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INSULIN AMELIORATES MIR-1-INDUCED INJURY IN H9C2 CELLS UNDER OXIDATIVE STRESS VIA AKT ACTIVATION

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Objectives Growing evidence indicates that aberrant up-regulation of microRNA-1 (miR-1) occurs in ischaemic myocardium. In addition, insulin elicits metabolism-independent cardioprotection against cardiovascular diseases. The aim of this study was to determine whether insulin could ameliorate miR-1-induced injury in H9c2 cells under oxidative stress and to investigate the underlying mechanisms.

Methods H9c2 cells were treated by hydrogen peroxide (H_2O_2). The expression level of miR-1 was detected by quantitative real-time PCR (qRT-PCR). Ad-GFP and Ad-miR-1 were transfected into H9c2 cells. MiR-1 transfected H9c2 cells were preincubated with LY294002 (10 μM), insulin (100 nM) alone or in combination for 1hr and subsequently were exposed to 200 μM H_2O_2. The cell viability, p-Akt/Total-Akt, and ROS production were detected by MTT, western-blot and DHE, respectively.

Results We show that miR-1 is upregulated in H9c2 cells after treatment with H_2O_2, and this effect is both dose- and time-dependent. Furthermore, expression of miR-1 decreased significantly after insulin treatment (4.5±0.1 vs 3.0±0.2, $p<0.05$). To determine the potential role of miR-1 in cellular injury and gene regulation, adenovirus-mediated overexpression of miR-1 was used. Overexpression of miR-1 decreased cell viability by 28±2% (n=6, $p<0.05$) and damaged Akt activation with or without H_2O_2 treatment. To further investigate the effect of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in miR-1-induced injury, H9c2 cells were pretreated with LY294002 (10 μM LY, a specific inhibitor of PI3K) with or without insulin (100 nM) and subjected to H_2O_2 treatment. LY pretreatment inhibited Akt activation, lead to increased reactive oxygen species (ROS), and further decreased cell viability induced by miR-1 (n=6, $p<0.05$, n=9–10 cells/group, $p<0.05$ and n=6, $p<0.05$) under oxidative stress. This effect was abolished by insulin.

Conclusions Our findings suggest that miR-1expression is sensitive to H_2O_2 stimulation. In addition, insulin decreases miR-1 expression and induces a marked protective effect on miR-1-induced injury under oxidative stress, which may be mediated by the Akt-mediated pathway. These results provide an important, novel clue as to the mechanism of the cardiovascular action of insulin.