period after AA administration, then declined thereafter. GR treatment significantly increased the biliary excretion rate of AA-glucuronide throughout the time range tested (0~120 min). The biliary excretion of AA-sulfate also peaked at 59.3 nmol/kg·min in the second 15 min collection period. GR treatment significantly decreased the biliary excretion rate of AA-sulfate from the first 15 min collection period (Fig. 1), resulting in a 63% reduction in the cumulative biliary excretion of AA-glucuronide (Table I). The graph for the excretion rate of AA-glutathione showed slight decrease in GR-treated rats at all time points. However, the difference between the two groups was

not statistically significant. The biliary excretion of AA, the parent compound, declined steadily over the course of the experiment. GR treatment produced no changes in the biliary excretion rate of AA.

The effect of GR on the cumulative biliary excretions of AA and its metabolites is shown in Table I. GR caused significant ($p<0.05$) changes in the amounts of AA-glucuronide and AA-sulfate excretions in bile when compared to controls. The cumulative biliary excretion of AA-glucuronide was significantly increased to 156%, whereas that of AA-sulfate was decreased to 63% by GR. GR treatment did not make significant differences in AA-glutathione and AA excretions into bile.

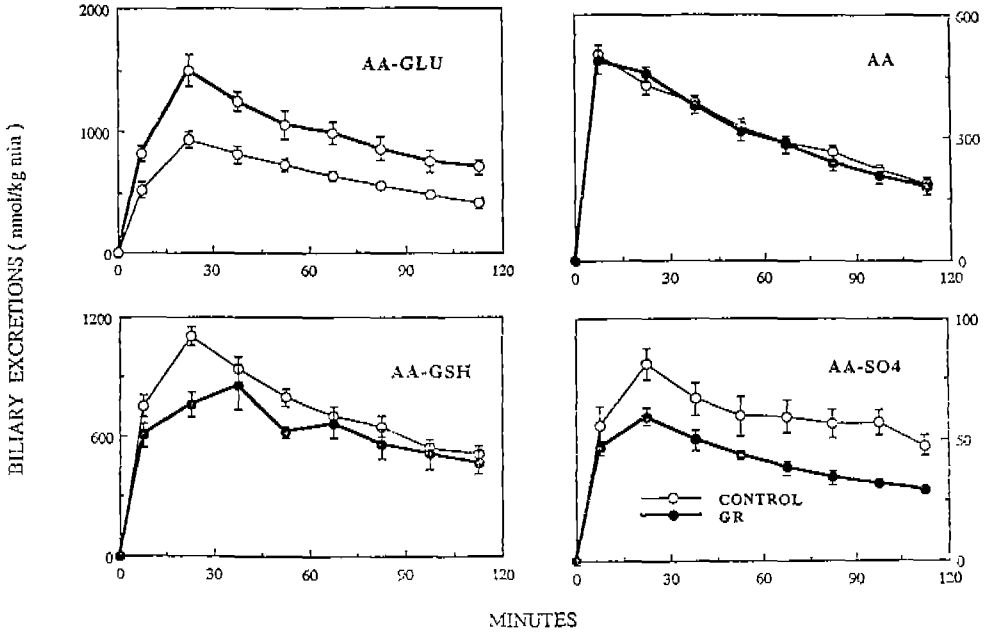

Fig 1. Effect of GR on biliary excretion of AA and its metabolites. Rats were treated with methanol extract of GR (500 mg/kg, p.o.) for 6 days before injection of AA (150 mg/kg, i.v.). Symbols represent mean values± SE of eight to ten rats.

Table I. Effect of GR on cumulative biliary excretion of AA and its metabolites[a]

Treatment	Bile flow	Biliary excretion[b]				Total
		AA-glucuronide	AA-sulfate	AA-glutathione	AA	
	μl/kg·min		μmol/kg			
Control	106.5± 4.4[c]	76.2± 5.9	7.3± 0.8	88.4± 5.6	39.3± 1.9	211.2± 14.2
GR	102.6± 4.4	119.0± 10.9*	4.6± 0.3*	76.9± 8.2	38.4± 0.3	238.9± 19.7

[a] Experimental conditions are the same as described in Fig. 1.
[b] Represents amount excreted into bile within 120 min after administration of AA (150 mg/kg, i.v.).
[c] Values are means± SE of eight to ten rats.
*significantly different from controls at $p<0.05$.

Table II. Effect of GR on cumulative urinary excretion of AA and its metabolites[a]

Treatment	Urine flow	Urinary excretion[b]				Total
		AA-glucuronide	AA-sulfate	AA-mercapturate	AA	
	μl/kg·min		μmol/kg			
Control	77.5± 5.0[c]	95.1± 11.9	133.9± 9.2	8.1± 2.5	13.3± 3.7	250.4± 27.3
GR	82.9± 3.5	126.0± 19.3*	162.4± 7.8	6.7± 1.0	13.1± 2.2	308.2± 30.3

[a] Experimental conditions are the same as described in Fig. 1.
[b] Represents amount excreted into urine within 120 min after administration of AA (150 mg/kg, i.v.).
[c] Values are means± SE of eight to ten rats.
*significantly different from controls at $p<0.05$.

Table III. Effect of GR on the total excretion (biliary plus urinary) of AA and its metabolites[a]

Treatment	Excretion[b]				
	AA-glucuronide	AA-sulfate	AA-thioethers[c]	AA	Total
			μmol/kg		
Control	171.3± 17.8[d]	141.2± 10.0	96.5± 8.1	52.6± 5.6	461.6± 41.5
GR	295.0± 30.2*	167.0± 8.1	83.6± 9.2	51.5± 2.5	597.1± 50.0

[a]Experimental conditions are the same as described in Fig. 1.
[b]Represents amount excreted into bile plus urine within 120 min after administration of AA (150 mg/kg, i.v.).
[c]Represents the sum of AA-glutathione and AA-mercapturate.
[d]Values are means± SE of eight to ten rats.
*significantly different from controls at $p<0.05$.

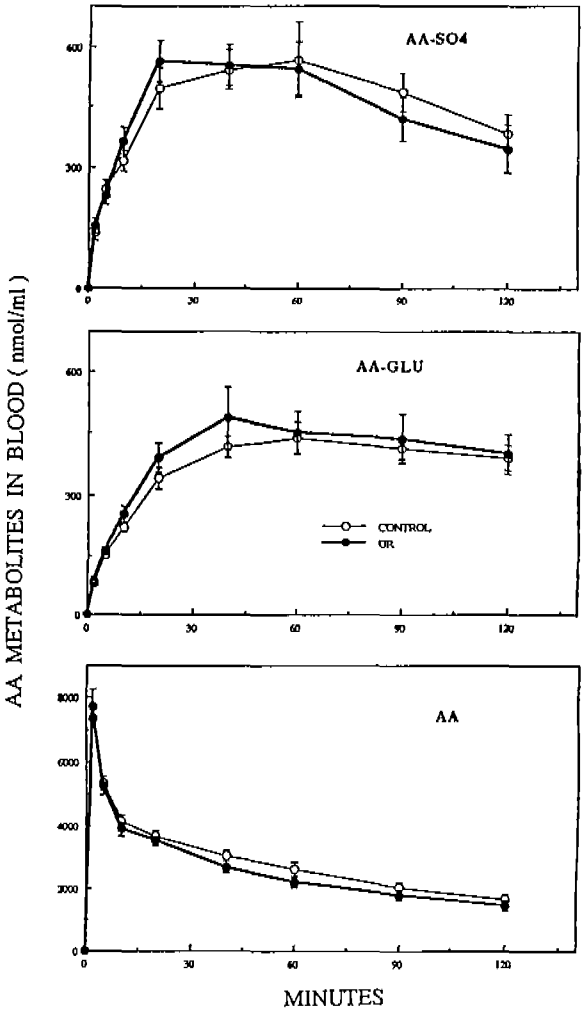

Fig. 2. Effect of GR on blood concentration of AA and its metabolites. Experimental conditions are the same as described in Fig. 2. Symbols represent mean values± SE of eight to ten rats.

The effect of GR on the cumulative urinary excretions of AA and its metabolites is shown in Table II. GR increased the urinary excretion of AA-glucuronide significantly (to 132%). The urinary excretions of AA-sulfate, AA-mercapturate and AA were not significantly affected by GR treatment. The majority of AA-thioether conjugates was excreted into bile as AA-glutathione and only a small amount was detected in urine as AA-mercapturate. The excretion of AA-mercapturate accounted for only 2~3% of the total urinary excretion of AA and its metabolites in GR-treated rats.

The effect of GR on the total excretions (biliary plus urinary) of AA and its metabolites is summarized in Table III. Relative to control rats, GR treatment resulted in 30% (statistically nonsignificant) increase in the total excretion of AA and its metabolites. The total excretions of AA-sulfate, AA-thioethers and AA were not significantly different from controls at $p<0.05$. However, marked change was noted in the excretion of AA-glucuronide. The total excretion of AA-glucuronide was increased by GR (172%). This suggested that GR enhanced the metabolic pathway *via* UDP-glucuronosyl transferase *in vivo*.

The time courses for the disappearance of AA and appearance of AA conjugates in the blood of control and GR-treated rats are depicted in Fig. 2. Blood concentrations of AA-sulfate and AA-glucuronide increased after administration of AA. The peak blood concentrations of AA-sulfate exceeded that of AA-glucuronide in both groups of rats. GR treatment did not affect the blood concentrations of AA and its metabolites.

Discussion

Treatment of rats with GR increased the total (biliary plus urinary) excretion of AA-glucuronide to 172 % without influencing the excretions of AA-sulfate and -thioethers conjugates. This finding suggests that GR enhances a detoxification pathway of AA mediated by UDP-glucuronosyl transferase in rat liver. One possible mechanism for this effect is that the treatment of GR increases hepatocellular levels of UDP-glucuronic acid, which is a substrate for UDP-glucuronosyl transferase,

since glycyrrhizin, one of the component of GR, is hydrolyzed to produce one molecule of glycyrrhetic acid and two molecules of glucuronic acid. It is also possible that GR treatment might increase UDP-glucuronosyl transferase activity in liver. We are currently studying those possibilities. Several compounds (butylated hydroxyanisole, pentobarbital, 3-methylcholanthrene, and pregnenolone-16α-carbonitrile) were reported to increase both UDP-glucuronosyl transferase activity and UDP-glucuronic acid concentration in rats and mice (Hjelle *et al.*, 1985; Hazelton and Klaassen, 1984; Watkins and Klassen, 1983).

GR treatment decreased the cumulative biliary excretion of AA-sulfate, whereas increased that of AA-glucuronide significantly without changing total amount of the excreted metabolites. However, a reduced amount of AA-sulfate by GR was only 1~2% of the total metabolites excreted in bile. The urinary excretion of AA-sulfate, the major urinary AA-metabolite, was not changed significantly by GR. These results suggest that GR may not affect sulfotransferase-mediated detoxification pathway in rat. Although small shift from biliary to urinary excretion was observed for AA-sulfate, it is not likely that GR changed the excretory routes for AA.

GR did not increase the excretion of AA-thioether conjugates, indicating that cytochrome P-450 isoenzymes responsible for the oxidation of AA to N-acetyl-*p*-benzoquinoneimine were not increased in rats by GR. A large difference has been reported to exist between animal species in the inducibility of cytochrome P-450 isoenzymes. Species that are sensitive to AA-induced hepatotoxicity (*i.e.*, mice and hamsters) excrete several times more AA-thioether conjugates into bile than species that are resistant to AA-induced liver injury (*i.e.*, rats, rabbits and guinea pigs). GR is known to have detoxifying effect on liver necrosis induced by chemicals. Thus it is of interest to determine whether GR reduces the formation of N-acetyl-*p*-benzoquinoneimine in mice or hamsters which are sensitive to AA-induced hepatotoxicity.

In summary, GR treatment significantly increased the cumulative biliary excretion of AA-glucuronide (156 %) and decreased that of sulfate (63%) compared to the controls. The cumulative urinary excretion of AA-glucuronide was also significantly increased to 132% of the control. The total (biliary plus urinary) excretion of AA-glucuronide was significantly increased to 172% of the control. The results presented here suggest that GR increases glucuronide conjugate by enhancing biotransformation in the elimination of xenobiotics.

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