Biochemical and Biophysical Research Communications 459 (2015) 220–226

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Zinc preconditioning protects against neuronal apoptosis through the mitogen-activated protein kinase-mediated induction of heat shock protein 70

Jeong-Min Lee [1], Jong-Min Lee [1], Ki-Ryeong Kim, Hana Im, Yang-Hee Kim*

Department of Molecular Biology, Sejong University, Seoul 143-747, South Korea

ARTICLE INFO

Article history:
Received 2 February 2015
Available online 21 February 2015

Keywords:
Ischemic preconditioning
Zinc
HSP70
ERK
p38 MAPK
Apoptosis

ABSTRACT

During brain ischemic preconditioning (PC), mild bursts of ischemia render neurons resistant to subsequent strong ischemic injuries. Previously, we reported that zinc plays a key role in PC-induced neuroprotection *in vitro* and *in vivo*. Zinc-triggered $p75^{NTR}$ induction transiently activates caspase-3, which cleaves poly(ADP-ribose) polymerase-1 (PARP-1). Subsequently, the PARP-1 over-activation-induced depletion of nicotinamide adenine dinucleotide (NAD^+)/adenosine triphosphate (ATP) after exposures to lethal doses of zinc or N-methyl-D-aspartate is significantly attenuated in cortical neuronal cultures. In the present study, zinc-mediated preconditioning (Zn PC) reduced apoptotic neuronal death that was caused by N,N,N′,N′-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), etoposide, or staurosporine in mouse cortical cells. We focused on heat shock protein 70 (HSP70) because NAD^+/ATP depletion does not directly cause apoptosis, and HSP70 can inhibit the activation of caspase-9 or caspase-3 by preventing apoptosome formation or cytochrome C release. Zn PC-mediated HSP70 induction was required for neuroprotection against neuronal apoptosis, and geldanamycin-induced HSP70 induction sufficiently blocked neuronal apoptotic cell death. Furthermore, Zn PC-mediated HSP70 induction was blocked by chemical inhibitors of extracellular signal-regulated kinase (ERK) or p38 mitogen-activated protein kinase (MAPK) signaling, but not c-Jun N-terminal protein kinase. Similarly, neuroprotection by Zn PC against TPEN-induced apoptosis was almost completely reversed by the blockade of ERK or p38 MAPK signaling. Our findings suggest that the ERK- or p38 MAPK-mediated induction of HSP70 plays a key role in inhibiting caspase-3 activation during Zn PC.

1. Introduction

Zinc plays an important role in synaptic neurotransmission and acts on the receptors of endogenous neuromodulators, such as 2-(aminomethyl)phenylacetic acid (AMPA)/kainate, *N*-methyl-D-aspartate (NMDA), and gamma-aminobutyric acid (GABA) [1]. Accumulation of excess zinc in neurons can lead to neuronal death after ischemia, epileptic seizures, and traumatic brain injury [2], and the severe depletion of intracellular zinc can induce neuronal apoptosis [3–5].

Brain ischemia is a common cause of death and the leading cause of adult disability worldwide. After ischemia, accumulation of labile zinc has been observed in degenerating neuronal cell bodies and nuclei, and inhibition of zinc accumulation with chelators can protect against ischemic neuronal death, suggesting the critical role of labile zinc in ischemic brain injury [6,7]. Ischemic preconditioning (PC) refers to a transient, mild ischemic condition, which results in tolerance to subsequent strong cerebral ischemia [8]. Previously, we showed that pre-exposure to a sublethal dose of zinc for 12–24 h exhibits a neuroprotective effect when cortical cultures are exposed to lethal levels of zinc or NMDA. However, the induction of PC by other metal ions, such as iron and copper, has no effect [9]. Thus, zinc plays a key role in ischemic PC-induced

Abbreviations: ERK, Extracellular signal-regulated kinases; ETPS, Etoposide; HSP70, Heat shock protein 70; JNK, c-Jun N-terminal kinase; MAPK, Mitogen-activated protein kinase; MEK, Mitogen-activated protein kinase kinase; NMDA, *N*-methyl-D-aspartate; PARP-1, poly(ADP-ribose) polymerase-1; PC, Preconditioning; STSP, Staurosporine; TPEN, N,N,N′,N′-tetrakis(2-pyridylmethyl)ethylenediamine; Zn PC, Zinc-mediated preconditioning.

* Corresponding author. 98 Gunja-Dong Gwangjin-Gu, Seoul 143-747, South Korea. Fax: +82 2 3408 4336.
E-mail address: yhkim@sejong.ac.kr (Y.-H. Kim).

[1] Contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.02.068
0006-291X/

neuroprotection and ischemic brain injury-induced neuronal death [10,11].

HSP70 is a chaperone protein that binds to other proteins to shepherd them across membranes and direct them to specific locations within a cell. It also prevents protein misfolding and inhibits aggregation, and there is accumulating evidence for its roles in other functions [12]. In the brain, HSP70 is neuroprotective against pathological conditions, including ischemia and neurodegenerative diseases [13]. The overexpression or systemic administration of HSP70 reduces brain damage and mitigates neuroinflammation after ischemic injury [14]. Furthermore, HSP70 attenuates apoptotic neuronal death by directly inhibiting caspase activity [15–18]. In neurodegenerative diseases with proteinopathies, including Alzheimer's disease, HSP70 induces protein conformational changes in favor of non-toxic and non-aggregated forms and increases protein degradation by modulating chaperone-mediated autophagy [12]. Thus, HSP70 is a well-known therapeutic target for overcoming diverse brain diseases.

Mitogen-activated protein kinases (MAPK) comprise an enzyme family of protein-serine/threonine kinases, which participate in signal transduction pathways that control a wide spectrum of intracellular events, including proliferation, differentiation, autophagy, cell death, and other stress responses [19,20]. MAPK largely consists of the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK families. The ERK signaling pathway controls the division and differentiation of cells by acting as a survival signal and is mainly activated by growth factors and tumor promoters. In contrast, JNK and p38 MAPK signaling pathways are mainly active in stress responses [21]. Ischemic brain injury predominantly activates JNK and p38 MAPK [22–24]. However, it was recently shown that neuronal death can be caused by prolonged ERK activation following the exposure to excess glutamate or zinc as well as ischemic injury [25–28]. Furthermore, ERK, p38 MAPK, and JNK are all involved in regulating the induction or suppression of apoptosis [20]. Because MAPK signals contribute to the regulation of various pathways, the control of MAPKs under specific conditions should be carefully and separately observed.

It has been suggested that HSP70, ERK, and p38 MAPK may contribute to the underlying mechanism of ischemic PC. However, the cascade of events leading to ischemic PC is not completely understood, although significant progress has been made. We have shown that zinc-triggered ischemic PC is mediated by $p75^{NTR}$ induction, followed by transient caspase-3 activation and PARP-1 cleavage in cortical neuronal cultures [9]. Because PARP-1 over-activation and nicotinamide adenine dinucleotide (NAD^+)/adenosine triphosphate (ATP) depletion constitute a major underlying mechanism of excitotoxicity, zinc neurotoxicity, and ischemic brain injury, PARP-1 cleavage during PC attenuates neurotoxicity. However, the mechanism by which ischemic PC reduces apoptotic neuronal death is unclear, as PARP-1 over-activation and NAD^+/ATP depletion do not mediate apoptosis. Hence, zinc (Zn) PC was conducted to determine which mechanism may be involved in the neuroprotection against apoptotic cell death.

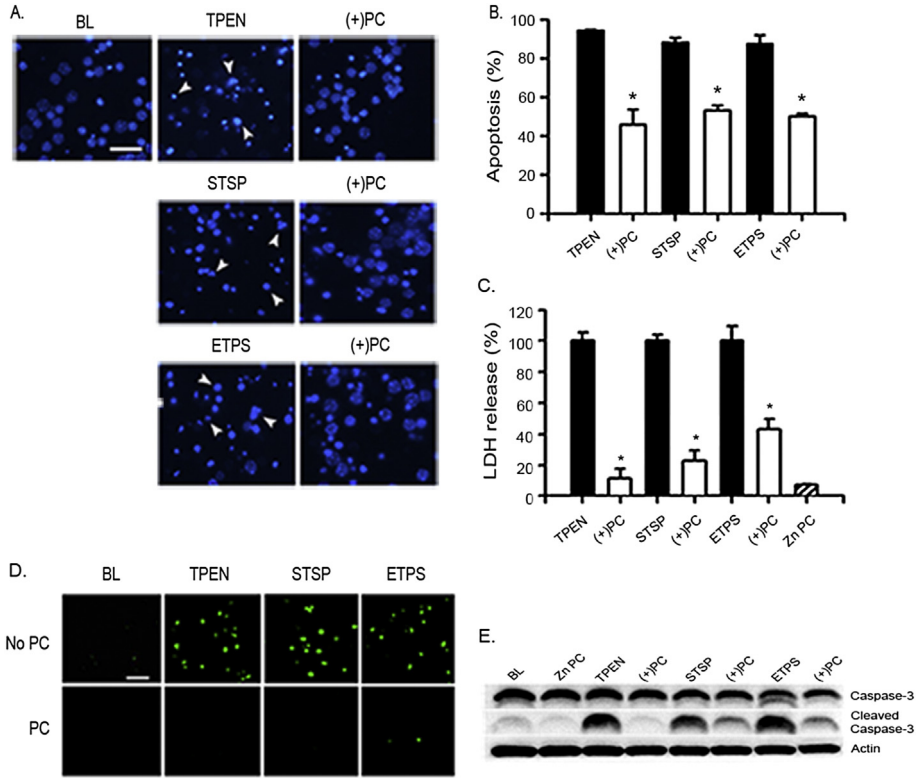

Fig. 1. Zinc preconditioning (Zn PC) attenuates neuronal apoptosis in cortical cultures. (A) Hoechst-stained photomicrographs of cortical cultures that were treated with or without Zn PC (+PC) and exposed to sham wash (BL), 2 μM N,N,N′,N′-Tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), 200 nM staurosporine (STSP), or 30 μM etoposide (ETPS) for 24 h. For Zn PC, cortical cultures were pre-treated with 20 μM zinc for 12 h. Arrows indicate apoptotic nuclei. Scale bar, 50 μm. (B) Shrunken, apoptotic nuclei were counted in Hoechst-stained cortical cultures (mean ± standard error of the mean [SEM], n = 4 cultures) after 24-h exposures to toxins (TPEN, STSP, or ETPS) with or without Zn PC. $^*P < 0.05$ compared to TPEN-, STSP-, or ETPS-treated cultures. Zn PC significantly attenuated neuronal apoptosis. (C) Lactate dehydrogenase (LDH) release (mean ± SEM, n = 4 cultures) in mouse cortical neuronal cultures was measured after 24-h exposures to toxins (TPEN, STSP, or ETPS) with or without Zn PC. $^*P < 0.05$ compared to TPEN-, STSP-, or ETPS-treated cultures. Zn PC alone did not show significant neuronal death. (D) Photomicrographs of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive, apoptotic cells in mouse cortical neuronal cultures after 24-h exposures to sham wash or toxins (TPEN, STSP, or ETPS) with or without Zn PC. Zn PC markedly reduced TUNEL-positive apoptotic cell death. Scale bar, 50 μm. (E) Western blotting of caspase-3. Zn PC reduced TPEN-, STSP-, or ETPS-induced caspase-3 activation.

2. Materials and methods

2.1. Mouse cortical neuronal cultures

Cultures were prepared from mice on embryonic days 14–15 [3,5]. Dissociated cortical cells were plated onto 24-well poly-L-lysine/laminin-coated plates (Nunc) in plating medium (Dulbecco's modified Eagle's medium [GibcoBRL], 20 mM glucose, 38 mM sodium bicarbonate, 2 mM glutamine, 5% fetal bovine serum, 5% horse serum [Cambrex Corp.]). There were ten hemispheres in each plate. All experiments were performed at 10–11 days *in vitro*.

2.2. In vitro PC and toxin exposure

Cortical neuronal cultures were washed with Eagle's minimum essential medium (MEM; GibcoBRL). PC was induced with 20 μM zinc ($ZnCl_2$) (Sigma) or 1 μM geldanamycin (Invitrogen) for 12 h. N-formyl-3,4-methylenedioxy-benzylidene-γ-butyrolaetam (KNK437, 100 μM; Calbiochem), 1,4-Diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene (U0126, 10 μM; Tocris), 4-(4-[4-Fluorophenyl]-5-[4-pyridinyl]-1*H*-imidazol-2-yl)phenol (SB202190, 15 μM; Tocris), or anthra(1,9-cd)pyrazol-6(2*H*)-one (SP600125, 10 μM; Tocris) were added during PC to inhibit HSP70, MEK, p38 MAPK, or JNK, respectively. After PC, the serum-containing medium was removed with multiple rinses and replaced with serum-free MEM. Neurons were then exposed to N,N,N′,N′-Tetrakis(2-pyridylmethyl)ethylenediamine (TPEN; 2 μM), 30 μM etoposide (ETPS), or 200 nM staurosporine (STSP) for 12–24 h (Sigma) in serum-free MEM.

2.3. Estimation of cell death

Cell death was estimated by measuring the release of lactate dehydrogenase (LDH) from irreversibly damaged cells into the medium 12–24 h after exposure to TPEN, ETPS, or STSP. The LDH value was scaled to the maximal value (=100) after a 24-h exposure to 100 μM NMDA in control cultures. To evaluate apoptotic cell death, cortical cultures were stained with Hoechst 33342 (Invitrogen) and then imaged on the EVOS fluorescence microscope. Cells exhibiting condensed chromatin nuclei were scored as apoptotic cells. All experiments were repeated at least three times, and cultures from different plates were used.

2.4. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

The detection of DNA fragmentation, which is a hallmark of apoptosis, in nuclear profiles was performed using a TUNEL assay on neurons based on the *In Situ* Cell Death Detection Kit (Roche), according to the manufacturer's recommendations. The TUNEL assay was followed by DAPI counterstaining, and nuclear profiles were examined under a fluorescence microscope.

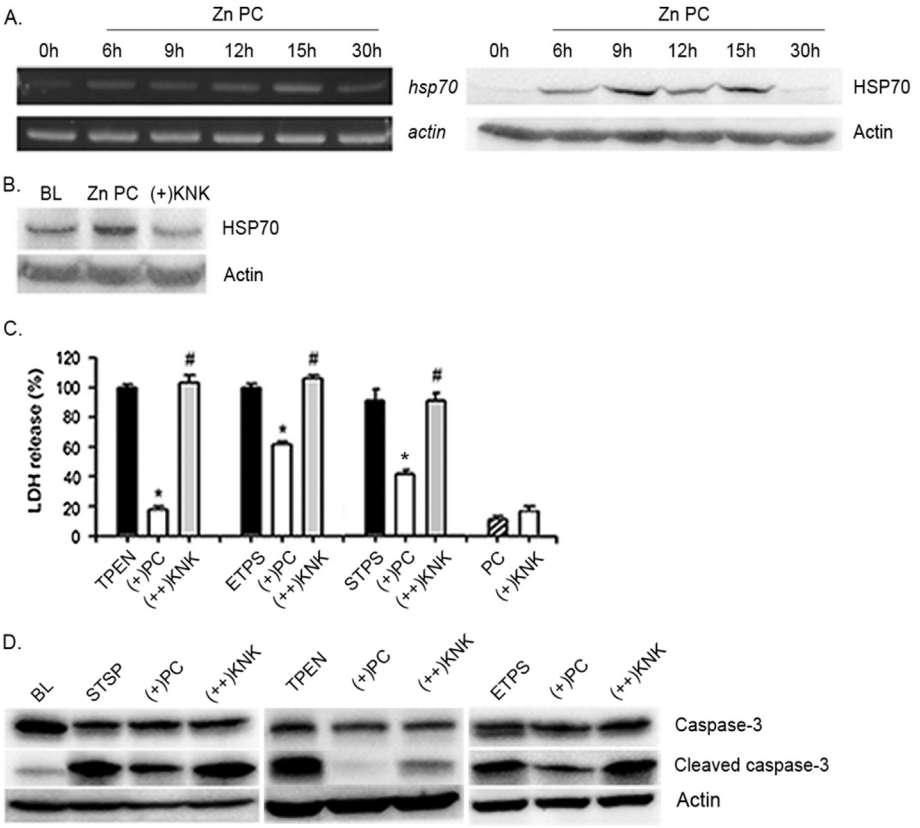

Fig. 2. Heat shock protein 70 (HSP70) induction contributes to the Zn PC-mediated protection against neuronal apoptosis. (A) Reverse transcription-polymerase chain reaction (RT-PCR) (left) and western blot analysis (right) of HSP70 expression. RNA and protein samples were prepared at the indicated time points after the onset of Zn PC. Increases in HSP70 expression levels were detected starting at 6 h after Zn PC onset. (B) Western blots showing HSP70 levels at the 12-h time point after Zn PC onset. The HSP70-specific inhibitor, KNK437 (++KNK; 100 μM), almost completely blocked HSP70 induction by Zn PC. (C) LDH release (mean ± SEM, n = 4 cultures) in mouse cortical neuronal cultures after 24-h exposures to toxins (TPEN, ETPS, or STSP) with or without Zn PC. Some cultures were co-treated with 100 μM KNK437. *$P < 0.05$ compared with toxin controls, #$P < 0.05$ compared with Zn PC controls. KNK437 abrogated the protective effect of Zn PC on neuronal apoptosis. (D) Western blots showing caspase-3 activation at the 12-h time point after exposures to toxins (STSP, TPEN, or ETPS) with or without Zn PC. Some cultures were co-treated with KNK437. KNK437 inhibited the Zn PC-mediated reduction of caspase-3 activation.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA from cortical neurons was isolated using Trizol (Invitrogen), and complementary DNA was synthesized and reverse transcribed using the oligo$(dT)_{14}$ primer (Promega). The PCR was conducted using the Maxime PCR PreMix Kit (Bioneer), and the following primers were used: HSP70, 5′-AACGTGCTGCGGATCAA-3′ (forward), 5′-GAAGTCCTGCAGCTTCT-3′ (reverse); and β-actin, 5′-TCTACAAATGTGGCTGAGGAC-3′ (forward), 5′-CCTGGGCCATTCA-GAAATTA-3′ (reverse).

2.6. Western blot analysis

Cell lysates were prepared in radioimmunoprecipitation assay lysis buffer (50 mM Tris; pH 7.5, 150 mM NaCl, 1% nonyl phenoxylpolyethoxylethanol-40, 0.5% Na-Doc, 0.1% sodium dodecyl sulfate [SDS], 5 mM ethylenediaminetetraacetic acid) with freshly prepared protease and phosphatase inhibitors (2 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na_3VO_4, 5 mM NaF, 10 mM $Na_4P_2O_7$). Total protein (30 μg) was separated by SDS-polyacrylamide gel electrophoresis (8–12%) under reducing conditions and immunoblotted with the appropriate antibody (anti-HSP70 [Stressgen], anti-phosphorylated (phospho)-ERK, anti-phospho-p38 MAPK, anti-p38 MAPK, anti-phospho-JNK, anti-JNK, anti-procaspase-3, anti-cleaved caspase-3 [Cell Signaling Technology], anti-ERK2 [Santa Cruz Biotechnology], or actin [Sigma]). Actin was used as a loading control.

2.7. Statistical analysis

All statistical comparisons were performed by the analysis of variance, followed by the Bonferroni correction for multiple comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Protective effects of zinc PC on neuronal apoptosis

During Zn PC, sublethal increases in zinc ion concentrations induce $p75^{NTR}$ and transiently activate caspase-3 to levels that do not induce apoptosis but are sufficient to promote PARP-1 cleavage. This subsequently blocks the depletion of NAD^+/ATP by lethal zinc- or NMDA-induced PARP-1 over-activation [9]. However, PARP-1 over-activation and NAD^+/ATP depletion are not characteristics of apoptosis [29]. Therefore, we examined whether Zn PC reduced TPEN-, ETPS-, or STSP-induced apoptotic neuronal death in mouse cortical cells. First, we observed morphological changes in nuclei. Hoechst 33258 staining was homogeneous throughout the nuclei of normal cells. However, TPEN-, STSP-, or ETPS-treated cultures showed chromatin condensation and nuclear fragmentation within the nuclei, as evidenced by bright, shrunken, fragmented staining (Fig. 1A). These morphological changes were inhibited by pre-treatment with sublethal doses of zinc for 12 h (Fig. 1A). The quantification of apoptotic cells showed significant reductions in TPEN-, STSP-, or ETPS-induced apoptosis by Zn PC (Fig. 1B). Furthermore, LDH assay results show that Zn PC significantly attenuated neuronal death in TPEN-, STSP-, and ETPS-treated cells (Fig. 1C). Furthermore, we assessed TUNEL staining and caspase-3 activation. Zn PC markedly reduced the number of TUNEL-positive cells (Fig. 1D) and caspase-3 activation in TPEN-, STSP-, or ETPS-treated cells (Fig. 1E). These results show that Zn PC has a neuroprotective effect and can prevent apoptotic cell death.

3.2. The role of HSP70 in the Zn PC-mediated protection against neuronal apoptosis

Previously, we demonstrated that prolonged caspase-3 activation is inhibited after HSP70 induction during Zn PC in mouse cortical cultures, thereby blocking possible caspase-mediated apoptosis by Zn PC itself [9]. Therefore, we focused on HSP70 as a key player in the Zn PC-mediated protection against apoptosis. Both HSP70 mRNA and protein levels increased starting at 6 h after Zn PC onset and diminished 30 h later (Fig. 2A). The addition of KNK437, which is a chemical inhibitor of HSP70 induction, markedly reduced HSP70 induction by Zn PC (Fig. 2B). The neuroprotective effects of Zn PC on TPEN, STSP, or ETSP toxicity were almost completely reversed by KNK437, thus indicating a critical role of HSP70 (Fig. 2C). Consistently, KNK437 reversed the Zn PC-mediated reduction of caspase-3 activation (Fig. 2D). Thus, HSP70 induction during Zn PC is essential for the protection against neuronal apoptosis.

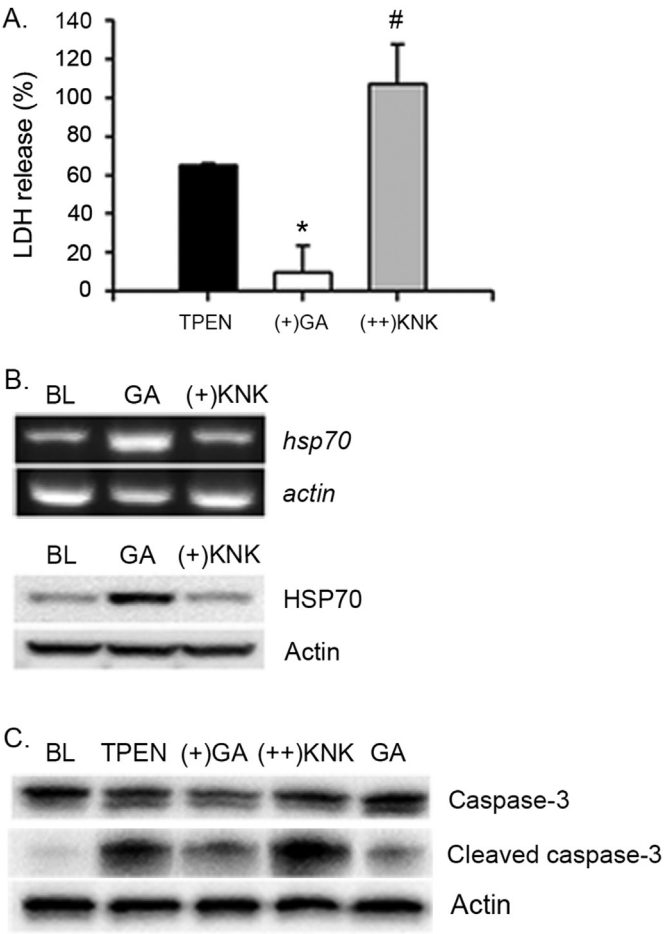

Fig. 3. HSP70 induction is sufficient for the Zn PC-mediated neuroprotection against neuronal apoptosis. (A) LDH release (mean ± SEM, n = 4 cultures) in mouse cortical neuronal cultures after 24-h exposures to TPEN with or without geldanamycin (GA)-mediated PC (GA PC; +GA) or GA PC plus KNK437 (++KNK). *$P < 0.05$ compared with TPEN, #$P < 0.05$ compared with GA PC. For GA PC, cortical cultures were pre-treated with 1 μM GA for 12 h. GA PC markedly attenuated TPEN-induced neuronal apoptosis, and this was reversed by KNK437. (B) RT-PCR (upper) and Western blot analysis (lower) of HSP70 expression. RNA and protein samples were prepared from neuronal cultures after 12-h exposures to sham wash (BL) or GA PC with or without KNK437. GA markedly increased HSP70 expression in a KNK437-dependent manner. (C) Western blot analysis of caspase-3 after TPEN treatment. Protein samples were prepared after 12-h exposures to sham wash (BL) or TPEN with or without GA PC or GA PC plus KNK437.

We next examined whether HSP70 expression itself could sufficiently exert cellular protective effects. Geldanamycin is an inducer of HSP70 that up-regulates HSP70 expression in the substep as the negative regulator of activated HSP90 [30]. As shown in Fig. 3A, pre-treatment with geldanamycin for 12 h markedly attenuates TPEN-induced neuronal apoptosis, and the effect of geldanamycin is completely reversed by KNK437. To test whether geldanamycin-mediated neuroprotection was mediated by HSP70 induction, we assessed HSP70 mRNA and protein levels after 12-h exposures to geldanamycin. HSP70 mRNA and protein levels increased noticeably, and both levels decreased with KNK437 treatment (Fig. 3B). Furthermore, geldanamycin PC reduced caspase-3 activation by TPEN, which was reversed by co-treatment with KNK437 (Fig. 3C). These results indicate that HSP70 induction is a critical factor for neuroprotection against apoptosis.

3.3. ERK and p38 MAPK play essential roles in HSP70 induction during Zn PC

The MAPK signaling pathway, which includes ERK, p38 MAPK, and JNK, is involved in the regulation of apoptosis after ischemia [19,20]. The ischemic PC-mediated neuroprotection against strong ischemia is mediated by activation of the MAPK signaling pathway [27]. Furthermore, the induction of HSP70 by ischemic PC is mediated by p38 MAPK and ERK signaling pathways [31,32].

We investigated whether these MAPK signaling components were involved in the up-regulation of HSP70 during Zn PC. ERK, p38 MAPK, and JNK activation began 0.5 h after the onset of Zn PC (Fig. 4A). ERK phosphorylation was prolonged to 8 h after Zn PC onset, whereas p38 MAPK and JNK phosphorylation showed two-phase activation patterns. The first activation was sustained for

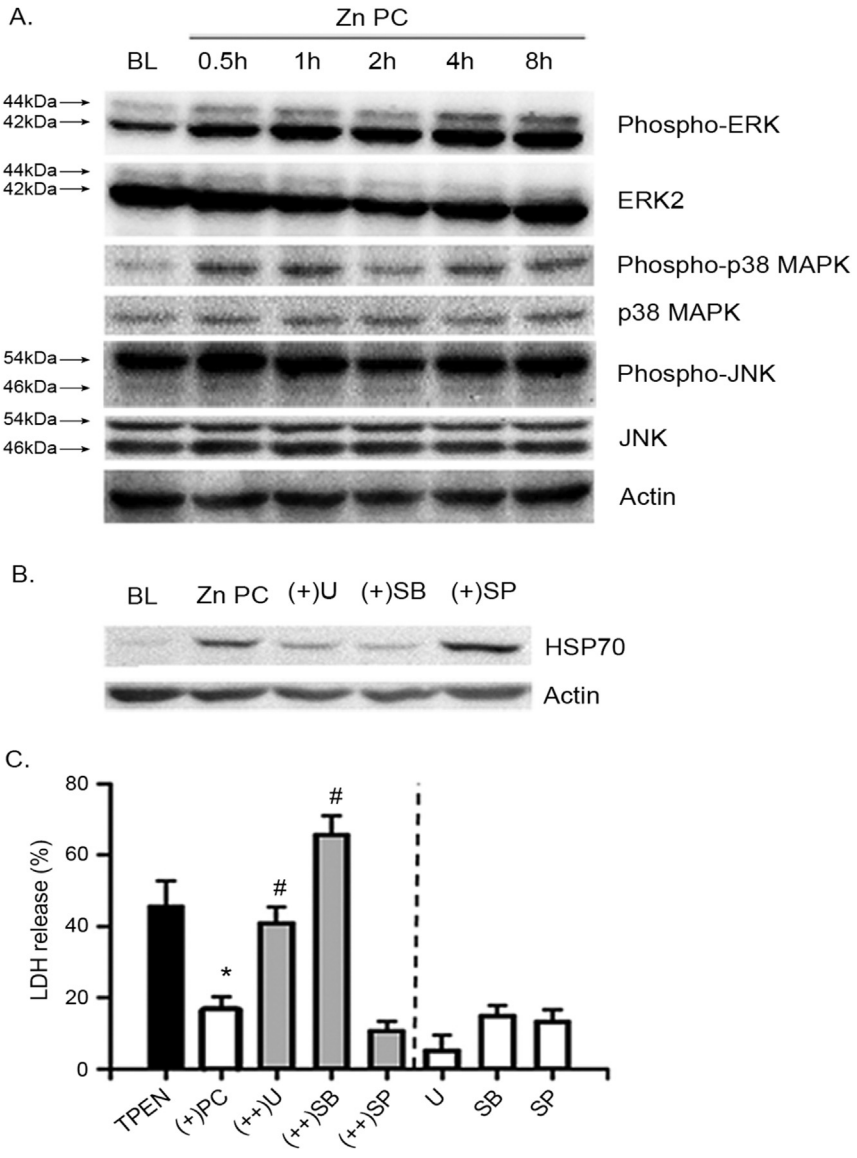

Fig. 4. Blockade of neuronal apoptosis by Zn PC is mediated by extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein kinase (MAPK). (A) Western blot analysis of phosphorylated (phospho)-ERK/ERK2, phospho-p38 MAPK/p38 MAPK, or phospho-c-Jun N-terminal kinase (JNK)/JNK at the indicated time points after the onset of Zn PC. (B) Western blot analysis of HSP70 12 h after the onset of Zn PC with or without 15 μM U0126, 15 μM SB202190, or 10 μM SP600125. Inhibitors of ERK or p38 MAPK, but not JNK, blocked Zn PC-mediated HSP70 induction. (C) LDH release (mean ± SEM, n = 3 cultures) in mouse cortical neuronal cultures after 18-h exposures to TPEN with or without PC alone (+PC), PC plus U0126 (++U), PC plus SB202190 (++SB), or PC plus SP600125 (++SP). *$P < 0.05$ compared with TPEN, #$P < 0.05$ compared with Zn PC controls. U0126, SB202190, or SP600125 did not show any neurotoxicity.

1 h, after which it returned to baseline levels, and then the second activation was induced starting at 4 h after Zn PC onset (Fig. 4A).

Next, we examined whether ERK, p38 MAPK, and JNK activation were associated with the neuroprotective effects of Zn PC. As shown in Fig. 4B, Zn PC-mediated HSP70 induction is reduced by inhibitors of ERK1/2 or p38 MAPK, but not JNK. Interestingly, treatment with the inhibitor of MEK (U0126) or p38 MAPK (SB202190) completely reversed the neuroprotection by Zn PC against TPEN-induced apoptosis. The JNK inhibitor, SP600125, had no effects (Fig. 4C).

These results strongly suggest that ERK and p38 MAPK activation occur earlier than HSP70 induction during Zn PC and contribute to the up-regulation of HSP70 in the neuroprotective effect of Zn PC on TPEN-induced apoptosis.

4. Discussion

Findings from this study demonstrate that Zn PC increased the expression of HSP70 in ERK- and p38 MAPK-dependent manners. Both ERK and p38 MAPK had neuroprotective effects against subsequent apoptosis in cultured cortical neurons.

Previously, we showed that zinc plays an important role in the neuroprotective effects of ischemic PC *in vitro* or *in vivo* [9]. Unlike other metal ions, such as iron and copper, zinc-triggered $p75^{NTR}$ induction transiently activates caspase-3 as a crucial factor in neuroprotection, which then cleaves PARP-1. Consistent with PARP-1 cleavage, the depletion of NAD^+/ATP by PARP-1 over-activation after exposure to toxins is significantly attenuated [9]. However, the mechanisms by which ischemic PC protects against apoptosis remain unclear. Therefore, we focused on the mechanisms involved in the effects of Zn PC on apoptosis.

HSP70 controls apoptosis by preventing both apoptosome formation and cytochrome c release from the mitochondria, thereby suppressing caspase-9 and caspase-3 activity [15–18]. We have shown that HSP70 induction inhibits prolonged caspase-3 activation during Zn PC and rescues neurons from Zn PC-induced apoptosis [9]. Despite the activation of caspase-3 during Zn PC, the subsequent induction of HSP70 prevents apoptotic cell death. This suggested that HSP70 exerts specific actions to prevent neuronal apoptosis after Zn PC. In the present study, HSP70 mRNA and protein levels increased over time with Zn PC. The neuroprotective effect disappeared after co-treatment with KNK437, indicating that increased HSP70 expression during Zn PC is a key factor. Geldanamycin is a benzoquinone ansamycin antibiotic that can inhibit the ATPase activity of HSP90 [33]. Because HSP90 is a major repressor of heat shock transcription factor-1 (HSF1), inhibition of HSP90 by geldanamycin induces HSP70 expression via HSF1 [34,35]. By demonstrating geldanamycin PC-mediated neuroprotection against TPEN-induced apoptosis and the reversal effect by KNK437, we verified the essential role of HSP70 in the Zn PC-mediated protection against neuronal apoptosis.

In comparison to HSP70 induction, ERK, JNK, and p38 MAPK activation occurred at a very early time point after Zn PC onset. However, only ERK and p38 MAPK signals, but not JNK, contributed to the HSP70-mediated neuroprotection by Zn PC. This is consistent with results from previous studies in animals [9]. Additionally, prolonged ERK activation was observed after Zn PC onset. Although sustained ERK activation may be related to neurotoxicity rather than survival, prolonged ERK activation during Zn PC exhibited protective actions. Thus, MAPK signaling-mediated cellular functions are dependent upon upstream or downstream signaling components, and the application of MAPK signals to control pathological conditions, such as brain ischemia, should be carefully considered.

Ischemic PC has received significant attention for a long time, because the PC process can protect cells from irreversible neurological damage. In clinical practice, strokes that occur after subtle presymptoms are associated with fewer complications than strokes that occur unexpectedly without presymptoms. Although the exact mechanism of this process is unknown, our results suggest that the ERK- or p38 MAPK-mediated induction of HSP70 may play a crucial role in inhibiting caspase-3 activation. Therefore, HSP70 could be potential therapeutic target for ischemic stroke.

Conflict of interest

None.

Acknowledgments

This work was supported by National Research Foundation of Korea grants from the Ministry of Science, ICT and Future Planning (NRF-2008-313-C00754, NRF-2009-0081490, and NRF-2011-0016050).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.068.

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