The molecular mechanism underlying Akt activation in zinc-induced cardioprotection

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Abstract

Our previous study demonstrated that zinc prevents cardiac reperfusion injury by targeting the mitochondrial permeability transition pore (mPTP) via Akt and glycogen synthase kinase 3β (GSK-3β). We aimed to address the mechanism by which zinc activates Akt.

Treatment of H9c2 cells with ZnCl₂ (10 µM) in the presence of the zinc ionophore pyrithione (4 µM) for 20 min enhanced Akt phosphorylation (Ser⁴⁷³), indicating that zinc can rapidly activate Akt. Zinc did not alter either PTEN (phosphatase and tensin homolog on chromosome ten) phosphorylation and total PTEN protein levels or PTEN oxidation, implying that PTEN may not play a role in the action of zinc. However, zinc-induced Akt phosphorylation was blocked by both the non-selective receptor tyrosine kinase (RTK) inhibitor genistein and the selective insulin-like growth factor-1 RTK (IGF-1RTK) inhibitor AG1024, indicating that zinc activates Akt via IGF-1RTK. Zinc-induced phosphorylation of protein tyrosine and serine/threonine was also abolished by AG1024. In addition, zinc markedly enhanced phosphorylation of IGF-1R, which was again reversed by genistein and AG1024. Confocal imaging study revealed that AG1024 abolished the preventive effect of zinc on oxidant-induced mPTP opening, confirming that IGF-1RTK plays a role in zinc-induced cardioprotection. Furthermore, zinc decreased the activity of protein phosphatase 2A (PP2A), a major protein serine/threonine (Ser/Thr) phosphatase, implying that protein Ser/Thr phosphatases may also play a role in the action of zinc on Akt activity. Taken together, exogenous zinc activates Akt via IGF-1RTK and prevents the mPTP opening in cardiac cells. Inactivation of Ser/Thr protein phosphatases may also contribute to zinc-induced Akt activation.

Key words: IGF-1RTK; PP2A; protein serine/threonine phosphatase
Introduction

In addition to its essential role in maintaining the structure and function of many proteins, enzymes, and transcriptional factors (4), zinc also regulates the activities of several important signaling kinases such as phosphatidylinositol 3-kinase (PI3K) (3), Akt/PKB (1), extracellular signal-regulated kinase (ERK) (1), and glycogen synthase kinase 3β (GSK-3β) (18). Since all these signaling kinases are involved in the mechanism underlying cardioprotection against reperfusion injury (15), treatment of cardiac cells with zinc may lead to cardioprotection against reperfusion injury. In support, Karagulova et al. documented that treatment of isolated rat hearts at reperfusion with zinc ionophore pyrithione induces cardioprotection against reperfusion injury by preserving protein kinase C isoforms (20). We have demonstrated that exogenous zinc protects cardiac cells from reperfusion injury by targeting the mitochondrial permeability transition pore (mPTP) through inactivation of glycogen synthase kinase 3β (GSK-3β) in cardiac H9c2 cells (8). Further, the effect of zinc on GSK-3β was reversed by the PI3K inhibitor LY294002 and zinc markedly enhanced Akt phosphorylation (8). Although these results suggest that the activation of the PI3K/Akt signaling is critical for zinc-induced cardioprotection, little is known regarding the mechanism by which exogenous zinc activates Akt, as mentioned by Mocanu and Yellon (29). Accordingly, it is intriguing to explore the mechanism underlying zinc-induced Akt activation.

The PI3K/Akt signaling is modulated by various phosphatases at several levels (2). It is regulated by the protein tyrosine phosphatase (PTPase) family at the level of receptor tyrosine kinase (RTK), lipid phosphatases such as PTEN (phosphatase and tensin homolog on chromosome ten) at the level of lipids such as phosphatidylinositol-3’,4’,5’-triphosphate (PIP3) generated by PI3K, and Ser/Thr phosphatases that inactivate Akt by reversing phosphorylation
of Thr\textsuperscript{308} and Ser\textsuperscript{473}. Since zinc can inhibit PTPase in rat glioma cells (14) and zinc-induced activation of the PI3K/Akt pathway is mediated by Src-dependent stimulation of the epidermal growth factor receptor (EGFR) (33), it is possible that exogenous zinc activates RTK by suppressing PTPase, which in turn leads to activation of PI3K/Akt. PTEN is a highly conserved phosphatase and negatively regulates the PI3K/Akt signaling pathway by dephosphorylating PIP\textsubscript{3} to PIP\textsubscript{2}. Although PTEN is a constitutively active enzyme, its activity can be down-regulated by phosphorylation or oxidation (25). Exposure of human airway epithelial cells to zinc for a relatively long period (4 h) can induce PTEN protein degradation and loss of function (34). However, it is unlikely that treatment of H9c2 cells with zinc for a short time (20 min) could markedly reduce PTEN protein levels in our study. Nevertheless, it is still interesting to test if zinc rapidly down-regulates PTEN activity to activate Akt. Since Ser/Thr phosphatases can inhibit Akt by dephosphorylating Thr\textsuperscript{308} and Ser\textsuperscript{473} residues (30), it is worthwhile to determine if zinc activates Akt by down-regulating Ser/Thr phosphatase activities.

In this study, we first examined if zinc could alter PTEN activity by evaluating both phosphorylation (Ser\textsuperscript{380/382/383}) and the total protein levels of PTEN in cardiac H9c2 cells. We next determined if zinc activates Akt through RTKs, focusing on the roles of insulin-like growth factor 1 receptor tyrosine kinase (IGF-1RTK) and epithelial growth factor receptor tyrosine kinase (EGFRTK). We then tested if inhibition of IGF-1RTK can block the preventive effect of zinc on the mPTP opening. Lastly, we tested if zinc can alter Ser/Thr protein phosphatase activity.
Materials and Methods

Cell Culture

Rat heart tissue-derived H9c2 cardiac myoblast cell line was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 U penicillin/streptomycin at 37 ºC in a humidified 5% CO2-95% air atmosphere.

Chemicals and antibodies

ZnCl2 and pyrithione were purchased from Sigma Chemical (St. Louis, MO). LY294002, wortmannin, and genistein were obtained from LC Laboratories (Woburn, MA). PP2, AG1024, and AG1478 were purchased from EMS Chemicals (Gibbstown, NJ). PP2A activity assay kit was purchased from Promega (Madison, WI). All antibodies were obtained from Cell Signaling Technology (Beverly, MA).

Western blotting analysis

Equal amount of protein lysates (whole cell or mitochondria or cytosol) were loaded and electrophoresed on SDS-polyacrylamide gel and transfected to a PVDF membrane. Membranes were probed with primary antibodies that recognize phosphorylation of Akt, GSK-3β, PTEN, IGF-1R, Serine (Ser)/Threonine (Thr), and tyrosine (Tyr). Each primary antibody binding was detected with a secondary antibody and visualized by the ECL method. Equal loading of samples were confirmed by reprobing membranes with anti-tubulin antibody.

Identification of reduced and oxidized forms of PTEN

The reduced and oxidized forms of PTEN were analyzed with Western blotting as previously described (22).

Confocal Imaging of ΔΨm
$\Delta \Psi_m$ was measured using confocal microscopy as reported previously (19). Briefly, cardiac cells cultured in a specific temperature-controlled culture dish were incubated with tetramethylrhodamine ethyl ester (TMRE, 100 nM) in standard Tyrode solution containing (mM) NaCl 140, KCl 6, MgCl$_2$ 1, CaCl$_2$ 1, HEPES 5, and glucose 5.8 (pH 7.4) for 10 min. Cells were then mounted on the stage of an Olympus FV 500 laser scanning confocal microscope. The red fluorescence was excited with a 543 nm line of argon-krypton laser line and imaged through a 560 nm long-path filter. Temperature was maintained at 37°C with Delta T Open Dish Systems (Bioptechs, Butler, PA). The images recorded on a computer were quantified using Image J.

**PP2A activity assay**

PP2A activity was determined using Serine/Threonine phosphatase assay kit (Promega) according to the manufacturer’s instruction. Briefly, cell lysates (25 µl) were centrifuged at 15,000x g for 1 h after a short sonication. Endogenous free phosphate was removed from the lysate supernatant with a Sephadex G-25 resin spin column. Serine/Threonine phosphate activity was measured colorimetrically by the capacity to dephosphorylate a synthetic -754 Da phosphopeptide through formation of molybdate. The total Serine/Threonine phosphatase activity for each sample was expressed as a percentage of the value measured in cell lysate without PP2A substrates.

**Experimental protocols**

Cultured cells were washed twice with Tyrode solution and then incubated in Tyrode solution for 2 h prior to experiments. To examine the effect of extracellular zinc on phosphorylation of kinases, receptors, and kinase residues, cells were exposed to 10 µM ZnCl$_2$ together with 4 µM zinc ionophore pyrithione for 20 min. Inhibitors were applied 20 min before exposure to ZnCl$_2$. In the study evaluating the effect of exogenous zinc on $\Delta \Psi_m$, cells were
exposed to 600 µM H₂O₂ for 20 min to cause mitochondrial oxidant damage. ZnCl₂ (10 µM) plus 4 µM pyrithione were given 20 min before exposure to H₂O₂. AG1024 was applied 20 min before exposure to ZnCl₂. To test if exogenous zinc can activate Akt and IGF-1R following ischemia, cells were exposed to a simulated ischemia solution (glucose-free Tyrode solution containing 10 mM 2-deoxy-D-glucose and 10 mM sodium dithionite (17)) for 90 min followed by 30 min of reperfusion with the normal Tyrode solution. ZnCl₂ (10 µM) plus 4 µM pyrithione were applied at the onset of reperfusion for 20 min.

Statistical analysis

Data are expressed as mean ± SEM and obtained from at least 6 experiments. Statistical significance was determined using one-way ANOVA followed by Tukey’s test. A value of \( P < 0.05 \) was considered as statistically significant.
Results

Treatment of H9c2 cells with 10 µM ZnCl₂ in the presence of the zinc ionophore pyrithione (4 µM) dramatically increased Akt phosphorylation at Ser⁴⁷³, an effect that was abrogated by the Zn²⁺ chelators ethylenediaminetetraacetic acid (EDTA, 10 mM) and N,N,N’,N’-tetrakis-(2-pyridylmethyl) ethylenediamine (TPEN, 10 µM), indicating that exogenous zinc can activate Akt (Fig. 1A). ZnCl₂ also markedly increased Akt and GSK-3β phosphorylation upon reperfusion in cardiac cells (Fig. 1B). To test if zinc activates Akt through inhibition of PTEN, the negative regulator of the PI3K/Akt pathway, we determined the effect of zinc on PTEN phosphorylation at Ser³⁸⁰/Thr³⁸²-³⁸³. As show in Fig. 2A, treatment of cells with 10 µM ZnCl₂ for 20 min did not alter PTEN phosphorylation, although phosphorylation of Akt and GSK-3β was significantly augmented by the treatment. In addition, 10 µM ZnCl₂ did not alter PTEN protein levels (Fig. 2B). Treatment of cells with H₂O₂ resulted in the appearance of the higher mobility form of PTEN (oxidized form) (Fig. 2C). In contrast, cells treated with ZnCl₂ did not show this form. These results indicate that exogenous zinc may not rapidly regulate PTEN activity in H9c2 cells.

To investigate if exogenous zinc activates Akt through receptor tyrosine kinases (RTKs), we tested effects of various RTK inhibitors on zinc-induced Akt phosphorylation. As shown in Fig. 3, the effect of ZnCl₂ (10 µM) on Akt phosphorylation at Ser⁴⁷³ was significantly inhibited by genistein (100 µM) (9), a non-selective RTK inhibitor, indicating that zinc may activate Akt via RTKs. Further experiments showed that the action of zinc was reversed by AG1024 (5 µM) (6), a selective inhibitor of IGF-1RTK, but not by the Src tyrosine kinase inhibitor PP2 (10 µM) (36) or AG1478 (10 µM) (28), a selective inhibitor of EGFRTK, suggesting that exogenous zinc activates Akt through activation of IGF-1RTK (Fig. 3).
To test if zinc activates Akt by entering cells, we determined the role of the zinc ionophore pyrithione in the action of zinc. As shown in Fig. 4A, in the absence of pyrithione, ZnCl₂ (10 µM) did not alter Akt and GSK-3β phosphorylation. Likewise, IGF-1R was activated by ZnCl₂ (10 µM) only when cells were co-treated with pyrithione (Fig. 4B). Pyrithione alone did not alter phosphorylation of Akt and IGF-1R (Fig. 4A and 4B). These results suggest that zinc activates Akt after entering cells.

To establish the role of IGF-1RTK in the action of zinc, we tested if exogenous zinc can activate IGF-1RTK by measuring phosphorylation levels of IGF-1R at Tyrⁱ¹³⁵/¹¹³⁶, since phosphorylation of Tyr¹¹³⁵/¹¹³⁶ residues is necessary for activation of IGF-1RTK (24). As shown in Fig 5A, ZnCl₂ (10 µM) significantly increased IGF-1R phosphorylation in cardiac cells, assuring that exogenous zinc indeed activates IGF-1RTK. ZnCl₂ (10 µM) also markedly enhanced IGF-1R phosphorylation in cells subjected to simulated ischemia/reperfusion (Fig. 5B). Zinc-induced IGF-1R phosphorylation was reversed by AG1024 (5 µM) and was partially inhibited by genistein (100 µM) (Fig. 5C). PP2 (10 µM) and AG1478 (10 µM) were not able to block the action of zinc. Genistein, PP2, AG1024, and AG1478 did not alter IGF-1R phosphorylation in the absence of Zn²⁺ (Fig. 5C). These results further support the essential role of IGF-1RTK in the action of zinc.

Having demonstrated the critical role of IGF-1RTK in zinc-induced Akt activation, we tested if inhibition of IGF-1RTK could reverse the preventive effect of zinc on oxidant-induced mPTP opening. As show in Fig. 6, ZnCl₂ (10 µM) prevented 600 µM H₂O₂-induced loss of TMRE fluorescence, indicating that exogenous zinc can modulate the mPTP opening. In contrast, zinc was not able to preserve TMRE fluorescence in the presence of the IGF-1RTK.
inhibitor AG1024 (5 µM), suggesting that IGF-1RTK contributes to the protective effect of zinc through activation of Akt.

Akt can be inactivated by dephosphorylation of Ser\textsuperscript{473} and Thr\textsuperscript{308} residues by Ser/Thr protein phosphatases. Thus, inhibition of Ser/Thr protein phosphatases may lead to Akt activation. To test if inactivation of Ser/Thr protein phosphatases accounts for zinc-induced Akt activation, we examined the effect of zinc on the activity of PP2A, a major Ser/Thr protein phosphatase. As shown in Fig. 7A, ZnCl\textsubscript{2} (10 µM) decreased PP2A activity, indicating that Ser/Thr protein phosphatases may also be involved in zinc-induced Akt activation. Calyculin A (12.5 nM), a cell permeable Ser/Thr protein phosphatase inhibitor, mimicked the effect of ZnCl\textsubscript{2} to increase Akt phosphorylation (Fig. 7B).
Discussion

The PI3K/Akt signaling pathway plays an essential role in protecting the heart from ischemia/reperfusion injury (16). Recently, we have also demonstrated that Akt mediates exogenous zinc-induced cardioprotection against reperfusion injury by inactivating GSK-3β (8). In this study, we sought to explore the mechanism by which exogenous zinc activates Akt and found that IGF-1RTK and Ser/Thr protein phosphatases may contribute to zinc-induced Akt activation.

Many growth-promoting ligands such as insulin, IGF-1, and EGF bind to RTKs and can activate Akt (21, 35, 37). In this study, the effect of exogenous zinc on Akt phosphorylation was blocked by the selective IGF-1RTK inhibitor AG1024 but not by the selective EGFRTK inhibitor AG1478. AG1024 also abolished the effect of zinc on phosphorylation of protein tyrosine and serine/threonine. Moreover, zinc increased IGFR phosphorylation, and this was reversed by AG1024 but not by AG1478. Furthermore, the preventive effect of zinc on the mPTP opening was reversed by AG1024. These observations clearly suggest that IGF-1RTK but not EGFRTK is responsible for the action of zinc on Akt activity. In support, a recent report has documented that zinc can modulate IGF-1 signaling in C6 rat glioma cells by regulating protein tyrosine phosphatase activities (14). However, our finding is inconsistent with a previous study by Wu et al. reporting that zinc activates Akt via a Src-dependent stimulation of EGFR in human bronchial epithelial cells (33). The fact that Wu et al. treated cells with a higher dose of Zn²⁺ (50 µM) for a longer time (120 min) may explain the discrepancy. It should be mentioned that although our observations adopting the specific inhibitors and antibody clearly indicate the essential role of IGF-1R in the action of zinc, future studies with IGF-1R and EGFR mutants or siRNAs will help establish their role in the action of zinc.
RTKs are integral membrane proteins that have a ligand-binding domain on the extracellular side and a tyrosine kinase domain on the cytosolic side. Although little is known whether exogenous zinc stimulates IGF-1RTK through its binding to the ligand-binding domain on the extracellular side, our data showed that exogenous zinc failed to activate either Akt or IGF-1R in the absence of the zinc ionophore pyrithione, indicating that zinc activates IGF-1RTK by entering cells rather than by binding to the extracellular binding domain. Thus, it is reasonable to speculate that zinc activates IGF-1RTK by directly or indirectly interacting with the tyrosine kinase domain on the cytosolic side. Auto-phosphorylation of RTKs within the kinase domain upregulates the kinase activity and the phosphorylation state of RTKs is modulated by protein tyrosine phosphatases (PTPs) (11). Zinc has been demonstrated to inhibit PTPs (5). Exogenous zinc can activate EGFR signaling by inhibiting PTP in airway epithelial cells (31). In addition, intracellular zinc fluctuation has also been reported to modulate insulin signaling by suppressing PTP activity (13). Therefore, it is likely that exogenous zinc activated IGF-1RTK through inhibition of PTP in cardiac cells in this study. While we did not directly measure PTP activity in the current study, our observation (see Data supplement) that zinc markedly enhanced tyrosine phosphorylation may imply that zinc activate IGF-1RTK by inhibiting PTPs. Thus, it would be intriguing to define the specific subtype (s) of PTPs that mediates the action of zinc in future studies.

PTEN is well known to negatively regulate the PI3K/Akt signaling by dephosphorylating PIP_3. Thus, suppression of PTEN activity could lead to cardioprotection through activation of the PI3K/Akt pathway (25). Indeed, it has been reported that loss of PTEN activity is responsible for induction of preconditioning in isolated rat hearts (7), although the protocol of ischemic preconditioning used in the study was questioned by other investigators (25). The importance of
PTEN in cardioprotection has also been demonstrated in a recent study showing that the loss of the cardioprotective effect of atorvastatin was associated with an increase in PTEN levels in the heart (26). Thus, it is intriguing to test if zinc activates Akt through inactivation of PTEN. Although the regulatory mechanism for PTEN activity remains unclear, phosphorylation and oxidation may deactivate PTEN (12). In this study, we found that zinc did not alter PTEN phosphorylation, indicating that zinc may not inactivate PTEN through phosphorylation. Since PTEN is a constitutively active phosphatase, its activity also depends on its cellular level (25). Indeed, Wu et al. reported that exogenous zinc decreases PTEN protein levels through proteasomal degradation in airway epithelial cells, leading to Akt activation (34). However, it should be noted that PTEN degradation was prominent after a long period (4 h) of treatment with a high dose (50 µM) Zn²⁺ (34). In the current study, we treated cardiac cells with a low dose of zinc (10 µM) for a short time (20 min) and found that zinc does not alter cellular PTEN protein levels. Thus, it is unlikely that zinc upregulated Akt activity by degrading PTEN in this study. Taken together, PTEN may not play an important role in zinc-induced Akt activation in this study in which cardiac cells experienced a short exposure to zinc. However, it is still possible that zinc may activate Akt by decreasing PTEN protein levels with longer treatments. Since PTEN is also reversibly inactivated through oxidation (23), we further tested if zinc could inactivate PTEN by oxidizing it. Our data exclude the possibility that zinc may inhibit PTEN through oxidation in cardiac cells.

Akt is activated by PDK-dependent phosphorylation of Ser⁴⁷³ and Thr³⁰⁸ residues (32). Dephosphorylation of the two residues by Ser/Thr protein phosphatases can inactivate Akt (2, 27). Therefore, it is not surprising that inhibition of Ser/Thr phosphatases will lead to Akt activation. The major Ser/Thr protein phosphatases in mammalian cells are PP1, PP2A, and
PP2B (10). In this study, we focused on the role of PP2A and found that exogenous zinc was able to decrease PP2A activity, which may result in up-regulation of Akt activity. We further demonstrated that inhibition of Ser/Thr protein phosphatases with calyculin A also mimicked the effect of zinc to activate Akt, reinforcing the critical role of PP2A in Akt activation. These findings suggest that in addition to IGF-1RTK, Ser/Thr protein phosphatases may also play a role in zinc-induced Akt activation. However, it should be mentioned that more studies are needed to characterize the exact role of Ser/Thr protein phosphatases in the action of zinc. In addition, it would be interesting to investigate the precise molecular mechanism by which zinc inhibits Ser/Thr protein phosphatases.

In summary (Figure 8), exogenous zinc upregulates Akt activity by activating IGF-1RTK and prevents the mPTP opening in cardiac cells. Inactivation of Ser/Thr protein phosphatases may also contribute to zinc-induced Akt activation. Zinc may not rapidly regulate PTEN activity.
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Figure legends

**Figure 1.** *A,* Western blot analysis of Akt (Ser\(^{473}\)) and GSK-3\(\beta\) (Ser\(^{9}\)) phosphorylation in cardiac H9c2 cells. H9c2 cells were treated with ZnCl\(_2\) (10 µM) in the presence of the zinc ionophore pyrithione (4 µM) for 20 min. ZnCl\(_2\) markedly enhanced phosphorylation of both Akt and GSK-3\(\beta\), an effect that was abolished by EDTA (10 mM) and TPEN (10 µM). *B,* Western blot analysis of Akt (Ser\(^{473}\)) and GSK-3\(\beta\) (Ser\(^{9}\)) phosphorylation in cardiac H9c2 cells subjected to simulated ischemia/reperfusion (I/R). ZnCl\(_2\) (10 µM) and pyrithione (4 µM) were given at the onset of reperfusion and cells were collected 20 min after exposure to ZnCl\(_2\). ZnCl\(_2\) significantly increased both Akt and GSK-3\(\beta\) phosphorylation upon reperfusion. I, ischemia.

**Figure 2.** *A,* Western blot analysis of Akt (Ser\(^{473}\)), GSK-3\(\beta\) (Ser\(^{9}\)), and PTEN (Ser\(^{380/382}\)/Thr\(^{382-383}\)) phosphorylation in cardiac H9c2 cells. ZnCl\(_2\) (10 µM) in the presence of the zinc ionophore pyrithione (4 µM) did not alter PTEN phosphorylation. *B,* Western blot analysis of PTEN protein levels in cardiac H9c2 cells. ZnCl\(_2\) (10 µM, 20 min) did not alter PTEN protein levels. *C,* Identification of reduced and oxidized forms of PTEN with Western blot. H9c2 cells were treated with H\(_2\)O\(_2\) (500 µM, 20 min) or ZnCl\(_2\) (10 µM) for 20 min. PTEN is oxidized by H\(_2\)O\(_2\), as indicated by the appearance of the higher mobility band (the oxidized form). In contrast, the oxidized form was not seen in cells exposed to ZnCl\(_2\).

**Figure 3.** Western blot analysis of Akt phosphorylation at Ser\(^{473}\) in cardiac H9c2 cells. ZnCl\(_2\) (10 µM) in the presence of the zinc ionophore pyrithione (4 µM) increased Akt phosphorylation, which was blocked by the non-selective RTK inhibitor genistein (Gen, 100 µM) and the selective IGF-1RTK inhibitor AG1024 (5 µM). The Src tyrosine kinase inhibitor PP2 (10 µM) or the EGFRTK inhibitor AG1478 (10 µM) did not alter the action of ZnCl\(_2\). The inhibitors themselves did not markedly alter Akt phosphorylation. * p < 0.05 vs. control; # p < 0.05 vs. Zn\(^{2+}\).
Figure 4. A, Western blot analysis of Akt (Ser^{473}) and GSK-3β (Ser^{9}) phosphorylation in cardiac H9c2 cells. H9c2 cells were treated with ZnCl₂ (10 µM) for 20 min in the presence or absence of the zinc ionophore pyrithione (4 µM). ZnCl₂ did not increase phosphorylation of Akt or GSK-3β in the absence of pyrithione. B, Western blot analysis of IGF-1R phosphorylation at Tyr^{1135/1136} in cardiac H9c2 cells. ZnCl₂ (10 µM, 20 min) did not enhance IGF-1R phosphorylation in the absence of pyrithione.

Figure 5. A, Western blot analysis of IGF-1R phosphorylation at Tyr^{1135/1136} in cardiac H9c2 cells. ZnCl₂ (10 µM, 20 min) in the presence of the zinc ionophore pyrithione (4 µM) significantly increased IGF-1R phosphorylation. * p < 0.05 vs. control. B, Western blot analysis of IGF-1R phosphorylation at Tyr^{1135/1136} in cardiac H9c2 cells subjected to simulated ischemia/reperfusion (I/R). ZnCl₂ (10 µM) in the presence of pyrithione (4 µM) markedly increased IGF-1R phosphorylation upon reperfusion. I, ischemia. C, Western blot analysis of IGF-1R phosphorylation at Tyr^{1135/1136} in cardiac H9c2 cells. ZnCl₂ (10 µM) in the presence of pyrithione (4 µM) markedly increased IGF-1R phosphorylation, an effect that was impeded by the non-selective RTK inhibitor genistein (Gen, 100 µM) and the selective IGF-1RTK inhibitor AG1024 (5 µM) but not by the Src tyrosine kinase inhibitor PP2 (10 µM) or EGFRTK inhibitor AG1478 (10 µM). The inhibitors did not alter phosphorylation of IGF-1R (the bottom panel).

Figure 6. Summarized data for TMRE fluorescence intensity measured with confocal microscopy 20 min after exposure to 600 µM H₂O₂. ZnCl₂ (10 µM) in the presence of the zinc ionophore pyrithione (4 µM) prevented oxidant-induced TMRE fluorescence induction compared to the control, an effect that was reversed by the selective IGF-1RTK inhibitor AG1024 (5 µM). * p < 0.05 vs. control; # p < 0.05 vs. Zn^{2+}. 

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Figure 7. A, ZnCl₂ (10 µM) in the presence of the zinc ionophore pyrithione (4 µM) significantly decreased PP2A activity compared to the control. * p < 0.05 vs. control. B, Western blot analysis of Akt phosphorylation at Ser^{473} in cardiac H9c2 cells. The Ser/Thr protein phosphatase inhibitor calyculin A (Caly A, 12.5 nM) increased Akt phosphorylation.

Figure 8. The signaling mechanism underlying zinc-induced Akt activation. TK, tyrosine kinase; PP2A, protein phosphatase 2A.
References


26. Mensah K, Mocanu MM, and Yellon DM. Failure to protect the myocardium against ischemia/reperfusion injury after chronic atorvastatin treatment is recaptured by acute


Fig. 1

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B

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- Akt
- P-GSK-3β
- GSK-3β
- tubulin
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