Effect of He-Ne laser irradiation on the cognitive function of neonatal rats following hypoxic-ischemic brain damage and its underlying mechanism

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Abstract Objective The aim of this study was to explore the effects of He-Ne laser irradiation on the cognitive function of neonatal rats following hypoxic-ischemic brain damage and its underlying mechanism. **Methods** Newborn Wistar rats aged 7 days were divided into control group、model group、He-Ne laser intervention group. Left common carotids of neonatal rats were ligated, and then oxygen at low concentration was breathed to establish ischemia-hypoxia brain injury rat model. The learning and memory ability of the rats were tested by Y-type maze, Immunohistochemistry was used to detect the expression of Nestin, microtubule-associated protein-2 and Choline Acetyltrans ferase. **Results** The learning and memory ability of rats with hypoxia-ischemia brain damage was obviously improved by He-Ne laser intervention($P<0.05$). Compared with the control group, the expression of Nestin and Choline Acetyltrans ferase was significantly increased in He-Ne laser intervention group($P<0.05$). **Conclusions** He-Ne laser can significantly improve cognitive function of neonatal rats following hypoxic-ischemic brain damage, which may be related to promoting proliferation of neural stem cells and increasing expression of Choline Acetyltrans ferase, then improving the number and function of neurons of neonatal rats following hypoxic-ischemic brain damage.

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1. Introduction

Hypoxic-ischemic brain damage (HIBD) is a common cause of neonatal brain dysplasia and mental retardation [1]. How to actively and effectively promote the rehabilitation of brain function in children following hypoxic-ischemic brain damage is the focus of the study [2-8]. The study found that He-Ne laser therapy can promote the proliferation of endogenous neural stem cells of neonatal rats following hypoxic-ischemic brain damage [9]. But little knowledge has been reported about its ability to improve cognitive function and its underlying mechanism. For this reason，this study adopted a generally accepted rat model with hypoxic-ischemic brain damage, selected "BaiHui"point; "DaZhui"point to study the effect of He-Ne laser irradiation on the learning and memory ability and the expression of Nestin, microtubule-associated protein-2 (MAP2) and Choline Acetyltrans ferase（ChAT) after hypoxia-ischemia in neonatal rats. The aim of this study was to find out the effects of He-Ne laser irradiation on the cognitive function of neonatal rats following hypoxic-ischemic brain damage and its underlying mechanism. We try to provide a new and safe therapeutic method for the clinical treatment of neonatal hypoxic-ischemic brain injury.

2. Material and Methods

2.1 Reagents and instruments

Mouse anti-rat Nestin monoclonal antibodies were purchased from Wuhan Boster Biological Engineering Co., Ltd. Rabbit anti-rat MAP2 and ChAT antibody monoclonal antibody, sp-9000 high-sensitivity kit, DAB chromogenic kit, purchased from Beijing Zhongshan Biological Engineering Co., Ltd. He-Ne laser multifunction therapeutic apparatus was produced by Guilin Electronic Instrument Factory. Y-type maze was produced by Zhangjiagang Bio-Medical Instrument Factory, Jiangsu, China.

2.2 Animals and grouping

36 healthy Wistar rats of 7 days old, weighting 12-16g, male and female informal, were provided by the Experimental Animal Center of Henan Province. The animals were randomly divided intocontrol group, model group and He-Ne laser intervention group. The study was performed in accordance with ethical guidelines for the use and care of animals.

2.3 Model Preparation

Use of internationally recognized modeling method of hypoxic-ischemic brain damage, that is, after left common carotid artery ligation in neonatal rats, which inhaled a low concentration of oxygen. Rats in the control group did not receive deligation of the left common carotid artery, but exposed to the air.

2.4 Interventional procedure

From the second day of model induction, rats in the He-Ne laser intervention group were subjected to He-Ne laser irradiation, at 632.8 nm wavelength, 4 mW laser power, 3 mm the diameter of light spot, 56.62 mV power density, 33.975 J/cm2 energy density, 10 minutes once, once a day, 7 days as a course, totally 2 courses, with an interval of 2 days between the two courses. Acupuncture point included Baihui and Dazhui.

2.5 Learning and memory testing

The rats were placed in a maze adapt to the 5-8min. The nature of rat is trend to dark. The rat will get electric shocks when he is inthe dark arm, only from the dark arm to the light arm (safety zone) which can not receive electric shocks. Random switching security arm, if the rats fled from dark arm to light arm at once by electric shock, which is the correct response. The learning and memory ability of the rats were tested by Y-type maze. 10 times of continuous electric shocks to test, 9 times of the correct response will be to reach the learning standards, then record the number of the formation of conditioned reflex of the animals, as indicators of the learning ability. After 24h rest, repeat the above experiment for the first two tests, then record the number of errors of the animals that occurred, as indicators of the memory ability.

2.6 Staining

Rats were abdominally anesthetized with 100 g/L hydrated ammonium aldehyde and sacrificed, performed with aortic cannlation of left ventricle to rapidly perfuse 100 mL saline in 37 ℃, and fixed with 40 g/L paraformaldehyde buffer (pH 7.2－7.4, 4 ℃) for 30 minutes. The left hemisphere tissue was fixed with 40 g/L paraformaldehyde buffer (4℃) for 12 hours, routinely dehydrated, embedded with paraffin, cut into serial coronal sections of hippocampal formation from coronal surface of optic chiasma with the thickness of 5 μm. One section was selected from every 5 sections, and 10 sections were selected from each sample. Paraffin sections were routinely dewaxed, dealt with 3% H2O2 for 10 minutes, repaired with hypertensive antigen, blocked with 10% normal goat serum for 20 minutes, added with antibody Ⅰ, incubated in wet box at 4 ℃ for overnight, washed with PBS, added with biotinylation antibody Ⅱ, incubated at 37 ℃ for 20 minutes, washed with PBS, incubated with horseradish peroxidase labeled chain enzyme synthetic prime at 37 ℃ for 20 minutes, colorized with DAB, mildly restained with sappan wood, routinely dehydrated, cleared and fixed with Neutral gum. Controlled experiment: normal goat serum instead of primary antibody, immunohistochemical staining simultaneously. The numbers of immune positive cells of each section were counted under microscope. Five adjacent sights were randomly selected from one brain section to calculate arithmetical average value of positive cells.

2.7 Statistical analysis

All data were analyzed using SPSS 10.0 software. Results were expressed as Mean ± SD. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1 Learning and memory ability

Compared with the control group, Learning and memory ability of the model group was significantly diminished ($P<0.05$). However, laser intervention group compared with the control group, the difference was not obvious ($p >0.05$), which means that the laser intervention can significantly improve the cognitive function of neonatal rats following hypoxic-ischemic brain damage. Learning and memory ability of the rats in the three groups are shown in Table 1.

Tab.1 Learning and memory ability of rats in the three groups

Group	n	Learning ability	Memory ability
Control	12	71.98±3.24	85.99±4.02
Model	12	62.58±2.69 ^{a}	74.43±3.92 ^{a}
Laser	12	74.00±4.68 ^{b}	87.80±4.00 ^{b}

Note: Compared with the control group, $^aP <0.05$; $^bP> 0.05$

Tab.2 Immune positive cells in hippocampal CA1 area of rats in the three groups

Group	n	Nestin	ChAT
Control	12	17.78±4.95	29.89±3.45
Model	12	24.63±3.87^a	14.71±6.08^b
Laser	12	32.15±4.39^c	27.12±5.38^d

Note: Compared with the control group, $^aP<0.05$, $^bP<0.05$; Compared with the model group, $^cP<0.05$, $^dP<0.05$.

3.2 Expression of Nestin and ChAT

Immune positive neurons of Nestin were mainly located in hippocampal area in each group. Under optic microscope, immune positive cells were brown yellow. Numbers of Nestin immune positive cells in hippocampal CA1 area of the model group were markedly increased than that of the control group ($P < 0.05$), which increased more obviously in the He-Ne laser intervention group ($P < 0.05$, Table 2). ChAT positive neurons distribute widely in hippocampal CA1 area in control group, decrease markedly in the CA1 subfield of the model group, there is marked difference compared with control group ($p<0.05$). Positive neurons increased markedly in the He-Ne laser intervention group, there is marked diference between the model group and the He-Ne laser intervention group ($p<0.05$, Table 2).

4. Discussions

Laser irradiation can also produce propagated sensation along meridian, as compared with ordinary acupuncture. He-Ne laser irradiation combine laser technology with traditional Chinese meridian theory and acupuncture, which is a non-invasive therapy and has soft, painless and sterile features, in addition which has a unique psycho and psychiatric rehabilitation function. So it is becoming an effective clinical therapeutic tool which has a better application prospect. Cognitive dysfunction is the main characteristics in the surviving newborn following hypoxic-ischemic brain damage [10-11]. So far, it is still no breakthrough in domestic and international research about treatment of this disease. The advanced features of the brain were the learning and memory ability. Studies have demonstrated that the hippocampus area is both ischemia and hypoxia vulnerable areas and learning and memory key parts [12]. The change in the number and function of neurons in the brain will be caused by hippocampal formation damage, which leads to body abnormal behavior. Therefore, the immature brain with hypoxic-ischemic brain injury will inevitably lead to changes in learning and memory function. In this study, learning and memory abilities change was tested by Y- type maze. The results show that learning and memory capacity in the laser intervention group significantly improved, compared with the model group, that is, laser treatment can improve learning and memory abilities of HIBD newborn rats. To a certain extent, the cognitive function of HIBD surviving patient can be improved, which can reduce the incidence of mental retardation and other neurological sequelae. But the underlying mechanism of laser treatment improving cognitive function rarely reported.

The abnormal changes of the structure and function of the hippocampus were pathology foundation, which lead to dementia, epilepsy and other central nervous system disease. Numerous studies have shown the neural stem cells have been isolated from the mammalian brain development in different periods and different regions[13-14]. Neural stem cells can differentiate into neurons and glial cells, and take part in repairing the injured neuronal web and function [15]. Therefore, the neural stem cells play an important role in the self-repair of the damaged brain tissue. Studies have shown that newborn neurons in the hippocampus can be extended to the CA3 region projected axons after mitosis completed 4-10d [16], which suggests that the newborn neurons will have a rapid impact on hippocampal function. This limits its ability to repair damaged central nervous system because of its producing the limited number of newborn nerve cells. The expression levels of Nestin in damaged brain tissue in pathological cases can be used as monitoring the level of neural stem cells, which is one of the most sensitivity signs [17]. Our studies have shown that Nestin immunoreactive cells of hippocampal CA1 area in the model group and the laser intervention group significantly increased, compared with the control group. And compared with the model group, Nestin positive cells in the laser intervention group obviously increased, which means He-Ne laser intervention significantly promote proliferation of neural stem cell in neonatal rat following hypoxic-ischemic brain damage, and can significant increase the number of nerve cells in the hippocampal formation, Then provide the morphological basis of cognitive function change. Central cholinergic neurons has a special role in learning, memory, emotional, et al. ChAT is closely related with the learning and memory of the brain, the change of which can be a direct reflection of the cognitive function of the brain. Therefore, the change in the number of ChAT positive cells in the brain tissue was detected, which can reflect the functional state of the brain cells [18]. This study shows that the number of ChAT positive neurons in the model group reduce, which means ChAT synthesis decreased because of hypoxic-ischemic brain damage.

After the intervention of He-Ne laser, the number of ChAT positive cells significantly increased compared with the model group. He-Ne laser intervention increased synthesis of neurotransmitters ChAT in the brain. Also it provides the basis of morphological brain changes in cognitive function.

After the intervention of He-Ne laser, the number of expression of Nestin and ChAT in hippocampal CA1 region was a positive correlation with its learning and memory abilities in neonatal rat following hypoxic-ischemic brain damage. That is,

He-Ne laser intervention promoted proliferation of neural stem cell in HIBD neonatal rats, and significant increased the number of nerve cells in the hippocampus structure. It increased synthesis of neurotransmitters ChAT in the brain, and improved learning and memory abilities. This may be one of the mechanisms that He-Ne laser intervention can improve the cognitive function of HIBD rats. Therefore, we hypothesized the mechanisms of He-Ne laser improvement cognitive function of brain are as following. By activating or promoting endogenous neural stem cell proliferation and differentiation, accelerating the growth of dendrites, and reconstruction and repair of damaged neural networks. By regulating the activity of nerve cells, promote the synthesis of brain neurotransmitters. Ultimately improve brain cognitive function of HIBD model rat. The mechanism of which He-Ne laser promote brain cognitive function still needs further investigation, which further reveals the regulatory mechanism of the cognitive function of the brain. It is important that makes full use of laser therapy to promote the rehabilitation of brain function. It is practical significance to improve neonatal quality and children's intelligence, and reduce the social and economic losses.

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References
1. Berger R, Garnier Y. Perinatal brain injury.J Perinat Med, 2000;28(4):261-285.
2. Yin X, Meng F, Wang Y, et al.Effect of hyperbaric oxygen on neurological recovery of neonatal rats following hypoxic-ischemic brain damage and its underlying mechanism[J].Int J Clin Exp Pathol, 2013;6(1):66-75.
3. Wang Y, Cao M, Liu A, et al. Changes of inflammatory cytokines and neurotrophins emphasized their roles in hypoxic-ischemic brain damage [J]. Int J Neurosci, 2012 Dec 7. [Epub ahead of print]
4. Yu L, Yi J, Ye G, et al. Effects of curcumin on levels of nitric oxide synthase and AQP-4 in a rat model of hypoxia-ischemic brain damage[J]. Brain Res, 2012; 1475:88-95.
5. Zhu WW, Ma XL, Guo AL, et al. Neuroprotective effects of NEP1-40 and fasudil on Nogo-A expression in neonatal rats with hypoxic-ischemic brain damage[J]. Genet Mol Res, 2011; 10(4):2987-95.
6. Hei M, Luo Y, Zhang X, et al. Tanshinone IIa alleviates the biochemical changes associated with hypoxic ischemic brain damage in a rat model[J].Phytother Res, 2011; 25(12):1865-1869.
7. Zhang YP, Huang QL, Zhao CM, et al. GM1 improves neurofascin155 association with lipid rafts and prevents rat brain myelin injury after hypoxia-ischemia [J]. Braz J Med Biol Res. 2011 Jun;44(6):553-61
8. Liu Y, Zou LP, Du JB. Nitric oxide-mediated neuronal functional recovery in hypoxic-ischemic brain damaged rats subjected to electrical stimulation [J]. Brain Res, 2011; 1383: 324-328.
9. Weihong Zhang, Wanqing Li, Lili Jiang, et al. Proliferation and differentiation of endogenous neural stem cells and brain functional reconstruction following laser irradiation [J]. Journal of Clinical Rehabilitative Tissue Engineering Research, 2010; 14(32):6077-6080.
10. Pietrini D, Piastra M, Luca E, et al. Neuroprotection and hypothermia in infants and children [J].Curr Drug Targets,2012;13:925–935
11. Verklan MT. The chilling details: hypoxic-ischemic encephalopathy [J]. J Perinat Neonatal Nurs, 2009;23:59–68.
12. Ikeda T, Mishima K, Yoshikawa T, et al. Selective and long-term learning impairment following neonatal hypoxic-ischemic brain insult in rats[J]. Behav Brain Res, 2001;118(1):17-25
13. Gould E, Reeves AJ, Graziano MS, et al. Neurogenesis in the neocortex of adult primates [J]. Science, 1999; 286(5439):548-552.
14. Gage FH. Mammalian neural stem cells [J]. Science, 2000; 287(5457): 1433-1438.
15. Ninomiya M, Yamashita T, Araki N, et al. Enhanced neurogenesis in the ischemic striatum following EGF-induced expansion of transit-amplifying cells in the subventricular zone[J]. Neurosci Lett,2006;403(1-2):63-67.
16. Hastings NB, Could E. Adult-generated granule cells rapidly extend axons into the CA3 region:a combined Brdu-labeling and retrograde tracer study[J]. Comp Neurol,1999,413:146-154.
17. Yamashita T, Ninomiya M, Hernández Acosta P, et al. Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum [J]. J Neurosci, 2006;26(24):6627-6636.
18. Tanaka K, Ogawa N, Asanuma M, et al. Relationship between cholinergic dysfunction and discrimination learning disabilities in Wistar rats following chronic cerebral hypoperfusion [J]. Brain Res, 1996;729(1):55-65.

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