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Insulin-like growth factor-1 inhibits colonic smooth muscle cell apoptosis in diabetic rats with colonic dysmotility

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ABSTRACT

Cellular apoptosis and colonic dysmotility are involved in diabetes mellitus (DM) complications. Insulin-like growth factor-1 (IGF-1) is known to affect apoptosis and proliferation. Here, we demonstrated that the treatment of 1500 ng/kg IGF-1 partly recovers the decrease of the muscle thickness, body weight and gastrointestinal transit rate in DM rats. The gastrointestinal transit rate is positively correlated with the IGF-I level, but negatively correlated with the level of colonic cellular apoptosis. The DM-induced colonic apoptosis is also attenuated by the IGF-1 stimulation. Moreover, IGF-1 inhibits the apoptosis of the isolated colonic SMCs in vitro via the activation of PI3K/Akt and ERK1/2 signaling pathways. Taken together, our data indicated that IGF-1 inhibits the DM-induced colonic SMC apoptosis and might be involved in the alleviation of colonic dysmotility in diabetic rats.

1. Introduction

Apoptosis takes part in the development of many diabetes mellitus (DM) complications, such as nephropathy, neuropathy and retinopathy [1–3]. Gastrointestinal dysfunction is another DM complication, which presents gastric/colonic dysmotility, and symptoms of early satiety, nausea, constipation or diarrhea [4–6]. There is growing evidence to support the notion that many factors, such as enteric neuronal loss, oxidative stress, gastric interstitial Cajal cells, and neuronal nitric oxide synthase, contribute to these DM complications [7–10]. Here, we aimed at studying the potential role of IGF-1 (insulin-like growth factor-1) in colonic dysmotility and apoptosis of colonic smooth muscle cells.

The IGF signaling system consists of IGFs, IGF receptors (IGF-R), IGF-binding proteins (IGFBPs), and so on [11–13]. IGF-1, a 70-amino acid polypeptide hormone, exhibits high structural similarity with proinsulin [12,13]. Circulating IGF-1 is synthesized by the liver, dependent on the growth hormone (GH) [13]. The IGF-IR, a member of the receptor tyrosine kinase family, comprises two α- and two β-subunits linked by disulfide bonds [13]. It was reported that the IGF-1/IGF-1R signaling axis could promote the proliferation and inhibit the apoptosis of peripheral tissues or cells via the phosphatidylinositol 3-kinase (PI3K) or mitogen-activated protein kinase (MAPK) signaling pathway [14–18]. Previously, the expression level of IGF-1 was reported to be reduced when DM occurs, and the block of IGF-1 signal transduction could result in the depletion of interstitial Cajal cells and the atrophy of gastric smooth muscle cells [19,20]. However, the role of apoptosis in gastrointestinal dysfunction, especially colonic dysmotility, has not yet been elucidated. It is thus interesting to study the association between colonic dysmotility and colonic smooth muscle cell (SMC) apoptosis in DM rats.

In this study, we investigated the effects of IGF-1 on the DM induced colonic dysmotility, the morphology of the smooth muscle tissue, the apoptosis level of SMCs and its related signaling pathways. The present data demonstrated that IGF-1 alleviates the colonic dysmotility in diabetic rats, stimulates cell proliferation and prevents the isolated colonic SMCs from apoptosis via the activation of the PI3K/Akt and ERK/MAPK signaling pathways.

2. Material and methods

1. Animals and treatments

96 male Sprague–Dawley rats (8 weeks old, purchased from the Academy of Military Medical Sciences in China) were used. Rats were randomly divided into 8 groups: 6 week old (6 W) normal group, 6 W

Abbreviations: IGF-1, insulin-like growth factor-1; DM, diabetes mellitus; SMCs, smooth muscle cells; GH, growth hormone; IGF-R, IGF receptors; IGFBPs, IGF-binding proteins; PI3K, phosphatidylinositol 3-kinase; MAPK, mitogen-activated protein kinase; H–E, hematoxylin–eosin; DMEM, Dulbecco's modified eagle medium; FBS, fetal bovine serum; TUNEL, terminal transferase dUTP nick end labeling; AI, apoptosis index; PBS, phosphate buffer solution; DAB, 3,3′-diamino-benzidine; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; ANOVA, one-way analysis of variance; InsR, insulin receptor

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DM group, 6 W DM + IGF-1 group, 6 W DM + insulin group, 10 W normal group, 10 W DM group, 10 W DM + IGF-1 group, and 10 W DM + insulin group, with n = 12 per group. Rats were given with streptozotocin (40 mg/kg body weight, Sigma Aldrich) by a single tail vein injection to induce DM. A Baianjie blood glucose meter was used to measure the fasting blood glucose levels. After 3 days, rats with blood glucose levels higher than 16.7 mmol/L were selected as DM rats [21,22]. The rats were untreated or treated with IGF-1 (1500 ng/kg body weight, i.p, qd, Pepro Tech) and insulin (16 U/kg body weight, Sigma Aldrich), respectively. Body weight was regularly recorded. After 6 or 10 weeks, all rats were sacrificed without anesthesia measures. The levels of IGF-1 and insulin in the blood were detected by the radioimmunoassay test kit (JiuDing, China). The gastrointestinal transit rate of each rat was detected as described previously [23]. Distal and proximal colon tissues were collected respectively. Three tissue sections of the same portion of colonic tissue either distal or proximal were stained with hematoxylin–eosin (H–E), and observed under a microscope (original magnification × 200). All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Tianjin Medical University in Tianjin, China.

2. Isolation of colonic SMCs and treatment

Colonic SMCs were isolated from the muscle tissue as described previously [18,24], and maintained at 37 °C with 5% CO_2 in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The SMCs were treated with 20 ng/mL IGF-1, 25 μmol/L LY294002 (Sigma Aldrich), 25 μmol/L PD98058 (Selleck Chemicals, Houston, TX), 20 ng/mL IGF-1 plus 25 μmol/L LY294002, or 20 ng/mL IGF-1 plus 25 μmol/L PD98058 for 30 min, respectively.

3. TUNEL staining assay

Terminal transferase dUTP nick end labeling (TUNEL) assay was performed to detect the apoptosis of the colonic smooth muscle tissue samples and isolated SMCs. Briefly, colonic smooth muscle tissue samples were fixed, dehydrated, embedded in paraffin, and sectioned into 4 μm slices. Isolated SMCs were cultured on coverslips and fixed in 4% formaldehyde. The TUNEL reaction mixture was applied to the slide using a peroxidase in situ cell death detection kit (Roche, Germany), according to the manufacturer's instructions. TUNEL-positive cells were identified as apoptosis, and the apoptosis index (AI) was calculated by the ratio of TUNEL-positive cells to total cells in at least 10 different high-power fields of a fluorescent microscope.

4. Western blotting assay

Western blotting assay was performed as described previously [25]. The following antibodies were used: mouse anti-Bax (1:100, Abcam), mouse anti-Bcl-2 (1:100, Abcam), rabbit anti-cleaved caspase-3 (1:1000, Abcam), mouse anti-β-actin (1:5000, Sigma Aldrich), rabbit anti-caspase-8 (1:1000, Abcam), rabbit anti-cleaved caspase-9 (1:1000, Abcam), rabbit anti-caspase-12 (1:1000, Abcam), rabbit anti-Akt (1:1000, Cell Signaling Technology), rabbit anti-Phospho-Akt-Ser473 (1:500, p-Akt, Cell Signaling Technology), rabbit anti-ERK1/2 (1:1000, Cell Signaling Technology) and rabbit anti-Phospho-ERK1/2 (1:500, p-ERK1/2, Cell Signaling Technology) antibodies.

5. Immunohistochemical analysis

Isolated colonic SMCs were subjected to immunohistochemical analysis. Briefly, tissue sections were deparaffinized, rehydrated, and treated with 3% hydrogen peroxide to quench endogenous peroxidase activity. Sections were then incubated at 4 °C overnight with rabbit anti-α-actin primary antibody (1:100, biosynthesis biotechnology, China), followed by incubation with horseradish peroxidase-labeled goat anti-rabbit IgG secondary antibody (Cell Signaling Technology). 10% normal goat serum was used to block nonspecific binding. After washing with phosphate buffer solution (PBS), tissue sections were stained with 3,3′-diamino-benzidine (DAB).

6. MTS assay

3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay was performed to analyze the proliferation of isolated colonic SMCs. Briefly, cells were cultured in 96-well plates and incubated with 20 μL MTS solution (Promega) for 4 h at 37 °C. The absorbance value was measured at 490 nm using a Multiskan ELISA microplate reader (Thermo Labsystems).

7. Statistical analysis

All experiments were repeated at least three times. The data are presented as means ± SE (Standard Error) and compared with one-way analysis of variance (ANOVA) followed by multiple mean comparisons by Student–Newman–Keul's test, using SPSS 16.0 software. Pearson correlation tests were used to examine the relationship between the gastrointestinal transit rate and blood IGF-1 level or colonic SMC apoptosis index. Differences were considered statistically significant if P values were less than 0.05.

3. Results

1. The effect of IGF-1 on the muscle thickness, body weight and gastrointestinal transit rate in DM rats

To investigate the role of IGF-1 in diabetic rats with colonic dysmotility, the DM rat model was made by the injection of streptozotocin. The DM rats were untreated or treated with IGF-1 or insulin. The level of blood IGF-1 and insulin was measured. As shown in Fig. 1, the levels of IGF-1 as well as insulin decreased remarkably in DM rats, compared with the normal rats (**, $P < 0.01$), and IGF-1 administration could recover the level of blood IGF-1, while the insulin also

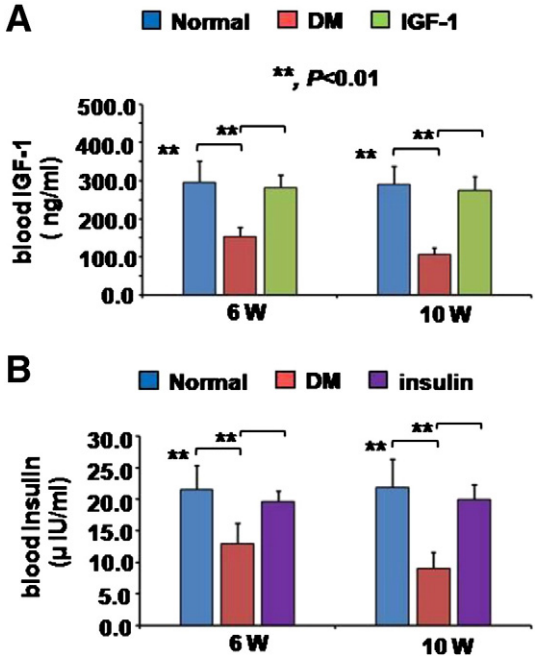

Fig. 1. The level of blood IGF-1 and insulin in DM rats. 6 W and 10 W DM rats were untreated or treated with 1500 ng/kg IGF-1 and 16 U/kg insulin, as indicated. The level of blood IGF-1 and insulin in DM rats was detected. One-way analysis of variance (ANOVA) was performed, and significant differences were indicated as follows: **, $P < 0.01$.