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Effect of RSCs combined with COP-1 on optic nerve damage in glaucoma rat model

Wei-Cai Fu, Yan Jiang, Lin Zhang*

Department of Ophthalmology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China

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ABSTRACT

Objective: To explore effect of retinal stem cells (RSCs) combined with copolymer-1 (COP-1) immunotherapy on optic nerve damage in glaucoma rat model. **Methods:** A total of 40 SD rats were selected for glaucoma model and were randomly divided into 4 groups to observe protective effects of RSCs transplantation combined with COP-1. **Results:** Brain-derived neurotrophic factor (BDNF) and insulin like growth factor-1 (IGF-1) were either positive in retina of RSCs transplanted or COP-1 immunological treated rat. Positive rate of BDNF and IGF-1 and expression of mRNA and protein were significantly higher in RSCs transplantation combined with COP-1 immunotherapy treated rats compared with the other 3 groups, in which amount of apoptotic RGCs was lowest. **Conclusions:** RSCs transplantation combined with COP-1 immunotherapy can promote the secretion of BDNF and IGF-1. They protect RGCs in glaucoma rats in coordination, significantly reduce the number of apoptosis RGCs so as to alleviate the optic nerve damage. It ponits a new research direction for treatment of glaucoma.

1. Introduction

Intraocular pressure of glaucoma patients stays intermittent or continuous high, and continuous high intraocular pressure can cause damage of eyeball tissue or visual function to finally cause vision loss even blindness with modality of less than 1%, however, it has become one of three blinding diseases universally[1,2]. Most researches consider that damage of retinal ganglion cells (RGCs) is the main reason for blindness of glaucoma patients, but recently there is no effective method to prevent progressive apoptosis of RGCs[3,4]. Thus, in recent years researchers start to apply retinal stem cells transplantation to treat this disease[5]. This study aims to analyze effects of RSCs combined with copolymer-1 (COP-1) on glaucoma rat model.

2. Materials and methods

2.1. Experiment materials

A total of 50 SD clean grade rats aged 8-10 weeks and weighted 180-220 g which were purchased from Institute of Laboratory Animal, Chinese Academy of Medical Sciences were selected. Animal qualified number was 01-3001. The rats were kept in clean animal house of experimental animal institute (Certification NO: SYXK11-00-0014) under environment temperature 20-23 ℃ and humidity 40%-80%. Male and female were separated and fed with water and food freely.

2.2. Establishment of glaucoma model

A total of 532 laser diodes were used to photocoagulate

*Corresponding author: Lin Zhang, Chief Physician, Professor, Department of Ophthalmology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China.
E-mail: 28906390 @ qq.com
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corneal limbus vascular network, temporal corneal limbus, superior temporal sclera superficial vein and inferior temporal sclera superficial vein of 40 rats. The rest 10 rats were not given any special treatment. Intraocular pressure, conjunctiva, cornea and aqueous water of rats were observed 21 days after photocoagulation[6,7].

2.3. Retina stem cell culture

Five SD rats from control group were selected and removed eye balls under general anesthesia, and other tissue of eyeball was removed under microscope only with marginal part of ciliary body tissue kept. The tissue was cut into pieces and digested by 0.25% trypsin plus 0.02% EDTA solution. After 7 minutes medium with 10% FBS was used to stop digestion and the sample was centrifuged for two times by 1 000 r/min. The cells were added with medium and inoculated in culture bottles after discarding supernatant. The cells were cultured under 37 ℃ and 5% CO_2. Immunofluorescent staining was used to identify RSCs and primary cells were kept for experiment[8,9].

2.4. Treatment of different groups

A total of 40 glaucoma model rats were numbered and randomly divided into A, B, C and D groups. Phosphate buffer solution (PBS) were injected in group A and C, COP-1 were injected in group B and D on d1. PBS were injected in group A and B, COP-1 were injected in group C and D on d7.

2.5. Indexes observation

Expression of brain-derived neurotrophic factor (BDNF) and insulin like growth factor-1 (IGF-1) was tested in groups 2-3 weeks after treatment by immunofluorescent assay. Polymerase chain reaction (PCR) was used to compare the mRNA expression difference[10]. Western blot was used to compare expression of BNDF and IGF-1[11]. Terminal-deoxynucleoitidyl transferase mediated nick end labeling was used to observe RGCs apoptosis of rat retina tissue frozen section[12,13].

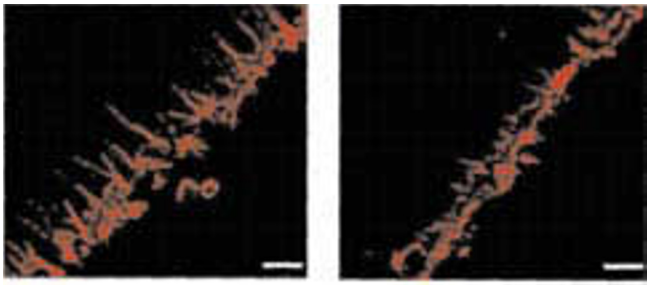
2.6. Statistical analysis

All data in our study were analyzed by SPSS13.0. Enumeration data was analyzed by Chi-square test and measurement data was analyzed by t test. The test level was set as $\alpha=0.05$. The difference was considered as statistically significant when $P<0.05$.

3. Results

3.1. Immunofluorescent staining results

BDNF and IGF-1 showed positive expression with red color in B, C and D groups and mainly concentrated at nerve fiber layer and retinal ganglion cell layer, as shown in Figure 1.



BDNF IGF-1

Figure 1. Expression of BDNF and IGF-1 under confocal microscope in group D.

3.2. mRNA expression difference

Expression of BDNF and IGF-1 was significantly higher in group D compared with the other three groups. Expression of BDNF and IGF-1 in group A was significantly lower than the other three groups ($P<0.05$), there was no significant difference between group B and group C ($P>0.05$), as shown in Table 1.

Table 1
BDNF and IGF-1 mRNA expression ($\bar{\chi}\pm$sd).

Group	Number of cases	BDNF	IGF-1
A	10	1.23 ± 0.12^{b}	1.00 ± 0.04^{b}
B	10	2.24 ± 0.37^{ab}	1.72 ± 0.16^{ab}
C	10	1.97 ± 0.23^{ab}	1.44 ± 0.57^{ab}
D	10	3.37 ± 0.45^{a}	2.21 ± 0.20^{a}

Note: [a] Compared with group A, $P<0.05$; [b] Compared with group D, $P<0.05$.

3.3. Protein expression

Western blot analysis showed that BDNF and IGF-1 protein expression in group D was significantly higher compared with the other 3 groups, BDNF and IGF-1 protein expression in group A was significantly lower compared with the other

three groups ($P<0.05$), there was no statistical difference between group B and group C ($P>0.05$), as shown in Table 2 and Figure 2.

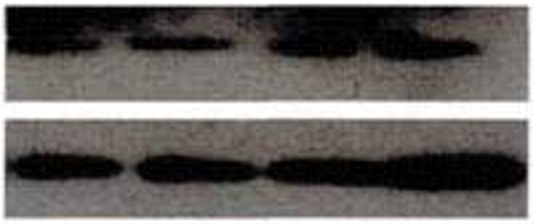


Figure 2. Protein expression of BDNF and IGF-1.

Table 2

Protein expression of BDNF and IGF-1 in 4 groups ($\bar{x}$±sd).

Group	Number of cases	BDNF grey value	IGF-1 grey value
A	10	0.74±0.07[b]	0.55±0.09[b]
B	10	1.35±0.09[ab]	0.86±0.15[ab]
C	10	0.83±0.25[ab]	0.68±0.10[ab]
D	10	1.60±0.13[a]	0.98±0.11[a]

Note: [a] Compared with group A, $P<0.05$; [b] Compared with group A, $P<0.05$.

3.4. Analysis of RGCs apoptosis

The average value of apoptosis cells in single vision was 11.2 in group A, 9.0 in group B, 9.5 in group C and 6.2 in group D. The amount of apoptotic RGCs was significantly lower in group D than the other 3 groups, The amount of apoptotic RGCs was significantly higher in group A than the other 3 groups ($P<0.05$), the amount had no statistical difference between group B and group C ($P>0.05$).

4. Discussion

In recent years, scholars have been looking for better methods to treat glaucoma from optimal nerve regeneration, RSCs transplantation and induction of RSCs directional differentiation. Stem cells are potential in self-renewal and differentiation. Researchers have verified that nerve stem cells can merge into rat tissue to promote formation of mature brain cells[10,11]. However there is less research about RSCs merging in rat retina. Besides, Niyadurupola *et al*[12-15] found that immune system showed significant effects on the development of glaucoma, and artificial COP-1 can protect the immune reaction to stop secondary damage of RGCs. To explore RSCs transplantation combined with COP-1 immune therapy on treating glaucoma we selected 50 SD rats for research.

Researchers have found that BDNF and IGF-1 showed positive expression in rat retina after RSCs transplantation or COP-1 immunotherapy, however, positive expression rate, mRNA and protein expression of BDNF and IGF-1 in rats which accepted RSCs transplantation combined with COP-1 immunotherapy was significantly higher than the other 3 groups, amount of apoptotic RGCs was also lowest, showing that nerve nutrition and growth factor recovered effectively in this group to promote the calcium ion concentration, decrease partial pressure of oxygen, and inhibit free oxygen radicals and release of harmful neurotransmitter to further stop RGCs apoptosis and decrease intraocular pressure[16-20], the mechanism is as follows: BDNF can be active in rats for a long term and release nutrition factor to supply nutrition and protection for glaucoma rats; As a self-reactive antigen, injection of COP-1 can activate local immune reaction and microglial cells to remove pieces of dead cells and glutamic acid. Besides, Werkmeister *et al*[21-30] found that RSCs transplantation combined with COP-1 could provide protection for central nerve cells and promote production of IGF-1, it's also critical in regulation of nerve formation.

In conclusion, RSCs transplantation combined with COP-1 immunotherapy can promote the secretion of BDNF and IGF-1, collaboratively protect RGCs in glaucoma rats, which significantly reduces the number of apoptosis RGCs so as to alleviate the optic nerve damage to open a new research direction for treatment of glaucoma.

Conflict of interest statement

We declare that we have no conflict of interest.

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