

The Influence of Excess Iron on Pancreatic Beta Cells

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Objective: To study the effect of iron overload on the viability, insulin secretion, mitochondria defect and oxidative stress change in the INS-1 cells.

Methods: INS-1 cells were cultured with ferric ammonium citrate (FAC) at different concentrations (0 as control and 5, 10, 20, 40, 80, 160, 320 $\mu\text{mol/L}$ respectively). Labile iron pool (LIP) were calculated by detecting calcein-AM fluorescence in 24 h, 48 h and 72 h, the cell viability was measured by CCK8 assay. Iron overload model was established by screening for the best combination to ensure both high LIP level and cell viability. Reactive oxygen species (ROS) level was further detected by flow cytometer after fluorescent probe staining. The function of insulin secretion was detected by ELISA. The mitochondrial membrane potential was analyzed by JC-1 kit, and the mitochondrial changes were observed by transmission electron microscopy.

Results: Intracellular LIP levels were significantly increased in the FAC groups with concentration dependent manner. The viability of INS-1 cells was suppressed ($P < 0.05$) with increasing in the FAC concentration or culture time. The highest LIP level and a cell viability of $>50\%$ were observed with the conditions of 48 h exposure of FAC, indicating that INS-1 iron overload cell model was established. With the increase in the FAC concentrations, the insulin secretion was also increased and then decreased, and that in 160 and 320 mol/L groups showed statistical difference compared with control group ($P < 0.05$). The ROS level was significantly increased by FAC exposure as compared with control group ($P < 0.05$). Mitochondrial membrane potential was decreased with the increase in the iron concentration ($P < 0.05$). After iron overload, the mitochondria of INS-1 cells were swollen, the internal cristae was expanded, and the normal structure was lost. With the increase in the FAC concentration, the mitochondrial structure was destroyed more obviously.

Conclusions: Co-culture of INS-1 cells with FAC for 48 h successfully establish the iron overload model. Iron overload significantly damages mitochondrial structure and increases intracellular ROS level. The viability of pancreatic beta cells is sensitive to iron, even lower doses of iron can damage beta cells. The insulin secretion is reduced when the number of beta cells is decreased to a certain extent.

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