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Alpha-lipoic acid attenuates cisplatin-induced acute kidney injury in mice by suppressing renal inflammation

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Abstract

Background. Cisplatin is a chemotherapeutic agent used in treatment of malignant tumours. However, cisplatin produces various side effects, such as nephrotoxicity, neurotoxicity, emetogenesis and ototoxicity. Inflammation is an important mechanism of cisplatin nephrotoxicity. Alpha-lipoic acid (α-LA) has anti-inflammatory effects that inhibit both adhesion molecule expression in human endothelial cells and monocyte adhesion by suppressing the nuclear factor-κB (NF-κB) signalling pathway. The goals of this study were to investigate the anti-inflammatory effects of α-LA during cisplatin-induced renal injury and to examine the mechanisms of protection.

Methods. C57BL/6 mice were given cisplatin (20 mg/kg) with or without α-LA treatment (100 mg/kg for 3 days). Renal function, histological changes, adhesion molecule expression and inflammatory cell infiltration were examined. The effect of α-LA on NF-κB activity was evaluated by examining nuclear translocation and phosphorylation of NF-κB p65 subunits in kidney tissue.

Results. Cisplatin-induced decreases in renal function, measured by blood urea nitrogen, serum creatinine level and renal tubular injury scores, were attenuated by α-LA treatment. α-LA decreased the tissue levels of tumour necrosis factor-α, the expression of intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1), and suppressed the infiltration of CD11b-positive macrophages. α-LA also attenuated the cisplatin-induced increases in the phosphorylation and nuclear translocation of NF- κB p65 subunits in kidney tissue.

Conclusions. These results suggest that α-LA treatment ameliorates cisplatin-induced acute kidney injury by reducing inflammatory adhesion molecule expression and NF-κB activity.

Keywords: acute; cisplatin; inflammation; kidney injury; nuclear factor-κB

Introduction

Cisplatin is a potent chemotherapeutic agent that has activity against solid tumours such as testicular, head and neck, ovarian and non-small cell lung cancers [1,2]. Despite the anti-tumour actions of this agent, major side effects such as nephrotoxicity, neurotoxicity, emetogenesis and

ototoxicity have limited its use in clinical treatment. Acute kidney injury can occur after high-dose cisplatin chemotherapy with ∼20% of patients experiencing various degrees of renal dysfunction [3]. Although several therapeutic strategies have been suggested for prevention of cisplatin-induced renal injury, no specific treatments have been recommended, except for vigorous hydration with saline [4,5]. Therefore, new and effective therapeutic strategies are needed for the prevention of cisplatin-induced renal injury.

Proposed mechanisms of cisplatin-induced nephrotoxicity include direct toxicity to renal tubular epithelial cells [6], apoptosis [7], dysregulation of cell-cycle proteins [8], activation of p53 tumour suppressor proteins [9], activation of the mitogen-activated protein kinase (MAPK)-signalling pathway [10], oxidative stress [11] and inflammation [12]. Cisplatin administration increases macrophage infiltration into damaged kidney tissues at 48–72 h, and also increases CX_3CL1 expression in damaged kidney and endothelial cells before renal dysfunction occurs [13]. Intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1) are also increased after cisplatin-induced renal injury [11,14,15]. Findings from previous studies suggest that inflammation may play an important pathophysiologic role in cisplatin-induced nephrotoxicity. Therefore, a modulation of the renal inflammatory reaction after cisplatin treatment may help to prevent cisplatin-induced renal injury.

Alpha-lipoic acid (α-LA) helps in the anti-oxidant effects of scavenging reactive oxygen species and metal chelation by acting as an essential cofactor for mitochondrial respiratory enzymes [16]. α-LA has also been shown to have anti-hyperglycaemic effects, to improve insulin resistance [17], to induce apoptosis in cancer cells [18] and to have an anti-obesity effect via regulation of hypothalamic AMP-activated protein kinase [19]. Clinically, α-LA is currently being used as treatment for diabetic polyneuropathy [20]. In addition, α-LA exerts anti-inflammatory actions by inhibiting nuclear factor-κB (NF-κB) activation and by decreasing adhesion molecule expression in endothelial cells [21]. We previously reported that α-LA inhibits fractalkine-mediated vascular inflammation during endotoxaemia [22]. α-LA also inhibits lipopolysaccharide-induced inflammatory reactions by activating the phosphoinositide 3-kinase/Akt signalling pathway [23].

On the basis of these previous studies, we hypothesized that α-LA may protect against cisplatin-induced renal injury by reducing renal inflammation in mice. We also examined whether α-LA inhibits NF-κB activation in kidney tissue, an action that would suppress immune and inflammatory responses. Our results showed that α-LA reduced cisplatin-induced functional and histological renal damage. Furthermore, α-LA lowered tissue tumour necrosis factor (TNF)-α levels and suppressed NF-κB activation in renal tubular epithelial cells, actions that should decrease cisplatin-induced ICAM-1 and tubular MCP-1 expression. α-LA also decreased cisplatin-induced CD11b-positive macrophage infiltration into the renal interstitium. These results demonstrate that α-LA protects against cisplatin-induced renal injury in mice, and suggest that it may have therapeutic potential for the prevention of cisplatin-induced renal injury.

Subjects and methods

Animals and drug treatments

Male C57BL/6 mice (Charles River Korea, Seoul, Korea) were given a standard laboratory diet and water *ad libitum* and were entered into a protocol approved by the Institutional Animal Care and Use Committee of the Chonbuk National University. At the start of the experiments, the mice were 8–9 weeks of age and weighed 20–23 g. The mice were divided into four groups: a control buffer-treated group (Con; $n = 10$), a α-LA group (LA; 100 mg/kg; VIATRIS GmbH & Co KG, Bad Homburg, Germany; $n = 10$), a cisplatin group (Cis; 20 mg/kg; Sigma Chemical Co., St Louis, MO, USA; $n = 10$) and a cisplatin plus α-LA group (Cis + LA; $n = 10$). The dose of cisplatin and the time of treatment were based on previous findings from our laboratory [14,15]. Maximal renal injury, as assessed by functional and histologic measurements, was observed at 72 h following intraperitoneal injections of 20 mg/kg cisplatin. Kidneys were harvested to evaluate tissue levels of TNF-α and MCP-1 using ELISA and to assess protein expression of ICAM-1 using immunoblot analysis at 24, 48 and 72 h after cisplatin administration. To obtain optimal concentrations of α-LA, we gave two doses of the drug (10 and 100 mg/kg), which were based on previous findings [24]. The 100 mg/kg dose was chosen because of its maximal anti-inflammatory effect. α-LA (100 mg/kg) was injected intraperitoneally once a day for 3 days, followed by intraperitoneal cisplatin injection.

Renal function analysis

On the day of sacrifice, mice were anaesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg), and blood was collected from the intracardiac puncture. Blood urea nitrogen and creatinine levels were measured with an automatic analyser using an enzymatic method (Hitachi 7180, Tokyo, Japan).

Histologic examinations

The kidneys were sectioned in blocks, fixed in 4% paraformaldehyde, dehydrated in graded concentrations of alcohols and then embedded in paraffin. Kidney blocks were cut into 5-μm sections and stained with periodic acid-Schiff (PAS). Renal tubular injury was assessed as previously described [25]. The magnitude of tubular epithelial cell loss, necrosis, intratubular debris and tubular cast formation was scored according to six levels on the basis of percentage of affected tubules under high-power field using a light microscope: 0, none; 0.5, <10%; 1, 10–25%; 2, 25–50%; 3, 50–75%; and 4, >75%. The morphometric examinations were performed in a blinded manner by two independent investigators as previously described [15].

Immunohistochemical analysis of ICAM-1, MCP-1 and NF-κB p65

Immunohistochemical staining for ICAM-1, MCP-1 and NF-κB p65 was performed as previously described [14,15]. In brief, isolated kidney tissues were fixed by immersion in 4% paraformaldehyde and embedded in paraffin. The tissue sections were then deparaffinized with xylene and rehydrated with ethanol. After treatment with the blocking buffer, the slides were incubated overnight at 4°C with either a hamster anti-mouse ICAM-1 antibody (dilution 1:100; BD Biosciences-Pharmingen, San Jose, CA, USA), a rabbit anti-mouse MCP-1 antibody (dilution 1:100; Fitzgerald, Concord, MA, USA) or a rabbit polyclonal antibody directed against NF-κB p65 (dilution 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The kidney sections were exposed to DAKO Chromogen (DAKO Cytomation, Glostrup, Denmark) to visualize the immunocomplexes and counterstained with haematoxylin (Sigma Chemical Co.). All of the slides were evaluated by two blinded observers using a Zeiss Z1 microscope (Carl Zeiss, Göttingen, Germany). The extent of ICAM-1 and MCP-1 immunostaining in kidney tissue was expressed as a percentage of the area of 10 random, non-overlapping fields per slide at a magnification of ×400 using a digital image analysis program (AnalySIS, Soft Imaging System, Münster, Germany). The numbers of NF-κB-activated cells in each section were calculated by counting the number of positively stained cells in 10 random and non-overlapping fields per slide at a magnification of ×400.

Immunofluorescence staining of kidney tissue

Immunofluorescence staining of kidney tissues was conducted as previously described [15]. Briefly, freshly frozen renal tissues were fixed with 4% paraformaldehyde, permeabilized in 1% Triton X-100 and then incubated with a blocking buffer. The samples were then incubated with the hamster anti-mouse CD11b antibody (dilution 1:1000; BD Biosciences-Pharmingen). The slides were exposed to the Cy3-labelled secondary antibody (Chemicon, Temecula, CA, USA). Nuclear staining was performed by using DAPI. Immunofluorescence staining for CD11b was visualized using a Zeiss Z1 microscope (Carl Zeiss). The numbers of CD11b-positive cells in each section were calculated by counting the number of positively stained cells in 10 random, non-overlapping fields per slide at a magnification of ×400.

Western blot analysis

Western blot analysis was performed as previously described [11]. Kidney tissues were homogenized in PBS with a protease inhibitor cocktail (Calbiochem, San Diego, CA, USA), and the protein concentration was quantitated. The samples (30 μg of protein per lane) were mixed with a sample buffer, boiled for 9 min, separated by SDS–polyacrylamide (8%) gel electrophoresis under denaturing conditions, and were electroblotted onto nitrocellulose membranes. The nitrocellulose membranes were blocked with 5% non-fat dry milk in a TRIS-buffered saline with the Tween 20 buffer [25 mmol/L Tris (pH 7.5), 150 mmol/L NaCl, 0.1% Tween 20] for 1 h and incubated overnight at 4°C with the goat anti-mouse ICAM-1 monoclonal antibody (dilution 1:1000; Santa Cruz Biotechnology) and the rabbit anti-mouse phospho-p65 antibody (dilution 1:1000; Cell Signaling Technology, Danvers, MA, USA). The blots were washed with PBS and incubated with horseradish peroxidase-conjugated anti-goat and anti-rabbit IgG. The signals were visualized with a chemiluminescent detection kit according to manufacturer instructions (Amersham Pharmacia Biotech, London, UK). The membranes were then reprobed with an anti-actin antibody to verify the equal loading of protein in each lane. All signals were visualized and analysed by densitometric scanning (LAS-3000; Fuji Film, Tokyo, Japan).

Measurement of tissue TNF-α and MCP-1 protein level

The TNF-α and MCP-1 protein concentrations in kidney tissues were measured in triplicate by using a Mouse Cytokine Lincoplex kit (Linco Research, Inc., St Charles, MO, USA). In all cases, a standard curve was constructed from the standards provided by the manufacturer.

Statistical analysis

Data were expressed as means ± S.E.M. Multiple comparisons were examined using ANOVA, followed by individual comparisons with the Tukey *post hoc* test. $P < 0.05$ indicated statistical significance.

Results

α-*LA ameliorates renal dysfunction during cisplatin-induced renal injury*

To investigate the protective effect of α-LA on cisplatin-induced renal injury, we treated mice with cisplatin, α-LA or cisplatin plus α-LA and measured blood urea nitrogen (BUN) and creatinine at 72 h after cisplatin and/or α-LA injection. Cisplatin administration significantly elevated BUN and serum creatinine levels compared with levels in the control buffer-treated mice (BUN, 132.4 ± 13.7 versus 24.6 ± 1.5 mg/dL, $P < 0.01$; creatinine, 1.0 ± 0.16 mg/dL versus 0.33 ± 0.03 mg/dL, $P < 0.01$). Treatment with α-LA significantly decreased cisplatin-induced elevations in BUN and creatinine levels (BUN, 96.9 ± 10.1 mg/dL, $P < 0.05$ and creatinine, 0.57 ± 0.06 mg/dL, $P < 0.05$; Figure 1). The BUN and creatinine levels after treatment with α-LA alone (BUN, 21.6 ± 0.88 mg/dL and creatinine, 0.34 ± 0.02 mg/dL) were not significantly different from those of control buffer-treated mice.

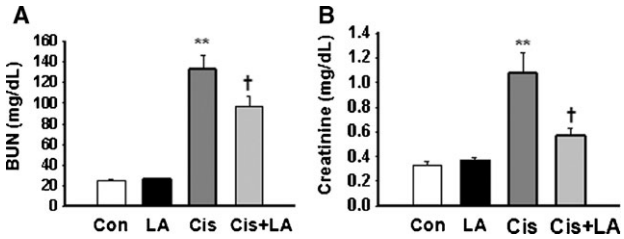

Fig. 1. Effect of α-lipoic acid on renal function during cisplatin-induced renal injury. Acute renal injury was induced by intraperitoneal injection of cisplatin (Cis; 20 mg/kg). Mice were treated with α-LA (LA; 100 mg/kg, IP) once a day for 3 days, followed by intraperitoneal cisplatin injection. Blood samples were collected 72 h after cisplatin treatment, and then BUN (**A**) and creatinine (**B**) levels were measured. Control mice (Con) were injected a control buffer. Data are expressed as means ± S.E.M. ($n =$ 10 mice per each group). $^{**}P < 0.01$ versus Con or LA; $^{\dagger}P < 0.05$ versus Cis.

α-*LA reduces renal tubular injury caused by cisplatin treatment*

We next examined whether α-LA helped to prevent cisplatin-induced renal tubular damage. At 72 h after cisplatin treatment, extensive renal tubular injury was observed, which included tubular cell necrosis, accumulation of the PAS-positive material in the tubular lumen, loss of brush–border membranes, tubular dilatation and inflammatory cell infiltration (Figure 2A). Cisplatin administration significantly increased tubular injury scores compared with the control buffer-treated group. Treatment with α-LA significantly reduced the cisplatin-induced renal tubular damage at 72 h after cisplatin administration (3.54 ± 0.15 in the cisplatin-treated group versus 1.91 ± 0.21 in the cisplatin plus α-LA group, $P < 0.01$; Figure 2B). Treatment with α-LA alone caused no significant morphologic alterations.

α-*LA decreases tissue TNF-α levels in cisplatin-treated mice*

TNF-α is a proinflammatory cytokine and is known to play an important role in cisplatin-induced renal injury [12]. Therefore, we evaluated kidney tissue protein levels of TNF-α by ELISA at 24, 48 and 72 h after cisplatin administration. Tissue levels of TNF-α were not changed at 24 h after cisplatin administration (5392.5 ± 304.8 pg/mg protein at 24 h) compared with control buffer treatment or with α-LA alone (5190 ± 290 pg/mg protein and 4460 ± 150 pg/mg protein, respectively). In contrast, TNF-α levels were significantly elevated at 48 and 72 h after cisplatin administration (21 675 ± 1406.7 pg/mg protein at 48 h, $P <$ 0.01; 24 125 ± 2780.3 pg/mg protein at 72 h, $P < 0.01$). α-LA treatment significantly lowered the elevated TNF-α tissue levels at 48 and 72 h after cisplatin administration (5557.5 ± 1003.1 pg/mg protein at 48 h, $P < 0.01$; 6225 ± 310.4 pg/mg protein at 72 h, $P < 0.01$; Figure 3). These findings demonstrate that α-LA reduces cisplatin-induced inflammatory mediators, such as tissue TNF-α, in the injured kidney.

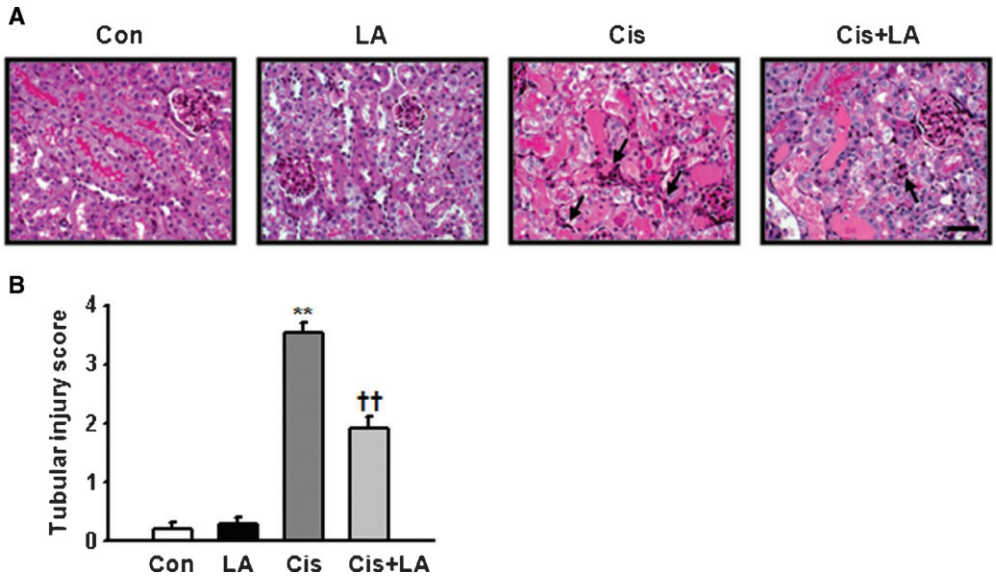

Fig. 2. Effect of α-lipoic acid on cisplatin-induced renal tubular damage. (**A**) Histologic sections from control (Con) or α-LA (LA) alone showed near normal glomeruli and tubules, whereas sections from the cisplatin-treated group (Cis) showed extensive renal tubular injury such as tubular cell necrosis, cast formation, loss of brush border, dilatation of tubules and inflammatory cell infiltration (arrows). These histologic changes were less pronounced in mice treated with cisplatin and α-LA (Cis + LA). Bar = 50 μm. (**B**) Semi-quantitative scoring of tubular injury was concomitant with histologic findings ($n = 10$ for each experimental group). Data are expressed as means ± S.E.M. ($n = 10$ mice per each group). $^{**}P < 0.01$ versus Con or LA; $^{††}P < 0.01$ versus Cis.

α-LA reduces ICAM-1 and MCP-1 expression during cisplatin-induced renal injury

Inflammatory reactions, such as the up-regulation of proinflammatory cytokines and chemokines, are major pathophysiologic mechanisms in cisplatin-induced nephrotoxicity [12,26]. To examine whether α-LA could suppress elevations in ICAM-1 and MCP-1 expression induced by cisplatin treatment, we evaluated kidney tissue for ICAM-1 protein expression by western blot analysis at 24, 48, and 72 h after cisplatin and/or α-LA treatment. ICAM-1 protein levels showed no significant changes at 24 h after cisplatin administration. In contrast, cisplatin treatment significantly increased the ICAM-1 protein levels by ∼2.0- and 3.9-fold at 48 h and 72 h, respectively, compared with either control buffer treatment or α-LA alone. Treatment with α-LA suppressed the cisplatin-induced increases in ICAM-1 expression by ∼25% at 48 h and 30.7% at 72 h after cisplatin administration (Figure 4A).

While evaluating the immunohistochemistry data, we found that ICAM-1 expression was increased in the tubular interstitial areas in the cisplatin-treated group compared with the control buffer-treated group (% of area fraction of ICAM-1, 8.13 ± 1.23% in the cisplatin-treated group versus 0.95 ± 0.16% in the control buffer-treated group, $P < 0.01$). Treatment with α-LA significantly reduced cisplatin-induced elevations in ICAM-1 expression in the tubular interstitial areas by ∼57% (% of area fraction of ICAM-1, 3.48 ± 0.8% in the cisplatin plus α-LA group, $P < 0.05$). Tubular interstitium ICAM-1 expression in the α-LA alone group was not different from controls (Figure 4B and C).

We also examined MCP-1 expression during cisplatin-induced renal injury. MCP-1 expression was increased in the damaged tubular cells in the cisplatin-treated group

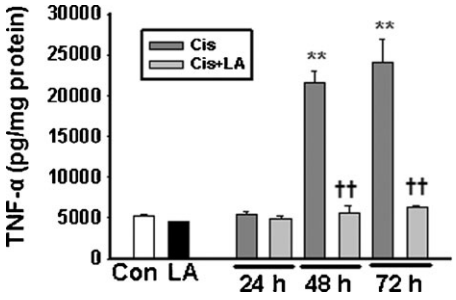

Fig. 3. Effect of α-lipoic acid on tissue TNF-α levels during cisplatin-induced renal injury. Kidney tissue TNF-α levels were measured by ELISA 24, 48 and 72 h after cisplatin administration ($n = 10$ per each group). Bar graphs represent tissue protein levels of TNF-α (pg/mg protein). Data are expressed as means ± S.E.M. of three independent experiments. $^{**}P < 0.01$ versus Con or LA; $^{††}P < 0.01$ versus Cis.

compared with the control buffer-treated group (% of area fraction of MCP-1, 11.4 ± 1.29% in the cisplatin-treated group versus 0.6 ± 0.05% in the control buffer-treated group, $P < 0.01$). Treatment with α-LA significantly reduced the cisplatin-induced increases in MCP-1 expression in the damaged tubular cells by ∼49% (% of area fraction of MCP-1, 6.28 ± 1.48% in the cisplatin plus α-LA group, $P < 0.05$; Figure 5A and B). There were no significant changes in MCP-1 expression in the tubular cells after treatment with α-LA alone. For evaluation of changes in tissue MCP-1 protein levels, we measured the tissue levels by ELISA at 24, 48 and 72 h after cisplatin administration. At 24 h after cisplatin-induced renal injury, there were no changes in the tissue MCP-1 levels compared with control buffer treatment or with α-LA alone. After 48 and 72 h of cisplatin administration, the tissue MCP-1 levels were

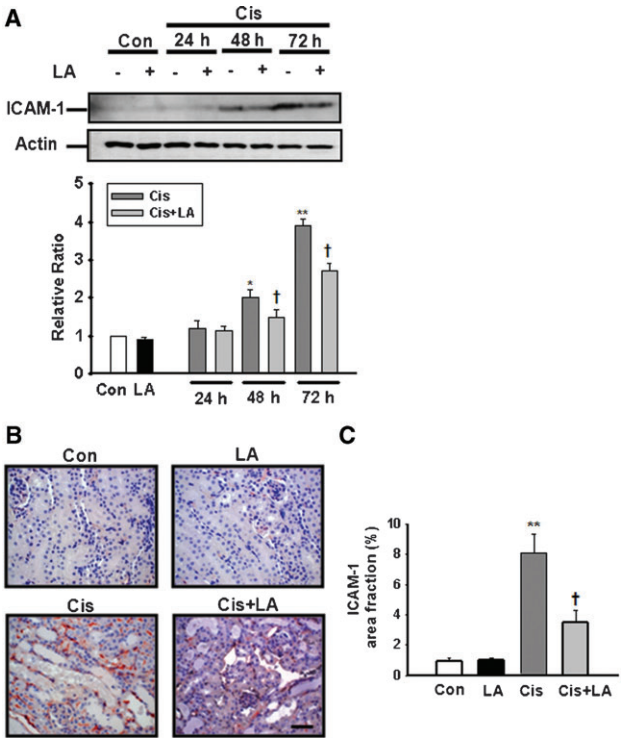

Fig. 4. Effect of α-lipoic acid on ICAM-1 expression during cisplatin-induced renal injury. Kidneys from mice treated with control (Con), α-LA (LA), cisplatin (Cis) and cisplatin plus α-LA (Cis + LA) were evaluated for ICAM-1 protein expression by western blot analysis and immunohistochemistry. (**A**) Western blot analysis of ICAM-1 expression in renal tissue. Densitometric analyses are presented as the relative ratio of each protein to actin. The relative ratio measured in the kidneys from control mice is arbitrarily presented as 1. Data are expressed as means ± S.E.M. of five independent experiments. $^{*}P < 0.05$ versus Con or LA; $^{**}P < 0.01$ versus Con or LA; $^{\dagger}P < 0.05$ versus Cis. (**B**) The expression of ICAM-1 in renal tubular interstitial areas was increased by cisplatin, whereas treatment with α-LA significantly reduced cisplatin-induced increases in ICAM-1 expression. Bar = 50 μm. (**C**) Quantitative scores of ICAM-1 in kidney ($n = 10$ per each group). Bar graph represents the percentage of ICAM-1positive stained area fraction to the total area (×400 magnification field). Data are expressed as means ± S.E.M. ($n = 10$ mice per each group). $^{**}P < 0.01$ versus Con or LA; $^{\dagger}P < 0.05$ versus Cis.

significantly increased (534.8 ± 46.6 pg/μg protein at 48 h and 920.6 ± 182.7 pg/μg protein at 72 h, $P < 0.01$) compared with control buffer treatment or with α-LA alone (100.5 ± 14.5 and 93.5 ± 7.3 pg/μg protein, respectively). Treatment with α-LA significantly suppressed the cisplatin-induced elevations in the tissue MCP-1 levels (123.6 ± 23.1 pg/μg protein at 48 h and 210.0 ± 35.0 pg/μg protein at 72 h, $P < 0.05$; Figure 5C) compared with levels in cisplatin-treated mice.

α-LA inhibits the infiltration of CD11b-positive macrophages during cisplatin-induced renal injury

We next examined macrophage infiltration at 72 h following cisplatin administration. We used CD11b as a macrophage marker, as in previous studies [27]. The number of CD11b-positive macrophages in the renal interstitium was significantly increased in the cisplatin-treated group (36.4 ± 6.5 in 10 fields at ×400 magnification, $P < 0.01$) compared with the control buffer-treated group (3.0 ± 1.5 in 10 fields at ×400 magnification) or the α-LA alone group (2.0 ± 1.3 in 10 fields at ×400 magnification). Treatment with α-LA significantly decreased the number of infiltrating CD11b-positive macrophages (14.6 ± 4.5 in 10 fields at ×400 magnification; $P < 0.01$; Figure 6A and B) induced by cisplatin administration.

α-LA inhibits NF-κB activation during cisplatin-induced renal injury

Finally, we tested a possible protective mechanism of α-LA in cisplatin-induced renal injury. We had previously reported that the NF-κB signalling pathway is activated during cisplatin-induced renal injury [11,15]. Therefore, we performed the western blot analysis of phospho-p65 of NF-κB in whole kidney tissue in cisplatin- and/or α-LA-treated mice. Phosphorylation of NF-κB p65 after cisplatin treatment was significantly increased by ∼1.56-fold compared with the control or α-LA alone group ($P < 0.05$). Treatment with α-LA decreased the cisplatin-induced p65 phosphorylation of NF-κB ∼26% ($P < 0.05$; Figure 7A) compared with the cisplatin-treated group. We also evaluated the nuclear translocation of NF-κB p65 during cisplatin-induced renal injury by immunohistochemical staining of the kidney. The number of nuclear translocations of NF-κB p65 was significantly increased in the cisplatin-treated group (12.4 ± 1.01 in 10 fields at ×400 magnification, $P < 0.01$) compared with the control or α-LA alone group. Treatment with α-LA significantly decreased the number of nuclear translocations of NF-κB p65 (5.18 ± 0.88 in 10 fields at ×400 magnification, $P < 0.01$; Figure 7B and C) induced by cisplatin administration.

Discussion

The present study demonstrated that (1) treatment with α-LA reduced cisplatin-induced renal injury, as assessed by functional and histologic measurements; (2) α-LA suppressed not only cisplatin-induced up-regulation of ICAM-1 and MCP-1 expression but also CD11b-positive macrophage infiltration; and (3) administration of α-LA significantly reduced the cisplatin-induced activation of NFκB.

Cisplatin induces a cascade of inflammatory reactions, which play an important pathogenic role in cisplatin-induced renal injury. Cisplatin treatment increases TNF-α production and NF-κB binding activity in kidney tissues [28]. α-LA not only exerts potent anti-oxidant activities but also has anti-inflammatory effects against inflammatory conditions such as Alzheimer's disease [29], rheumatoid arthritis [30] and lipopolysaccharide-induced endotoxaemia [22]. In cultured endothelial cells, α-LA administration inhibits the TNF-α-induced up-regulation of E-selectin, vascular cell adhesion molecule-1 (VCAM-1), ICAM-1 and MCP-1 mRNA expression [21]. In a model of experimental autoimmune encephalomyelitis, α-LA effectively decreased the expression of ICAM-1 and VCAM-1 in the spinal cord, which are both necessary for inflammatory

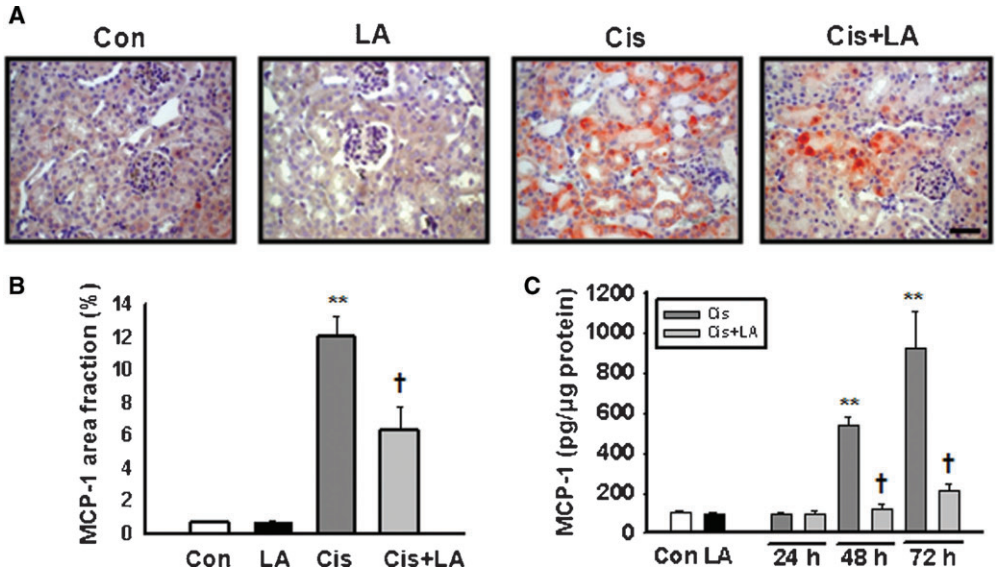

Fig. 5. Effect of α-lipoic acid on MCP-1 expression during cisplatin-induced renal injury. (**A**) Kidneys from mice treated with control (Con), α-LA (LA), cisplatin (Cis) and cisplatin plus α-LA (Cis + LA) were immunostained for MCP-1. The expression of MCP-1 was increased by cisplatin in damaged tubular cells, whereas α-LA treatment significantly reduced the cisplatin-induced increase in MCP-1 expression. Bar = 50 μm. (**B**) Quantitative score of MCP-1 in kidney ($n = 10$ per each group). Bar graph represents the percentage of MCP-1 positive stained area fraction to the total area (×400 magnification field). Data are expressed as means ± S.E.M. ($n = 10$ mice per each group). $^{**}P < 0.01$ versus Con or LA; $^{\dagger}P < 0.05$ versus Cis. (**C**) Tissue level of MCP-1 protein following a control buffer, cisplatin and/or α-LA administration ($n = 10$ per each group) measured by ELISA at 24, 48 and 72 h after cisplatin administration. Bar graph represents the tissue protein level of MCP-1 (pg/μg protein). Data are expressed as means ± S.E.M. of three independent experiments. $^{**}P < 0.01$ versus Con or LA; $^{\dagger}P < 0.05$ versus Cis.

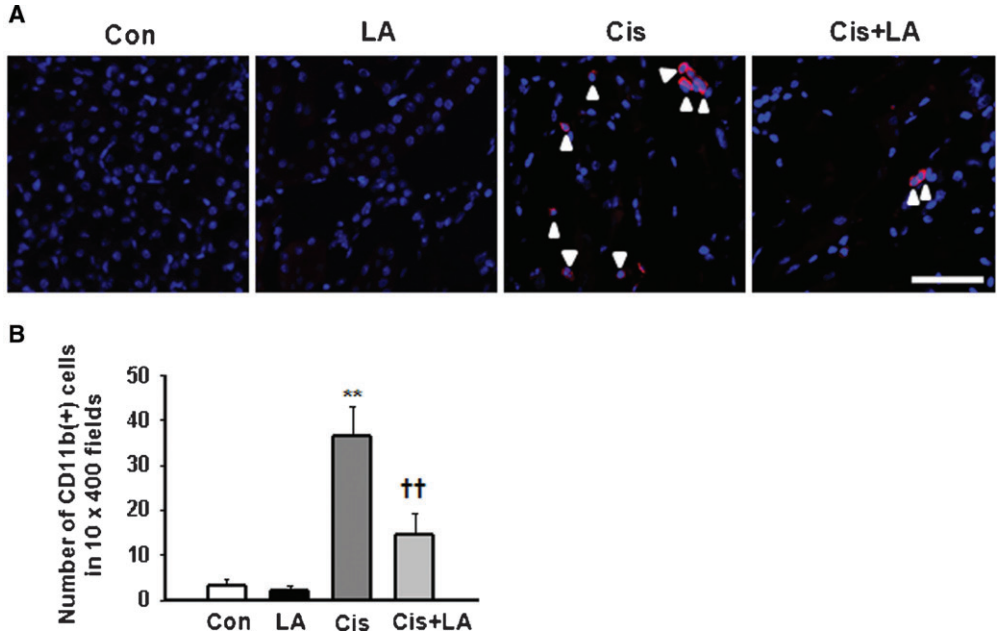

Fig. 6. Effect of α-lipoic acid on CD11b-positive macrophage infiltration during cisplatin-induced renal injury. (**A**) Kidneys from mice treated with control (Con), α-LA (LA), cisplatin (Cis) and cisplatin plus α-LA (Cis + LA) were immunofluorescence stained for CD11b-positive macrophages. The number of CD11b-positive cells (arrowheads) was highest in the cisplatin-treated kidney section, whereas treatment with α-LA decreased the cisplatin-induced infiltration of CD11b-positive cells. Bar = 50 μm. (**B**) Number of CD11b-positive cells in 10 fields at × 400 magnification. Data are expressed as means ± S.E.M. ($n = 10$ mice per each group). $^{**}P < 0.01$ versus Con or LA; $^{\dagger\dagger}P < 0.01$ versus Cis.

cell infiltration [31]. The present study showed that cisplatin treatment induced ICAM-1 expression in the damaged renal tubulointerstitial area, specifically in peritubular endothelial cells, and that this increased expression was effectively suppressed by α-LA treatment. Sung *et al*. [15] also suggested that ICAM-1 expression after cisplatin administration may be localized in peritubular capillary endothelial cells. Therefore, this localization of ICAM-1 suggests that the mechanism of inflammation may be juxtacrine during cisplatin-induced acute kidney injury. We evaluated the

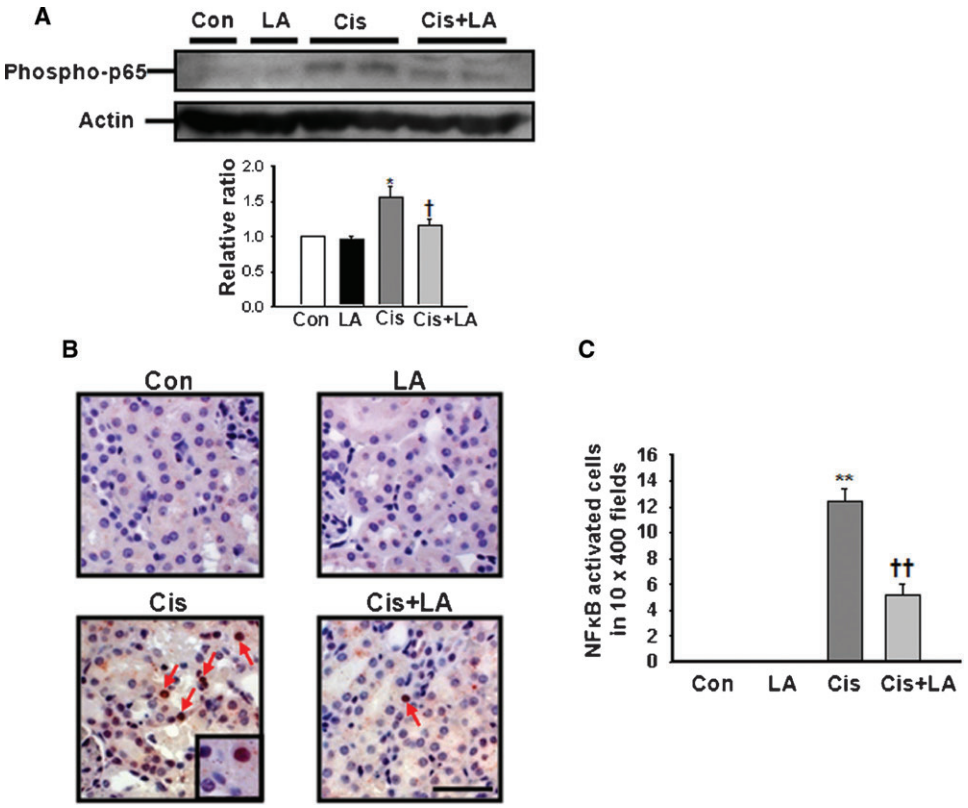

Fig. 7. Effect of α-lipoic acid on NF-κB activation during cisplatin-induced renal injury. Kidneys from mice treated with control (Con), α-LA (LA), cisplatin (Cis) and cisplatin plus α-LA (Cis + LA) were evaluated for phosphorylation of p65 of NF-κB by western blot analysis and nuclear translocation of NF-κB p65 by immunohistochemistry. (**A**) Western blots analysis of phospho-p65 expression in whole kidney tissue. Densitometric analyses are presented as the relative ratio of each protein to actin. The relative ratio measured in kidneys from control mice is arbitrarily presented as 1. Data are expressed as means ± S.E.M. of three independent experiments. $^{*}P < 0.05$ versus Con or LA; $^{\dagger}P < 0.05$ versus Cis. (**B**) The number of renal tubular cells with nuclear staining for NF-κB p65 (arrows) was significantly increased in the cisplatin group, whereas treatment with α-LA decreased the number of cells having nuclear staining for NF-κB p65 after cisplatin administration. Inset shows the nuclear staining of NF-κB p65 at ×1000 magnification. Control buffer or α-LA alone had no effect of nuclear translocation of NF-κB p65. Bar = 50 μm. (**C**) Number of renal tubular cells with nuclear staining for NF-κB p65 in 10 fields at ×400 magnification are expressed as means ± S.E.M. ($n = 10$ mice per each group). $^{**}P < 0.01$ versus Con or LA; $^{\dagger\dagger}P < 0.01$ versus Cis.

expression time course of inflammatory mediators such as TNF-α, ICAM-1 and MCP-1 at 24 and 48 h after cisplatin administration when histologic changes are not yet visible. Interestingly, kidney tissue levels of TNF-α, ICAM-1 and MCP-1 were elevated before tubular necrosis occurred, and α-LA effectively inhibited their expression. Therefore, these data suggest that a dissociation of the activation of inflammation from cellular injury is the pathophysiologic mechanism of cisplatin-induced renal injury, and that α-LA may exert anti-inflammatory effects through the inhibition of the cisplatin-induced increases in inflammatory cytokine expression before tubular injuries are apparent.

Inflammatory cell infiltration into damaged kidney tissue may be an important process in cisplatin-induced renal injury. Infiltration of macrophages into the kidney tissue is increased at 24 h–72 h after cisplatin administration [11,13]. The present study showed that the amount of CD11b-positive macrophages was increased at 72 h after cisplatin administration and that α-LA treatment effectively inhibited CD11b-positive macrophage infiltration. In possible disagreement, Lu *et al*. [13] reported that cisplatin administration increased both infiltration of macrophages and fractalkine expression, but the depletion of macrophages did not halt the cisplatin-induced renal injury. Therefore, an understanding of how macrophage infiltration is modulated will help in the prevention of cisplatin-induced renal injury.

Infiltrating inflammatory cells may be reservoirs of inflammatory cytokines and chemokines, and as such may release these molecules into damaged kidney tissues [12,13]. Following cisplatin treatment, we screened for the expression of cytokines, such as MCP-1, interferon-γ (IFN-γ), interleukin (IL)-4 and IL-10, in kidney using tissue protein ELISA. While tissue levels of IFN-γ and IL-4 were not significantly altered following cisplatin, MCP-1 protein expression was significantly increased compared to control buffer-treated mice. α-LA treatment effectively reduced the cisplatin-induced increases in tissue MCP-1 protein expression. The kidney tissue levels of IL-10, a well-known anti-inflammatory cytokine [32], were decreased after cisplatin administration in tissue protein ELISA studies. However, α-LA administration did not restore the cisplatin-induced decreases in tissue IL-10 protein levels (data not shown). These data suggest that the anti-inflammatory effect of

α-LA may be related in part to a suppression of MCP-1 expression during cisplatin-induced renal injury.

Cisplatin-induced renal injury also activates the NF-κB signalling pathway. Importantly, suppressed NF-κB activation by either anti-oxidants or a peroxisome proliferator-activated receptor-γ agonist improved damaged kidney function and morphology after cisplatin administration [11,14,15,33]. The present study demonstrated that α-LA suppressed NF-κB activation following cisplatin administration. We also evaluated the upstream signals of NFκB activation in cisplatin-induced renal injury. Elevated TNF-α is known as an important step for activation of the NFκB signalling pathway [34]. After cisplatin administration, kidney tissue levels of TNF-α were increased, but were effectively lowered by α-LA treatment. We demonstrated that cisplatin treatment increased nuclear translocation of NF-κB p65 subunits in the damaged tubules and stimulated phosphorylation of NF-κB p65 subunits in whole kidney tissue; both of these were inhibited by treatment with α-LA. Both increased phosphorylation and nuclear translocation of NF-κB p65 subunits after cisplatin administration were reduced by α-LA treatment. Therefore, an important protective mechanism against cisplatin-induced renal injury may be through the modulation of inflammatory cytokine and chemokine expression and their effects on inflammatory cell infiltration in damaged kidney tissue. Our findings suggest that α-LA has potent anti-inflammatory effects through the regulation of tissue TNF-α levels and nuclear translocation of NF-κB p65 subunits during cisplatin-induced renal inflammatory reactions.

Human pharmacokinetic studies have demonstrated that a once-daily formulation of α-LA (10 mg/kg) can be safely administered to healthy volunteers or patients with renal insufficiency [35,36]. In animal studies, high-dose α-LA (121 mg/kg) was relatively safe for long-term administration in rats [16]. We therefore used a high dose of α-LA (100 mg/kg) and found that it safely and effectively suppressed cisplatin-induced renal inflammation in mice; subjects treated with α-LA alone showed no specific side effects or toxicity.

In conclusion, we found that α-LA decreases cisplatin-induced NF-κB activation and renal inflammation, and therefore represents a potential therapeutic strategy for renal injury caused by cisplatin.

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Conflict of interest statement. None declared.

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Ischaemia/reperfusion in rat renal cortex: vesicle leakiness and Na^+, K^+-ATPase activity in membrane preparations

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Abstract

Background. Despite the central role of Na^+, K^+-ATPase (NKA) in ischaemic renal injury (IRI), cortical NKA activity values during renal ischaemia remain controversial. In this study, we explore why cortical NKA activity shows such behaviour during ischaemia in rats.

Methods. Ischaemia was induced by unilateral renal artery clamping (40 min, I) followed or not by reperfusion (60 min, IR). NKA α- and β-subunit abundance was analysed by western blot. We studied the NKA detergent sodium dodecyl sulphate (SDS) enzymatic activation in isolated membrane preparations from control and ischaemic kidneys.

Results. NKA activity was diminished in *I* cortical homogenates ($C = 9.3 \pm 1.1$, $I = 4.7 \pm 1.1^*$ μmol Pi/h mg Prot, $n = 4$–6, $^*P < 0.05$ versus *C*). This was rapidly recovered after reperfusion (IR = 9.9 ± 1.2 μmol Pi/h mg Prot). α-subunit levels were increased, while β-subunit was unchanged. At SDS 0.9 mg/ml (maximal detergent activation), the activities were indistinguishable ($C = 90.5 \pm 2.2$, $I = 91.4 \pm 15.1$ μmol Pi/h mg Prot). The analysis of detergent activation of NKA activity is widely used to estimate membrane leakiness in plasma membrane preparations. Our results suggest a higher population of sealed impermeable vesicles in preparations from ischaemic renal tissue.

Conclusion. The well-known effect of ischaemia on renal cell cytoskeleton could explain the observed changes in the leakiness of membrane vesicles.

Keywords: ischaemia/reperfusion; K^+-ATPase; Na^+; renal cortex; SDS

Introduction

Na^+, K^+-ATPase (NKA) is an integral membrane protein located in the basolateral membrane by direct interactions with membrane-associated cytoskeletal proteins [1]. The functional enzyme unit is a heterodimer of two subunits: α (∼110 kDa, catalytic) and β (∼55 kDa) [2]. Following an ischaemic insult, proximal tubular cells exhibit a disruption of the actin-based cytoskeleton [3,4], NKA dissociates from its cytoskeletal anchorage [5–7] and relocates into the apical domain in proximal tubular cells [5,8]. These