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Platycodin D, a triterpenoid sapoinin from *Platycodon grandiflorum*, ameliorates cisplatin-induced nephrotoxicity in mice

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ABSTRACT

Platycodin D (PD) is well known as a potent triterpenoid saponin having various pharmacological activities isolated from the root of *Platycodon grandiflorum* (Jacq.) A. DC. (Campanulaceae). We aimed to evaluate protective effect of PD on cisplatin (CDDP)-induced nephrotoxicity. Male ICR mice were allocated into five groups as follows: Negative control, CDDP alone and CDDP with PD (0.1, 1 and 5 mg/kg) treated group. PD was given for three consecutive days before CDDP injection. Increased blood urea nitrogen (BUN) and creatinine (CRE) levels in CDDP alone treated mice were decreased to normal range by pretreatment with PD. It also decreased nitric oxide (NO) and lipid peroxidation with increased antioxidant enzymes such as glutathione (GSH), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in PD pretreated mice. In histopathological examination, pretreatment with PD showed ameliorated renal injury such as intraluminal cast formation and epithelial desquamation. Furthermore, over-expression of nuclear factor-kappa B p65 and apoptotic cells were suppressed by PD pretreatment. Taken together, PD pretreatment might be beneficial to CDDP-induced nephrotoxicity.

1. Introduction

Cisplatin (cis-diamminedichloroplatinum [II], CDDP), is a potent platinum-containing anticancer drug and still used for the treatment of testicular, head and neck, ovarian, cervical, nonsmall cell lung carcinoma, and many other types of cancer (Yao et al., 2007; Pabla and Dong, 2008). However, its use is mainly limited by severe side effects in normal tissues including neurotoxicity, ototoxicity, nausea and vomiting, and nephrotoxicity (Pabla and Dong, 2008). Especially, the prevalence of cisplatin nephrotoxicity is high, occurring in about one-third of patient undergoing cisplatin treatment (Arany and Safirstein, 2003).

The pathophysiological basis of cisplatin nephrotoxicity has been studied for the last three decades. Oxidative stress, inflammation and apoptosis are the main factors of the CDDP-induced renal injury (Yao et al., 2007). In CDDP-induced nephrotoxicity, platinum-glutathione (GSH) conjugates formed in cells were metabolized through a gamma-glutamyl transpeptidase (GGT) to a reactive thiol, which is a potent nephrotoxin, and depleted GSH impairs regulation of reactive oxygen species (ROS) (Townsend and Hanigan, 2002). In addition, oxidative stress is well known to stimulate transcription factors, including nuclear factor-kappa B (NF-κB) (Bubici et al., 2006). Consequently, NF-κB activation leads to expression of many gene involved in the renal damages such as inducible nitric oxide synthase (iNOS) and proinflammatory cytokine gene, resulting in excessive nitric oxide (NO) generation (Xie et al., 1994; Coimbra et al., 2007; Pabla and Dong, 2008).

Many herbal extracts were studied for reducing renal injury in CDDP-induced nephrotoxicity (Shirwaikar et al., 2004; Hung et al., 2007). The root of *Platycodon grandiflorum* has been traditionally used as a medicine for various respiratory diseases including bronchitis, asthma and pulmonary tuberculosis in East Asian countries (Takagi and Lee, 1972). Triterpenoid saponins were found in the roots of *Platycodon grandiflorum* such as platycosides (A, B, C, D, E and F), platycodins (A, D, D2 and D3), polygalacin (D, D2) and platyconic acid A (Kim et al., 2007). These triterpenoid saponins from *Platycodon grandiflorum* exhibited a variety of pharmacological activities, such as anti-inflammatory, anti-cancer and immune enhancing effects (Kim et al., 2008; Shin et al., 2009; Xie et al., 2009). In addition, they are believed to have a wide range of health benefits and prevent chemicals-induced hepatotoxicity (Lee et al., 2008; Khanal et al., 2009). Furthermore, Ahn et al. (2005) reported that *Platycodon grandiflorum* saponins inhibited iNOS and cyclooxygenase 2 (COX-2) by NF-κB suppression and platycodin D (PD) has most potent biological effect among the triterpenoid saponins from *Platycodon grandiflorum*. However, the protective effects of triterpenoid saponins from *Platycodon grandiflorum* on CDDP-induced nephrotoxicity were not evaluated.

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In this view, the main objective of the present study was to investigate the protective effect of PD from *Platycodon grandiflorum* roots on the CDDP-induced renal injury in mice.

2. Materials and methods

2.1. Chemicals

PD (approximately 98% purity) from *Platycodon grandiflorum* roots was purchased from Innoten (Seoul, Korea). Assay kits for SOD, TBARS, GSH and GPx were obtained from Cayman (Ann Arbor, MI, USA) and assay kit for NO was purchased from BioAssay Systems (Hayward, CA, USA). Serum creatinine (CRE) and blood urea nitrogen (BUN) test kits were obtained from IDEXX (Westbrook, ME, USA). TUNEL staining kit and immunostaining kit were purchased from Millipore (Billerica, MA, USA) and Vector Laboratories (Burlingame, CA, USA), respectively. Anti-NF-κB p65 antibody was bought from Abcam (Cambridge, UK). All other reagents were from Sigma Chemicals (St. Louis, MO, USA).

2.2. Animals and treatment

Male ICR mice, 5–6 weeks old, were obtained from Orient Bio (Seongnam, Korea) and acclimated to the laboratory condition (25 ± 0.2 °C, 50% relative humidity, 12 h light/dark cycle) for 1 week before experiments. All animals were supplied with standard chow (Charles River Inc., Richmond, IN, USA) and water ad libitum.

Healthy mice were randomly allocated into five groups as follows: group 1 (negative control, $n = 5$), group 2 (CDDP alone, $n = 5$), group 3 (CDDP with 0.1 mg/kg of PD, $n = 5$), group 4 (CDDP with 1 mg/kg of PD, $n = 5$) and group 5 (CDDP with 5 mg/kg of PD, $n = 5$). PD was prepared in distilled water and all animals were administered 5 mL/kg of body weight as a dosing volume. Negative and CDDP alone groups were orally administered with distilled water and the others were orally administered with PD (0.1, 1 and 5 mg/kg) for three consecutive days. At 3 h after the last treatment, 0.9% of saline (negative control group) or CDDP solution (20 mg/kg, diluted in 0.9% saline) was intraperitoneally injected.

Animals were anaesthetized with carbon dioxide at 3 days after CDDP and blood obtained by cardiac puncture under light carbon dioxide anaesthesia. Serum was separated by centrifuging at the 800g for 15 min and stored at −70 °C until the following markers of renal damage, serum CRE and BUN were measured. The left kidney was quickly removed to obtain samples for histopathological and immunohistochemical studies. The other kidney was removed and stored at −70 °C until assay for the renal oxidative and antioxidative activity. The experimental protocols were approved by the Institutional Animal Care and Use Committee of Chungnam National University (Daejeon, Korea).

2.3. Serum biochemical examination

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

2.4. Kidney lipid peroxidation, NO, GSH, GPx and SOD content

The right kidney was homogenized in PBS (50 mmol/l, pH 7) with a protease inhibitor (Sigma–Aldrich, St. Louis, MO, USA). For lipid peroxidation, the content of malondialdehyde (MDA), a terminal product of lipid peroxidation, was measured by the thiobarbituric acid reduction method using a commercially available kit (TBARS Assay Kit, Cayman Chemical Co., Ann Arbor, MI, USA). The nitrite concentration was measured according to the Griess reaction (QuantiChrom NO Assay Kit, BioAssay Systems, Hayward, CA, USA). Tissue levels of GSH were determined by the improved DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)] method (Glutathione Assay Kit, Cayman Chemical Co., Ann Arbor, MI, USA). GPx activity was assessed by monitoring the oxidation of reduced NADPH at 340 nm (Glutathione Peroxidase Assay Kit, Cayman Chemical Co., Ann Arbor, MI, USA). Total SOD was measured by the disappearance of superoxide detected using a tetrazolium salt (Superoxide Dismutase Assay Kit, Cayman Chemical Co., Ann Arbor, MI, USA).

2.5. Histopathological examination

The left kidney was taken, fixed immediately in a 10% buffered formalin phosphate solution, embedded in paraffin and cut into 5 μm sections. These serial tissue sections were either stained with haematoxylin and eosin (H&E) for histopathological examination or subjected to immunohistochemical staining. Apoptotic nuclei were detected with the TUNEL method using an apoptosis detection kit (ApopTag Peroxidase In Situ Apoptosis Detection Kit; Millipore, Billerica, MA, USA) and immunostaining for NF-κB p65 was performed with anti-NF-κB p65 antibody (Abcam, Cambridge, UK), according to the manufacturer's protocol. All of the stained slides were analyzed under the light microscope. The histopathological alterations were scored on the basis of the semi-quantitative percentage of damaged area: 0, none; 0.5, <10%; 1, 10–25%; 2, 25–50%; 3, 50–70%; and 4, >75% (Kang et al., 2011).

Image analysis for the quantification of positive stained cell number and area was performed using Image J (Rasband WS, ImageJ, US National Institutes of Health, Bethesda, MD, USA) in 10 randomly-selected microscopic fields per specimen.

2.6. Statistical analysis

Results were expressed as mean ± standard error (SEM). The significances of differences among experimental groups were determined using the one way analysis of variance (ANOVA) test or the non-parametric Kruskal–Wallis test. 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.

3. Results

3.1. Serum BUN and CRE levels

The levels of BUN and CRE in CDDP alone group were significantly increased to 126.2 ± 5.22 and 2.14 ± 0.63 Unit/L compared to the negative control group. However, PD (1, 5 and 10 mg/kg) pretreatment dose-dependently attenuated these CDDP-induced elevation in serum BUN and CRE levels by 85% and 83%, respectively ($p < 0.05$; Fig 1).

3.2. Renal lipid peroxidation, NO, GSH, GPx and SOD levels

Antioxidant effect of PD on CDDP induced renal injury were evaluated by GSH, GPx, SOD, TBARS and NO levels in kidney homogenates (Figs 2 and 3). In the CDDP alone group, the activities of GSH, GPx and SOD were significantly decreased, whereas the levels of NO and TBARS were increased, as compared to the nega-

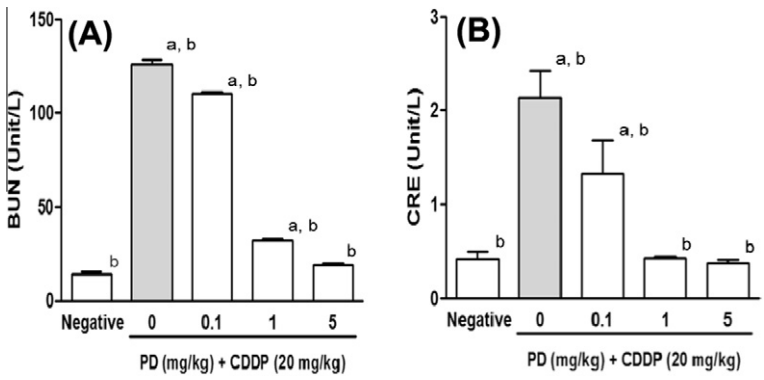

Fig 1. Effect of PD on the renal function in the cisplatin-induced nephrotoxicity (BUN, A; CRE, B) ($n = 5$/group). Mice were given orally PD (0, 0.1, 1, and 5 mg/kg) once daily for three consecutive days prior to cisplatin injection. Values are expressed as means ± SEM. [a]$p < 0.05$, a significant difference in comparison with the negative control group. [b]$p < 0.05$, a significant difference in comparison with the positive control group.