Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical Pharmacology 70 (2005) 1408–1416
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Serum thymic factor, FTS, attenuates cisplatin nephrotoxicity by suppressing cisplatin-induced ERK activation

Yuka Kohda [a,*], Yoshiko Kawai [a], Noriaki Iwamoto [a], Yoshiko Matsunaga [a], Hiromi Aiga [a], Akira Awaya [b], Munekazu Gemba [a]

[a] *Division of Pharmacology, Osaka University of Pharmaceutical Sciences, Nasahara, Takatsuki, Osaka 569-1094, Japan*
[b] *Japan Science and Technology, Tachikawa, Tokyo 190-0012, Japan*

Received 3 June 2005; accepted 8 August 2005

Abstract

Serum thymic factor (FTS), a thymic peptide hormone, has been reported to attenuate the bleomycin-induced pulmonary injury and also experimental pancreatitis and diabetes. In the present study, we investigated the effect of FTS on *cis*-diamminedichloroplatinum II (cisplatin)-induced nephrotoxicity. We have already demonstrated that cephaloridine, a nephrotoxic antibiotic, leads to extracellular signal-regulated protein kinase (ERK) activation in the rat kidney, which probably contributes to cephaloridine-induced renal dysfunction. The aim of this study was to examine the effect of cisplatin on ERK activation in the rat kidney and also the effect of FTS on cisplatin-induced nephrotoxicity in rats. In vitro treatment of LLC-PK_1 cells with FTS significantly ameliorated cisplatin-induced cell injury. Treatment of rats with intravenous cisplatin for 3 days markedly induced renal dysfunction and increased platinum contents in the kidney cortex. An increase in pERK was detected in the nuclear fraction prepared from the rat kidney cortex from days 1 to 3 after injection of cisplatin. FTS suppressed cisplatin-induced renal dysfunction and ERK activation in the kidney. FTS did not influence any Pt contents in the kidney after cisplatin administration. FTS has been shown to enhance the in vivo expression of heat shock protein (HSP) 70 in the kidney cortex. The beneficial role of FTS against cisplatin nephrotoxicity may be mediated in part by HSP70, as suggested by its up-regulation in the kidney cortex treated with FTS alone. Our results suggest that FTS participates in protection from cisplatin-induced nephrotoxicity by suppressing ERK activation caused by cisplatin.

Keywords: *cis*-Diamminedichloroplatinum II (cisplatin); Nephrotoxicity; Serum thymic factor (FTS); ERK activation; HSP70

1. Introduction

cis-Diamminedichloroplatinum II (cisplatin) is one of the most effective anti-cancer drugs, and is widely used for the treatment of a variety of human solid tumors, including cancers of the ovary, testis, bladder, head and neck, lung, cervix and endometrium [1–4]. The efficacy of cisplatin is limited, however, by its dose-limiting nephrotoxicity [5–10]. Cisplatin is toxic to the renal proximal tubules, especially to the S3 segments [11–13]. Evidence has been provided that oxidative stress, DNA damage, apoptosis and inflammation are involved in the pathogenesis of cisplatin-induced nephrotoxicity [14–25].

Anti-cancer platinum compounds that do not induce renal toxicity, and replace cisplatin, have been developed [26]. Carboplatin, a second-generation platinum-containing anti-cancer drug, is currently being used against human cancers. However, high-dose carboplatin chemotherapy can cause renal tubular injury in cancer patients [27,28]. Nedaplatin, a platinum compound, has anti-tumor activity equivalent to that of cisplatin; however, renal toxicity is relieved [29,30]. We reported the relationship between cisplatin or nedaplatin-induced nephrotoxicity and renal accumulation in rats [31]. In the present, cisplatin and its analogs carboplatin and nedaplatin are widely used anti-tumor drugs. The mechanism of nephrotoxicity from cisplatin, as a platinum compound, is still worth investigating.

Serum thymic factor (FTS) is a nonapeptide thymic hormone, which was first isolated from pig serum and

* Corresponding author. Tel.: +81 72 690 1053; fax: +81 72 690 1053.
E-mail address: kohda@gly.oups.ac.jp (Y. Kohda).

0006-2952/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2005.08.002

then from the thymus [32]. FTS is secreted by thymic epithelial cells and is involved in functional activation and differentiation of T cells [33,34]. In addition, FTS has been reported to exert a variety of biological activities both in vivo and in vitro, including an immunobiological effect [35–38] and preventive effect on experimental pancreatitis and diabetes induced by alloxan or streptozotocin and also myocarditis caused by the encephalomyocarditis virus in mice [39,40]. Other studies have reported that FTS suppresses acute experimental allergic encephalomyelitis and skin fibrosis during wound repair [41,42].

Heat shock proteins (HSP) are known to confer cellular protection from a variety of insults, which include temperature elevation, ischemia, anoxia and certain heavy metal ions in both in vitro and in vivo models [43–46]. HSP70 has been shown to be present at low levels in glomerular and tubular epithelial cells of normal rat kidneys [47]. It has been reported that over expressed HSP70 plays a direct role in protecting renal tubular cells against cisplatin toxicity [48].

Mitogen-activated protein kinases (MAPKs) are important mediators of signal transduction processes that serve to coordinate cellular responses to a variety of extracellular stimuli. Mammals express at least four distinctly regulated groups of MAPKs, the extracellular signal-regulated protein kinase (ERK) 1/2, c-Jun N-terminal kinase (JNK), p38 MAP kinase and ERK5 cascades which are capable of responding to different stimuli, such as cellular stress and growth factors [49,50]. Cisplatin can activate the ERK, JNK, and p38 MAP kinase pathways in various systems including cultured tubular cells [51–55], but a specific role for MAPKs in mediating renal dysfunction remains unknown. Numerous studies have demonstrated that the ERK pathway is mainly activated by a variety of growth factors and is known to be associated with cellular proliferation and differentiation [56,57]. The protective role of suppressing the ERK pathway in cisplatin-induced renal injury was suggested by a study that demonstrated the pharmacological inhibition of ERK [58]. Several in vivo studies have also shown that the ERK cascade is phosphorylated in the damaged brain caused by ischemia and hypoglycemia [59,60]. However, the ERK pathway has been known to play a role in giving a survival advantage to cells [61,62]. The biological outcome of ERK activation may be attributed to differences in stimuli and cell types. A preliminary study from our laboratory has demonstrated that FTS attenuates cephaloridine-mediated renal dysfunction by suppressing the ERK activation induced by cephaloridine.

In the present study, we investigated whether FTS prevents the development of cisplatin-induced renal injury in vivo and in vitro. The aim of this study was also to examine if the protective effect of FTS treatment on cisplatin-induced nephrotoxicity is associated with the suppression of the ERK pathway and the enhancement HSP70 expression in rat kidney.

2. Materials and methods

2.1. Chemicals

Cisplatin was supplied by the Sigma Chemical Co. (St. Louis, MO, USA). FTS was supplied by Carlbiotech (Copenhagen, Denmark). Dulbecco's Modified Eagle Medium (D-MEM) and nutrient medium F-12 (1:1) were purchased from Invitrogen Co. (Carlsbad, CA. USA). Fetal bovine serum was obtained from Trace Scientific, Ltd. (Melbourne, Australia). The anti-phospho MAP kinase (pERK) and α-tubulin antibodies was purchased from the Sigma Chemical Co. Antibodies specific to ERK, HSP70 and HSP90 were from BD Transduction Laboratories (Lexington, KY, USA). Secondary anti-mouse IgG (H&L, horseradish peroxidase-linked) was from Amersham Pharmacia Biotech (NJ, USA). Protease inhibitor was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals used were of the highest purity available (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

2.2. Cell culture

LLC-PK_1 cells, a cultured renal epithelial cell line derived from the porcine kidney, were cultured in Dulbecco's Modified Eagle Medium containing nutrient mixture F-12 (D-MEM/F-12) supplemented with 5% fetal bovine serum (FBS) in a humidified atmosphere of 95% air and 5% CO_2 at 37° for 4 days. Two hours after the cells being fed fresh medium without FBS on the fourth day, cisplatin was added to the fresh medium.

2.3. Determination of cell injury

The index of renal cell injury was lactate dehydrogenase (LDH) leakage from the cells into the medium. The medium containing the cells was centrifuged at 4° and 3000 rpm for 10 min to isolate the supernatant. Thereafter, the precipitating cells were fused with 1% Triton X-100, and ultrasonic treatment was performed for 15 s to homogenize the cell suspension. We measured LDH activity in the supernatant and cell suspension using a commercial kit (Wako Pure Chemical Industries, Ltd.) and calculated the rate at which LDH was released from the cells to the medium as an index of cell injury. LDH release was calculated as follows:

$$\text{LDH release}\,(\%) = [\text{LDH activities of the medium}/\text{total (medium and cell) LDH activities}] \times 100$$

2.4. Cisplatin-induced nephrotoxicity in rats

Sprague–Dawley rats were used in all experiments. They were the progeny of rats obtained from the SLC,

Inc. Shizuoka, Japan) and were maintained in the central animal facility of our university. The animals were provided with a commercial diet and water ad libitum under temperature-, humidity- and lighting-controlled conditions (22 ± 2°, 55 ± 5% and a 12-h light:12-h dark cycle, respectively). Six-week-old male rats received a single intravenous injection of 5 mg/kg cisplatin. On days 1 and 0 before cisplatin administration, FTS was injected intravenously via the tail vein. The animals were sacrificed under pentobarbital anesthesia (50 mg/kg, i.p.), and their kidneys were removed on days 1–3 after the injections. Blood samples were drawn from the abdominal aorta after the above days of cisplatin treatment. Urine was collected into bottles on ice after cisplatin treatment by placing the rats in metabolic cages for 18 h. These samples were used to measure serum creatinine, blood urea nitrogen (BUN) and urinary glucose, *N*-acetyl-β-D-glucosamidase (NAG) and protein levels by a colorimetric method using a spectrophotometer.

2.5. Platinum contents as an index of cisplatin accumulation in the renal cortex

On days 1–3, kidney tissue accumulations of cisplatin were investigated by atomic absorption analysis, regarding the platinum contents of the renal cortex as the drug level. The renal cortex (100 mg) was placed in a dryer at 100° overnight, and kept in 1 ml of 61% HNO_3. Platinum levels were quantified by flameless atomic absorption spectrometry (Solaar A-880, Jarrell-Ash). The platinum standard solution (Wako Pure Chemical Industries, Ltd.) included equivalent amounts of HNO_3.

2.6. Preparation of the nuclear fraction

Nuclear fractions were prepared from the kidney cortex of rats treated with cisplatin and FTS by a modification of the method of Dignam et al. [63]. Kidneys were rapidly placed in ice-cold hypotonic buffer, which consisted of 10 mM HEPES (pH 7.6), 15 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol and 0.2% Nonidet P-40 with protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin and 1 μg/ml pepstatin) to prevent proteolysis and/or dephosphorylation. The kidneys were homogenized at 0° in a glass homogenizer and centrifuged at 850 × *g* for 10 min at 4°. The resultant pellets were suspended in hypotonic buffer, and recentrifuged at 850 × *g* for 10 min at 4°. The nuclear pellet was disrupted in hypertonic buffer, which consisted of 25 mM HEPES (pH 8.0), 50 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, 0.4 M NaCl and protease inhibitors, for 30 min on ice for use as the nuclear fraction and recentrifuged at 18,000 × *g* for 15 min at 4°. Its supernatant was used as the soluble nuclei fraction and for Western blot analysis. The PIERCE protein estimation kit was used to determine the protein concentration with bovine serum albumin as the standard.

2.7. Determination of ERK phosphorylation in the nuclear fraction by Western blotting analysis

Equal amounts of the soluble nuclear protein (10 μg protein) in the above fraction were separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were transferred to nitrocellulose membranes using a semi-dry blotting system. The membranes were blocked with 3% bovine serum albumin (BSA) in buffer containing 50 mM Tris–HCl (pH 7.5), 150 mM NaCl and 0.1% Tween 20 (TBST) overnight at 4°, and then incubated with the primary antibody specific against phospho-ERK (pERK) in 1% BSA–TBST for 30 min at 37°. The membranes were washed three times in TBST to remove unbound antibodies and then incubated with a horseradish peroxidase-conjugated secondary antibody in 1% TBST for 30 min at 37°. Enhanced chemiluminescence (ECL) Western blotting detection reagents (Amersham Pharmacia Biotech, NJ, USA) were used to detect the immunoreactive bands of pERK.

2.8. Determination of HSP70 and HSP90 expression levels in the kidney cortex by Western blotting analysis

Cortical tissues were homogenized in ice-cold mammalian tissue lysis/extraction reagent (CelLytic-MT) (Sigma Chemical Co.) supplemented with protease inhibitor cocktail (Nacalai, Tesque), followed by centrifugation at 18,000 × *g* for 30 min. The supernatant was collected, and the protein concentration was measured by the PIERCE protein estimation kit. Equal amounts of homogenate protein were separated by 10% SDS-PAGE gel electrophoresis under reducing conditions. The separated proteins were electrotransferred to nitrocellulose membranes (Amersham International, Buckingham, UK). The membranes were blocked with 5% skimmed milk in phosphate-buffered saline containing 1% Tween 20 (PBS-T) overnight at 4°, and then incubated with the primary antibody specific against the anti-HSP70 or anti-HSP90 for 30 min at 37°. After extensive washing with PBS-T, the bound antibodies were detected with a horseradish peroxidase-conjugated secondary antibody in 1% PBS-T for 30 min at 37°. ECL Western blotting detection reagents were used to detect the immunoreactive bands of HSP70 and HSP90.

2.9. Statistical analysis

All numerical data are presented as means ± S.E.M. One-way ANOVA followed by Scheffe's test was used to determine differences between more than two groups for all continuous parameters while the Student's *t*-test was used for two group data. The significance level was set at $P < 0.05$ for all tests.

3. Results

3.1. Effect of FTS on cisplatin-induced renal cell injury in LLC-PK$_1$ cells

To investigate whether FTS prevents cisplatin-induced cell injury, LDH leakage was examined as an index of renal cell injury. LLC-PK$_1$ cells were treated with cisplatin at a concentration of 30 μM for 24 h. Cisplatin markedly increased LDH leakage from the cells after 24 h of exposure. LLC-PK$_1$ cells were treated with various concentrations of FTS 2 h before exposure to 30 μM cisplatin for 24 h, and LDH leakage was determined after the culture for 24 h. FTS at 0.1 and 10 nM ameliorated the cisplatin-induced increase in LDH leakage (Fig. 1). These results indicated that FTS significantly attenuated cisplatin-induced renal cell injury.

3.2. Effect of FTS on cisplatin-induced renal dysfunction

Next, in vivo experiments were carried out using rats treated with the dose of cisplatin (5 mg/kg body weight) in the basis of above in vitro findings. Cisplatin treatment of rats caused the prominent increases in BUN and plasma creatinine after day 3 in cisplatin-treated rats (Fig. 2A and B). In addition, the urinary excretion rates of glucose, NAG and protein were remarkably higher in cisplatin-administered rats compared with normal controls (Fig. 3A–C).

The effect of FTS on the above indices caused by cisplatin was investigated in cisplatin-treated rats. Pretreatment with FTS (200 μg/kg, i.v.) on days 1 and 0 before cisplatin injections significantly decreased cisplatin-induced increases in BUN and plasma creatinine levels (Fig. 2A and B). No significant differences in BUN and plasma creatinine levels were observed after the injection of FTS alone. Treatment of rats with FTS also prevented the increases in glucose, NAG and protein levels in urinary

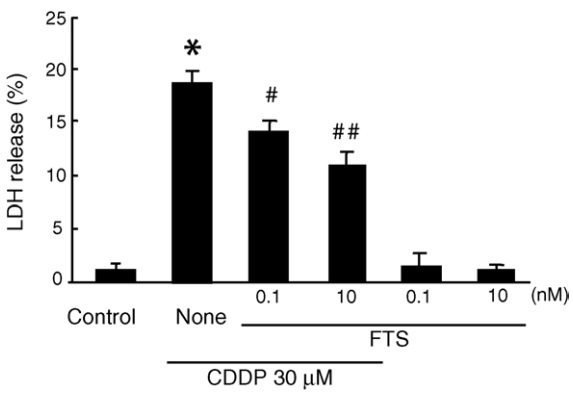

Fig. 1. Effect of serum thymic factor, FTS, on cisplatin (CDDP)-induced renal cell injury. LLC-PK$_1$ cells were cultured in the medium with 30 μM CDDP at 37° for 24 h. Each value represents the mean ± S.E.M. of three experiments. $^{*}P < 0.01$, compared with the control; $^{\#\#}P < 0.01$; $^{\#}P < 0.05$, compared with "CDDP".

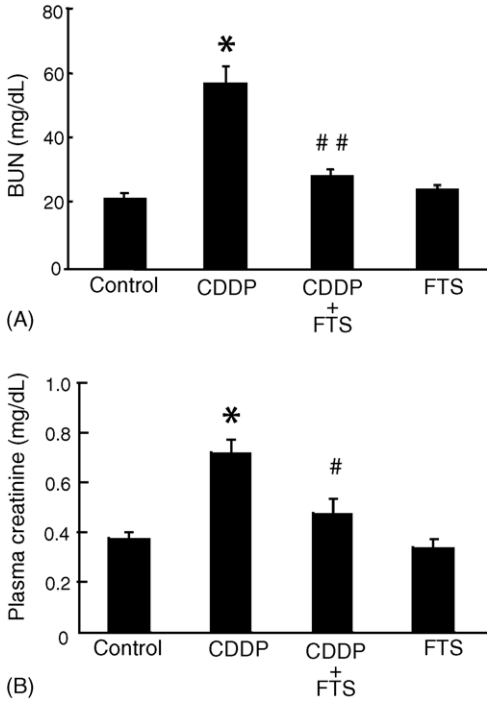

Fig. 2. Effects of serum thymic factor, FTS (200 μg/kg, i.v.), on blood urea nitrogen (BUN) (A) and plasma creatinine (B) in rats 3 days after one i.v. injection of cisplatin (CDDP, 5 mg/kg). Each value represents the mean ± S.E.M. $^{*}P < 0.01$, compared with the control; $^{\#\#}P < 0.01$; $^{\#}P < 0.05$, compared with "CDDP".

excretion (Fig. 3A–C). None of the changes were significantly different from those observed in rats given FTS alone.

3.3. Effect of FTS on cisplatin accumulation in the rat renal cortex

We investigated whether FTS inhibits nephrotoxicity by reducing the amount of platinum retained by the kidney as an index of renal accumulation of cisplatin. The platinum concentrations in the kidney cortex of cisplatin-treated rats were determined by graphite furnace atomic absorption spectroscopy. Kidney cortices from rats 3 days after treatment were analyzed for their platinum contents. Platinum levels in the kidney cortex were no significant differences in CDPP-treated rats (8.0 ± 0.2 μg/g wet weight) and CDDP and FTS-treated rats (8.3 ± 0.3 μg/g wet weight). Treatment with FTS did not affect the concentration of platinum in the kidney cortex.

3.4. ERK activation in the kidney cortex of cisplatin-treated rats

Rats treated intravenously with a single dose of 5 mg/kg cisplatin were sacrificed 1, 2 or 3 days after treatment. The phosphorylation status of ERK was determined by Western blotting. Our results showed that ERK was activated by cisplatin compared with the control rat kidney (Fig. 4). The

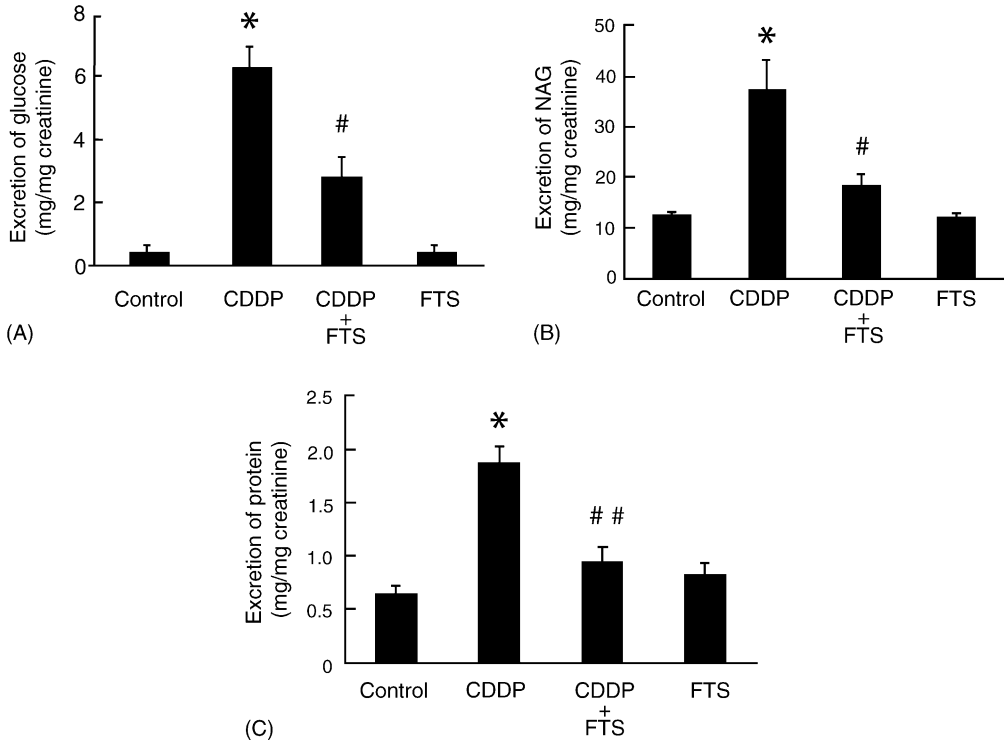

Fig. 3. Effects of serum thymic factor, FTS (200 μg/kg, i.v.), on urinary excretion of glucose (A), *N*-acetyl-β-D-glucosamidase (NAG) (B) and protein (C) in rats 3 days after one i.v. injection of cisplatin (CDDP, 5 mg/kg). Each value represents the mean ± S.E.M. $^{*}P < 0.01$, compared with the control; $^{\#\#}P < 0.01$; $^{\#}P < 0.05$, compared with "CDDP".

activation was sustained during the observation period and preceded the development of renal dysfunction (Fig. 4).

3.5. Effect of FTS on cisplatin-induced ERK phosphorylation in the nuclei

To investigate the effect of FTS on cisplatin-mediated ERK phosphorylation, nuclear fractions prepared from the kidney cortex of rats treated with cisplatin were subjected to Western blotting with anti-phospho ERK. The phosphorylation of ERK was clearly observed in nuclei prepared from the kidney cortex of rats on days 1–3 after cisplatin administration. FTS significantly suppressed the cisplatin-induced increase in phosphorylated ERK during the observation period in the nuclei (Fig. 4).

3.6. FTS induces an increase in HSP70 expression in the kidney cortex

HSP70 and HSP90 were presented at low levels in the cortex of both control and cisplatin-treated rat kidneys (Fig. 5). Three days after the FTS treatment, expression of HSP70 was markedly observed in the kidney cortex prepared not only from rats treated with cisplatin and FTS but also from rats treated with FTS alone (Fig. 5A and B), although, on the contrary, there was no significant induction of HSP90 (Fig. 5C and D).

4. Discussion

This study investigated the effect of FTS on cisplatin-induced renal injury in vivo and in vitro. Some studies have reported that FTS prevents experimental pancreatitis and diabetes induced by alloxan or streptozotocin [39,40]. Previous reports have also shown that FTS prevents bleomycin-induced pulmonary fibrosis [64]. However, the exact mechanisms by which FTS protects against tissue injury are still unknown. While preliminary, results obtained with FTS indicated that it attenuates cephaloridine-mediated renal dysfunction by suppressing the ERK activation induced by cephaloridine. We hypothesize that FTS, having a variety of biological activities, might modify tissue injury, and possibly attenuate cisplatin-induced nephrotoxicity. In the present study, the administration of FTS markedly attenuated cisplatin-induced renal dysfunction in rats. The data in this study reveal that pretreatment with FTS does not block the uptake of platinum into the kidney, nor does FTS reduce the concentration of platinum retained by the kidney. We also showed that the treatment of LLC-PK_1 with FTS in vitro significantly ameliorated cisplatin-induced cell injury. From the present data, it is clear that FTS attenuates cisplatin-mediated renal cell injury and nephrotoxicity.

In many kinds of cells, several studies have indicated that MAPK activation and ERK are involved in cell proliferation and survival [56,57]. However, previous studies

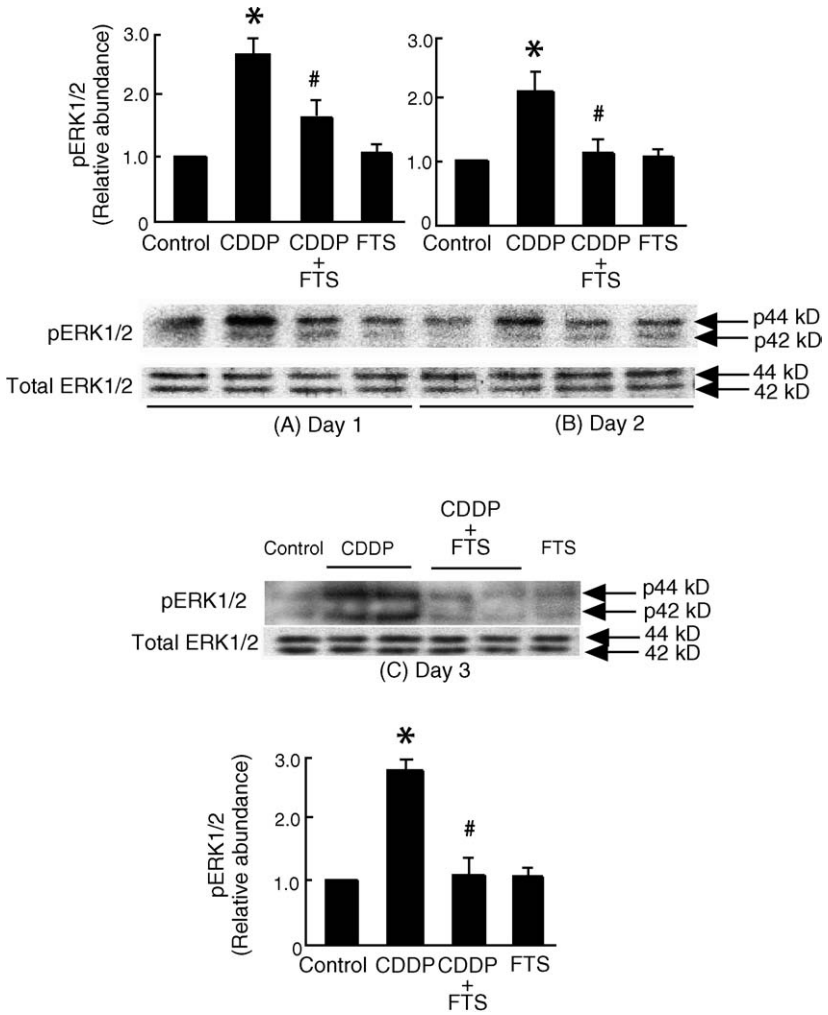

Fig. 4. Effect of serum thymic factor, FTS (200 μg/kg, i.v.), on cisplatin (CDDP)-induced phosphorylation of ERK in the nuclei prepared from the kidney cortex of rats treated with CDDP on day 1 (A), day 2 (B) and day 3 (C). The phosphorylated ERK (pERK) and total ERK bands were detected by Western blot analysis using each specific antibody against pERK1/2 and total ERK1/2 and were quantitated by densitometric scanning. Blots are representative of three similar experiments. $^{*}P < 0.01$, compared with the control; $^{\#}P < 0.01$, compared with "CDDP".

from our laboratory demonstrate a crucial role of ERK activation in cephaloridine-induced renal cell injury [65]. The importance of ERK activation, which results in renal cell damage, has been illustrated by the observation that MEK inhibitors ameliorate cephaloridine-induced renal cell injury and ERK activation in rat renal cortical slices [65]. Matsunaga et al. suggested the involvement of ERK activation in zinc-related renal cell injury [66]. Jo et al. have observed that an MEK inhibitor, U0126, effectively attenuates cisplatin-induced renal injury [58]. Our present findings, considered together with those mentioned above, indicate the involvement of ERK activation in cisplatin-induced nephrotoxicity.

The exact mechanisms by which the cisplatin induces renal dysfunction only occurred after 3 days already the pERK is elevated earlier remain to be clarified. After the cisplatin injection pERK was elevated earlier at day 1, while renal dysfunction only occurred after 3 days. In this study, pretreatment with FTS on days 1 and 0 before cisplatin injections significantly ameliorated renal dysfunction caused by cisplatin, and FTS suppressed the cisplatin-induced ERK activation. We have gotten data that post-treatment with FTS on days 1 and 2 after cisplatin injections did not affect not only the cisplatin-induced nephrotoxicity but also rapid ERK activation in the kidney cortex. It has been reported that the gradually increased and sustained ERK activation mediates the signal transduction pathways responsible for the renal regeneration [67]. We hypothesize that ERK activation, which elevated earlier are involved in the cisplatin-induced renal damage. While further studies are required to characterize the role of ERK activation involved in the pathogenesis of acute renal failure.

It has been reported that inhibition of ERK activity enhances sensitivity to cisplatin cytotoxicity in ovarian cancer cell line [68]. It has also shown that ERK signal cascades may play a considerable role in cisplatin resistance in squamous cell carcinoma [69]. Cisplatin activates

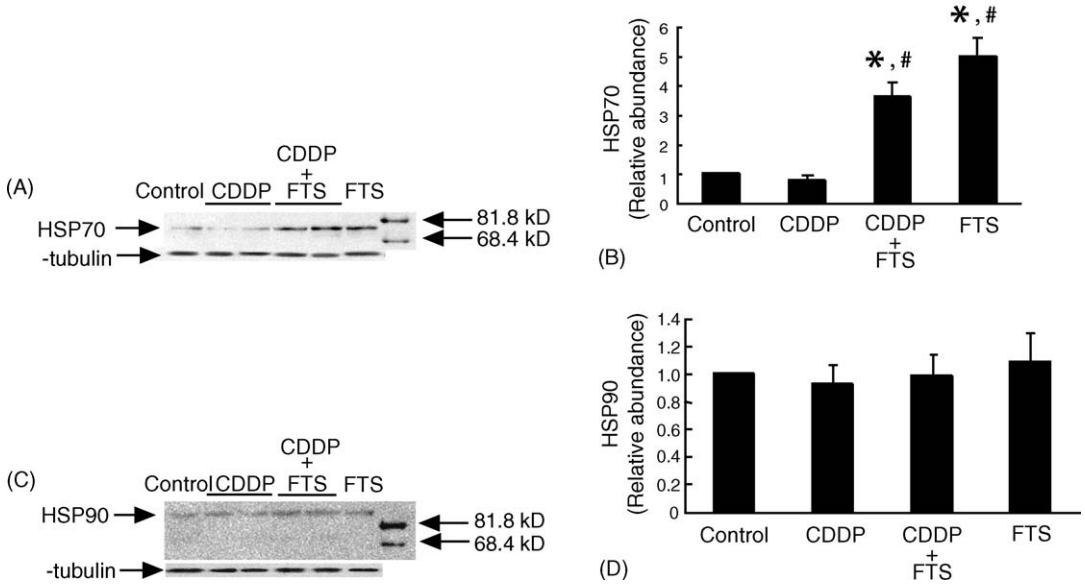

Fig. 5. Effects of serum thymic factor, FTS (200 μg/kg, i.v.), on heat shock protein (HSP) 70 and HSP90 expressions in the kidney cortex 3 days after treating rats with FTS. The HSP70 and HSP90 bands were Western blot analysis using each specific antibody against HSP70 (A) and HSP90 (C) and were quantitated by densitometric scanning (B and D). Blots are representative of three similar experiments. $^{*}P < 0.01$, compared with the control; $^{\#}P < 0.01$, compared with "CDDP".

apoptosis signals in addition to survival signal pathway via ERK signaling pathway in various cell types. The biological outcome of ERK activation, resulting in the induction of apoptosis or cell survival, attributed to differences in cell types, that is, the tumor cells or normal cells. We hypothesize that FTS may reduce the nephrotoxicity would not reduce the anti-tumor effect of the drug at the same time. In a further study, we will try to investigate FTS, suppressing the activation of the ERK signaling pathway induced by cisplatin, make it clinically effective for the anti-tumor therapy.

In the present study, the data indicated that treatment with FTS stimulated the expression of HSP70 in the kidney cortex. HSPs are highly conserved proteins that are expressed in both physiological and pathological conditions. HSPs are classified on the basis of their molecular weight and the 70 kDa proteins, referred to collectively as the HSP70 family, represent the most prominent group [70–72]. Members of the HSP70 family have been identified in protecting against cellular damage from stressful stimuli by acting as chaperone molecules, binding to and preventing the aggregation of denatured or abnormal proteins, and facilitating the restoration of normal protein function. The present findings indicate that increases in HSP70 levels in the kidney by FTS administration are probably reno-protective, and would serve as a mechanism of FTS's cytoprotection in cisplatin nephrotoxicity.

It is not known whether there are FTS receptors on the kidney. It has been reported that there are FTS receptors like on human lymphoblastoid T cells [73]. It remains to be clarified the receptors for FTS on the proximal tubule cells. Our preliminary experiments have shown that FTS inhibites the cisplatin-induced increase in the ERK activation, and FTS induces HSP70 expression in the LLC-PK_1 cells, suggesting the direct effect of FTS on the kidney. It is not definite that whether FTS has secondary effect on the kidneys be mediated by the immune system in vivo in this study. The role of the inflammatory mechanism in renal damage induced by cisplatin became available very recently [74,75]. These effects of cisplatin may make the damage worse. FTS is a multifunctional thymic hormone that regulates a variety of biological activities both in vivo and in vitro, including an immunobiological effect [27–32]. It is still speculative that FTS might modify renal damage by having not only direct but also secondary effect on the kidney.

In conclusion, this study demonstrated that cisplatin-induced renal injury is mediated by the activation of the ERK signaling pathway, although further studies are required to characterize the exact mechanisms by which ERK activation causes renal dysfunction. Moreover, we demonstrated that FTS significantly ameliorated cisplatin-induced renal damage and sustained ERK activation in the kidney. These results suggest that FTS participates in protection from cisplatin-mediated nephrotoxicity, at least in part, by suppressing the activation of the ERK signaling pathway induced by cisplatin and also by inducing the increased level of HSP70 in rat kidney.

Acknowledgment

This research was supported by a Grant-in-Aid for High Technology Research from the Ministry of Education, Science, Sports and Culture, Japan.

References

[1] Einhorn LH. Curing metastatic testicular cancer. Proc Natl Acad Sci USA 2002;99:4592–5.

[2] Borch RF. The platinum antitumor drugs. In: Powis G, Prough RA., editors. Metabolism and action of drugs, vol. 163. London: Taylor&-Francis; 1987. p. 193.

[3] Rosenberg B. Platinum complex for the treat of cancer. Interdisciplinary Sci Rev 1978;3:134–47.

[4] Lippman AJ, Helson C, Helson L, Krakoff IH. Clinical trials of *cis*-diammine-dichloro platinum (NSC-119875). Cancer Chemother Rep 1973;57:191–200.

[5] Kim YK, Byun HS, Kim YH, Woo JS, Lee SH. Effect of cisplatin on renal function in rabbits: mechanism of reduced glucose reabsorption. Toxicol Appl Pharmacol 1995;130:19–26.

[6] Daugaard G. Cisplatin nephrotoxicity: experimental and clinical studies. Dan Med Bull 1990;37:1–12.

[7] Ries F, Klastersky J. Nephrotoxicity induced by cancer chemotherapy with special emphasis on cisplatin toxicity. Am J Kidney Dis 1986;8:368–79.

[8] Madias NE, Harrington JT. Platinum nephrotoxicity. Am J Med 1978;65:307–14.

[9] Leonard BJ, Eccleston E, Jones D, Todd P, Walpole A. Antileukemic and nephrotoxic properties of platinum compounds. Nature 1971;234:243.

[10] Berns JS, Ford PA. Renal toxicities of antineoplastic drugs and bone marrow transplantation. Semin Nephrol 1997;17:54–66.

[11] Chopra S, Kaufman JS, Jones TW. *cis*-Diammine-dichloro platimum-induced acute renal failure in the rat. Kidney Int 1982;21:54–64.

[12] Arany I, Safirstein RL. Cisplatin nephrotoxicity. Semin Nephrol 2003;23:460–4.

[13] Dobyan DC, Levi J, Jacobs C, Kosec J, Weiner MW. Mecahnism of *cis*-platinum nephrotoxicity. II. Morphological observations. J Pharmacol Exp Ther 1980;213:551–6.

[14] Gemba M, Yamaguchi T, Kambara K, Suzuki A, Kawai Y. Increases in urinary enzyme excretion in rats depleted of glutathione inhibited by scavenger of oxygen free radicals. Pharmacobiodynamics 1992;15(9):513–8.

[15] Kameyama Y, Gemba M. The iron chelator deferoxamine prevents cisplatin-induced lipid peroxidation in rat kidney cortical slices. Jpn J Pharmacol 1991;57(2):259–62.

[16] Schaaf GJ, Maas RF, Groene EM. Management of oxidative stress by heme oxygenase-1 in cisplatin-induced toxicity in renal tubular cells. Free Radic Res 2002;36:835–43.

[17] Xiao T, Choudhary S, Zhang W. Possible involvement of oxidative stress in cisplatin-induced apoptosis in LLC-PK1 cells. J Toxicol Environ Health 2003;66:469–79.

[18] Vermeulen NP, Baldew GS. The role of lipid peroxidation in the nephrotoxicity of cisplatin. Biochem Pharmacol 1992;44(6):1193–9.

[19] Zhang JG, Lindup WE. Role of mitochondria in cisplatin-induced oxidative damage exhibited by rat renal cortical slices. Biochem Pharmacol 1993;45(11):2215–22.

[20] Deng J, Kohda Y, Chiao H. Interleukin-10 inhibits ischemic and cisplatin-induced acute renal injury. Kidney Int 2001;60:2118–28.

[21] Ramesh G, Reeves WB. TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. J Clin Invest 2002;110:835–42.

[22] Cummings BS, Schnellmann RG. Cisplatin-induced renal cell apoptosis: caspase 3-dependent and -independent pathway. J Pharmacol Exp Ther 2002;302:8–17.

[23] Okuda M, Masaki K, Fukatsu S, Hashimoto Y, Inui K. Role of apoptosis in cisplatin-induced toxicity in the renal epithelial cell line LLC-PK_1: implication of the functions of apical membranes. Biochem Pharmacol 2000;59:195–201.

[24] Lau AH. Apoptosis induced by cisplatin nephrotoxic injury. Kidney Int 1999;56:1295–8.

[25] Lieberthal W, Triaca V, Levine J. Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: apoptosis vs. necrosis. Am J Physiol 1996;270:F700–8.

[26] Desoize B, Madoulet C. Particular aspects of platinum compounds used at present in cancer treatment. Crit Rev Oncol Hematol 2002;42:317–25.

[27] English MW, Skinner R, Pearson ADJ, Price L, Wyllie R, Craft AW. Dose-related nephrotoxicity of carboplatin in children. Br J Cancer 1999;81:336–41.

[28] Poirier MC, Reed E, Litterst CL, Kats D, Gupta-Burt S. Persistence of platinum–ammine–DNA adducts in gonads and kidneys of rats and multiple tissues from cancer patients. Cancer Res 1992;52:149–53.

[29] Alberts DS, Fanta PT, Running KL, Adair LP, Garcia DJ, Liu-Stevens R, et al. In vitro phase II comparison of the cytotoxicity of a novel platinum analog, nedaplatin (254-S), with that of cisplatin and carboplatin against fresh, human ovarian cancers. Cancer Chemother Pharmacol 1997;39:493–7.

[30] Uchida N, Takeda Y, Hojo K, Maekawa R, Sugita K, Yoshioka T. Sequence-dependent antitumour efficacy of combination chemotherapy of nedaplatin, a novel platinum complex, with 5-fluorouracil in an in vivo murine tumour model. Eur J Cancer 1998;34:1796–801.

[31] Kawai Y, Taniuchi S, Okahara S, Nakamura M, Gemba M. Relationship between cisplatin or nedaplatin-induced nephrotoxicity and renal accumulation. Biol Pharm Bull 2005;28(8):1385–8.

[32] Bach JF, Dardenne M, Pleau JM. Biological characterization of a serum thymic factor. Nature 1977;266:55–6.

[33] Kaufman DB. Maturational effects of thymic hormones on human helper and suppressor T cells: effect of FTS ('Factuer Thymique Serique') and thymosin. Clin Exp Immunol 1980;39:722–7.

[34] Bach JF. Thymulin (FTS-Zn) Clin Immunol Allergy 1983;3:133–56.

[35] Bach MA, Beaurain G. Respective influence of extrinsic and intrinsic factors on age-related decrease of thymic secretion. J Immunol 1979;122:1056–8.

[36] Bach JF, Dardenne M, Pleau JM, Bach MA. Isolation biochemical characteristic and biological activity of circulating thymic hormone in the mouse and in the human. Ann N Y Acad Sci 1975;249:186–93.

[37] Dardenne M, Nabama B, Lefancier P, Denien M, Choay J, Bach JF. Contribution of zinc and other metals to the biological activity of the serum thymic factor. Proc Natl Acad Sci USA 1982;79:5370–3.

[38] Savino W, Dardenne M. Neuroendocrine control of thymus physiology. Endocr Rev 2000;21:412–43.

[39] Mizutani M, EI-Fotoh M, Mori M, Ono K, Doi K, Awaya A, et al. In vivo administration of serum thymic factor (FTS) prevents EMC-D virus-induced diabetes and myocarditis in BALB/cAJ cl mice. Arch Virol 1996;141:73–83.

[40] Yamanouchi T, Moromizato H, Kojima S, Shinohara T, Sekino N, Minoda S, et al. Prevention of diabetes by thymic hormone in alloxan-treated rats. Eur J Pharmacol 1994;257:39–46.

[41] Nagai Y, Osanai T, Sakakibara K. Intensive suppression of experimental allergic encephalomyelitis (EAE) by serum thymic factor and therapeutic implication for multiple sclerosis. Jpn J Exp Med 1982;52(4):213–9.

[42] Barbul A, Shawe T, Frankel HL, Efron JE, Wasserkrug HL. Inhibition of wound repair by thymic hormones. Surgery 1989;106:373–7.

[43] Welch W, Kang HS, Mizzen LA. Response of mammalian cells to metabolic stress: changes in cell physiology and structure/function of stress proteins. Curr Top Microbiol Immunol 1994;137:31–5.

[44] Langer T, Neupert W. Heat shock proteins hsp60 and hsp70: their roles in folding, assembly and membrane translocation of proteins. Curr Top Microbiol Immunol 1991;167:3–29.

[45] Ang D, Liberek K, Skowtra D. Biological role and regulation of the universally conserved heat shock proteins. J Biol Chem 1991;266(36):24233–6.

[46] Andrés D, Bautista M, Cascales M. Attenuation of cyclosporine A toxicity by sublethal heat shock: role of catalase. Biochem Pharmacol 2005;69(3):493–501.

[47] Komatsuda A, Wakui H, Imai H. Renal localization of the constitutive 73-kDa heat-shock protein in normal and PAN rats. Kidney Int 1992;41:1204–12.
[48] Komatsuda A, Wakui H, Oyama Y, Imai H, Miura A. Overexpression of the human 72 kDa heat shock protein in renal tubular cells confers resistance against oxidative injury and cisplatin toxicity. Nephrol Dial Transport 1999;14:1385–90.
[49] Cobb MH, Goldsmith EJ. How MAP kinase are regulated. J Biol Chem 1995;270:14843–6.
[50] Lewis TS, Shapiro PS, Ahn NG. Signal transduction through MAP kinase cascades. Adv Cancer Res 1998;74:49–139.
[51] Nowak G. Protein kinase C and ERK1/2 mediate mitochondrial dysfunction, decreases in active Na^+ transport, and cisplatin-induced apoptosis in renal cells. J Biol Chem 2002;277:43377–88.
[52] Persons DL, Yazlovitskaya EM, Cui W, Pelling JC. Cisplatin-induced activation of mitogen-activated protein kinases in ovarian carcinoma cells: inhibition of extracellular signal-regurated kinase activity increases sensitivity to cisplatin. Clin Cancer Res 1999;5:1007–14.
[53] Sanches-Perea I, Murguia JR, Perona R. Cisplatin induces a persistent activation of JNK that is related to cell death. Oncogene 1998;16:533–40.
[54] Wang X, Martindale JL, Holbrook NJ. Repuirement for ERK activation in cisplatin-induced apoptosis. J Biol Chem 2000;275:39435–43.
[55] Arany I, Megyesi JK, Kaneto H, Price PM, Safirstein RL. Cisplatin-induced cell death is EGFR/src/ERK signaling dependent in mouse proximal tubule cells. Am J Physiol Renal Physiol 2004;287:F543–9.
[56] Boulton TG, Nye SH, Robbins DJ, Ip NY, Radziejewska E, Morgenbesser SD, et al. ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. Cell 1991;65:663–75.
[57] Davis RJ. The mitogen-activated protein kinase signal transduction pathway. J Biol Chem 1993;268:14553–6.
[58] Jo SK, Cho WY, Sung SA, Kim HK, Won NH. MEK inhibitor, U0126, attenuates cisplatin-induced renal cell injury by decreasing inflammation and apoptosis. Kidney Int 2005;67:458–66.
[59] Campos-Gonzalez R, Kindy MS. Tyrosine phosphorylation of microtubule-associated protein kinase after transient ischemia in the gerbil brain. J Neurochem 1992;59:1955–8.
[60] Kindy MS. Inhibition of tyrosine phosphorylation prevents delayed neuronal death following cerebral ischemia. J Cereb Blood Flow Metab 1993;13:372–7.
[61] Bonni A, Brunet A, West AE, Datta SR, Takasu MA, Greenberg ME. Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. Science 1999;286:1358–62.
[62] Cobb MH. MAP kinase pathways. Prog Biophys Mol Biol 1999;71:479–500.
[63] Dignam JD, Lebovitz RM, Roeder RG. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res 1983;11(5):1475–89.
[64] Yara S, Kawakami K, Kudeken N, Tohyama M, Teruya K, Chinen T, et al. FTS reduces bleomycin-induced cytokine and chemokine production and inhibits pulmonary fibrosis in mice. Clin Exp Immunol 2001;124(1):77–85.
[65] Kohda Y, Hiramatsu J, Gemba M. Involvement of MEK/ERK pathway in cephaloridine-induced injury in rat renal cortical slices. Toxicol Lett 2003;143:185–94.
[66] Matsunaga Y, Kawai Y, Kohda Y, Gemba M. Involvement of activation of NADPH oxidase and extracellular signal-regulated kinase (ERK) in renal cell injury induced by zinc. J Toxicol Sci 2005;30(2):135–44.
[67] Ishizuka S, Yano T, Hagiwara K, Sone M, Nihei H, Ozasa H, et al. Extracellular signal-regulated kinase mediates renal regeneration in rats with myoglobinuric acute renal injury. Biochem Biophys Res Commun 1999;254(1):88–92.
[68] Wei SQ, Sui LH, Zheng JH, Zhang GM, Kao YL. Role of ERK1/2 kinase in cisplatin-induced apoptosis in human ovarian carcinoma cells. Chin Med Sci J 2004;19:125–9.
[69] Aoki K, Ogawa T, Ito Y, Nakashima S. Cisplatin activates survival signals in UM-SCC-23 squamous cell carcinoma and these signal pathways are amplified in cisplatin-resistant squamous cell carcinoma. Oncol Rep 2004;11:375–9.
[70] Villar J, Mendez-Alvarez S. Heat shock proteins and ventilator-induced lung injury. Curr Opin Crit Care 2003;9:9–14.
[71] Fekete A, Treszl A, Toth-Heyn P. Association between heat shock protein 72 gene polymorphism and acute renal failure in premature neonates. Pediatr Res 2003;54:452–5.
[72] Schroder O, Schulte KM, Ostermann P. Heat shock protein70 genotypes HSPA1B and HSPA1L influence cytokine concentrations and interfere with outcome after major injury. Crit Care Med 2003;31:76–9.
[73] Gastinel LN, Pleau JM, Goldstein G, Bach JF. Interaction of thymopoietin peptides with the specific receptor of facteur thymique serique (FTS). Thymus 1983;5(2):78–87.
[74] Deng J, Kohda Y, Chiao H, Wang Y, Hu X, Hewitt SM, et al. Interleukin-10 inhibits ischemic and cisplatin-induced acute renal injury. Kidney Int 2001;60:2118–28.
[75] Ramesh G, Reeves WB. TNF-alpha mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. J Clin Invest 2002;110:835–42.