Protection of INS-1 Cells from Free Fatty Acid-induced Apoptosis by Inhibiting the Glycogen Synthase Kinase-3

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Summary: To examine the role of glycogen synthase kinase 3 (GSK-3) in the apoptosis of pancreatic β-cells to better understand the pathogenesis and to find new approach to the treatment of type 2 diabetes, apoptosis was induced by oleic acid (OA) in INS-1 cells and the activity of GSK-3 was inhibited by LiCl. The PI staining and flow cytometry were employed for the evaluation of apoptosis. The phosphorylation level of GSK-3 was detected by Western blotting. The results showed that OA at 0.4 mmol/L could cause conspicuous apoptosis of INS-1 cells and the activity of GSK-3 was significantly increased. After the treatment with 24 mmol/L of LiCl, a inhibitor of GSK-3, the OA-induced apoptosis of INS-1 cells was lessened and the phosphorylation of GSK-3 was increased remarkably. It is concluded that GSK-3 activation plays an important role in OA-induced apoptosis in pancreatic β-cells and inhibition of the GSK-3 activity can effectively protect INS-1 cells from the OA-induced apoptosis. Our study provides a new experimental basis and target for the clinical treatment of type-2 diabetes.

Key words: glycogen synthase kinase 3; apoptosis; oleic acid

With juvenile obesity becoming endemic worldwide, the incidence of obesity-related type-2 diabetes in children is on rise and its long-term consequences make the treatment of type-2 diabetes more challenging. The apoptosis of β-cells plays an important role in the pathogenesis of type-2 diabetes. Recent studies demonstrated that glycogen synthase kinase-3 (GSK-3) is implicated in the sugar metabolism, transduction of insulin signals and the secretion of β-cells and the increased activity of GSK-3 can result in insulin resistance, inhibition of insulin secretion and is closely related with the development and progression of type-2 diabetes. In this study, oleic acid (OA) was used to induce the apoptosis of INS-1 cells to explore the role of GSK-3 in the regulation of apoptosis to further explore the pathogeneses of type-2 diabetes and to find new alternatives for the treatment of the condition.

1 MATERIAL AND METHODS

1.1 Materials

RPMI 1640 media and fetal calf serum were bought from Gibco, USA. OA, β-thioglycol, hydroxyethyl piperazine ethanesulfonic acid (HEPES), glutamine, sodium pyruvate were obtained from Wuhan Lingfei Co., China. Total GSK antibody was obtained from Calbiochem, USA. Phospho-GSK-3α/β antibody (Ser21/9) was product of Cell Signaling Technology Inc., USA. Lithium chloride (LiCl) and propidium iodide (PI) were procured form Sigma Co., USA. The other products were of analytic purity.

1.2 Methods

1.2.1 Culture of INS-1 Cells INS-1 cells were kindly provided as gift by Prof. XU Tao of the College of Life Sciences, Huazhong University of Science and Technology, Wuhan, China. The cells were cultured in complete medium (containing 2 mmol/L glucose, 10% fetal calf serum, 50 µmol/L sodium pyruvate, 100 U/mL penicillin, 100 U/mL streptomycin) at 37°C in 5% CO₂ at saturated humidity. The cells adhered to walls with 48 h and began to proliferate on the third day after the culture. The cells grew at the bottle bottom in patches and were become 80% confluent. The cells were then digested with 0.25% trypsin and passaged for continuous culture.

1.2.2 OA-induced Apoptosis of INS-1 Cells After digestion with 0.25% trypsin, the cells were plated into 6-well plates at a concentration of 1×10⁵/L. After culture for 3 days in complete medium, the cells were divided into 4 groups and the cells were cultured with OA of various concentrations (0, 0.2, 0.4 and 0.6 mmol/L). After culture in medium containing OA for another 24 h, the cells in each well were harvested and completely washed with PBS (pH 7.4). Finally, the cells were co-cultured with PI for 30 min. The cells were examined for apoptosis by flow cytometry.

1.2.3 Determination of the Level of GSK-3 Phosphorylation during OA-induced Apoptosis of INS-1 Cells The INS-1 cells were plated into 6-well plates and cultured for 24 h till the cells were 70%–80% confluent. The cells were cultured for 24 h with 0.4 mmol/L OA or without OA and the culture medium was discarded. The cells were washed twice with cold PBS and
mild lysate containing 50 mmol/L HEPES (pH 7.0), 10 mmol/L EDTA, 1.0 mmol/L β-glycerophosphoric acid (pH 7.0), 1.0 mmol/L Na3VO4 (pH 7.0), 50 mmol/L NaF (pH 7.0), 1 mmol/L PMSF, 2 mmol/L benzamidine, 1 mg/mL leupeptin, 1 mg/mL pepstatin A, 1 mg/mL aprotonin was added. After homogenization of the cells, the cells were collected into an EP tube. The cells were then centrifuged at 12 000 r/min at 4°C for 5 min and afterwards, the supernatant was harvested for determination of protein concentration and electrophoresis. The GSK-3 phosphorylation level of the samples were detected by using Western blotting.

1.2.4 Detection of the Effect of LiCl on the OA-induced Apoptosis of INS-1 Cells
After digestion with 0.25% trypsin, the cells were plated into 6-well plates at a concentration of 1×105/L. After culture for 3 days in complete medium, the cells were then cultured in medium containing 0.4 mmol/L OA. The cells were divided into 4 groups, with LiCl of various concentrations (0, 12, 24 and 36 mmol/L) added into the medium separately. After culture for another 24 h, the cells in each well were harvested and detected for apoptosis by employing flow cytometry.

1.2.5 The Change in Phosphorylation Level of GSK-3 in the OA-induced Apoptosis under the Effect of LiCl
After digestion with 0.25% trypsin, the cells were plated into 6-well plates at a concentration of 1×105/L. After culture for 3 days in complete medium, the cells were then cultured in medium containing 0.4 mmol/L OA. The cells were then divided into 3 groups (control group, OA group and OA+LiCl group) and cultured for another 24 h. The cells were lysed with cell lysate and the total protein was extracted. The phosphorylation level of GSK-3 was determined by employing Western blotting.

1.2.6 Statistical Analysis
One-way ANOVA was used and Student t-test was employed for intra-group comparison. A P value less than 0.05 was considered to be statistically significant. With the results of Western blotting, the data were first processed by Image Quant software package (GE Health Care, USA) and then analyzed by utilizing SPSS12.0 program.

2 RESULTS

2.1 Cell Growth
The INS-1 cells adhered to bottle walls within 48 h after the culture and began to proliferate from the third day. The cells were 80% confluent on the tenth day. The growing cells aggregated assuming patchy pattern.

2.2 The OA-induced Apoptosis of INS-1 Cells
At the OA concentration of 0.2 mmol/L, the apoptotic INS-1 cells began to increase and at 0.4 mmol/L, the percentage of apoptotic INS-1 cells reached a peak. With the further increase of OA concentration, the number of apoptotic cells ceased to rise, suggesting that 0.4 mmol/L was the optimal OA concentration for inducing apoptosis (fig. 1).

2.3 Level of GSK-3 Phosphorylation during OA-induced Apoptosis of INS-1 Cells
At the concentration of 0.4 mmol/L the GSK-3 phosphorylation level was lower than that of the control group but the total GSK-3 content experienced no conspicuous change (fig. 2). Quantitative analysis showed that the relative GSK-3 phosphorylation level in OA-treated group was lower than that of control group.