

Research Paper

Puerarin decreases lens epithelium cell apoptosis induced partly by peroxynitrite in diabetic rats

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Abstract: The present study was designed to observe if puerarin decreases lens epithelium cell (LEC) apoptosis induced partly by peroxynitrite (ONOO⁻). One hundred and eight rats were randomly divided into control group ($n=36$), streptozotocin (STZ) group ($n=36$) and STZ + puerarin group ($n=36$). The rats in the control group intraperitoneally (i.p.) received 0.5 ml of saline. The rats in STZ group and STZ + puerarin group received intraperitoneal injection of STZ (45 mg/kg). Three days later, the rats in STZ + puerarin group were given puerarin (140 mg/kg per day, i.p.). On days 20, 40 and 60 of the experiment, morphologic changes of lenses were observed with slit lamp. Then the animals were sacrificed for further analysis. The amount and percentage of apoptotic LECs were determined by flow cytometry. Nitrotyrosine (NT, the foot print of ONOO⁻) was examined by immunohistochemistry. Apoptosis-related genes (iNOS, etc.) were analyzed by gene array. The results showed that in the control group, all the lenses were clear. In STZ group, gradually severe opacity of the lens was observed on days 20, 40 and 60. But in STZ + puerarin group, mild opacity of the lens was observed on day 20 and more severe on day 40, but markedly decreased on day 60. In the control group, mild apoptosis of LECs was observed. In STZ group, time-dependent increase in apoptosis of LECs was observed. In STZ + puerarin group, mild apoptosis of LECs was observed on day 20, significantly increased on day 40, but markedly decreased on day 60. There was no expression of NT in the lens in the control group, but an increased expression of NT in STZ group. In STZ + puerarin group, mild expression of NT was observed on day 20, significantly increased on day 40, but markedly decreased on day 60. There was no expression of iNOS in the lens in the control group, but continuous up-regulation of iNOS expression in STZ group. In STZ + puerarin group, mild expression of iNOS was observed on day 20, significantly increased on day 40, but markedly decreased on day 60. Except the changes of iNOS related to NO production, the other apoptosis-related genes, including BCL-2 and SOD were down-regulated, while NF- κ B and TNFR1-FADD-caspase signal transduction way were up-regulated in STZ group. The results were opposite in STZ + puerarin group and the control group. These findings show that NT is expressed in diabetic rat lens, which proves that LEC apoptosis in diabetic lens is partly induced by ONOO⁻ which may be a new oxidative damage way to form cataract. Puerarin partly decreases LEC apoptosis induced by ONOO⁻ and is a potential medicine for therapy of diabetic cataract. The mechanism of puerarin dealing with diabetic cataract may be related to its direct inhibition of LEC apoptosis and antagonism of ONOO⁻ in diabetic rats.

Key words: puerarin; lens epithelium cell; diabetes mellitus; cataract

葛根素减轻部分由过氧亚硝基阴离子导致的糖尿病大鼠晶状体上皮细胞凋亡

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摘要: 本研究观察葛根素是否减轻部分由过氧亚硝基阴离子(peroxynitrite, ONOO⁻)导致的糖尿病大鼠晶状体上皮细胞(lens epithelium cell, LEC)凋亡。采用大鼠腹腔注射链脲佐菌素(streptozotocin, STZ)的方法建立糖尿病动物模型。36只大鼠作为对照组, 腹腔注射生理盐水; 其他72只大鼠腹腔注射STZ (45 mg/kg)后分为STZ组和STZ + 葛根素组, 每组36只。STZ注射3 d后, STZ + 葛根素组大鼠每天腹腔注射葛根素(140 mg/kg)。于实验开始后第20、40和60天用裂隙灯检查晶状体的形态学变化后处死动物。用流式细胞仪检测LEC凋亡, 用免疫组化方法检测晶状体中ONOO⁻的标志物——硝基酪氨酸

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(nitrotyrosine, NT)的表达,用基因芯片分析技术检测 LEC 凋亡相关基因 iNOS 等的表达。结果发现,对照组大鼠晶状体均透明,各项指标基本正常;STZ 组大鼠第 20 天时即出现晶状体混浊,40~60 d 期间混浊不断加重;STZ + 葛根素组大鼠 20~40 d 时晶状体混浊呈加重趋势,但 40~60 d 以后明显减轻。对照组 LEC 轻度凋亡,而 STZ 组凋亡细胞呈持续性增长,STZ + 葛根素组大鼠 20~40 d 时细胞凋亡呈增长趋势,但 40~60 d 以后明显下降。对照组大鼠晶状体 NT 未见明显表达;STZ 组大鼠 NT 表达明显加强;STZ + 葛根素组大鼠 20~40 d 时 NT 表达呈增长趋势,但 40~60 d 以后明显下降。对照组凋亡相关基因未见明显变化,STZ 组凋亡相关基因 iNOS 表达明显上调。其他凋亡相关基因如 BCL-2、SOD 表达明显下调,但 NF- κ B 和 TNFR1-FADD-caspase 信号转导途径明显上调;STZ + 葛根素组凋亡相关基因表达则呈相反改变。上述结果表明,在糖尿病大鼠晶状体中有 ONOO⁻ 的标志物 NT 表达,证明糖尿病大鼠 LEC 凋亡部分由 ONOO⁻ 诱导,这可能是氧化损伤导致白内障形成的新途径。葛根素能够部分逆转 ONOO⁻ 对 LEC 的致凋亡作用,提示葛根素可能是治疗糖尿病性白内障的有效药物,其治疗机制可能与葛根素直接抑制凋亡和对抗 ONOO⁻ 对糖尿病大鼠 LEC 的损伤有关。

关键词: 葛根素;晶状体上皮细胞;糖尿病;白内障

中图分类号: R683.42

Defects in diabetes mellitus result in clouding of the lens or cataract^[1,2]. Therefore, cataract is one of the most significant vision-impairing complications of diabetes. It has been reported that peroxynitrite (ONOO⁻), a reactive oxygen species (ROS), causes a dose-dependent toxicity in retinal pigmented epithelial cells. Cell toxicity is partially mediated by apoptosis as demonstrated by nuclear fragmentation and TdT-mediated dUTP nick-end labeling assay^[3]. ONOO⁻ exists in many areas of the eyes^[4,5]. Likely, ROS and oxidative stress are involved in the pathophysiology of ocular inflammation in the anterior segment of the eye and retina^[6-10]. Puerarin is an extract of natural Chinese medicine (Fig.1) and has been extensively used in the treatment of coronary disease, hypertension, diabetes, etc. It has also been reported that puerarin can decrease oxidative damage and inhibit cell apoptosis^[11]. On the basis of our previous works^[12-14] that lens was oxidized by ONOO⁻ and antagonized by puerarin, the present study was aimed to examine the feasibility of inhibiting cataract formation by puerarin, a chemical known to effectively scavenge ROS and inhibit protein glycation, both involved in the genesis of diabetic cataracts. We used flow cytometry, immunohistochemistry and gene array techniques to explore if puerarin de-

creases lens epithelium cell (LEC) apoptosis induced partly by ONOO⁻ in diabetic rats.

1 MATERIALS AND METHODS

1.1 Animals

Pathogen-free male Sprague-Dawley (SD) rats (5~6 weeks old) were used. All animals were treated in accordance with ARVO Statement for the use of Animals in Ophthalmic and Vision Research in China.

1.2 Antibodies and reagents

Monoclonal mouse anti-nitrotyrosine (NT) antibody, goat anti-mouse fluorescein isothiocyanate (FITC) antibody and streptozotocin (STZ) were purchased from Sigma (St. Louis, MO, USA). Array slides were obtained from Qiagen (Valencia, CA, USA). Puerarin was the product of Kangenbei Pharmaceutical Limited Corporation, China.

1.3 Groups and animal model

Animals were divided into three groups: control group, STZ group and STZ + puerarin group, each containing 36 animals. SD rats in STZ and STZ + puerarin groups were intraperitoneally (i.p.) injected with STZ (45 mg/kg) to establish the diabetic animal model. The rats in the control group received the same amount of saline. Three days after starting of the experiment, the rats in STZ + puerarin group received puerarin 140 mg/kg per day. Animals were examined by slit lamp on days 20, 40 and 60 after STZ injection for clinical signs of cataract. The opacities of the lens were divided into six stages. 0: clear of the lens; I: some peripheral opacity cortex of the lens was present with bubbles; II: peripheral opacity cortex of the lens was present with concentrating bubbles; III: part of cortex was flaked and cloudy besides above; IV: nuclear and peripheral cortex of the lens were spotted in the turbid area besides above;

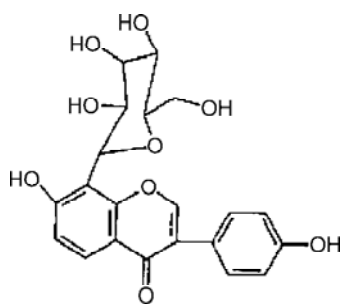


Fig.1. Structure of puerarin.

V: turbid area in nuclear and peripheral cortex of the lens had severely degenerated and some had developed whole cataracts. The lenses were then enucleated for further study.

1.4 Flow cytometry

Twelve rats (totally 36 rats for days 20, 40 and 60) were sacrificed and 24 eyes (totally 72 eyes for days 20, 40 and 60) were enucleated immediately on days 20, 40 and 60 respectively. The eyeballs were dissected via posterior incision under a dissecting microscope and the lenses were taken out and fixed with 70% ethanol for 24 h to get LEC, then the apoptotic cell number and percentage in rat LEC were examined. Cells were washed 3 times in phosphate-buffered saline (PBS) and re-suspended in PBS at 2×10^7 /ml. After staining with primary and secondary antibodies, cells were analyzed by FACScan. The optical system was 2 W laser producer with an output of 300 mW and exciting wavelength at 488 nm, emitting wavelength at 605 nm. The data were input into an HP-300 Cosort 30 model computer and processed with related software. The DNA content distribution square pattern and dual parameter dimension pattern were obtained in this way. The cell number was calculated with cell circle analyzing sequence. Chicken blood erythrocyte was taken as a standard sample to adjust the instrument coefficient (CV) <5.0%.

1.5 Immunohistochemistry

Seventy-two samples on days 20, 40 and 60 were fixed with 10% buffer formalin, embedded with paraffin and cut into slices of 5 μ m. After washed with PBS, the slides were incubated with hydrogen peroxide (peroxidase blocking reagent; Daco, Carpinteria, CA) to block endogenous peroxidase activity, then with 10% goat serum for 30 min at room temperature to block non-specific antigen. After rinsed and washed in PBS the block slides were incubated with anti-NT antibody (1:200 dilution), then in goat biotinylated anti-rat IgG (LSAB2 System; Dako, as a secondary antibody). After washed with PBS, the slides were incubated in streptavidin conjugated with horseradish peroxidase. The color was developed with streptavidin and biotin chromogen (Liquid DAB + Substrate-Chromogen System; Dako).

1.6 Gene arrays

Seventy-two lenses used in arrays were dissected free of any contaminating tissue and homogenized in TRIzol reagent. RNA extraction was carried out according to the manufacturer's protocol. The concentration and quality of RNA were assessed via spectrophotometry and formaldehyde gel electrophoresis. Amplified mRNA was then

labeled with Cy3 or Cy5 (Random Primer DNA Labeling Kit). The control and experimental targets were successfully labeled with 12 housekeeping genes and 12 artificially synthesized 70 mer oligo DNA which served as positive and negative controls were then combined and prepared for hybridization. Array slides were incubated for pre-hybridization for 1 h at 42 °C. Targets were dried via vacuum centrifugation and then re-suspended in 50 μ l hybridization solution with 1 μ l Cot1 DNA and 1 μ l polyA oligonucleotide as blocking agents, heated to 95 °C for 5 min and then added to the face of one slide. The printed face of the second slide of the pair was then placed face to face with the first, using the same probe. Slide pairs were then placed on a level plastic cover on $1 \times$ SSC moistened tissue in a slide box, which was sealed and floated in a water bath, hybridized for 24~48 h at 42 °C. Following hybridization, slides were washed for 20 min and repeated for another 20 min and then dipped in nuclease-free water and sprayed dry. Finally, the backs of the slides were cleaned with ddH₂O, wiped with 100% ethanol, then wiped dry and scanned by Scan Array Express Scanner (Packard Bioscience Corporation).

1.7 RT-PCR array confirmation

RT-PCR was performed using 2 μ g of total RNA for the first-strand synthesis followed by amplification in the presence of specific primers for iNOS: 5'-CGCCCTTCCGCA-GTTCT-3' and 5'-TCCAGGAGGACATGCAGCAC-3'; for β -actin: 5'-GAGACCTTCAACACCCAGCC-3' and 5'-GCGGGGCATCGGAACCGCTCA-3'. The amplification consisted of 29 cycles of denaturation for 1 min at 94 °C, annealing for 1 min at 60 °C, and extension for 1 min at 72 °C.

1.8 Statistical analyses

Statistical analyses of the data were performed on computer (SPSS 10.0). The results were expressed as mean \pm SD. Statistical significance was determined by one-factor analysis of variance (ANOVA) followed by the Fisher *post hoc* test for multiple comparisons. $P < 0.05$ was considered significant. GenePix Pro 4.0 software (Axon Instruments Corporation) was used for clustering analysis. Divergence higher than two folds was regarded as divergence expression of genes.

2 RESULTS

2.1 Animal model

Comparing with that in the control and STZ + puerarin groups, there were typical diabetic symptoms in the rats in STZ group. There were also remarkable differences in glu-

Table 1. Effects of puerarin on blood glucose (mmol/L) and body weight (g) in diabetic rat cataract

Day	Control group		STZ group		STZ + puerarin group	
	Blood glucose	Body weight	Blood glucose	Body weight	Blood glucose	Body weight
0	3.37±0.17	318.06±2.04	3.39±0.21	319.23±2.12	3.31±0.19	321.06±2.08
3	3.47±0.11	319.09±2.12	21.74±0.10**	271.08±1.34**	21.98±0.16**	226.08±1.44**
20	3.57±0.07	321.16±2.14	20.98±0.04**†	226.08±1.44**†	10.79±0.07*	311.27±2.88*
40	3.56±0.05	381.23±2.29	22.32±0.03**†	191.27±2.61**†	9.79±0.08*	367.33±2.65*
60	3.49±0.06	456.32±2.19	23.59±0.06**†	151.25±2.29**†	7.63±0.07*	459.77±2.33*

* $P<0.05$, ** $P<0.001$ vs the control group; † $P<0.01$ vs STZ + puerarin group. mean±SD, $n=12$.

glucose concentration and body weight among the three groups on days 20, 40 and 60 (Table 1).

The lenses of rats were examined with a slit lamp (Table 2, Fig.2). Lenses were clear in the control group during the whole experiment period. On day 20, lenses developed opacity in STZ group (10 lenses in grade 2 accounting for 83%, and 2 lens in grade 3 accounting for 17%) but clear in STZ + puerarin group. On day 40, continuous opacities occurred in STZ group (9 lenses in grade 3 accounting for 75%, and 3 lenses in grade 4 accounting for 25%), while lenses also developed opacity in STZ + puerarin group (8 lenses in grade 1 accounting for 66%, and 4 lenses in grade 2 accounting for 34%). On day 60, severe opacities could be seen in STZ group (3 lenses in grade 3 accounting for 25%, and 9 lenses in grade 4 accounting for 75%). While opacities in STZ + puerarin group were decreased (8 lenses in grade 1 accounting for 66%, and 4 lenses in grade 0 accounting for 34%).

Table 2. Comparison of lens opacities (gray value) among three groups

Day	Control	STZ	STZ + puerarin
0	139.51±2.13	138.98±2.10	138.76±2.16
20	140.46±2.11	189.46±2.11**†	179.46±2.18*
40	139.14±2.17	248.78±2.16**†	208.14±2.17*
60	139.66±2.19	299.03±2.14**†	187.56±2.09*

* $P<0.05$, ** $P<0.001$ vs the control group; † $P<0.01$ vs STZ + puerarin group. mean±SD, $n=12$.

2.2 Flow cytometry

There were rare apoptotic cells in the control group. As time passed, apoptotic cells continued to be increased in STZ group. On day 20, there were mildly apoptotic cells in STZ + puerarin group, but apparently increased on day 40. While on day 60, apoptotic cells were decreased. There were distinct differences among the three groups ($P<0.05$, Table 3).

2.3 Immunohistochemistry

Immunohistochemical staining revealed that the specific expression of NT was yellow, brown-yellow or brown staining in the cell nucleus and cytoplasm. In the control group, very faint yellow could be observed. At different times in STZ group, staining ranged from yellow to brown-yellow, then to brown in the cell nucleus and cytoplasm. On day 20, there was mild staining in STZ + puerarin group, and the staining gradually increased on day 40 but decreased on day 60. With computer photo-analysis, there were significant differences at different stages of the experiment between the control, STZ and STZ + puerarin groups ($P<0.001$, Table 4, Fig.3).

2.4 Gene arrays

Cy3 and Cy5 fluorescent intensity values were taken as standard for the X and Y-axis. Every data represented each hybridization signal in the microarray. If the scatter spot appeared in red it indicated that the ratio between X and Y was 0.5~2.0, meaning no difference in the expression. If the scatter spot appeared in yellow, it indicated that the ratio between X and Y was beyond 0.5 or 2.0, probably meaning difference in the expression of NT.

Taking advantage of gene array technique, we observed that there was no expression of iNOS in the control group, but there was continuous up-regulation of iNOS and nNOS genes in STZ group compared with that in the control and STZ + puerarin groups. There was minor up-regulation of iNOS (NOS2) gene with 2.0-fold increase and nNOS (NOS1) gene with 1.6-fold increase on day 20 in STZ + puerarin group and continued to 5.0-fold up-regulation of iNOS gene and 3.5-fold of nNOS gene on day 40, but decreased to 2.2-fold of iNOS gene and 2.0-fold of nNOS gene on day 60. Except the changes of iNOS related to NO production, the other apoptosis-related genes including BCL-2, SOD were down-regulated and NF- κ B as well as tumor necrosis factor receptor, I type TNF receptor (TNFR1)-Fas associating protein with death domain

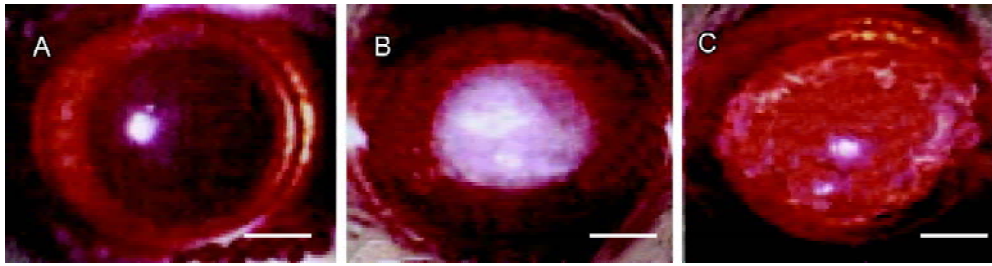


Fig.2. Examination of lens opacity with slit lamp. A: Clear lens was present in the control group. B: Severe opacity of the lens was present on day 60 in STZ group. C: Mild opacity of the lens was present on day 60 in STZ + puerarin group. Scale bar, 2 mm.

Table 3. Number and percentage of apoptotic cell at different times

Day	Control		STZ		STZ + puerarin	
	Number	Percentage (%)	Number	Percentage (%)	Number	Percentage (%)
20	474±156	2.21±0.79	1045±145 ^{***†}	13.15±0.67 ^{***†}	536±156 [*]	3.91±0.64 [*]
40	476±154	2.23±0.89	1879±139 ^{***†}	17.98±0.479 ^{***†}	995±185 ^{**}	8.01±0.57 ^{**}
60	475±155	2.22±0.79	2667±103 ^{***††}	20.95±0.84 ^{***††}	617±179 [*]	4.58±0.44 [*]

* $P < 0.05$, ** $P < 0.001$ vs the control group; † $P < 0.05$, †† $P < 0.001$ vs STZ + puerarin group. mean±SD, $n=12$.

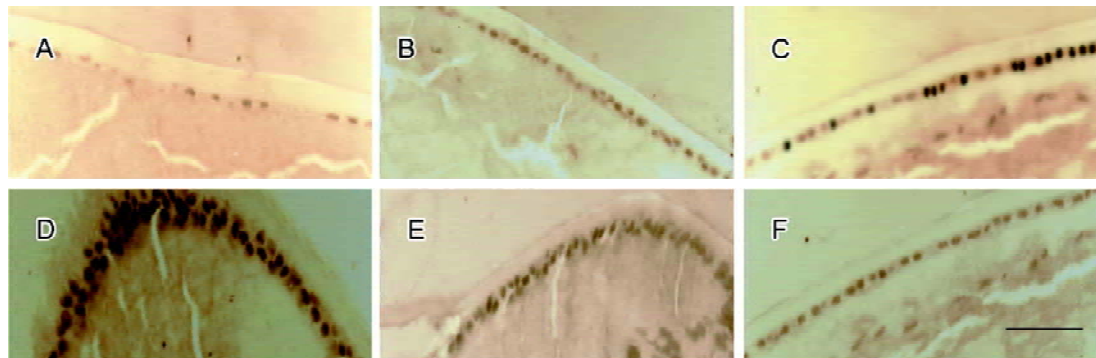


Fig.3. Expression of NT in LEC was detected with immunohistochemical staining technique. A: Faint NT expression in the control group. B, C, D: Gradual increase of NT expression could be observed on days 20, 40 and 60 in STZ group. E, F: Gradual decrease of NT expression could be observed on days 40 and 60 in STZ + puerarin group. Scale bar, 100 μm .

Table 4. Comparison of expression of NT (gray value) among three groups

Day	Control	STZ	STZ + puerarin
20	32.33±2.35	74.44±3.00 ^{***†}	72.78±2.64 [*]
40	34.33±1.73	77.22±2.44 ^{***†}	145.00±3.94 ^{**}
60	33.44±1.94	235.78±5.97 ^{***††}	74.44±3.00 [*]

* $P < 0.05$, ** $P < 0.001$ vs the control group; † $P < 0.05$, †† $P < 0.001$ vs STZ + puerarin group. mean±SD, $n=12$.

(FADD)-caspase signal transduction way were up-regulated in STZ group. There were opposite results in STZ + puerarin group (Fig.4).

2.5 RT-PCR confirmation

There was no expression of iNOS mRNA in the control group, but there was distinct up-regulation of iNOS mRNA in STZ group as time passed. Expression of iNOS mRNA in STZ + puerarin group gradually up-regulated from day 20 to 40, then down-regulated on day 60 (Fig.5). With computer photo-analysis, there were significant differences among the three groups ($P < 0.001$, Fig.6).

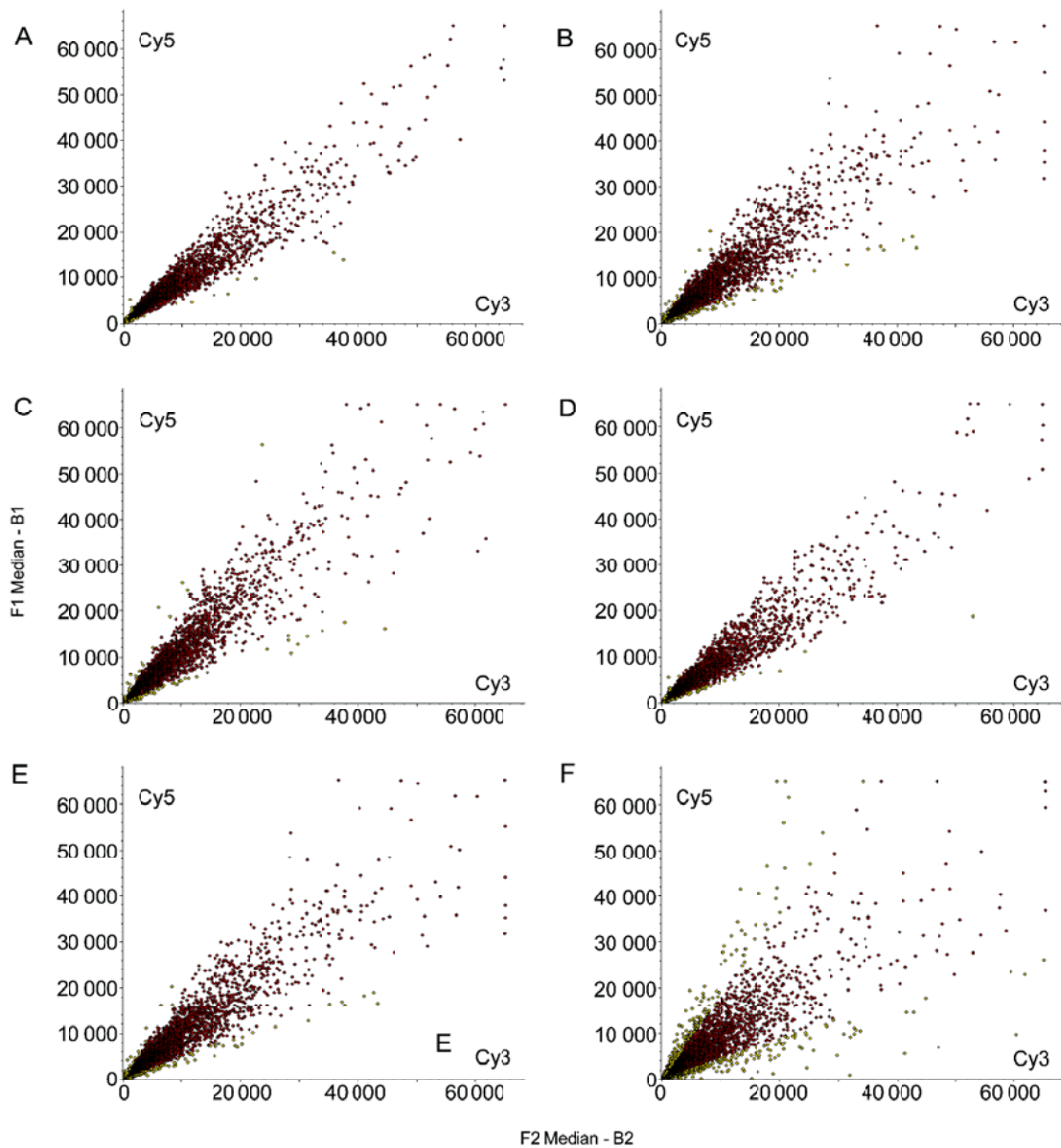


Fig. 4. Gene array in STZ + puerarin and STZ groups. The red spots indicate the ratio between 0.5 and 2.0, meaning no difference; the yellow spots indicate the ratio beyond 0.5 or 2.0, meaning difference. A, B, C: Decrease of apoptosis was shown in STZ + puerarin group on days 20, 40 and 60, respectively. D, E, F: Increase of apoptosis was shown in STZ group on days 20, 40 and 60, respectively.

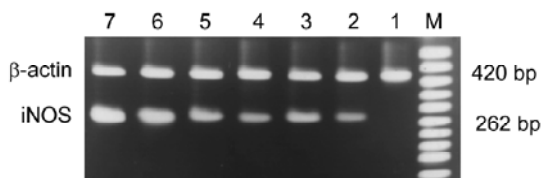


Fig.5. RT-PCR confirmation of iNOS mRNA expression in the lens of diabetic cataract. M, DNA marker; 1, control; 2~4, STZ + puerarin group on days 20, 40 and 60, respectively. 5~7, STZ group on days 20, 40 and 60, respectively.

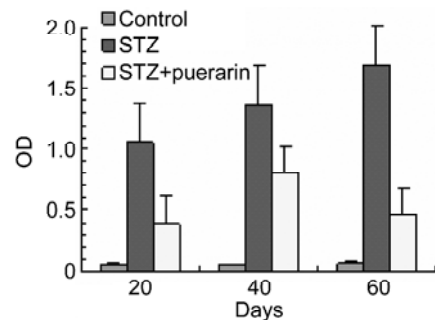


Fig.6. RT-PCR confirmation of iNOS mRNA expression in the lens of diabetic cataract. There were significant differences among the three groups ($P < 0.001$).

3 DISCUSSION

In this study, we investigated the role of puerarin in the apoptosis of LEC in diabetic cataract. Apoptosis is thought to be a critical determiner for the normal condition of the lens^[15,16]. Apoptotic cells continued to be increased in STZ group. In STZ + puerarin group, apoptotic cells were increased from day 20 to 40, but decreased from day 40 to 60, which may indicate a protective role of puerarin on LEC. These results are consistent with our previous work^[12-14].

The traditional oxygen-free radical stress mechanism pays more attention to the role of hydrogen-peroxide (H_2O_2), nitric-oxide (NO) and superoxide-anion (O_2^-), while the new theory includes ONOO⁻, a product from rapid reaction of NO and O_2^- , which may be an important mediator of cytotoxicity in oxidation^[17,18]. It is also highly reactive and interacts with cellular constituents inflicting damage on cells^[19]. Our study supports the new theory. We observed that the ONOO⁻-mediated protein nitration product, NT, was located in LEC and decreased under the intervention of puerarin.

We found that expression of NT greatly increased in LEC of diabetic rats, which indicated that high glucose could induce the production of NT. Due to high blood glucose, there was no enzyme-glucose reaction took place in blood plasma and tissue protein. Therefore the structure and role of protein changed and hemoglobin-associated-crystals-glucose (HbA1C) was developed. Increase of HbA1C enhanced the combination of hemoglobin and oxygen and decreased their separation, leading to the lack of oxygen in plasma and tissue but increase of free radical, which is one of main complications causing chronic diabetic. On one hand, puerarin alleviated the oxidation of LEC and other tissues induced by ONOO⁻ in diabetic rat, which would decrease the production of HbA1C and free radical. On the other hand, puerarin could inhibit the expression of iNOS, therefore decreased the formation of ONOO⁻.

It is likely that iNOS may contribute to oxidation stress by helping develop more powerful oxidative agents such as ONOO⁻^[20]. Karabiyikoglu *et al.*^[21] examined the influence of mild hypothermia on early expression of NOS isoforms and ONOO⁻ generation after experimental stroke. With gene array analysis, we found that iNOS divergence genes appeared in six microarray slides (NOS2, 10400U12611). They were up-regulated in STZ group and down-regulated in STZ + puerarin group. These results verified our previous work again^[12-14]. Meanwhile, nNOS (NOS1, 10573U67309) showed the same results as iNOS.

nNOS is expressed under normal physiological conditions and mainly locates in the brain and its activation depends on Ca^{2+} and calmodine (CaM). Many studies have shown that iNOS affects and regulates apoptosis^[22,23]. Therefore, the relationship between iNOS and nNOS still needs to be studied and clarified.

Cell apoptosis is the result of cascade gene expression. Up to date, more genes contribute to production and regulation of cell apoptosis. It is believed that genes in the inner layer of cell directly regulate the production and development of apoptosis, while related elements in the outer layer of cell affect the expression of genes through signal transduction way^[24]. At the same time, we also observed the expression of some apoptosis-related genes and signal transduction ways in this study.

BCL-2 gene (Bnip31, 827NM-080888), BCL-2/adenovirus E1B 19 kDa-interacting protein 3 and nuclear gene for mitochondrial product gene (Bnip3, 2060AF243515) inhibited LEC apoptosis. BCL-2 was down-regulated in STZ group but up-regulated in STZ + puerarin group. BCL-2 exists in the outer membrane of the mitochondria and overexpression of BCL-2 can inhibit apoptosis. BCL-2 blocks damage to mitochondria resulting in release of cytochrome C, thus unable to promote apoptosis^[25]. But the one can promote apoptosis in BCL-2 family is Bax (Bax, NM-017059, Bcl-2 associated X protein) subclass and its overexpression can induce cell apoptosis. Bax and BCL-2 are highly the same originated. Bax can react with BCL-2 to form different origin double form so as to inhibit activity of BCL-2 and lead to apoptosis. We infer that puerarin can strengthen the anti-apoptosis role of BCL-2 and therefore inhibits activity of Bax and alleviates the apoptosis of LEC.

NF- κ B (NFKB1, L26267) was up-regulated in STZ group. Our research verified^[26-29] that NF- κ B can enhance apoptosis in LEC by activating A1/BFI-1, which belongs to BCL-2 family. We observed that NF- κ B induced apoptosis in LEC but showed strong and weak up-regulation in STZ and STZ + puerarin groups. Puerarin decreased the apoptotic effect of NF- κ B with its anti-oxidative ability. Lacking of puerarin's anti-oxidative ability, the strong apoptotic effect of NF- κ B was shown in STZ group. The activation of NF- κ B could also induce the iNOS transcription^[30]. That is probably the reason why ONOO⁻ was expressed in both two groups.

It was also found that SOD was the target gene of NF- κ B^[31]. As we all know, SOD induced by NF- κ B can clear off O_2^- and express specific cell protection. Otherwise, SOD1 and 3 (SOD1, NM-017050, soluble; SOD3, NM-012880) in macroarray up-regulated in STZ + puerarin

group and down-regulated in STZ group showed the facts that NF- κ B induced the production of SOD.

We also observed that the less of TNFR1-FADD-caspase-8 signal transduction way in microarray slides. TNFR1 (NM-013091) is the member of TNF receptor. It combines with FADD (NM-152937) first, then reacts caspase-8 (NM-022277) and activates the latter. FADD and caspase-8 contain death effector domain (DED) and react with each other through DED-DED. The activation of the caspase-8 starts caspase family enzyme cascade, leading to the cell apoptosis through executing death proteinase caspase-3,6,7. Compared with our results, TNFR1-FADD-caspase-8 was down-regulated in STZ + puerarin group but up-regulated in STZ group. These facts indicated puerarin delayed apoptosis in LEC.

In conclusion, our findings demonstrate that experimentally induced diabetic cataract is mediated by many apoptotic factors. Like other medicine^[32], puerarin regulates the expression of anti-apoptotic genes, which inhibit the production of iNOS and production of ONOO⁻, resulting in increase of LEC survival and decrease of LEC apoptosis.

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