Endoplasmic Reticulum Stress-Activated PERK-eIF2α-ATF4 Signaling Pathway is Involved in the Ameliorative Effects of Ginseng Polysaccharides against Cisplatin-Induced Nephrotoxicity in Mice

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ABSTRACT: Although previous studies have reported that saponins (ginsenosides, the major active and most representative ingredients in Panax ginseng C.A. Meyer) exerted a good ameliorative effect on cisplatin (CP)-induced acute kidney injury in animal models, little attention has been paid to a large number of polysaccharides isolated and purified from ginseng. This work aimed to investigate the protective effect and the possible molecular mechanism of ginseng polysaccharide (WGP) on CP-induced kidney toxicology in mice. The results from biomarker analysis including serum creatinine (CRE) and blood urea nitrogen (BUN) confirmed the protective effect of WGP at 200 and 400 mg/kg on CP-induced renal-toxicology. We found that WGP reduces the apoptosis of kidney cells by inhibiting endoplasmic reticulum (ER) stress caused by CP, which is manifested by increased phosphorylation of PERK. In addition, the apoptosis-associated with caspase 3 activation in renal cells induced by CP was inhibited after administration of WGP, and the phosphorylation levels of PI3K and AKT were also reduced significantly. We also demonstrated that after exposure to CP, the unfolded protein response signaling pathway PERK-eIF2α-ATF4 axis was significantly activated, manifested by increased phosphorylation of eIF2α and increased expression of ATF4 and CHOP. Interestingly, the WGP administration improves this situation. Furthermore, the supplement of WGP inhibited the overexpression of nuclear factor-kappa B p65 (NF-κB p65) and tumor necrosis factor-α (TNF-α) caused by CP exposure. In short, for the first time, our findings indicated that WGP could effectively prevent CP-induced ER stress, inflammation, and apoptosis in renal cells, in part, by regulating the PI3K/AKT and PERK-eIF2α-ATF4 signaling pathways.

1. INTRODUCTION

Acute kidney injury (AKI) is reported to be a heterogeneous clinical syndrome, with nephrotoxic drugs accounting for 15% of AKI cases. The pathophysiology of AKI includes inflammation, immune disorders, oxidative damage, and microcirculation. Cisplatin (cis-dichlorodiammine platinum, CP) is a commonly used antitumor drug. However, nephrotoxicity and ototoxicity are common adverse reactions. In recent years, great headway has been made in the mechanism of CP-induced AKI, but effective treatments are still lacking to relieve this renal toxicity. Therefore, there is an urgent need to develop drugs to prevent CP-induced AKI. Endoplasmic reticulum (ER) and mitochondria are two important organelles in living organisms. Mitochondria are involved in cell differentiation, cell signaling, apoptosis, and furnishing energy. The ER is a place for cell signal transduction, protein synthesis, folding, and secretion. It plays an irreplaceable role in cell physiological regulation and pathological regulation. ER stress refers to the accumulation of erroneous folded and unfolded proteins in the ER caused by multiple causes (such as oxidative stress, inflammation, hypoxia, and calcium metabolism disorders), resulting in cell balance disorders. Continuous ER stress activates the apoptotic pathway, which eventually causes cell apoptosis. According to the literature, some unfolded proteins bind to sensory proteins on the ER membrane, which activates three ER stress signal transduction pathways: protein kinase R-like ERK kinase (PERK), eukaryotic initiation factor-2 α (eIF2α), activating transcription factor-4 (ATF4), IRE1α-X-box binding protein 1 (XBP1), and activating transcription factor 6 (ATF6). Notably, recent studies have shown that PERK can regulate the apoptosis of human hepatocellular carcinoma cell
lines HepG2, Bel-7402, and SMMC-7721, and can also modulate the apoptosis of damaged IEC-6 in necrotizing enterocolitis models. However, the role of PERK activation in CP-induced nephrotoxicity is still unknown. Based on previous studies, the present study will provide further evidence to elucidate the possible interaction mechanism of action between activated PERK pathways and apoptosis in CP-exposed nephrotoxicity.

Numerous reports showed CP could regulate the PI3K/AKT signaling pathway to induce apoptosis in kidney tissues. The PI3K/AKT pathway plays an important role in kidney protection. After knocking out the PI3K gene in mice, treatment with CP could increase the apoptosis number of mouse renal tubular epithelial cells. In the present study, we identified the protective effect of WGP on CP-induced renal injury, indicating that regulating the PI3K/AKT pathway is a potential strategy for AKI.

Generally, saponins (ginsenosides) are considered to be the major functional and indicative components of *Panax ginseng* C.A. Meyer. Growing scientists have confirmed ginsenosides can reproduce almost all of the biological activities of ginseng itself as a whole. However, it is undeniable that the pharmacological activity of ginsenosides is not all. Among the active ingredients of *P. ginseng*, ginseng polysaccharide (WGP) accounts for about 40–50%, which has been proven to exert many important physiological activities. In addition to...
ginsenosides, the activities of WGP including improving the body’s immunity,\textsuperscript{11} antitumor, antidepressant,\textsuperscript{12} and anti-fatigue\textsuperscript{13} properties have been reported in the past decades. Extensive studies have focused on the role of the main active ingredients of ginsenosides, such as ginsenoside Rg5,\textsuperscript{14} ginsenoside Rk3,\textsuperscript{15} and ginsenoside Rh2,\textsuperscript{16} which have good protective effects on cisplatin-induced kidney injury in animal models. Although previous studies have reported that saponins (ginsenosides, the major active and most representative ingredients in \textit{P. ginseng} C.A. Meyer) exerted a good ameliorative effect on cisplatin (CP)-induced acute kidney injury in animal models, little attention has been paid to a large number of polysaccharides isolated and purified from ginseng. Moreover, the small molecular substances, maltol and arginine,\textsuperscript{17} produced by ginseng have a good protective effect on AKI caused by CP, but the protective effect of WGP on AKI induced by CP has not been reported. Therefore, based on previous research, we assume that WGP has a protective effect on AKI. This experiment verifies this conjecture by testing the protective effect of WGP on AKI.

At present, there is little research on the impact of CP on ER stress, and the relationship between ER stress and WGP is still

Figure 2. WGP alleviated renal inflammation response in mice. Expression of TNF-\(\alpha\) (a) and NF-\(\kappa\)B p65 (b) in kidneys of different groups by IF staining. Quantification of the fluorescence intensity of TNF-\(\alpha\) (c) and NF-\(\kappa\)B p65 (d) (red fluorescence, 200\(\times\)). DAPI was applied to nuclear counterstaining. All data are expressed as mean \(\pm\) SD (\(n=8\)). Compared with the normal control group (N) **\(p<0.01\) and ***\(p<0.001\); compared with the cisplatin group (CP) **\(p<0.01\).
unknown. Although CP was reported to play a role in ER stress progress, the downstream signaling mechanisms were not fully understood. Overall, the protective effects of WGP on AKI are poorly understood. From our current research, we found that WGP could have the potential to protect kidney toxicity in mouse models. Interestingly, we demonstrated the renal protection of WGP in CP-treated mice for the first time.

2. RESULTS

2.1. WGP Reduced CP-Induced Renal Insufficiency and Histopathological Changes in Mice. The protective effect of WGP on CP-induced AKI was evaluated by observing the histopathological changes in the kidneys of mice. As depicted in Figure 1d,e, optical microscopy of the kidney tissue of normal mice showed that the glomerular structure was intact and renal tubule interstitium was without cell necrosis and inflammatory infiltration. In the CP-treated group, glomerular cell nucleus outflowed, renal tubular epithelial cells showed necrosis, renal cortical vacuole worsened, and inflammatory infiltration increased. The number of apoptotic cells and inflammatory infiltration was significantly reduced in the WGP pretreatment group. Tubule necrosis scores were consistent with the results obtained from hematoxylin and eosin (H&E) staining, suggesting that WGP reversed the pathological changes of renal tissue induced by CP.

In addition, serum BUN and CRE as basic indicators for evaluating renal function were evaluated in this work. Compared with the normal control group, the serum BUN and CRE in mice injected with CP only increased significantly, which indicated that CP caused severe renal damage ($p < 0.001$). Nevertheless, as depicted in Figure 1b,c, compared with the CP-treated group, these two renal function indicators such as BUN and CRE in the WGP pretreatment group were significantly decreased significantly ($p < 0.05$, $p < 0.01$, and $p < 0.001$), indicating that pretreatment of WGP can significantly suppress CP-induced renal damage.

2.2. WGP Alleviated CP-Induced Renal Inflammation Response in Mice. To analyze the effect of WGP on the inflammatory response in CP-induced renal toxicity in mice, immunofluorescence staining was used to detect the inflammation-related proteins TNF-$\alpha$ and NF-$\kappa$B p65. Compared with the normal control group, the expression of TNF-$\alpha$ and NF-$\kappa$B p65 was significantly increased after exposure to the CP injection. As shown in Figure 2a,b, pretreatment with WGP at 200 and 400 mg/kg inhibited the overexpression of TNF-$\alpha$ and NF-$\kappa$B in mice induced by CP.
WGP alleviated CP-induced ER stress in mice. The expression of key ER stress proteins PERK, eIF2α, ATF4, CHOP, and cl-caspase 12 was analyzed by western blotting with specific primary antibodies (a–c). β-Actin levels serve as load control. All data are expressed as mean ± SD (n = 3). Compared with the normal control group (N), *p < 0.05 and **p < 0.01; compared with the cisplatin group (CP) #p < 0.05 and ##p < 0.01.

2.3. WGP Reduced CP-Induced Apoptosis Via the PI3K/AKT Signaling Pathway in Mice. Through western blotting and Hoechst 33258 staining to explore the mechanism of CP-induced renal tissue apoptosis, we analyzed apoptosis-related proteins, such as Bax and Bcl-2. At the same time, the number of apoptotic cells in the kidney slices was detected. The images of kidney tissues were observed under a fluorescent microscope, the nuclei of renal cells in CP group were blue and the nuclei of apoptotic cells were dense and bright blue. As shown in Figure 3a,b, no bright blue kidney cells were observed in the normal group of mice. In fact, the bright blue kidney cells in the CP group increased significantly, which was 3.9 times that in the normal control group (p < 0.001). In contrast, the WGP pretreatment group significantly reduced renal cell apoptosis (p < 0.001).

The expressions of apoptotic upstream related proteins such as PI3K/AKT and apoptotic downstream proteins Bax and Bcl-2 in renal samples were detected by western blotting analysis. Compared with the normal control group, the expression levels of p-PI3K, p-AKT, and Bcl-2 in the kidneys of the CP group were significantly reduced (p < 0.01). Compared with the normal control group, the level of Bax in the kidneys of the CP group was significantly increased (p < 0.01). However, WGP pretreatment significantly reversed these changes in the kidneys (p < 0.05 or p < 0.01) (Figure 3c,d), indicating clearly that WGP effectively prevents cisplatin-induced apoptosis of renal cells.

2.4. WGP Inhibited CP-Induced ER Stress in Mice. Generally, ER stress as a way of apoptosis is involved in regulating cell growth and death. The purpose of detecting ER stress-related proteins in kidney tissues was to evaluate the regulatory effect of WGP on ER stress. The expressions of PERK, eIF2α, caspase 12, and chop were detected by western blotting analysis. As indicated in Figure 4a–c, compared with the normal control group, the expression levels of p-PERK, eIF2α, ATF4, and CHOP in the kidneys of the CP group were significantly increased (p < 0.05 or p < 0.01). Compared with the normal control group, WGP pretreatment can significantly improve the changes of the kidneys (p < 0.05 or p < 0.01). It was worth noting that WGP effectively inhibits ER stress through the PERK-eIF2α-ATF4 pathway, indicating that WGP has a protective effect on ER stress caused by CP.

3. DISCUSSION

CP is a commonly used antitumor drug for patients receiving chemotherapy, which accumulates in the kidney after entering the systemic circulation and produces nephrotoxicity. Clinical application of CP for the long-term is often limited by the occurrence of AKI or renal failure. According to reports, the mechanism of cisplatin causing kidney damage is related to apoptosis, autophagy, oxidative stress, and inflammation. Ginseng per se and its ginsenosides have been revealed to provide excellent renal protection. There has been no literature to prove that WGP protects CP-induced AKI and its molecular mechanism. In this study, the potential protective effect and possible mechanisms of action of WGP on CP-induced nephrotoxicity were investigated for the first time.

The main excretory organ of CP is the kidneys and is reabsorbed into urine after glomerular filtration. However, studies have shown that only a small portion of CP is excreted from the urine a few days after injection, and most of CP is irreversibly bound to protein and accumulated in the kidneys. CP-induced renal injury is prone to occur in renal blood vessels, glomeruli, and tubules, with tubular damage being the most common. In this study, administration of WGP for 10 consecutive days could improve CP-induced renal histopathological changes and inhibit the increases of serum CRE and BUN in mice. In addition, we found that protective potentials of WGP in kidney tissues were shown obviously in histopathological examinations. Mice administered with CP alone showed histopathological changes in kidney tissue. However, WGP added to CP can alleviate the aforementioned changes.
In the pathogenesis of CP-induced AKI, inflammatory response plays an important role. CP can activate NF-κB p65, increase the expression of inflammatory factors such as TNF-α, induce renal tissue damage, and even induce acute renal failure. Inhibition of NF-κB activation and reduction of the inflammatory factor TNF-α levels can significantly reduce the inflammatory response in renal tissue, suggesting that anti-inflammatory is the key to reducing CP nephrotoxicity. In addition, the inflammatory response increases the oxidative stress in cells and induces ER stress by increasing the expression level of ER sensors (such as PERK). The expressions of NF-κB and TNF-α in kidney tissue of mice were significantly reduced after WGP administration for 10 days, suggesting that WGP exerted an anti-inflammatory effect in CP-induced AKI.

The PI3K/AKT pathway is a classic antiapoptotic pathway that can transmit extracellular activation signals to the intracellular area and participate in cell metabolism, growth, survival, and apoptosis. AKT is one of the important downstream factors of the PI3K promoter. Activated PI3K can activate AKT through phosphorylation of the AKT regulatory region Ser473 and catalytic region Thr308, and then activate its important downstream molecular targets. CP upregulates the expression of proapoptotic protein Bax, opens mitochondrial permeability transition pores, releases proapoptotic factors such as cytochrome c into the cytoplasm, and cytochrome c can activate caspase 9 and initiate the caspase level. This reaction could induce caspase-dependent apoptosis. Recent studies have confirmed that PI3K/AKT activation may be associated with kidney injury and apoptosis. Current results indicate that CP exposure results in the inhibition of protein expression levels of PI3K/AKT in vivo, which is consistent with the recent literature. However, we found here that administration of WGP enhances the PI3K/AKT phosphorylation cascade that is suppressed by CP, suggesting that increased expression of the PI3K/AKT pathway is possibly related to renal protection. The regulatory effect of WGP on the PI3K/AKT signaling pathway is unclear, and polysaccharides extracted from other medicinal materials can regulate apoptosis through the PI3K/AKT signaling pathway. Therefore, we speculated that WGP may regulate necrotic renal cell apoptosis through the PI3K/AKT signaling pathway. Furthermore, WGP can reduce the expression of proapoptotic protein Bax, increase the expression of inhibitory apoptosis protein Bcl-2, and inhibit caspase family proteins from being cleaved. The results indicate that WGP may protect renal cells by partly regulating the PI3K/AKT signaling pathway.

The ER is an important organelle related to protein synthesis, modification, and processing in cells. As the bearer of cell life activities, protein's correct synthesis and folding is the guarantee of cell function. When the internal environment is unstable, unfolded proteins or misfolded proteins accumulate in the ER, disrupting their normal organelle functions and triggering ER stress. ER stress is a new way of apoptosis, which can regulate the toxic effect of drugs in cells. Recent studies have shown that CP can induce ER stress, upregulate multiple ER stress markers, activate caspase 3, and ultimately promote cell damage and apoptosis. CP-induced apoptosis of renal tubular cells can be regulated by an ER pathway. Caspase 12 on the surface of the ER is a pivotal promoter of ER stress. CP can activate caspase 12 in the epithelial cell line of the proximal tubule of pig kidney and induce apoptosis. When a large number of misfolded or unfolded proteins accumulate in the ER, PERK dissociates from GRP78/BIP and activates the unfolded protein response signal cascade. After dissociation of PERK and GRP78, it autophosphorylates and oligomerizes, activates eIF2α, reduces protein synthesis, and reduces the amount of protein entering the ER. Subsequently, eIF2α phosphorylation can increase the expression of ATF4. ATF4 can activate the expression of CHOP. During ER stress, the expression of CHOP increases significantly and is mainly distributed in the nucleus. As a transcription factor, CHOP is involved in regulating the expression of the downstream target gene Bcl-2 protein, affecting cell growth and death. Under the action of ER stress, the activation of caspase 12 is transferred to the cytoplasm and the downstream signaling protein caspase 9 is shear-activated, which, in turn, activates the core protein caspase 3 and executes apoptosis, finally completing the caspase 12-mediated apoptotic response. The protective effect of WGP on renal cells, on the one hand, is to reduce the apoptosis of renal cells and, on the other hand, to reduce the amount of unfolded and misfolded proteins retained in the ER stress. It was informational in the literature that ginsenosides could protect multiple organs from injury by regulating ER stress. For example, ginsenoside Rh2 (a minor protopanaxadiol saponin) promotes apoptosis of lung cancer cells by inhibiting ER stress. Ginsenoside Rg3 induces ER stress to promote gallbladder apoptosis of cancer cells and ginsenoside Rg1 prevents doxorubicin-induced cardiotoxicity by inhibiting ER stress. However, the effect of polysaccharides from ginseng on ER stress is still less studied. To further explore the effect of WGP on ER stress in CP-induced renal toxicity in mice, this study examined ER stress-related proteins and apoptotic proteins. The results showed that CP promoted the accumulation of unfolded proteins by ER stress, and the expression of p-PERK, p-eIF2α, eIF2α, ATF4, and CHOP decreased significantly compared with the CP group after WGP administration. It can be proved that the protective effect of WGP on CP-induced AKI in mice is related to the inhibition of ER stress.

Current research shows that WGP has a potential therapeutic effect on CP-induced AKI by inhibiting apoptosis, inflammatory response, and ER stress. The mechanisms of WGP were involved in the activation of PI3K/AKT and suppression of ER stress. As a consequence, WGP is capable of preventing CP-induced kidney injury in mice. CP triggers ER stress by inducing protein misfolding or nonfolding, thereby activating the PERK-eIF2α-ATF4 signaling pathway. A new treatment option for AKI may be related to the mechanisms we have discovered here. For the first time, our research team has shown that the protective effect of WGP on CP-induced AKI is related to the inhibition of ER stress, and studies involving the ER stress-activated PERK-eIF2α-ATF4 signaling pathway may provide the basis for the development of AKI therapy.

4. MATERIALS AND METHODS

4.1. Preparation of WGP. Ginseng polysaccharide (WGP) with a purity of 99% was isolated and purified from white ginseng purchased from ginseng market Fusong County (Baishan, Jilin Province, China). The specific separation and purification processes are as described previously in our lab. In brief, ginseng roots were extracted with distilled water, precipitated with ethanol, and then deproteinized in Sevag reagent. After lyophilization, WGP was obtained.
4.2. Chemicals and Reagents. CP was purchased from Sigma-Aldrich (St. Louis, MO) with a purity of more than 99%. The hematoxylin and eosin (H&E) dye kit and some commercial kits including urea nitrogen (BUN) and creatinine (CRE) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu Province, China). Primary antibodies against PI3K, p-PI3K, Akt, p-Akt, p-PERK, PERK, eIF2α, p-eIF2α, caspase 12, caspase 9, cl-caspase 9, caspase 3, cl-caspase 3, and CHOP antibodies were purchased from Cell Signaling Technology (Danvers, MA). Primary antibody against ATF4 was provided by Bioss Biotechnology Co. Ltd. (Beijing, China). All of the other reagents were obtained from Beijing Chemical Works (Beijing, China), all of which were analytically pure.

4.3. Animals and Experimental Design. Thirty-two male ICR mice (body weight 18–22 g, 8 weeks old) were purchased from Changchun YISI Experimental Animal. The quality certificate number for Experimental Animal is No. SCXK (JL) 2016-0003 (Changchun, China). The feeding environment remained unchanged with a temperature of 25 ± 2 °C, humidity of 60.0 ± 10.0%, constant illumination, maintenance at 12 h light, 12 h dark cycle, and a free supply of food and water. All experimental animals’ processing projects were strictly executed according to the Guide for the Care and Use of Laboratory Animals (2016).

Mice were divided into four groups after 1 week of adaptive rearing, normal control group (N, group 1), CP model group (CP, group 2), and two WGP + CP groups (groups 3 and 4). Based on preliminary experiments and existing studies, the dose of WGP and the dose of CP-induced AKI were determined.23 WGP was dissolved with 0.9% physiological saline and administered intragastrically to mice for 10 consecutive days. In the WGP + CP groups, the WGP groups were fed 200 and 400 mg/kg. The other two groups were given 0.9% saline. On the 7th day of intragastric administration (mice rearing on the 14th day), the mice in groups 2, 3, and 4 were intraperitoneally injected with CP (20 mg/kg, diluted with 0.9% warm saline) for 1 h after the final WGP treatment. The injection site in the abdominal cavity of mice. In clinical applications, it has been found that CP causes toxic reactions such as nephrotoxicity, liver toxicity, cardiotoxicity, ototoxicity, intestinal injury, and spinal cord inhibition. At present, there is no clinically used drug to cooperate with CP to alleviate its toxicity. Therefore, a group of positive drugs has not been established in current work. The experiment was stopped 72 h after CP injection. The serum samples were collected from the eyeball. Then, after anesthesia, mice were sacrificed by executing cervical dislocation, and after dissection, kidneys of mice were collected for subsequent experiments. The serum samples were separated by centrifugation (3500 rpm for 10 min) and collected for subsequent measurement.

4.4. Estimation of CRE and BUN in Serum. As described previously,41 serum samples were collected for evaluating the levels of BUN and CRE according to the protocols of the biochemical assay kits from the manufacturer (Jiancheng, Nanjing, China).

4.5. H&E and Hoechst 33258 Staining. Prior to paraffin embedding, kidney tissues were fixed with 10% formaldehyde for 48 h. After that, the tissues were removed and cut into 5 μm thick sections. Paraffin sections were dewaxed in xylene for 20 min, absolute ethanol for 10 min, 95% ethanol for 6 min, 70% ethanol for 3 min, 30% ethanol for 3 min, and distilled water for 5 min. The renal tubular injury in the renal cortex was scored according to the previous literature.42 As for Hoechst 33258 staining, paraffin sections of kidney tissues with a thickness of 5 μm were dewaxed and transparent to water, washed with phosphate-buffered saline (PBS) for 6 min, then added with 0.5 mL of Hoechst 33258 for 5 min, and finally washed with PBS three times. The blue nucleus was detected with a fluorescence microscope (Leica TCS SP8, Germany).

4.6. Immunofluorescence (IF) Staining. Paraffin sections were dewaxed with xylene for 10 min, twice dehydrated with gradient ethanol, and bovine serum albumin (BSA) was blocked at ambient temperature for 20 min. After all, slices were hatched with primary antibodies TNF-α (1:200) and NF-κB (1:100) at 4 °C 12 h, then were incubated by DyLight 488-labeled or CY3-labeled secondary antibodies (Boster Biological Technology, Wuhan, China).

4.7. Western Blotting Analysis. Western blotting analysis was executed as mentioned earlier.43 First, the kidney tissues were ruptured using radioimmunoprecipitation assay (RIPA) buffer. Second, a bicinchoninic acid (BCA) protein assay kit was used to detect protein concentrations. These proteins were separated by 15 or 10% sodium dodecyl sulfate (SDS) polyacrylamide gel and dispersed to a poly(vinylidene fluoride) (PVDF) membrane. These membranes were blocked with 5% nonfat milk for 2 h at room temperature and incubated with primary antibodies including PI3K (1:1000), p-PI3K (1:1000), Akt (1:1000), p-Akt (1:1000), p-PERK (1:1000), PERK (1:1000), eIF2α (1:1000), p-eIF2α (1:1000), cleaved caspase 9 (1:1000), cleaved caspase 3 (1:1000), CHOP (1:1000), ATF4 (1:500), caspase 12 (1:1000), caspase 9 (1:1000), cl-caspase 9 (1:1000), cl-caspase 3 (1:1000), caspase 3 (1:2000), and β-actin (1:5000) at 4 °C overnight. Next, these membranes were washed three times with TBS-T for 5 min each time and then incubated with secondary antibodies at room temperature for 2 h. Finally, the membranes were visualized with the emitter-coupled logic (ECL) plus western blot detection system (Bio-Rad Laboratories, Hercules, CA).

4.8. Statistical Analysis. All data are expressed as mean ± standard deviation (mean ± SD) and analyzed by SPSS 19.0 (SPSS, Chicago, IL). The differences between the experimental groups were analyzed by one-way variance (ANOVA). A difference of p < 0.05 or p < 0.01 was statistically significant.

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X.-m.W. drafted the manuscript and performed the experiments under the supervision of W.L. S.J. planned the project. S.-s.L. worked on the extraction and purification of ginseng polysaccharides and interpretation of results. All authors carefully read and approved the final manuscript.

Notes
The authors declare no competing financial interest.

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■ ABBREVIATIONS

CP, cisplatin; ER, endoplasmic reticulum; CRE, creatinine; BUN, urea nitrogen; TNF-α, tumor necrosis factor-α; AKI, acute kidney injury; eIF2α, eukaryotic initiation factor-2 α; XBP1, IRE1α-X-box binding protein 1; ER, endoplasmic reticulum; P13K, phosphatidylinositol-3-kinase; NF-κB, nuclear factor-kappa B; WGP, ginseng polysaccharide; ATF4, activating transcription factor 4; PERK, protein kinase R-like ERK kinase; ATF6, activating transcription factor 6

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