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Original Article

Effects of resveratrol on spermatogenic function in rats with type 2 diabetes

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Abstract: Objective: The aim of this study was to investigate the effects of resveratrol on Bcl-2, Bax, and SOD expression and spermatogenic function in testicular tissues of rats with type 2 diabetes. Methods: A total of 40 pathogen-free, mature, male SD rats were selected. Ten rats were randomly selected as the control group using a random number table. The remaining 30 rats were induced to develop type 2 diabetes. The model was successfully established. The rats were then randomly divided into the model group and treatment group. Expression of Bcl-2, Bax, and SOD protein and spermatogenic function were determined in testicular tissues. Effects and mechanisms of resveratrol on spermatogenic function in rats with type 2 diabetes were analyzed. Results: After 13 weeks of treatment, expression of Bcl-2, Bax, and SOD was significantly different in the three groups (P<0.001). Testicular index (P=0.001, P=0.042), testicular volume (P=0.001, P=0.045), sperm concentrations (all P<0.001), capacity of action (P<0.001, P=0.005), survival rates (P<0.001, P=0.010), and malformation rates (all P<0.001) in the model and treatment groups were lower than those in the control group. Values were higher in the treatment group than in the model group (P=0.046, P<0.001, P<0.001, P<0.001, P<0.001, P<0.001). Conclusion: Resveratrol improved the reproductive function of rats with type 2 diabetes. Underlying mechanisms were likely that it protects against oxidative stress damage and spermatogenic cell apoptosis in testicular tissues.

Keywords: Resveratrol, type 2 diabetes, testicular tissue, oxidative stress, spermatogenic function

Introduction

Type 2 diabetes accounts for approximately 95% of all types of diabetes [1]. Improvement in living standards, the aging population, and changes in modern lifestyles have resulted in high incidence of diabetes. It is expected to become the seventh most common cause of death, worldwide, by the year 2030 [2, 3]. The principle outcomes of type 2 diabetes are acute or chronic complications, including reproductive system damage. As type 2 diabetes develop with a decreasing trend, a lot of attention has been given to its impact on reproductive function. Reproductive function damage is currently a critical problem faced by clinicians [4, 5].

The specific mechanisms which cause reproductive function damage in patients with type 2 diabetes are not clear. Many studies have shown that spermatogenic functions of patients with type 2 diabetes are generally weakened, with reproductive function impairment [6, 7]. Based on animal experiments, it was proven that the sperm quality and reproductive abilities of male animals were decreased [8, 9].

Resveratrol is a non-flavonoid polyphenolic com-pound and a natural antioxidant that improves the metabolic function of pregnant women. It is often are used to treat diabetes. In recent years, it was shown that resveratrol is effective for male reproductive system disorders [10, 11]. However, there are few studies on the treatment of reproductive function in patients with type 2 diabetes. Type 2 diabetes is often accompanied by oxidative stress damage, an important cause of spermatogenic dys-

function [12]. The formation of sperm is also an important part of spermatogenic function, closely linked to proliferation and apoptosis of sper-matogenic cells [13].

The current study established a model of type 2 diabetes using male SD rats to investigate the effects of resveratrol on spermatogenesis and explore possible mechanisms of action.

Materials and methods

Subjects

Forty male SD rats purchased from the Peking University Medical School Laboratory Animal Center (SCXK (Beijing) 2011-0001) were fed with an ordinary rat diet (Beijing Zhecheng Technology Co., Ltd.). Drinking water was autoclaved acidified water between pH 2.5 and 3. The average age of the rats was 42.6±2.4 days and the average body weight was 224.1±8.5 g. The animals were kept at 18°C-22°C, with a relative humidity of 40%-70%. All rats were housed in separate cages and the padding was replaced twice a day. Ambient noise ≤ 85 db, ammonia concentration ≤ 20 ppm, and ventilation was performed 8-12 times per hour. Cages housing the rats were replaced, cleaned, and disinfected each week. Noise ≤ 60 dB and ammonia concentration ≤ 14 ppm. Rats were ventilated 15 times per hour and fluorescent lamps were set to a 12-hour cycle. Ten rats were allocated to the control group using a random number table, while the remaining 30 rats were used to establish a model of type 2 diabetes. After the model was successfully established, the rats were randomly allocated to the model group or the treatment group. All procedures were approved by the Animal Care and Use Committee of the Qilu Hospital, Shandong University, and were in conformity with the Guidelines of National Institute of Health (No81004).

Rat model establishment

Thirty SD rats were used to establish a model of type 2 diabetes. They were first fed a high-fat diet (Wuhan Cloud-Clone Diagnostic Corp.) for 8 weeks, then fasted for 12 hours. A single intraperitoneal injection of streptozotocin solution (45 mg/kg; Shanghai Baoman Biotechnology Co., Ltd.) was administered. After 72 hours, blood sugar levels were measured in the tail veins. Fasting blood sugar ≥ 7.0 mmol/L and postprandial blood glucose ≥ 11.1 mmol/L indicated successful modeling. Rats in the control group were fed with ordinary nutrient feed.

Treatments

Resveratrol (Shanghai Baoman Biotechnology Co., Ltd.) was prepared as a 10 mg/mL solution in 0.9% physiological saline. Rats in the treatment group were treated with 10 mg/kg/day resveratrol solution by intragastric administration, while rats in the normal control group and model group were treated with 0.9% saline by intragastric administration. Treatment continued for 13 weeks. Rats in the control group were fed with ordinary nutrient feed. Those in the model group and treatment group were continuously fed with a high-fat diet.

Detection of Bcl-2 and Bax protein expression

Expression of Bcl-2 and Bax in rat testes was detected by ELISA. After 13 weeks, the rats were sacrificed. Testicular tissues were removed, cut, and homogenized. Next, 50 μL of sample analysis buffer and 50 μL of tissue homogenate were added to each well and incubated for 2 hours at 18°C-21°C. After incubation, the plate was washed five times, 100 μL of biotinylated antibody was added, and the plate was sealed. After incubation for 1 hour at room temperature, the plate was washed again, 100 μL of horseradish peroxidase-labeled antibody was added, and the plate was sealed. The plate was incubated in the dark for 20 minutes at room temperature. Subsequently, 100 μL of TMB was added and the incubation was repeated, 50 μL of the termination solution was added, and the plate was read by using a microplate reader after 15 minutes to determine the maximum absorption at 450 nm. Three groups of duplicate wells were used. The experiment was repeated three times. Kits for the measurement of Bcl-2 and Bax were purchased from Shanghai Jingkang Biological Engineering Co., Ltd.

Detection of SOD levels

SOD levels in the testes tissues of rats were detected by the xanthine oxidase method. The specimen was the testis tissue homogenate, described above. The specific experimental procedure followed kit instructions. Absorbance of

Table 1. Analysis of basic animal data in the three groups of rats

	Control group	Model group	Treatment group	F	*P*-value
Number	10	14	14		
Age (day)	42.2±2.9	42.5±2.1	43.1±1.9	0.503	0.609
Premodeling weight (g)	221.7±21.2	225.5±13.4	225.1±13.2	0.196	0.823
Pretreatment weight (g)	271.2±27.3*	391.4±35.9*,#	382.7±36.3*,#	43.863	<0.001
Premodeling FBG (mmol/L)	4.19±0.73	4.22±0.65	4.17±0.69	0.019	0.981
Pretreatment FBG (mmol/L)	4.18±0.72	12.63±4.32*,#	12.56±4.27*,#	18.855	<0.001
Premodeling PBG (mmol/L)	4.23±0.73	4.18±0.66	4.21±0.67	0.016	0.984
Pretreatment PBG (mmol/L)	4.25±0.67	13.33±1.36*,#	13.45±1.34*,#	209.498	<0.001
Premodeling insulin (μg/L)	35.26±15.43	34.86±15.17	35.04±15.13	0.024	0.976
Pretreatment insulin (μg/L)	35.31±15.13	20.32±14.98*,#	21.34±15.09*,#	3.422	0.044

Note: *comparison within the group, P<0.05 before modeling; #P<0.05 compared with control group.

SOD at 469 nm was measured within 3 minutes. Triplicate tests were used for each specimen. The SOD kit was purchased from Shanghai Lianmai Bioengineering Co., Ltd.

Spermatogenic function testing

Testicular index and testicular volume were measured. Testicular index was calculated based on the following formula: testicular index (g/kg) = testicular weight (g)/rat body weight before sacrificing (g) × 100%. Testicular volume was measured by a testicular volume meter. An automatic sperm analyzer, purchased from Nanning Songjing Tianlun Biotechnology Co., Ltd., was used to measure epididymis sperm concentrations and to observe the sperm motility, survival rates, and deformity rates.

Statistical analysis

SPSS 19.0 (Asia Analytics, formerly SPSS China) was used for statistical analyses. Count data are expressed as a percentage and the rates were compared by χ^2 test. Measurement data are expressed as mean ± SD. Comparisons among multiple groups were performed using ANOVA and comparisons between each pair of groups was performed using an LSD test. P<0.05 indicates statistical significance.

Results

Modeling results

Thirty male Sprague-Dawley rats were induced as the model of type 2 diabetes. The model was successfully established in 28 rats (93.33%). Before induction, body weight, fasting blood sugar, postprandial blood glucose, and insulin levels of the three groups were not significantly different (P>0.05). After successful modeling and before treatment, fasting blood glucose, postprandial blood glucose, and insulin levels in the normal group were not different than those before modeling, but body weight, fasting blood glucose, and postprandial blood glucose in the model group and treatment group were significantly different (P<0.05). Insulin levels were also decreased (P<0.05). Body weights of rats in the normal group before intervention were higher than those before modeling (P<0.05). Vital signs, before and after modeling, in the three groups of rats are presented in **Table 1**.

Detection of apoptosis-related indicators

After 13 weeks of treatment, expression of Bcl-2 in the three groups was significantly different (P<0.05). Expression in the model group and treatment group was lower than that of the control group (P<0.05), but higher in the treatment group than in the model group (P<0.05). Bax protein expression in the three groups was significantly different (P<0.05). Expression in the model and treatment groups was higher than that of the control group (P<0.05), but lower in the treatment group than in the model group (P<0.05) (**Figure 1**).

Detection of SOD levels

After 13 weeks of treatment, SOD levels were detected by the xanthine oxidase method. In the testis tissues of rats in the control, model, and treatment groups, SOD levels were

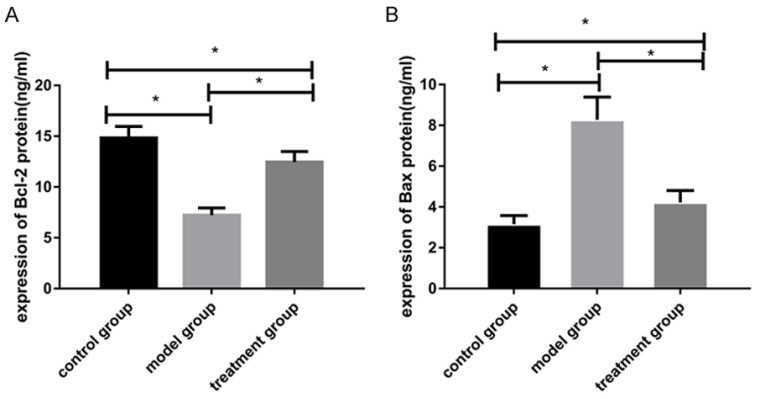

Figure 1. Expression levels of Bcl-2 and Bax in the testis of the three groups after 13 weeks of treatment. A: Bcl-2 protein detection results. B: Bax protein detection results. *: P<0.05.

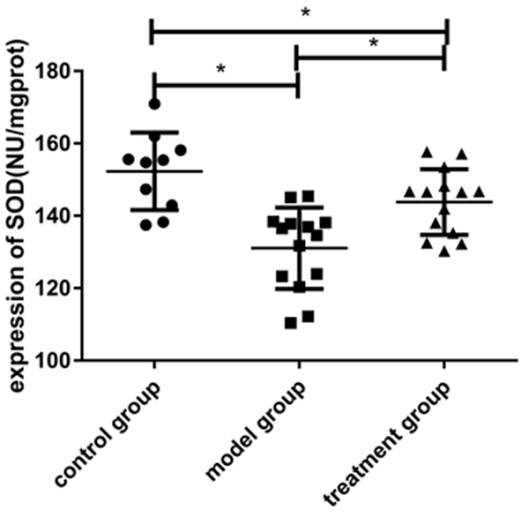

Figure 2. SOD levels detection results. *: P<0.05.

152.33±11.59, 127.45±9.83, and 144.72±10.66 NU/mg protein, respectively. SOD levels in the three groups were significantly different (P<0.05). Levels in the model and treatment groups were lower than those of the control group (P<0.05), but higher in the treatment group than in the model group (P<0.05) (**Figure 2**).

Detection of rat spermatogenic function

After 13 weeks of treatment, testicular index, testicular volume, sperm concentrations, motility, survival rates, and deformity rates of the three groups were significantly different (P<0.05). Testicular index, testicular volume, sperm concentrations, sperm motility, survival rates, and deformity rates in the model and treatment groups were lower than those in the control group (P<0.05). Conversely, these values in the treatment group were higher than those in the model group (all P<0.05) (**Figure 3**).

Discussion

Patients with type 2 diabetes often suffer from hypogonadism and approximately 30%-40% of male patients with type 2 diabetes are infertile [14, 15]. Studies have reported that resveratrol improves the testicular function of rats [16]. However, there are few reports concerning the therapeutic effects of resveratrol on reproductive function of patients with type 2 diabetes. Therefore, in this study, resveratrol was administered to rats with type 2 diabetes. Therapeutic effects of resveratrol on the reproductive function of rats with type 2 diabetes were examined, aiming to provide reference for clinical treatment.

This study used rat models of type 2 diabetes, which were easily constructed and reproducible. The established models showed similar clinical features. Disease conditions were controlled and the model was stable, which were ideal for experiments [17]. Before modeling, the three groups were not significantly different in age, body weight, fasting blood glucose, postprandial blood glucose, and insulin levels, indicating that the study was reliable and that results were comparable. After modeling, there were significant differences in body weight, fasting blood glucose, postprandial blood glucose, and insulin levels, suggesting successful modeling. This study administered resveratrol to rats in the treatment group and observed the effects of resveratrol on the reproductive function of rats with type 2 diabetes. Testicular volume is an important indicator of spermatogenic function. Individuals with larger testicular volume generally have better spermatogenic function [18], as supported by the results in this study. After 13 weeks of treatment, the rats were sacrificed and the testicular index and testicular volume of the three groups of rats were measured. Results indicated that rats with type 2 diabetes had a significantly lower

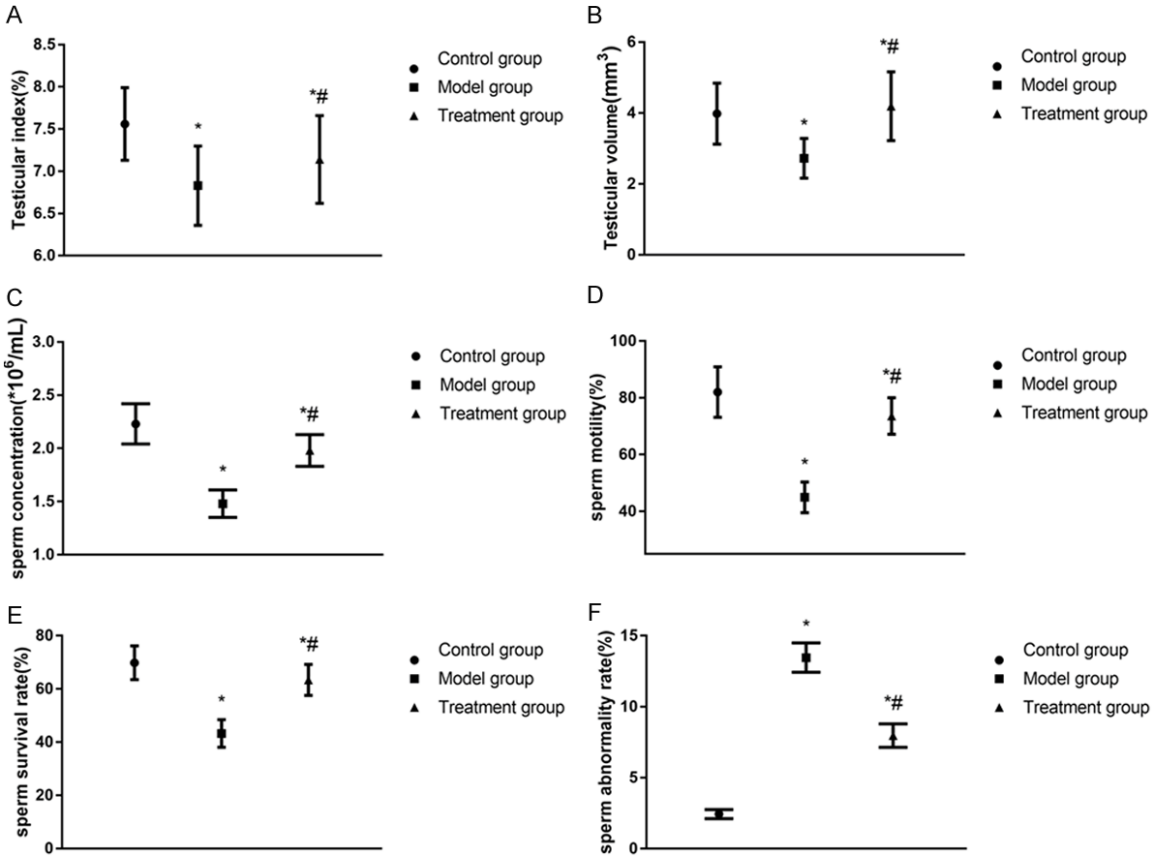

Figure 3. Differences in spermatogenic function related indexes among the three groups after 13 weeks of treatment. A: Testicular index. B: Testicular volume. C: Sperm concentration. D: Sperm motility. E: Sperm survival rate. F: Sperm abnormality rate. *P<0.05 compared with the control group; #P<0.05 compared with the model group.

testicular index and significantly smaller testicular volume than healthy rats. After treatment, the testicular index and testicular volume of the rats were restored, indicating that resveratrol may improve the spermatogenic function of rats with type 2 diabetes. Sperm concentrations, motility, and survival rates of rats with type 2 diabetes were significantly lower than those of the control rats. Deformity rates were significantly higher, consistent with previous reports [19, 20]. After 13 weeks of treatment, sperm concentrations, motility, survival rates, and deformity rates of the model rats were generally improved, indicating that resveratrol contributed to improving the spermatogenic function of rats with type 2 diabetes.

Spermatogenic function is also closely associated with the proliferation and apoptosis of spermatogenic cells [13]. Rats with type 2 diabetes are often accompanied by oxidative stress dysfunction, which results in oxidative damage to cells [12]. This study detected SOD, which is closely associated with oxidative stress, and expression of Bcl-2 and Bax proteins, which are closely related to apoptosis [21, 22]. SOD is an important enzyme that is able to scavenge oxygen free radicals [21] and, consequently, catalyze the disproportionation reaction of oxygen free radicals, which counteracts oxidative stress injury and scavenges free radicals in the human body [22]. Bax acts to promote apoptosis and Bcl-2 counteracts apoptosis [23]. Results of this study showed that Bax expression in the testicular tissues of the model rats was significantly higher than that of the control rats. Bcl-2 expression and SOD levels were significantly lower than those of the control rats. Present results were supportive of those in related reports [24, 25]. After 13 weeks of treatment with resveratrol, protein expression of each factor was improved, indicating that resveratrol ameliorated oxidative stress and reduced apoptosis in the testis tis-

sues of model rats. These changes were associated with the changes in spermatogenic function discussed above. After 13 weeks of treatment, sperm concentrations, capacity of action, survival rates, and deformity rates of model rats were improved. Thus, it was speculated that resveratrol may reduce apoptosis of spermatogenic cells in the testes of rats with type 2 diabetes. However, due to the limitations of experimental conditions, these results were not confirmed. Therefore, the hope is that this study will act as a platform through which other researchers can explore the therapeutic effects of resveratrol on the spermatogenic function of rats with type 2 diabetes. This study had other limitations as well. Rats are different than humans, thus the model is not a complete simulation of the pathological processes in patients with type 2 diabetes patients. Therefore, more clinical data is required to confirm present results.

In conclusion, resveratrol ameliorated the reproductive function in rats with type 2 diabetes. The underlying mechanisms of this improvement may be the reduction in oxidative stress damage to testicular tissues and the reduction of apoptosis of spermatogenic cells.

Disclosure of conflict of interest

None.

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