Protective Effects of Acetylbergenin against Carbon Tetrachloride-Induced Hepatotoxicity in Rats

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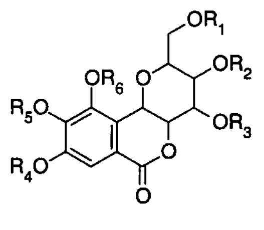
The present study was undertaken to investigate whether or not the hepatoprotective activity of acetylbergenin was superior to bergenin in carbon tetrachloride (CCl_4)-intoxicated rat. Acetylbergenin was synthesized by acetylating bergenin, which was isolated from *Mallotus japonicus*. The hepatoprotective effects of acetylbergenin were examined against CCl_4-induced liver damage in rats by means of serum and liver biochemical indices. Acetylbergenin was administered orally once daily for 7 successive days, then a 0.5 ml/kg mixture of CCl_4 in olive oil (1:1) was intraperitoneally injected at 12 h and 36 h after the final administration of acetylbergenin. Pretreatment with acetylbergenin reduced the elevated serum enzymatic activities of alanine/aspartate aminotransferase, sorbitol dehydrogenase and γ-glutamyltransferase in a dose dependent fashion. Acetylbergenin also prevented the elevation of hepatic malondialdehyde formation and depletion of glutathione content dose dependently in CCl_4-intoxicated rats. In addition, the decreased activities of glutathione S-transferase and glutathione reductase were restored to almost normal levels. The results of this study strongly suggest that acetylbergenin has potent hepatoprotective activity against CCl_4-induced hepatic damage in rats by glutathione-mediated detoxification as well as having free radical scavenging activity. In addition, acetylbergenin doses of 50 mg/kg showed almost the same levels of hepatoprotective activity as 100 mg/kg of bergenin, indicating that lipophilic acetylbergenin is more active against the antihepatotoxic effects of CCl_4 than those of the much less lipophilic bergenin.

Key words: Acetylbergenin, Bergenin, Hepatoprotective activity, Carbon tetrachloride

INTRODUCTION

The hepatoprotective effects of a water extract of the *Mallotus japonicus* cortex containing 11-18% bergenin against carbon tetrachloride (CCl_4) and galactosamine (GalN) were reported previously (Lim *et al.*, 1999). It has been also shown that bergenin, an active component of *Mallotus japonicus,* protected against the hepatocyte damage induced by both CCl_4 and GalN both *in vitro* as well as *in vivo* (Kim *et al.*, 2000a; Lim *et al.*, 2000). In addition, it is generally known that lipophilic drugs are easily absorbed due to their ability to cross the bilayer of cell membranes, which results in an increase of physiological activity. Because of this, acetylbergenin (penta-acetylbergenin) was synthesized to increase both the lipophilic and physiological activities of bergenin (Fig. 1). It has been demonstrated that acetylbergenin has hepatoprotective



Bergenin	$R_1 = R_2 = R_3 = R_4 = R_6 = H$ $R_5 = -CH_3$
Acetylbergenin	$R_1 = R_2 = R_3 = R_4 = R_6 = -COCH_3$ $R_5 = -CH_3$

Fig. 1. Chemical formula of bergenin and acetylbergenin

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activity against CCl_4- and GalN-induced cytotoxicity in cultured rat hepatocytes (Kim *et al.*, 2000b). Accordingly, the present study was undertaken to investigate whether the hepatoprotective activity of acetylbergenin would be improved *in vivo* using CCl_4-intoxicated rats as an experimental model.

MATERIALS AND METHODS

Animals

Sprague-Dawley rats (150 ± 20 g) were supplied from the Samyuk Laboratory Animal Inc., Osan, Korea. They were housed in polyacrylic cages and maintained at 22 ± 1°C and humidity 60 ± 5%. They were fed a solid diet and tap water *ad libitum*. The animals were starved overnight prior to sacrifice in order to reduce variations in hepatic metabolism.

Preparation of acetylbergenin

Bergenin was isolated from the bark of *Mallotus japonicus* by the method described previously (Kim *et al.*, 2000a). The bark was collected in Chungbuk Province, Korea and identified by Dr. K. S. Lee, College of Pharmacy, Chungbuk National University. The voucher specimens were deposited in the same university.

Acetylbergenin was synthesized from bergenin by the method outlined by Ramaiah *et al.* (1979). Bergenin (10 g) was dissolved in acetic acid anhydrate (500 ml) and dry pyridine (100 ml), heated in a water bath for 6 h, and worked up in the usual manner. The acetylbergenin (12 g) was recrystallized from benzene. Acetylbergenin and bergenin were confirmed by comparing the physical-chemical properties and spectral (UV and NMR) data. Both bergenin and acetylbergenin were dissolved in 10% carboxymethylcellulose.

CCl_4-induced hepatotoxicity in rats

The present animal experiments were conducted using the same method described previously. Liver damage was induced in rats by an intraperitoneal injection of a 0.5 ml/kg mixture of CCl_4 in olive oil (1:1). Acetylbergenin was pretreated in the aspect of hepatoprotection before the administration of hepatotoxic CCl_4. The rats were administered acetylbergenin (25, 50 and 100 mg/kg) and bergenin (100 mg/kg) orally once a day for 7 days, and then the CCl_4/olive oil mixture was injected at 12 h and 36 h after the final administration of acetylbergenin.

Assessment of liver function

The rats were anaesthetized with CO_2 gas 12 h after the final administration of CCl_4 (Park *et al.*, 1996 and 1997), and blood was collected from the abdominal aorta of each rat. The blood was centrifuged at 3,000 rpm for 15 min to separate the serum and stored at 4°C. The activities of alanine/aspartate aminotransferase (ALT/AST) were deter-mined by the methods reported by Reitman and Frankel (1957). Sorbitol dehydrogenase (SDH) and γ-glutamyltransferase (γ-GT) were measured by the methods described by Gerlach (1965) and Szasz (1969), respectively.

After the collection of the blood, the liver was exhaustively perfused with ice-cold 0.15 M sodium chloride through the portal vein. It was then removed, minced and homogenized with 4 volumes of an ice-cold 0.1 M potassium phosphate buffer (pH 7.5) solution. The malondialdehyde (MDA) and glutathione (GSH, one of the endogenous protective biomolecules) concentrations were determined in the liver homogenate by the methods described by Ellman (1959) and Ohkawa *et al.* (1979), respectively. The Ellman method is relatively non-specific to GSH, but the results obtained with the Ellman method were similar to that with an HPLC method reported by Reed *et al.* (1980). Therefore, the Ellman method was used for convenience in this study. The glutathione S-transferase (GST) and glutathione reductase (GR) activities in the liver cytosol fraction were determined by the method reported by Habig *et al.* (1974) and Mize and Langdon (1962), respectively. The protein content was measured by the methods outlined by Lowry *et al.* (1951) with bovine serum albumin as a standard.

Statistical analysis

The data is expressed as a mean ± SD. The statistical significance of the drug effects in all experiments was assessed by a one-way analysis of the variance followed by Duncan's new multiple range test for post-hoc comparisons (Tallarida *et al.*, 1986). A p-value <0.05 was considered statistically significant.

RESULTS

Effects of acetylbergenin on ALT, AST, SDH and g-GT activities

The hepatoprotective effects of acetylbergenin on CCl_4-intoxicated rats are shown in Table I. In the CCl_4-treated control group, the serum ALT, AST, SDH and γ-GT activities increased significantly when compared with the normal group. In contrast, the groups treated with 25, 50 and 100 mg/kg of acetylbergenin decreased these elevated enzyme activities toward normal levels when compared to the CCl_4 control group ($P<0.05$).

Effects of acetylbergenin on hepatic MDA and GSH levels

MDA production in the CCl_4-treated group increased

Table I. Effects of acetylbergenin on activities of ALT, AST, SDH and γ-GT in CCl_4-intoxicated rats

Group	ALT (Unit/ml)	AST (Unit/ml)	SDH (U/ml)	γ-GT (mU/ml)
Control	39.6 ± 4.21[a]	56.7 ± 4.18[a]	18.7 ± 1.07[a]	24.7 ± 3.16[a]
CCl_4 Control	100.3 ± 7.69[b]	188.7 ± 7.97[b]	78.3 ± 11.16[b]	202.3 ± 13.47[b]
AB 25 + CCl_4	82.8 ± 5.72[c] (28.8%)	158.7 ± 7.85[c] (22.7%)	53.0 ± 2.46[c] (42.4%)	165.0 ± 7.18[c] (21.0%)
AB 50 + CCl_4	79.6 ± 5.88[c] (34.1%)	138.8 ± 4.51[d] (37.8%)	42.4 ± 3.32[d] (60.2%)	130.0 ± 12.70[d] (40.7%)
AB100 + CCl_4	65.1 ± 5.15[d] (58.0%)	99.4 ± 5.52[e] (67.7%)	37.0 ± 2.89[e] (69.3%)	107.7 ± 7.80[e] (53.3%)
B 100 + CCl_4	73.1 ± 5.01[e] (44.8%)	105.9 ± 12.20[e] (62.7%)	33.5 ± 4.28[e] (75.2%)	110.8 ± 16.40[e] (51.5%)

The rats were administered acetylbergenin 25, 50, and 100 mg/kg orally once a day for 7 days, and then a mixture 0.5 ml/kg (ip) of CCl_4 in olive oil (1:1) was injected at 12 h and 36 h after the final administration of acetylbergenin. Rats were decapitated 12 h after the final administration of CCl_4. Data is expressed as mean ± SD (n=8). The values in the parenthesis are % of protection calculated as 100 × (values of CCl_4 control values of sample)/(Values of CCl_4 control values of normal). The values having the same superscript are not significantly different each other by Duncan's new multiple range test ($p<0.05$). AB; acetylbergenin, B; bergenin.

3.2-fold when compared with the normal group. Pretreatment with 25, 50 and 100 mg/kg of acetylbergenin reduced CCl_4-induced MDA production in a dose-dependent manner, when compared with the CCl_4 control group ($P<0.05$). The administration of CCl_4 decreased the hepatic GSH levels by 59%. The GSH levels after pretreatment with acetylbergenin decreased towards normal levels with increasing dosage ($P<0.05$) (Table II).

Effects of acetylbergenin on GR and GST activities

Both the GR and GST activities were significantly lower in the CCl_4-intoxicated rats compared with that of the normal group. Meanwhile, pretreatment with acetylbergenin prevented this reduction in enzyme activity that is caused by CCl_4 ($P<0.05$) (Table II).

DISCUSSION

Previous investigations have shown that bergenin, a major component of *Mallotus japonicus*, protected hepatocytes against hepatic damage induced by either CCl_4 or GalN both *in vitro* as well as *in vivo* (Kim *et al.*, 2000a; Lim *et al.*, 2000, Hikino *et al.*, 1985). The present study has demonstrated that acetylbergenin *in vivo* has more active hepatoprotective activity against liver injury induced by CCl_4 than bergenin.

CCl_4 is metabolically activated by the cytochrome P450-dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radicals. The free radical combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation (Recknagel and Glende, 1977; De Groot and Noll, 1986). This results in changes in the structure of the endoplasmic reticulum and other membranes, a loss of metabolic enzyme activation, a reduction in protein synthesis and a loss of glucose 6-phosphate activation, which leads to liver damage (Recknagel and Glende, 1973; Gravela *et al.*, 1979; Wolf *et al.*, 1980; Azri *et al.*, 1992). This is generally reflected through the marked changes in the enzymatic and non-enzymatic indices in both the serum and the livers of CCl_4-treated animals. Significant increases in the activities of ALT, AST, SDH and γ-GT were observed in CCl_4-intoxicated rats, which is consistent with previous studies (Lim *et al.*, 1999). Pretreatment with acetylbergenin attenuated the increased enzyme activities produced by CCl_4, indicating that acetylbergenin can prevent the liver injury

Table II. Effects of acetylbergenin on levels of MDA and GSH and activities of GR and GST in GalN-intoxicated rats

Group	MDA (nmole/g of tissue)	GSH (μmole/g of tissue)	GR (GSH formed nmole /min/mg protein)	GST (CDNB nmole /min/mg protein)
Control	22.7 ± 2.07[a]	5.67 ± 0.32[a]	25.8 ± 1.73[a]	241.6 ± 4.85[a]
CCl_4 Control	71.2 ± 3.96[b]	2.33 ± 0.20[b]	12.4 ± 1.50[b]	104.3 ± 11.15[b]
AB 25 + CCl_4	55.7 ± 1.73[c] (32.0%)	3.10 ± 0.09[c] (23.1%)	18.4 ± 1.17[c] (44.8%)	191.0 ± 5.79[c] (63.1%)
AB 50 + CCl_4	47.5 ± 0.70[d] (48.9%)	3.81 ± 0.16[d] (44.3%)	20.8 ± 0.58[d] (62.7%)	210.0 ± 9.60[d] (77.0%)
AB100 + CCl_4	42.7 ± 2.84[e] (58.8%)	4.23 ± 0.16[e] (56.9%)	22.0 ± 1.13[d] (71.6%)	220.1 ± 14.98[d] (84.3%)
B 100 + CCl_4	48.2 ± 2.91[d] (47.4%)	3.87 ± 0.31[d] (46.1%)	18.6 ± 1.23[c] (46.0%)	179.0 ± 17.22[c] (54.4%)

The experimental protocol is the same as in Table I. Data is expressed as mean ± SD (n=8). The values in the parenthesis are % of protection calculated as 100 × (values of CCl_4 control values of sample)/(Values of CCl_4 control values of normal). The values having the same superscript are not significantly different each other by Duncan's new multiple range test ($p<0.05$). AB; acetylbergenin, B; bergenin.

induced by CCl_4.

It has been hypothesized that one of the principal causes of CCl_4-induced liver injury is lipid peroxidation by the free radical derivatives of CCl_4 (Recknagel *et al.*, 1974). In the state of oxidative stress, GSH is converted to oxidized glutathione (GSSG) and the depletion of GSH leads to lipid peroxidation. Therefore, the role of GSH as a reasonable maker for evaluating oxidative stress is important (Recknagel *et al.*, 1991). GSH has been reported to preserve cytochrome P450 by blocking lipid peroxidation (Reiner *et al.*, 1972). GSH plays a fundamental role in protecting against electrophilic attack by xenobiotics such as free radicals (Mitchell *et al.*, 1973). Accordingly, to prevent lipid peroxidation, it is very important to maintain the GSH levels. GR plays a role in maintaining adequate levels of GSH by reducing GSSG to glutathione (Recknagel *et al.*, 1991). In the present study, the increase in liver MDA, a typical parameter of lipid peroxidation, and the depletion of hepatic GSH are serious indicators in CCl_4-intoxicated rats. Acetylbergenin prevented CCl_4-induced MDA production and hepatic GSH depletion in a dose-dependent manner. Acetylbergenin also restored the decreased activity of GR to normal levels. In addition, GST is a soluble protein located in cytosol, which plays an important role in the detoxification and excretion of xenobiotics (Boyer *et al.*, 1984; Masukawa and Iwata, 1986). The activity of GST was markedly lower in CCl_4-intoxicated rats, but acetylbergenin restored the decreased GST activity induced by CCl_4 toward normal levels. Therefore, the effects of acetylbergenin might be related to normalization mechanisms by maintaining adequate levels of GSH to detoxify xenobiotics and by diminishing lipid peroxidation through a free radical scavenging activity.

In addition, it has been demonstrated that in primary cultured rat hepatocytes *in vitro*, the hepatoprotective effects of norbergenin as a hydrophilic polyphenol compound shows greater activity than that of acetylbergenin as a lipophilic compound (Kim *et al.*, 2000b). Furthermore, polyphenol compounds have been reported to show hepatoprotective effects in primary cultured rat hepatocytes (Hikino *et al.*, 1985; Miyagawa *et al.*, 1997). However, in the present study, the hepatoprotective activity of 50 mg/kg acetylbergenin showed almost the same level of hepatoprotective activity as 100 mg/kg bergenin. This suggests that lipophilic acetylbergenin shows greater activity against CCl_4-induced hepatotoxicity in rats than that the much less lipophilic bergenin. These results suggest that acetylbergenin is more easily absorbed due to its ability to cross the bilayer of the intestinal cell membrane, which results in increases of activity after being hydrolyzed into hydrophilic polyphenol compounds such as norbergenin and bergenin. In view of this aspect, it could be said that acetylbergenin in this study also showed greater activity than bergenin.

From the above results, we conclude that acetylbergenin showed hepatoprotective activity against CCl_4-intoxicated rats. It is assumed that effects of acetylbergenin on liver protection are related to glutathione-mediated detoxification as well as having free radical scavenging activity. In addition, it was demonstrated that acetylbergenin provides greater hepatoprotection than bergenin, supporting the fact that the more lipophilic drugs are usually the more active.

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