Original Article
The effect of Exendin-4 on apoptosis of islet cells in rats with type I diabetes

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Abstract: Incidence rate of type I diabetes showed an increasing trend. Lifelong exogenous insulin treatment is essential for type I diabetes patients due to insulin-secretion cells dysfunction. This severely affects life quality of patients. Our study is focused to explore the effect of Exendin-4 on apoptosis of type I diabetes rat β-cells and provide data support for clinical treatment. 120 male Sprague-Dawley rats were randomly assigned into three groups with equal number, including Sham group, type I diabetes group (DM group) and Exendin-4 intervention group (treated group). Type I diabetes rat model was induced with intraperitoneal injection of streptozotocin (STZ, 60 mg/kg). Fasting blood-glucose of rat model was assessed at 72 h after STZ injection to verify type I diabetes rat model, and successful model is defined as fasting blood-glucose >16.67 mmol/L and maintained four weeks with no significant glucose fluctuation. Rats in Sham group were normally fed. Rats in treated group received 2 ng/kg Exendin-4 intervention, at 2, 4, 6 and 8 weeks after intervention, TUNEL staining and qRT-PCR were performed to examine apoptosis of β-cells and insulin expression, respectively. Compared with DM group, insulin expression of treated group increased significantly (P<0.05). 80 rats were successfully performed with type I diabetes, fasting blood-glucose was maintained at 30 mmol/L for four consecutive weeks. Results at different time showed apoptosis of β-cells was reduced gradually after Exendin-4 intervention. Exendin-4 intervention inhibits apoptosis of β-cells effectively in type I diabetes, elevates expression of insulin, and its effect increases with intervention time. This suggested Exendin-4 had promising efficacy and benefit for type I diabetes treatment and was worth of being generalized.

Keywords: Exendin-4, type I diabetes, sprague-dawley rats, islet cell, apoptosis

Introduction

Type I diabetes (T1DM) is known as insulin dependent diabetes mellitus. Because of insulin-secretion cells dysfunction, type I diabetes patients need exogenous insulin treatment to maintain insulin level. Once patients suffer from type I diabetes, they must receive lifelong medication, which brings huge mental pressure and economic burden. On the other hand, type I diabetes is also called Youth type diabetes for most of type I diabetes onsets are under the age of 35 [1, 2]. Pathophysiology mechanism of type I diabetes is dysfunction of islet insulin-secretion β-cells abrogated production of insulin so as to cause absolute absence of insulin in such patients, accordingly, patients had persistently increasing level of blood glucose and finally suffered from diabetes [1, 3]. Therefore, in the field of type I diabetes treatment, it has very important practical significance to protect function of unmarred β-cells and slow down apoptosis.

So far, prevention of β-cells apoptosis is one of the hotspots and researchers have achieved some progress in this field, for example, Insulin, insulin-like growth factor and glucagon-like peptide-1 (GLP-1) were proved with promising effect to prevent β-cells apoptosis [4-6]. Exendin-4 is well-established high potent GLP-1 receptor agonists, and has the same effecy as GLP-1 in controlling blood glucose and reducing fast blood glucose. Exendin-4 has a remarkably longer half-life period in vivo than GLP-1, and was named as augmented GLP-1 [7, 8]. However, Exendin-4 is mainly used to controlling blood glucose and there is little study in its

Table 1. Primer used in this paper

Name	Sequence
F	ACCTTTGTGGTCCTCACCTG
R	GTGCTGCACTGATCCACAA
β-actin F	ACGAGGCCCAGAGCAAG
β-actin R	TTGGTTACAATGCCGTGTTCA

effect on apoptosis of islet cells. For further research about mechanism, we should confirm the effect of Exendin-4 on β-cells. Consequently, our study explored whether Exendin-4 influenced β-cells with STZ intraperitoneal-injected type I diabetes rat model.

Methods

General materials

80 male SD rats with 5 weeks old were offered by Fujian Medical University experimental animal center, after adaptive feed for 1 week, all experimental animals weight of 220±18.65 g were randomly divided into sham group and treated group, the TUNNEL kit for cell apoptosis was produced by Beyotime, total RNA extraction kit (Trizol) for tissue and cells was from TAKARA, RNA reverse transcription kit (Tian Gen), all the qRT-PCR Reagents and consumables were purchased from Promega, real time PCR was IQ5 from BioRad, data was analyzed by version 2.1 software, the fluorescent dye was produced by Roche, all the rest of reagents were the lab self-saved. Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Zhangzhou Hospital Affiliated to Fujian Medical University.

Experimental animal model: type 1 diabetes rats

Remove all the substandard rats (weight >200 g) after weighing, then made up to ensure the same number of each group, all animals were fed in SPF-class housing of laboratory after randomized caging. Rearing condition: room temperature (25±1°C), humidity range (50±2%), sterilized drinking water and diet all adopted freely, after 1 w adaptive feeding and animal body weight increased to more than 220 g, injected STZ with the dose of 60 mg/kg intraperitoneally in the case of animal empty stomach for 12 h, collecting tail vein blood after 72 h injection, the glycemic index were monitored to confirm the further study, the empty stomach glycemic index which exceed 16.67 mmol/L,

maintained for above 4 weeks and displayed no difference, means the successful animal model.

TUNEL assay for islet cell apoptosis

Injecting Exendin-4 (2 ng/g) for successful models. Treated group and sham group islet samples were collected at week 2, 4, 6, 8. After anesthesia animal with ethyl carbamate, took 5 mg of sample into liquid nitrogen, and the rest of tissue fixed in 4% paraformaldehyde quickly. Paraffin sections were prepared by routine method, and the islet cell apoptosis was assessed by the TUNEL assay.

qRT-PCR assay for insulin express in islet

After cutting the 5 mg sample mentioned above into pieces in mortar, added liquid nitrogen and grinded the specimen into powder, then transfered those powder into nucleic acid-free and 1 ml Trizol added EP tubes, mixed sufficiently, after placing at room temperature for 5 minutes, adding 200 ul chloroform, covering EP tube tightly and rocking violently for 15 s, centrifuged 20 minutes at 12000 rpm, 4°C, taking the upper water phase into a new EP tube, adding 500 ul pre-cooled chloroform, mixed up and down, after storing in -20°C for 2 h, centrifuged 10 minutes at 12000 rpm, discarded the supernatant, added 1 ml 75% ethanol, vortex mixing, centrifuged 5 minutes at 12000 rpm, 4°C, discarded supernatant, drying 10 minutes at room temperature, precipitation were dissolved with 35 ul RNA enzyme-free water, RNA stored in -80°C freezer after detecting concentration.

After detecting the RNA concentration, took 1 ug RNA for cDNA reserve transcription, specific steps were described in RNA reverse transcription kit, complemented with nucleic acid-free water for the different concentration in different specimen. After the reverse transcription, took 1 ul sample for qRT-PCR to detect the expression of insulin. Procedure of qRT-PCR is shown as follow: 95°C 3 min; 95°C 10 s, 55°C 30 s, 40 cycles, primer used in this experiment was designed and synthesized by Genomics company, sequences are shown in **Table 1**.

Statistical analysis

All test results were summarized and analyzed by SPSS 16.0 software, the results were shown as mean value ± standard deviation. Intra-

Table 2. Fast blood glucose levels of modeled rats in different period (mmol/L)

Group	Pre-administration	72 h	1 week	2 week	3 week	4 week
Sham	5.6±0.8	5.5±0.5	5.6±0.5	5.4±0.9	5.7±1.0	5.5±0.7
STZ-T1DM	5.5±0.6	26.3±2.8*	29.6±2.6*	30.2±2.4*	29.9±2.5*	30.3±3.1*

*P>0.05.

Table 3. Comparison of apoptosis index of three groups at different period (%) ($\bar{x}$±s)

Time	Sham	Exendin-4	Type I diabetes
2-week (n=10)	2.08±0.34	38.08±3.34*	46.22±4.55
4-week (n=10)	2.42±0.23	32.08±4.2*	44.18±3.14
6-week (n=10)	2.11±0.31	20.08±3.42*	46.67±5.84
8-week (n=10)	2.25±0.21	8.08±1.23*	55.73±6.24

*P<0.05.

group comparison of prior treatment and post-treatment was analyzed by t test. Comparison between groups was performed with One-way ANOVA analysis. *P* value <0.05 was considered to be statistically significant.

Result

TypeI diabetes rat

80 male Sprague-Dawley rats were randomly raised in separate cages. Intraperitoneal injection was performed with 60 mg/kg STZ at 12 hours fasting. 40 rats in sham group were normally bred, and all other food and drinking condition is the same among three groups. Caudal venous sampling was obtained to assess fast blood glucose. Examination result was showed in **Table 2**. To guarantee model stability, we cofirmed fast blood glucose levels of all modeled rats were higher than 16.67 mmol/L after 72 hours STZ administration. During 4-week observation period (weekly observation), all modeled rats had onset of typical diabetes "three polys and one little" symptom after 24 hours STZ administration, including clinical polydipsia, polyuria, and more food and weight loss. There were no significant differences among fast blood glucose levels of all modeled rats in observation period (P>0.05). All above confirmed STZ-Type I diabetes rat model was successful.

TUNEL staining to assess apoptosis

Maintenance rats-feeding condition after model was confirmed successful. 40 rats were randomly selected to receive Exendin-4 inter-

vention among Type I diabetes modeled rats. Exendin-4 intervention rats were harvest for pancreatic specimen under isoflurane anesthesia at 2-week, 4-week, 6-week and 8-week after Exendin-4 intervention, respectively. Examine apoptosis of pancreatic specimen by TUNEL apoptosis assays kit protocol. Use apoptosis index to assess apoptosis of islet cells, especially β-cells. Result of TUNEL staining was showed in **Table 3**. Exendin-4 intervention decreased apoptosis index of islet cells, and apoptosis index of treated group was significantly lower than DM group (P<0.05). Moreover, there was a time-dependent relationship between apoptosis and Exendin-4 intervention period, and this suggested Exendin-4 intervention could slow down apoptosis of islet cells.

qRT-PCR to examine insulin expression

Relative expression level of insulin was examined with qRT-PCR (**Table 4**). Compared with DM group, treated group had a higher level of insulin mRNA (P<0.05). Moreover, insulin expression in treated group increased gradually after Exendin-4 intervention with a time-dependent manner, and such increment is negative with islet apoptosis. This proved Exendin-4 intervention reduced β-cells apoptosis so as to increase transcription of insulin.

Discussion

As one kind of non-infectious diseases, diabetes significantly affects the daily lives and health of patients alongside changing lifestyles and dietary habit, and the number of diabetes patients is on rise year by year. China has become one of countries most impacted by diabetes, which brings huge mental pressure and economic burden to the society and patients family. Therefore, it is particularly important to explore diabetes mechanism and formulate effective therapeutic plan [9, 10]. Diabetes can be classified into two categories, including Type I diabetes and Type II diabetes. As previously mentioned, Type II diabetes patients still have ability to produce insulin, but there are insulin resistance in Type II diabetes so that relative

Table 4. Result of qRT-PCR for insulin

Time	Sham	Exendin-4	Type I diabetes
2-week (n=10)	162.08±13.34	88.08±3.34*	76.25±4.55
4-week (n=10)	158.42±9.13	92.08±4.2*	74.23±3.14
6-week (n=10)	164.11±11.13	94.08±3.42*	73.37±5.84
8-week (n=10)	162.35±6.71	98.08±1.23*	76.23±6.24

*$P<0.05$.

absence of insulin occurs, moreover, medication therapy can effectively improve insulin secretion and restore insulin sensitive [11-13]. In Type I diabetes patients, insulin-secretion cells are deteriorated and cannot produce insulin, so such patients need lifelong exogenous insulin treatment. Compared with Type II diabetes, Type I diabetes patients have more severe prognosis [14, 15]. Consequently, studies about clinical medication and mechanism of Type I diabetes should be focused on restoration of insulin-secretion function.

GLP-1 and gastrin releasing peptide (GRP) are two kinds of incretin hormones with abilities to enhance insulin secretion. GLP-1 is produced through degradation of proglucagon expression product in Langerhans cell of intestinal mucosa, and has similar protein structure and nucleotide sequence with glucagon. In addition, GLP-1 is generally found in various organs with highly conserved nucleotide sequence [16, 17]. Recent studies showed Exendin-4 had nucleotide sequence homology and function consistency with GLP-1, and proved Exendin-4 was high potent GLP-1 receptor agonists with mimicking blood glucose regulation function so as to decrease fast blood glucose and postprandial blood glucose. Specifically, Exendin-4 binds pancreas GLP-1 receptors and stimulates glucose-dependent insulin production via cAMP-dependent and β-cells differentiation mechanism, and finally extends antidiabetic time. As a novel antidiabetic agent, Exendin-4 has great potential in diabetes treatment as a member of incretin family [18-20].

Although studies proved Exendin-4 can be used as insulin-secreting agent, there is scarce evidence illustrating its effect on β-cells of Type I diabetes. Therefore, we made Type I diabetes rat models to explore Exendin-4 effect. In detail, 2 ng/kg Exendin-4 was injected, and molecular-biological indicators were observed after 2-week, 4-week, 6-week, 8-week Exendin-4

intervention, respectively, including TUNEL staining and qRT-PCR.

In conclusion, STZ intervention is an effective method for typeI diabetes model. Meanwhile, Exendin-4 intervention inhibits apoptosis of β-cells effectively and elevates expression of insulin. We also found longer intervention period of Exendin-4 intervention was associated with higher insulin level. All these results suggested Exendin-4 effectively inhibited apoptosis of β-cells and enhances insulin secretion during type I diabetes progression. Exendin-4 has promising efficacy and benefit for type I diabetes treatment and was worth of being generalized.

Disclosure of conflict of interest

None.

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