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Antioxidative Effects of *Lycium chinense* Miller on Cisplatin-induced Nephrotoxicity in Rats

Yu-sun Jung[1], Chan-hum Park[2], Hyeon-cheol Shin[1]

[1]Dept. of Internal Medicine of Korean Medicine, College of Korean Medicine, Dae-gu Haany University
[2]Dept. of Herbal Medicine, College of Korean Medicine, Dae-gu Haany University

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ABSTRACT

Objectives : Cisplatin is a widely used cancer therapy drug. However, nephrotoxicity resulting in increased oxidative stress is a major side effect of cisplatin chemotherapy, thereby limiting its chemotherapeutic use. *Lycium chinense* Miller (LCM) has been used as a traditional herbal medicine in various febrile and inflammatory diseases such as night sweat, cough, nosebleed, bronchitis, pulmonary tuberculosis, etc. In this study we investigated the protective and antioxidative potential of LCM against cisplatin-induced nephrotoxicity in rats.

Methods : Twenty-four 8-week-old male Wistar rats were divided into four groups: normal untreated: cisplatin treatment only: LCM 10 mg/kg plus cisplatin treatment: and LCM 30 mg/kg plus cisplatin treatment. Twenty-four hours after the last cisplatin injection, all the rats were sacrificed, and serological changes were evaluated. The levels of NF-κB activity and NOX-4, $p47^{phox}$, $p22^{phox}$, COX-2, iNOS, SOD, catalase expressions were analyzed in Western blot analysis.

Results : Cisplatin injection caused an increase in the BUN level, which is a reliable indicator of renal toxicity. The levels of BUN, renal ROS, and renal TBARS were significantly reduced in the LCM groups compared with the cisplatin-only groups. The levels of $p47^{phox}$ and $p22^{phox}$, which are NADPH oxidase subunits, were increased in the cisplatin-only groups, whereas they were decreased in the LCM groups. The levels of renal NF-κB activity and COX-2, iNOS expressions were increased significantly in the cisplatin-only groups compared with the normal groups, whereas they were decreased in the LCM groups. Compared with the cisplatin-only groups, renal GSH and GSH/GSSG increased in the LCM groups. Also, the administration of LCM increased levels of SOD and catalase as compared with the cisplatin-only groups.

Conclusions : These results suggest that LCM protects cisplatin-induced nephrotoxicity via a mechanism that may involves the inhibition of oxidative stress by the activation of antioxidants.

Key words : *Lycium chinense* Miller, cisplatin, nephrotoxicity, NF-κB, GSH

· Correspondence to: Hyeon-cheol Shin 411, Seacheonyeon-daero, Nam-gu, Pohang-si, Gyeongsangbuk-do, Korea
Dept. of Internal Medicine of Korean Medicine, College of Korean Medicine, Dae-gu Haany University
TEL: 054-281-0055 FAX: 054-281-7464
E-mail: ungaeshin@naver.com
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Ⅰ. Introduction

Lycium chinense Miller (LCM), the root bark of solanaceous *Lycium chinense*, has been used as a traditional herbal medicine for various febrile and inflammatory diseases[1-5]. Previous studies[6,7] demonstrated

inhibitory effects of LCM on the generation of reactive oxygen species (ROS) and inflammatory processes by suppression of pro-inflammatory nuclear factor-κB (NF-κB), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) in H_2O_2-treated renal epithelial cells of mouse and protective effects of LCM on oxidative stress in rats skin. However, the effect of LCM on the antioxidants activity has not been studied.

Cisplatin is one of the most widely used and most effective cytotoxic agents in the treatment of epithelial malignancies such as lung, head-and-neck, ovarian, bladder, and testicular cancer[8]. However, nephrotoxicity, with a significant decline in renal function, occurs in about 25-35% of patients after a single dose of cisplatin. This major side effect limits the use of cisplatin[9]. It has been suggested that oxidative stress plays a critical role in the pathogenesis of cisplatin-induced nephrotoxicity[10]. Earlier experimental findings also suggested that free radicals and ROS are involved in cisplatin-induced renal damage because of the depletion of glutathione (GSH) and the reduction in the antioxidant enzyme activity in the kidney[11]. The decreased concentration of GSH increases the sensitivity of the organ to oxidative and chemical injury. Thus reduced renal GSH can markedly increase lipid peroxidation and the toxicity of cisplatin[12].

Reduced glutathione (GSH), a ubiquitous tripeptide thiol, is a vital intracellular and extracellular protective antioxidant. Also, GSH is considered one of the most important scavengers of ROS, and the GSH ratio to oxidized glutathione (GSSG) is used as a marker of oxidative stress[13].

The purpose of this study was to investigate the protective effect of LCM on renal function and to examine the mechanism of its protection in a well-established rat model of cisplatin-induced nephrotoxicity, especially focusing on antioxidants activity.

Ⅱ. Materials and methods

1. Animals and treatment

8-week-old Wistar rats were obtained from Samtako (Osan, Korea) and fed NIH#3 diet (Samtako). All the rats were allowed food and water *ad libitum*. The rats were housed individually in polycarbonate cages with wood chip bedding, maintained in controlled animal room (23±1 ℃, 12 h light/dark cycle). They were divided into four groups (n=6/group): two LCM groups that received LCM (10 or 30 mg/kg body weight/day) orally for two days and a vehicle-treated and normal group, which were given normal saline orally. After two days of the administration period, two LCM groups and vehicle-treated group were received two cisplatin injections (7.5 mg/kg/day i.p.). All the rats were sacrificed 24 h after last injection of cisplatin. Blood samples were collected by cardiac puncture from anesthetized rats, and the serum was immediately separated from blood samples by centrifugation. Subsequently, the rats were perfused with ice-cold saline after cardiac puncture, and the kidney was harvested, snap- frozen in liquid nitrogen, and stored at -80 ℃ until analyses.

2. Preparation of LCM

LCM 50 g was purchased from Human Herb (Daegu, Korea) and extracted with boiled water (600 ml) for 3 h. After filtration (Whatman No. 2, Maidstone, England), the solution was evaporated (BUCHI, Switzerland) under reduced pressure and

then freeze dried.

3. Materials

All chemical reagents were obtained from Sigma -Aldrich (St. Louis, MO, USA). Polyvinylidene difluoride (PVDF) membranes were obtained from Millipore Corporation (Bedford, MA, USA). Western blotting antibodies to NOX-4, $p47^{phox}$, $p22^{phox}$, NF-κB, COX-2, iNOS, superoxide dismutase (SOD), catalase, histone, and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). 2', 7'-Dichlorodihydrofluorescein diacetate (DCFDA) and dihydrorhodamine (DHR) 123 were obtained from Molecular Probe, Inc. (Eugene, OR, USA), and cis-diammineplatinum(II) dichloride (cisplatin) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All the serum assays were measured using a commercial kit (Shinyang Chemical, Busan, Korea).

4. Assessment of renal function

The blood urea nitrogen (BUN) level was measured with assay kits, following the manufacturer's protocols (Shinyang Chemical, Busan, Korea). Briefly, a serum sample (5 μl) was loaded, and the reagent (200 μl) was added. This mixture was incubated at 37 ℃ for 5 min. The optical density was determined at 540 nm.

5. Measurement of ROS and TBARS

A fluorescence assay was used to determine levels of ROS, and DCFDA was oxidized to the highly fluorescent 2', 7'-dichlorofluorescin (DCF) in the presence of ROS, including lipid peroxides. Utilizing menadione as the source of superoxide, a working solution of 25 mM DCFDA diluted from stock solution was placed on ice in the dark immediately prior to the study. Changes in fluorescence in density were measured for 30 min on a fluorescence plate reader (TECAN, Salzburg, Austria) with the emission wavelengths set at 485 and 535 nm.

For quantification of the end products of lipid peroxidation, specifically MDA, we performed a thiobarbituric acid reactive substances (TBARS) assay in mouse kidney homogenates. Tissue samples (100 μl) were mixed in e-tubes containing 40 μl of 8.1% SDS solution, 300 μl of 20% acetic acid and 200 μl of 1.2% thiobarbiturate solution. The tubes were heated in boiling water for 1 h, cooled to room temperature, and 300 μl of butanol were added. The mixtures were centrifuged at 1,500 rpm for 10 min. The absorbance of the butanol layer was measured at 532 nm. Using the MDA standard, TBARS was calculated as μM/mg protein.

6. Measurement of GSH and GSSG

To measure the GSH level, 1 mM of EDTA-50 mM phosphate solution was added to the supernatant of trichloric acid (TCA)-treated homogenates, followed by *o*-phthaldehyde and then incubated for 25 min at room temperature. To measure the GSSG level, *N*-ethylmaleimide was added to the supernatant of the TCA-treated homogenates. After 30 min at room temperature, 0.5 N NaOH and *o*-phthaldehyde were added to the sample and then incubated for 25 min. The levels of both GSH and GSSG were measured with the excitation and emission set at 360 and 460 nm.

7. Preparation of cytosol extract

The renal tissue (100 mg) was homogenized with 1ml homogenate buffer (10 mM HEPES (pH 7.8), 10 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.1 mM EDTA, and 0.1 mM PMSF) containing 100 mM Tris-HCl (pH 7.4), 20 mM β-glycerophosphate, 20 mM NaF, 2 M Na_3VO_4, 1 mM EDTA, 0.5

mM PMSF, 1 μM pepstatin, and 80 mg/L trypsin inhibitor and homogenized in an ice bath using a tissue homogenizer (Bio Spec Product, USA). The supernatants were centrifuged at 12,000 rpm at 4 ℃ for 15 min to yield precipitate mitochondrial fraction. The supernatants were used as cytosol fractions.

8. Preparation of nuclear extract

The renal tissue (100 mg) was placed in 500 μl of hypotonic buffer A (10 mM HEPES (pH 7.8), 10 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.1 mM EDTA, and 0.1 mM PMSF) and homogenized in ice using a tissue homogenizer. A total of 62.5 μl of 10% NP-40 solution was added to the homogenates, and the mixture was centrifuged at 12,000 rpm for 30 sec. The pelleted nuclei were washed once with 100 μl of buffer A added 10% NP-40, centrifuged at 12,000 rpm, resuspended in 100 μl of a solution consisting of buffer B (50 mM HEPES, 50 mM KCl, 0.3 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF, and 10% glycerol), mixed for 30 min, and centrifuged at 4 ℃, 12,000 rpm for 10 min. The supernatant containing nuclear proteins was collected and stored at -80 ℃ in a deep freezer.

9. Western blot analysis

To investigate changes in the expression of NOX-4, $p47^{phox}$, $p22^{phox}$, COX-2, iNOS, SOD, catalase proteins and NF-κB activity, Western blot experiments were perfomed to examine the cytosolic and nuclear fractions of kidney tissue. Samples were boiled for 5 min with a gel loading buffer (125 mM Tris-Cl, 4% SDS, 10% 2-mercaptoethanol, pH 6.8, and 0.2% bromophenol blue) at a ratio of 1:1. Total protein equivalents for each sample were separated on 8% SDS-polyacrylamide mini-gels using a Laemmli buffer system. The samples were then transferred to polyvinylidene difluoride (PVDF) membranes at 90 V for 1.5 h. Blots were blocked using 5% skim milk solution for 1 h and then incubated overnight at 4 ℃ with primary antibodies to NOX-4, $p47^{phox}$, $p22^{phox}$, NF-κB, COX-2, iNOS, SOD, catalase, histone, and β-actin. After the blots were washed, they were incubated with secondary antibody (horseradish peroxidase-conjugated donkey anti-rabbit antibody; 1:5,000) for 1 h at room temperature. Antibody labeling was detected by enhanced chemiluminescence (Amersham) as per the manufacturer's instructions, and the blots were then exposed to Hyperfilm (Amersham). Band densities were determined using ATTO Densitograph Software (ATTO Corporation, Tokyo, Japan) and quantified as the ratio to histone or β-actin.

10. Statistical analysis

Data were expressed as means±SD. The statistical significance of the difference between the groups was determined by one-factor analysis of variance (ANOVA). Values of $p<0.05$ were considered statistically significant.

Ⅲ. Results

1. Effects of LCM on renal function in cisplatin -induced acute renal failure

To investigate the effect of LCM on cisplatin -induced renal dysfunction, levels of BUN were measured 24 h after the last cisplatin injection. The BUN level in the vehicle-treated group (58.32 ±1.86 mg/dl) was significantly increased compared with that of the normal group (41.06±2.96 mg/dl). The administration of 30 mg/kg LCM significantly reduced the increase in the BUN level (47.54±4.47 mg/dl), whereas the administration of 10 mg/kg

LCM slightly reduced the level (54.68±1.88 mg/dl) (Fig. 1).

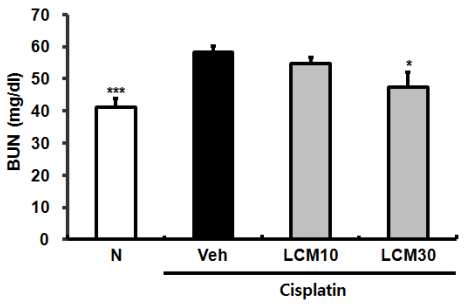

Fig. 1. Effects of LCM on BUN levels in cisplatin-induced acute renal failure in rats.

N : normal group, Veh : vehicle-treated group, LCM10 : LCM 10 mg/kg treated group, LCM30 : LCM 30 mg/kg treated group. Bars represent means±SD. * $p<0.05$, *** $p<0.001$ versus vehicle-treated group value.

2. Effects of LCM on the inhibition of renal oxidative stress

The level of ROS in the kidney of the vehicle-treated group was significantly increased compared with that of the normal group. The administration of 30 mg/kg LCM significanlty inhibited the level of ROS compared with the vehicle-treated group (Fig. 2). The TBARS level, a marker of the level of lipid peroxidation[14], was increased significantly in the kidney of the vehicle-treated group, whereas it was significantly reduced in the LCM 30 mg/kg group compared with that of the vehicle-treated group (Fig. 3).

Protein levels of NADPH oxidase subunits were also assessed. Cisplatin injection induced a significant increase in the expression levels of renal NOX-4, $p47^{phox}$, and $p22^{phox}$ in the vehicle-treated group compared to the normal group (Fig. 4). The NOX-4 level was not altered significantly in the LCM-administered groups (Fig. 4A). However, the expression level of $p47^{phox}$ showed a concentration-dependent decrease in the LCM-administered groups, and the expression of $p22^{phox}$ significantly decreased in the LCM 30 mg/kg group (Fig. 4B, C).

These results indicate that cisplatin induces the generation of ROS and stimulates oxidative stress in the kidney and that LCM effectively inhibits them.

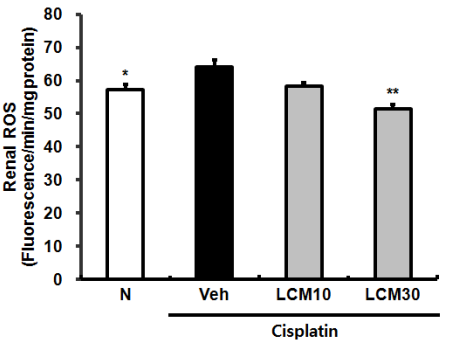

Fig. 2. Effects of LCM on renal ROS generation in cispatin-induced nephrotoxicity in rats.

N : normal group, Veh : vehicle-treated group, LCM10 : LCM 10 mg/kg treated group, LCM30 : LCM 30 mg/kg treated group. Bars represent means±SD. * $p<0.05$, ** $p<0.01$ versus vehicle-treated group value.

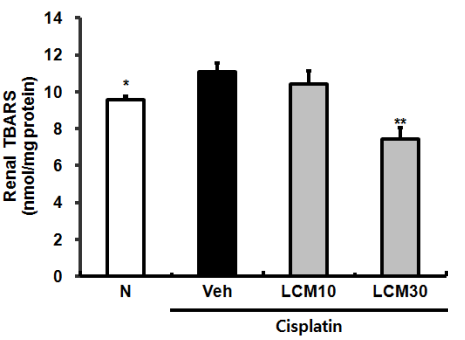

Fig. 3. Effects of LCM on renal TBARS generation in cisplatin-induced nephrotoxicity in rats.

N : normal group, Veh : vehicle-treated group, LCM10 : LCM 10 mg/kg treated group, LCM30 : LCM 30 mg/kg treated group. Bars represent means±SD. * $p<0.05$, ** $p<0.01$ versus vehicle-treated group value.

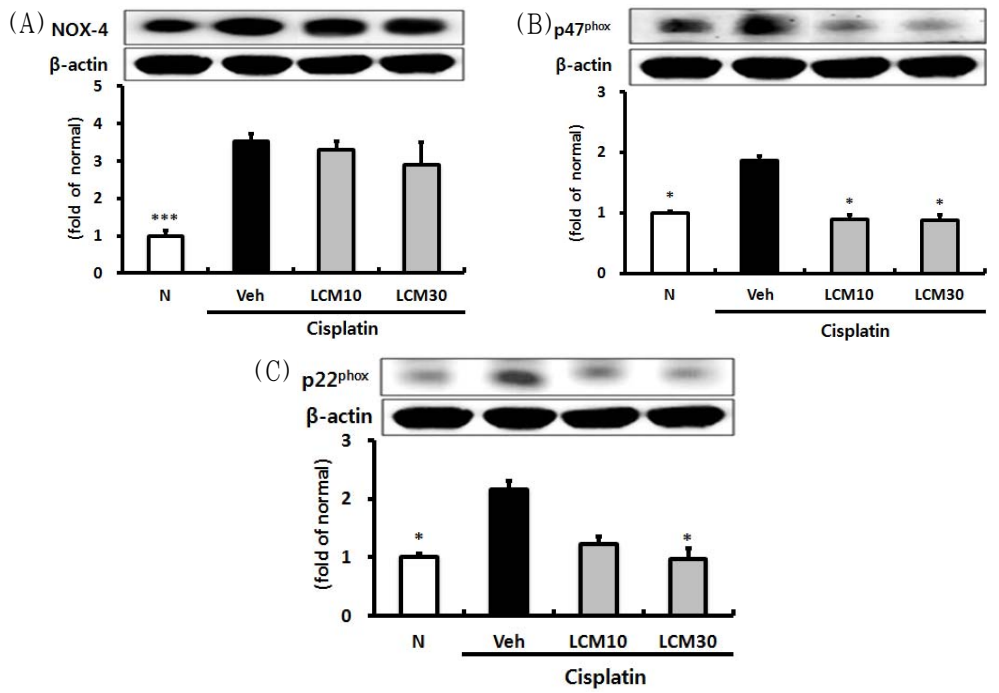

Fig. 4. Effects of LCM on the inhibition of renal NADPH oxidase subunits expression in cisplatin-induced nephrotoxicity in rats.

NOX-4 (A), $p47^{phox}$ (B), $p22^{phox}$ (C) protein expressions in kidney. N : normal group, Veh : vehicle-treated group, LCM10 : LCM 10 mg/kg treated group, LCM30 : LCM 30 mg/kg treated group. β-actin was used for loading control. Bars represent means±SD. * $p<0.05$, *** $p<0.001$ versus vehicle-treated group values.

3. Effects of LCM on the inhibition of renal NF-κB activity and COX-2, iNOS expressions

To confirm the effect of LCM on oxidative stress-induced inflammatory response to cisplatin in the kidney, we investigated the expression of various pro-inflammatory proteins by Western blotting. The vehicle-treated groups showed significantly increased levels of NF-κB and NF-κB-mediated inflammatory factors such as COX-2 and iNOS, as compared with the normal group. In contrast, the LCM-administered groups showed reduced expression of these inflammatory factors in renal tissue (Fig. 5). NF-κB activity was significantly downregulated in the LCM 30 mg/kg group (Fig. 5A). The expression levels of COX-2 and iNOS showed a concentration-dependent reduction (Fig. 5B, C).

These results indicate that LCM has a inhibitory effect on the inflammatory response signaling pathway through the regulation of NF-κB, COX-2, and iNOS.

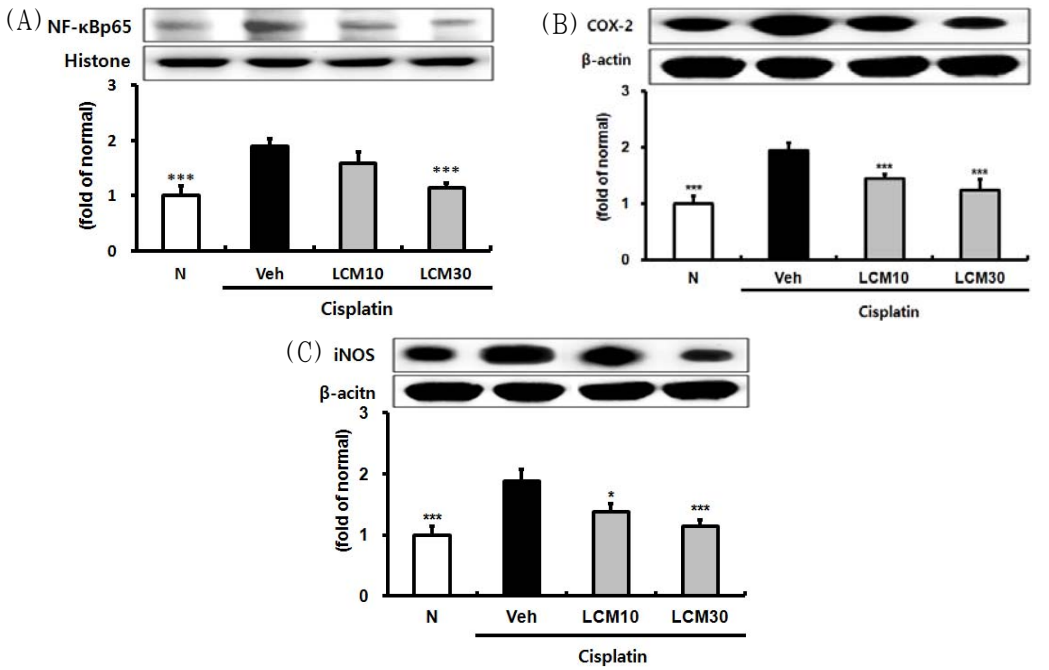

Fig. 5. Effects of LCM on the inhibition of renal NF-κB activity and COX-2, iNOS expressions in cisplatin-induced nephrotoxicity in rats.

NF-κBp65 (A) activity, COX-2 (B), iNOS (C) protein expressions in kidney. N : normal group, Veh : vehicle-treated group, LCM10 : LCM 10 mg/kg treated group, LCM30 : LCM 30 mg/kg treated group. Histone or β-actin was used for loading control. Bars represent means±SD. * $p<0.05$, *** $p<0.001$ versus vehicle-treated group values.

4. Effects of LCM on the antioxidants activity in kidney

To assess the anti-oxidant effects of LCM on cisplatin-induced oxidative stress, we measured the GSH, GSSG, SOD, and catalase levels in the kidney.

Compared with the normal group, the levels of GSH and GSH/GSSG were significantly decreased in the vehicle-treated group (Fig. 6A, C). In contrast, the levels of GSH and GSH/GSSG were significantly increased in the LCM 30 mg/kg group compared with the vehicle-treated groups (Fig. 6A, C). The level of GSSG, the oxidized form of GSH, was not significantly altered by the cisplatin injection and the LCM administration (Fig. 6B).

SOD and catalase expression levels were decreased in the vehicle-treated group, but the decrease was not statistically significant (Fig. 7). The LCM 30 mg/kg group showed a significant increase in the level of SOD compared with the vehicle-treated group (Fig. 7A). Catalase expression levels showed a significant dose-dependent increase (Fig. 7B).

These results indicate that LCM has potent effects on the antioxidant activity in the kidney.

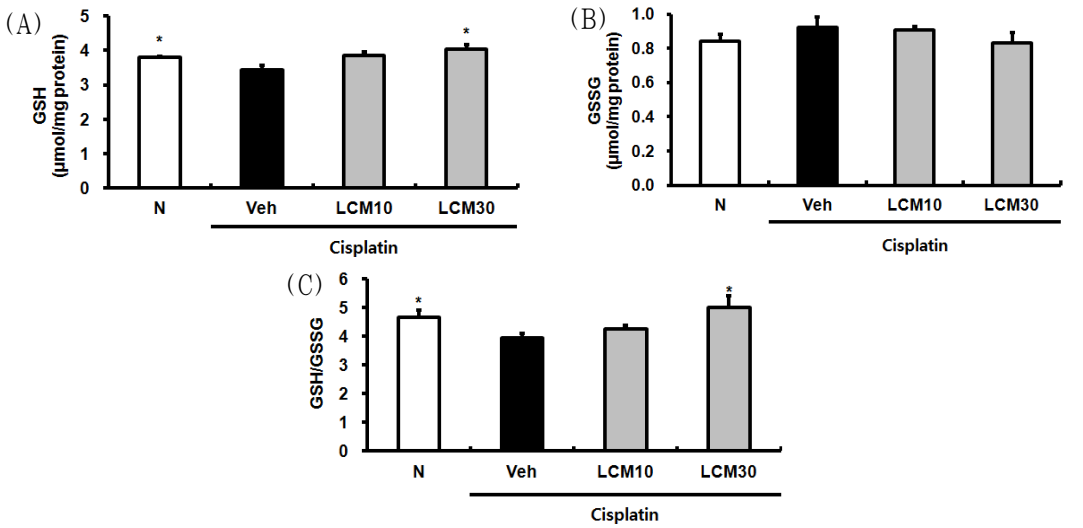

Fig. 6. Effects of LCM on GSH and GSSG levels in cisplatin-induced nephrotoxicity in rats.

GSH (A), GSSG (B), GSH/GSSG (C) levels. N : normal group, Veh : vehicle-treated group, LCM10 : LCM 10 mg/kg treated group, LCM30 : LCM 30 mg/kg treated group. Bars represent means±SD. * $p<0.05$ versus vehicle-treated group values.

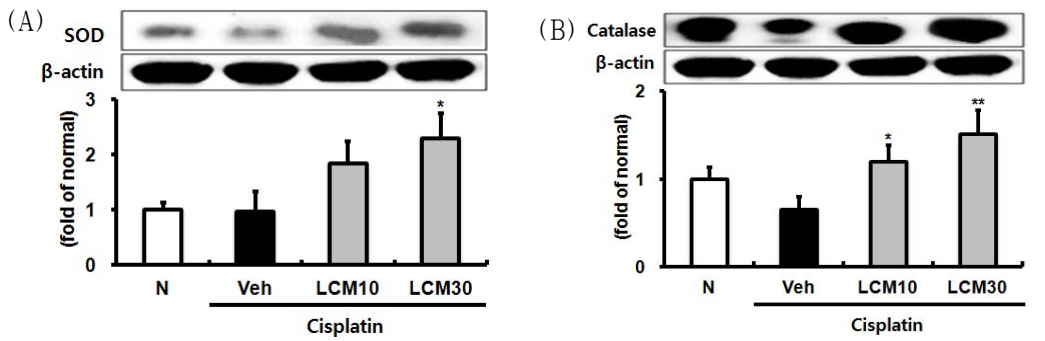

Fig. 7. Effects of LCM on the antioxidants activity in cisplatin-induced nephrotoxicity in rats.

SOD (A), Catalase (B) protein expressions in kidney. N : normal group, Veh : vehicle-treated group, LCM10 : LCM 10 mg/kg treated group, LCM30 : LCM 30 mg/kg treated group. β-actin was used for loading control. Bars represent means±SD. * $p<0.05$, ** $p<0.01$ versus vehicle-treated group values.

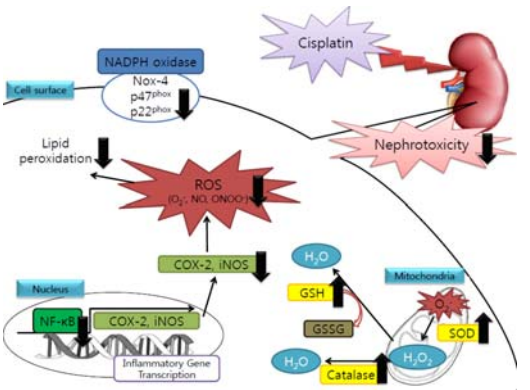

Fig. 8. Predictable action mechanisms of LCM on cisplatin-induced oxidative stress in renal tissue.

LCM inhibited ROS and TBARS generations in the kidney. Also, LCM decreased the expression of COX-2 and iNOS proteins regulated by NF-κB. Futher, LCM activated the antioxidants such as GSH, SOD, and catalase.

Ⅳ. Discussion

The aim of this study was to determine the renal protective effect of LCM against cisplatin-induced nephrotoxicity and to examine the anti-oxidative potential of LCM. LCM has been commonly used as a traditional oriental herbal medicine for treating febrile diseases that include night sweat, cough, nosebleed, hemoptysis, and inflammatory diseases such as pneumonia, chronic bronchitis, pulmonary tuberculosis, and gingivitis[1-5]. Previous studies revealed the inhibitory effects of LCM on oxidative stress *in vivo*[6,7]. However, the effect of LCM on the antioxidants activity was not examined.

Nephrotoxicity is a major side effect that limits the use of cisplatin which is an effective chemotherapeutic agent for many cancers[8,9]. In the present study, there was a significant increase in the BUN level of vehicle-treated group compared with that of the normal group following the injection of cisplatin. In agreement with prior reports, cisplatin induced marked renal dysfunction. However, the administration of LCM significantly inhibited the increase in BUN.

These results demonstrate that LCM has potential to protect renal function against acute renal failure induced by cisplatin.

Several mechanisms, including hypoxia, oxidative stress, inflammation, and apoptosis are thought to be involved in ciplatin-induced nephrotoxicity[15]. Among those mechanisms, oxidative stress and inflammation have been suggested to be the major mechanisms in the pathogenesis of cisplatin-induced nephrotoxicity[16]. Oxidative stress reflects an imbalance between cellular generation of ROS and the cellular ability for effective defense against ROS[17-19]. Oxidative stress can cause cellular damage and subsequent cell death due to the oxidation of vital cellular components such as lipids, proteins, and DNA[20]. Our data indicate that the levels of renal ROS and TBARS were increased significantly by cisplatin injection in the vehicle-treated group compared to the normal group and that these levels were significantly decreased in the LCM 30 mg/kg group. NADPH oxidase (NOX) which is a membrane-bound enzyme complex found in the plasma membrane and the cytoplasm, is a major source of ROS generation[21]. Therefore, we measured the expression of NADPH oxidase subunits such as NOX-4, $p47^{phox}$, and $p22^{phox}$ in renal tissue. Cisplatin resulted in significant increase in the expression levels of NOX-4, $p47^{phox}$, and $p22^{phox}$ in the vehicle-treated group compared to the normal group. In contrast, it significantly inhibited the expression of $p47^{phox}$ and $p22^{phox}$ levels in the LCM 30 mg/kg group. The NOX-4 level was also slightly decreased in the LCM-administered

groups, but the decrease was not statistically significant.

These results show that the administration of LCM can inhibit ROS generation and prevent cisplatin-induced nephrotoxicity and renal damage induced by oxidative stress.

As the redox potential is modulated by oxidative stress, various cellular processes related to signaling and gene expression can be activated by oxidative changes in the intracellular environment[22]. In particular, a redox imbalance caused by oxidative stress can exert a substantial effect on redox-sensitive gene regulation, including upregulating the expression of genes that are positively associated with NF-κB[23]. NF-κB is one of the most ubiquitous transcription factors and regulates genes involved in cellular proliferation, cell adhesion, and the inflammatory response[24]. NF-κB activates the transcriptions of several inflammatory enzymes such as COX-2 and iNOS[25]. Furthermore, prostaglandin E_2 (PGE_2), which is synthesized by COX-2, and nitric oxide (NO), which is derived from iNOS, play a pivotal role in the pathogenesis of acute and chronic inflammation[25]. Therefore, the inhibition of the abnormal upregulation of NF-κB, COX-2, and iNOS provides a molecular mechanism for the therapeutic and preventive effect on inflammation conditions induced by oxidative stress. In this study, cisplatin significantly increased the levels of NF-κB activity and COX-2, iNOS expressions in the vehicle-treated group compared to the normal group. These results demonstrate that cisplatin has a potent effect on the induction of inflammatory response to oxidative stress. In contrast, the administration of LCM significantly inhibited those levels.

These results confirm that LCM has inhibitory effects on the inflammatory response signaling pathway induced by oxidative stress.

Cisplatin-induced oxidative stress is involved in the depletion of GSH and antioxidant enzymes activity in the kidney[11]. GSH plays a central role in antioxidant defense, and irreversible cell damage occurs when the cell is unable to maintain the intracellular GSH concentration[26]. GSH is present in the cell in reduced (GSH) and oxidized (GSSG) forms[13]. GSH serves as a substrate for enzymes that scavenge ROS, inactivate electrophilic species, and restores reduced cysteine-thiol moieties on proteins, with the concomitant generation of oxidized GSSG[27]. As alterations in the redox balance by exposure to ROS cause dose-dependent changes in the GSH:GSSG ratio, the GSH:GSSG ratio within cells is often used as a marker of cellular toxicity[13,28]. The results of the present study showed that levels of GSH and GSH/GSSG were significantly decreased in the vehicle-treated group compared with the normal group, whereas they were significanlty increased in the LCM 30 mg/kg group. Protection against increased ROS generation can be achieved by the antioxidant enzymes such as SOD, dismutates O_2 into oxygen and H_2O_2, and catalase, decomposes H_2O_2 into water and oxygen[29]. Our data indicated that the expressions of both SOD and catalase were significantly increased by the administration of 30 mg/kg LCM. Cisplatin decreased the expression levels of SOD and catalase, but the decrease was not statistically significant.

These results show that cisplatin reduces levels of antioxidants, particularly GSH, and that LCM has a potent stimulatory effect on antioxidants, including GSH, SOD, and catalase.

Based on the findings of the present study, we conclude that LCM can prevent renal damage and dysfunction in cisplatin-induced nephrotoxicity through the inhibition of oxidative stress and the activation of antioxidants.

Ⅴ. Conclusion

In the present study, we examined the protective effect of LCM against cisplatin-induced acute renal failure. We investigated the antioxidative potential of LCM by examining its effect on the generation of ROS, the expression of oxidative stress-related proteins, and the activation of antioxidants.

The results demonstrate that the administration of LCM can preserve renal function and protect against cisplatin-induced nephrotoxicity by down-regulation of NF-κB, COX-2, and iNOS, thereby inhibiting oxidative stress and inflammatory signaling pathway. Also LCM increased the induction of antioxidants such as GSH, SOD, and catalase in kidney.

Therefore, the present study provides evidences that LCM has renal protective potential, mainly through the antioxidants activation, and that it may be a potential therapeutic and protective agent for cisplatin-induced nephrotoxicity.

Cisplatin으로 유도된 급성신부전증에 대한 地骨皮의 항산화효과

정유선[1], 박찬흠[2], 신현철[1]
[1]대구한의대학교 한의과대학 내과학교실, [2]대구한의대학교 한의과대학 본초학교실

초 록

목 적 : 본 연구는 淸虛熱藥으로서 肺, 肝, 腎經에 歸經하여 凉血除蒸, 淸肺降火의 효능으로 陰虛潮熱, 骨蒸盜汗, 肺熱咳嗽, 喀血, 衄血, 內熱消渴 등의 치료에 상용되는 地骨皮에 대해, 산화적 스트레스를 유발하여 신 독성을 일으키는 것으로 알려진 cisplatin을 투여한 Wistar rats에서의 신기능 손상 방지 효능을 관찰하고, 산화적 스트레스 및 그로 인한 염증반응 관련 전사인자와 효소들에 대한 억제효과와 항산화제를 촉진하는 효능을 확인하였다.

방 법 : Cisplatin으로 급성신부전증이 유도된 Wistar rats에서 地骨皮의 복용으로 인한 혈중 BUN 수치 변화를 관찰함으로써 신기능 보호효과를 확인하였다. Cisplatin으로 인한 신 손상의 주요 기전으로 알려진 산화적 스트레스에 대한 억제 효과를 확인하기 위하여 신 조직에서의 ROS, TBARS 수치를 관찰하고, ROS를 생성시키는 NADPH oxidase의 subunits인 NOX-4, $p47^{phox}$, $p22^{phox}$와 산화적 스트레스로 유도되는 염증반응과 관련한 NF-κB의 활성 및 COX-2, iNOS의 단백질 발현정도를 Western blotting을 통해 확인하였다. 또한 주요 항산화제인 glutathione의 환원형과 산화형 수치를 각각 확인하고 그 비율을 조사하였으며, 또 다른 항산화제인 SOD, catalase의 발현정도를 Western blotting을 통해 확인함으로써 地骨皮의 항산화제 촉진 효능을 관찰하였다.

결 과 : 地骨皮는 cisplatin으로 유도된 급성신부전증 모델에서 증가한 혈중 BUN 수치를 감소시켜 신기능 손상을 유효하게 방지하였다. 또한 cisplatin 투여는 Wistar rats에서 산화적 스트레스 및 그로 인한 염증반응 관련 전사인자와 효소들의 발현을 항진시켜 cisplatin의 신 독성 기전이 산화적 스트레스로 초래됨을 확인할 수 있었으며, 地骨皮 투여군의 경우 신조직 ROS, TBARS, NADPH oxidase를 유의하게 감소시켰고 NF-κB의 활성과 COX-2, iNOS 발현 또한 억제하는 것을 관찰하였다. 나아가 주요 항산화제로 알려진 GSH, SOD 및 catalase에 대한 地骨皮의 촉진효과를 확인하였다.

결 론 : 이상의 결과로 地骨皮는 신기능 손상을 방지하고, 항산화제 활성을 촉진시켜 산화적 스트레스와 염증반응을 효과적으로 저해함으로써 cisplatin으로 유발되는 급성신부전증의 치료 및 예방에 활용될 수 있음이 시사되었다.

중심단어 : 지골피, 신독성, 급성신부전증, 항산화효과

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