·Basic Research·

HIF-1α expression and retinal cell apoptosis in rat retina ischemia-reperfusion injury

Wei Zhang, Ning-Ning Liu, Jian-Hua Xu, Zhe-Li Liu

Department of Ophthalmology, the First Affiliated Hospital of China Medical University, Shenyang 110001, Liaoning Province, China

Correspondence to: Zhe-Li Liu. Department of Ophthalmology, the First Affiliated Hospital of China Medical University, Shenyang 110001, Liaoning Province, China. zheli_liu @yahoo.com.cn

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Abstract

• AIM: To investigate the expression of hyporia inducing factor-1α (HIF-1α), apoptosis of retinal cells and the role of HIF-1α in apoptosis in rats' retinal ischemia-reperfusion injury.

• METHODS: The rat model of experimental retinal ischemia-reperfusion injury was established by increasing the intraocular pressure to 110mmHg (1kPa =7.5mmHg) in rat eyes. At different time points of post ischemia, the expression of HIF-1α of the retina was detected by immunohistochemical staining, and the apoptosis of retinal cell was detected by terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphatebiotin nick end labeling (TUNEL).

• RESULTS: HIF-1α appeared in the cells of retinal ganglion layer and inner nuclear layer at 2 hours after ischemia .The expression reached to a peak, 12 hours after retinal ischemia-reperfusion, then, the expression was declined. The apoptotic cells were mainly in inner nuclear layer and could be detected at the 12th, 24th and 48th hour after ischemia, the peak value was the group of 24th hour.

• CONCLUSION: The expression of HIF-1α in rats' retina is greatly enhanced after ischemia-reperfusion, which may be involved in the retinal injury; the injury of retinal neurons occurs partly in the form of apoptosis. The expression of HIF-1α may play an important role in cell apoptosis.

• KEYWORDS: ischemia-reperfusion; retina; HIF-1α ; apoptosis

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INTRODUCTION

Ischemia-reperfusion injury in retina can initiate a series of pathological changes and serious consequences. It is a common situation in ophthalmology. The pathogenesis and therapy method attract a great interest domestically and abroad. Recently emerging data have been obtained from studies focused on hyporia inducing factor-1α (HIF-1α). Apoptosis is a programmed cell death. Studies show a close relationship between ischemia-reperfusion injury and apoptosis in the retina [1]. The present study is aimed at detecting the role of HIF-1α in ischemia-reperfusion injury in rat retina, the presence of cell apoptosis in retina and the relationship between them, which will further establish a new clue for the management of glaucoma and other retinal ischemia diseases.

MATERIALS AND METHODS

Animal Model Fifty-four healthy adult Wistar rats (sex is not limited; weighting 170-220g, offered by experimental animal department of China Medical University) were randomly divided into 3 groups. Group A: normal control group (n=6); Group B: ischemia-reperfusion group (n=42), the anterior chamber was punctured in right eye of each rat, these rats were further divided into 7 sub-groups according to the different time point of reperfusion (0, 2, 6, 8, 12, 24, 48 hours), each sub-group had 6 rats; Group C: experimental control group (n=6), 6 rats were randomly selected to perform the puncture of anterior chamber in left eyes as sham procedure controls. To make the model of ischemia-reperfusion injury in retinal of rats the animals were anesthetized by peritoneal injection 200mL/L chloral hydrate. In addition, Decicaine was used for superficial anesthesia. Rats' heads were fixed on the table in order to perform the puncture of anterior chamber. 4 1/2 injection needle was entered through anterior chamber from temple limbus of cornea. The distal end of the pinhead was connected with Saline bottle which of height was adjustable. When the Saline bottle was elevated at 150cm above the eye of rats[2], the intraocular pressure (IOP) was 110mmHg. This higher

IOP was kept for 60 minutes. Overdose narcotic was used to execute the mice after anterior chamber was filled at different time, then the eyes were removed and fixed in 40g/L paraformaldehyde fluids. Tissue was then anhydrated and paraffinized. Section was made 4μm in thickness.

Immunohistochemistry and TUNEL For immunohistochemistry, sections were deparaffinized, rehydrated and washed in phosphate-buffered saline solution (PBS). The sections were heated twice at 95℃ for 5 minutes each in 0.01mol/L of citric acid buffer at pH 6.0, then cooled to room temperature and washed three times with PBS for 5 minutes. The endogenous peroxidase activity was inactivated with 3% hydrogen peroxide in ethanol, and binding was suppressed with 1.5% normal blocking serum in PBS. Sections were incubated with rabbit anti-rat HIF-1α antibody (Boster biotechnology limited company) in a humidified chamber for 2 hours at room temperature. With intervening washes in PBS, sections were then incubated for 30 minutes with a biotin-conjugated secondary antibody. The avidin-biotin-immunoperoxidase complex (ABC staining system, Santa Cruz) was then applied. Peroxidase activity was visualized with diaminobenzidine (DAB). As controls, sections from all samples were stained with the above procedures, without the use of the primary or secondary antibodies. The distribution of apoptosis was detected by using TUNEL with *in situ* cell death staining kit (Boster Biotechnology Limited Company), the experiment procedures were according to instructions of the agentia. Immunohistochemical staining and TUNEL staining image were evaluated by analysis assay of HIF-1α integrated optical density average (IOD) and apoptosis IOD.

Statistical Analysis The Image Analysis System (UIC, USA) was used for analyzing the pictures. SPSS 13.0 software was used for statistical analysis. Variance analysis and t-test were used to deal with the date. All values were expressed as means±SE. Statistical significance was accepted when the $P<0.05$.

RESULTS

HIF−1α Expression There was no HIF-1α expression in normal control group and experimental control group. HIF-1α was expressed in ischemia-reperfusion group at 2, 6, 8, 24, 48 hours. The expression reached to a peak at the 12 hours mark after retinal ischemia-reperfusion, the expression of HIF-1α declining was detected in 12, 24, 48 hours group in gangling cell layer of retina and inner nuclear layer (Figures 1,2). Buffy-yellow drop located in cell nucleus and cytoplasm has shown the positive expression (Table 1).

Table 1 Expression of HIF-1α integrated optical density average (IOD) in retina at different time after ischemia-reperfusion

Time	*n*	HIF-1α(IOD)	*P*
Normal control group	6	0.27±0.073	
Experimental control group	6	0.30±0.12	0.949
0h	6	0.29±0.02	0.987
2h	6	0.68±0.12	0.449
6h	6	1.91±0.43	0.018
8h	6	5.061±0.69	0.00
12h	6	7.48±1.35	0.00
24h	6	4.14±1.49	0.00
48h	6	2.01±0.43	0.00

P: The afterward group compared with the forward group

Table 2 TUNEL positive cells integrated optical density average (IOD) in retinal at different time after ischemia-reperfusion

Time	*n*	TUNEL (IOD)	*P*
Normal control group	6	0.23±0.07	
Experimental control group	6	0.258±0.1	0.943
0h	6	0.22±0.13	0.922
2h	6	0.43±0.1	0.556
6h	6	0.74±0.09	0.963
8h	6	0.99±0.29	0.135
12h	6	5.44±0.51	0.00
24h	6	8.3±1.0	0.00
48h	6	3.54±0.81	0.00

P: The afterward group compared with the forward group

TUNE Labeling With TUNEL test, positive cells were increased in number after ischemia 60 minutes and 12, 24 hours reperfusion in GCL (ganglion cell layer) and INL (inner nuclear layer). But only few TUNEL positive cells were seen after 48 hours (Figures 3,4). There were no positive cells of apoptosis in normal control group, experiment control group and ischemia-reperfusion 2, 6 hours group. There was a significant difference among the normal control group, control group and ischemia-reperfusion 2,6 hours group and ischemia-reperfusion 12, 24, 48 hours group ($P<0.01$). In ischemia-reperfusion 12, 24, 48 hours group apoptosis in GCL and INL layers (Figures 3,4). The comparison about IOD of positive cells apoptosis was shown in Table 2. Compare Table 1 with Table 2, the expression of HIF-1α is correlated with TUNEL (The correlation coefficient is 0.602).

DISCUSSION

Retinal ischemia is frequent problem, such as in central retinal artery occlusion, the grand of angle-closure glaucoma and some ophthalmologic operation that affect the blood stream of the retinal. The common therapeutic method is to

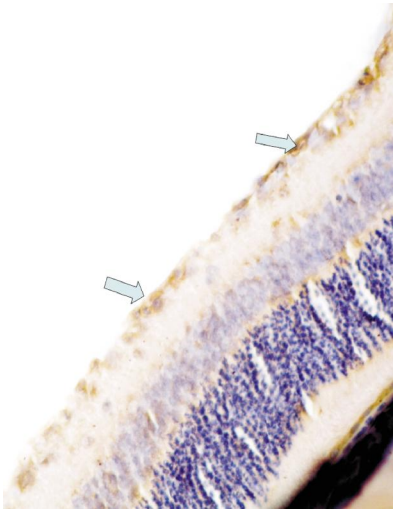

Figure 1 With HIF-1α immunohistochemical staining, the HIF-1α expression has shown in gangling cell layer of retina and inner nuclear layer after ischemia-reperfusion 8 hours (×400)

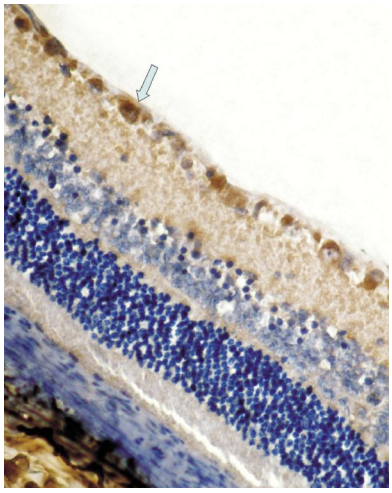

Figure 2 With HIF-1α immunohistochemical staining, the HIF-1α expression has shown in gangling cell layer of retina and inner nuclear layer, Buffy-yellow drop located in cell nucleus and cytoplasm after ischemia-reperfusion 12 hours (×400)

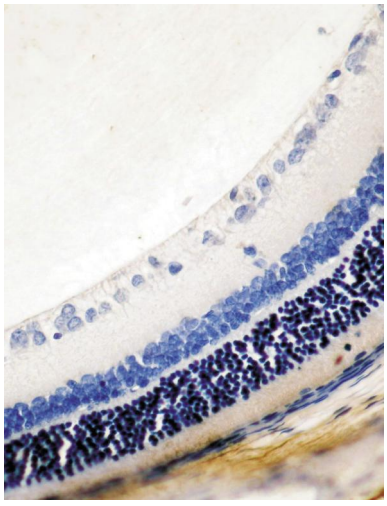

Figure 3 No TUNEL positive cells in experiment control group after 24 hours (×400)

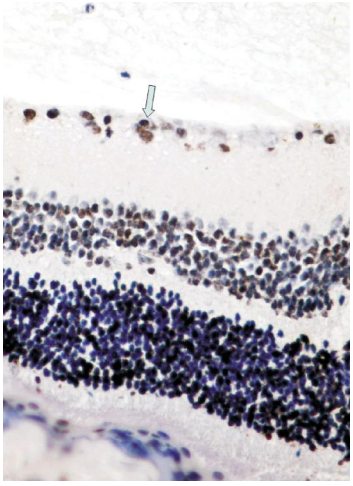

Figure 4 TUNEL positive cells were seen form GCL and INL in the experiment group after ischemia 60 minutes and 12 hours reperfusion (×400)

recover blood supply of retina. This can repair the impaired tissue and improve the patient's vision acuity [1-3]. We have found that there is evidently functional disturbance even inconvertible injure after reperfusion. However, there is no obvious functional disturbance after certain time ischemia but without reperfusion. This is called the ischemia-reperfusion injury in clinical. Hypoxia inducible factor-1 (HIF-1) has emerged recently as a key regulator of maintaining oxygen homeostasis, mediating a wide range of cellular and physiological responses necessary to adapt to changes in oxygen tension. HIF-1 is composed of two subunits: HIF-1α and HIF-1β. HIF-1α is the oxygen-regulated component that determines HIF-1 activity. HIF-1α is generally resided in many kinds of cells. The half-life time of HIF-1α is very short (about 30 seconds at normoxia).When ischemia and hypoxia occurred the decompose pathway of HIF-1α was blocked, and HIF-1α increased [4]. Our finding indicates that there is not HIF-1α's expression in normal retinal. It appears after 2 hours ischemia-reperfusion, we presume the expression of HIF-1α is induced by the transient ischemia of retina leads to hypoxia in cells. The expression of HIF-1α increased gradually with the injury exacerbation and it achieved to the peak at 12 hours and decreased gradually. The expression of HIF-1α is correlated with apoptosis.

Recently with extensive investigation of ischemic retina disease, a close relation of ischemia- reperfusion injury with apoptosis has been found[5]. In this experiment, the apoptosis in ischemia-reperfusion injury in retina has been disclosed. Little apoptosis in GCL and INL layers before 12 hours

ischemia-reperfusion was observed. Apoptosis cells reached to the peak after 24 hours ischemia-reperfusion especially. Apoptosis cells decreased after 48 hours ischemia-reperfusion gradually. It suggests that the death of some retinal cells appears to be the cell apoptosis during retinal ischemia-reperfusion and apoptosis procedure may be control by many factors. HIF-1α is only one of them.

Our results are consistent with those findings. We have also found that the appearance of apoptosis cells and the change tendency is coincident with the expression of HIF-1α. They all gradually increase to the peak and decrease gradually. Apoptosis cells appear in GCL and INL layers, the position is same with the expression of HIF-1α. All these indicate there is a close relationship between them. The activity of HIF-1α reached to the peak after 12 hours that is the same as the apoptosis arrive to the peak. The expression of HIF-1α and apoptosis were obvious association on time order. However, the mechanism that HIF-1α leads to apoptosis remains to be investigated. The death pattern of most retinal cell of mice are displayed as apoptosis, many kinds of factors are play roles in retinal ischemia-reperfusion. So it can be seen that anti-apoptosis and other means therapeutic alliance is becoming the main content in treating the ischemia-reperfusion diseases [5,6]. Ischemia results in cells damage, reperfusion leads to the damage heavy. The mechanism of the injury is not very clear, HIF-1α not only regulated EPO, VEGF, EMP pathway factor but also regulated heme oxygenase, tyrosine hydrxylase, glucose transporter, adenylate kinase, the promoter, enhancer and other control region of these genes all include HIF-1α idiocombination sequence [7,8]. Investigation of the relationship between the expression of HIF-1α and cell damage is profitable to make it clear that the role HIF-1α played in ischemia-reperfusion injury.

REFERENCES

1 Semenza G. Signal transduction to hypoxia-inducible factor 1. *Biochem Pharmacol*2002;64(5-6):993-998

2 Buchi ER, Suivaizdis I. Pressure induced retinal ischemia in rats: an experimental model for quantitative study. *Ophthalmological*1991;203(3):138-147

3 Lando D, Peet DJ, Whelan DA. Asparagine hydroxylation of the HIF transactivation domain: a hypoxic switch. *Science*2002;295(5556):858-861

4 Deng AJ, Jiang DY. Sequential changes of HIF-1 protein and mRNA in hypoxic bovine retinal microvessel endothelial cells. *Int J Ophthalmol (Guoji Yanke Zazhi)* 2005;5(2):225-228

5 Lam TT, Abler AS, Tso MO. Apoptosis and caspases after ischemia-reperfusion injury in rat retina. *Invest Ophthalmol Vis Sci*1999;40(5):967-975

6 Brune B, Zhou J. The role of nitric oxide (NO)in stability regulation of hypoxia inducible factor-1alpha (HIF-1 alpha). *Curr Med Chem*2003;10(10):845-855

7 Chun YS, Kim MS, Park JW. Oxygen dependent and independent regulation of HIF-1alpha. *Korean Med Sci*2002;17(5):581-588

8 Bu ER. Cell death in the rat retina after a pressure induced ischemia reperfusion injury: an electron microscopic study. I. Ganglion cell layer and inner nuclear layer. *Exp Eye Res*1992;55(4):605-613