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Expression and significance of HIF−1 α and VEGF in rats with diabetic retinopathy

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ABSTRACT

Objective: To investigate the expression of hypoxia inducible factor−1 α (HIF−1 α) and vascular endothelial growth factor (VEGF) in diabetic retinopathy (DR) rats and its effect on the DR occurrence and development. **Methods:** A total of 120 SD rats were randomly divided into trial group and control group with 60 in each. STZ *i.p.* was used in the trial group to establish the DM model, citrate buffer salt of same amount was used *i.p.* to the control group. 1, 3 and 6 months after injection, respective 20 rats were sacrificed in each group to observe expression of HIF−1 α and VEGF in the rat retina tissue at different time points. **Results:** Expression of HIF−1 α and VEGF were negative in the control group; expression of HIF−1 α and VEGF protein in retinal tissue were weak after 1 month of DR mold formation. It showed progressive enhancement along with the progression in different organizations, differences between groups were significant (*P*<0.05). **Conclusions:** Expressions of HIF−1 α and VEGF were correlated with disease progression in early diabetic retinopathy. Retinal oxygen can induce over−expression of HIF−1 α and VEGF. It shows that HIF−1 α and VEGF play an important role in the pathogenesis of DR.

1. Introduction

Diabetes is a clinical common disese. Lifestyle changes and the aging process induce a rising trend of diabetes year by year, which has become a global public health problem[1−3]. Diabetic retinopathy (DR) is a serious complication of diabetes. Its pathogenesis is not fully clear. It is said that DR is the result of many factors, and can lead permanent vision loss. The important step is the hyperglycemia, which causes the pathological change of hypoxia[4]. It have been reported that hypoxia inducible factor−1 α (HIF−1 α) and vascular endothelial growth factor (VEGF) play an important role in the pathogenesis of DR[5−7]. This study aimed to observe the expression of HIF−1 α and VEGF in DR induced by STZ. We selected 120 SD rats to set up DM model, then observed pathological change of lens and retina respectively in 1, 3 and 6 months after injection. The results reported as follows.

2. Materials and methods

2.1. Experimental animals

A total of 120 male and clean SD rats were selected, which were purchased from Laboratory Animal Center, Tianjin Medical University. They weighted (241.3±16.5) g, and were fed free with food and water. The experimental process strictly followed "Regulations of Experimental Animals".

2.2. Reagent and instrument

Rabbit anti rat HIF−1 α antibodies, rabbit anti mouse VEGF antibodies and STZ (Sigma company, USA); UltraSensitive SP allergic kit (Fuzhou Wallace New Biological Company); RNA extraction kit and Trizol Reagent,

Taq DNA polymerase, dNTPs, (Fermentas company, USA); PCR Marker of New England [New England biological technology (Beijing) co., LTD.] production. PCR primers (Invitrogen company, USA); Optical microscope BH–2 OLMPUS; Uv spectral analyzer (DU–800) (American beckman coulter co., LTD.); GeneAmp type 9700 PCR amplification (Applied Biosystems, USA); Gel imaging analysis system for Gel DOC2000 (Bio–Rad company, USA); One Touch Ⅱ J&J blood glucose meter (Shanghai).

2.3. Method

A total of 120 SD rats were randomly divided into trial group and control group with 60 in each. STZ (pH 4.4, 1%) *i.p.* (60 mg/kg) was used in the trial group to establish the DM model, after starving for 10 h. Citrate buffer salt of same amount was used i.p to the control group, success criteria of DM mold is tail blood $\geqslant$16.7 mmol/L 72 h. 1, 3 and 6 months after injection. A total of 20 rats were sacrificed in each group to exact the eyes. The eyeball wall was 0.5 mm behind the blade side in left eye, followed by separation of the retina, formalin fixing, conventional embedding and sectioning. Blunt dissection detachment was performed in the right eye retina at −80 ℃ liquid nitrogen for further total RNA extraction. The expression of HIF–1 α and VEGF in the rat retina tissue were observed using immunohistochemistry, strictly following the operation instruction. Slices were dewaxed conventionally, hydrated followed by washed in PBS for 3 min. After heating repair, it was washed again for 5 min. Each section was added with 1 drop peroxidase blockers, washed with PBS for 3 min; 1 drop of goat serum blocking buffer was added, and it was placed for 20 min at room temperature. Excess serum was removed, and added with 1:100 dilution of HIF–1 α and VEG at 37 ℃ for 1 h. It was added with 1 drop of biotin labeled antibody after PBS washing; then was added with 1 drop of Streptomyces, anti– biotin peroxidase solution at room temperature for 10 min. 2 drops of DAB solution was added at room temperature for 3 to 10 min for the microscopic observation.

2.4. Criteria

Four splices from each specimen was selected, and five vision areas were randomly taken. At least 100 cells was calculated. Positive cells positive expression rate was evaluated, the ratio less than 5% was expressed as negative. HIF–1 α mainly is located in nucleus as tan particles, VEGF is mainly expressed in the cytoplasm as tan particles.

2.5. Statistical analysis

SPSS12.0 statistics software was used for processing data, and they were expressed as ($\bar{x} \pm s$). It was analyzed by *t* test. $P < 0.05$ was considered as statistically significant difference.

3. Results

3.1. Expression of HIF–1 α and VEGF in different time points

Expression of HIF–1 α and VEGF were negative in the control group; expression of HIF–1 α and VEGF protein in retinal tissue were weak after 1 month of DR mold formation in observation group, and it showed a progressive enhancement along with the progression in different parts. Differences between groups were significant ($P<0.05$), the results are shown in Table 1.

3.2. Absorbance ratio of HIF–1 α mRNA and VEGF mRNA at different time points

Absorbance ratio of HIF–1 α and VEGF were negative or weak in the control group; absorbance ratio of HIF–1 α and VEGF protein in retinal tissue in observation group showed

Table 1
Expression of HIF–1 α and VEGF in different time points.

Groups	1 month		2 months		3 months	
	HIF–1 α	VEGF	HIF–1 α	VEGF	HIF–1 α	VEGF
Observation group	10.49±2.12[*]	9.71±1.29*	35.66±3.3*	40.78±4.11[*]	88.73±6.22[*]	92.87±5.12[*]
Control group	2.61±0.43	2.32±0.58	2.09±0.53	1.82±0.59	2.12±0.45	1.78±0.63

Note: * compared with controls, *P*< 0.05.

Table 2
Absorbance ratio of HIF–1 α mRNA and VEGF mRNA at different time points.

Groups	1 month		2 months		3 months	
	HIF–1 α	VEGF	HIF–1 α	VEGF	HIF–1 α	VEGF
Observation group	0.46±0.19[*]	0.49±0.17[*]	0.55±0.19[*]	0.59±0.21[*]	0.77±0.23[*]	0.91±0.19*
Control group	0.75±0.05	0.35±0.01	0.69±0.15	0.41±0.10	0.64±0.06	0.36±0.01

Note: * compared with controls, *P*< 0.05.

a progressive enhancement with significant differences between groups (*P*<0.05), the results are shown in Table 2.

3.3. Immunohistochemical results of rats retinal tissue between two groups

There were no positive expression of HIF−1 α and VEGF in the control group, expression of HIF−1 α and VEGF in retinal tissue in observation group showed a progressive enhancement with significant differences between groups (*P*<0.05), the results are shown in Figure 1.
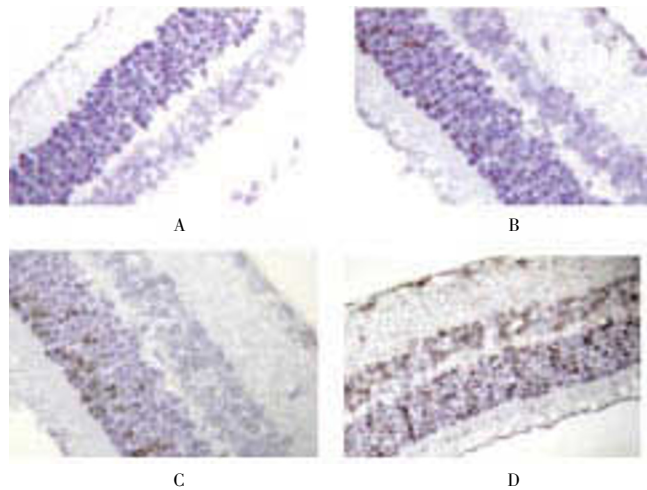
Figure 1. Immunohistochemical results of rats retinal tissue between two groups.
A: Control group, B: Observation group (1 month), C: Observation group (3 months), D: Observation group (6 months).

4. Discussion

DR is a serious fundus complications of diabetes during late phase. Its pathogenesis basis is hyperglycemia, which can induce the capillary basement membrane thicken, chronic ischemic retinal tissue hypoxia[8–13], lextracellular matrix and basement membrane dissolved, fade, and cell migration, proliferation and lead to new retinal angiogenesis[14–16].

Studies have found that, in the process of starting and development of diabetic retinopathy, HIF−1 α and VEGF play an important role in different phases[17–21]. HIF−1 α is the key factor of regulating metabolism of oxygen in cells, which has stable effect on cell hypoxia[22–25]. Hypoxia can trigger the cells signaled by receptor molecules on the cell membrane. It s many changes in gene expression in the cell, inducing complex stress responses to hypoxia, such as coding VEGF, erythropoietin, tyrosine hydroxylase and enzyme gene. They were involved in glycolytic activity during hypoxia, in order to make the body to adapt to the body's internal environment. This study found that in the

control group rats, in normal retina tissue, HIF−1 α is not expressed. HIF−1 α was expressed clearly in observation group rats. It shows that oxygen deficit plays an important role in the process of the onset of DR. Expression of HIF−1 α is enhanced with the progression of DR. It shows that hypoxia develops along with the progression of DR. VEGF is the most powerful angiogenic factor, which can induce formation of retinal neovascularization. Its expression is regulated by the multiple factors, hypoxia is the most powerful inducing factors[26–29]. In this study, the expression of VEGF in rat retina is obvious in the observation group. It is enhanced with the course development[30,31]. It shows that VEGF plays an important role in the pathogenesis of DR. The ischemic retinal HIF−1 α showed that its expression increased temporarily and space−specific with expression increment of VEGF. So this result match the assumption that HIF−1 α will play an important role in the increment of VEGF expression[32–34].

This study results showed that expression of HIF−1 α and VEGF was positively correlated with DR severity. HIF−1 α and VEGF play an important role in the process of in DR induction and vascularization. Moderate adjustment expression of HIF−1 α and VEGF has positive significance to the prevention and treatment of DR.

Conflict of interest statement

We declare that we have no conflict of interest.

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