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EXENATIDE ATTENUATE H9C2 CELLS DAMAGES INDUCED BY HYPOXIA/REOXYGENATION VIA P38MAPK γ NOT P38MAPK α AND β

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Objectives The glucagon-like peptide-1 analogue Exenatide (Ex-4) acts as a protective factor in the cardiomyocytes damages induced by hypoxia/reoxygenation(H/R) treatment, and Ex-4 could enhance the glucose uptake of cardiomyocytes. But the relationship between those two effects was unclear. We carried out this experiment to detect Ex-4 effects on H9c2 cells damages induced by H/R, and to clarify its correlation with glucose uptake, and explore the underlying signal transduction passway.

Methods H9c2 cells were cultured at a series of concentrations (50 nM, 100 nM, 200 nM) of Ex-4, and then subjected to H/R treatment (10/5 h). The cell vitality and the glucose concentration in the medium were measured with cell counting kit-8 (CCK-8) and glucosemeter. H9c2 cells were divided into four groups: control group (NC group), Ex-4 group, Ex-4 and p38MAPK α and β inhibitor SB203580 group (Ex-4+SB203580 group), Ex-4 and p38MAPK inhibitor BIRB796 group (Ex-4+ BIRB796 group). The cells were processed with 2-NBDG, Ex-4 and inhibitors for 8 h before H/R treatment. Intracellular rate of 2-NBDG was used as a fluorescent probe for direct glucose uptake measurement. Western blot was used to analyse p38MAPK subunits proteins expression.

Results The most suitable Ex-4 concentration and preincubation period for H9c2 cells were 200 nM and 45 min. Cells treated with Ex-4 better survived with following H/R treatment and the glucose levels decreased more than that without Ex-4 incubation. The fluorescence intensity of Ex-4 group was higher significantly than the NC group ($p < 0.05$). However, the function of Ex-4 was abolished by p38MAPK inhibitor BIRB796 at a very low concentration of 0.5 $\mu\text{mol/l}$ (fluorescence intensity: 415.9 ± 57.9 vs 363.2 ± 66.8 , $n=5$, $p < 0.05$) and the glucose uptake of Ex-4 groups was also attenuated by BIRB796 (316.3 ± 52.8 au vs 386.8 ± 30.2 au, $n=5$, $p < 0.05$). While, the p38MAPK α and β inhibitor SB203580 showed no inhibition of Exenatide effects on H9c2 (405.7 ± 45.6 vs. 415.9 ± 57.9 , $n=5$, $p > 0.05$) and the glucose uptake of two groups did not make a difference (521.2 ± 75.3 au vs 491.0 ± 41.8 au, $n=5$, $p > 0.05$). Western blot proved that p38MAPK γ expression decreased in Ex-4+ BIRB796 group (0.62 ± 0.03 vs 0.83 ± 0.04 , $n=3$, $p < 0.05$), but not in Ex-4+ SB203580 group (0.79 ± 0.03 vs. 0.83 ± 0.04 , $n=3$, $p > 0.05$), when compared with Ex-4 group.

Conclusions Ex-4 could attenuate H9c2 cells damages induced by H/R and promote glucose uptake. The p38MAPK pathway was involved in cell signal transduction, but it is p38MAPK γ , not p38MAPK α and β mediate Ex-4's effects.