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The role of endoplasmic reticulum in cadmium-induced mesangial cell apoptosis

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ABSTRACT

Cd is an industrial and environmental pollutant that affects many organs in humans and other mammals. However, the molecular mechanisms of Cd-induced nephrotoxicity are unclear. In this study, we show that endoplasmic reticula (ER) played a pivotal role in Cd-induced apoptosis in mesangial cells. Using Fluo-3 AM, the intracellular concentration of calcium ($[Ca^{2+}]_i$) was detected as being elevated as time elapsed after Cd treatment. Co-treatment with BAPTA-AM, a calcium chelator, was able to significantly suppress Cd-induced apoptosis. Calcineurin is a cytosolic phosphatase, which was able to dephosphorylate the inositol-1,4,5-triphosphate receptor (IP_3R) calcium channel to prevent the release of calcium from ER. Cyclosporine A, a calcineurin inhibitor, increased both $[Ca^{2+}]_i$ and the percentage of Cd-induced apoptosis. However, EGTA and the IP_3R inhibitor, 2-APB, were able to partially modulate Cd cytotoxicity. These results led us to suggest that the extracellular and ER-released calcium plays a crucial role in Cd-induced apoptosis in mesangial cells. Following this line, we further detected the ER stress after Cd treatment since ER is one of the major calcium storage organelles. After Cd exposure, GADD153, a hallmark of ER stress, was upregulated (at 4 h of exposure), followed by activation of ER-specific caspase-12 and its downstream molecule caspase-3 (at 16 h of exposure). The pan caspase inhibitor, Z-VAD, and BAPTA-AM were able to reverse the Cd-induced cell death and ER stress, respectively. Furthermore, the mitochondrial membrane potential ($\Delta\Psi_m$) was depolarized significantly and cytochrome *c* was released after 24 h of exposure to Cd and followed by mild activation of caspase-9 at the 36-h time point, indicating that mitochondria stress is a late event. Therefore, we concluded that ER is the major killer organelle in Cd-induced mesangial cell apoptosis and that calcium oscillation plays a pivotal role.

1. Introduction

Cadmium is an occupational and environmental pollutant with high cytotoxicity, which accumulates in the liver and kidneys after exposure through routes, such as oral ingestion or inhalation. It was reported that occupational exposure to Cd is associated with glomerular damage [1,2]. Exposure to Cd leads to renal dysfunction [3–5], which is characterized by an increased urinary excretion of low molecular weight proteins, like β_2-microglobulin, *N*-acetyl-β-D-glucoseaminidase, retinol-binding protein, and α_1-microglobulin [5]. Furthermore, renal glomeruli and mesangial cells were contracted after treatment with Cd [6], suggesting that glomeruli and mesangial cells may be significant targets of Cd nephrotoxicity. The cytotoxic mechanism of Cd is through the induction of apoptosis or necrosis in cells, such as leukemia cells [7], C6-glioma cells [8], MRC-5 fibroblasts [9], rat Sertoli cells [10], and liver cells [11]. Furthermore, Cd-induced apoptosis occurs through a caspase-dependent pathway [12,13] or through caspase-independent cell death accompanied by translocation of the apoptosis-inducing factor (AIF) [9,14–16]. A recent report has demonstrated that Cd induces a calcium-dependent apoptosis in mesangial cells [17]. A previous report of ours also

Abbreviations: AIF, apoptosis-inducing factor; BAPTA-AM, 1,2-bis(2-aminophenoxy)ethane-N,N,N,N-tetraacetic acid; Cd, cadmium; ER, endoplasmic reticula; IP_3R, inositol-1,4,5-triphosphate receptor; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazol-carbocyanine iodide; FITC, fluorescein isothiocyanate; PI, propidium iodide; ROS, reactive oxygen species; Z-VAD-fmk, n-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone; $\Delta\Psi_m$, mitochondrial membrane potential.

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demonstrated that Cd induces apoptotic and autophagic cell death through a calcium-mediated signaling pathway [18]. However, the molecular mechanism responsible for Cd-induced apoptosis in mesangial cells remains unclear and merits further investigation.

The endoplasmic reticulum (ER) is an organelle responsible for the synthesis and post-translational modification of proteins, and storage of calcium. ER dysfunction results in a stressful condition, called ER stress, which is characterized by an increase in unfolded proteins, the upregulation of chaperones, and the activation of caspase-12 [19]. So that activation of caspase-12 and upregulation of GADD153 are considered as markers of ER stress [20]. ER stress is induced by treatment with toxic insults, including calcium ionophores, by the inhibition of glycosylation, and by chemical toxicants and oxidative stress [21–28]. Prolonged ER stress leads to cell death and is linked to neurodegenerative conditions like Alzheimer's disease [29]. However, the role of calcium in the Cd-induced ER stress in mesangial cells is unclear.

In this study, we observed that Cd-induced apoptosis was through caspase-dependent manner, accompanied with ER and mitochondria stress. Furthermore, scavenging of cytosolic calcium significantly decreased Cd-induced apoptosis, ER stress and mitochondria depolarization, suggesting Cd-induced apoptosis is through calcium-mediated ER and mitochondria stress.

2. Materials and methods

2.1. Cell culture, treatment, and chemicals

Mesangial (MES-13) cells (ATCC, CRL-1927, Manassas, VA) were maintained in the medium containing Dulbecco's modified Eagle's medium (DMEM) and F-12 (3:1) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin (pH 7.4) in a humidified atmosphere containing 5% CO_2. Since MES-13 cells are normal mouse mesangial cells, all of the experiments were performed at 5–15 passages. In experiments of this study, cells were starved in the medium containing 0.1% FBS for 24 h, followed by treatment with Cd for another 24 h. DMEM, FBS, penicillin, and streptomycin were purchased from HyClone (Logan, UT). F-12 medium was from Invitrogen Corporation (Grand Island, NY). Cadmium chloride, bovine serum albumin (BSA), BAPTA-AM, 5,5′,6,6′,-tetrachloro-1,1′,3,3′,-tetraethylbenzimidazolylcarbocyanine iodide (JC-1), Fluo-3 AM, and cyclosporine A were from Sigma Chemical (St. Louis, MO). 2-Aminoethoxydiphenyl borate (2-APB) was from Calbiochem (San Diego, CA). Rabbit anti-caspase-3, -9, and -12 antibodies were from Cell Signaling Technology (Beverly, MA). Mouse anti-GADD153 antibody was from Abcam (Cambridge, MA). Mouse monoclonal anti-GAPDH antibody was from Chemicon International (Temecula, CA). Annexin V-FITC reagent and n-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (Z-VAD-fmk) were supplied by Biovision (Mountain View, CA). The secondary antibodies, including horseradish peroxidase (HRP)-conjugated goat anti-mouse and -rabbit immunoglobulin G (IgG), were from Pierce (Rockford, IL) and Jackson ImmunoResearch Laboratories (West Pine, PA), respectively. The polyvinylidene difluoride (PVDF) membrane was from Millipore (Bedford, MA). The Protein Assay Dye Reagent was from Bio-Rad Laboratories (Hercules, CA).

2.2. Measurement of cell death

The percentage of necrosis and apoptosis was determined by flow cytometry with annexin V/PI double-staining according to a previous publication [9]. In brief, total cells, including adhered cells and the suspended cells, were collected in HEPES buffer (10 mM HEPES [pH 7.4], 140 mM NaCl, and 2.5 mM $CaCl_2$). Subsequently, cells were stained with annexin V (1 μg/ml)/PI (0.2 ng/ml) for 15 min and then 1×10^4 cells were collected and analyzed by flow cytometry using CellQuest software (Becton Dickinson, San Jose, CA). The four quadrants of annexin V/PI dot plots were distinguished between living cells (annexin V−/PI−), early apoptotic cells (annexin V+/PI−), late apoptotic cells (annexin V+/PI+), and necrotic cells (annexin V−/PI+). The percentage of total apoptosis was summed up from early apoptosis and late apoptosis.

2.3. Measurement of intracellular calcium

The mesangial cells were harvested and incubated with 500 nM Fluo-3 AM dye for a total of 30 min at 37 °C and then 1×10^4 cells were collected and analyzed on a flow cytometer (Becton Dickinson) using FL-1 as a detector. The relative intracellular calcium concentrations were calculated from the ratio of the geographic mean values of the FL-1 peak generated from Cd-treated cells over each respective control as indicated in the figure legend.

2.4. Immunoblot analysis

Cells were lysed with lysis buffer (25 mM HEPES, 1.5% Triton X-100, 0.1% sodium dodecylsulfate (SDS), 0.5 M NaCl, 5 mM EDTA, and 0.1 mM sodium deoxycholate) [30] containing a protease inhibitor cocktail (Roche, Boehringer Mannheim, Germany). After a 10-min incubation on ice, sampling buffer (60 mM Tris–HCl (pH 6.8), 2% SDS, 10% glycerol, and 140 mM β-mercaptoethanol) was added to each lysate, and this combination was subsequently boiled and centrifuged. The concentration of the collected supernatant was determined using the Bio-Rad Protein Assay Dye Reagent and subjected to electrophoresis on an SDS-polyacrylamide gel (50 μg protein/lane). Proteins were electrotransferred onto PVDF membranes and immunoblotted with antibodies. Detection was performed with appropriate HRP-conjugated secondary antibodies (1:10,000 dilution) and enhanced chemiluminescence reagent (Pierce Biotechnology). The band intensity was quantified with Gel-Pro Analyzer densitometry software (Media Cybernetics).

2.5. Measurement of the mitochondrial membrane potential ($\Delta\Psi_m$)

JC-1, a lipophilic cationic fluorescence dye with the ability to enter mitochondria, was used as an indicator of $\Delta\Psi_m$ [31]. Cells were incubated with JC-1 (5 μg/ml) dissolved in DMSO for 30 min at 37 °C after treatment with Cd. The total cells were trypsinized and collected in 0.5 ml PBS and 1×10^4 cells were collected and analyzed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). The percentage of upper-left quadrant was determined as normal cells.

2.6. Statistical analysis

Three independent experiments were performed. The results are expressed as the mean ± standard deviation (SD), and the statistical analysis was evaluated by Student's *t*-test, in which a value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Cd triggers Ca^{2+}-ER stress-mediated apoptosis in mesangial cells

To examine whether Cd induces apoptotic cell death in mesangial cells, we investigated the effects of Cd on phosphatidylserine externalization, a hallmark of apoptosis, using an annexin V/PI double-staining method. As revealed in Fig. 1A, we observed that

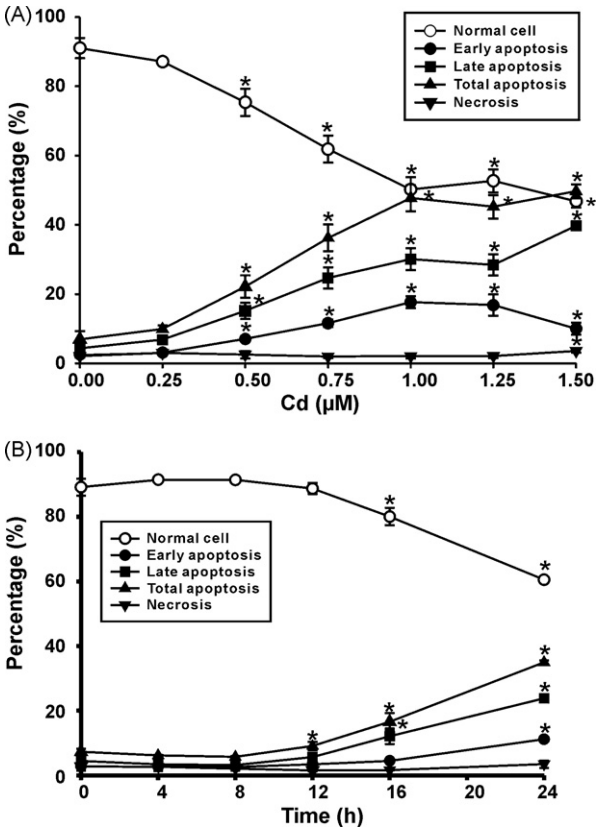

Fig. 1. Dose- and time-dependent experiments on cell death induced by Cd in mesangial cells using flow cytometry with annexin V and propidium iodide (PI) staining. Mesangial cells were treated with various concentrations of Cd for 24 h (A) or with 0.75 μM Cd for different time courses (B) as indicated. Cells were collected and stained with annexin V and PI to analyze the extent of cell death. The statistical results are presented in panels A and B. Con, Control ($^{*}p < 0.05$ vs. the control).

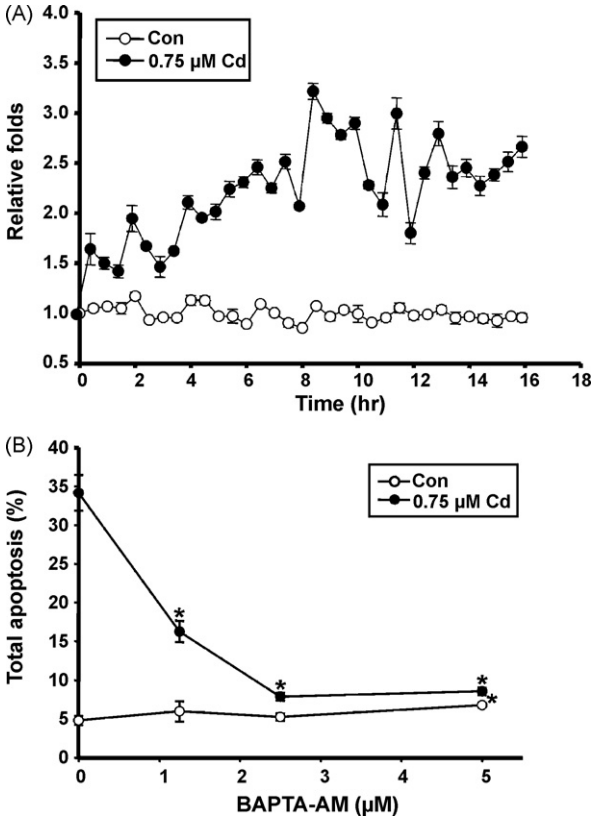

Fig. 2. Elevation of intracellular calcium is a major mediator in the Cd-induced apoptosis of mesangial cells. (A) Cd induced intracellular calcium oscillations in mesangial cells using flow cytometry with Fluo-3 AM staining. (B) The intracellular calcium chelator, BAPTA-AM, prevented cell death induced by Cd. The statistical results are presented in panel B. Con, Control. $^{*}p < 0.05$ vs. the control.

the percentage of total apoptosis increased in a dose-dependent manner, and plateaued at 47.7% ± 3.8% ($p < 0.05$). A transition from early apoptosis to late apoptosis was observed at concentrations of over 1.25 μM Cd. However, necrosis showed no significant change. To further detect the effects of exposure time on apoptosis after treatment with Cd, mesangial cells were treated with 0.75 μM Cd (IC_{50} of cell viability = 0.72 μM, see Suppl. 1) and analyzed with flow cytometry using annexin V/PI double-staining. As revealed in Fig. 1B, the ratio of apoptosis was elevated in a time-dependent manner. These results suggested that Cd-induced cytotoxicity in mesangial cells might be exerted through apoptotic cell death, but not necrosis. Elevation of cytosolic calcium has been demonstrated to serve as an important mediator in the induction of apoptosis. To detect the effect of Cd on cytosolic calcium in mesangial cells, $[Ca^{2+}]_i$ was measured over time using flow cytometry with a calcium indicator dye, Fluo-3 AM. In Fig. 2A, we observed that $[Ca^{2+}]_i$ was significantly elevated after treatment with Cd, at as early as 0.5 h, and oscillated to a peak of around 3.2-fold at 8.5 h after exposure to Cd. Next, to investigate the role of calcium in Cd-induced apoptosis, cells were pretreated with BAPTA-AM, a chelator of cytosolic calcium, before treatment with Cd. As shown in Fig. 2B, pretreatment with BAPTA-AM before exposure to Cd resulted in a markedly reduced percentage of apoptosis, suggesting that an increase in intracellular calcium is a major cause of Cd-induced apoptosis. To detect the role of ER in the Cd-induced augmentation of intracellular calcium, we inhibited the ER calcium channel (IP_3R) by treatment with 2-APB [32], to explore its effects on Cd-induced cell death. As shown in Fig. 3A, 2-APB had the ability to reduce Cd-induced apoptosis. Furthermore, calcineurin is a phosphatase with the ability to dephosphorylate IP_3R and decrease the release of calcium from ER [33]. Inhibition of calcineurin by treatment with cyclosporine A resulted in enhancement of Cd-induced apoptosis (Fig. 3A). Using flow cytometry with Fluo-3 AM, we further investigated the effect of BAPTA-AM, 2-APB and cyclosporine A on cytosolic calcium. As shown in Fig. 3B, BAPTA-AM significantly decreased Cd-induced elevation of cytosolic calcium. 2-APB was with a mild ability to decrease the levels of cytosolic calcium. However, the Cd-induced increase of cytosolic calcium was further elevated in mesangial cells by pretreatment with cyclosporine A. All the above results suggested that ER-released calcium plays a pivotal role in Cd toxicity toward mesangial cells. To further determine the ER stress in Cd-induced apoptosis, we used immunoblotting to detect the induction of GADD153, a hallmark of ER stress. As shown in Fig. 4, GADD153 increased within 4 h after treatment with Cd, but BAPTA-AM abrogated this phenomenon, suggesting that Cd-induced ER stress may result from an increase in cytosolic calcium.

3.2. Cd induces ER-mediated apoptosis

Caspases-12 and -9 are downstream molecules of ER and mitochondrial stress, respectively. To detect the effect of Cd on caspase activation, caspases were analyzed using immunoblotting. As shown in Fig. 5A, caspases-12 and -3, were activated after treatment with Cd within 24 h. Cleaved form of caspase-9 was observed in mesangial cells exposed to 0.75 μM Cd for 36 and 48 h as well as treated with 2 μM Cd for 24 h (Fig. 5A(a)). However, caspase-3 activation was induced by treat-

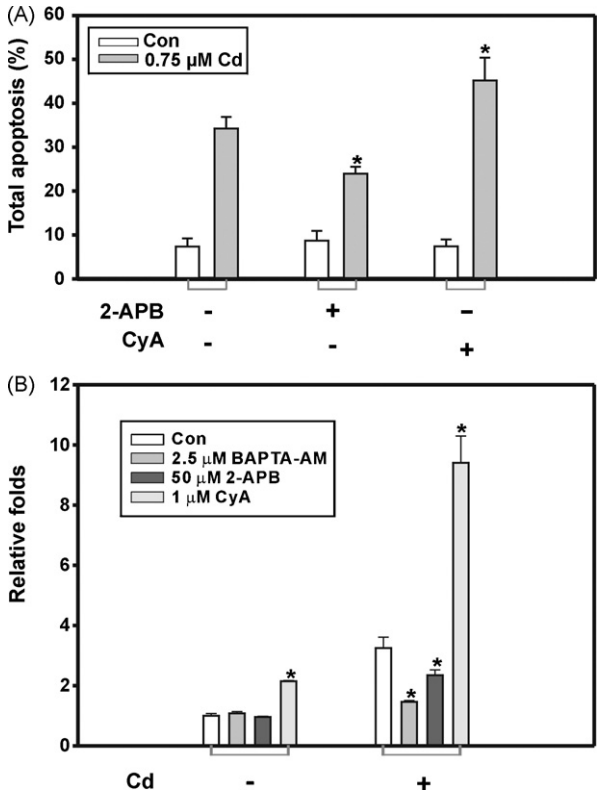

Fig. 3. The IP_3R inhibitor (2-APB) and calcineurin inhibitor (cyclosporine A) modulate cell death induced by Cd in mesangial cells. (A) Pretreatment of mesangial cells with 50 μM 2-APB or 1 μM cyclosporine A modulated cell death induced by Cd. The statistical results are presented in panel A. (B) 2-APB and cyclosporine A modify the variation in $[Ca^{2+}]_i$ after Cd treatment as determined using flow cytometry with Fluo-3 AM staining. Data are presented as described in Section 2. Con, Control; CyA, cyclosporine A; 2-APB, 2-aminoethoxydiphenyl borate. $^*p < 0.05$ vs. the control.

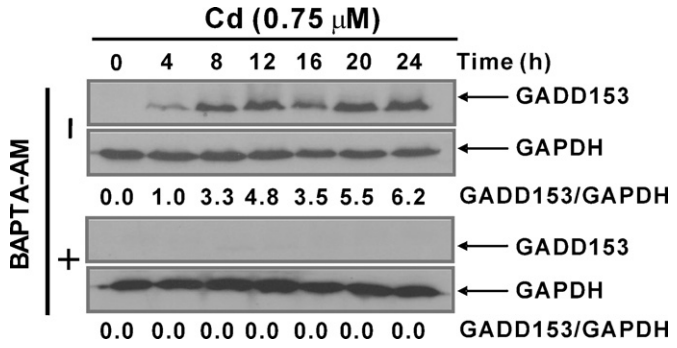

Fig. 4. Endoplasmic reticulum (ER) stress marker, GADD153, is induced by Cd and reversed when co-treated with BAPTA-AM. Mesangial cells were treated with Cd with or without 5 μM BAPTA-AM for the indicated time period. Cell lysates (50 μg/lane) were analyzed using immunoblots with an antibody against GADD153 or GAPDH. GAPDH was used as an internal control to normalize the amount of proteins applied to each lane. The intensity of GADD153/GAPDH was indicated.

ment with Cd for 16 h, suggesting caspase-9 activation is a late event in Cd-induced apoptosis. To further investigate the role of mitochondria in Cd-induced apoptosis, mitotracker green and antibodies against cytochrome *c* was employed with confocal microscope using mesangial cells exposed to Cd. As shown in Suppl. 2, the release of cytochrome *c* was observed after treatment with Cd for 24 h, suggesting mitochondria-mediated apoptosis is involved in Cd-induced apoptosis. Furthermore, a broad caspase inhibitor (Z-VAD-fmk) effectively decreased Cd-induced apoptosis (Fig. 5B), suggesting that Cd induces caspase-mediated apoptosis. Moreover, chelation of cytosolic calcium by treatment with BAPTA-AM suppressed the activation of caspases-12 and -3 (Fig. 6), suggesting that Cd induced an elevation of intracellular calcium that is associated with caspase activation. The mitochondrion has been considered a major organelle in the regulation of cell death [34,35]. To determine the effects of Cd on mitochondria, we monitored the mitochondrial membrane potential using JC-1 dye. As revealed in Fig. 7, the membrane potential declined after treatment with Cd (Fig. 7A), and BAPTA-AM was able to restore it (Fig. 7B), suggesting that intracellular calcium also contributes to the collapse of mitochondria. However, it is worth noting that mitochondrial membrane potential began declining at the 16-h time point and became significant at the 24-h time point after Cd treatment (Fig. 7A). Caspase-9 was

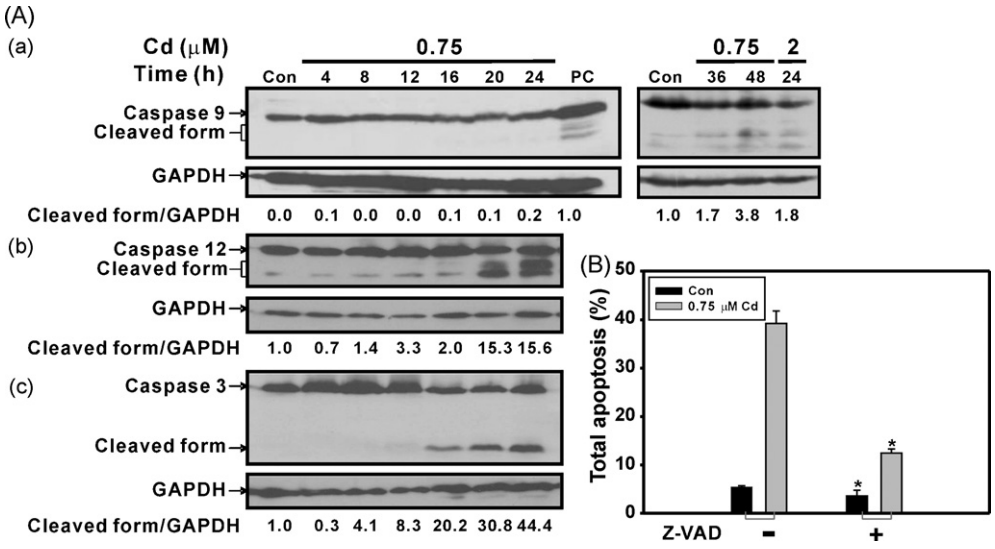

Fig. 5. Caspases are activated during Cd-induced apoptosis, and a broad caspase inhibitor, Z-VAD-fmk, reverses Cd-induced apoptosis. (A) Mesangial cells were treated with 0.75 μM Cd and subjected to analysis of the caspase activation points at the indicated times. Cell lysates (50 μg/lane) were analyzed using immunoblots with anti-caspase-12, anti-caspase-9, anti-caspase-3, or anti-GAPDH antibodies. GAPDH was used as an internal control to normalize the amount of proteins applied to each lane. H_2O_2-treated Raw 264.7 cells undergoing caspase-9-dependent apoptosis were used as a positive control to locate caspase-9. The intensity of cleaved form/GAPDH is indicated (B) Z-VAD-fmk (100 μM, 1 h)-pretreated mesangial cells escape from Cd (0.75 μM, 24 h)-induced apoptosis. Three independent experiments were performed, and statistical results are expressed as the mean ± SD. Z-VAD, Z-Val-Ala-Asp-fluoromethylketone. $^*p < 0.05$ vs. the control.

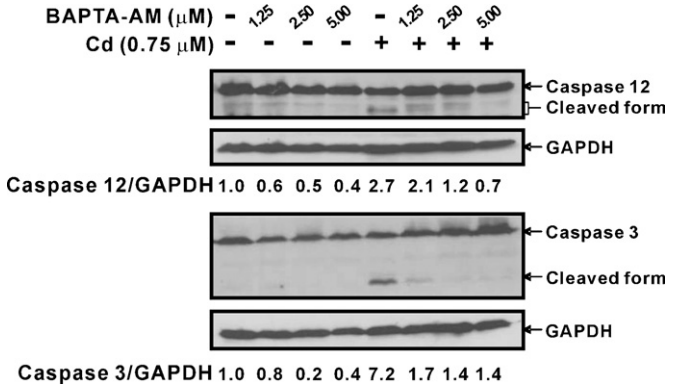

Fig. 6. BAPTA-AM inhibits Cd-activated caspases-12 and -3. Cell lysates (50 μg/lane) were analyzed using immunoblots with anti-caspase-12, anti-caspase-3, or anti-GAPDH antibodies. GAPDH was used as an internal control to normalize the amount of proteins applied to each lane. The intensity of cleaved form/GAPDH is indicated.

only mildly activated after 36 h of Cd treatment (Fig. 5A(a)). This time frame was a sequel to the activation of caspases-12 and -3 (Fig. 5A), indicating that mitochondrial depolarization is more likely a consequence than a primary cause of Cd-induced apoptosis. Therefore, we proposed a model to demonstrated Cd toxicity toward mesangial cells (Fig. 8).

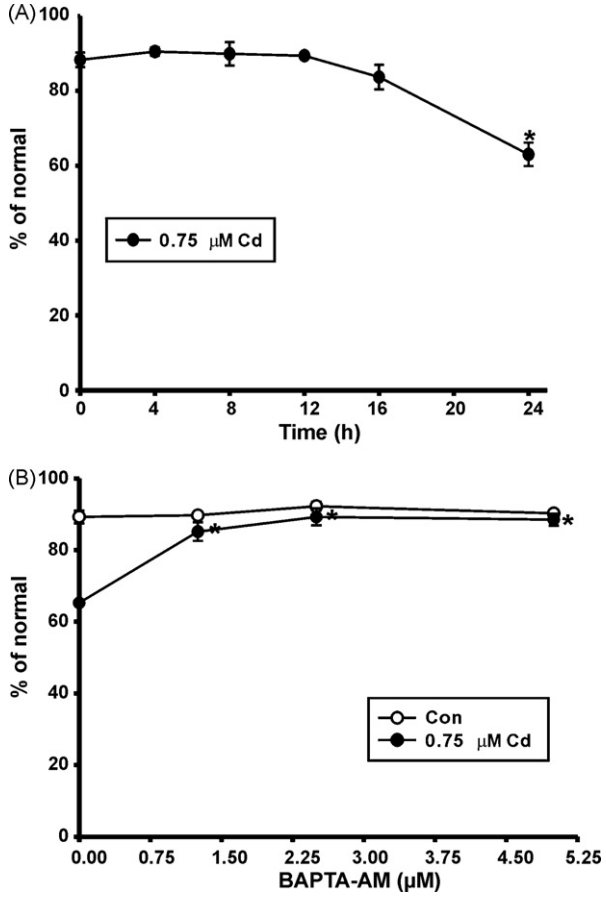

Fig. 7. Cd induces calcium-mediated mitochondria depolarization. Cells were treated with Cd for 24 h. The mitochondrial membrane potential was analyzed by JC-1 staining using flow cytometry. Three independent experiments were performed, and the statistical results are presented in panel A. $^{*}p < 0.05$ vs. the control. Cells were pretreated with the indicated concentrations of BAPTA-AM for 1 h, followed by treatment with 0.75 μM Cd for 24 h, and then the mitochondrial membrane potential was detected using flow cytometry with JC-1 dye. Three independent experiments were performed, and the statistical results are presented in panel B. $^{*}p < 0.05$ vs. the experiment with 0.75 μM Cd but without BAPTA-AM.

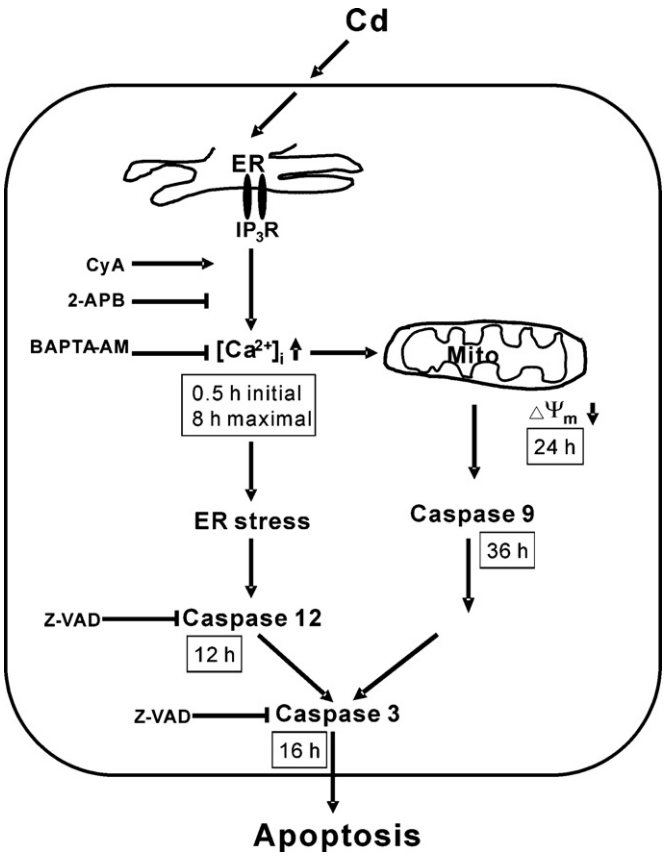

Fig. 8. A proposed model of Cd toxicity toward mesangial cells.

4. Discussion

In this study, Cd induced a caspase-dependent apoptosis which occurred in a dose and time-dependent manner and was accompanied by ER stress, which was confirmed by the elevation of the GADD153 protein level, a hallmark of ER stress, and activation of caspase-12. Furthermore, cytosolic calcium released from ER served as a major mediator of Cd-induced apoptosis and ER stress, since BAPTA-AM decreased the extent of apoptosis and induction of GADD153. Collectively, these data suggest that the cytotoxicity of Cd toward mesangial cells results from an increase in cytosolic calcium leading to caspase activation and ER stress.

It was reported that Cd may induce apoptosis through the generation of ROS, induction of mitochondria-mediated cell death and caspase activation, and the alteration of protein kinases and/or phosphatases [36,37]. In this study, Cd-induced GADD153 expression and caspase-12 activation followed by mitochondria depolarization in mesangial cells. Consistent with our results, the ROS-mediated upregulation of ER stress markers, such as Grp78, Grp94, and GADD153, was observed in Cd-treated renal epithelial cells [38], suggesting that ER is an important organelle in Cd toxicity. Moreover, we also observed that the chelation of cytosolic calcium by treatment with BAPTA-AM inhibited Cd-induced GADD153 induction. Furthermore, blocker of IP_3R, a calcium channel on ER, and extracellular calcium chelator both decreased the proportion of Cd-induced apoptosis, suggesting that intracellular and extracellular calcium is involved in Cd-induced apoptosis. BAPTA-AM also reduced Cd-induced apoptosis, indicating that calcium plays a crucial role in it. However, 5F-BAPTA was reported to decrease Cd toxicity through binding with Cd directly [39]. It is conceivable that calcium served a major regulator in Cd-induced apoptosis, since modulating the function of the ER calcium channel, IP3R by 2-APB or

cyclosporine A, resulted in decrease or increase of cytosolic calcium and apoptosis. A recent report also demonstrated that Cd-induced apoptosis was mediated by calcium elevation induced by inhibition of sarcoplasmic-ER calcium ATPases (SERCA) [40]. Therefore, we could not exclude the possibility that Cd-induced cytosolic calcium elevation was through other mechanism. Recent report demonstrated that EGTA could reduce Cd-induced cell death in astrocytes [41], suggesting that extracellular calcium may play a role in Cd-induced cell death. In this study, EGTA also had an ability to suppress Cd-induced apoptosis (Suppl. 3). However, nifedipine, an L-type calcium channel blocker, had no effect on apoptosis (Suppl. 3). Therefore, the effect of EGTA on Cd-induced apoptosis may indicate that the extracellular calcium plays a role in Cd-induced apoptosis. However, we could not exclude the possibility that EGTA is able to chelate Cd directly.

Prolonged ER stress would be expected to lead to cell death. In the present study, the chelating of cytosolic calcium by BAPTA-AM decreased not only the percentage of cells undergoing apoptosis, but also the expression of GADD153. Furthermore, activation of caspase-12, another marker of ER stress, was also detected after treatment with Cd within 24 h. However, caspase-9 was mildly activated after treatment with Cd exceeded 24 h, suggesting ER stress is the major cause of Cd-induced apoptosis. Recent reports demonstrated that overexpression of an ER-associated apoptosis-involved proteins containing the PH and FYVE domains, (EARF)/Phafin-2, enhances TNF-α induced apoptosis through ER signaling accompanied by mitochondrial depolarization. However, caspases-12 and -3 were activated, and AIF was translocated after treatment with TNF-α, but no effect on caspase-9 was observed [42], suggesting that ER stress is as a major cause of cell death in some systems. In this study, induction of GADD153 and activation of caspase-12 were earlier events than mitochondrial depolarization, suggesting that mitochondrial collapse is more likely a downstream consequence of Cd-induced ER stress. Moreover, chelation of cytosolic calcium resulted in a reduction in mitochondrial depolarization, suggesting that ER-released calcium may serve as a major mediator between ER and mitochondria.

Mitochondria and ER in living cells do interact, such as in the modulation of calcium signaling during cell activation and cell death [43]. The key mediator in connecting ER and mitochondria is the elevation of calcium leading to its prolonged accumulation of calcium in the mitochondria which results in the opening of MPTP and release of apoptotic factors like AIF and cytochrome *c* [44]. In this study, mitochondria depolarization induced by Cd was suppressed by BAPTA-AM, suggesting calcium released from ER may be a mediator connecting ER and mitochondria. It was reported that Bcl-2 family proteins can affect ER and mitochondria-mediated cell death [43]. Furthermore, overexpression of Bcl-2 [45–48] or downregulation of Bax and Bak [49–51] can modulate the concentration of calcium in ER, suggesting that Bcl-2 family proteins may regulate cell death through modulating the concentration of calcium. However, the detailed interaction between ER and mitochondria needs to be further investigated.

Finally, in this study, we demonstrate that Cd induces ER stress, followed by activation of caspases-12, -3 and -9, eventually culminating in apoptotic cell death, suggesting ER and mitochondria stress both are involved in Cd-induced apoptosis. Based on the time frame, we suggest that, in the initial stage, ER is the major killer organelle in Cd-induced mesangial cell apoptosis.

Conflict of interest

None.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cbi.2009.05.004.

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