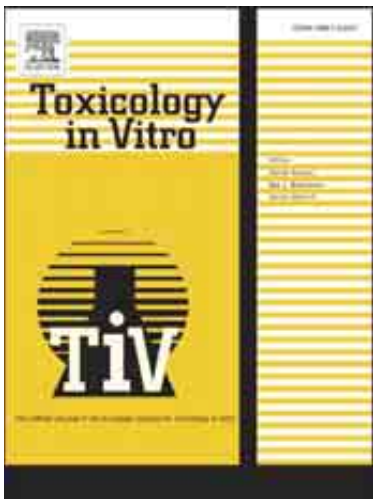

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Cytoprotective effects of selenium on cadmium-induced LLC-PK_1 cells apoptosis by activating JNK pathway

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Running title: Selenium prevents Cadmium-induced apoptosis

Abbreviations*:* $CdCl_2$, cadmium chloride; Na_2SeO_3, sodium selenite; JNK, c-Jun N-terminal kinase; SAPK, stress-activated protein kinase; MAPK, mitogen-activated protein kinases; ROS, reactive oxygen species; GSH, glutathione; NAC, N-acetyl-L-cysteine; PBS, phospate buffered saline; FITC, Annexin V-fluorescein isothiocyanate; PI, propidium iodide

Abstract

Extensive studies have indicated that the apoptosis pathway appears to be associated with intracellular reactive oxygen species (ROS) production in cadmium-induced nephrotoxicity, however, the precise cellular mechanism remains unclear. The purpose of this study was to determine the relationships between the activation of phosphorylated c-jun N-terminal kinase (JNK) and cadmium-induced apoptosis, and assess the possible cytoprotective mechanism of selenium. Our study clearly revealed cadmium treatment caused apoptosis in LLC-PK_1 cells, which was partially suppressed by pretreatment with selenium, an antioxidant nutrient. Further studies found the phosphorylation of JNK kinase increased with exposure to cadmium for 3 h, even remained elevated at 9 h in the time course study, and the activation of phosphorylated JNK was detected in a dose-dependent manner. In addition, a concomitant time-dependent increase in caspase-3 activities was observed by cadmium treatment. During the process, selenium played the same role as N-acetyl-L-cysteine (NAC), a free radical scavenger. Pretreatment of cells with selenium partially suppressed of the phosphorylation of JNK, coupled with caspase-3 activation involved in cadmium-induced apoptosis. In conclusion, our studies provided a molecular linkage between the phosphorylation of JNK and cadmium-induced LLC-PK_1 cells apoptosis, and demonstrated selenium also contributed a potentially protection to prevent cadmium-cytotoxicity.

Keywords: Cadmium; Apoptosis; Selenium; JNK; Caspase-3

1. Introduction

Cadmium is a widespread environmental and industrial pollutant, which is classified by International Agency for Research on Cancer (IARC) as Group I carcinogen to humans (IARC, 1993). It has been well established that chronic exposure to cadmium causes irreversible kidney damage and renal tubular dysfunction (Nishijo et al., 2006; Horiguchi et al., 2006). Despite being one of the major routes for cadmium absorption, the toxic mechanism of cadmium on renal tissue is still poorly understood.

During the last decade, a number of studies have shown that cadmium induces apoptosis of the proximal tubular cells (Lee et al., 2005; Ishido et al., 1998). Although extensive research has been undertaken to elucidate signal pathways in apoptosis, at present, oxidative stress has been considered an important possible mechanism of cadmium toxicity (Kim and Sharma, 2006; Filipic et al., 2006). Accumulated evidence has also shown that cadmium increased cellular reactive oxygen species (ROS) levels (Valko et al., 2006; Pathak and Khandelwal, 2006; Oh and Lim, 2006), lipid peroxidation and alteration in glutathione (GSH) levels in many cell types (Pathak and Khandelwal, 2006; Shaikh et al., 1999), suggesting that cadmium-induced apoptosis may be connected with oxidative stress. Our previous studies showed that cadmium can induce apoptosis in LLC-PK_1 cells, increased cellular ROS production (data not shown), and that selenium has a protective effect against cadmium cytotoxicity (Ren et al., 2004). Nevertheless, the exact mechanism in oxidative stress of selenium protective effect against cadmium-induced apoptosis has not been clarified.

The c-Jun N-terminal kinase (JNK), also known as stress-activated protein kinase (SAPK), belongs to the mitogen-activated protein kinases (MAPK) superfamily. Based on substantial

evidence, JNK signaling is also sensitive to a decreased or increased oxidative environment (Gomez et al., 1996; Wang et al.,1998), which is frequently involved in mediating stress responses induced from a diverse array of factors (Barr and Bogoyevitch, 2001; Pearson et al., 2001). In response to various environmental stresses including heavy metals, the c-Jun N-terminal kinase (JNK) is activated by dual phosphorylation on Thr183 and Thr185 (Derijard et al., 1994; Kyriakis and Avruch, 1996). Resent evidence suggests that JNK activity may play an important role in triggering apoptotic signaling (Chen et al., 1996; Eilers et al., 1998). For example, studies indicated that the JNK/c-Jun signaling cascade plays a crucial role in cadmium-induced neuronal cell apoptosis and apoptosis in CL3 human lung adenocarcinoma cells (Chuang et al., 2000; Kim and Sharma, 2004). In contrast to the above reports, Lag M et al demonstrated the MAPK p38, more than JNK pathway seemed to be involved in the cadmium-induced apoptosis in Clara cells and type 2 cells (Lag et al., 2005). Considering the above results, JNK signaling pathway may be responsible for cadmium-induced apoptosis in LLC-PK_1 cells and the relationships between protective mechanism of selenium against cadmium-induced apoptosis and JNK pathway activation need further characterization.

In order to gain further insights into the toxic mechanism response to cadmium, the objectives of this study were to examine the relationships between the phosphorylation of JNK and cadmium-induced apoptosis in LLC-PK_1 cell lines and to explore the protective mechanism of selenium.

2. Materials and methods

2.1. Materials.

Cadmium chloride (Wako PureChemical Ind., Osaka, Japan) and Sodium selenite (Shanghai Zhenxin Chemical CO., China) were dissolved in water, sterilized with 0.22μm filters, and added to cultures at the indicated time and concentrations. Cell culture reagents were obtained from GIBCO Life Technology (Grand Island, NY, USA). Antibodies specific for the total and phospho-SAPK/JNK (Thr183/Tyr185), procaspase-3 and anti-rabbit IgG, HRP-linked Antibody were purchased from Cell Signaling Technology (Beverly, MA, USA). β-actin antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Hoechst 33258 Staining and caspase-3 activity kit were obtained from Beyotime Institute of Biotechnology (Haimen, China), and Annexin V-FITC Apoptosis Detection kit was purchased from Pharmingen (Becton Dickinson Company, San Jose, CA, USA). N-acetyl-L-cysteine (NAC) and other reagents in the molecular studies were supplied from Sigma (St. Louis, MO, USA).

2.2. Cell culture

LLC-PK_1 cells, a porcine renal epithelial cell line, were generously provided by Dr Xiao-Ming Zhou (Uniformed Services University, USA). Cells were cultured in RPMI-1640 medium supplemented with 10 % fetal bovine serum, 100 units/ml penicillin G, 100 μg/ml streptomycin in a humidified atmosphere of 5% CO_2 at 37℃. For each experiment, exponentially growing LLC-PK_1 cells were plated at 5×10^4 cells / well in 6-well culture plates, cultured for 1 day, and maintained in serum-free medium for 12 h before being used for subsequent experiments.

2.3. Hoechst 33258 Staining

Apoptotic morphological changes in the nuclear chromatin of cells were detected by Hoechst

33258 staining. LLC-PK_1 cells were seeded on sterile cover glasses placed in the 6-well plates. After overnight growth, cells were pre-treated with NAC (500 μM) or selenium (20 μM) for 30 min, then treated with 40 μM cadmium for 12 h, cells were washed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde for 10 min, and then incubated with 50 μM Hoechst 33258 staining solution for 10 min. After three washes with PBS, the cells were viewed under a fluorescence microscope (Olympus, IX-70, Japan).

2.4. Flow cytometric assessment of apoptosis

The measurement of phosphatidylserine redistribution in a plasma membrane was conducted according to the protocol outlined by the manufacturer of the Annexin V-FITC Apoptosis Detection kit (Becton Dickinson Company, San Jose, CA, USA). Briefly, After pre-treatment with NAC (500 μM) or selenium (20 μM) for 30 min, then with 40 μM cadmium for 12 h, harvested cells were suspended in a binding buffer (1×). An aliquot of 100 μl was incubated with 5 μl of Annexin V-FITC and 5 μl of PI for 15 min in dark, and 400 μl binding buffer (1×) was added to each sample. The stained cells were analyzed directly by flow cytometry using the Cell Quest program (Becton Dickinson, Franklin, NJ).

2.5. Western blotting

Cells were grown at 5×10^4 cells / well in 6-well microplates and incubated with NAC, selenium and cadmium for indicated time. Following treatment, cells were washed with PBS, and total cells were prepared by scraping in 200 μl of lysis buffer [20 mM Tris–HCl (pH 8.0), 1 mM sodium orthovanadate, 10% glycerol, 1mM phenylmethylsulfonyl fluoride, 2 mM ethylenediaminetetraacetate (EDTA), 1% Triton X-100, 50 mM β-glycerolphosphate, and 10 mg/ml each of aprotinin, leupeptin, and pepstatin]. Fifty micrograms of proteins determined by

Bradford assay were electrophoretically separated using a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to polyvinylidence fluoride (PVDF) membrane and then immunoblotted with the corresponding antibodies. Immunodetection was performed with enhanced chemiluminescence (ECL) detection kit (Cell Signaling Technology, Beverly, MA). The protein bands were followed by densitometry scanning using a Chemilmager^{TM} 5500 Fluorescence system equipped with the analysis software AlphaEase FC^{TM} (Alpha Innotech Corporation, San Leandro, CA 94577, USA).

2.6. Caspase-3 activity assay

The activity of caspase-3 was determined using the Caspase-3 activity kit (Beyotime Institute of Biotechnology, Haimen, China).To evaluate the activity of caspase-3, cell lysates were prepared after their respective treatment with various designated treatments. Assays were performed on 96-well microtitre plates by incubating 10 μl protein of cell lysate per sample in 80 μl reaction buffer (1% NP-40, 20 mM Tris-HCl (PH 7.5), 137 mM Nad and 10% glycerol) containing 10 μl caspase-3 substrate (Ac-DEVD-pNA) (2 mM). Lysates were incubated at 37℃ for 4 h. Samples were measured with an ELISA reader at an absorbance of 405nm. The detail analysis procedure was described in the manufaturer's protocol.

2.7. Statistical analysis

Results are expressed as means ± SD. Data were evaluated with one-way analysis of variance (ANOVA) procedures among treatment groups. Statistically significant differences were reported as * $p<0.05$ or ** $p<0.01$. Data with values of $p<0.05$ were generally accepted as statistically significant.

3. Results

3.1. Protective effects of selenium against cadmium-induced apoptosis

To explore the protective effects of selenium involved in cadmium-induced LLC-PK_1 cells apoptosis, we tested apoptosis by using Hoechst 33258 staining and AnnexinⅤ-FITC / PI methods. As shown in Fig. 1, the cells treated with 40 μM cadmium for 12 h showed nuclear morphological changes with Hoechst 33258 staining (Fig. 1B), while almost no apoptotic nuclei were observed in control cells (Fig. 1A). When cells were pretreated with 500 μM NAC and 20 μM selenium for 30 min, then exposed to 40 μM cadmium for 12 h, the morphological changes of apoptosis were effectively inhibited compared with cadmium treatment group (Fig. 1C, D).

By flow cytometry analysis with annexin V-FITC and PI double staining, Fig. 2 shows, when exposed to 40 μM cadmium for 12 h, the number of apoptosis cells increased almost 87.66 % as compared to 0.10 % in the control population (Fig. 2 A, B). when cells were pretreated with 500 μM NAC and 20 μM selenium for 30 min, then exposed to 40 μM cadmium for 12 h, the proportion of apoptosis of cells was 10.97 % in NAC group and 16.50 % in selenium group, respectively (Fig. 2 C, D). The results of two assays provide substantial evidence that selenium plays a protective role during cadmium-induced apoptosis.

3.2. Relationships between selenium and cadmium-induced phosphorylation of JNK

To examine if the phosphorylation of JNK is involved in the cadmium induced apoptosis by western blot analysis, we found the levels of phosphorylated forms of p54 (JNK2) and p46 (JNK1) increased clearly when LLC-PK_1 cells were treated with 40 μM cadmium for 3 h, and remained elevated even at 9 h, then declined at 12 h. In contrast, the levels of total (phosphorylation-independent) JNK were not changed during the incubation period of 15 h (Fig. 3

A). When cells were incubated with cadmium concentrations ranging from 20 to 50 μM for 9 h, the levels of phosphorylated JNK were all significantly increased in a dose-dependent manner while those of total JNK were not changed (Fig. 3 B). The densitometric analysis showed the increases in the ratio of phosphorylated JNK / total JNK band densities reached statistical significance at 3 h (3.2-fold, $P < 0.05$), 9 h (5.8-fold, $P < 0.01$), and 15 h (3.6-fold, $P < 0.05$) compared with the control group, when cells were exposed to 40 μM cadmium (Fig. 3 A). And the ratio of phosphorylated JNK / total JNK band densities was observed in a significant concentration-dependent increase (Fig. 3 B).

In addition, the relationships between the phosphorylation of JNK and oxidative stress in cadmium-induced apoptosis were examined using NAC, a free radical scavenger and selenium, an antioxidant nutrient to further observe the change in phosphorylation of JNK in LLC-PK_1 cells. As shown in Fig. 4, the results revealed NAC at 500 μM significantly blocked cadmium-induced JNK phosphorylation compared with the cells treated with cadmium only. Also, selenium effectively inhibited the levels of phosphorylated JNK in a dose-dependent manner. The levels of total JNK were not changed after cadmium or antioxidant treatments. Results obtained by densitometric analysis showed that the ratio of phosphorylated JNK / total JNK band densities was significantly decreased in the groups of pre-treatment with NAC at 500 μM (3.0-fold) and pre-treatment with selenium at 10 μM (4.7-fold), 20 μM (4.3-fold), 30 μM (2.8-fold) compared with the cells exposed to cadmium only (5.8-fold) (Fig. 4). These results suggest an early response in ROS production followed by a subsequent phosphorylation of JNK during cadmium-induced apoptosis in LLC-PK_1 cells.

3.3. Effects of selenium in cadmium-induced caspase-3 activation

We first examined the levels of procaspase-3 in cadmium-treated LLC-PK_1 cells. As shown in Fig. 5, the cells were harvested with 40 μM cadmium for 0-15 h. The activation of procaspase-3 was initiated over 6 h of cadmium treatment, after which, its intensity decreased in a time-dependent manner (Fig. 5A). Pre-treatment with NAC and selenium increased the levels of procaspase-3 in cadmium exposed cells (Fig. 5B). As shown by densitometric analysis in Fig. 5, when LLC-PK_1 cells were treated with 40 μM cadmium, a significant decrease in the ratio of phosphorylated JNK / total JNK band densities was easily detectable after 6 h (0.8-fold, $P < 0.05$) and was minimal after 12 h (0.3-fold, $P < 0.01$) compared with the control group. However, the ratio of phosphorylated JNK / total JNK band densities was significantly elevated by pre-treatment with NAC and selenium, suggesting that NAC and selenium inhibit cadmium-induced procaspase-3 degradation.

Since caspase-3 has been shown to play a pivotal role in the execution phase of apoptosis induced by diverse stimuli (Lockshin RA. 2005). In addition, the activation of caspase-3, analyzed by measuring the levels of *p*-nitroanilide cleaved from the substrate N-Ac-DEVD-pNA, increased over a similar time course to that the western blot results (Fig. 6). As presented, LLC-PK_1 cells treated with 40 μM cadmium for 0-12 h, showed significant time-dependent increases in caspase-3 activity, and activation of caspase-3 could be significantly inhibited under exposure conditions with selenium (20 μM) and NAC (500 μM). These results implicated that cadmium-induced apoptosis occurs through the activation of common executors of apoptosis such as caspase-3, and selenium and NAC inhibited caspase-3 activity appeared to participate in preventing cadmium-induced apoptosis pathway.

4. Discussion

Cadmium-induced apoptosis has been described in various cells including renal cells (Thevenod et al., 2000; Nordberg et al., 1992; Xie and Shaikh, 2006), and so far no uniform molecular mechanism has been proposed. Our previous studies also showed that cadmium induced apoptosis, lipid peroxidation and selenium protected against cadmium-cytotoxicity in LLC-PK_1 cells (Ren et al., 2004). In the present study, we have attempted to determine the relationships between phosphorylation of c-jun N-terminal kinase (JNK) pathway and cadmium-induced apoptosis, and to explore whether selenium plays a protective role during this process.

Several studies demonstrated that cadmium-induced apoptosis was associated with ROS production (Risso-de Faverney, et al., 2001; Watanabe et al., 2003), including the superoxide (O_2-), hydroxyl radicals and hydrogen peroxide (H_2O_2) (Tan et al., 1998; Simon and Haj-Yehia, 2000). Although ROS has been implicated to act as a signal molecule in the cadmium-induced apoptosis pathway, its role in cadmium-induced phosphorylation of JNK remains unclear. In this study, we used NAC, a free radical scavenger, to explore the roles of ROS in the phosphorylation of JNK and cadmium-induced apoptosis. The present results showed that cadmium-induced apoptosis was mediated by the phosphorylation of JNK implicated in oxidative stress in LLC-PK_1 cells, which was similar to the study that showed cadmium generated hydrogen peroxide and free radical scavenger or antioxidant clearly reduced cadmium-induced toxicity (Lopez et al., 2006; Valko et al., 2006). In addition, these results suggested that the generation of ROS occurs upstream of JNK phosphorylation and is the major mediator for the cadmium-induced apoptosis.

Concerning the protective effects of NAC on cadmium-induced apoptosis, we further observed cytoprotective mechanism of selenium, an antioxidant nutrient, which also inhibited

cadmium-induced apoptosis mediated by the phosphorylation of JNK. Several studies reported that selenium has also been recognized to have a capacity for conferring tolerance to the toxic manifestation of various metal exposures (Diplock et al, 1986). Mechanisms for the antagonistic behavior have been proposed such as the cadmium-selenium complex formation (Bozkurt and Smith, 1981) and redistribution of cadmium in the different protein fractions on selenium feeding (Viljoen and Tapple, 1988). In addition, cadmium-induced apoptosis has been associated with the depletion of glutathione (Rikans and Yamano, 2000; Hart et al., 1999; Stohs et al., 2000) and NAC has been shown to raise intracellular glutathione levels (Lavrentiadou et al., 2001; Schafer and Buettner, 2001), thereby protecting the cells from the effects of ROS. Furthermore, NAC and selenium also include the –SH groups, which may be related to direct reaction with radicals and reducing the level of GSH-PX (Gillissen et al., 1997). Therefore, our findings demonstrated that selenium as an antioxidant agent played a cytoprotective role, which was mediated by the phosphorylation of JNK in cadmium-induced apoptosis.

Cysteine aspartases (caspases), a protease family, are known to be required for apoptosis induced by various stimuli (Krepela 2001). Among mammalian caspases, comprising at least 14 known members, caspase-3 is thought to be the main effecter of caspases and has been identified as being activated in response to cytotoxic drugs (Krepela 2001). Activation of caspase-3 is an important step in the execution phase of apoptosis and its inhibition blocks cell apoptosis (Budihardjo et al., 1999). In our study, we found that caspase-3 activation persisted in a time-dependent manner when exposed to cadmium, and caspase-3 activation was inhibited by pre-treatment with NAC or selenium, suggesting that cadmium-induced apoptosis was required for caspase-dependent pathway in LLC-PK_1 cells.

In conclusion, the findings of the current study suggest that cadmium-induced apoptosis in LLC-PK_1 cells through ROS production linked to JNK phosphorylation activation, followed by their downstream molecular, caspase-3 activation involved in oxidative stress. Alternately, Selenium provided an alternative pathway for preventing cadmium-nephrotoxicity. Hence, delineating for cadmium induced apoptosis will help us to better understand the protective mechanisms of oxidative stress.

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LEGENDS

Fig. 1. Effects of selenium on cadmium-induced LLC-PK_1 cells apoptosis by Hoechst 33258 staining.

Fig. 2. Inhibition of cadmium-induced apoptosis by selenium and N-acetyl-L-cysteine (NAC).

Fig. 3. Effects of cadmium on phosphorylation of JNK in LLC-PK_1 cells.

Fig. 4. Effects of selenium on the cadmium-induced JNK phosphorylation.

Fig. 5. (A) Cadmium-induced procaspase-3 activation in LLC-PK_1 cells.

Fig. 5. (B) Effects of selenium on cadmium-induced procaspase-3 activation in LLC-PK_1 cells.

Fig. 6. (A) Cadmium-induced caspase-3 activation in LLC-PK_1 cells.

Fig. 6. (B) Effects of selenium and NAC on cadmium-induced caspase-3 activation.

Figure 1.

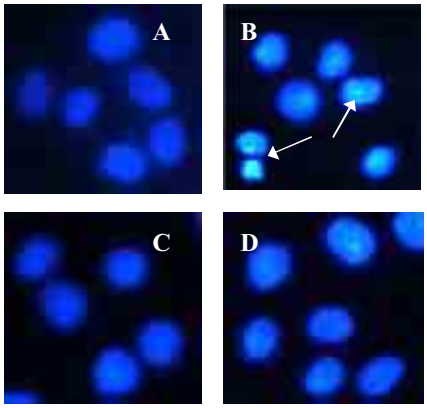

Fig. 1. Effects of selenium on cadmium-induced LLC-PK_1 cells apoptosis by Hoechst 33258 staining. (A) In controls, the majority of cells had uniformly stained nuclei. (B) After exposure to 40 μM cadmium for 12 h showed morphological changes typical of apoptosis (ie nuclei fragmentation with condensed chromatin). (C) Cells pre-treated with 500 μM NAC for 30 min, then exposed to 40 μM cadmium for 12 h, the apoptosis cells have been clearly decreased compared to cadmium treatment groups. (D) Cells pre-treated with 20 μM selenium for 30 min, then exposed to 40 μM cadmium for 12 h, the apoptosis cells have also been observed to drop compared to cadmium treatment groups.

Figure 2.

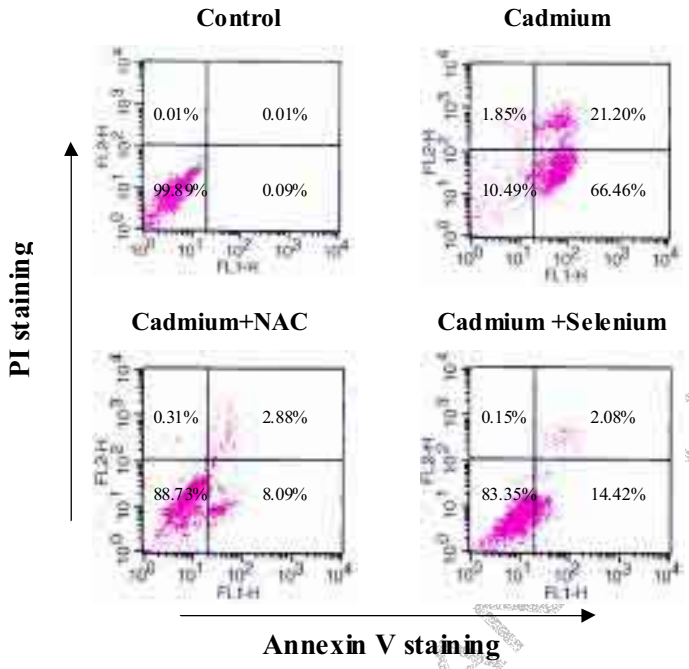

Fig. 2. Inhibition of cadmium-induced apoptosis by selenium and N-acetyl-L-cysteine (NAC). The LLC-PK_1 cells were treated with 40 μM cadmium for 12 h or pretreated with 500 μM NAC or 20 μM selenium for 30 min, then exposed to 40 μM cadmium for 12 h, cell distribution was analyzed with Annexin V / FITC and propidium iodide (PI) uptake. The FITC and PI fluorescence measured using flow cytometer with FL-1 and FL-2 filters, respectively. LL, living cells (Annexin V negative / PI negative); LR, early apoptotic / primary apoptotic cells (Annexin V positive / PI negative); UR, late apoptotic / secondary apoptotic cells (Annexin V positive / PI positive); UL, necrotic cells (Annexin V negative / PI positive). Numbers in the respective quadrant profiles indicate the percentage of the cells present in this area.

Figure 3.

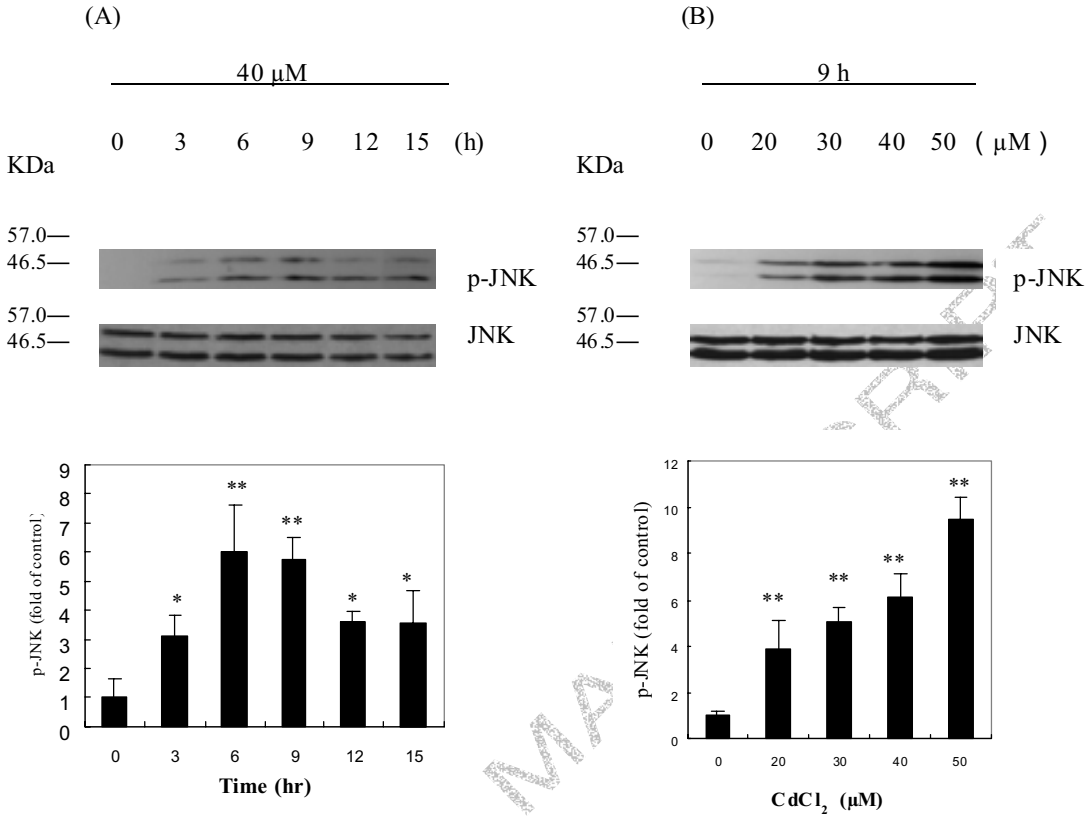

Fig. 3. Effects of cadmium on phosphorylation of JNK in LLC-PK_1 cells.
(A) Time course of cadmium-induced accumulation of phosphorylated JNK. LLC-PK_1 cells were exposed to 40 μM cadmium for 0-15 h. (B) Dose effects of cadmium on the accumulation of phosphorylated JNK. LLC-PK_1 cells were incubated with 20-50 μM cadmium for 9 h, and proteins (50 μg) prepared from whole cell extracts were analyzed by western blot using phospho-specific JNK antibody (upper panel) or phophorylation state-independent JNK antibody (lower panel). Densitometric analysis of the phosphorylated JNK and total JNK protein was calculated from the average of three experiments. Each value was expressed as the ratio of phosphorylated JNK level to total JNK level, and the value of control was set to 1. Each column and bar represents mean±SD. *indicates significant difference compared to control group at $p<0.05$ analyzed using one-way ANOVA test.

Figure 4.

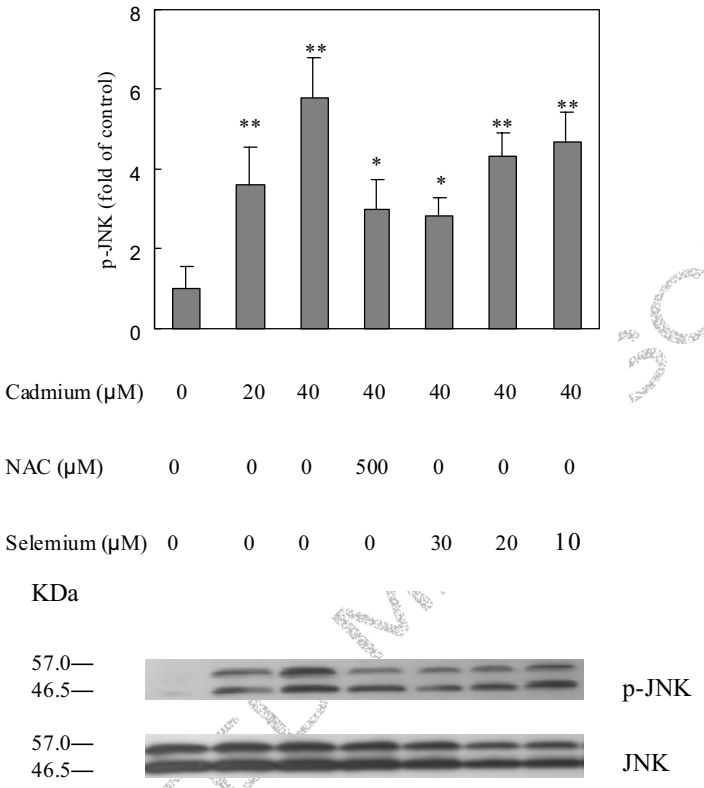

Fig. 4. Effects of selenium on the cadmium-induced JNK phosphorylation.
LLC-PK$_1$ cells were pre-incubated with 500 μM NAC or 10, 20, 30 μM selenium for 30 min, then treated with 40 μM cadmium for 9 h, phosphorylation of JNK was analyzed by the Western blot. Densitometric analysis of the phosphorylated JNK and total JNK protein was calculated from the average of three experiments. Each value was expressed as the ratio of phosphorylated JNK level to total JNK level, and the value of control was set to 1. Each column and bar represents mean± SD. *indicates significant difference compared to control group at $p<0.05$ analyzed using one-way ANOVA test.

Figure 5.

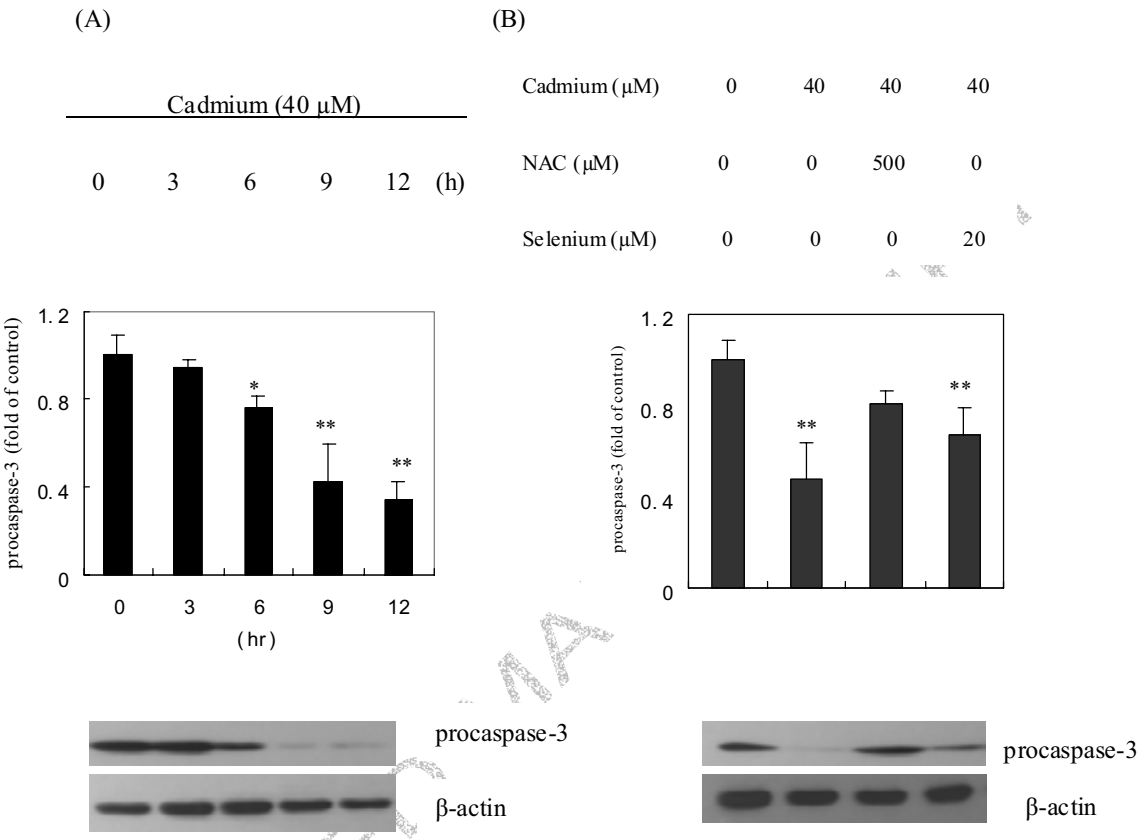

Fig.5. (A) Cadmium-induced procaspase-3 activation in LLC-PK_1 cells. The cells were incubated with 40 μM cadmium for the indicated times. The cells were harvested and lysed. 50 μg of protein lysate was subjected to 12 % SDS-PAGE gel and then immunoblotted with procaspase-3 antibodies.β-actin was used as loading control. (B) Effects of selenium on cadmium-induced procaspase-3 activation in LLC-PK_1 cells. Cells were pretreated with 500 μM NAC and 20 μM selenium for 30 min and then incubated with 40 μM cadmium for 9 h. The cells were harvested and lysed. 50 μg of protein lysate was subjected to 12 % SDS-PAGE gel and then immunoblotted with procaspase-3 antibodies.β-actin was used as a loading control. Densitometric analysis of procaspase-3 protein was calculated from the average of three experiments. Each value was expressed as the ratio of procaspase-3 level toβ-actin level, and the value of control was set to 1. Each column and bar represents mean±SD. *indicates significant difference compared to control group at $p<0.05$ analyzed using one-way ANOVA test.

Figure 6.

(A) (B)

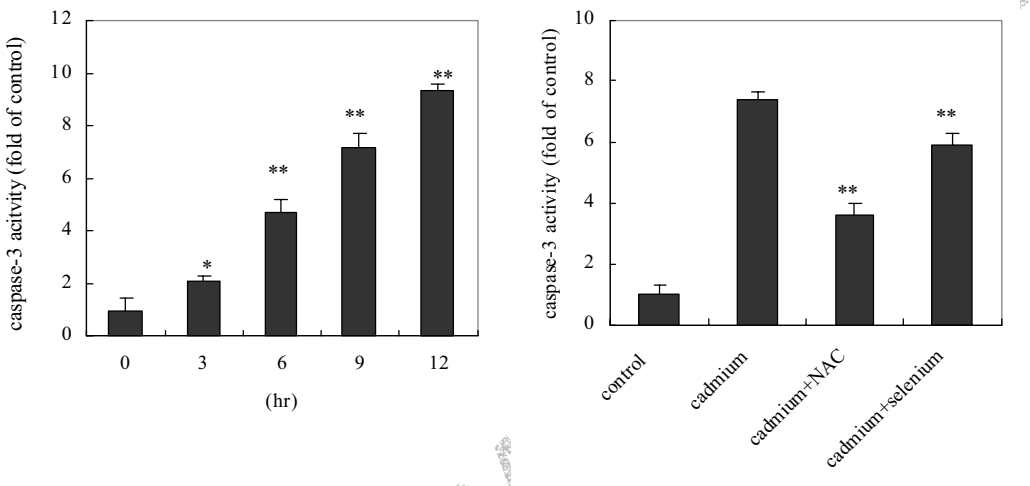

Fig.6. (A) Cadmium-induced caspase-3 activation in LLC-PK_1 cells. The cells were incubated with 40 μM cadmium for 0-12 h. Data are the mean±SD. *$p<0.05$ compared with control group. (B) Effects of selenium and NAC on cadmium-induced caspase-3 activation. The LLC-PK_1 cells pre-treated with 500 μM NAC and 20 μM selenium for 30min, then incubated with 40 μM cadmium for 9 h. Data are the mean±SD. *$p<0.05$ compared with cadmium treatment group. The relative activities of caspase-3 shown are calculated from the average of three experiments. Each value was expressed as the ratio of caspase-3 activation level to control level, and the value of control was set to 1.