Diallyl trisulfide (DATS) Suppresses HG-induced Cardiomyocyte Apoptosis by Targeting ROS- mediated HIF-1α-IGFBP-3 Activation

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Abstract

Background:

Diabetes is one of the most common diseases to lead death in Taiwan and more than 80% patients are dead due to cardiovascular diseases. In our previous study, it is demonstrated that cardiac activation of HIF-1α-IGFBP-3 signaling mediated by ROS-regulated PHD is involved in HG-induced apodosis. Diallyl trisulfide (DATS) is the component in garlic oil with the strongest inhibitory effect on dilated cardiomyopathy (DCM). In this study, we will further investigate whether HIF-1α-IGFBP-3 signaling governs the anti-apoptotic effect of DATS on HG-exposed cardiomyocyte.

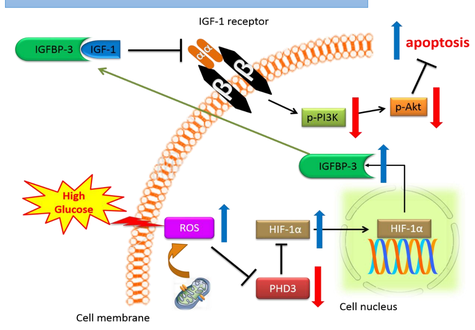
Methods and Results:

H9c2 cells were treated with 5.5 mM and 33mM glucose for 36 hr. It was observed that significant increased levels of the cell apoptosis and down-regulated phosphorylated Akt phosphorylation induced by HG were reversed by the treatment of DATS in a dose-dependent manner. The treatment of H_2O_2 and PHD siRNA increased HIF-1α and IGFBP-3 protein levels which was decreased by DATS. The overexpressed HIF-1α and IGFBP-3 reversed the level of cell apoptosis which was suppressed by the treatment of DATS in HG-exposed cells. We also observed HIF-1α, IGFBP-3 and PHD protein levels in animal model and data showed the same results as H9c2 cell model. Results of co-immunoprecipitation (Co-IP) assay not only pointed out that DATS suppressed the extracellular association of IGF-1 with IGFBP-3 on H9c2 cardiomyoblast exposed to HG but also had the same results in animal serum. Neonatal cardiomyocytes, Flow cytometry assay and TUNEL assay were used to sum up the experiments did before.

Conclusion:

Taken together, these findings indicate that the mediation of ROS-regulated PHD on HIF-1α-IGFBP-3 signaling activation governs the anti-apoptotic effect of DATS on HG-exposed cardiomyocyte.

Previous Studies



Schematic illustrating extracellular downregulation of cardiac IGF-1 survival signaling through binding and sequestering activity of increased secreted-IGFBP-3 induced by HG. Additionally, ROS-regulated PHD mediated the intracellular activation of HIF-1a - IGFBP-3 signaling to increase IGFBP-3 secretion, involving gin HG-induced cardiomyocyte apoptosis.

Results

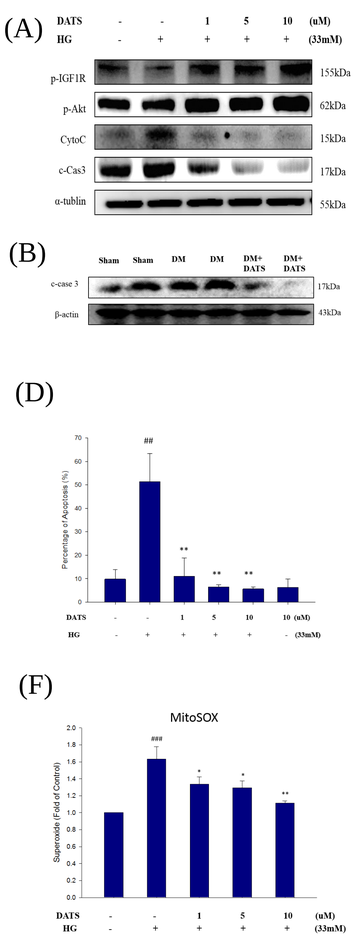


Fig.1.The DATS treatment mediated the apoptotic effect and ROS generation of HG-exposed H9c2 cardiomyoblast cells. Cell were culture in high glucose medium (33mM) for 36 hours. DATS were treated at doses 1, 5 and 10 (μM), and (A) the survival and apoptosis marker were detected by western blotting in H9c2 cells, (B) apoptosis marker were also detected in animal tissue, (C)(D) apoptosis detection and quantitative results by Flow Cytometry under different doses of DATS, (E) $p22^{phox}$, $p47^{phox}$ and $NOX2\ gp91^{phox}$ were detected by western blotting, (F) MitoSOX analysis the superoxide in different doses of DATS, (G) the treatment of H_2O_2 in 50 and 100 mM then detected by western blotting to prove that DATS reversed the apoptotic effects through regulating ROS production.

Fig.2 DATS reverses PHD to down-regulate HIF-1α and IGFBP-3 expression reduced apoptotic effect in cardiac cells exposed to HG. Cell were culture in high glucose medium (33mM) for 36 hours. (A) DATS were treated in different concentration and PHD3 protein level were detected by western blotting, (B) PHD3 siRNA were cotreat with HG 33mM and DATS 10 μM. Protein levels were detected by Western Blotting. Data indicated DATS reduces the HG-induced apoptosis through upregulating PHD expression then further activating its downstream.

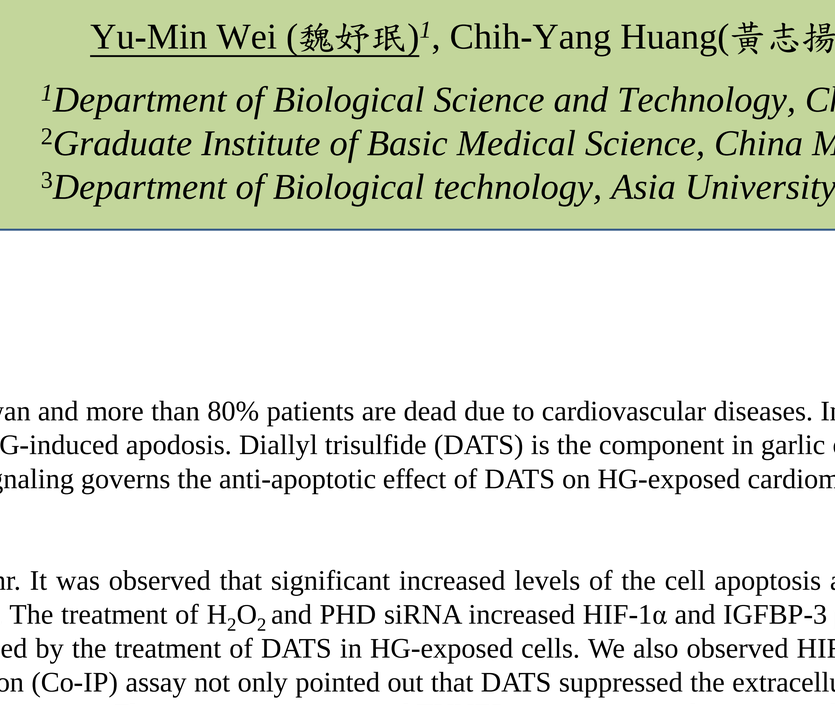


Fig.3 The anti-apoptotic effects of DATS on High glucose-treated H9c2 cells is through inhibiting HIF-1α expression. Cell were culture in high glucose medium (33mM) for 36 hours. (A) DATS were treated in different concentration and HIF-1α protein level were detected by western blotting, (B) under STZ-treated diabetes rats, HIF-1α protein level were also detected by western blotting and it showed the same results as H9c2 cells, (C) Nuclear extraction assay were treated with HG 33mM and DATS in 1, 5 and 10 μM, (D) successful transfection of HIF-1α plasmid (0.6, 0.8 1 and 1.2 μg/ml) examined by western blotting, (E) HIF-1α plasmid (0.5, 1.0 μg/ml) were co-treat with HG 33mM and DATS 10 μM. Protein levels were detected by Western Blotting.

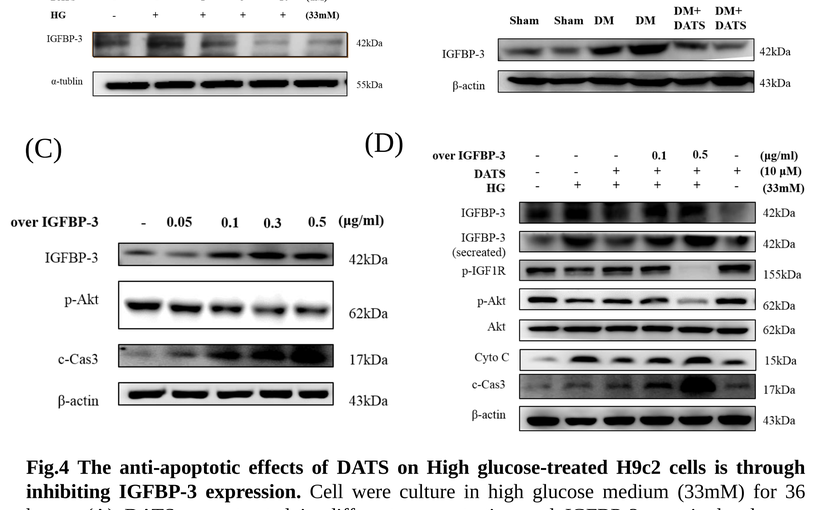
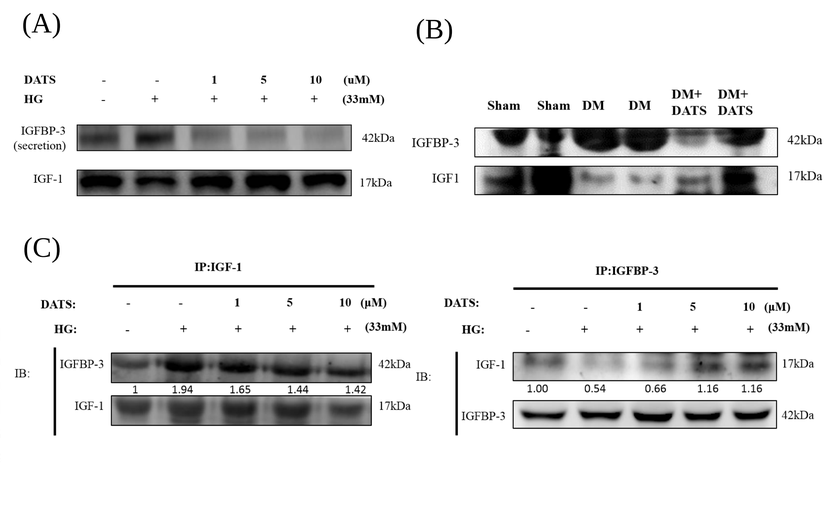


Fig.4 The anti-apoptotic effects of DATS on High glucose-treated H9c2 cells is through inhibiting IGFBP-3 expression. Cell were culture in high glucose medium (33mM) for 36 hours. (A) DATS were treated in different concentration and IGFBP-3 protein level were detected by western blotting, (B) under STZ-treated diabetes rats, IGFBP-3 protein level were also detected by western blotting and it showed the same results as H9c2 cells, (C) successful transfection of IGFBP-3 plasmid (0.05, 0.1 0.3 and 0.5 μg/ml) examined by western blotting, (D) IGFBP-3 plasmid (0.1, 0.5 μg/ml) were co-treat with HG 33mM and DATS 10 μM. Protein levels were detected by Western Blotting.



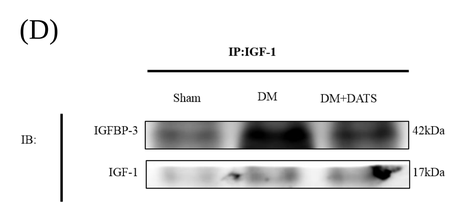


Fig.5 DATS reduced the extracellular binding of IGFBP3 and IGF1 caused by high glucose. Cell were culture in high glucose medium (33mM) for 36 hours. (A) DATS were treated in different concentration and the secreted-IGFBP-3 and IGF-1 protein levels in medium were detected by western blotting, (B) under STZ-treated diabetes rats, IGF-1 and secreted-IGFBP-3 protein levels in animal serum were also detected by western blotting and it showed the same results as H9c2 cells, (C) coimmunoprecipitation assay were done by the treatment of anti-IGF-1 and anti-IGFBP-3 in H9c2 cell medium and the protein levels of IGFBP-3 and IGF-1 were detected by western blotting, (D) in animal serum also we did the coimmunoprecipitation assay to detect the protein levels of IGFBP-3 and IGF-1 by western blotting

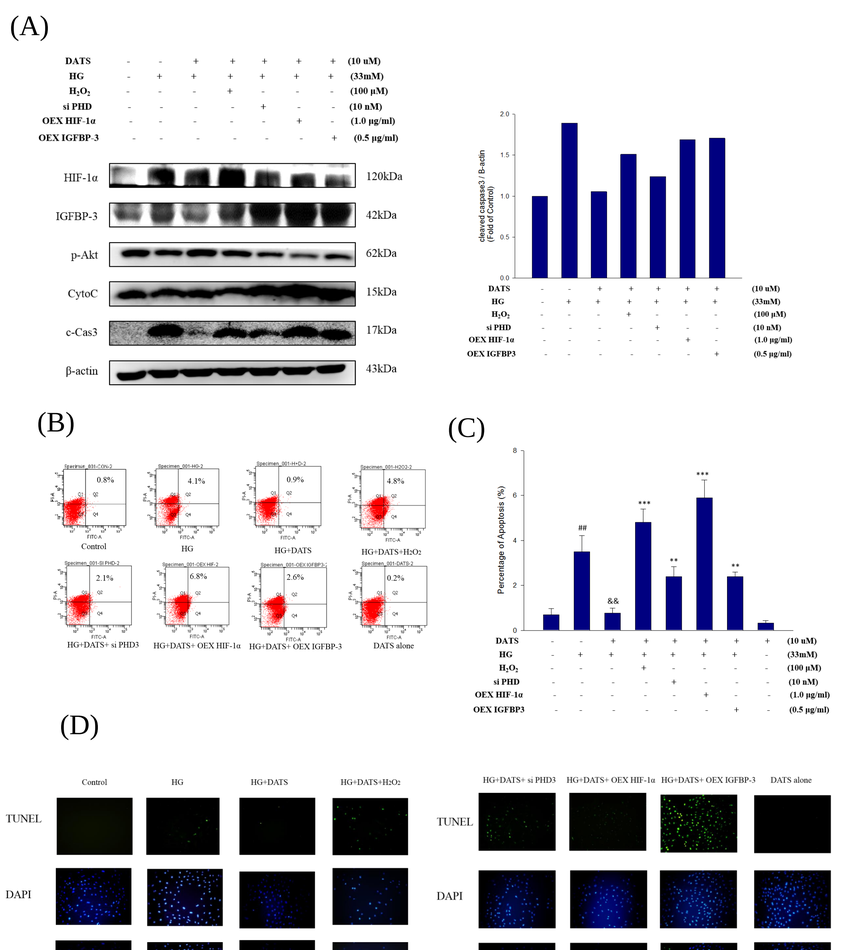
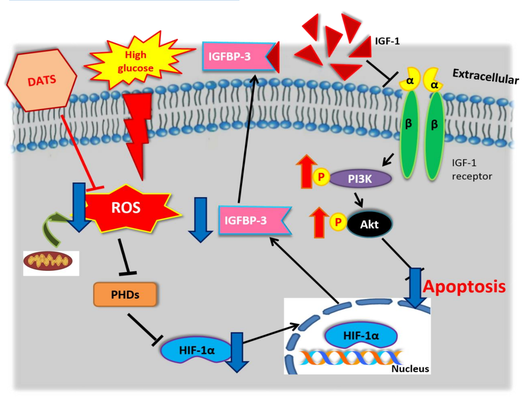


Fig.6 In order to sum up, combined all of the treatment done before to give the evidence that DATS can attenuate the apoptotic effect caused by HG. Cell were culture in high glucose medium (33mM) and DATS (10μM) for 36 hours (A) Neonatal cardiomyocyte were cotreat with H_2O_2 (100 mM), si PHD3 (10 ng), HIF-1α plasmid (1.0 μg/ml) and IGFBP-3 plasmid (0.5 μg/ml). Protein level were detected by western blotting, (B)(C) apoptosis detection and quantitative results by Flow Cytometry under cotretment of H_2O_2 (100 mM), si PHD3 (10 ng), HIF-1α plasmid (1.0 μg/ml) and IGFBP-3 plasmid (0.5 μg/ml), (D) H9c2 cells were treated with H_2O_2 (100 mM), si PHD3 (10 ng), HIF-1α plasmid (1.0 μg/ml) and IGFBP-3 plasmid (0.5 μg/ml). The DNA damage level were detected by TUNEL assay.

Summary



Inhibitions of ROS generation, PHD protein reduction and HIF-1α-IGFBP-3 signaling activation are involved in anti-apoptotic effects of DATS on cardiomyocyte exposed to HG. Furthermore, decreased secreted IGFBP-3 by DATS enhances more extracellular unbound IGF-1 to promote cardiac cell survival under HG condition.