Original Article

Effect of resveratrol intervention on renal pathological injury and spermatogenesis in type 2 diabetic mice

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Received January 12, 2021; Accepted February 23, 2021; Epub May 15, 2021; Published May 30, 2021

Abstract: Background: Type 2 diabetes (T2D) is a clinically common cardiovascular disease that can lead to kidney damage and adversely affect male fertility and sperm quality. Resveratrol (Res) is a natural product that has a wide range of effects in animals and cell models. Objective: This research is designed to observe the effect of resveratrol (Res) intervention on renal pathologic injury and spermatogenesis in mice with type 2 diabetes (T2D). Methods: Sixty healthy male SD mice without specific pathogens (SPF grade) were selected, and numbered by statistical software to randomize into control group (CG; n=20), model group (MG; n=20) and research group (RG; n=20). Mice in CG were given regular diet, while those in MG and RG were fed with high fat diet. Subsequently, RG was given Res intervention while MG received no treatment. Biochemical indexes [triglyceride (TG), total cholesterol (TC), fasting blood glucose (FBG), 24-hour urinary albumin excretion rate (24h-UAER)] of mice in the three groups before and after intervention were observed and recorded. The effect of Res on oxidative stress, kidney histopathological structure, spermatogenic function, sperm density and viability of mice, as well as spermatogenic cell cycle of testis were determined. Results: Res reduced hyperlipidemia and hyperglycemia in T2D mice. By reducing malondialdehyde (MDA) and increasing superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), Res relieved oxidative stress and alleviated kidney tissue damage. In addition, Res improved the spermatogenic function of T2D mice by increasing the sperm density and survival rate and restoring the percentage of spermatogenic cells at all levels. Conclusions: Res intervention in T2D mice can reduce kidney tissue damage, lower blood glucose (BG), and improve spermatogenic function by increasing sperm density and restoring the percentage of spermatogenic cells at all levels.

Keywords: Resveratrol, type 2 diabetes, mice, renal pathological injury, spermatogenic function, effect investigation

Introduction

Type 2 diabetes (T2D) is a clinically common cardiovascular disease [1], whose increasing incidence is driven by unfavorable changes of people’s living habits and population aging [2]. With hyperglycemia as the main feature of the disease, most patients will suffer from metabolic disorders or low immune function, resulting in higher disability rate and mortality [3]. In addition, patients with diabetes will develop endothelial dysfunction, which can lead to kidney damage [4]. It is also indicated that diabetes can trigger reproductive system disorder in males, and that glucose metabolism is an important event in spermatogenesis, while T2D can adversely affect male fertility and sperm quality [5]. Hence, it is of utmost importance to find therapeutic drugs to treat T2D to control and delay disease progression.

Resveratrol (Res) is a natural product that has a wide range of functions in animal and cell models, including prolonging life [6]. It can be found in many natural plants such as grapes, pine trees, peanuts and mulberry, and has potent therapeutic effects on a wide range of diseases, especially those related to oxidative stress [7]. It is a potent antioxidant phytochemical that plays a significant role in inflammatory diseases, diabetes and cancer [8]. Oxidative stress injury can trigger abnormal changes in renal hemodynamics, leading to renal injury, which is related to increased mortality [9]. Evidence has shown that Res can reduce high-fat diet induced kidney injury in obese rats, and relieve...
oxidative stress [10]. Sajadi revealed that the structural and functional changes of testicular cells after diabetes induction will lead to changes in the spatial arrangement of Sertoli cells (SC) and spermatogonia, which will eventually reduce the number of testicular cells and total sperm count [11]. It is well documented that Res benefits spermatogenesis. For example, Guo pointed out that Res can improve the reproductive dysfunction of rats caused by high-intensity exercise, and enhance the reproductive function by increasing testosterone secretion, reducing inflammation and improving antioxidant capacity as well as regulating spermatogenic regulatory proteins [12].

In this study, a mouse model of T2D was established to observe the effect of Res on renal pathologic injury, blood glucose (BG), and spermatogenesis in mice.

Methods

Experimental animals

Sixty healthy male SD mice without specific pathogens (Junke Bioengineering Co., Ltd., Nanjing, China, J006) were selected and acclimatized for a week in a clean environment with good ventilation and following environmental adaptation conditions: 12-hour light-dark cycles, room temperature 22±2°C, humidity 50-65%. Food and water were available ad libitum according to the standard procedures of animal care. This study was conducted following the guiding principles for the Protection and Use of Experimental Animals [13] after the approval by the hospital ethics committee.

Modeling and grouping [14]

After numbering by statistical software, 60 mice were randomly divided into control group (CG), model group (MG), and research group (RG), with 20 mice each. Mice in CG were routinely fed during the experiment, while the rest were fed with a high-sugar and high-fat diet. Thirty days later, a mouse model of T2D was induced by intraperitoneal injection of 35 mg/kg STZ (Saihongrui Biotechnology Co., Ltd., Nanjing, China, S817944-1 g). The mice developed diabetes symptoms such as polydipsia and polyuria after intraperitoneal injection of STZ injection for 16 h, and were continued to be fed with a high-calorie diet. Two weeks later, glycosuria test paper was used for continuous measurement for 3 days, and the T2D model was deemed successfully established if the fasting blood glucose (FBG) of mice was higher than 11.1 mmol/L. The mice in RG were treated with Res, while those in CG were not treated with any intervention.

Treatment and intervention methods

Mice in RG were given 150 mg/kg Res administered intragastrically, while those in CG and MG were given the same volume of normal saline at 10 mL/kg every day for 6 days every week, with a total of 4 weeks of intervention.

Outcome measures

1. Biochemical indicators: Before and 4 weeks after treatment, 5 mL of femoral artery blood was drawn from each mouse, which was then centrifuged at 1500 ×g at 4°C for 10 min and stored at -70°C until use. The contents of triglyceride (TG), total cholesterol (TC) and FBG were measured by an automatic biochemical analyzer. 24-hour urine was collected 4 weeks after intervention in the three groups of rats, and part of the supernatant was collected after centrifugation to detect urinary albumin (UA).

2. Oxidative stress: The contents of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were determined by enzyme linked immunosorbent assay (ELISA) [15].

3. Renal histopathology: The mice were anesthetized with ether, and the abdominal cavity was cut open to expose the bilateral kidneys after blood collection. Next, the renal envelope and surrounding adipose tissue were gently removed on ice, and the residuals were fixed in 4% neutral formaldehyde, embedded and fixed, and made into 3 μm thick paraffin sections. H&E staining was performed after dewaxing, and the morphologic changes of kidney tissue in each group were observed under the ordinary light microscope.

4. Detection of spermatogenic function: Estimated glomerular filtration rate (EGFR) was detected by routine avidin-biotin complex (ABC) method, and the content of serum testosterone was determined. Blood was extracted from mice through the inner canthus, and the supernatant was collected after centrifugation at 1500 ×g and 4°C for 10 min. Testicular con-
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5. Sperm density and viability: 5 mL sperm nutrient solution was heated to 37°C in a thermostatic water bath. Then, one side of the epididymis was removed, shredded, shaken in a test tube and kept at 37°C for 10 min to make the sperm fully swim away. Next, 1 drop was dripped into the Neubauer hemocytometer, and the sperm count per milliliter was calculated. Finally, 10 μL sperm suspension was absorbed by a washer, and the sperm survival rate was determined by a semen analyzer.

6. Testicular spermatogenic cell cycle: One testicle was selected to remove the capsule, cut into pieces with scissors, and placed in a PBS test tube. After mixing, the mixture was filtered into the test tube with a nylon net and centrifuged at 1500 xg and 4°C for 4 min to discard the resultant supernatant. After suspension, the precipitate was added into 3 mL PBS for 1 min and washed twice to make a single-cell suspension. Cells of 10⁶-10⁷ cells/mL were then fixed with ethanol for 1 h, and centrifuged to remove the fixed solution, followed by two washes with PBS and immersion in 400 μL RNase (40 μg/ml). After a water bath at 37°C for 20 min, the computer was used to calculate the percentage of different ploidy cells.

Statistical processing

SPSS 18.0 and GraphPad 6 software package was used for data analysis and visualization respectively. All the data were presented as mean ± SD; independent sample t test was used for inter-group comparisons between groups, one-way analysis of variance (denoted by F) was utilized for multi-group comparisons (denoted by F), and LSD-t test for post-hoc comparisons. Differences with p-values <0.05 were considered significant.

Results

Effect of Res on biochemical indexes in mice

The effect of Res on mouse biochemical indexes was explored. The results revealed evidently elevated serum TG, TC, FBG and 24h-UAER in MG as compared to CG (P<0.05). After Res treatment, evidently reduced serum TG, TC, FBG and 24h-UAER were observed in RG, with significant differences (P<0.05) Figure 1.

Effect of Res on oxidative stress in mice

The effect of Res on mouse oxidative stress was investigated. Compared with the CG, serum MDA in MG was observably increased, while SOD and GSH-Px were notably decreased (P<0.05). Res intervention resulted in statistically decreased MDA and notably elevated SOD and GSH-Px in RG (P<0.05) Figure 2.

Effect of Res on histopathological structure of mouse kidney

The effect of Res on the pathological structure of mouse kidney was observed. In CG, the glomeruli were plump and had no space within the wall of the capsule; the size and morphology of renal tubules were normal, with no inter-
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interstitial inflammatory cell infiltration, necrosis, or fiber proliferation. In MG, glomerular atrophy, cystic cavity enlargement and extensive necrosis of renal tubular cells occurred. After Res treatment, the pathologic changes of mice in RG were alleviated to varying degrees; however, the glomeruli were still atrophied, the cystic space was slightly larger than normal, and the area of renal tubular cell necrosis was smaller than that in MG Figure 3.

Diabetes is a metabolic disorder characterized by hyperglycemia and a heavy burden associated with microvascular and macrovascular complications [16]. T2D, the most prevalent type most diabetic patients experience, is a polygenic metabolic disorder caused by oxidative stress, insulin resistance, β cell dysfunction, and low glucose tolerance [17]. Therefore, it is time to find new treatments to intervene in T2D.

Figure 2. Effect of Res on oxidative stress in mice. A: Effect of Res on MDA in type 2 diabetic mice. B: Effect of Res on SOD in type 2 diabetic mice. C: Effect of Res on GSH-Px in type 2 diabetic mice. Note: *P<0.05, **P<0.01.

Effect of Res on spermatogenesis in mice

We examined the effect of Res on spermatogenesis in mice. ECFR, testicular mass, testosterone, and LH in the MG were noticeably decreased as compared to CG (P<0.05). Following Res intervention, notably elevations in ECFR, testicular mass, testosterone and LH were observed in RG (P<0.05) Figure 4.

Effect of Res on sperm density and viability in mice

Changes in Res-intervened sperm density and sperm viability in mice were also observed. Sperm density and sperm viability were distinctly less in the MG than in the CG (P<0.05). After Res treatment, the sperm density and viability of mice in RG increased evidently, and the difference was significant (P<0.05) Figure 5.

Effect of Res on percentage of spermatogenic cells in mice

The effect of Res on the percentage of spermatogenic cells in mice was determined. The percentage of 1C, 2C, and 4C spermatogenic cells decreased dramatically in the MG as compared to CG (P<0.05), while it increased notably in RG after Res intervention (P<0.05) Figure 6.

Discussion

Figure 3. Effect of Res on histopathologic structure of mouse kidney. A: H&E staining images of mouse kidney tissue in control group. B: H&E staining images of mouse kidney tissue in model group. C: H&E staining images of mouse kidney tissue in research group.
A previous study showed that Res can promote bone formation and enhance biomechanical fixation in T2D [18]. Given the current lack of research on the effect of Res on renal pathologic damage and spermatogenesis of T2D, we conducted this investigation for clarification. The results determined a significantly decreased BG in T2D mice, controlled kidney injury, and improved spermatogenic function following Res intervention. Research has shown indicators such as BG and 24h-UAER, as the criteria for early diabetic nephropathy in clinical stud-

ies, can reflect the damage course of renal structure and function [19]. Res can ameliorate kidney injury caused by high glucose, and reduce hyperglycemia and 24h-UAER [20]. Unsurprisingly, serum TG, TC, FBG, and 24h-UAER in MG were notably increased, and these measures in RG were markedly reduced after Res intervention, suggesting that Res can lower BG in T2D mice, reduce hyperlipidemia, and protect the kidney. Diabetes-induced kidney injury stems from multiple factors, among which the role of oxidative stress is particularly prominent. This is because the presence of oxidative stress in diabetic nephropathy will lead to the cleavage and oxidation of antioxidant enzymes in kidney tissue, which leads to a significant decrease in antioxidant capacity [21]. In vitro and animal studies have shown that Res exerts antioxidant effect on blood of patients with T2D [22]. Similarly, the present research found increased MDA and decreased SOD and GSH-Px in serum of mice in MG, but decreased MDA and elevated SOD and GSH-Px in RG after Res treatment. This indicates that Res intervention plays an active and effective role in regulating the metabolism of oxygen free radicals in kidney, and can reduce oxidation products and stress injury in mice. We also observed the improvement of kidney tissue of the three groups of mice after H&E staining. The kidney tissue of mice was found to be seriously damaged in the MG, while was improved to varying degrees in RG after Res intervention. This shows that Res, as a bioactive substance, can prevent against kidney injury through antioxidation.

T2D, which affects testosterone synthesis, is increasing rapidly among young people [23]. Evidence has shown that spermatogenic dys-

Figure 4. Effect of Res on spermatogenesis in mice. A: Effect of Res on ECFR in T2D mice. B: Effect of Res on testicular mass in T2D mice. C: Effect of Res on testosterone in T2D mice. D: Effect of Res on luteinizing hormone in T2D mice. Note: *P<0.05, **P<0.01.

Figure 5. Effect of Res on sperm density and viability in mice. A: Effect of Res on sperm density in T2D mice. B: Effect of Res on sperm viability in T2D mice. Note: *P<0.05, **P<0.01.
function, a common complication of diabetic men, is also the most important manifestation of diabetes-related reproductive injury in men, and increasing antioxidant enzyme activity and inhibiting inflammation have beneficial effects on spermatogenic dysfunction caused by diabetes [24]. Omur reported that Res inhibited testicular injury and lipid peroxidation resulted from aflatoxin, and was protective of sperm motility and viability [25]. Similarly, ECFR, testicular mass, testosterone, LH, sperm density, and sperm viability were observed to be decreased in the MG in the present study, and were significantly increased in the RG after Res treatment, demonstrating that Res can reduce T2D-induced testicular injury, and can effectively improve sperm density and viability in mice. Moreover, the percentage of 1C, 2C, and 4C spermatogenic cells was decreased in the MG, while elevated markedly in the RG after Res treatment, indicating that Res can significantly restore the percentage of spermatogenic cells at all levels in mice, which is effective in improving spermatogenic function. However, the study still has some limitations. First, the effect of Res with different doses must be further explored. Second, the clinical application of Res needs to be verified in clinical practice.

In summary, Res intervention in T2D mice can reduce kidney injury and lower BG, as well as improve spermatogenic function by increasing sperm density and restoring the percentage of spermatogenic cells at all levels.

Disclosure of conflict of interest

None.

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