Protective Effects of Platycodon grandiflorum Aqueous Extract on Thioacetamide-induced Fulminant Hepatic Failure in Mice

Jong-Hwan Lim¹, Tae-Won Kim², Sang-Jin Park¹, In-Bae Song², Myoung-Seok Kim³, Hyo-Jung Kwon⁴, Eun-Sang Cho², Hwa-Young Son², Sang-Wook Lee¹, Joo-Won Suh⁵, Jong-Woo Kim¹, and Hyo-In Yun²

¹B&C Biopharm, Advanced Institutes of Convergence Technology, Gyeonggi-Do 443-270, South Korea
²College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, South Korea
³Jeollanamdo Development Institute for Traditional Korean Medicine, Jeollanam-Do 529-851, South Korea
⁴Laboratory of Liver Diseases, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, MD 20892, USA
⁵Division of Bioscience and Bioinformatics, Myongji University, Gyeonggi-do 449-728, South Korea

Abstract: The aim of the present study was to evaluate the protective activity of aqueous extract from Platycodon grandiflorum (BC703) on thioacetamide (TA)-induced hepatotoxicity in mice. We found that BC703 significantly decreased mortality and the change in serum transaminase following TA administration. The group treated with BC703 at doses of 1, 5, and 10 mg/kg produced significant hepatoprotective effects against TA-induced liver damage by decreasing the activities of serum enzymes, nitric oxide and lipid peroxidation in dose-dependent manners. Histopathological studies further substantiated the protective effect of BC703. These results show the hepatoprotective activity of aqueous extract from Platycodon grandiflorum on thioacetamide-induced fulminant hepatic failure. (DOI: 10.1293/tox.24.223; J Toxicol Pathol 2011; 24: 223–228)

Key words: fulminant hepatic failure, hepatotoxicity, Platycodon grandiflorum, thioacetamide

Introduction

The roots of Platycodon grandiflorum have been consumed as a foodstuff and as a folk remedy for diseases such as bronchitis, asthma, pulmonary tuberculosis and inflammation¹. It was reported to contain a lot of carbohydrates (at least 90%), protein (2.4%), lipids (0.1%) and ash (1.5%)¹,². Polysaccharides obtained from many natural sources represent a structurally diverse class of macromolecules, and are known to affect a variety of biological responses. It has been reported that the polysaccharides isolated from the root of

Platycodon grandiflorum prevent obesity, hypercholesterolemia, hypertension, diabetes and hyperlipidemia²,³. The inulin-type polysaccharides, (1→2)-β-D-fructan, isolated from the roots of Platycodon grandiflorum demonstrated the selective immune-modulating effects on B cells and macrophages⁴. Triterpenoid saponins, such as platycodins (A, D, D₂, and D₃), 2 and 3-O-acetyl polygalacin D₂, platyconic acid and platycosides (A, B, C, D, E, and F), were also found in the roots of the Platycodon grandiflorum⁴. These saponins are believed to have a wide range of health benefits and to prevent chemicals-induced hepatotoxicity⁵,⁶.

Rodent models of toxin-induced hepatotoxicity are used to elucidate the biochemical processes involved in many forms of liver disease and to evaluate the potential candidates for hepatoprotectants⁷. An animal model of fulminant hepatic failure induced by the thioacetamide (TA) has been described previously⁷,⁸. The animal model for TA-induced fulminant hepatic failure was characterized by severe liver function impairment, massive hepatocyte necrosis and apoptosis⁹. Within a short period of administration, TA is converted to thioacetamide sulfoxide (TASO) by the oxidase system. It is further metabolized by cytochrome P450 monooxygenases to thioacetamide sulfone (TASO₂), a

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Mailing address: Hyo-In Yun, College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, South Korea, TEL / FAX: 82-42-821-6759; J-H Lim, B&C Biopharm, Advanced Institutes of Convergence Technology, Gyeonggi-Do 443-270, South Korea, TEL / FAX: 82-31-888-9474
E-mail address: hiyun@cnu.ac.kr (Yun HI); jhlim99@bncbio.com (Lim JH)
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highly reactive electrophilic metabolite\(^{10,11}\). Covalent binding of this polar product to cellular macromolecules exerts hepatotoxicity, leading to generation of reactive oxygen species (ROS)\(^{10-12}\).

In the present study, we aimed to investigate the hepatoprotective effect of the standardized *Platycodon grandiflorum* aqueous extract on TA-induced fulminant hepatic failure in mice.

### Materials and Methods

#### Chemicals and reagents

Assay kits for thiobarbituric acid reactive substances (TBARS), glutathione (GSH), nitric oxide (NO) were purchased from BioAssay Systems (Hayward, CA, USA). Test kits for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrage (BUN), creatinine (CRE) were obtained from IDEXX (Westbrook, ME, USA). TA and other chemicals were obtained from Sigma chemicals (St. Louis, MO, USA). HPLC-grade acetonitrile and methanol were obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA).

#### Preparation of standardized *Platycodon grandiflorum* aqueous extract

The standardized *Platycodon grandiflorum* aqueous extract (BC703) was manufactured by SkyHerb Pharmaceuticals (Zhejiang, China) in accordance with good manufacturing practices (GMP). Briefly, the dried roots of *Platycodon grandiflorum* (100 g) were cut into slices and extracted in distilled water with occasional shaking at 60–90 °C for 6–10 hours. The aqueous extract was then filtered, concentrated under reduced pressure in a rotary evaporator and spray-dried into powder (36 g). It has previously been reported that the aqueous extracts of the roots of *Platycodon grandiflorum* contain an inulin-type polyfructose, (1–2)-\(\beta\)-D-fructan\(^{2,3,11}\). It also includes deapio-platycoside E, platycoside E, deapio-platycodin D\(_4\), platycodin D\(_4\), deapio-platycadin D, platycadin D, polygalacin D, 3″-O-acetylpolygalacin D, platycadin A and 2″-O-acetyl polygalacin D. Therefore, BC703 was standardized in reference to platycodin D (at least 0.8%) using a validated HPLC assay method as previously described with some modifications\(^{14}\).

#### Animals and treatments

Male ICR mice, weighing between 32 and 36 g at the age of 5–6 weeks, were used for this study. They were obtained from Orient Bio (Sungnam, Korea) and acclimated for one week before experiments. Healthy ICR mice were randomly allocated into 5 groups of 6 male mice each. The mice in the negative and positive control groups were orally given distilled water. The others were orally administered with BC703 (1, 5 and 10 mg/kg) for three consecutive days. At 3 h after the last oral administration, mice were intraperitoneally injected with saline or 200 mg/kg of TA. Twenty-four hours after TA administration, the surviving mice were killed by cardiac puncture after being anesthetized lightly with carbon dioxide. The dead animals were necropsied as soon as they were found and excluded from the histopathological and biochemical examination. Collected blood samples were separated by centrifugation at 800 g for 15 min, and the serum samples were subjected to biochemical investigations. Liver samples from each mouse were removed for histopathological and biochemical examination. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Chungnam National University (Deajeon, Korea).

#### Serum biochemical examination

The serum ALT, AST, BUN and CRE activities were determined on a dry chemistry system, the VetTest 8008 blood chemistry analyzer (IDEXX Laboratories, Westbrook, ME, USA).

#### Hepatic lipid peroxidation, nitric oxide and glutathione content

The content of malondialdehyde (MDA), a terminal product of lipid peroxidation, was measured by the thiobarbituric acid reduction method using a commercially available kit (QuantiChrom TBARS Assay Kit, BioAssay systems, Hayward, CA, USA). The hepatic GSH levels were determined using the improved DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)] method (QuantiChrom GSH Assay Kit; BioAssay Systems, Haywad, CA, USA).

#### Histopathological examination

Liver slices were made from a part of the left lobe and fixed immediately in a 10% buffered formalin phosphate solution, embedded in paraffin, and cut into 5 μm sections. Four random samples for each liver sample were stained with hematoxylin and eosin (H&E) to evaluate the portal inflammation, hepatocellular necrosis and Kupffer cell hyperplasia. To quantify the degree (%) of hepatic necrosis, liver H&E sections were digitally photographed, and the percentage of necrotic areas was quantified as the mean of 10 randomly selected fields within each slide.

#### Statistical analysis

Results were expressed as means ± standard error (SEM). The Kaplan-Meier test was applied to determine the significance of the survival rate differences. The significance of differences among experimental groups was determined using one-way analysis of variance (ANOVA) or the corresponding nonparametric (Kruskal-Wallis) test, as required. Where significant effects were found, post hoc analysis using the Tukey’s multiple comparison test or Mann-Whitney *U*-test was performed, and *p*<0.05 was considered to be statistically significant.
Results

Effect of BC703 on survival rate and serum liver enzymes

After intraperitoneal TA injection, the percentage of mortality in the positive control group was 66% compared with 50, 44 and 44% in mice treated with BC703 at 1, 5 and 10 mg/kg, respectively. Although mortality was highest in the positive control group, there were no significant differences. Marked increases in the levels of ALT, AST, BUN and CRE were shown in TA-treated mice (Table 1). This increase was attenuated in mice that received BC703 in a dose-dependent manner. Moreover, the group treated with 10 mg/kg of BC703 showed reduced liver enzyme and renal function marker (BUN and CRE) levels to a greater extent than the positive control group (p<0.05).

### Table 1. Effect of BC703 on Serum Biochemical Parameters in TA-induced Fulminant Hepatic Failure in Mice

| Groups                  | AST (Unit/L) | ALT (Unit/L) | ALP (Unit/L) | BUN (mg/dL) | CRE (mg/dL) |
|-------------------------|--------------|--------------|--------------|-------------|-------------|
| Negative control        | 97.33 ± 21.55* | 71.50 ± 8.63* | 106.8 ± 9.02* | 15.40 ± 1.54* | 0.32 ± 0.09* |
| Positive control (TA alone) | 4308.33 ± 246.24 | 8351.67 ± 890.48 | 167.50 ± 42.50 | 72.50 ± 12.50 | 0.81 ± 0.17 |
| TA+1 mg of BC703/kg     | 3425.83 ± 722.42 | 7465.00 ± 700.58 | 166.25 ± 9.44 | 57.50 ± 17.50 | 0.60 ± 0.11 |
| TA+5 mg of BC703/kg     | 2912.50 ± 435.66* | 7275.00 ± 1130.29 | 150.00 ± 10.80 | 46.25 ± 10.28* | 0.55 ± 0.06* |
| TA+10 mg of BC703/kg    | 2301.17 ± 510.10* | 6312.50 ± 1351.87* | 156.25 ± 11.43 | 32.50 ± 12.50* | 0.45 ± 0.05* |

Values are expressed as means ± SEM (in survival animals). The mice in the negative and positive control groups were orally given distilled water. The others were orally administered BC703 (1, 5, and 10 mg/kg) for three consecutive days. At 3 h after the last oral administration, mice were intraperitoneally injected saline or 200 mg/kg of TA. * Significantly different compared with the positive control group (p<0.05)
Protective Effects of BC703 on TA-induced Hepatotoxicity

Hepatic levels of GSH, TBARS, NO and SOD

The liver homogenate showed significantly reduced activities of GSH and SOD, whereas increased levels of NO and TBARS were shown in positive group (Fig. 1). The hepatic TBARS level in the positive control group was significantly increased by 1.9-fold compared with the level in the negative control group.
negative control group after TA injection \((p<0.05)\). BC703 attenuated the increased TBARS level in BC703 pretreated group at 5 and 10 mg/kg \((p<0.05)\). The level of hepatic NO was remarkably decreased by BC703 pretreatment in dose-dependent manners, compared with the positive control group. Moreover, the significant decrease in hepatic GSH, which was observed in the positive control group as compared with the negative control group, was significantly reversed by BC703 pretreatment \((p<0.05)\).

**Histopathological observations**

The livers of animals in the negative control group had normal hepatic cells with well-preserved cytoplasm, a prominent nucleus, a nucleolus and visible central veins, whereas the liver collected from TA-administered mice showed severe hepatic injury characterized by centrilobular necrosis, perportal hepatocytic vacuolation and infiltration of inflammatory cells (Fig. 2). Most of the parenchyma of the liver was destroyed in this group of mice, resulting in hepatic failure. Confluent submassive necrosis was noted around the central vein in positive control group mice. Also, numerous neutrophil infiltrations were seen adjacent to necrotic areas. The lower dose of BC703 (1 mg/kg) did not prevent the toxic effect of TA, with large necrotic areas still present. The other doses of BC703 (5 and 10 mg/kg) produced a more or less normal lobular pattern with a mild degree of hepatic injury. Scattered spotty necrosis was found, and some inflammatory cells surrounded spotty necrotic foci in mice treated with 5 and 10 mg/kg of BC703 (Fig. 2). The necrotic area was significantly reduced in BC703-treated mice in a dose-dependent manner compared to the positive control group mice (Fig. 2).

**Discussion**

TA is a centrilobular hepatotoxicant, widely used as a model compound to induce acute and chronic liver diseases\(^{16,17}\). Liver injury begins with bioactivation of TA to TASO and further to TASO\(_2\). An unstable and reactive metabolite, TASO\(_2\), is thought to initiate necrosis by covalently binding to liver macromolecules\(^{11,12}\). Although TA showed high specificity for the liver as a target organ, it also produced a marginal and transient renal injury at excessively high doses\(^{15}\). In this study, elevated serum BUN and CRE levels were observed in the positive control group, but these renal dysfunctions were ameliorated pretreatment of mice with BC703. Meanwhile, TA administration caused severe acute liver damage in mice, demonstrated by remarkable elevation of serum AST and ALT levels in the present study. The increased levels of hepatic enzymes in the present study are consistent with the findings of several TA-induced liver injuries\(^{16,17}\). The increased serum levels of AST and ALT are attributed to the damaged structural integrity of the liver. Administration of BC703 prevented TA-induced lethality, elevation of ALT, AST and lipid peroxidation in a dose-dependent manner. TA is bioactivated by hepatic microsomal CYP2E1 to sulfoxide and further to TASO\(^{10,11}\). CYP2E1 metabolizes a large number of low-molecular-weight compounds, many of which are industrial solvents, chemical additives, halogenated anesthetics, and drugs\(^{16,17}\). CYP2E1 activates many xenobiotics to hepatotoxic or carcinogenic substances.\(^{8,16}\) An inhibition of CYP2E1 was associated with a decrease in toxicity induced by some chemicals, such as acetaminophen, benzene, carbon tetrachloride and chloroform\(^{17,18}\). In previous studies, a crude saponin extract from *Platycodon grandiflorum* resulted in a significant decrease in the CYP2E1-dependent hydroxylation of aniline and showed a hepatoprotective effects on CCl\(_4\)-induced hepatic injury in mice\(^{5,6}\). Thus, these results suggest that BC703 would attenuate the TA-induced hepatic injury in mice by suppression of CYP2E1 in the metabolism of TA, and the same evidence has been reported by Kang et al.\(^{11}\). In addition, this assumption was also confirmed by histological observation. Perivenous cells contained a higher level of CYP2E1 compared to perportal cells, which reflected zonal differences in the toxicity of acetaminophen, carbon tetrachloride, chloroform and ethanol\(^{17,18}\). In the histopathological examination, perivenous cells were much more severely damaged than perportal cells in positive control group mice, whereas the pretreatment with BC703 produced only a very mild degree of liver injury in a dose-dependent manner.

GSH is the major nonenzymatic antioxidant and regulator of intracellular redox homeostasis and is ubiquitously present in all cell types\(^{32}\). In this study, pretreatment with BC703 prior to TA-induced hepatic injury inhibited lipid peroxidation, and reduces TA-induced hepatic GSH depletion and restored hepatic Cu/Zn SOD activities in the liver. A sufficient regenerative response of BC703 by reducing the intensity of liver damage could be expected.

In conclusion, BC703 ameliorated hepatic damage and improved survival in mice suffering from TA-induced fulminant hepatic failure. Although further studies are necessary, BC703 may be a beneficial agent for the management of fulminant hepatic failure.

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