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THE MECHANISM BY WHICH ERS INHIBITION LEADS TO CARDIOPROTECTION

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Objectives Purpose While it is well known that endoplasmic reticulum stress (ERS) plays an important role in myocardial ischaemia/reperfusion (I/R) injury and inhibition of ERS leads to cardioprotection against I/R injury, the precise mechanism by which inhibition of ERS induces cardioprotection remains unclear. Inhibition of mitochondrial permeability transition pore (mPTP) opening is critical for the prevention of reperfusion injury and that ERS is implicated in the mechanism underlying reperfusion injury and induces mitochondrial stress. The purpose of this study was to determine if inhibition of ERS can prevent the mPTP opening and to explore the signalling mechanism whereby ERS inhibition leads to the protection against the mPTP opening.

Methods All experiments were conducted using H9c2 cells. First, we tested if TUDCA, an inhibitor of ERS, could modulate the mPTP opening. To detect the mPTP opening, cells were loaded with the mitochondrial specific fluorescent dye TMRE and imaged with confocal microscopy. After treatments with TUDCA at different concentrations, cells were exposed to 800 μM H_2O_2 to induce the mPTP opening. To determine the roles of the PI3K/Akt and PKG pathways in the action of TUDCA, cells were treated with the PI3K inhibitor LY294002 and the PKG inhibitor KT5823 prior to the application of TUDCA. To define the signalling mechanism responsible for the protective effect of TUDCA, phosphorylation status of Akt, VASP, and GSK-3 β were measured with Western blotting. Finally, to corroborate the protective effect of TUDCA, subcellular structures and cell viability were detected with transmission electron microscopy (TEM) and flow cytometry, respectively.

Results

- (1) Exposure of cells to 800 μM H_2O_2 for 20 min caused a marked decrease in TMRE fluorescence, indicating mPTP opening by oxidative stress. Compared to the control, 20, 30, and 40 μM TUDCA prevented the loss of TMRE fluorescence ($63.2 \pm 4.8\%$, $75.3 \pm 2.7\%$, $63.6 \pm 2.4\%$), pointing to that inhibition of ERS leads to the prevention of mPTP opening.
- (2) The effect of TUDCA on TMRE fluorescence was inhibited by LY294002 and KT5823 (fluorescence intensity decreased to

$60.7 \pm 4.6\%$ and $53.2 \pm 4.2\%$), implying that the PI3K/Akt and PKG signalling pathways may mediate the action of TUDCA.

- (3) TUDCA at different concentrations significantly increased GSK-3 β phosphorylation at Ser9 with the peak at 30 μM ($285.6 \pm 9.9\%$). The expression level of ERS marker GRP78 was also most prominent with 30 μM TUDCA ($37.3 \pm 5.7\%$).
- (4) TUDCA-induced increases in Akt and GSK-3 β phosphorylation were inhibited by LY294002 ($63.6 \pm 3.7\%$, $84.1 \pm 3.5\%$), whereas KT5823 could suppress phosphorylation of VASP and GSK-3 β by TUDCA ($50.6 \pm 4.0\%$, $78.7 \pm 3.9\%$).
- (5) Experiments with TEM revealed that TUDCA prevented H_2O_2 -induced swelling of ER and mitochondrial damages.
- (6) Studies with flow cytometry showed that TUDCA given at reperfusion but not during ischaemia improved cell viability in cells subjected to ischaemia followed by reperfusion.

Conclusions

- (1) Inhibition of ERS leads to the prevention of mPTP opening and reperfusion injury.
- (2) Inhibition of ERS results in the modulation of mPTP opening through inactivation of GSK-3 β .
- (3) The PI3K/Akt and PKG pathways may mediate inactivation of GSK-3 β .