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Preventive effect of gomisin A, a lignan component of shizandra fruits, on acetaminophen-induced hepatotoxicity in rats

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Abstract—The preventive effect of gomisin A, a lignan component of shizandra fruits, on acetaminophen-induced hepatotoxicity in rats was examined by histological and biochemical analysis. Acetaminophen at a dose of 750 mg/kg was administered to male Wistar rats with or without pretreatment with 50 mg/kg of gomisin A. Gomisin A inhibited not only the elevation of serum aminotransferase activity and hepatic lipoperoxides content, characteristic of acetaminophen administration, but also the appearance of histological changes such as degeneration and necrosis of hepatocytes. However, gomisin A did not affect the decrease in liver glutathione content. These results suggest that gomisin A protects the liver from injury after administration of acetaminophen through the suppression of lipid peroxidation.

Shizandra fruits have been used in Japan and East Asia to produce herbal medicine for the treatment of elevated serum aminotransferase activity in acute hepatitis. Shizandra fruits contain several lignans, including gomisin A, B, C, F and G [1]. Gomisin A (Go*) is the most active component among these lignans [2]. As shown in Fig. 1, the structure of Go is (+)-(6*S*,7*S*,*R*-Biar)-5,6,7,8-tetrahydro-1,2,3,12-tetramethoxy-6,7-dimethyl-10,11-methylenedioxy-6-dibenzo[*a*,*c*]cyclooctenol [1]. It has been previously shown that Go prevents some experimental acute liver injuries [3]. However, the nature of the protective mechanisms involved remains to be elucidated.

Liver injury caused by acetaminophen (AAP) administration is due to the formation of a reactive toxic metabolite by the hepatic cytochrome P450 system [4]. The mechanism of hepatocellular necrosis depends not only on the amount of toxic metabolite formed and consequent detoxification by reduced glutathione (GSH), but also on secondary events occurring after lipid peroxidation in the hepatocyte membrane [5, 6]. To clarify the preventive effect of Go on AAP-induced hepatotoxicity and elucidate possible protective mechanisms, we simultaneously measured serum aminotransferase activities, hepatic lipoperoxide content and hepatic GSH content in rats 3, 6, 18 and 24 hr after administration of AAP and/or Go.

Materials and Methods

Male Wistar rats, weighing 220–240 g, were fed a chow diet (Oriental Kobo Co., Tokyo, Japan) and water *ad lib*. Food but not water was removed overnight before AAP and/or Go administration. A solution of 5% AAP (Wako Pure Chemical Ind. Ltd, Osaka, Japan) dissolved in 0.25% tragacanth gum was administered by metal-gavage to rats at a dose of 750 mg/kg (AAP group, N = 24) [7]. A solution of 2% Go (Tsumura Ltd, Tokyo, Japan) suspended in 1% (v/v) Tween 80–saline was administered intraperitoneally to rats at a dose of 50 mg/kg (Go group, N = 25). A third group of rats was administered intraperitoneally 50 mg/kg of Go 1 hr before AAP administration (AAP + Go group, N = 28).

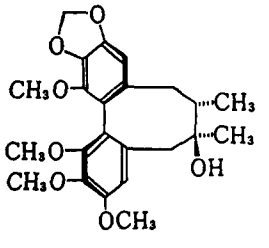

Fig. 1. Chemical structure of Go.

Rats were killed by exsanguination from the abdominal aorta at 3, 6, 18 and 24 hr after AAP administration. Serum was obtained by centrifugation, and the livers were immediately removed, weighed and kept at −80° until further analysis. Liver tissues were fixed in 10% formalin and routinely processed for histological analysis. Preparations were stained with hematoxylin and eosin, and histological changes of liver tissues were examined microscopically.

Serum activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by the method of Wroblewski-Karmen and Ladue [8]. Hepatic content of GSH was determined by the method of Kaplowitz [9]. Hepatic lipoperoxide content was measured by the method of Uchiyama and Mihara [10] using the thiobarbituric acid (TBA) test. In brief, an aqueous solution of 1% H_3PO_4 and 0.6% TBA (Wako Pure Chemicals) was added to 10% liver homogenate, and the mixture heated in a boiling-water bath for 45 min. After cooling, *n*-butanol was added and the optical densities determined at 520 and 535 nm, using a spectrophotometer (Hitachi U-3200, Hitachi Ltd, Tokyo, Japan). The difference between the optical densities was used to calculate the TBA value, expressed as the malondialdehyde (MDA) value [11]. The protein content was assayed by the method of Lowry *et al.* using bovine serum albumin as a standard [12].

Results were expressed as a mean ± SEM. Data was analysed according to the Student's *t*-test. $P < 0.05$ was considered to be significant.

Results

As shown in Table 1, in untreated age-matched normal rats, the mean liver weight (expressed as a percentage of body weight) was 3.4 ± 0.1%, and the mean hepatic protein content was 172 ± 3 mg/g liver. Compared to normal rats the liver weight increased in AAP group rats 24 hr after AAP administration, but remained unchanged in other groups. The hepatic protein content did not significantly change in any of the three groups.

As shown in Fig. 2, Go did not affect the serum aminotransferase activity in the time course experiment (Go group). In the AAP group of rats, serum levels of

* Abbreviations: Go, gomisin A; AAP, acetaminophen; GSH, glutathione, AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBA, thiobarbituric acid; MDA, malondialdehyde.

Table 1. Effect of Go on liver weight and liver protein content after AAP treatment in rats

Group	Time after treatment (hr)	No. of cases	Liver weight (g/100 g body weight)	Liver protein (mg/g liver)
Go	3	7	3.6 ± 0.2	172 ± 7
	6	5	3.6 ± 0.1	150 ± 7
	18	6	3.6 ± 0.1	171 ± 2
	24	7	3.5 ± 0.2	188 ± 8
AAP	3	6	3.7 ± 0.1	192 ± 22
	6	6	3.3 ± 0.3	172 ± 16
	18	6	3.7 ± 0.3	220 ± 23
	24	6	4.2 ± 0.3	164 ± 18
AAP + Go	3	7	3.7 ± 0.3	206 ± 14
	6	6	3.5 ± 0.1	191 ± 14
	18	6	3.9 ± 0.4	183 ± 18
	24	9	4.2 ± 0.1	196 ± 12
Control		4	3.4 ± 0.1	172 ± 3

Data are means ± SEM.

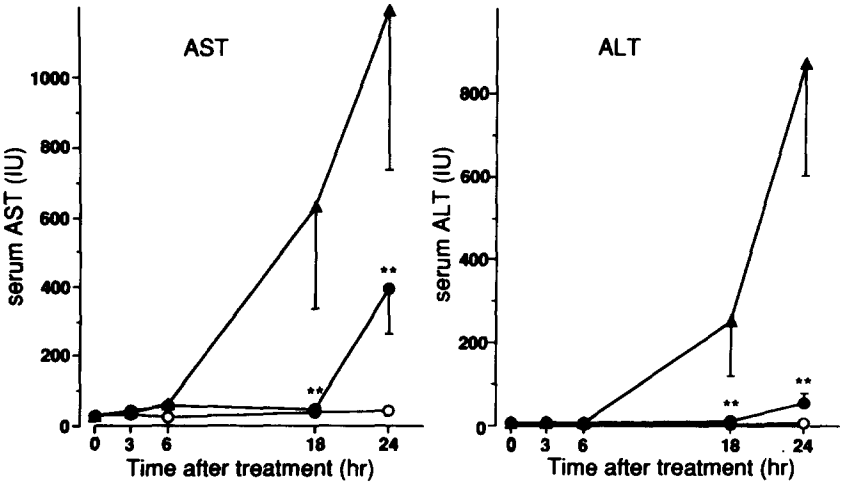

Fig. 2. Time course of serum transaminase activities. (○) Go group, (▲) AAP group, (●) AAP + Go group, **$P < 0.01$ as compared to AAP group.

AST and ALT had increased relative to their initial levels by 16-fold and 31-fold, respectively at 18 hr after AAP administration, and by 31-fold and 108-fold at 24 hr. However, in AAP + Go-treated rats, only slight increases were recorded, even 24 hr after treatment. Thus, significant differences in serum aminotransferase activity were observed between the AAP-treated group and AAP + Go-treated rats at 18 and 24 hr after AAP administration.

Figure 3 shows the histological findings in the rat liver 24 hr after AAP administration. Massive necrosis and vacuolization in the centrilobular area were observed after AAP (Fig. 3a). Administration of Go appeared to suppress these effects (Fig. 3b).

As shown in Fig. 4, Go did not affect hepatic lipoperoxide content in the time course experiment (Go group). Hepatic lipoperoxide content in the rats given AAP had increased relative to its initial level by 1.7-fold, 2.6-fold and 3.4-fold at 6, 18 and 24 hr, respectively. However, in the AAP + Go group, no increase in hepatic lipoperoxide content was found. Thus, significant differences in hepatic lipoperoxide content were observed between AAP- and AAP + Go-treated rats at 18 and 24 hr after AAP administration.

As shown in Fig. 5, Go did not affect the hepatic GSH content in the time course experiment (Go group). The hepatic GSH content in the AAP group had decreased to 47% of its initial level at 3 hr after AAP administration, and then recovered to 89% of its initial level at 24 hr. In the AAP + Go group, hepatic GSH content had decreased to 42% of its initial level at 3 hr after AAP administration, and then recovered as in AAP-treated rats. However, the hepatic GSH content at 18 hr was higher in the AAP + Go group of rats than in the AAP group.

Discussion

Go is a lignan component of shizandra fruits with LD_{50} values in mice of 390 mg/kg, i.p., 500 mg/kg, s.c. and 777 mg/kg, p.o. [13]. In this experiment, 50 mg/kg of Go were administered intraperitoneally, with all the rats surviving throughout the study period. Go-treated rats showed no change in body weight, liver weight or liver protein content compared to untreated rats. Liver protein content in the AAP group and the AAP + Go group was higher than in the Go group of rats, but not significantly so.

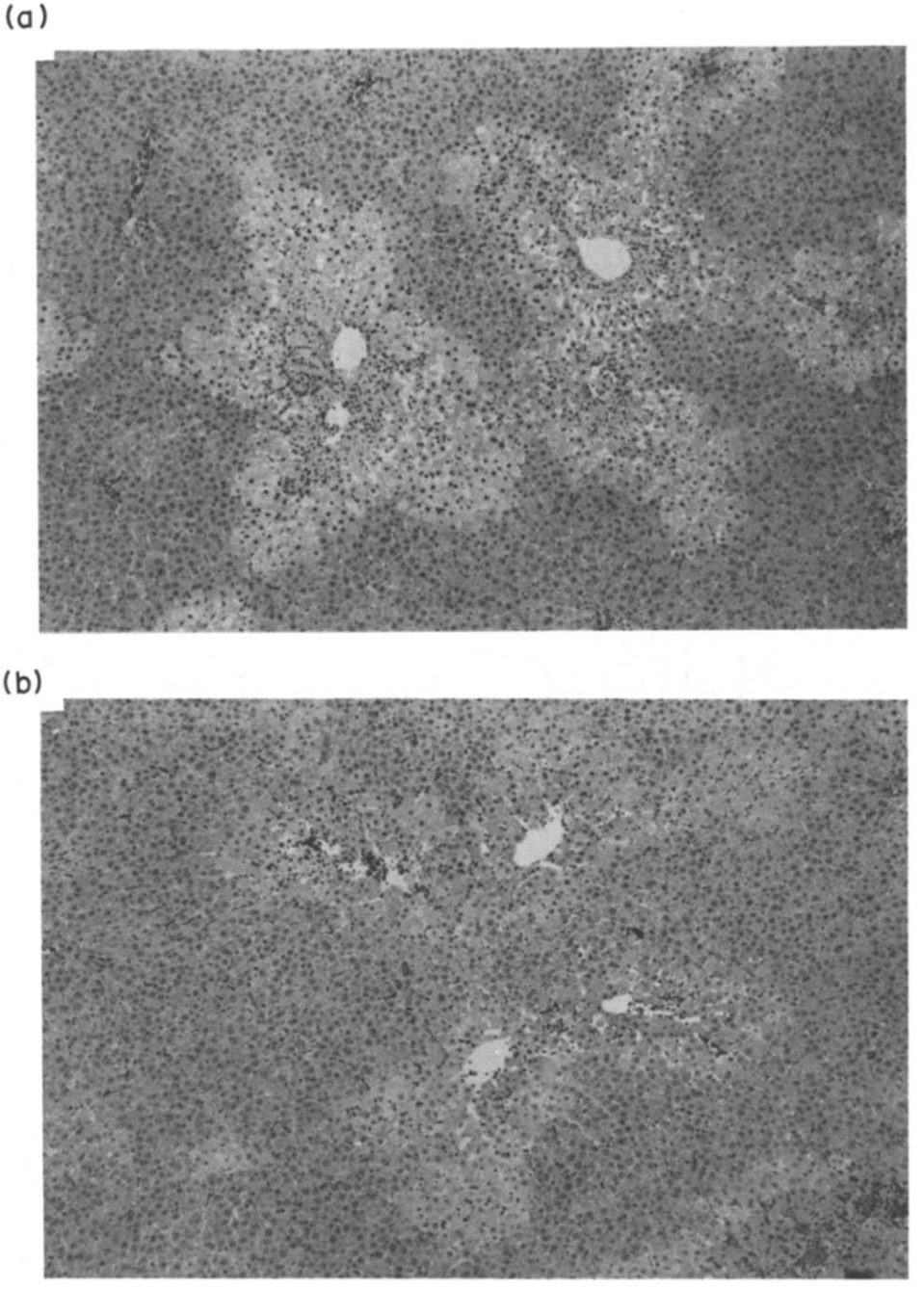

Fig. 3. Histological findings in the rat liver 24 hr after AAP administration with and without Go. (a) Rat received 750 mg/kg of AAP alone. (b) Rat received 50 mg/kg of Go 1 hr before AAP administration.

The data presented suggest that AAP-induced hepatotoxicity in rats is markedly suppressed by prior administration of Go. The protective effect of Go manifested itself in all indices of toxicity. It reduced the histological extent of hepatocellular necrosis as assessed by light microscopy and markedly inhibited the elevation in serum aminotransferase activity and hepatic lipoperoxide content that is characteristic of AAP treatment.

Although the protective effect of Go was evident in this and other studies, the mechanism(s) underlying the protection is less clear [2, 3]. If the protective effect of Go, like thiol compounds, is due to the direct scavenging of toxic reactive metabolites, the level of hepatic GSH would be expected to be increased in Go-treated rats. However, the GSH levels in the AAP + Go group of rats were similar to those in the AAP group. Thus, Go does not appear to restore the hepatic GSH content, in contrast to some protective thiol compounds [5, 7].

In this study, Go suppressed the elevation of hepatic lipoperoxides after AAP administration. It is well known that AAP-induced hepatotoxicity leads to an accumulation of neutrophils and macrophages in the liver [14].

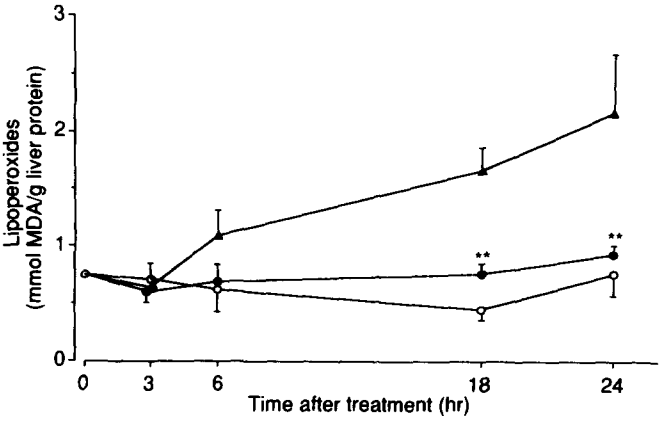

Fig. 4. Time course of rat liver lipoperoxide content. (○) Go group, (▲) AAP group, (●) AAP + Go group, $^{**}P < 0.01$ as compared to AAP group.

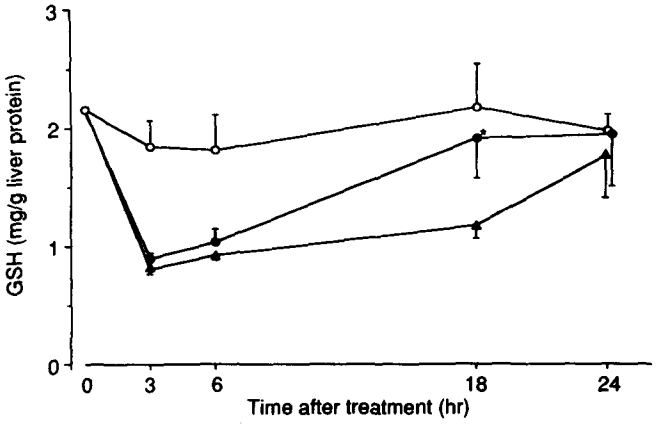

Fig. 5. Time course of rat liver GSH content. (○) Go group, (▲) AAP group, (●) AAP + Go group, $^{*}P < 0.05$ as compared to AAP group.

Macrophage and neutrophil products such as reactive oxygen species, leukotrienes, prostaglandins, tumor necrosis factor and cytokines may impair the microcirculation, resulting in ischemic damage. Additionally, these products may cause direct hepatocyte injuries, including lipid peroxidation [15–18]. Thus, Go may protect the liver from injury after AAP administration through the suppression of lipid peroxidation. Indeed, some lignans are known to inhibit formation of liver MDA *in vivo* and *in vitro* [19, 20]. The phenolic hydroxy group or the methoxy group may be responsible for any anti-lipid peroxidation activity [20].

In conclusion, our study suggests that Go, a lignan component of shizandra fruits, protects the liver from injury after administration of AAP through the suppression of lipid peroxidation.

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