Tissue Oxidative Stress in Diabetic Mice Induced by High-fat Diet Combined with STZ

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Abstract: Purpose: To investigate the oxidative stress of diabetic mouse model established byhigh-fat diet combined with STZ. Methods: Twenty male C57 bl/6 mice were randomly divided into normal group and model group. Model group mice received a single intraperitoneal injection of streptozotocin(STZ) at a dose of 100 mg / kg after 4 weeks ofhigh-fat diet. Model group mice continue to take ahigh-fat diet for 1 week, then measure weight, fasting blood glucose (FPG), and take samples to measure total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), Malondialdehyde(MDA)and Superoxide dismutase(SOD) in serum; MDA, SOD, glutathione peroxidase(GSH-Px) and catalase(CAT) in tissues ofheart, liver, kidney, pancreas,hippocampus and cortex. Results: Compared with the normal group, FPG, TC, TG and LDL-C in the diabetic mice were significantly increased ($P<0.05$). In serum, the SOD activity of the diabetic mice was significantly lower than that of the normal group ($P<0.05$); the MDA content was significantlyhigher than that of the normal group ($P<0.05$). The MDA content in theheart tissues of mice in the diabetic group was significantlyhigher than that in the normal group ($P<0.05$); the CAT activity was significantly lower than that in the normal group ($P<0.05$). MDA content in liver tissues of mice in the diabetic group was significantlyhigher than that in the normal group ($P<0.05$); SOD and GSH-Px activities were significantly lower than those in the normal group ($P<0.05$). MDA content in kidney tissues of mice in diabetes group was significantlyhigher than that in normal group ($P<0.05$). The MDA content and GSH-Px activity in pancreatic tissue of mice in the diabetic group were significantlyhigher than those in the normal group ($P<0.05$); SOD and CAT activities were significantly lower than those in the normal group ($P<0.05$). Conclusion: Diabetic mice induced byhigh fat diet combined with STZ showed obvious tissue oxidative stress.

1. Introduction

In recent years, the role of abnormalities in oxidative defense on diabetes complicationshas received increasing attention. Oxidative stress injury of organs may be a common channel for the pathogenesis of multiple complications of diabetes [1, 2]. The pancreas is directly related to the occurrence of diabetes. Increasing studies [3, 4, 5] indicated that the impairment of pancreatic function was largely related to oxidative stress. The liver is the main organ of glucose and fat metabolism, and is also the main place for ingestion, storage, synthesis and metabolism of glucose. The liver damage induced by persistenthyperglycemia was mainly manifested by fatty liver infiltration with fatty liver, part of which can develop into steatohepatitis, liver fibrosis and even cirrhosis [6, 7]. Diabetic nephropathy is one of the chronic microvascular complications of diabetes, and its pathogenesishas not been elucidated. Some studies suggest that oxidative stress may play an important role [8, 9]. Studieshave also found that the increased incidence of cardiovascular disease in diabetic patients is associated with increased free radical activity [10, 11]. There are also a few researchs reported on the relationship between the changes in the antioxidant defense function of thehippocampus and cortical tissues and the oxidative stress of the tissues of diabetic mice [12, 13]. This study established a diabetic mouse model by combining ahigh-fat diet with STZ to study the oxidative stress levels in theheart, liver, kidney, pancreas,hippocampus, and cortical tissues of diabetic mice and to provide a basis for exploring the status of the tissue oxidative damage of diabetic mice and for the prevention and treatment of diabetes.

2. Materials and Methods

2.1.Animals

Male C57BL/6J mice (weight 16-18g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Animal certificate: SCXK (Beijing) 2016-0011. The mice werehoused in cages in SPF-level animalhouses, and they were allowed to eat water and feed freely. The initial body weight was measured after 1 week.

2.2.Main Reagents and Instruments

Streptozotocin (Sigma Inc., USA), MDA, SOD, GSH-Px, CAT and tissue protein kits were purchased from Nanjing Jiancheng Biological Research Institute. Blood glucose test paper (Johnson Medical Equipment Co., Ltd.), blood glucose meter (Johnson Medical Equipment Co., Ltd.), full-wavelength microplate reader (Thermo Scientific), constant temperature water bath, desktop low speed centrifuge (Hunanhexi Instrument Equipment Co., Ltd.).

2.3.Model Preparation and Grouping

The mice in the model group were given 60%high-fat diet, and the mice in the normal group were given normal diet for 4 weeks. After 4 weeks, all mice were fasted for 12hours. The mice onhigh-fat diet were given a one-time intraperitoneal injection of STZ 100 mg/kg, and the mice on normal diet were given the same volume of 100 mmol/L sodium citrate buffer. Before STZ injection, all mice were weighed and measured fasting plasma glucose (FPG). STZ is prepared with 100 mol/L sodium citrate buffer solution in pH=4.5, then filtered and sterilized with 0.22μm micropores filter under ice bath and in dark conditions. Twohours after the STZ injection, the mice continued to be fed withhigh-fat diet. In order to prevent the death of mice with low blood sugar, we also gave 5% glucose water for 24h. Seven days after intraperitoneal injection of STZ, mice were fasted for

12hours, weighed and tested FPG. The blood glucose value>=13.9mmol/L was regarded as successful modeling [14].

2.4.Sample Collection and Preservation

The mice were fasted for 12h, and anesthetized with 10% chloralhydrate. Eye blood of the mice was collected and allowed to stand overnight, centrifuged at 3000r/min for 10 minutes. The serum was collected and stored at -80℃ for detection of TC, TG, LDL-C, SOD and MDA.heart, liver, kidney, pancreas,hippocampus and cortical tissues were washed with normal saline and absorbed water with filter paper, then were quickly froze with liquid nitrogen and store at -80 ℃ for detection of MDA, SOD, GSH-Px and CAT.

2.5.Detection of TC, TG and LDL-C in Mouse Serum

The detection of total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) in the serum of mice was commissioned by Jianghan Universityhospital to use automatic biochemistry Detector detection.

2.6.Detection of Oxidation Indexes of Serum and Tissues

Serum and 10% tissuehomogenate were centrifuged at low temperature and low speed, and the supernatant was taken for testing. MDA content was detected by thiobarbituric acid (TBA) condensation method; SOD activity was detected by xanthine oxidase method; GSH-Px activity was detected by dithiodinitrobenzoic acid colorimetry; CAT activity was detected by ultraviolet spectrophotometry; tissue protein content was determined by Coomassie brilliant blue method. The specific measurement method is strictly in accordance with the requirements of the kit.

2.7. Statistical Analysis

The experimental data were expressed as mean ± standard error($\overline{X}$ ±SEM), and SPSS 18.0 statistical software was used for independent sample t test analysis to determine whether the two groups of data were statistically different.

3. Results

3.1.General Status of Animals

During the test, the normal group grew well, while the diabetic group showed increased food intake, increased drinking water, increased urine output, decreased activity, and fluffy and dull fur.

3.2. Weight, Blood Sugar and Serum Related Indicators

As shown in Table 1, the difference in initial body weight between the two groups of mice was not statistically significant ($P>0.05$); the weight of the mice in the diabetic group at the fourth week was significantlyhigher than that of the normal group ($P<0.05$); compared with the pre-modeling period, the diabetic micehad a downward trend but no significant difference ($P>0.05$). Compared with the normal group, the FPG, TC, TG and LDL-C of the diabetic group were significantly increased ($P<0.05$); the serum SOD activity was significantly lower than the normal group ($P<0.05$); the MDA content was significantlyhigher than the normal group ($P<0.05$).

Table 1: Comparison of mouse body weight, blood glucose and serum related indicators($\overline{X}$ ±SEM).

Group	n	Initial weight (g)	Modeling weight (g)	7 days after modeling weight (g)	Modeling FPG (mmol/L)	7 days after modeling FPG (mmol/L)	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	Serum SOD (U/ml)	Serum MDA (nmol/ml)
NC	10	20.82±0.21	24.35±0.45	25.05±0.39	6.60±0.44	6.46±0.38	2.62±0.23	1.34±0.14	0.60±0.05	33.12±1.43	6.30±0.74
DM	10	20.77±0.34	28.77±0.46*	27.32±0.52*	6.41±0.18	23.58±1.15*	5.34±0.31*	5.85±1.30*	1.24±0.15*	22.26±3.75*	9.02±0.62*

Compared with NC group, *P<0.05

3.3. The Status of Oxidative Stress in Diabetic Mice

As shown in Table 2, the content of MDA in theheart tissue of the diabetic group was significantlyhigher than that of the normal group (P<0.05); the activity of CAT was significantly lower than that of the normal group (P<0.05); the activities of SOD and GSH-Px of the diabetic group were not significantly different than those of the normal group(P>0.05). The MDA content in the liver tissue of the diabetic group was significantlyhigher than that of the normal group (P<0.05); the activities of SOD and GSH-Px were significantly lower than those of the normal group (P<0.05); the CAT activity of the diabetic group was not significantly different than that of the normal group (P>0.05). The MDA content in the kidney tissue of the diabetic group was significantlyhigher than that of the normal group (P<0.05); there was no significant difference in the activities of SOD, GSH-Px, and CAT of the diabetic group than those of the normal group (P>0.05). The MDA content and GSH-Px activity in the pancreas tissue of the diabetic group were significantlyhigher than those of the normal group (P<0.05); the SOD and CAT activities were significantly lower than those of the normal group (P<0.05). There was no significant difference in MDA content, SOD, GSH-Px, CAT activity inhippocampus and cortical tissues of the diabetic group than those of the normal group (P> 0.05).

Table 2: Comparison of oxidative stress indexes ofheart, liver, kidney, pancreas,hippocampus and cortical tissues($\overline{X}$ ±SEM).

Tissues	Group	MDA (nmol/mgprot)	SOD (U/mgprot)	GSH-Px (Vitality Unit)	CAT (U/mgprot)
heart	NC	2.35±0.066	48.89±1.15	251.52±34.16	8.76±1.04
	DM	3.34±0.060*	51.16±1.34	254.53±33.68	5.83±0.84*
liver	NC	1.89±0.049	123.56±4.31	1370.10±144.94	18.57±1.71
	DM	2.80±0.032*	102.82±4.38*	1057.01±43.33*	15.97±1.61
kidney	NC	2.88±0.068	91.37±1.84	1223.52±27.55	64.88±4.25
	DM	4.14±0.086*	80.23±3.89	1287.08±44.39	53.68±3.55
pancreas	NC	0.59±0.053	36.08±1.89	715.06±250.01	54.56±7.71
	DM	0.99±0.070*	23.87±2.59*	1196.56±174.74*	30.63±6.54*
hippocampus	NC	0.98±0.057	58.10±9.52	381.10±69.11	23.93±3.99
	DM	1.07±0.066	48.70±1.75	247.97±45.66	18.58±5.91
cortical	NC	0.10±0.055	45.62±1.28	80.63±14.00	15.54±2.51
	DM	1.05±0.044	46.57±1.62	121.93±24.06	13.54±1.86

Compared with NC group, *P<0.05

4. Conclusions

The experimental results showed that FPG, TC, TG and LDL-C of the diabetic mice were significantly increased, suggesting that the glucose and lipid metabolism of the diabetic micehad been disturbed. In addition, the SOD activity in the serum of the diabetic mice was significantly reduced; the MDA content was significantly increased, suggesting that this disorder may be related to oxidative stress. Because diabetes is a disease of abnormal glucose metabolism which could generate more free radicals during the metabolism in cellular mitochondria, it may cause abnormalities of free radicals in tissue cells [15]. This study showed that the oxidative and anti-oxidative balance inheart, liver, kidney, and pancreas tissues of diabetic mouse models is severely disturbed, and the oxygen free radical reaction, oxidation, peroxidation, and lipid peroxidation reaction were intensified, causing oxidative stress in the main tissues of diabetic mice.It suggested that various tissue complications of diabetic patients may be related to abnormal levels of free radicals or oxidative stress in tissue cells.This study provides a theoretical basis for investigating the oxidative damage in the tissues of diabetic mice, preventing diabetes and complications.

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