Renoprotective Effects of Fermented Black Ginseng through Ameliorating Oxidative Stress Associated with Cisplatin-Induced Acute Nephrotoxicity in Mice

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Abstract

We aimed to evaluate the renal protective capacity of Fermented Black Ginseng (FBG) and its mechanism to reduce the cisplatin-induced nephrotoxicity. Nephrotoxicity was induced by a single intraperitoneal injection of cisplatin (20 mg/kg) and treated with white ginseng (WB) and FBG (200 mg/kg/day, orally) for 4 days before cisplatin treatment. Biochemical results showed that WB and FBG pretreatment significantly reduced the increase of blood urea nitrogen (BUN) and creatinine, and histopathological changes were meaningfully ameliorated. Cisplatin increased the production of reactive oxygen species (ROS) and depletion of Glutathione (GSH) in serum and kidney, whereas, WB or FBG administration markedly down-regulated. Moreover, the expression of nuclear factor-kappaB (NF-κB), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), and Interleukin-1 β (IL-1β) was markedly suppressed by both WB and FBG. However, FBG pretreatment was more effective than those of WB in COX-2, iNOS, and IL-6 levels. Moreover, FBG treated mice significantly up-regulated the antioxidative enzymes. In HPLC analysis, the increasing ginsenoside contents that include Rg1, Re, Rb1, Rc, Rb3, Rd, Rg3, Rk1, and Rg5 by heat-processing were greater about 5.7 fold when fermentation additionally. Taken together, FBG may be a worthwhile candidate for the prevention of nephrotoxicity in patients receiving cisplatin.

Keywords: Fermented black ginseng; Cisplatin; Oxidative stress; Inflammation; Nephrotoxicity

Introduction

Cisplatin is one of the most effective and potent antineoplastic agent, 25~30% of patients receiving cisplatin experience acute kidney injury formally called acute renal failure (ARF) [1,2]. Moreover, cisplatin use can also result in a variety of clinical complications such as hypomagnesemia, hypocalcemia, distal renal tubular acidosis, thrombotic microangiopathy [3].

The etiology of ARF is associated to the accumulation of cisplatin in renal proximal tubular cells and cisplatin is well-known to permeate proximal tubular cells through apical and basolateral organic cation transporters [4,5]. Especially, the global burden of ARF is calculated to be 13.3 million cases per year, 11.3 million of which are in low-income countries. According to the UK’s National Institute for Health and Care Excellence (NICE), adequate care of ARF could avoid 42,000 deaths every year [6]. The various efforts which include intravenous volume expansion, avoidance of other potential nephrotoxins, renal replacement therapy have been tried to prevent nephrotoxicity. However, none of them have demonstrated definite positive clinical results. Ultimately, the clinical use of cisplatin has been limited because of this side effect [3] and is also greatly demanded the development of new therapeutic approaches to support and improve renal function.

Panax ginseng (P. ginseng) has been used as traditional herbal medicine in Korea, China, and Japan and especially roots have been provided a health food or various remedies. P. ginseng has been reported to possess multiple biological activities including antioxidant, anti-inflammatory, anti-tumor, antiaging, antidiabetic, anti-fatigue effects [7-9]. Ginsenosides, saponin molecules that are characteristic of Panax species, are commonly considered to be the major active pharmacological components of P. ginseng. The bioactive components in ginseng root include more than 60 ginsenosides such as Rg1, Re, Rb1, Rg2(s), Rg2(r), Rc, Rb3, Rd, Rg6, Rg3(s), Rg3(r), Rk1, Rg5, etc. [10,11].

Fresh ginseng is easily degraded at room temperature and consequentially decreases its efficacy. Therefore, fresh ginseng is processed into red ginseng or black ginseng through the process of steaming and drying or processed into white ginseng by a simple drying process [12]. These processing methods increase their pharmacological effects and decrease their cytotoxicity through the chemical alteration of components and the content change [13]. Thus, recent many studies have examined the enhancement of active components and physiological activities by fermenting ginseng. Moreover, fermentation-processed ginseng was absorbed and digested more easily [14,15] and also improves their pharmacological efficacy [16].

However, virtually no studies have investigated the changes in chemical and biological activities according to fermentation of P. ginseng, which is undergone 9 times steaming and drying repeatedly, was called generally ‘Black Ginseng’ in Korea. Therefore, we separated the major ginsenosides and analyzed their contents by high-performance liquid chromatographic (HPLC) and compared it with white ginseng by a simple drying process. Moreover, we evaluated the antioxidant and anti-inflammatory effects of fermented black ginseng on Cisplatin-induced nephrotoxicity in mice.
Materials and Methods

Materials

Cisplatin (cis-diaminedichloroplatinum(II) (CDDP)), phenylmethylsulfonyl fluoride (PMSF), dithiothreitol (DTT), N-1-naphthylenediamine dihydrochloride, sulphanalidam, phosphoraciced, diethylaminoethanepenta-acetic acid (DTPA), and dihydrotruhomadium 123 (DHR 123) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The protease inhibitor mixture and ethylenediaminotetraacetic acid (EDTA) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 2', 7'-Dichlorofluorescein diacetate (DCF-DA) was obtained from Molecular Probes (Eugene, OR, USA). The Pierce bichinchonic acid (BCA) protein assay kit was obtained from Thermo Scientific (Rockford, IL, USA). Goat polyclonal antibodies against tumor necrosis factor-α (TNF-α; 1:1,000) and interleukin-6 (IL-6; 1:1,000); rabbit polyclonal antibodies against nuclear factor-κB (NF-κB; 1:1,000), superoxide dismutase (SOD; 1:1,000), catalase (1:1,000), glutathione peroxidase (GPX; 1:1,000); mouse monoclonal antibodies against phosphor-inhibitor of nuclear factor kappa B (p-IkBα; 1:1,000), cyclooxygenase-2 (COX-2; 1:1,000) and inducible nitric oxide synthase (iNOS; 1:1,000), and histone (1:1,000) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Rabbit anti-goat and goat anti-mouse immunoglobulin G (IgG) horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5,000) were acquired from Santa Cruz Biotechnology, Inc. ECL Western Blotting Detection Reagents were supplied by GE Healthcare (Piscataway, NJ, USA). The all agent were used for the cultivation of the microorganisms were purchased from BD Difco (Detroit, MI, USA) and Streptococcus thermophilus obtained from prof. Lee CY (Microbiology and Biotechnology, Daejeon University, Korea). Complex enzyme liquid was manufactured using Cellulac, HTEc2, and Amyloglucosidase and used when black ginseng (BG) was fermented. Here, each ratio of three agents is 1:1:1. Complex enzyme liquid obtained from the Korea Research Institute of Chemical Technology (Daejeon, Korea).

Plant materials and Strain used for fermentation

The fresh ginseng roots used standardized 6-yr-old Korean ginseng harvested in 2013 (Yeouju-si, Gyeonggi-do, South Korea). A voucher herbarium specimen has been deposited at the Herbarium of Daejon University and was identified by Prof. Y.B. Seo, the herbarium leader of Daejon University. White ginseng (WG) was experienced a simple steaming process (steaming temperature; 90ºC, steaming time; 4 h) using non-pneumatic steaming machine (Ace groundbreaking, Seoul, Korea) and dried repeatedly, thereby was fermented by Streptococcus thermophilus. This fermented WG was called FBG hereafter. WG and BG are showed Figure 1A and 1B. First, mix WG 50 g and distilled water (250 mL) (20% w/v) in air-tight containers. Second, reacts complex enzyme liquid (2 mL) for 24 h. Third, inoculates Streptococcus thermophilus (2 mL) and incubates at 35ºC. Fourth, inculcates the strain for the solid fermentation (about 105-107 cells/mL) and measure pH at 0 day, 4 days, 7 days. Fifth, inoculates the strain for the solid fermentation (about 105-107 cells/mL) and measure pH at 0 day, 4 days, 7 days, according to fermentation days. The change of pH is showed in Figure 2A-C.

Animals and experimental design

Six-week-old ICR mice were purchased from Orient Bio (Gyeonggi-do, Korea). The animals were maintained under a 12 h light/dark cycle, and housed with a controlled temperature (24°C) and humidity (55 ± 5%). All animal experimental protocols were approved by the Institutional Animal Care and Use Committee of the Daejeon Haany University (Ethical approval number: 2015-050). For the main study, 36 mice were randomly divided into four groups. The group 1 (Normal group, n=9) received water; group 2 (Control group, n=9) received only a single injection of cisplatin (20 mg/kg) intraperitoneally; group 3 (WG group, n=9) received WG (200 mg/kg/day) orally for 4 successive days thereafter injected cisplatin; group 4 (FBG group, n=9) received FBG (200 mg/kg/day) orally for 4 successive days thereafter injected cisplatin. At 24 h after cisplatin challenge, blood samples were collected by cardiac puncture from anesthetized mice. The serum was immediately separated from the blood samples by centrifugation. Subsequently, the kidney was perfused through the artery with ice-cold physiological saline (0.9% NaCl, pH 7.4), removed, quickly frozen, and kept at -80°C until analysis.

pH Measurement

The pH values were measured using Oakton 35634-40 pH Spear (Varmon hills, IL, USA) at 0 day, 4 days, 7 days according to fermentation days. The change of pH is showed in Figure 2A-C.

HPLC analysis

We injected 20 µL of WG or FBG into a reverse-phase high-performance liquid chromatography (HPLC) using a XbridgeTM RP18 (4.6 × 250 mm, 5-µm pore size), with a column temperature of 40°C. Mobile phase component A = water and B =acetonitrile. The gradient conditions were as follows: 0-42 min, 18% B; 42-46 min, 24% B; 46-79 min, 40% B; 79-115 min, 65% B; 115-135 min, 85% B; 150-151 min, 18% B. The flow rate was 1.0 mL/min. The UV absorbance from 203 nm was monitored using PDA (Waters 1525, detector 2998, USA). All peaks were assigned by carrying out co-injection tests with authentic samples and comparing them with the UV spectral data. The components of major compounds (Rg1, Re, Rb1, Rc, Rb3, Rd, Rg3, Rk1, and Rg) were detected from WG, BG, and FBG. The measurement was repeated three times for each sample. Representative HPLC results are illustrated in Figure 3.

Figure 1: Morphology change from WG to BG. WG (A) BG (B) WG, White ginseng; BG, 9 times steaming and drying repeatedly. This figure is showed that WG is undergone 0, 1, 3, 6, 9 times processing and is changed in its morphology.
Measurement of renal functional parameters

Renal functional parameters, blood urea nitrogen (BUN) and Creatinine assay kits were conducted spectrophotometrically using commercially available kits from Asan Pharm. Co., Ltd., (Seoul, Korea).

Measurement of ROS and GSH in serum and kidney

ROS level was measured employing the method of Ali et al. [17]. DCF-DA was added to serum or kidney tissue. After incubation for 30 min, the changes in fluorescence values were determined at an excitation wavelength of 486 nm and emission wavelength of 530. GSH assay was
carried out by employing the method of Hessin and Hill [18]. Renal tissues were homogenized on ice with 1 mM EDTA-50 mM sodium phosphate buffer (pH 7.4). Then, 25% metaphosphoric acid was added for protein precipitation. The homogenate was centrifuged at 4°C for 30 min to obtain the supernatant for the assay of GSH. To assay, 1 mM EDTA-50 mM sodium phosphate buffer (pH 7.4) was added to the supernatant, followed by α-phthalaldehyde. After 20 min at room temperature, fluorescence was estimated at an excitation wavelength at 360 nm and emission wavelength of 460 nm. Protein assay was carried out using a Bio-Rad protein kit (Bio-Rad Laboratories, Hercules, CA, USA).

Preparation of cytosol and nuclear fractions

Protein extraction was performed according to the method of Komatsu with minor modifications [18,19]. Esophageal tissues for cytosol fraction were hemogenized with ice-cold lysis buffer A (250 mM NaCl) containing 10 mM HEPES (pH 7.8), 10 mM KCl, 2 mM MgCl₂, 1 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, and 1,250 μL protease inhibitor mixture solution. The homogenate incubated at 4°C for 20 min. And then 10% NP-40 was added and mixed well. After centrifugation (13,400 × g for 2 min at 4°C) using Eppendorf 5415R (Hamburg, Germany), the supernatant liquid (cytosol fraction) was separated as new e-tube. The left pellets were washed twice by buffer A and discard the supernatant. Next, the pellets were suspended with lysis buffer C (20 mM NaCl) containing 50 mM HEPES (pH 7.8), 50 mM KCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, 1% (v/v) glycerol, and 100 μL protease inhibitor mixture solution suspended and incubated at 4°C for 30 min. After centrifugation (13,400 × g for 10 min at 4°C), the nuclear fraction was prepared to collect the supernatant. Both cytosol and nuclear fractions were kept at -80°C before the analysis.

Immunoblotting analyses

For the estimation of NF-κBp65, 10 μg of protein from each nuclear fraction was electrophoresed through an 8% sodium dodecylsulfate polyacrylamide gel (SDS-PAGE). Separated proteins were transferred to a nitrocellulose membrane, blocked with 5% (w/v) skim milk solution for 1 h, and then incubated with primary antibodies to NF-κBp65 and histone, respectively, overnight at 4°C. After the blots were washed, were incubated with anti-mouse or anti-rabbit IgG HRP-conjugated secondary antibody for 1 h at room temperature. Also, 10-15 μg of protein of each cytosol fraction of SOD, catalase, GPx, p-IκBα, COX-2, iNOS, TNF-α, and IL-6 was electrophoresed through 8-15% SDS-PAGE. Each antigen-antibody complex was visualized using ECL Western Blotting Detection Reagents and detected by chemiluminescence with ATTO Densitograph Software (ATTO Corporation, Tokyo, Japan) and quantified as the ratio to histone and/or β-actin. The protein levels of groups are expressed relative to those of normal mice (represented as 1).

Statistical analysis

The data are expressed as the mean ± SEM. Significance was assessed by one-way analysis of variance (ANOVA) followed by least-significant differences (LSD) test (SPSS 22.0 for Windows, SPSS Inc., Chicago, IL, USA). Values of p < 0.05 were considered significant.

Results and Discussion

HPLC analysis for WG and FBG and pH change

Traditional herbal medicines have been processed to enhance their therapeutic effects, remove or reduce toxicity and side effects, and make storage higher. Heat-processed Korean ginseng (Panax ginseng) is one of the representative examples of a processed herbal medicine [20]. Besides, the lactic acid fermentation is considered as one of the appropriate tool to enhance the functional potential or bioactive compounds [21]. Especially, Streptococcus thermophilus, a gram-positive facultative anaerobe, is one of the crucial lactic acid bacteria in the manufacture of yogurt widely. In addition, it is considered as the most important industrial dairy starter during fermentation processes [22].

The major 15 ginsenosides such as Rg1, Re, Rb1, Rg2(s), Rg2(r), Rb3, Rd, Rg6, Rg3(s), Rg3(r), Rk1, and Rg5 and total ginsenoside contents were determined after comparison with standards Figure 2. In this study, we investigated the heat-processing and fermentation-induced chemical changes of Panax ginseng. As shown in Table 1, WG was high Rg1, Re, Rb1, Rc, and Rb3 levels, however, BG was high Rd, Rg3(s), Rg3(r), Rk1, and Rg5 levels. When BG was fermented, Rd, Rg3(s), Rg3(r), Rk1, and Rg5 levels increases much higher than those of BG. Above all, the Rd level showed the noticeable rise (5.7 fold compared with BG, and 11.7 fold compared with WG). Total ginsenosid content of WG, BG, and FBG was 4.360, 7.857, and 9.252, respectively. These results suggest that fermentation of BG could exert the potent pharmacological activities than WG or BG. As shown in Figure 3, pH level according to fermentation days of BG is reduced. pH of WG is 5.37 and pH of BG is 3.93 at fermentation 7 days. FBG (pH 3.93) was used in the current study.

Effect of FBG in Body and Kidney weights and serum parameters

Cisplatin (cis-diammine-dichloroplatinum II, CDDP) is a widely used cancer chemotherapeutic agent to treat various types of solid tumors in the head and neck, lung, ovaries, uterus, testicles, and bladder [23]. The well-known side effects of cisplatin are nephrotoxicity, neurotoxicity, ototoxicity, electrolyte disturbances, nausea and vomiting, myelotoxicity, and hemolytic anemia. Hence, nephrotoxicity is the most common adverse effect and it is a major issue [24]. High concentrations of cisplatin and its toxic metabolites related to the risk of nephrotoxicity, therefore cisplatin has been used limitedly. Cisplatin induced nephrotoxicity consists of change in kidney weight, histological changes in kidney and increase BUN and creatinine in

<table>
<thead>
<tr>
<th>Ginkenoside</th>
<th>WG</th>
<th>BG</th>
<th>FBG</th>
</tr>
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<tbody>
<tr>
<td>Rg1</td>
<td>0.846</td>
<td>0.077</td>
<td>0.094</td>
</tr>
<tr>
<td>Re</td>
<td>0.386</td>
<td>0.033</td>
<td>0.027</td>
</tr>
<tr>
<td>Rb1</td>
<td>1.368</td>
<td>0.714</td>
<td>0.012</td>
</tr>
<tr>
<td>Rg2(s)</td>
<td>0.049</td>
<td>0.115</td>
<td>0.146</td>
</tr>
<tr>
<td>Rg2(r)</td>
<td>N/D</td>
<td>0.039</td>
<td>0.05</td>
</tr>
<tr>
<td>Rc</td>
<td>0.513</td>
<td>0.32</td>
<td>0.355</td>
</tr>
<tr>
<td>Rb3</td>
<td>0.452</td>
<td>0.042</td>
<td>0.062</td>
</tr>
<tr>
<td>Rd</td>
<td>0.063</td>
<td>0.129</td>
<td>0.736</td>
</tr>
<tr>
<td>Rg6</td>
<td>N/D</td>
<td>0.27</td>
<td>0.292</td>
</tr>
<tr>
<td>F4+Rk3</td>
<td>N/D</td>
<td>0.239</td>
<td>0.269</td>
</tr>
<tr>
<td>Rh4</td>
<td>N/D</td>
<td>0.432</td>
<td>0.5</td>
</tr>
<tr>
<td>Rg3(s)</td>
<td>N/D</td>
<td>0.401</td>
<td>0.473</td>
</tr>
<tr>
<td>Rg3(r)</td>
<td>N/D</td>
<td>0.165</td>
<td>0.272</td>
</tr>
<tr>
<td>Rk1</td>
<td>0.046</td>
<td>1.096</td>
<td>1.273</td>
</tr>
<tr>
<td>Rg5</td>
<td>0.034</td>
<td>3.364</td>
<td>4.085</td>
</tr>
<tr>
<td>Etc (R, Rb2, F1, F2, CK)</td>
<td>0.603</td>
<td>0.421</td>
<td>0.604</td>
</tr>
<tr>
<td>Total</td>
<td>4.360</td>
<td>7.857</td>
<td>9.252</td>
</tr>
</tbody>
</table>

Table 1: Comparison of composition content of WG, BG, and FBG.
serum [25]. Recent report showed that Ginsenoside-Rd was proved to decrease the severity of renal injury induced by cisplatin [26]. In the present study, we evaluated the kidney weight, BUN and creatinine which are renal function biomarker. Cisplatin-control mice showed a significant decrease in final body weight (33.3 ± 0.25 vs. 35.5±0.45 g) and increase in the kidney weight (12.25 ± 0.52 vs. 5.47 ± 0.41 mg/g b.w.); which were alleviated with WG or FBG pretreatment Table 2. In addition, cisplatin raised both serum BUN and creatinine levels compared to the normal group. However, BUN and creatinine levels both WG and FBG were decreased significantly. Herein, pretreatment with WG and FBG attenuated cisplatin-induced renal dysfunction as demonstrated by normalization of BUN and creatinine level compared to cisplatin-control mice [27-30].

ROS and GSH levels in Serum and Kidney

Recently, studies in vitro and in vivo have been accumulated that the pathogenesis of nephrotoxicity is closely related to reactive oxygen metabolites [30,31]. Oxidative stress initially triggered by cisplatin treatment directly acts on cell components, including lipids, proteins, and DNA and destroys their structure. The induction of ROS contributes to the development of nephrotoxicity. Most of free radicals rapidly impair biological functions of cellular constituent. Thus, cisplatin-induced nephrotoxicity was protected by radical scavengers [32,33]. GSH is the major antioxidative tripeptide and plays important role in the detoxification of toxicants to disturb cellular homeostasis.

Table 3 shows the effect of WG and FBG on the level of ROS and GSH in serum and kidney. The serum ROS level of cisplatin-control mice was significantly elevated to 3802 ± 352 fluorescence/min/mL in comparison with that of normal mice of 2106 ± 155 fluorescence/min/mL. However, in WG or FBG-treated mice, it was significantly decreased to 2859 ± 252 or 2902 ± 199 fluorescence/min/mL, respectively. The renal ROS level of cisplatin-control mice was significantly elevated to 3635 ± 61 fluorescence/min/mg protein in comparison with that of normal mice of 3076 ± 77 fluorescence/min/mg protein. Besides, in WG or FBG-treated mice, it was remarkably decreased to 2950 ± 153 (p<0.001) or 29676 ± 64 (p<0.001) fluorescence/min/mg protein, respectively. Moreover, the oral administration of WG or FBG significantly up-regulated the GSH level in serum when it compared with cisplatin-control mice. Similarly, the renal GSH level by WG or FBG pretreatment was increased higher than it of normal mice. Especially, FBG administration showed a significant increase (p<0.05) [34,35].

Renal oxidative and inflammatory protein expressions

Compared to normal mice, renal expressions of SOD, catalase, and GPx were markedly reduced in cisplatin-control mice [36]. The administration of WG or FBG led to a significant up-regulation of these protein expressions. The SOD level of FBG was superior to it of WG Figure 4A-C.

An early response to cisplatin-induced nephrotoxicity includes the activation of NF-kB signaling pathway [34]. NF-kB is a major pro-inflammatory transcriptional factor sequestered in the cytoplasm by the inhibitor protein IκBα. Phosphorylation of IκBα leads to the release of NF-κB and then NF-κB translocates to the nucleus and promotes the transcription of target genes including inflammatory mediators and cytokines such as COX-2, iNOS, TNF-α and IL-6 [35,37].

Figure 5 shows p-IκBα and NF-κBp65 protein expressions. In cisplatin-control mice, the phosphorylation of IκBα was significantly increased compared with the normal group. Pretreatment of WG or FBG significantly suppressed under the level of normal mice Figure 5A. Moreover, cisplatin-control mice showed up-regulation of the nuclear NF-Bp65 protein compared to normal mice. On the other hand, the administration of WG or FBG led to reduce nearly too normal levels Figure 5B.

We quantified renal COX-2, iNOS, TNF-α, and IL-6 protein expressions, whose activations are involved in ROS and related to inflammatory responses, presented in Figure 6A-D, respectively. COX-2, iNOS, TNF-α, and IL-6 protein expressions in cisplatin-control mice were significantly increased compared to those in normal mice, whereas these increased protein expressions were down-regulated by the administration of WG or FBG. Especially, COX-2, iNOS, and IL-6 protein expressions in FBG pretreated groups were similar to those in normal mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight</th>
<th>Kidney weight</th>
<th>BUN (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (g)</td>
<td>Final (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal mice</td>
<td>32.7 ± 0.54</td>
<td>35.5 ± 0.45***</td>
<td>5.47 ± 0.41*</td>
<td>22.83 ± 0.74</td>
</tr>
<tr>
<td>Cisplatin-treated</td>
<td></td>
<td></td>
<td></td>
<td>1.90 ± 0.01***</td>
</tr>
<tr>
<td>mice</td>
<td>32.7 ± 0.50</td>
<td>33.3 ± 0.25</td>
<td>12.25 ± 0.52</td>
<td>24.74 ± 1.48</td>
</tr>
<tr>
<td>Con</td>
<td>32.7 ± 0.50</td>
<td>34.0 ± 0.37</td>
<td>10.24 ± 0.46</td>
<td>20.44 ± 0.53*</td>
</tr>
<tr>
<td>WG</td>
<td>32.6 ± 0.43</td>
<td>34.0 ± 0.34</td>
<td>11.27 ± 0.50</td>
<td>21.07 ± 0.20*</td>
</tr>
<tr>
<td>FBG</td>
<td>32.6 ± 0.43</td>
<td>34.0 ± 0.34</td>
<td>11.27 ± 0.50</td>
<td>1.89 ± 0.02***</td>
</tr>
</tbody>
</table>

Con: Cisplatin-treated mice; WG, Cisplatin-treated and WG 200 mg/kg pretreated mice; FBG, Cisplatin-treated and FBG 200 mg/kg pretreated mice. Significance: *p<0.05, **p<0.01 vs. Con. (n=9).

<table>
<thead>
<tr>
<th>Item</th>
<th>Normal mice</th>
<th>Cisplatin-treated mice</th>
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<tbody>
<tr>
<td>Serum</td>
<td></td>
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</tr>
<tr>
<td>ROS (fluorescence/min/mL)</td>
<td>2106 ± 155***</td>
<td>3802 ± 352</td>
</tr>
<tr>
<td>GSH (µ/mL)</td>
<td>0.40 ± 0.01</td>
<td>0.38 ± 0.01</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
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<tr>
<td>ROS (fluorescence/min/mg protein)</td>
<td>3076 ± 77***</td>
<td>3635 ± 61</td>
</tr>
<tr>
<td>GSH (µ/mL/mg protein)</td>
<td>441.5 ± 9.4</td>
<td>417.9 ± 8.7</td>
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Con: Cisplatin-treated mice; WG, Cisplatin-treated and WG 200 mg/kg pretreated mice; FBG, Cisplatin-treated and FBG 200 mg/kg pretreated mice. Significance: *p<0.05, **p<0.01 vs. Con. (n=9).
Figure 4: Renal SOD (A), Catalase (B), and GPx (C) protein expressions. N, normal mice; Con, Cisplatin-treated mice; WG, Cisplatin-treated and WG 200 mg/kg pretreated mice; FBG, Cisplatin-treated and FBG 200 mg/kg pretreated mice. Significance: *p<0.05, **p<0.01 vs. Con. (n=9).

Figure 5: Renal p-IkB (A) and NF-κBp65 (B) protein expressions. N, normal mice; Con, Cisplatin-treated mice; WG, Cisplatin-treated and WG 200 mg/kg pretreated mice; FBG, Cisplatin-treated and FBG 200 mg/kg pretreated mice. Significance: *p<0.05 vs. Con. (n=9).

Figure 6: Renal COX-2 (A), iNOS (B), TNF-α (C), and IL-6 (D) protein expressions. N, normal mice; Con, Cisplatin-treated mice; WG, Cisplatin-treated and WG 200 mg/kg pretreated mice; FBG, Cisplatin-treated and FBG 200 mg/kg pretreated mice. Significance: *p<0.05, **p<0.01, ***p<0.001 vs. Con. (n=9).
levels in the mice receiving FBG were significantly reduced nearly to those of normal mice or below, as shown Figure 6.

Effect of FBG on renal tubular damage

Cisplatin is filtered at the glomerulus and taken up into renal tubular cells mainly by a transport-mediated process. Accordingly, nephrotoxicity by cisplatin is characterized by renal electrolyte disturbances and by acute fall in glomerular filtration rate (GFR) [27,28]. Cisplatin treatment was found to cause diffuse tubular necrosis and desquamation and parenchyma degeneration in the cortex [29]. In the current study, tubular damage was similar to control kidney of a previous reportd study. However, WB or FBG treatment partially was reduced the histological features of renal injury. Cisplatin-control mice Figure 7A and 7B showed a meaningful tubular damage compared to the normal group Figure 7A. Pretreatment with WG or FBG decreased tubular damage, as shown Figure 7C and 7D.

Conclusions

Data from previous studies demonstrated key roles for inflammatory and oxidative pathways in nephrotoxicity induced by cisplatin. Based on these reports, cisplatin nephrotoxicity observed in our study can be attributed to the concomitant deterioration in the...
antioxidant tendency (reduced SOD, catalase, and GPx), elevations in inflammatory mediators (COX-2 and iNOS), and increases in inflammatory cytokines (TNF-α and IL-6) in renal tissues. Herein, pretreatment both WG and FBG reversed significantly these protein expressions. Especially, antioxidative effect of FBG treatment was more superior than those of WG treatment. These results showed that FBG may more effectively develop the renoprotection through the strong suppression of the oxidative stress. FBG could effectively protect more than it of BG Figure 8.

In this study, the administration of FBG effectively ameliorates nephroptoxicity in Cisplatin-treated mice through the inhibition of NF-κB activation and the up-regulation of antioxidative enzyme, thus, FBG would result in the improvement of renal disorders caused by the ROS system.

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References  


