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Original Paper

Kidney Protection Effect of Ginsenoside Re and Its Underlying Mechanisms on Cisplatin-Induced Kidney Injury

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Key Words
Cisplatin • Ginsenoside Re • Oxidative stress • Inflammation • Apoptosis

Abstract
Background/Aims: Cisplatin (CDDP) was the first platinum-containing anti-cancer drug. However, CDDP causes nephrotoxicity as a side effect, which limits its clinic application. The aim of this study was to investigate the renoprotective effect of ginsenoside Re (G-Re) in a murine model of CDDP-induced acute kidney injury. ***Methods:*** Male ICR mice were divided into 4 groups. G-Re was administered to the mice by oral gavage once a day at a dose of 25 mg/kg for 10 days. On the 7th day, a single injection of CDDP (25 mg/kg) was given at 1 h after G-Re treatment. ***Results:*** CDDP administration resulted in renal dysfunction, as evidenced by an increase in the serum levels of creatinine and urea nitrogen. Oxidative stress in the CDDP group was reflected by an increase of malondialdehyde and a depletion of reduced glutathione and catalase in renal tissue. These findings were supported by increased 4-hydroxynonenal expression, which was significantly reduced by G-Re. Simultaneously, the overexpression of cytochrome P450 E1 was inhibited. G-Re inhibited the inflammatory response by the reduction of the protein expression of cyclooxygenase-2 and inducible nitric oxide synthase. Furthermore, CDDP increased the expression of Bax and decreased Bcl-2 expression in renal tissue. Hematoxylin and eosin, Hoechst 33258, and TUNEL staining also confirmed the presence of acute tubular necrosis and apoptosis. G-Re significantly decreased the levels of indicators of renal dysfunction, inflammatory cytokines, apoptosis, and malondialdehyde in the kidney and also significantly attenuated the histopathological changes associated with acute renal failure. ***Conclusions:*** Collectively, the results of this study suggest that the nephroprotective potential of G-Re may, in part, be related to its anti-oxidant, anti-inflammatory, and anti-apoptotic effects.

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Introduction

Cisplatin (CDDP) is one of the most important antineoplastic drugs for the treatment of solid tumors, including those of the head, neck, lung, breast, ovary, and testis [1, 2]. CDDP induces the formation of DNA intra-strand crosslinks, which cause cytotoxic lesions in both tumors and other normal dividing cells [3]. Hence, the clinical application of CDDP is limited by tumor cell resistance and a myriad of adverse effects such as nephrotoxicity, ototoxicity, neurotoxicity, and emetogenicity [4, 5]. CDDP nephrotoxicity is recognized as a complex process involving various factors and signaling pathways, and the identification of beneficial strategies to prevent CDDP nephrotoxicity has primarily focused on reducing renal CDDP accumulation or activation, anti-oxidants, anti-inflammation, or anti-apoptosis.

At present, several therapeutic strategies, including intensive hydration and the application of alternative CDDP analogs, have been proposed as possible approaches for preventing or reducing CDDP-induced nephrotoxicity [6-8]. However, clinical outcomes using such treatments have been unsatisfactory. In fact, hydration therapy did not resolve renal dysfunction in a significant percentage of treated patients, and CDDP analogs were not as effective as CDDP [9-11]. The discontinuation of CDDP therapy remains the only option for patients with progressive renal failure. Therefore, the identification of an effective method for preventing CDDP-induced renal injury is a critical issue in cancer therapeutic research. Recently, several chemical agents have been examined for their ability to prevent CDDP nephrotoxicity, but were not suitable for application in clinical practice [12]. In the course of drug development, a large number of natural compounds have been harvested from herbal medicines as useful pharmacological agents owing to their safety and efficacy [13-15].

Natural sources, including plants, animals, and minerals, provide plenty of resources for the discovery of antioxidants. Studies have shown that dietary antioxidants can reduce the side effects of several cytotoxic anti-cancer drugs by detoxifying reactive oxygen species [16-19]. Ginseng, the root and rhizome of *Panax ginseng* C. A. Meyer, has been used as a medicine or dietary supplement for thousands of years in Asian countries such as China, Korea, and Japan [20-22]. Numerous studies have reported its chemical constituents and biological effects. The major biological active components of ginseng are ginsenosides [23], which have shown various types of biological activity, including antioxidant, anti-inflammatory, and anti-tumor effects [24, 25]. Ginsenoside Re (G-Re), one of the major protopanaxatriol-type ginsenosides isolated from *P. ginseng*, accounts for approximately 30% of total ginsenosides (Fig. 1A). Among all of the ginsenosides in ginseng, G-Re is relatively easy to isolate and purify.

A large number of studies have found that G-Re possesses multiple types of pharmacological activity, including anti-diabetes, cardioprotection [26, 27], immune response enhancement [28, 29], and neuroprotection [30]. A recent study reported the nephroprotective effects of heat-processed mixtures of G-Re with serine or leucine against CDDP-induced renal damage *in vivo* and *in vitro* [31, 32]. However, the effects of G-Re itself on CDDP-induced acute kidney injury (AKI) and the possible molecular mechanisms of its action remain unclear. Therefore, this study was designed to evaluate the kidney protection effects of G-Re on CDDP-induced AKI and to elucidate its molecular mechanisms.

Materials and Methods

Chemicals and reagents

G-Re was isolated from the leaves of *P. ginseng* with a purity greater than 98% using high-performance liquid chromatography in our laboratory. CDDP (purity > 99.0%) was obtained from Sigma-Aldrich Co., Ltd. (St. Louis, MO). Commercial assay kits for serum creatinine (CRE), blood urea nitrogen (BUN), malondialdehyde (MDA), catalase (CAT) and reduced glutathione (GSH) were purchased from Nanjing Jiancheng Institute of Biotechnology (Nanjing, China). A commercial assay kit for bicinchoninic acid (BCA) was purchased from Thermo Fisher Scientific, Inc. (Waltham, MA). Primary antibodies against cyclooxygenase-2 (COX-

2), inducible nitric oxide synthase (iNOS), cytochrome P450 E1 (CYP2E1), Bax, and Bcl-2 were obtained from Cell Signaling Technology, Inc. (Boston, MA). Primary antibodies against 4-hydroxynonenal (4-HNE) were purchased from Beijing Biosynthesis Biotechnology Co., Ltd. (Beijing, China). All other chemicals and reagents used were of high quality and obtained from commercial sources.

Animals

Male ICR mice, weighing 22–25 g (6–8 weeks old), were provided by Yi-Si Experimental Animal Technical Co., Ltd. (Changchun, China; Certificate of Quality No. SCXK (JI) 2016-0003). All mice were housed in specific standard laboratory conditions for at least 1 week, including a temperature-controlled environment (25 ± 2°C), a relative humidity of 60 ± 5%, and with a regular 12-h light/dark cycle. All animals were fed with a standard chow diet and water *ad libitum*. The animal experiments were conducted according to the Guide for the Care and Use of Laboratory Animals (Ministry of Science and Technology of China, 2006). All experimental procedures were approved by the Ethics Committee for Laboratory Animals of Jilin Agricultural University.

Experimental design

All mice were assigned randomly into 4 experimental groups with 8 mice in each group as follows: normal group, G-Re group, CDDP group, and CDDP+G-Re group. The G-Re and CDDP+G-Re groups were administered G-Re at a dose of 25 mg/kg, i.g. for 10 days, while the normal and CDDP groups received saline for the same time period [33]. The dose of G-Re was based on our preliminary experiments and referred to previous work. On the 7th day, the animals in the CDDP and CDDP+G-Re treatment groups received a single intraperitoneal injection of CDDP at a dose of 25 mg/kg to induce nephrotoxicity. After the last treatment and overnight fasting, the animals were sacrificed at 72 h after CDDP injection.

Blood samples were collected from retrobulbar vessels, and serum was separated by centrifugation (3500 rpm, 10 min, twice) and stored at -80°C before analysis. Both kidneys were harvested: one of the kidneys was placed immediately in a 10% buffered formalin solution for histopathological analysis; the other kidney was snap frozen in liquid nitrogen and stored at -80°C until biochemical analysis.

Detection of renal function

The serum was separated by centrifugation twice at 3500 rpm for 10 min. BUN and CRE levels were measured using commercial kits according to the manufacturer's instructions (Nanjing Jiancheng Institute of Biotechnology).

Measurement of lipid peroxidation and antioxidants

Kidney tissue was dissociated in 9 volumes of ice-cold 0.9% NaCl and centrifuged twice at 3500 rpm for 10 min at 4°C. Total protein concentration was used for normalization, which was measured using a BCA protein assay kit (Thermo Fisher Scientific, Inc.). MDA levels in kidney tissue were determined using the thiobarbituric acid method with a commercially available kit from Nanjing Jiancheng Institute of Biotechnology. CAT activity and GSH content were measured using commercial kits (Nanjing Jiancheng Institute of Biotechnology).

Histopathological examination

Kidney tissue was fixed in 10% phosphate-buffered formalin for longer than 24 h, dehydrated in an alcohol series, cleared with xylene, and embedded in paraffin. Paraffin sections (5 μm) were stained with H&E and examined by light microscopy. Tubular damage in H&E-stained kidney sections was evaluated and scored based on the percentage of cortical tubular necrosis: 0 = no damage, 1 = 0–10%, 2 = 11–25%, 3 = 26–45%, 4 = 46–75%, and 5 = greater than 75%. Five sections from each sample were selected randomly for scoring by 2 independent investigators, and 3 mice were used in each group.

Immunohistochemistry and immunofluorescence

Immunohistochemical analysis was performed as described previously with minor modifications [34]. Briefly, 5-μm-thick paraffin sections were deparaffinized and rehydrated with a series of xylene and aqueous alcohol solutions. The deparaffinized renal slices were treated with 3% H_2O_2 for 30 min at 37°C to quench endogenous peroxidase activity, followed by rinsing 3 times in phosphate-buffered saline (PBS) for 3 min.

The sections were then irradiated in 0.1 mol/L sodium citrate buffer (pH 6.0) in a microwave oven (medium high temperature) for 10 min and then with 5% bovine serum albumin for 10 min. The deparaffinized renal slices were incubated overnight at 4°C with primary antibodies against COX-2 (1:400), iNOS (1:100), Bax (1:200), and Bcl-2 (1:200). The sections were rinsed in PBS, incubated in horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at 37°C, and rinsed in PBS. Immunoreactivity was detected using diaminobenzidine (DAB) [35], followed by counterstaining with hematoxylin. The sections were rinsed in tap water for 1 min, dehydrated with an alcohol series, cleared with xylene, mounted with neutral balsam, and coverslipped. Slides prepared for each case were examined by light microscopy (Olympus BX-60, Tokyo, Japan).

Immunofluorescence was carried out on tissue sections processed as described for immunohistochemistry. The sections were incubated overnight at 4°C with primary antibodies against CYP2E1 (1:100) and 4-HNE (1:100). The next day, the slides were covered with DyLight 488-labeled goat anti-rabbit IgG and examined under a fluorescence microscope equipped with a camera.

Western blot analysis

Western blot analysis was performed as described previously [36]. Frozen kidney tissue was homogenized with a lysis buffer and centrifuged at 12, 000 × *g* for 15 min at 4°C. Supernatants containing total protein were analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. After blocking, the membranes were incubated with a primary antibody against Bax (1:2000 dilution), Bcl-2 (1:2000 dilution), and GAPDH (1:1000 dilution) overnight at 4°C, washed 3 times for 10 min to remove the buffer, incubated with an HRP-conjugated secondary antibody (1:4000 dilution, anti-rabbit) for 1.5 h at room temperature, washed 3 times, and detected with an electrochemiluminescence solution.

Hoechst 33258 and TUNEL assay

Hoechst 33258 analysis was performed as described previously [37]. Three samples were selected randomly from each group, and these samples were cut into 5-μm sections and stained with Hoechst 33258. After being washed 3 times with PBS, stained nuclei were visualized under UV excitation and photographed under a fluorescence microscope.

TUNEL analysis was carried out using an In Situ Cell Death Detection Kit, POD according to the manufacturer's directions. The sections were deparaffinized and rehydrated using xylene and a graded series of ethanol. Proteinase K was added and incubated for 10 min to enhance tissue permeability. Endogenous peroxidase activity in the tissue sections was annulled with 3% H_2O_2 in methanol for 20 min, and the sections were incubated with an equilibration buffer and terminal deoxynucleotidyl transferase. Finally, the sections were incubated with anti-digoxigenin-peroxidase conjugate. Peroxidase activity in each tissue section was shown by the application of DAB. The sections were counterstained with hematoxylin prior to analysis by light microscopy.

Statistical analysis

Experimental data were analyzed using SPSS version 21.0 software (SPSS, Inc., Chicago, IL). Differences among the groups were assessed with one-way analysis of variance, followed by the least significance difference multiple comparison test. Values are expressed as means ± standard deviation. *P*-values less than 0.05 or 0.01 were considered statistically significant.

Results

Effect of G-Re on body weight and organ indices

As shown in Table 1, body weight and relative tissue weight were measured at 72 h after CDDP administration. The body weight and relative organ indices of the mice in the normal and G-Re groups were

Table 1. Effects of G-Re on body weight and organ index in mice. Data represent the mean ± S.D. Significant differences were indicated by ** $p < 0.01$ and * $p < 0.05$ vs. normal group. $^{\#}$ $p < 0.05$ vs. CDDP group

Groups	Dosage	Weights (g)		Organ indices (mg/g)		
	(mg/kg)	Initial	Final	Liver	Spleen	Kidney
Normal	—	25.25±1.78	31.58±2.06	5.01±0.21	0.44±0.08	1.61±0.10
G-Re	25	26.11±1.52	31.75±1.95	5.12±0.31	0.39±0.08	1.59±0.12
CDDP	20	25.82±1.02	29.77±1.08**	5.43±0.65	0.19±0.06**	1.41±0.08*
CDDP+G-Re	25	25.86±1.42	30.17±1.21$^{\#}$	5.47±0.34	0.27±0.04$^{\#}$	1.55±0.09$^{\#}$

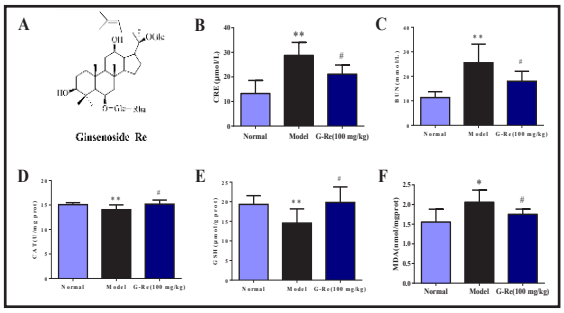

Fig. 1. The chemical structure of G-Re (A). Effects of G-Re on the serum levels of CRE (B) and BUN (C) in CDDP-induced AKI. Effects of G-Re on the levels of kidney CAT (D), GSH (E) and MDA (F). Data represent the mean ± S.D. Significant differences were indicated by $^{**}p<0.01$ and $^{*}p<0.05$ vs. normal group. $^{\#\#}p<0.01$ and $^{\#}p<0.05$ vs. CDDP group.

similar, indicating that G-Re had no negative influence on the experimental animals. Body weight was reduced after CDDP injection ($P < 0.01$), and the organ indices of the spleen and kidney decreased significantly compared with the normal group, providing evidence for the induction of AKI by CDDP ($P < 0.01$ and $P < 0.05$, respectively). However, G-Re pretreatment for 10 consecutive days blocked the CDDP-induced decrease in the indices of both organs and body weight ($P < 0.05$). In addition, the liver index in the CDDP-treated group did not change in comparison with the normal and CDDP+G-Re groups.

Effect of G-Re on renal function

To evaluate whether G-Re exerted a protective effect on renal function, serum CRE and BUN levels were measured in all groups (Fig. 1B, C). Following a single CDDP injection, there was a significant increase in the serum levels of CRE ($P < 0.01$) and BUN ($P < 0.01$) when compared with the normal group, reflecting the successful establishment of AKI. However, pretreatment with G-Re improved renal function and led to the significant normalization of serum CRE ($P < 0.05$) and BUN levels ($P < 0.05$). The above findings indicate that AKI was reduced with G-Re treatment for 10 days.

Effect of G-Re on antioxidants and lipid peroxidation

Oxidative stress plays a critical role in CDDP-induced AKI. Here, we evaluated the effect of G-Re on CDDP-induced oxidative stress injury in kidney tissue. As summarized in Fig. 1D, CAT activity in the kidney was significantly decreased following exposure to CDDP ($P < 0.01$), which was attenuated by G-Re pretreatment ($P < 0.05$). In the CDDP group, GSH content was decreased when compared to the normal group (Fig. 1E). The administration of G-Re prevented the reduction of GSH ($P < 0.05$). As shown in Fig. 1F, MDA levels in kidney homogenate were measured, and the results showed that treatment with CDDP increased MDA levels ($P < 0.05$). Treated with G-Re significantly inhibited the overproduction of renal MDA ($P < 0.05$).

Furthermore, the degree of oxidative stress injury in the kidney was confirmed by measuring the expression of 4-HNE, an end product of lipid peroxidation and an indicator of free radical production. Kidney tissue of mice in the normal group showed low 4-HNE expression (Fig. 2A). CDDP administration markedly increased 4-HNE expression, which was attenuated by pretreatment with G-Re. The expression of the metabolizing enzyme CYP2E1 was increased after CDDP challenge and reduced in CDDP+G-Re mice (Fig. 2B). These results clearly suggested that the administration of G-Re exerted a protective effect against CDDP-induced oxidative stress injury in the kidney.

Effect of G-Re on inflammation

To evaluate the effect of G-Re on the CDDP-induced inflammatory response in the kidney, the expression levels of COX-2 and iNOS were measured in the kidney by immunohistochemistry and western blot analysis. As shown in Fig. 3, CDDP exposure caused a significant increase of COX-2 and iNOS expression in the kidney when compared with mice in the normal group. Moreover, treatment with G-Re significantly decreased the relative

protein expression levels of COX-2 and iNOS in the kidney when compared with the CDDP group. These data indicated that G-Re attenuates the inflammatory response in renal tissue caused by CDDP administration.

Effect of G-Re on apoptosis

In order to determine the extent of apoptosis in renal tissue, the levels of the apoptotic protein Bax and anti-apoptotic protein Bcl-2 were analyzed by using immunohistochemical staining in all experimental groups. As shown in Fig. 3, CDDP induced apoptosis in tubular cells, which was evidenced by the increased expression of Bax and decreased expression of Bcl-2. Interestingly, G-Re significantly inhibited apoptosis as shown by the increased expression of Bcl-2 and decreased expression of Bax protein.

Similar to the results from immunohistochemistry, western blot analysis also showed that the mice in the CDDP-treated group had increased Bax protein expression ($P < 0.05$) and decreased Bcl-2 protein expression ($P < 0.05$) in renal tissue in comparison with the normal group (Fig. 3). However, pretreatment with G-Re significantly inhibited the up-regulation of Bax ($P < 0.05$) and down-regulation of Bcl-2 caused by exposure to CDDP ($P < 0.05$).

Generally, the pathophysiology of CDDP-induced renal injury can be classified into 4 types of toxicity: tubular toxicity, vascular damage, glomerular injury, and interstitial injury [35]. We examined CDDP-induced tubular damage by H&E staining (Fig. 4). The mice in the normal group exhibited normal

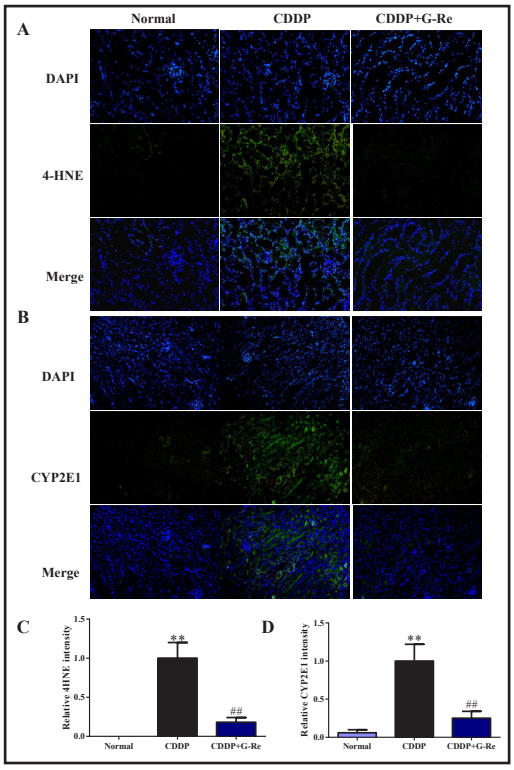

Fig. 2. Effects of G-Re on the expression levels of 4-HNE (A) and CYP2E1 (B). The protein expression were examined by immunofluorescence. Data represent the mean ± S.D. Significant differences were indicated by ** p<0.01 and * p<0.05 vs. normal group. $^{\#\#}$ p<0.01 and $^{\#}$ p<0.05 vs. CDDP group.

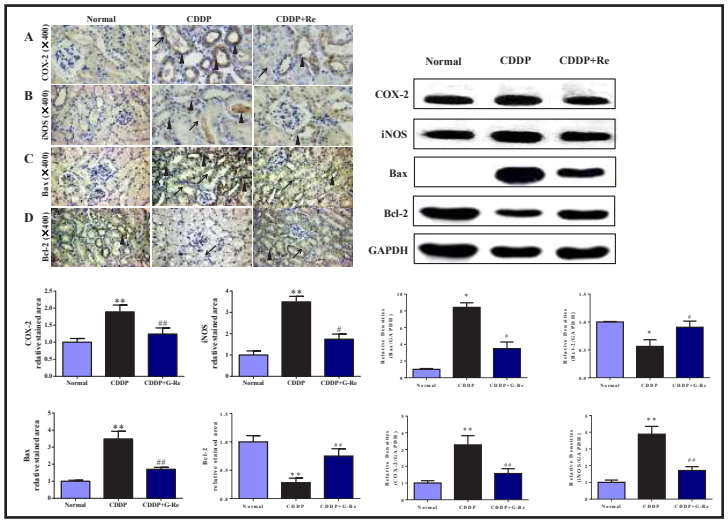

Fig. 3. Effects of G-Re on the expression levels of COX-2 (A), iNOS (B), Bax (C) and Bcl-2 (D), the protein expression was examined by immunohistochemistry and western blot analysis. Data represent the mean ± S.D. Significant differences were indicated by ** p<0.01 and * p<0.05 vs. normal group. $^{\#\#}$ p<0.01 and $^{\#}$ p<0.05 vs. CDDP group.

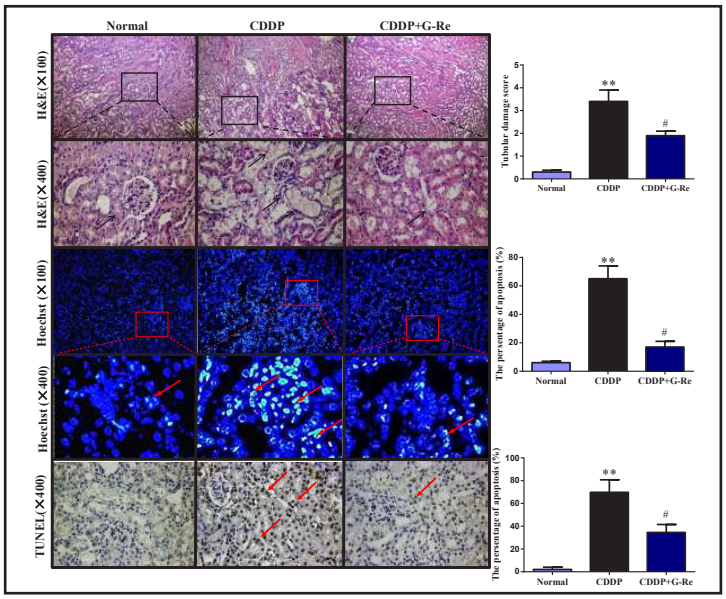

Fig. 4. Histological examination of morphological changes. Kidney tissues stained with H&E. The arrows show renal pathological changes including renal tubular epithelial cell necrosis, cell structure collapse and inflammatory infiltration. Tubular damage score was scored using the quantitative evaluation method. Effects of G-Re on CDDP-induced apoptosis stained with Hoechst 33258 and TUNEL and the graph showing the percentage of apoptosis within group. The arrows show renal tubular epithelial cell apoptosis. Data represent the mean ± S.D. Significant differences were indicated by ** $p<0.01$ and * $p<0.05$ vs. normal group. $^{\#\#}$ $p<0.01$ and $^{\#}$ $p<0.05$ vs. CDDP group.

renal tissue architecture, whereas the mice in the CDDP group demonstrated obvious tubular damage, including tubular degeneration, swelling, vacuole formation, and necrosis. However, these changes were markedly attenuated by pretreatment with G-Re for 10 days. As shown in Fig. 4, the tubular damage score of the CDDP group was significantly higher than that of the normal group ($P < 0.01$), while the score of the CDDP+G-Re group was significantly lower than that of the CDDP group ($P < 0.05$), suggesting that the administration of G-Re could be used to prevent CDDP-induced tubular injury.

Hoechst 33258 staining showed the absence of blue fluorescent nuclei in kidney tissue from the mice of the normal group, indicating no apoptosis. However, the mice in the CDDP group showed a large number of evenly distributed blue fluorescent nuclei, and the nuclear structure was broken with intense fluorescent signals (Fig. 4). In the CDDP+G-Re group, blue fluorescent nuclei were significantly decreased, indicating that G-Re significantly inhibited CDDP-induced kidney cell apoptosis ($P < 0.05$).

Furthermore, there were a large number of TUNEL-positive cells (Fig. 4) in the CDDP group ($P < 0.01$) and only a few TUNEL-positive cells ($P < 0.05$) in G-Re-treated mice, indicating that G-Re inhibits cell apoptosis in renal tissue after CDDP exposure.

These results from immunohistochemistry, western blotting, Hoechst 33258, and TUNEL analyses indicate that G-Re ameliorates the apoptosis of tubular cells, which is one of the main mechanisms for CDDP-induced nephrotoxicity.

Discussion

Nephrotoxicity is one of the most significant and dose-limiting side effects encountered in CDDP-based chemotherapy, placing a considerable health and economic burden on patients. Unfortunately, there are no effective nephroprotective agents to counter CDDP-induced renal toxicity. Although many renoprotective approaches are currently being evaluated, the protective effects of such agents are limited and not perfect, providing the rationale for developing combined strategies. In the present study, we found that G-Re, a major ginsenoside in *P. ginseng*, significantly suppresses CDDP-induced renal injury in mice by attenuating renal oxidative stress injury, the inflammatory response, and apoptosis.

CDDP is mainly excreted by kidney tissue, and its concentration is much higher in

the epithelium of the renal tubules than in blood, thereby causing serious renal toxicity [38]. Generally, injury to renal tubules with low doses of CDDP is reversible; however, the accumulation of CDDP under high doses can cause irreversible injury, kidney failure, or even death [39, 40]. Thus, improving chemotherapy quality, alleviating kidney injury, and increasing the antitumor efficiency of CDDP has become the focus of research.

In this study, we successfully established a CDDP-induced AKI model by a single injection of CDDP in mice according to previous reports. Our findings indicated that the body weight and organ indices of the kidney were significantly decreased after CDDP exposure, and G-Re reversed the decline of body weight and organ indices of the kidney when compared to the CDDP group. In addition, the serum levels of CRE and BUN (two main kidney indicators) were increased, reflecting renal damage in the CDDP-induced injury model. However, treatment with G-Re for 10 days lowered CRE and BUN levels in CDDP-exposed mice, indicating that G-Re improved renal function.

Although the mechanism of CDDP-induced nephrotoxicity is not well characterized [41], numerous studies have confirmed that oxidative stress injury is involved in the pathological process. First, the depletion of renal GSH may markedly aggravate lipid peroxidation and nephrotoxicity induced by CDDP [42]. In this study, treatment with CDDP alone markedly increased the formation of MDA in the kidney, whereas it decreased the levels of antioxidant parameters, such as GSH content and CAT activity. However, these changes were significantly reversed after G-Re treatment compared with the normal group. These results showed that the kidney protection effect of G-Re may be, in part, by its action against CDDP-induced oxidative stress injury. Lipid peroxidation is a hallmark of oxidative stress in tissues and cells [43]. Previous studies showed increased lipid peroxidation in the kidney after single CDDP exposure, which was markedly attenuated by natural antioxidants isolated from herbal medicines [44, 45]. In this study, G-Re treatment suppressed the CDDP-induced overproduction of the lipid peroxidation product 4-HNE, which was accompanied by the reduced expression of another oxidative stress mediator (CYP2E1).

Inflammatory cytokines play an important role in the pathogenesis of renal damage [1]. COX-2 is an inducible component of the prostaglandin synthesis cascade and is inducible in normal cells by various proinflammatory cytokines and mitogens [46]. Recent studies have demonstrated that COX-2 is highly overexpressed in response to inflammation in kidney tissue. Therefore, it may be intimately involved in prostaglandin-dependent renal inflammatory processes [47]. Thus, the development of COX-2 inhibitors serves as a paradigm for molecularly targeted renoprotective agents. The results from immunohistochemical staining showed that COX-2 protein expression was significantly increased after CDDP injection, but CDDP co-treatment with G-Re resulted in its almost complete inhibition. iNOS is typically synthesized in response to inflammatory stimuli at 3 days after CDDP injection, and we found that iNOS levels were increased significantly compared with the normal group. The administration of G-Re led to a significant reduction in iNOS expression. These results imply that G-Re may alleviate inflammation in the kidney.

CDDP-induced renal injury is caused by the accumulation of platinum in the tubules [48]. Tubular cell apoptosis is a characteristic feature of CDDP nephrotoxicity and results in the loss of renal endothelial cells and renal dysfunction [35]. In order to measure the extent of apoptosis in renal tissue, the levels of an apoptotic protein (Bax) and an anti-apoptotic protein (Bcl-2) were measured in all experimental groups. Immunohistochemical staining showed CDDP induced apoptosis in tubular cells, as evidenced by the increased expression of Bax and decreased expression of Bcl-2. G-Re suppressed apoptosis as it increased Bcl-2 expression and decreased Bax expression. The decreased level of Bax protein and increased level of Bcl-2 protein in western blot analysis confirmed the findings from the immunohistochemical analysis.

There were an increased number of Hoechst-positive cells in the CDDP group, but G-Re treatment reduced their number and attenuated apoptosis in renal tubular cells. Furthermore, there were an increased number of TUNEL-positive cells in the CDDP group. G-Re also reduced the number of TUNEL-positive cells. Hence, we think that G-Re mitigated

CDDP-induced nephrotoxicity, in part, by attenuating apoptosis in CDDP-injected mice.

In addition, histological analysis showed obvious tissue damage in the kidney, including vacuolization, severe necrosis, degenerative changes in the epithelial lining of renal tubules, and desquamation of degenerated cells present in the lumen of the tubules, which were markedly attenuated by pretreatment with G-Re.

Conclusion

The renal protection effect of G-Re was evaluated using a mouse model of CDDP-induced nephrotoxicity for the first time. The findings of this study clearly indicated that pretreatment with G-Re for 10 days reduces CDDP-induced oxidative stress, inflammation, and apoptosis. Furthermore, G-Re did not show any influence on body weight or the function of organs in normal mice, suggesting that G-Re might be considered as a novel renoprotective agent against CDDP-induced nephrotoxicity in the near future.

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Disclosure Statement

The authors declare they have no conflict of interests.

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