Effects of Gomisin A on Liver Functions in Hepatotoxic Chemicals-Treated Rats[†]

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Abstract—The effects of gomisin A, which is a lignan component of schizandra fruits, on liver functions in various experimental liver injuries and on bile secretion in CCl₄-induced liver injury were studied. Gomisin A weakly accelerated the disappearance of plasma ICG by itself at a high dose (100 mg/kg, i.p.). All of the hepatotoxic chemicals used in this study inhibited the excretion of ICG from plasma. Gomisin A showed a tendency to prevent the delays of the disappearance of plasma ICG induced by CCl₄, d-galactosamine and orotic acid, but not that by ANIT. Bile flow and biliary outputs of total bile acids and electrolytes (Na⁺, K⁺, Cl⁻ and HCO₃⁻) were decreased in CCl₄-treated rats. Gomisin A maintained bile flow and biliary output of each electrolyte nearly to the level of the vehicle-treated group, but did not affect biliary output of total bile acids. These findings suggest that gomisin A possesses a liver function-facilitating property in normal and liver injured rats and that its preventive action on CCl₄-induced cholestasis is due to maintaining the function of the bile acids-independent fraction.

We previously reported that gomisin A, schizandrin and their analogues, which are lignan components of schizandra fruits, showed preventive effects on some experimental liver injuries in serum-biochemical and histopathological examinations (1). The efficacy of gomisin A was the most prominent among the lignan components. The contents of Gomisin A in schizandra fruits is ca. 0.24 dry wt.% (2).

In the present study, the effects of gomisin A on liver functions lowered by various hepatotoxic chemicals (carbon tetrachloride, d-galactosamine, α -naphthylisothiocyanate and orotic acid) and on

bile secretion in carbon tetrachloride-induced liver injury were investigated.

Materials and Methods

Animals

Male Wistar strain rats weighing 200–250 g were used.

Materials

Gomisin A (Fig. 1) was isolated from schizandra fruits according to the method of Ikeya et al. (3). This compound was suspended in 2% (v/v) Tween 80-saline solution

Fig. 1. Structure of gomisin A.

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just before the administration.

Chemicals

Carbon tetrachloride (CCl_4) was purchased from Wako Pure Chemical Ind. (Osaka, Japan), d-galactosamine hydrochloride from Sigma Chemical Co. (St. Louis, U.S.A.), α -naphthylisothiocyanate (ANIT) from Eastman Kodak Co. (Rochester, U.S.A.), orotic acid from Tokyo Kasei Co. (Tokyo, Japan) and indocyanine green (ICG, Diagnogreen® injection) from Daiichi Seiyaku Co. (Tokyo, Japan).

Methods

1. Effects of gomisin A on liver functions (ICG-clearance) in various experimental liver injuries

1-a) Effects on plasma ICG in normal rats:

Rats were anesthetized with intraperitoneal (i.p.) administration of pentobarbital-Na (50 mg/kg) 24 hr after the i.p. administration of the material (50 and 100 mg/kg) or vehicle. Two ml/kg of 0.5% (W/V) ICG-distilled water solution was injected via the femoral vein, and about 120 μl of blood was collected from the tail vein using a hematocrit tube (Terumo, Tokyo, Japan) at 5, 10, 20, 30, 40, 50 and 60 min after the injection. Each blood sample was centrifuged at 10,000 rpm for 5 min, using a centrifuge for hematocrit determination (Kubota/Model KH-120), to obtain the plasma. Forty μl of plasma was added with 2 ml of distilled water, and the absorbance at 805 nm was measured by using a spectrophotometer (Hitachi/Model 101). The amount of ICG was calculated from the previously constructed calibration curve.

1-b) Effects on plasma ICG in CCl_4 -treated rats:

The material (12.5–50 mg/kg) was administered i.p. and 4 ml/kg of 50% (V/V) CCl_4 -olive oil solution was orally (p.o.) given 30 min later. Twenty-four hr after the administration of CCl_4 , ICG was injected intravenously, and blood was periodically collected under anesthesia with pentobarbital-Na. The amount of plasma ICG was measured according to the procedure described in 1-a).

1-c) Effects on plasma ICG in d-galactosamine-treated rats: D-Galactosamine (400 mg/kg) was injected i.p. and the material (50 mg/kg) was also given i.p. 2 hr

later. The measurement of the amount of plasma ICG was performed with the same procedure as 1-a) 24 hr after the administration of d-galactosamine.

1-d) Effects on plasma ICG in ANIT-treated rats: Three ml/kg of 1.5% (W/V) ANIT-olive oil solution was given p.o. daily for 4 days. The material (50 mg/kg) was injected i.p. 30 min before every administration of ANIT. The amount of plasma ICG was measured using the same procedure as 1-a) 24 hr after the last administration of ANIT.

1-e) Effects on plasma ICG in orotic acid-treated rats: Two % (W/W) orotic acid-containing food (Oriental Kobo Co., Japan) was given for 12 days. The material was administered by adding it to the orotic acid-mixed food at the rate of 50 mg per 100 g. The measurement of the amount of plasma ICG was performed 24 hr after the last administration.

2. Effects of gomisin A on bile secretion in CCl_4 -treated rats

The material (50 mg/kg) was administered i.p. and 4 ml/kg of 50% (V/V) CCl_4 -olive oil solution was given p.o. 30 min later. Rats were laparotomized under anesthesia with pentobarbital-Na 24 hr after the administration of CCl_4 . A polyethylene tube (PE-10) was inserted into the common bile duct to drain bile off freely, and after 10 min, the bile was collected for 60 min to measure the volume. Biliary concentration of total bile acids was measured using Sterognost-3 α (Daiichi Chemical Pharmaceutical Co., Tokyo, Japan) and that of electrolytes (Na^+ , K^+ , Cl^- and HCO_3^-) using Stat/Ion II (Technicon Instruments Co., New York, U.S.A.).

Statistical analysis

The values were expressed as the mean $\pm$ S.E. Statistical analysis was carried out using Student's *t*-test between the hepatotoxic chemical-treated group and the gomisin A and hepatotoxic chemical-treated group.

Results

1. Effects of gomisin A on liver functions (ICG-clearance) in various experimental liver injuries: a) Gomisin A slightly accelerated the disappearance of plasma ICG in normal rats

24 hr after the administration of 100 mg/kg, i.p., but not at 50 mg/kg, i.p. (Fig. 2).

b) CCl_4 (2 ml/kg, p.o.) markedly inhibited

the excretion of ICG from plasma. This was dose-dependently prevented by the pre-treatment with gomisin A (12.5–50 mg/kg,

Fig. 2. Effects of gomisin A on disappearance of ICG from blood in normal rats. —●—: Vehicle-treated group (n=8), —▲—: Gomisin A (50 mg/kg, i.p.)-treated group (n=9), ---▲---: Gomisin A (100 mg/kg, i.p.)-treated group (n=9). Data are expressed as the mean±S.E.

Fig. 3. Effects of gomisin A on disappearance of ICG from blood in CCl_4 -treated rats. —○—: CCl_4 (2 ml/kg, p.o.)-treated group (n=11), ---▲---: Gomisin A (12.5 mg/kg, i.p.) and CCl_4 -treated group (n=10), ---▲---: Gomisin A (25 mg/kg, i.p.) and CCl_4 -treated group (n=9), —▲—: Gomisin A (50 mg/kg, i.p.) and CCl_4 -treated group (n=8), —●—: Vehicle-treated group (n=8). Data are expressed as the mean±S.E. *, ** and ***: Statistically significant difference from the CCl_4 -treated group at $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

i.p.) (Fig. 3).

c) Gomisin A (50 mg/kg, i.p.) showed a tendency to prevent the delay of the disappearance of plasma ICG which was caused

by 400 mg/kg, i.p. of d-galactosamine (Fig. 4).

d) The repeated administration of ANIT (45 mg/kg, p.o., daily for 4 days) delayed the

Fig. 4. Effects of gomisin A on disappearance of ICG from blood in d-galactosamine-treated rats. —○—: d-Galactosamine (400 mg/kg, i.p.)-treated group (n=11), —▲—: d-Galactosamine and gomisin A (50 mg/kg, i.p.)-treated group (n=9), —●—: Vehicle-treated group (n=8). Data are expressed as the mean±S.E.

Fig. 5. Effects of gomisin A on disappearance of ICG from blood in ANIT-treated rats. —○—: ANIT (45 mg/kg, p.o., daily for 4 days)-treated group (n=13), —▲—: Gomisin A (50 mg/kg, i.p., daily for 4 days) and ANIT-treated group (n=14), —●—: Vehicle-treated group (n=11). Data are expressed as the mean±S.E.

Fig. 6. Effects of gomisin A on disappearance of ICG from blood in orotic acid-treated rats. —○—: 2% Orotic acid-mixed food (for 12 days)-treated group ($n=6$), —▲—: Gomisin A and orotic acid-mixed food-treated group ($n=5$), —●—: Normal food-treated group ($n=5$). Data are expressed as the mean $\pm$ S.E.

disappearance of plasma ICG. Gomisin A (50 mg/kg, i.p., daily for 4 days) showed little influence on the ANIT-induced change (Fig. 5).

e) A tendency to inhibit the excretion of ICG was observed in rats fed the orotic acid-containing food. The ICG level in plasma was maintained nearly to that of the normal food-treated group by the combined administration of gomisin A with orotic acid (Fig. 6).

2. Effects of gomisin A on bile secretion in CCl_4 -treated rats: Bile flow and biliary outputs of total bile acids and electrolytes (Na^+ , K^+ , Cl^- and HCO_3^-) were decreased by the administration of CCl_4 (2 ml/kg, p.o.). Bile flow and biliary output of each of the electrolytes in the gomisin A (50 mg/kg, i.p.)-pretreated group showed values near to those in the vehicle-treated group, but the decrease of biliary output of total bile acids in CCl_4 -treated rats was not affected by gomisin A (Tables 1 and 2).

Discussion

The effects of gomisin A, which is a lignan component of schizandra fruits, on liver functions in experimental liver injuries induced by CCl_4 , d-galactosamine, ANIT

and orotic acid were studied using the ICG-clearance test. Also, its effect on bile secretion in CCl_4 -induced liver injury was examined using bile flow and biliary total bile acids and electrolytes (Na^+ , K^+ , Cl^- and HCO_3^-) as parameters.

LD50 values of gomisin A in mice are 390 mg/kg, i.p., 500 mg/kg, s.c. and 777 mg/kg, p.o., as reported in our previous paper (4).

Dyes such as ICG are actively taken up into the hepatocytes through the cell membranes from blood. This uptake mechanism is considered to be carrier-mediated (5). Therefore, the disappearance rate of ICG from blood indicates the condition of a liver function. Gomisin A slightly accelerated the disappearance of plasma ICG by itself at a high dose (100 mg/kg, i.p.). This fact indicates that gomisin A possesses a liver function-facilitating property. Each of the hepatotoxic chemicals used in this study depressed a liver function. CCl_4 , d-galactosamine and ANIT markedly inhibited the excretion of ICG from plasma. Orotic acid also showed a similar effect, but it was less pronounced as compared with those of the aforementioned three chemicals. CCl_4 is considered to

Table 1. Effects of gomisin A on bile flow and biliary total bile acids in CCl₄-treated rats

Treatment	No. of rats	Bile flow (μ l/100 g. b.w./hr)	Total bile acids	
			Concentration (mM)	Output (μ mole/100 g. b.w./hr)
CCl ₄	23	175.3 $\pm$ 12.0	31.7 $\pm$ 1.7	5.3 $\pm$ 0.6
Gomisin A + CCl ₄	19	291.6 $\pm$ 21.1*	20.4 $\pm$ 1.0*	5.1 $\pm$ 1.1
Vehicle	32	300.7 $\pm$ 12.7	26.4 $\pm$ 1.1	7.7 $\pm$ 0.5

Gomisin A (50 mg/kg) was given i.p. 30 min before the administration of CCl₄ (2 ml/kg, p.o.). Bile was collected for 60 min 24 hr after CCl₄-administration. Each value represents the mean $\pm$ S.E. *: Statistically significant difference from the CCl₄-treated group at $P < 0.001$.

Table 2. Effects of gomisin A on biliary electrolytes in CCl₄-treated rats

Treatment	No. of rats	Na ⁺		K ⁺		Cl ⁻		HCO ₃ ⁻	
		Conc. ^a	Output ^b	Conc.	Output	Conc.	Output	Conc.	Output
CCl ₄	6	161.3 $\pm$ 1.6	29.5 $\pm$ 3.2	4.1 $\pm$ 0.2	0.7 $\pm$ 0.1	101.7 $\pm$ 1.5	18.4 $\pm$ 1.7	24.1 $\pm$ 1.6	4.4 $\pm$ 0.6
Gomisin A + CCl ₄	12	154.4 $\pm$ 0.9	43.6 $\pm$ 4.6*	3.9 $\pm$ 0.2	1.1 $\pm$ 0.1*	102.8 $\pm$ 0.9	28.9 $\pm$ 1.2*	28.4 $\pm$ 1.2	8.4 $\pm$ 1.3*
Vehicle	8	158.3 $\pm$ 1.5	45.6 $\pm$ 6.0	4.6 $\pm$ 0.1	1.4 $\pm$ 0.2	99.5 $\pm$ 0.8	29.2 $\pm$ 3.4	24.8 $\pm$ 1.9	7.4 $\pm$ 1.1

Gomisin A (50 mg/kg) was given i.p. 30 min before the administration of CCl₄ (2 ml/kg, p.o.). Bile was collected for 60 min 24 hr after CCl₄-administration. Each value represents the mean $\pm$ S.E. ^a: Concentration (mM). ^b: Output (μ mole/100 g. b.w./hr). *: Statistically significant difference from the CCl₄-treated group at $P < 0.01$.

induce a hepatocellular injury and a fatty degeneration by CCl_3 free radical (6, 7); d-galactosamine causes a necrosis by the depletion of hepatic uridine store (8); ANIT causes an intrahepatic cholestasis by cholangitis (9); and orotic acid causes a fatty liver by the repression in transport of lipoproteins (10). Gomisin A showed a tendency to prevent the delays of the disappearance of plasma ICG which was caused by CCl_4 , d-galactosamine and orotic acid, but not that by ANIT. These findings suggest that gomisin A may protect a lowered liver function in parenchymal injuries, and such preventive effects of gomisin A on hepatotoxicities may have partly resulted from the protective action on the hepatocytes (1).

A cholestasis and the decreases in biliary output of total bile acids and electrolytes (Na^+ , K^+ , Cl^- and HCO_3^-) were observed after the administration of CCl_4 . The pretreatment with gomisin A in CCl_4 -treated rats maintained bile flow and biliary output of each electrolyte nearly to the levels of the vehicle-treated group, but did not affect biliary output of total bile acids. Besides, gomisin A has no choleretic action in normal rats (4). Canalicular bile consists of at least two fractions, one of which (bile acids-dependent fraction) is related to a secretion of bile acids. The other fraction (bile acids-independent fraction) is considered to result from active transport of Na^+ into the bile canaliculi (11–13). From our results, it is probable that gomisin A has little effect on the biliary output of total bile acids and that its preventive effect on cholestasis is due to maintaining the function of the bile acids-independent fraction.

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References

- 1 Maeda, S., Sudo, K., Miyamoto, Y., Takeda, S., Shinbo, M., Aburada, M., Ikeya, Y., Taguchi, H. and Harada, M.: Pharmacological studies on schizandra fruits. II. Effects of constituents of schizandra fruits on drug induced hepatic damage in rats. *Yakugaku Zasshi* **102**, 579–588 (1982) (Abs. in English)
- 2 Nakajima, K., Taguchi, H., Ikeya, Y., Endo, T. and Yoshioka, I.: The constituents of *Schizandra chinensis* Baill. XIII. Quantitative analysis of lignans in the fruits of *Schizandra chinensis* Baill. by high performance liquid chromatography. *Yakugaku Zasshi* **103**, 743–749 (1983) (Abs. in English)
- 3 Ikeya, Y., Taguchi, H., Yoshioka, I. and Kobayashi H.: The constituents of *schizandra chinensis* Baill. I. Isolation and structure determination of five lignans, gomisin A, B, C, F and G, and the absolute structure of schizandrin. *Chem. Pharm. Bull. (Tokyo)* **27**, 1383–1394 (1979)
- 4 Maeda, S., Sudo, K., Aburada, M., Ikeya, Y., Taguchi, H., Yoshioka, I. and Harada, M.: Pharmacological studies on schizandra fruits. I. General pharmacological effects of gomisin A and schizandrin. *Yakugaku Zasshi* **101**, 1030–1041 (1981) (Abs. in English)
- 5 Fox, I.J. and Wood, E.H.: Indocyanin green: Physical and physiologic properties. *Proceedings of the Staff Meeting of Mayo Clinic* **35**, 732–744 (1960)
- 6 Recknagel, R.O.: Carbon tetrachloride hepatotoxicity. *Pharmacol. Rev.* **19**, 145–208 (1967)
- 7 Robinson, D.S. and Seakins, A.: Observations relating to the mechanism of action of carbon tetrachloride. *Biochem. J.* **82**, 9 (1962)
- 8 Decker, K. and Keppler, D.: Galactosamine induced liver injury. *In* *Progress in Liver Disease*, Edited by Popper, H. and Schaffner, F., Vol. IV, p. 183–199, Grune & Stratton, New York (1972)
- 9 Fukumoto, Y., Okita, K., Kodama, T., Noda, K., Harada, T., Mizuta, M. and Takemoto, T.: Studies of α -naphthylisothiocyanate-induced hepatic disturbance. *Hepatogastroenterology* **27**, 457–464 (1980)
- 10 Lombardi, B.: Considerations on the pathogenesis of fatty liver. *Lab. Invest.* **15**, 1–20 (1966)
- 11 Boyer, J.L.: Canalicular bile formation in the isolated perfused rat liver. *Am. J. Physiol.* **221**, 1156–1163 (1971)
- 12 Forker, E.L.: Mechanisms of hepatic bile formation. *Annu. Rev. Physiol.* **39**, 323–437 (1977)
- 13 Erlinger, S., Dhumeaux, D., Berthelot, P. and Dumont, M.: Effects of inhibitors of sodium transport on bile formation in the rabbit. *Am. J. Physiol.* **219**, 416–422 (1970)