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Coenzyme Q10 treatment ameliorates acute cisplatin nephrotoxicity in mice

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ABSTRACT

The nephroprotective effect of coenzyme Q10 was investigated in mice with acute renal injury induced by a single i.p. injection of cisplatin (5 mg/kg). Coenzyme Q10 treatment (10 mg/kg/day, i.p.) was applied for 6 consecutive days, starting 1 day before cisplatin administration. Coenzyme Q10 significantly reduced blood urea nitrogen and serum creatinine levels which were increased by cisplatin. Coenzyme Q10 significantly compensated deficits in the antioxidant defense mechanisms (reduced glutathione level and superoxide dismutase activity), suppressed lipid peroxidation, decreased the elevations of tumor necrosis factor-α, nitric oxide and platinum ion concentration, and attenuated the reductions of selenium and zinc ions in renal tissue resulted from cisplatin administration. Also, histopathological renal tissue damage mediated by cisplatin was ameliorated by coenzyme Q10 treatment. Immunohistochemical analysis revealed that coenzyme Q10 significantly decreased the cisplatin-induced overexpression of inducible nitric oxide synthase, nuclear factor-κB, caspase-3 and p53 in renal tissue. It was concluded that coenzyme Q10 represents a potential therapeutic option to protect against acute cisplatin nephrotoxicity commonly encountered in clinical practice.

1. Introduction

Cisplatin is an effective chemotherapeutic agent that is widely used for treatment of malignant tumors including head and neck, ovarian, testicular, lung and breast cancers (Delord et al., 2009). Despite the antineoplastic efficacy, the optimal clinical usefulness of cisplatin is usually limited due to its dose-related nephrotoxicity (Ali and Al Moundhri, 2006; Pabla and Dong, 2008). Acute renal injury can occur after an initial dose of cisplatin with about 20% of patients experiencing various degrees of renal dysfunction (Yao et al., 2007). Cisplatin exerts its nephrotoxic effect mainly in the proximal tubular cells where it is preferentially accumulated (Ramesh and Reeves, 2005). The precise mechanisms underlying this toxicity are not fully elucidated. However, oxidative stress with increased generation of reactive oxygen species, and inflammation with increased production of proinflammatory cytokines seem to play a crucial role (Behling et al., 2006; Yano et al., 2007). Several antioxidants and anti-inflammatory agents were proved effective in protecting the kidney against the deleterious effects of cisplatin (Do Amaral et al., 2008; Kang et al., 2009; Khan et al., 2009).

Coenzyme Q10 (CoQ10) is an endogenous lipid-soluble benzoquinone compound that functions as a diffusible electron carrier in the mitochondrial respiratory chain (Lenaz et al., 2007). CoQ10 also acts as a powerful antioxidant which scavenges free radicals, prevents the initiation and propagation of lipid peroxidation in cellular biomembranes, and helps regeneration of α-tocopherol (Crane, 2001; Bentinger et al., 2007). In addition, CoQ10 has anti-inflammatory properties decreasing the production of proinflammatory cytokines as tumor necrosis factor-α (Schmelzer et al., 2007, 2008). Previous studies demonstrated the protective effects of CoQ10 in various models of oxidative and inflammatory tissue damage (Upaganlawar et al., 2006; Sohet et al., 2009; Spindler et al., 2009). Therefore, CoQ10 has the potential to protect against renal tissue injury and renal dysfunction induced by cisplatin.

This was encouraging to conduct the present study in order to evaluate the protective effect of CoQ10 in mice exposed to acute cisplatin nephrotoxicity. Also, the possible mechanisms underlying this effect were investigated.

2. Materials and methods

2.1. Animals

Male albino mice, weighing 25–30 g were obtained from the Animal House, College of Medicine, Al-Ahsa, King Faisal University. The animals were kept at standard housing facilities (24 ± 1 °C, 45 ± 5% humidity and 12 h light/dark cycle). They were supplied with standard laboratory chow and water ad libitum, and left to acclimatize for 1 week before the experiments. The experimental protocol was approved by the Local Animal Care Committee and the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

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Table 1
Effects of coenzyme Q10 (CoQ10) treatment on kidney/body-weight ratio, blood urea nitrogen (BUN) and serum creatinine levels in mice exposed to acute cisplatin (CP) nephrotoxicity.

	Control	Vehicle + CP	CoQ10 + CP	CoQ10
Kidney/body-weight ratio (1000×)	14.86 ± 0.65	18.55 ± 0.91[a]	15.63 ± 0.55[b]	15.11 ± 0.49
BUN (mg/dl)	13.31 ± 1.15	55.61 ± 4.11[a]	25.18 ± 1.66[a,b]	12.83 ± 1.47
Serum creatinine (mg/dl)	1.41 ± 0.05	2.77 ± 0.14[a]	1.69 ± 0.07[b]	1.55 ± 0.04

All the values are expressed as mean ± S.E.M., $n = 8$ in each group.
[a] $P < 0.05$ vs. control group.
[b] $P < 0.05$ vs. vehicle + CP group.

Table 2
Effects of coenzyme Q10 (CoQ10) treatment on renal malondialdehyde (MDA), reduced glutathione (GSH), tumor necrosis factor-α (TNF-α) and nitric oxide (NO) levels, and catalase and superoxide dismutase (SOD) activities in mice exposed to acute cisplatin (CP) nephrotoxicity.

	Control	Vehicle + CP	CoQ10 + CP	CoQ10
MDA (nmol/g tissue)	55.12 ± 2.41	97.29 ± 4.53[a]	68.36 ± 2.77[a,b]	50.61 ± 3.02
GSH (mmol/g tissue)	6.33 ± 0.42	1.89 ± 0.08[a]	4.26 ± 0.35[a,b]	5.19 ± 0.39
Catalase (U/g tissue)	5.15 ± 0.41	2.52 ± 0.17[a]	3.21 ± 0.27[a]	6.03 ± 0.47
SOD (U/mg protein)	41.76 ± 2.68	21.18 ± 1.99[a]	36.53 ± 2.78[b]	46.12 ± 4.06
TNF-α (pg/100 mg tissue)	ND	63.65 ± 4.72[a]	40.22 ± 2.99[a,b]	ND
NO (nmol/100 mg tissue)	96.27 ± 7.25	181.53 ± 11.64[a]	132.12 ± 9.94[b]	108.41 ± 9.06

All the values are expressed as mean ± S.E.M., $n = 8$ in each group, ND = non-detectable.
[a] $P < 0.05$ vs. control group.
[b] $P < 0.05$ vs. vehicle + CP group.

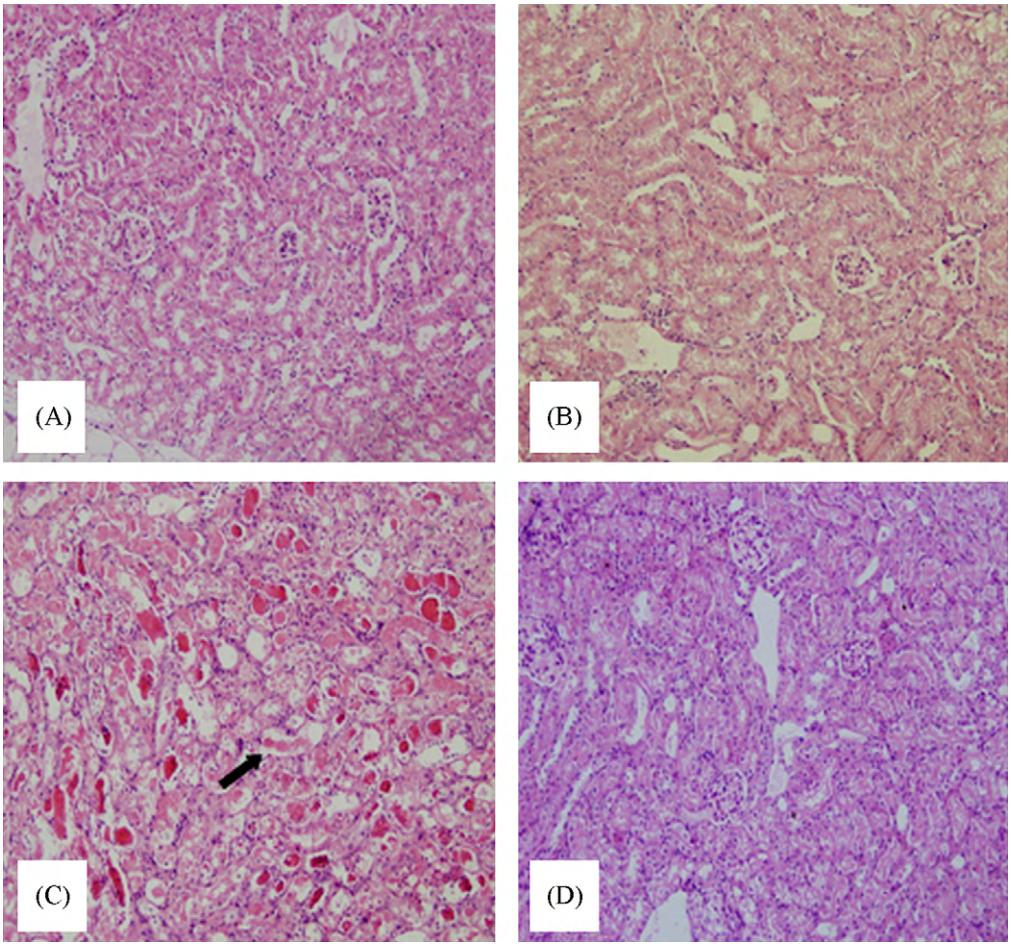

Fig. 1. Photomicrographs of mice kidney (H&E, 200×) from: (A) control group and (B) CoQ10 alone treated group showing normal renal architecture; (C) cisplatin group without CoQ10 treatment showing extensive necrosis with dilatation, vacuolar degeneration, epithelial desquamation and intraluminal cast formation (black arrow) in the proximal tubules; (D) CoQ10 plus cisplatin group displaying marked improvement in the histological picture which is comparable to that of the control group.

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Table 3
Effects of coenzyme Q10 (CoQ10) treatment on renal platinum, selenium and zinc ion concentrations in mice exposed to acute cisplatin (CP) nephrotoxicity.

	Control	Vehicle + CP	CoQ10 + CP	CoQ10
Platinum ion (μg/g tissue)	ND	13.77 ± 1.16[a]	9.54 ± 0.64[a,b]	ND
Selenium ion (μg/g tissue)	8.12 ± 0.63	3.37 ± 0.25[a]	6.81 ± 0.44[b]	8.59 ± 0.71
Zinc ion (μg/g tissue)	49.58 ± 3.41	21.66 ± 1.94[a]	37.13 ± 2.85[a,b]	52.73 ± 4.05

All the values are expressed as mean ± S.E.M., $n = 8$ in each group, ND = non-detectable.
[a] $P < 0.05$ vs. control group.
[b] $P < 0.05$ vs. vehicle + CP group.

2.2. Drugs

Cisplatin powder and CoQ10 powder were obtained from Sigma Chemical Company, USA. Cisplatin was dissolved in normal saline, while CoQ10 was prepared in 1% aqueous solution of Tween 80.

2.3. Experimental protocol

The mice were randomly divided into four equal groups ($n = 8$, each). The first group received a single i.p. injection of normal saline (vehicle of cisplatin) and served as control. Nephrotoxicity was induced in animals of the second and third groups by a single i.p. injection of cisplatin at a dose of 5 mg/kg (Behling et al., 2006). The second and third group animals received a daily i.p. injection of the vehicle of CoQ10 (1% aqueous solution of Tween 80) or CoQ10 at a dose of 10 mg/kg (Upaganlawar et al., 2006), respectively, for 6 consecutive days starting 1 day before cisplatin administration. The mice of the fourth group received CoQ10 for 6 consecutive days without induction of cisplatin nephrotoxicity. The body weight of each animal was recorded after completion of drug administration.

2.4. Sample preparation and biochemical studies

The animals were sacrificed 5 days following cisplatin administration. Blood samples were collected and centrifuged for 10 min at 5000 rpm to obtain clear sera which were stored at −20 °C for subsequent measurement of blood urea nitrogen (BUN) and serum creatinine levels using colorimetric assay kits according to the recommendations of the manufacturer (Stanbio Laboratory, USA).

The kidneys were isolated from each animal and their fresh weight was recorded. The renal cortex was separated, kept at −80 °C and subsequently homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The homogenates were centrifuged at 5000 rpm for 10 min at 4 °C. The resulting supernatant was used for determination of malondialdehyde (MDA) and reduced glutathione (GSH) levels, and catalase and superoxide dismutase (SOD) activities using colorimetric assay kits according to the manufacturer's instructions (Biodiagnostic, Egypt). The level of nitric oxide (NO) was assayed using colorimetric assay kit as indicated by the manufacturer (Cayman Chemical Company, USA). Also, the level of tumor necrosis factor-α (TNF-α) in renal homogenates was determined by enzyme-linked immunosorbent assay (ELISA) using rat TNF-α immunoassay kit according to the recommendations of the manufacturer (R&D Systems, USA).

In addition, parts of the renal cortical tissue were dried overnight at 80 °C and the dry weight was recorded. The samples were then digested with equal volumes of 30% (w/v) H_2O_2 and 70% (w/v) nitric acid, and the clear digest was diluted with ultrapure water (1:3). Renal platinum, selenium and zinc ion concentrations were analyzed using inductively coupled plasma optical emission spectrometer (Optima 2100 DV, PerkinElmer, UK) at 265.94, 196.02 and 206.2 nm, respectively, with sample-based standards.

2.5. Histopathological examination of renal tissue

The left kidneys were fixed in 10% formalin solution and then dehydrated in ascending grades of alcohol and embedded in paraffin. Sections at 4 μm-thickness were taken, stained with hematoxylin and eosin (H&E) and examined under light microscope by a pathologist unaware of the treatment protocol.

2.6. Immunohistochemical examinations of renal tissue

Four micrometer thick sections were prepared from different animal groups and immunohistochemistry was performed. Sections were deparaffinised, rehydrated, and endogenous peroxidase activity was blocked with H_2O_2 in methanol. Sections were pre-treated in citrate buffer (pH 6.0) in a microwave. Sections were incubated at room temperature with monoclonal anti-inducible nitric oxide synthase (iNOS), anti-nuclear factor-κB (NF-κB), anti-caspase-3 and anti-p53 antibodies (Thermo Scientific, USA, dilution 1:100). UltraVision detection System (Thermo Scientific) was used as follows; sections were incubated with biotinylated goat anti-polyvalent, then with streptavidin peroxidase and finally with DAB plus chromogen. Slides were counterstained with hematoxylin. The slides were visualized under light microscope and the extent of cell immunopositivity was assessed.

2.7. Statistical analysis

All values are expressed as mean ± S.E.M. The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test for multiple comparisons using SPSS for Windows (version 11). Differences were considered significant at $P < 0.05$.

3. Results

3.1. Effects of CoQ10 on BUN, serum creatinine and kidney/body-weight ratio

Mice received a single dose of cisplatin (5 mg/kg, i.p.) showed significant increases in BUN and serum creatinine levels, and kidney/body-weight ratio as compared to the control group. CoQ10 treatment resulted in a significant reduction in BUN, and restored normal serum creatinine level and kidney/body-weight ratio (Table 1).

3.2. Effects of CoQ10 on renal biochemical analysis

Treatment with CoQ10 significantly attenuated the depletion of the antioxidant defense mechanisms (GSH level and SOD activity), suppressed lipid peroxidation, and decreased the elevations of TNF-α and NO levels in renal tissue resulted from cisplatin administration. However, CoQ10 treatment did not significantly alter the cisplatin-induced reduction in renal catalase activity (Table 2).

3.3. Effects of CoQ10 on renal platinum, selenium and zinc ion concentrations

A significant rise in platinum ion concentration associated with significant reductions in selenium and zinc ion concentrations was observed in the kidney tissue of mice received cisplatin as com-

Table 4
Effects of coenzyme Q10 (CoQ10) treatment on the percentage expression of inducible nitric oxide synthase (iNOS), nuclear factor-κB (NF-κB), caspase-3 and p53 in the kidney of mice exposed to acute cisplatin (CP) nephrotoxicity.

	Control	Vehicle + CP	CoQ10 + CP	CoQ10
iNOS	ND	12.47 ± 1.16[a]	3.12 ± 0.29[a,b]	ND
NF-κB	ND	14.58 ± 1.27[a]	5.11 ± 0.35[a,b]	ND
Caspase-3	ND	15.36 ± 1.24[a]	2.24 ± 0.19[b]	ND
p53	ND	10.21 ± 1.08[a]	ND[b]	ND

All the values are expressed as mean ± S.E.M., $n = 8$ in each group, ND = non-detectable.
[a] $P < 0.05$ vs. control group.
[b] $P < 0.05$ vs. vehicle + CP group.

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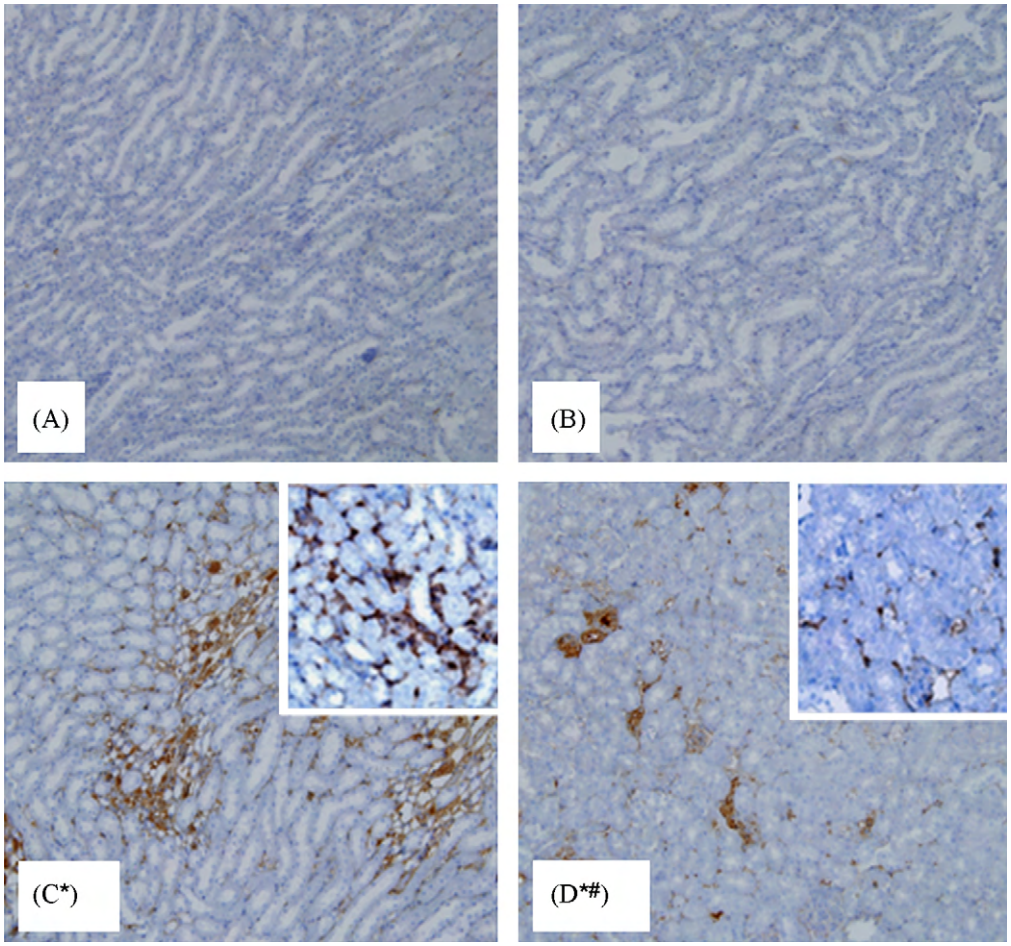

Fig. 2. Immunohistochemical staining of inducible nitric oxide synthase (iNOS) in mice kidney (200×) from: (A) control group and (B) CoQ10 alone treated group showing no expression of iNOS; (C) cisplatin group without CoQ10 treatment showing a significant increase in iNOS immunoreactivity in the cytoplasm of proximal tubular cells (inserted figure 400×); (D) CoQ10 plus cisplatin group showing a significant decrease in iNOS immunostaining (inserted figure 400×). Brown color indicates iNOS positivity. Values are expressed as mean ± S.E.M. ($n = 8$), $^{*}P < 0.05$ vs. control group, $^{\#}P < 0.05$ vs. cisplatin group without CoQ10 treatment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

pared to the control animals. However, CoQ10-treated mice had a significantly lower renal platinum level and higher selenium and zinc levels in comparison with the cisplatin group non-treated with CoQ10 (Table 3).

3.4. Effects of CoQ10 on renal histopathology

Cisplatin administration caused severe and widespread necrosis with dilatation, vacuolar degeneration, epithelial desquamation and intraluminal cast formation in the proximal convoluted tubules. The histopathological renal damage induced by cisplatin was minimal in animals received CoQ10 treatment (Fig. 1).

3.5. Effects of CoQ10 on renal immunohistochemistry

Immunohistochemical examinations of mice kidney revealed that cisplatin administration caused significant increases in the immunoreactivity of iNOS, NF-κB and caspase-3 in the cytoplasm of proximal tubular cells as compared to the control group. This was associated with a significant rise in p53 immunopositivity in the nuclei of proximal tubular cells as compared to the control group. On the other hand, CoQ10-treated mice showed significant reductions in the cisplatin-induced overexpression of iNOS, NF-κB, caspase-3 and p53 in the kidney tissue as compared to the cisplatin group non-treated with CoQ10 (Figs. 2–5, and Table 4).

4. Discussion

Recent evidence suggests that cisplatin nephrotoxicity occurs as a result of oxidative stress and increased generation of superoxide anion, hydrogen peroxide and hydroxyl radicals due to increased activity of NADPH oxidase, xanthine oxidase and adenosine deaminase (Gulec et al., 2006; Chirino et al., 2008). Increased production of reactive oxygen species decreases the activity of the antioxidant enzymes (catalase, SOD and glutathione peroxidase), depletes GSH and protein thiols, and enhances lipid peroxidation in renal tissue (Ali et al., 2007). Also, cisplatin induces a cascade of inflammatory reactions with increased production of proinflammatory cytokines, particularly TNF-α which is responsible for further kidney tissue injury (Yano et al., 2007; Kang et al., 2009).

In addition, increased NO production in the renal tissue is involved in the pathogenesis of cisplatin nephrotoxicity. This can be explained by the ability of TNF-α to up-regulate the iNOS enzyme (Mukhopadhyay et al., 2010). Excess NO reacts with superoxide anion to generate peroxynitrite radical that causes further cell damage by oxidizing and nitrating cellu-

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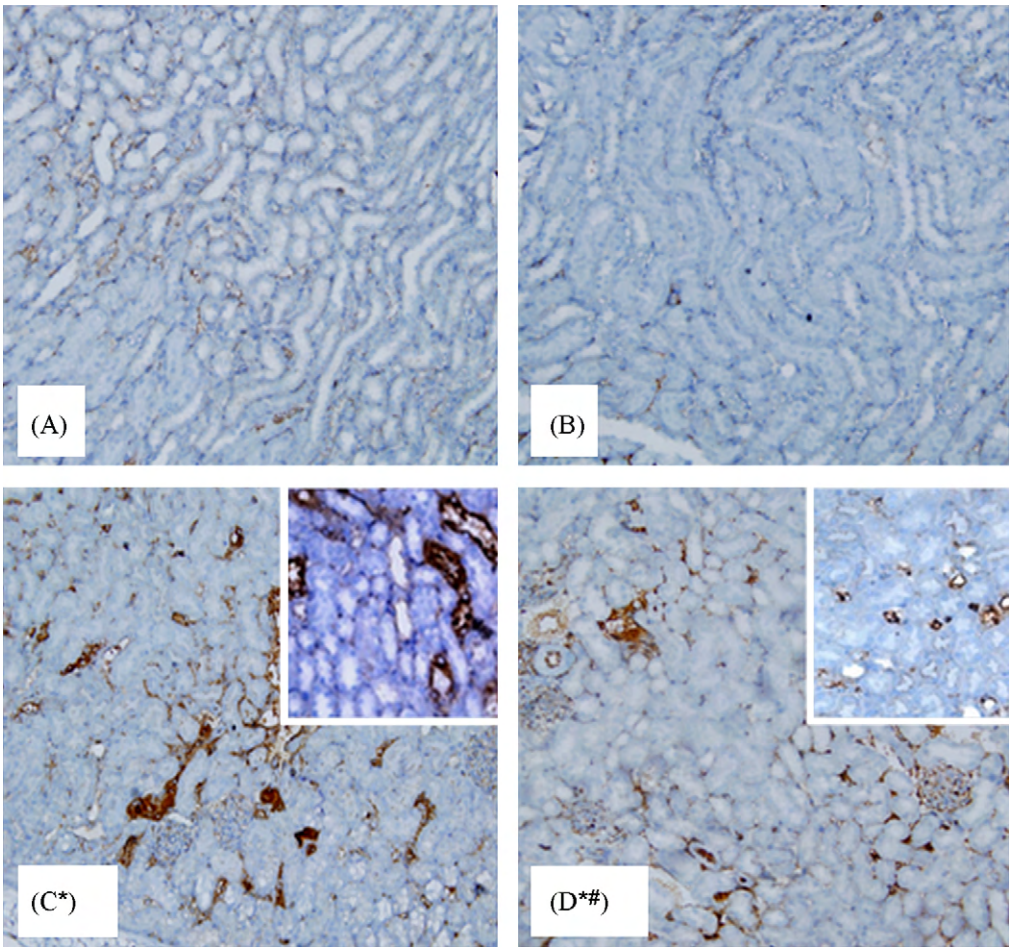

Fig. 3. Immunohistochemical staining of nuclear factor-κB (NF-κB) in mice kidney (200×) from: (A) control group and (B) CoQ10 alone treated group showing no expression of NF-κB; (C) cisplatin group without CoQ10 treatment showing a significant increase in NF-κB immunoreactivity in the cytoplasm of proximal tubular cells (inserted figure 400×); (D) CoQ10 plus cisplatin group demonstrating a significant reduction in NF-κB immunostaining (inserted figure 400×). Brown color indicates NF-κB positivity. Values are expressed as mean ± S.E.M. ($n = 8$), $^{*}P < 0.05$ vs. control group, $^{\#}P < 0.05$ vs. cisplatin group without CoQ10 treatment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

lar macromolecules. Also, excess NO depletes intracellular GSH increasing the susceptibility to oxidative stress (Jung et al., 2009a).

In agreement with previous studies, the present one confirmed that oxidative stress, increased lipid peroxidation, depletion of antioxidant defenses and increased production of proinflammatory mediators are implicated in the pathogenesis of cisplatin-induced acute renal injury. Also, the present work demonstrated that CoQ10 treatment provided a significant protective effect in mice exposed to acute cisplatin nephrotoxicity as indicated by improvement of the disturbed biochemical parameters and amelioration of renal tissue damage observed by histopathological and immunohistochemical examinations.

CoQ10 is a naturally occurring hydrophobic compound that is not only a critical component of the mitochondrial respiratory chain, but also a powerful antioxidant. CoQ10 suppresses the generation of reactive oxygen species by blunting the expression of NADPH oxidase (Sohet et al., 2009), and scavenges lipid peroxidation products during free radical reactions (Tsuneki et al., 2007). CoQ10 also suppresses excess NO production and prevents nitrative tissue stress (Jung et al., 2009b). In addition, CoQ10 exhibits anti-inflammatory properties reducing the release of proinflammatory cytokines during inflammatory injury (Schmelzer et al., 2007, 2008).

It was reported that cisplatin administration caused NF-κB activation with subsequent inflammatory reactions responsible for renal injury (Sung et al., 2008; Kang et al., 2009). Elevated TNF-α is known as an important step for activation of the NF-κB signaling pathway (Li and Verma, 2002). Previous studies showed that agents which inhibit TNF-α production and NF-κB activation effectively ameliorated cisplatin nephrotoxicity (Sung et al., 2008; Kang et al., 2009). The present results revealed that CoQ10 treatment significantly suppressed lipid peroxidation, restored the antioxidant defense mechanisms, attenuated the overproduction TNF-α and NO, and reduced the expression of NF-κB and iNOS in the kidneys of mice exposed to acute cisplatin nephrotoxicity. The nephroprotective effect of CoQ10 can be attributed to its ability to inhibit the activation of NF-κB signaling pathway which promotes the transcription of NADPH oxidase, TNF-α and iNOS genes (Takaya et al., 2006; Morishima et al., 2009).

Also, it was demonstrated that cisplatin treatment induced p53 phosphorylation and accumulation mainly in the proximal tubular cell nuclei, and this correlated with the development of renal injury and dysfunction (Wei et al., 2007; Jiang and Dong, 2008). Induction of p53 mediates cell apoptosis through transcriptional up-regulation of target proapoptotic genes leading finally to activation of caspase family of proteases and apoptotic cell death (Jiang et al., 2006; Yano et al., 2007). This was evidenced by the fact

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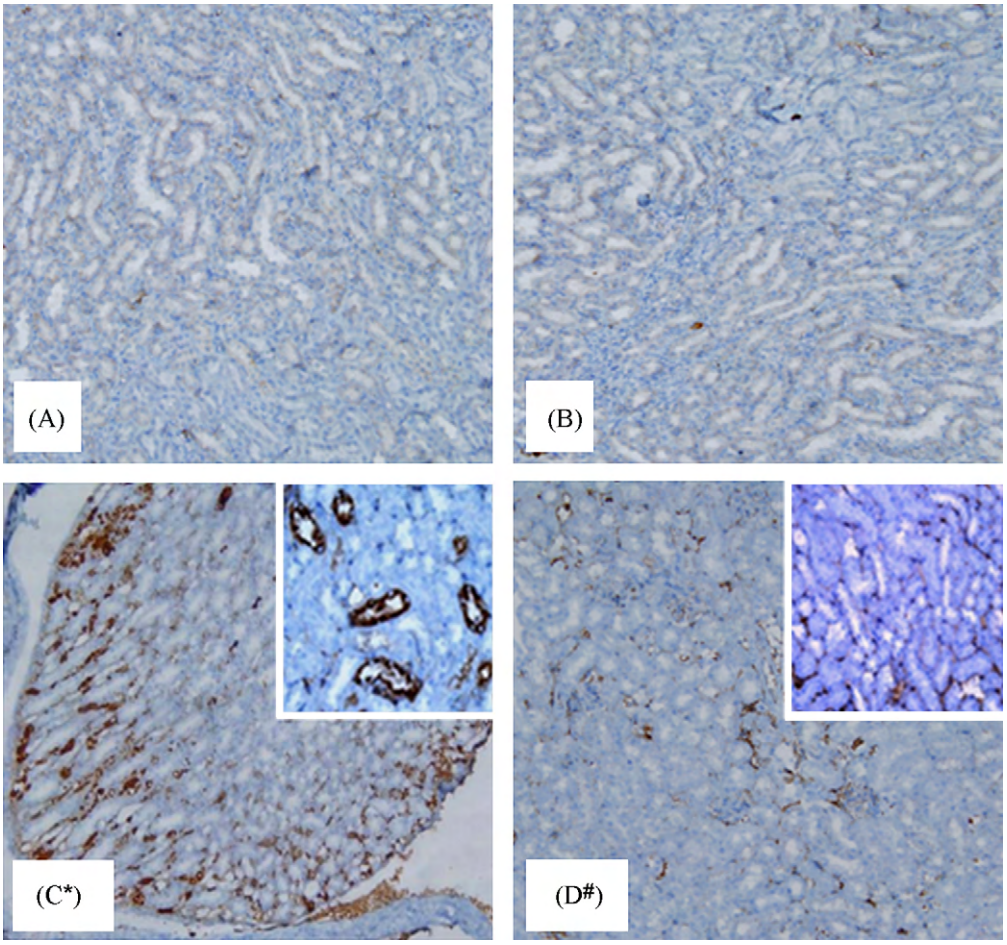

Fig. 4. Immunohistochemical staining of caspase-3 in mice kidney (200×) from: (A) control group and (B) CoQ10 alone treated group showing no expression of caspase-3; (C) cisplatin group without CoQ10 treatment showing a significant increase in caspase-3 immunoreactivity in the cytoplasm of proximal tubular cells (inserted figure 400×); (D) CoQ10 plus cisplatin group showing a significant decrease in caspase-3 immunostaining (inserted figure 400×). Brown color indicates caspase-3 positivity. Values are expressed as mean ± S.E.M. ($n = 8$), $^{*}P < 0.05$ vs. control group, $^{\#}P < 0.05$ vs. cisplatin group without CoQ10 treatment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

that cisplain-induced renal cell apoptosis was significantly ameliorated by the use of pifithrin-α, a pharmacological inhibitor of p53, and in p53-deficient mice (Wei et al., 2007). The exact mechanisms leading to p53 activation in response to cisplatin are not well understood. However, oxidative stress with increased generation of reactive oxygen species, particularly hydroxyl radicals, correlated well with p53 activation following cisplatin incubation of renal tubular cells. Scavengers of reactive oxygen species attenuated the induction of p53 and protected the renal tubular cells from cisplatin-mediated apoptosis (Jiang et al., 2007). In addition, NF-κB could be another molecule causing p53 induction in cisplatin nephrotoxicity. The activated NF-κB has been shown to act upstream of p53 to modulate its transcription activity and induce cell apoptosis (Aleyasin et al., 2004; Sung et al., 2008).

The present study revealed that CoQ10 treatment significantly decreased the cisplatin-induced overexpression of p53 protein and caspase-3, an executioner of apoptosis, in renal tubular cells. Therefore, it could be stated that CoQ10 protected against cisplatin-induced renal cell apoptosis. The reduced p53 and caspase-3 activities observed with CoQ10 treatment may be due to its free radical scavenging activity, anti-inflammatory action with reduced TNF-α production, and attenuation of NF-κB expression. However, this needs to be clarified by further investigations.

It was recognized that inhibition of p53 may limit apoptosis of cancer cells and thus reduce the therapeutic effect of cisplatin. However, over 50% of cancers have p53 mutations, yet cisplatin still effective in treating these tumors, indicating that cisplatin therapy is not entirely p53-dependent (Gudkov and Komarova, 2005). Also, CoQ10 was found effective in preventing doxorubicin cardiotoxicity without interfering with its antineoplastic activity, although p53 activation is known to be involved in the anticancer effect of doxorubicin (Conklin, 2000; Fujiwara et al., 2006). However, this point warrants further investigation.

In the present study, CoQ10 significantly attenuated the increase in platinum concentration, and the reductions in selenium and zinc concentrations in renal tissue resulted from cisplatin administration. Selenium is an essential component of glutathione peroxidase, while zinc acts as a cofactor for SOD. Also, both elements preserve GSH and induce metallothionein which have antioxidant and metal-chelating properties (Rooney, 2007). The decreased renal platinum overload in response to CoQ10 treatment observed in the present study may be due to the fact that platinum can be bound with high affinity to the thiols in GSH and metallothionein molecules which are essential for intracellular heavy metal detoxification (Satoh et al., 2000; Rooney, 2007). It could be stated that CoQ10, through its antioxidant activity, restored the depleted selenium and zinc, and decreased the platinum burden in renal tissue which results in an additional protective effect against cisplatin nephrotoxicity.

The results of the present study indicate that CoQ10 effectively protected the kidney tissue against cisplatin-induced acute nephrotoxicity in mice. The antioxidant and anti-inflammatory activities

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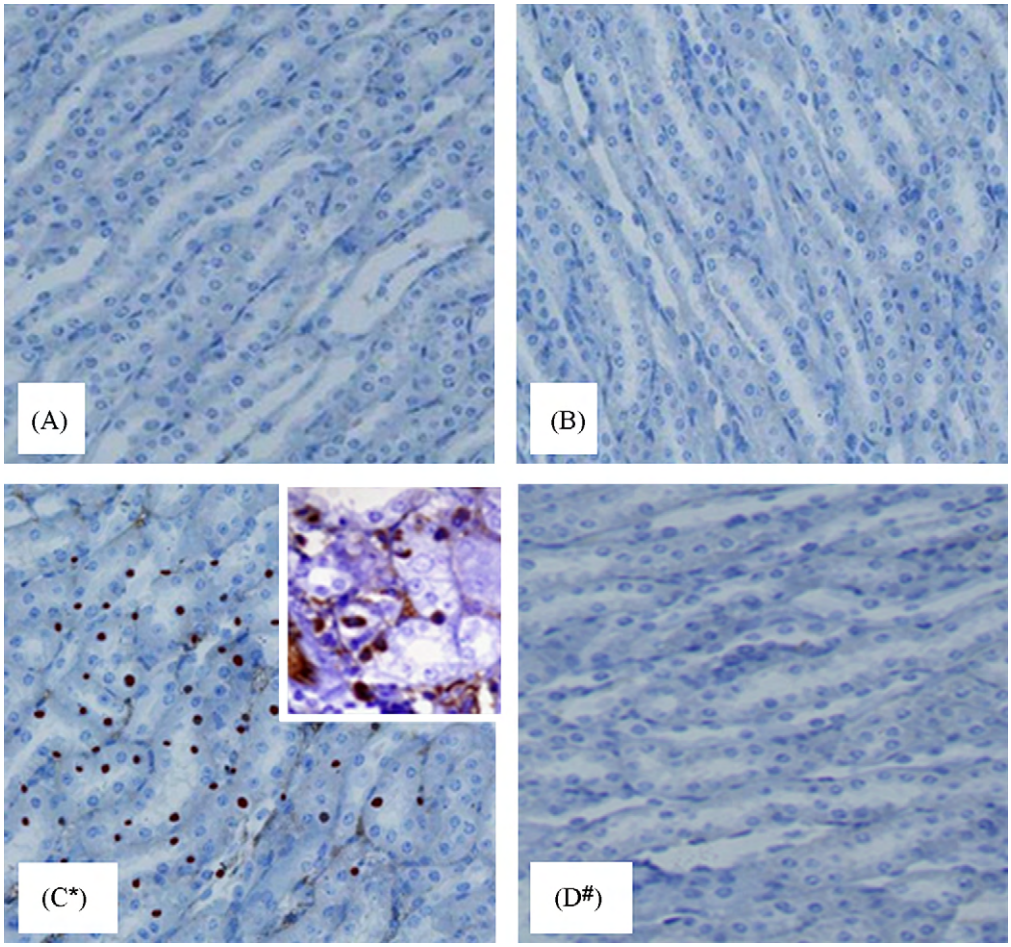

Fig. 5. Immunohistochemical staining of p53 in mice kidney (400×) from: (A) control group and (B) CoQ10 alone treated group showing no expression of p53; (C) cisplatin group without CoQ10 treatment showing a significant increase in p53 immunoreactivity in the nuclei of proximal tubular cells (inserted figure 1000×); (D) CoQ10 plus cisplatin group demonstrating negative p53 immunostaining. Brown color indicates p53 positivity. Values are expressed as mean ± S.E.M. ($n = 8$), $^{*}P < 0.05$ vs. control group, $^{\#}P < 0.05$ vs. cisplatin group without CoQ10 treatment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

can be considered the main factors responsible for the nephroprotective effect of CoQ10. Therefore, CoQ10 represents a potential candidate to prevent renal injury and dysfunction which is a major and dose-limiting problem during cisplatin therapy.

Conflict of interest

There are none.

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