Danshen (*Salvia miltiorhiza*) mediated through estrogen receptor to suppress the cardiac apoptosis effect induced by IGF2R signaling activation

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Abstract

Danshen (*Salvia miltiorrhiza*) are widely used in China for the treatment of cardiovascular disorders, including coronary heart disease. Tanshinone IIA, a lipid-soluble biologically active component isolated from Danshen, this compound chemical structure similar to 17β-estrodiol (E2). Therefore, pretreatment of cardiomyoblast cells with ICI 182,780 (ICI), an estrogen receptor antagonist to investigated the estrogenic activity of Danshen. The aim of the present study was to assess whether the cardioprotection exerted by Danshen is mediated through the ER is involved within H9c2 cardiomyoblast cells. To further identity the effect of Danshen extracts on Leu27IGF-II pathway-induced apoptosis by analyzing the TUNEL assay, JC-1 staining and Western blot were used to detect IGF-IIR signaling pathways in H9c2 cardiomyoblast cells. We found that treatments Danshen extracts significantly phophorylated Akt that mediated through estrogen receptor to inhibited Leu27IGF-II-induced apoptosis analyzed by TUNEL assay, and Western blot for IGF-IIR signaling proteins and JC-1 staining to detect mitochondrial membrane stability and relative proteins. However, the cardioprotective properties of Danshen to inhibit Leu27IGF-II-induced apoptosis and promote cell survival were attenuated by applying ICI. The findings suggest that cardio protective effect of Danshen is mediated through estrogen receptors. All data suggest that Danshen exerts estrogenic activity against IGF2R signaling induced cardiac apoptosis, and acts as a potential cardioprotctive agent.

Results

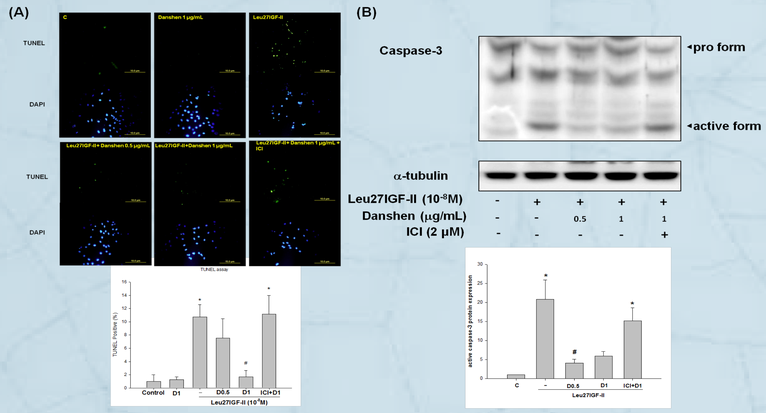

Figure 1. Effect of Danshen extract on Leu27IGF-II-induced H9c2 cell apoptosis.

(A) Leu27IGF-II-induced apoptosis of H9c2 cells were determined by TUNEL assay. DAPI staining (blue) in the lower panel represents cell nuclei. TUNEL stating (green) spots in the upper represent apoptotic. bodiesBars represent the percentage of TUNEL-positive cells based on total stained cells by DAPI. Graph represented the percentage of TUNEL-positive cells and indicated mean values ± SE, n = 3. * p <0.05; ** p <0.01 represent significant differences from the control group; # p<0.05; ## p<0.01 value were based on comparisons with Leu27IGF-II. **(B)** Total protein of cell extracts was separated by 12% SDS-PAGE, transferred to PVDF membranes, and further analyzed by Western blot with specific antibody against caspase-3**.** Equal loading was verified with an anti-α-tubulin antibody.

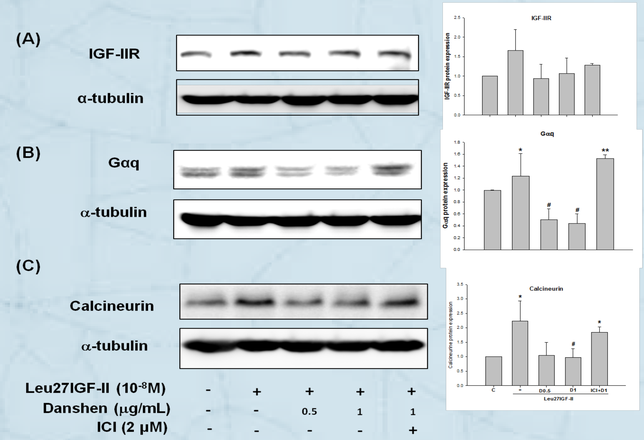

Figure 2. Danshen extract inhibited IGF-IIR signaling pathway on Leu27IGF-II-induced H9c2 cell apoptosis.

H9c2 cells at 80% confluence were pretreated with ICI 182,780 (2 μM) for 1h and followed by incubation with danshen extracts 0.5 and 1 μg/mL for 2h in the presence of Leu27IGF-II (10^{-8}M) administration for 24h and then were harvested for the following analyses. Total protein of cell extracts was separated by 12% SDS-PAGE, transferred to PVDF membranes, and further analyzed by Western blot with specific antibody against IGF-IIR, Gαq and Calcineurin.Equal loading was verified with an anti-α-tubulin antibody.

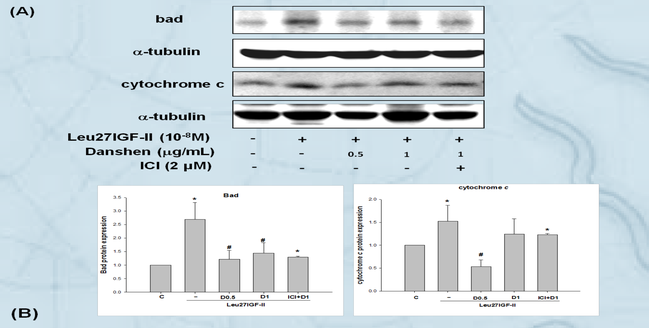

Figure 3. Danshen extract prevent the mitochrodrial potential loss of H9c2 cells induced by Leu27IGF-II.

H9c2 cells were pretreated with ICI 182,780 (2 μM) for 1h and followed by incubation with Danshen extracts 0.5 and 1 μg/mL for 2h in the presence of Leu27IGF-II (10^{-8}M) administration for 24h. (**A**) Total protein of cell extracts was separated by 12% SDS-PAGE, transferred to PVDF membranes, and further analyzed by Western blot with specific antibody against Bad and cytochrome **c**. Equal loading was verified with an anti-α-tubulin antibody. (**B**) The $\Delta\Psi_m$ of H9c2 cells was measured by immunofluorescence microscopy after JC-1 staining.

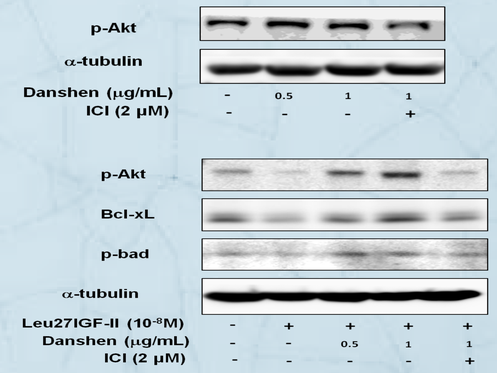

Figure 4. Danshen extract active survival p**athway on Leu27IGF-II-induced H9c2 cell.**

H9c2 cells at 80% confluence were pretreated with ICI 182,780 (2 μM) for 1h and followed by incubation with danshen extracts 0.5 and 1 μg/mL for 2h in the presence of Leu27IGF-II (10^{-8}M) administration for 24h and then were harvested for the following analyses. Total protein of cell extracts was separated by 12% SDS-PAGE, transferred to PVDF membranes, and further analyzed with an anti-α-tubulin antibody.

Summary

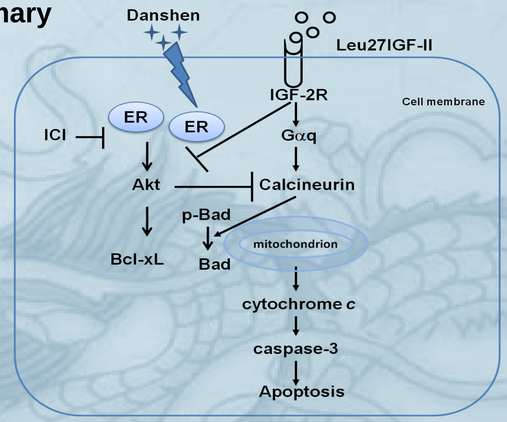

Figure 5. A schematic representation showing Danshen extract inhibit Leu27IGF-II-induced IGF-IIR singnalling and cardiomyocyte apoptosis through activation of Akt.

Activation of Insullin-like growth factor (IGF-IIR) by specific Leu27IGF-II binding induces IGF-IIR activation which then leads to the activation of Gαq and Calcineurin. calcineurin then directly induce cell apoptosis in a mitochondrial-dependent mannar. Danshen extract could block Leu27IGF-II-induced apoptosis and administration of ICI could totally reverse effect of Danshen, thereby Danshen is mediated through estrogen receptors inhibiting Leu27IGF-II-induced calcineurin activation by specifically activating PI3K-Akt pathway, thereby inhibiting cardiomyocyte apoptosis.