Effect of human umbilical cord blood stem cells on flash visual evoked potential in traumatic optic neuropathy in rats

ZHU Xinghua[1], JIANG Bing[1,2], ZHANG Pu[1], ZHOU Dan[1]

(1. *Department of Ophthalmology, Second Xiangya Hospital, Changsha* 410011;
2. *Institute of Clinical Pharmacology, Central South University, Changsha* 410078, *China*)

Abstract: Objective To investigate the effect of human umbilical cord blood stem cells on flash visual evoked potentials (F-VEP) of the traumatic optic neuropathy rats. **Methods** Forty-eight Sprague-Dawley rats were randomly divided into an injury group (Group A) and 3 treatment groups (Groups B, C, and D). A traumatic optic neuropathy model was built in Group A, and the rats in Groups B, C, and D were injected with the neurotrophic factor, human umbilical cord blood stem cells, and the mixture of the neurotrophic factor and human umbilical cord blood stem cells, respectively. F-VEP was recorded in both eyes of rats at the 1st h, 1st week, 2nd week, 3rd week, and 4th week after the optic nerve injury. **Results** At all time points, there were significant difference in the wave latency and amplitude between Group A and normal control eyes ($P<0.01$). The differences of the wave latency and amplitude between Group A and Groups B, C, and D were statistically significant at various time points after the injury except for the wave latency at the 1st h post-operation ($P>0.05$). The amplitude in Group D was higher while the latency was shorter than those of Group B at all time points since the 1st week ($P<0.05$). The comparisons at the same point in the remaining treatment groups were not significantly different ($P>0.05$). **Conclusion** The mixture of human umbilical cord blood stem cells and neurotrophic factor has a promotion effect for the recovery of F-VEP of optic nerve in traumatic optic neuropathy in rats to some degrees.

Key words: human umbilical cord blood stem cell; optic neuropathy; flash visual evoked potentials

人脐血干细胞对大鼠外伤性视神经病变闪光视觉诱发电位的影响

朱兴华[1], 江冰[1,2], 张谱[1], 周丹[1]

(中南大学 1.湘雅二医院眼科, 长沙 410011; 2.临床药理研究所, 长沙 410078)

[摘要] **目的:**观察人脐血干细胞对大鼠外伤性视神经病变闪光视觉诱发电位(flash visual evoked potentials, F-VEP)的影响。**方法:**将48只SD大鼠左眼制成外伤性视神经病变模型,A组不治疗,B,C和D组分别予以玻璃体腔内注射神经营养因子、人脐血干细胞、人脐血干细胞+神经营养因子混合液。记录多个时间点F-VEP的波幅及峰潜时,并进行统计分析。**结果:**损伤组与正常对照眼、治疗组之间相同时间点的比较,波幅和峰潜时的差异有统计学意义(除损伤后1 h的峰潜时);各治疗组相同时间点之间的比较,D组与B组之间

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Biography ZHU Xinghua, B. A., surgeon, mainly engaged in clinical visual electrophysiology.

Corresponding author JIANG Bing, E-mail:jiangbing82@yahoo.com.cn

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的波幅与峰潜时的差异均有统计学意义($P<0.05$),其余各组间的差异无统计学意义($P>0.05$)。**结论**:人脐血干细胞和神经营养因子的混合液对大鼠外伤性视神经病变后 F-VEP 的恢复有一定的促进作用。

[**关键词**] 脐血干细胞; 外伤性视神经病变; 闪光视觉诱发电位
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Traumatic optic neuropathy is a kind of serious disease that can cause blindness. Current therapeutic methods include pharmacotherapy, surgical therapy, and medicine and surgery combination therapy. However, these therapies make different effects. Recently, some scholars put forward the idea to treat optic nerve injury with the method of stem cell transplantation and made some progress in animal tests. Flash visual evoked potentials (F-VEP) examination is a relatively mature, objective, and effective method to evaluate the functional status of optic nerve, mainly reflecting the functional status of retinal ganglion cells (RGCs) to visual cortex through stabilized waveform, good repeatability, and zero difference in gender or dominant eyes[1]. Because F-VEP examination can show the severity of traumatic optic neuropathy, it is necessary to monitor the variation of optic nerve from repair to regeneration after the injury using visual electrophysiology and demonstrate the variation according to the function of optic nerve.

1 MATERIALS AND METHODS

1.1 Animals and grouping

1.1.1 Animal source and feeding environment

All of Sprague-Dawley (SD) rats, SPF grade, weighing 200 – 300 g, were provided by the Animal Laboratory Center of the Second Xiangya Hospital, Central South University.

1.1.2 Animal grouping

Altogether 48 rats were randomly divided into 4 groups: A, B, C, and D groups, 12 in each. All of them had optic nerve injury in left eyes and normal right eyes as control eyes. Group A was the optic nerve injury group; Group B was the neurotrophic factor group; Group C was the human umbilical cord blood stem cell group; and Group D was the human umbilical cord blood stem cell + neurotrophic factor combination group. F-VEP examination was performed on injured eyes and normal control eyes at the 1st h, 1st week, 2nd week, 3rd week, and 4th week after the injury to record wave amplitude and peak latency.

1.2 Methods

1.2.1 Establishment of rat traumatic optic neuropathy model and inclusion criteria

The SD rat partial optic nerve injury model was built by reference to the method adopted by Jiang Bing, et al.[2]. After intraperitoneal injection of anesthetics (10% chloral hydrate 3.5 mL/kg) on rats, upper eyelid skin and upper bulbar conjunctiva were vertically cut off under the microscope and made the optic nerve exposed. The optic nerve at 2 mm behind the eyeball was clamped using a special purpose clamp (provided by scholars visiting to Japan) with the clamping force of 40 g for 30 s. Immediately after clamping, if pupil dilation, absence of pupil reactions to direct light but presence of pupil reactions to indirect light, no bleeding or infarction in retinal vessel and recovery of blood flow in 5 min were observed in injured eyes, the rat could be included into the test.

1.2.2 Medicine intervention

Immediately when the partial optic nerve injury model was built, following measures were adopted for treatment groups. A 30 g syringe needle was used to pierce through conjunctiva and sclera at 1 mm behind corneal limbus above tempus and immediately withdrawn under the microscope. Human umbilical cord blood stem cells (about 10^6 per cell, Shenzhen Beike Biotechnology Co. Ltd.), brain-derived neurotrophic factors (BDNF, Shenzhen Beike Biotechnology Co. Ltd.), and their mixed liquid (10 μL each) were absorbed using a micropipette and dripped into eyes at a 45-degree angle through a re-perforated pinhole under the microscope. We put the needle tip towards optic nerve and slowly injected the fluid through the pinhole, and immediately observed bubbles in vitreous cavity after injection. The pinhead was withdrawn after held for 60 s.

1.2.3 F-VEP examination

Electrophysiological examination referred to international clinical visual electrophysiological criteria[3]. After intraperitoneal injection of anesthetics, a rat was fixed on the test table and compound tropicamide was applied for a mydriatic test. The recording electrode of the visual electrophysiological exami-

nation system (Chongqing Medical Equipment Factory) was inserted subcutaneously into occipital protuberance; the reference electrode was inserted subcutaneously into the midpoint between 2 eyes; and the grounding electrode was inserted subcutaneously into the right ear. F-VEP values were recorded after dark adaptation for 15 min. Three stabilized waveforms were recorded for each rat. When one eye was checked, the other eye was covered by a self-made black lightproof eyeshade. The normal eye was checked at first and then the injured eye.

1.2.4 Observation indicators

Harding's nomenclature[3] was adopted. During the test, we recorded relatively stable N-P-N waves and named them N1, P1, and N2. Latent periods of the initial wave N1 and positive waves P1 and N2 were in milliseconds (ms), and sizes of wave amplitudes N1 – P1 and P1 – N2 were in μV. Measuring method: the peak latency N1 was measured from the beginning to peak N1; the peak latency P1 was measured from the beginning to valley bottom P1 and so forth; the wave amplitude N1 – P1 was measured from peak N1 to valley bottom P1 and so forth. Indicator values were automatically gotten by the computer. Each indicator was the mean value of 3 measured values.

1.3 Statistical analysis

SPSS10.0 statistical software package was used for the statistical analysis. Data was expressed as mean ± standard deviation ($\bar{x} \pm s$). The matched-pairs *t*-test was conducted for comparison in each group, and the LSD-*t* test was performed for comparison among groups. The variance analysis was made for differences among indicators, and the paired comparison was made for differences among groups. A *P* value of less than 0.05 was considered significantly different.

2 RESULTS

2.1 F-VEP graph of each group

Normal control eyes of all rats showed a stable F-VEP waveform (Fig. 1) and had no significant change as time went by. The wave amplitude was fluctuated between (12.1 ± 1.5) μV and (13.5 ± 1.6) μV, and the peak latency was fluctuated between (72.9 ± 6.7) ms and (74.2 ± 6.1) ms. In the injured group, F-VEP of optic nerve showed lowered wave amplitude and a widening waveform. The more seriously injured optic nerve, the lower wave amplitude in F-VEP examination. The changes were more significantly as time went by (Fig. 2A – 2C). At the same time point, F-VEP waveform could be observed in treatment groups. Compared with the injured group, the treatment groups showed narrower wave amplitudes and shorter latent periods (Fig. 3 – 5). It changed more significantly as time went by.

2.2 Comparison of F-VEP's P1 wave amplitudes between the injured group and treatment groups

Wave amplitudes of the injured group and treatment groups were lowered to varying degrees as time went by. However, treatment groups showed a lowered trend compared with the injured group, most obviously in the combined medicine treatment group. Starting from the 2nd week, the treatment groups showed obviously higher wave amplitudes than the injured group. At the 3rd weeks after the injury, the injured group showed the most different wave amplitudes to each treatment group. At each time point after the injury, the paired comparison between the injured group and each treatment group showed statistically significant differences ($P < 0.05$); whereas the paired comparison among treatment groups showed no statistically significant differences ($P > 0.05$, Tab. 1).

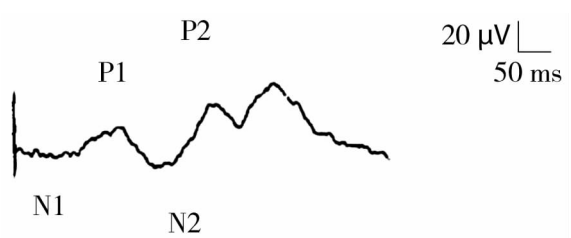

Fig. 1 F-VEP in normal eye of rats.

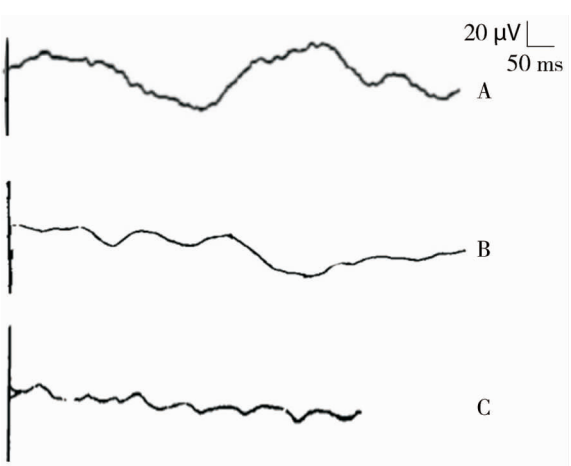

Fig. 2 F-VEP in injured rats. A: First hour after the injury; B: Third week after the injury; C: Forth week after the injury.

Fig. 3 F-VEP in neurotrophic factor treatment rats (3rd week after the injury).

Fig. 4 F-VEP in cord blood stem cell treatment rats (3rd week after the injury).

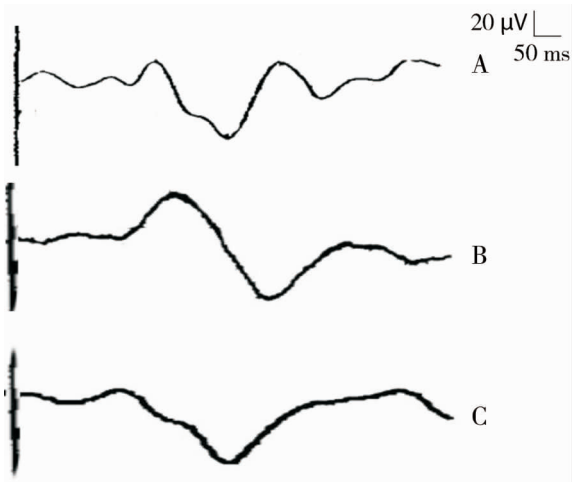

Fig. 5 F-VEP in neurotrophic factor and cord blood stem cell treatment rats. A: First hour after the injury; B: Third week after the injury; C: Forth week after the injury.

2.3 Comparison of F-VEP's P1 peak latencies between the injured group and treatment groups

Peak latencies of the injured group and treatment groups showed changes as time went by. The peak latency of each treatment group was shorter than the injured group from the 1st week. At 2nd week after the injury, the injured group had the most different peak latency compared with each treatment group, especially in the combined medicine treatment group. At the 3rd week after the injury, all groups showed the longest peak latency. At the 4th week after the injury, all groups showed lowered peak latency P1. The paired comparison between the injured group and each treatment group showed statistically significant differences (1st week $P<0.05$, 2nd, 3rd, and 4th weeks $P<0.01$) at all time points, except at the 1st h after the injury ($P>0.05$). At all time points starting from the 1st week, the combined medicine treatment group showed lowered peak values compared with the neurotrophic factor treatment group ($P<0.05$). The paired comparison showed no statistically significant differences among other treatment groups ($P>0.05$, Tab. 2).

Tab. 1 Comparision of amplitude of P1 between 4 groups ($\bar{x}\pm s$)

Time points post-injury	Group A	Group B	Group C	Group D
1st h	6.41±0.61*	6.46±0.92#	6.54±1.22	6.61±1.43
1st week	5.38±0.63*	6.00±0.71#	6.08±1.01	6.15±1.23
2nd week	4.56±0.76**	5.89±0.83#	5.97±1.10	6.04±1.32
3rd week	3.78±0.53**	5.29±0.65#	5.37±0.91	5.67±1.10
4th week	3.17±0.75**	4.27±0.57#	4.39±0.65	4.82±0.95

Compared with Group B, C, or D at the same time point, $*P<0.05$, $**P<0.01$; compared with Group D, $\#P<0.05$.

Tab. 2 Comparision of latency of P1 between 4 groups ($\bar{x}\pm s$)

Time points post-injury	Group A	Group B	Group C	Group D
1st h	81.44±1.00	82.03±1.24#	82.72±2.04	82.92±2.21
1st week	95.51±3.14*	90.74±2.52#	86.24±1.81	84.52±1.61
2nd week	104.53±7.02**	92.31±6.53#	88.72±6.31	86.64±6.03
3rd week	106.11±8.32**	97.53±4.31#	93.61±3.53	91.61±3.02
4th week	99.34±3.92**	94.24±3.50#	89.83±2.73	85.53±2.02

Compared with Group B, C, or D at the same time point, $*P<0.05$, $**P<0.01$; compared with Group D, $\#P<0.05$.

3 DISCUSSION

Traumatic optic neuropathy (TON) refers to the indirect injury on optic nerve caused by external force through movement of bones and eyeballs. As the injury may result in no clinical manifestation but visual loss[4], objective and sensitive electrophysiological indicators are generally adopted for evaluation in clinical and experimental studies. F-VEP is a relatively mature and effective non-invasive method[5] for the study of visual functional status in normal and sick conditions. As an important method to evaluate optic nerve injury, it can be adopted to evaluate the transmission performance between retina and visual cortex and observe the lowered trend of wave amplitudes and the latent period of wave peaks in F-VEP. In our previous findings[2], RGCs could be partially lost after the optic nerve was clamped by an optic nerve clamp with the clamping force of 40 g for 4 s to

build a traumatic optic neuropathy model. In this study, injured eyes of rats in the injured group showed a significant change in F-VEP's waveform compared with their normal control eyes at the 1st h after the injury. At all time points, there were statistically significant difference in the wave amplitude and peak latency between injured eyes and normal control eyes.

In recent years, the effect of neurotrophic factor and stem cell transplantation on clinical treatment has attracted more attentions. It was reported in some Chinese literatures[6-8] that many stem cells such as retina progenitor cell stem cells, human embryonic neural stem cells, and neural stem cells can be transplanted into retina or eye to treat partially injured optic nerve. However, there is not any report about the application of cord blood stem cell in traumatic optic neuropathy to date. Cord blood contains abundant hemopoietic stem cells and mesenchymal stem cells[9]. Mesenchymal stem cells are capable of renewing and proliferating by themselves and differentiating to be various cells or tissues under the impact or induction of special factors. According to reports[10-13], stem cells, including neuronal precursor cells and bone marrow stem cells, can play an active role in various central nervous system injury models. In a study, researchers[14] cut off optic tract at lateral geniculate body of newborn rats and put gelfoam containing mesenchymal cells (separated from human umbilical cord blood stem cell) in the injured part. During the transplantation of mesenchymal cells, it can be observed that host nerve cords became protuberant in the injured part, and number of endogenous neural precursor cells increased. At the 4th week after transplantation, transplanted mesenchymal cells took effect to protect nerve and improve RGCs with cutting-off axon to regenerate and transmit the effect to superior colliculus. According to findings of Naoko[15], human umbilical cord blood stem cells can be differentiated to retina nerve cells at the 2nd week after being transplanted to retina of rats. These studies revealed the therapeutical effect of human umbilical cord blood stem cell on retina and optic nerve degenerative diseases and raised probable mechanisms. In this study, immediately after the rat traumatic optic neuropathy model was built, BDNF, cord blood stem cells or their mixed liquid, which could significantly delay the optic nerve injury, were injected into vitreous cavity in treatment groups. We found that the wave amplitude in treatment groups is declined more gently than that of the injured group, and their peak latency was shorter than that of the injured group, most obviously in the combined medicine treatment group. Meanwhile, the significant waveform could still be performed in the treatment groups 4 weeks after injury, whereas the result in the injured group was the exact opposite, indicating that optic nerve in the injured group is irreversibly injured and transplant of treatment groups plays a pivotal role. Another finding of this study morphologically proved the protective effect of human umbilical cord blood stem cells on RGCs (the article was accepted by *Chinese Journal of Experimental Ophthalmology* and will be published recently). HE stain and TUNEL detection showed the number of normal RGCs in treatment groups decreased more gently than that of the injured group, and treatment groups showed less apoptotic cells than the injured group. After the injury, the paired comparison of RGC number and TUNEL positive cell number between both groups showed statistical significance ($P<0.01$).

In this study, immediately after the rat traumatic optic neuropathy model was built with a special purpose optic nerve clamp, abnormal F-VEP wave amplitudes and peak latencies were found in injured eyes, indicating the declining function of optic nerve. At the moment, the transplantation of neurotrophic factor, human umbilical cord blood stem cells or human umbilical cord blood stem cell + neurotrophic factor mixed liquid into vitreous cavity helps enhance rats' F-VEP wave amplitudes and shorten their peak latenceis after partial injury of optic nerve. In particular, human umbilical cord blood stem cells or human umbilical cord blood stem cell + neurotrophic factor mixed liquid had the most significant effect on the treatment of optic nerve injury, possibly because neurotrophic factors play an active role in differentiating cord blood stem cells. According to our findings, human umbilical cord blood stem cells and neurotrophic factors are able to restoring optic nerve's function to varying degrees. This proves that cord blood stem cells have obvious therapeutic effect on treating traumatic optic nerve injury from the view of functionalism and provides new thoughts to the clinical treatment of traumatic optic neuropathy.

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