Contents lists available at ScienceDirect IF: 0.926

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60307-2

Protective effect of resveratrol on lens epithelial cell apoptosis in diabetic cataract rat

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ARTICLE INFO

Article history:
Received 20 October 2014
Received in revised form 10 November 2014
Accepted 15 December 2014
Available online 20 February 2015

Keywords:
Resveratrol
Diabetic cataract
Lens epithelial ce11
Cell apoptosis

ABSTRACT

Objective: To study the protective effect of resveratrol on lens epithelial cell apoptosis in diabetic cataract rat. **Methods:** A total of 84 Wistar rats were divided into 4 groups: 12 in Group A (control group), 24 in Group B (diabetic cataract group), 24 in Group C (therapeutic-dose of resveratrol group) and 24 in Group D (low-dose of resveratrol group). Rats in Group B-D were given with 60 mg/kg streptozotocin through intraperitoneal injection. Rats in Group C were given with 100 mg/kg resveratrol and rats in Group D were given with 20 mg/kg resveratrol. The caspase-3 expression levels and apoptosis ratios of LEC among each group were observed; the degrees of lens opacity in Group B-D after 12 weeks were compared. **Results:** There were significant differences in caspase-3 expression levels, apoptosis ratios of LEC among groups at 4 w, 8 w and 12 w (P<0.05). After 12 weeks, in Group B the degree of lens opacity was as follow: 0 (0.00%) in grade Ⅰ, 3 (37.50%) in grade Ⅱ,2 (25.00%)in grade Ⅲ, 2 (25.00%)grade Ⅳ, and 1 (12.50%) in grade Ⅴ; in Group C: 2 (25.00%)in grade Ⅰ, 4 (50.00%) in grade Ⅱ, 2 (25.00%)in grade Ⅲ, 0 (0.00%)grade Ⅳ, and 0 (0.00 %) in grade Ⅴ; in Group D: 1 (12.50%)in grade Ⅰ, 4 (50.00%) in grade Ⅱ, 2 (25.00%) in grade Ⅲ, 1 (12.50%) grade Ⅳ, and 0 (0.00%) in grade Ⅴ. The difference among Group B-D was statistically significant (P<0.05). **Conclusions:** Resveratrol has protective effect on lens epithelial cell apoptosis in diabetic cataract rat, and the effect is relative to its dose.

1. Introduction

With the improvement of the economic level and national consumption level, people's diet manner has changed, and the incidence of diabetes has increased year by year[1]. As a complication of diabetes, diabetic cataract (DC) seriously affects patients' vision and quality of life. The patients are presented mainly with blurred vision and decreased visual acuity, and show progressive lens opacity under slit lamp[2]. The pathogenesis of DC is complex, in which lens epithelial cell (LEC) apoptosis plays an important role in the occurrence and development of the disease[3]. Resveratrol, a polyphenol with powerful bioactive substance, is a natural antioxidant. It has been found that resveratrol can effectively remove free radicals. Currently, clinical studies have shown that resveratrol have significant clinical efficacy when were used to treat coronary heart disease and cancer, but the report about application in DC is few[4,5]. In this study, we observed the protective effect of resveratrol on LEC apoptosis in DC rat.

2. Materials and methods

2.1. Experimental animals and grouping

A total of 84 healthy male Wistar rats, each weighing 240-260 g with an average of (250.4±6.2) g, were provided by Experimental Animal Center of Chongqing University, with a Certificate No. of 14-0212. All rats were divided into 4 groups: 12 in Group A (control group), 24 in Group B

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Foundation project: It is supported by Wenzhou Science and Technology Project (Y20080087).

(diabetic cataract group), 24 in Group C (therapeutic-dose of resveratrol group) and 24 in Group D(low-dose of resveratrol group).

2.2. Drugs and reagents

Drugs: resveratrol(Sigma, purity>99%), streptozotocin (STZ, Sigma, purity>98%), 0.5% atropine sulfate ophthalmic solution (Alcon Labratories Inc, 20140103).

Reagents: rabbit anti-rat caspase-3 polyclonal antibody (Santa Cruz); horseradish peroxidase-marked goat anti-rabbit IgG antibody (Beijing Saichi Biotechnology Co., Ltd.); ECL chemiluminescence kit (Pierce); Annexin-Ⅴ apoptosis detection kit (U.S. BD); other biochemical reagents were mainly from Nanjing Shengxing biological Co Ltd.

2.3. Model establishment and drugs treatment

All rats were adaptively fed for one week. Group B-D were given with 60 mg/kg STZ (dissolved in 0.1 mol/L citrate buffer solution, pH=4.5) through intraperitoneal injection[6]. The blood of rat tail vein was drawn after 72 h to measure the fasting blood glucose, and when the blood glucose was higher than or equal to 16.7 mmol/L, the diabetic model was established successfully; Group A was given with the same dose of citrate buffer solution through intraperitoneal injection. Four groups of rats were fed routinely, while rats in Group C were given with 100 mg/kg resveratrol at 3:00 pm every day and rats in Group D were given with 20 mg/kg resveratrol at the same time.

2.4. Obtaining of the LEC

At 4 w, 8 w and 12 w 4 rats from Group A and 8 rats from Group B-D were randomly selected to observe the caspase-3 expression levels and apoptosis ratios of LEC. Cell preparation method was as follow: rats were anesthetized with 10% chloral hydrate and executed under aseptic condition, and then bilateral lens were removed to tear anterior lens capsule under a microscope for producing single cell suspension with 300 mesh.

2.5. Detection of the Caspase-3 expression level of LEC by Western blot

The single cell suspension was centrifuged for 5 min at a speed of 1 000 r/min, and cells were washed with PBS to obtain total protein after cell lysis. After SAS-PAGE electrophoresis, transfer film, sealing and cutting film, the quantified protein was respectively added with anti-rat caspase-3 polyclonal antibody and β-actin antibody and incubated at 4 ℎ overnight. After washing, horseradish peroxidase-marked secondary antibody was added, and after incubation for 1.5 h at room temperature, the film was washed before exposure and development by ECL kit.

2.6. Detection of apoptosis ratio of LEC by flow cytometry

The single cell suspension was centrifuged for 5 min at a speed of 1 000 r/min, and cell concentration was adjusted to 1×10^{9}/L after washing with PBS. Precooled 1×binding bugger, 5×10^{-6}/L Annexin-Ⅴ FITC solution and 10×10^{-6}/L PI solution were added into the cell suspension, which was placed avoiding light for 15 min at room temperature after mixing for detecting apoptosis ratio by flow cytometry.

2.7. Observation of degree of lens opacity

All rats accepted eye examinations at the end of the third week, and the appearance of opacity indicated the successful establishment of diabetic model. At the end of the twelfth week, after mydriasis with 0.5% atropine sulfate eye drops, the degrees of lens opacity of rats were observed under slit lamp. Evaluation criteria were as follows[7]: Grade Ⅰ, lens was transparent and clear, without opacity; grade Ⅱ, lens was mildly opacified and a small amount of vacuoles scattered in the surrounding; grade Ⅲ, lens was moderately opacified and vacuoles spread to center area, making part of lens nucleus cloudily opacified; grade Ⅳ, lens was highly opacified and vacuoles spread to the lens nucleus, making the cloudy opacity obviously; grade Ⅴ, lens nucleus was opacified, namely complete cataract.

2.8. Statistical analysis

All data was processed using SPSS15.0 data processing system. Measurement data are shown as (mean±sd) using *t* test; measurement data used *Chi*-square test, and ranked data with rank-sun test. P value<0.05 was considered as statistically significant difference.

3. Results

3.1. Caspase-3 expression level of LEC

At 4 w, 8 w and 12 w, the caspase-3 expression levels of rats in Group B-D were significantly higher than that in

Table 1
Apoptosis ratio of caspase-3 in LEC (%).

Time	Group A	Group B	Group C	Group D
4 w	4.82±0.13	13.12±1.78*	7.11±1.23*#※	10.25±1.36*#
8 w	4.91±0.22	16.55±2.01*	9.89±1.35*#※	12.88±1.75*#
12 w	5.03±0.35	19.32±2.54*	13.17±1.56*#※	15.94±2.03*#

*P<0.05 compared to Group A; #P<0.05 compared to Group B; ※P<0.05 compared to Group D.

Group A (P<0.05); the caspase-3 expression levels of rats in Group C and D were lower than Group B (P<0.05); the caspase-3 expression level in Group C was lower compared to Group D (P<0.05) (Figure 1).

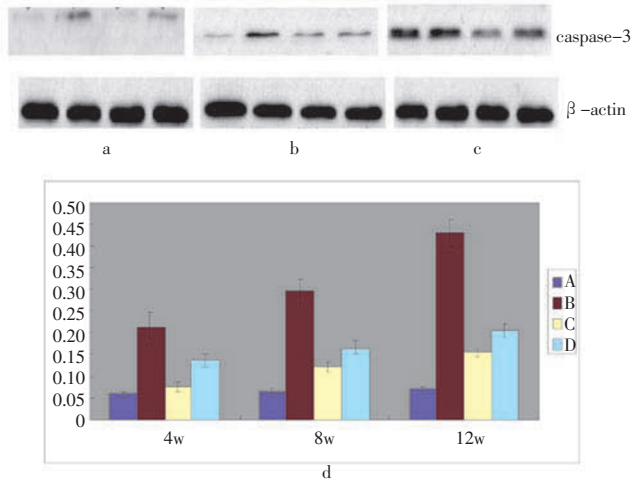

Figure 1. Expression level of caspase-3 in LEC.
a: 4w; b: 8w; c: 12w.

3.2. Apoptosis ratio of LEC

At 4 w, 8 w and 12 w, the cell apoptosis ratios of rats in Group B-D were significantly higher than that in Group A (P<0.05); the cell apoptosis ratios of rats in Group C and D were significantly lower than Group B (P<0.05); the cell apoptosis ratio in Group C was significantly lower than Group D (P<0.05) (Table 1).

3.3. Degree of lens opacity

After 12 weeks, the lenses of 4 rats in Group A were clarified; in Group B the degrees of lens opacity was as follow: 0 (0.00%) in grade Ⅰ, 3 (37.50%) in grade Ⅱ, 2 (25.00%) in grade Ⅲ, 2 (25.00%) grade Ⅳ, and 1 (12.50%) in grade Ⅴ; in Group C: 2 (25.00%) in grade Ⅰ, 4 (50.00%) in grade Ⅱ, 2 (25.00%) in grade Ⅲ, 0 (0.00%)grade Ⅳ, and 0 (0.00%) in grade Ⅴ; in Group D: 1 (12.50%)in grade Ⅰ, 4 (50.00%) in grade Ⅱ, 2 (25.00%) in grade Ⅲ, 1 (12.50%) grade Ⅳ, and 0 (0.00%) in grade Ⅴ. The difference among Group B-D was statistically significant (Z=5.112, P<0.05).

4. Discussion

With the improvement of living standard and the change of diet structure, the incidence of diabetes in China increases gradually, bringing great burden to our medical and health undertakings. The pathogenesis of DC, a complication of diabetes, is complex, in which lens LEC apoptosis plays an important role in the occurrence and development of the disease. In this study, we observed the protective effect of resveratrol on LEC apoptosis in DC rat.

In the study, we established the rat model of diabetic retinopathy by intraperitoneal injection of STZ, a DNA alkylation reagent with high cell toxicity on pancreatic islet β cell. STZ can induce diabetic models of a variety of animals, and was often used to establish the diabetic models of rats and mice[8,9]. In the study, 60 mg/kg STZ was given through intraperitoneal injection, leading to damage of islet cell and reduced insulin secretion. When all blood glucose of Group B-D were higher than or equal to 16.7 mmol/L, the diabetic models was established successfully. All rats accepted eye examinations at the end of the third week, and the appearance of opacity in Group B-D meaned the successful establishment of DC models. After 12 weeks, lenses were clarified in Group A and opacified in varying degree in Group B-D, and it showed grade Ⅱ or above in Group B; the degrees of lens opacity were lower significantly in Group C and D compared to Group B, while it was higher in Group C than in Group D. It is indicated that resveratrol can control the progress of cataract in diabetic rats effectively, and its effect is related to the dose. The expression and activation of caspase-3, a terminal shear enzyme, is closely related to apoptosis[10]; Annexin Ⅴ, a calclum dependent phospholipid binding protein, can be combined with the cell membrane of early apoptosis cell by phosphatidylserine exposed outside of the cell, so Vnnexin Ⅴ has higher sensitivity for detection of apoptosis[11]. Therefore, in this study, we detected the caspasse-3 expression level by western blot and the apoptosis ratio by flow cytometry as the indexes of apoptosis. After 4 w, 8 w and 12 w, the caspase-3 expression levels and apoptosis ratios of rats in Group B-D were significantly higher than

that in Group A; the caspase-3 expression levels and apoptosis ratios of rats in Group C and D were lower than Group B; the caspase-3 expression level and apoptosis ratio in Group C were lower compared to Group D. It was shown that resveratrol had protective effect on lens epithelial cell apoptosis in diabetic cataract rat, and the effect was relative to its dose.

It has been found that as a natural antioxidant, resveratrol has strong antioxidant activity in the cells and can activate the expression of *SIRT1* gene. The expression product of *SIRT1* gene is MAD+ dependent histone deacetylase, which can effectively enhance the activity of peroxlsome proliferator-activiated recepter-γ coactivator-1α, thereby playing a role in the body's energy metabolism and process of oxidation-reduction reaction[12,13]; at the same time, the product of *SIRT1* gene can also enhance the activity of FOXO to protect islet β cells[14]. Through the mechanism, resveratrol can effectively improve the blood glucose level in diabetic rat, thus contributing to the control of the occurrence and development of cataract. The apoptosis of LEC is a core link of DC, and the apoptosis ratios of rats in Group B-D were significantly higher than that in Group A, which indicated that high blood glucose can increase the apoptosis of LEC. It has been found that the expression of p53 protein and Bcl-2/Bax pathway were involved in the regulation of apoptosis process of LEC, while the resveratrol can effectively reduce the expression of p53 and Bcl-2, and increase the expression of Bax, which plays a role in protecting LEC[15,16]. How resveratrol participated in the regulation of apoptosis process of LEC is complex, and the specific mechanism need to be further studied.

Conflict of interest statement

We declare that we have no conflict of interest.

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