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Pathologic Changes of Pancreatic Endothelial Cells in Diabetic Rat

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Abstract

Aim: The study is to explore the relationship between pancreas endothelial cell injury and diabetes.

Methods: To induce with a single intraperitoneal injection of streptozotocin (STZ) for diabetic animal models, and collected the blood after 8 weeks, the level of Angiogenin-1 (Ang-1) and its receptor (Tie-2), vascular cell adhesion molecule-1 (VCAM-1) were determinated by ELISA method, histopathological examination of the pancreas, and observed pathological conditions of the pancreas endothelial cell injury in the models.

Results: The results showed that the level of ang-1, tie-2 and VCAM-1 were 585.04 ± 45.32 (pg/ml), 2399.40 ± 124.85 (pg/ml), 117.69 ± 12.34 (μg/L) in the Diabetes groups which were higher than that in control groups 551.29 ± 31.86 (pg/ml), 2103.27 ± 152.89 (pg/ml) and 93.43 ± 10.48 (μg/L), (t=3.78, 3.52, 7.52, P<0.05).

Conclusion: The Vascular endothelial cell factors of e NOS, VCAM-1, SMA, VEGFR and ICAM-1 were higher expressed in Diabetes mellitus rats than that in the normal control groups. We found that there is a certain correlation between the development of pancreatic endothelial cells and the development of diabetes, and the treatment of diabetes can provide some ideas for the future.

Keywords: Pancreas; Streptozotocin; Diabetes mellitus

Introduction

Diabetes is one of the most common metabolic diseases in the world. The micro vessel disorders, which could result in vascular homeostasis imbalance and vascular dysfunction, is the main causes of morbidity in the diabetic patients [1,2]. Diabetes mellitus is a vascular systemic disease, which can lead to systemic vascular injury, especially pancreatic vascular injury associated with hyperlipidemia. Therefore, it is important to study the mechanism of vascular injury of the pancreas. In this study, the Diabetes models were constructed to investigate the condition of pancreatic vascular pathological changes in Diabetes mellitus rat, which provides an experimental basis for the diagnosis and treatment of diabetes.

Materials and Methods

Animal model

The adult male Sprague-Dawley rats with ages of 10 weeks and weight of 200 ± 20 g, (Shanghai slack laboratory animal co, LTD), were randomly divided into two groups, the diabetic group (n=6) and the normal control group (n=6). the temperature was 20-25°C, with the air circulation, the relative humidity was 35%~55%, and all the freely feeding water rats were breeding in the Laboratory Animal Center of Fujian Medical University.

Diabetes models were induced with a single intraperitoneal injection of streptozotocin (STZ) (Sigma-purchased), the 1% of STZ solution by 0.1 mmol/L citric acid buffer (pH=4.4), 60 mg/kg of the Sprague-Dawley rat body weight, and the normal control group were injected the same volume of the citric acid-sodium citrate buffer in the same way. After injecting STZ for 72 h, the rats which were appearing drink more with stomach blood sugar concentration>16.7 mmol/L, that tendency for successful diabetic model. The weight was measured once a week and the sugar levels were determinated by the trace glucose meter from the rat caudal vein blood. The changes in the parameters were examined at 8 weeks after injection of STZ. After inhalation of the ether anesthesia, the blood collection was placed in a vacuum to collect blood vessels, and the tissue was soaked in formaldehyde for the immunohistochemical examination.

Observation indexes and methods

The serum of ang1, tie2 and VCAM-1 were detected by enzyme-linked immunosorbent assay kits which purchased from Shanghai LI-su biotechnology co. LTD. Operation steps were in strict accordance with the kit instructions. The tests were tested by automatic washing machine (model RT-3000, Rayto Company) and enzyme standard instrument (model RT-6100, Rayto Company). Pathological samples were taken from the fixed organization. Next, paraffin embedding,

sectioning and immunohistochemical staining were implemented to prepare the pathological section, and then it's observed by the microscope. Antibodies were purchased from abeam British company.

Data processing and statistical analysis

The experimental data measured data to mean ± standard deviation. Data statistics used Graph Pad Prism 7.5 software, the single factor analysis of variance between groups, when $P<0.05$ as the difference statistically significant.

Results

The level of ang-1, tie-2, VCAM-1 in serum of different groups

The level of ang-1, tie-2, VCAM-1 were 585.04 ± 45.32 (pg/ml), 2399.40 ± 124.85 (pg/ml), 117.69 ± 12.34 (μg/L) in the diabetes groups which were higher than that in control groups 551.29 ± 31.86 (pg/ml), 2103.27 ± 152.89 (pg/ml), 93.43 ± 10.48 (μg/L), (t=3.783.52, 7.52P<0.05), as shown in Table 1.

Groups	N	ang1 (pg/ml)	tie2 (pg/ml)	VCAM-1 (μg/L)
DM	6	585.04 ± 45.32	2399.40 ± 124.85	117.69 ± 12.34
NC	6	551.29 ± 31.86	2103.27 ± 152.89	93.43 ± 10.48
t		3.78	3.52	7.52
p		0.0236	0.025	0.0167

Table 1: The level of ang-1,tie-2,VCAM-1 in serum of different groups.

Immunohistochemical result

The vascular endothelial cell factors of eNOS, VCAM-1, SMA, VEGFR and ICAM-1 were higher expressed in diabetes mellitus rats than that in the normal control groups, as shown in Figure 1.

Discussion

Diabetes is a chronic systemic metabolic disease, the genetic and environmental factors which contribute to biochemical characteristics of sustained high blood sugar. Due to the lack of insulin secretion, or due to the increased demand of islet cells because of the relative lack of insulin, sugar, protein, fat and electrolyte metabolism disorder, eventually lead to serious complications of the tissuesorgans, and syndrome is a serious injury to the body's endocrine metabolism [3,4]. Insulin secretions from the pancreas, and the pancreatic islet cells proliferated in the pancreas to maintain dynamic balance and regulate glucose in the internal environment. The damage of pancreatic and islet cell apoptosis will accelerate the process of diabetes. On the one hand, long-term high blood sugar can weaken the synthesis of islet cells and secretion of insulin, and reduce insulin secretion or loss of glucose. On the other hand, long-term high blood sugar decreases the number of islet cells and increases failure islet beta-cell function in patients with diabetes [5-7].

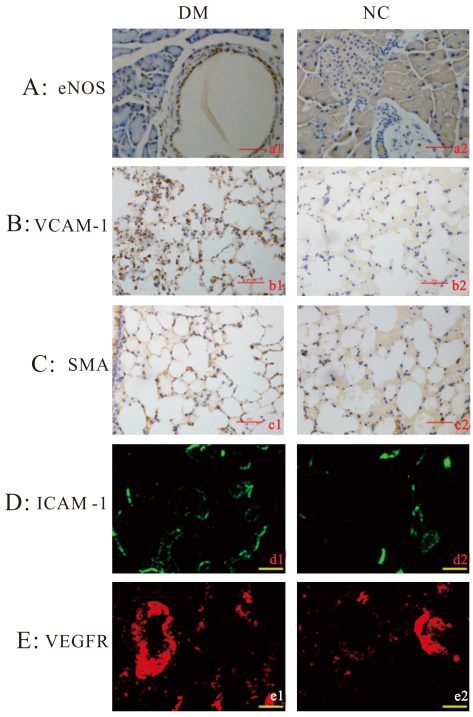

Figure 1: Immunohistochemical result A: a1-DM group of eNOS in high expression of vascular endothelial cells, a2: NC group of eNOS lower expression; B: b1-DM group of epithelial tissue high expression of VCAM-1, b2-NC group expression of VCAM-1 weak; C: c1-SMA high expression of DM group, c2-SMA lower expression NC group; D: d1-DM group high expression of ICAM-1, d2-lower expression ICAM-1 NC group; E: e1-DM group VEGFR- high expression, e2-NC group VEGFR-lower expression.

STZ-induced diabetic rat model is similar to human diabetes, which has the same pathological and physiological changes. The STZ was selective to damage islet beta-cell, which leads to diabetes. Studies have reported that the damage in male rat model was obviously higher than that of female rat model, so the male rats were chosen for the research objects [8]. STZ was carried by glucose transporters-2 into pancreatic islet beta cells through DNA alkylation and ADP ribose base. Depletion of DNA and ATP content in beta-cells induced degeneration necrosis, resulting in the occurrence of diabetes [9]. With intraperitoneal injection of STZ in the SD rat body with weight of 60 mg/kg, the diabetes model could be successfully constructed.

In the Diabetes rat showed that pancreatic angiogenesis microcirculation disorder, chronic inflammation around blood vessels and focal acini. In the early, the vascular lesions was damaged, endothelial cell injury and vascular endothelial loss of diastolic function [9,10]. The endothelial cells play an important role in many aspects, because it is an important screen between blood and vascular smooth muscle barrier, and can accomplish the metabolism of exchange between blood and tissue fluid, merger secrete a variety of biological active substances and maintain the body's coagulation in keeping the blood vessels blood fibrinolytic system balance, inhibiting platelet aggregation and reducing the endothelial permeability, the adhesion molecule expression and the proliferation vascular smooth muscle cell.

More and more research has been found that vascular cell adhesion molecule-1 (VCAM-1), angiogenin (Ang) and its receptor (Tie) in the development of the disease have promoted the angiogenesis, reshaping, maturity and stability [10]. Diabetes is a common metabolic disease around the world. Ang is a kind of indispensable vascular growth factors in the formation of new blood vessels, and its family members include Ang-1, Ang-2, Ang-3 and Ang-4. Ang-1 and Ang-2 are characteristic of vascular growth factors, and the Ang-3 and Ang-4 are found in the source gene between the mice and the human. Ang family common receptor is Tie-2. Ang-1 is composed of 498 amino acids, and its gene location is in 8q22. Through a paracrine effect, the Tie is a type of highly homologous tyrosine kinase receptor and its gene location is in 9p21, encoding the 22 amino acids [11]. Ang-1 combine with Tie-2 to be a polymer formation, and then induced the Tie-2 itself phosphorylation and activation, which was through the PIP3/AKT signal pathways to regulate/apoptosis of endothelial cell proliferation, regulating the endothelial cells of budding, migration, chemotaxis and aggregation, etc [12-15]. In the study, the level of ang-1, tie-2 and VCAM-1 were 585.04 ± 45.32 (pg/ml), 2399.40 ± 124.85 (pg/ml) and 117.69 ± 12.34 (μg/L) in the diabetes groups which were higher than that in control groups 551.29 ± 31.86 (pg/ml), 2103.27 ± 152.89 (pg/ml) and 93.43 ± 10.48 (μg/L), $P<0.05$, has different statistically significance. Study with others show that the ang1, tie2 and VCAM-1 involved in endothelial injury, and diabetes the body's vascular injury has certain diagnostic value.

STZ, being a kind of NO donor, NO can adjust the production of mitochondrial ATP; combine with iron aconitase to inhibit the enzyme activity, causing the damage of pancreatic islet cells. STZ can inhibit the Krebs cycle, reduce the oxygen consumption. Limit of the mitochondria ATP has caused the loss of nucleoside in β-cell and the damage of DNA. Due to the stimulation of STZ, the polymerization of more ADP ribose base happened, further reducing the ATP number and inhibiting the synthesis and secretion of insulin [16-18]. These study shows that the pancreatic acinar epithelial cells and islet structure in the diabetes rats damaged and degenerated, islet edge was not neat, and the disordered arrangement of cells appeared. The vascular endothelial cell factors of eNOS, VCAM-1, SMA, VEGFR and ICAM-1 were highly expressed in diabetes mellitus rats. The research results show that the STZ can lead to the injury of pancreas endothelial cells, resulting in diabetes eventually [19,20].

The etiology and pathogenesis of diabetes is complicated, from genetic, infection factors and diabetes, obesity and pregnancy, drugs, environmental factors, such as any kind of factors leading to loss of pancreatic cells, the destruction of the pancreatic beta cells, which can lead to abnormal insulin secretion, leading to the development of diabetes. In order to explore the changes of endothelial function of pancreatic vascular endothelial cells, this study provides a new idea for the treatment of diabetes mellitus.

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