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Differences in Cognitive Function of Rats with Traumatic Brain Injuries Following Hyperbaric Oxygen Therapy

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Background: Hyperbaric oxygen (HBO) is a historical therapeutic option in the treatment of various types of brain damage. At present, clinical treatment of hypoxic-ischemic injury is giving priority to cognitive training. The effects of HBO on cognitive dysfunction were observed in a controlled cortical impact (CCI) rat model.





Material/Methods: Seventy male SD rats were randomly divided into control (n=10) and intervention (n=60) groups. All rats underwent baseline water maze testing 1 day before modeling, and were retested 8 weeks after modeling. The percentage of residence time during escape latency in the target quadrant and the total time were recorded. Data were analyzed by SPSS 16.0 software. P<0.05 was considered statistically significant.

Results: After 8 weeks, no statistical difference (P>0.05) existed in spatial learning ability in the 3-day and 5-day groups when compared with baseline. The other groups were statistically different by auto-comparison (P<0.05). No statistical difference (P>0.05) in spatial memory existed in the 5-day and 1-week groups when compared with baseline, while a significant difference was noted in the other groups by self-comparison (P<0.05). No statistical difference (P>0.05) was noted in the level of expression of growth-associated protein-43 (GAP-43) and synaptophysin (Syn) in the 1-day group compared with the control group. The remaining groups and the control group were statistically different (P<0.05), while the level of expression of GAP-43 and Syn in the 5-day, 1-week, and 2-week groups was significantly different compared with that in the control group (P<0.01).

Conclusions: If HBO therapy was provided 5-7 days after craniocerebral trauma, there was apparent improvement in cognitive function and neuroplasticity.

MeSH Keywords: **Brain Injuries • Hyperbaric Oxygenation • Neuronal Plasticity**

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Background

The cerebrum is the most complex and important organ of the human body. Even though the quantity of brain tissue accounts for only 2–3% of the total body weight, it requires 20–25% of the total oxygen supply. Because of the limited oxygen and glucose reserves, brain tissue is susceptible to hypoxic-ischemic injury. Indeed, tolerance of brain tissue for hypoxic-ischemic injury is poor. Young and middle-aged patients are a high-risk population for traumatic brain injury, and cognitive impairment is one of the most common symptoms of patients with moderate and severe brain injuries. Further, hypoxic-ischemic brain injury may seriously affect quality of life and prognosis. At present, clinical treatment of hypoxic-ischemic injury is giving priority to cognitive training. No convincing evidence exists that the cognitive impairment can be improved by medications.

Hyperbaric oxygen (HBO) is a historical therapeutic option in the treatment of various types of brain damage. HBO functions as follows in the treatment of brain injuries: (1) by correcting local brain tissue hypoxia, the oxygen partial pressure increases nearly 20-fold and strengthens the ability of oxygen to infiltrate local brain tissue; (2) HBO can improve the oxygen supply and blood supply in the brainstem reticular system and induce the excitability of brain cells, which plays a role in the promotion of consciousness; (3) by eliminating cerebral edema and protecting brain cells; and (4) by promoting the recovery of neural functionality and reducing sequelae [1–4].

Material and Methods

Laboratory animals and grouping

All procedures were in accordance with the Chinese Capital Medical University Animal Welfare Guidelines and approved by the Institutional Animal Care and Use Committee of the Chinese Capital Medical University. Seventy clean and healthy male Sprague Dawley (SD) rats (3 months old; 275±25 g) were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). The rearing conditions were as follows: temperature, 21±1°C; light/dark cycle, 12 h/12 h; illumination time, 19:00–7:00; sufficient moisture and food were provided daily pre- and post-operatively; and there was an 8-hour fast before surgery. The SD rats were randomly divided into control (n=10) and intervention (n=60) groups. Randomized SD rats in the intervention groups had HBO treatment for 1, 3, and 5 days and 1, 2, and 4 weeks (n=10 in each group), while routine breeding resumed in the control group. All rats were given 7 days of treatment.

Modeling

Described by Dixon [5], the models were established by ECC16.3. Abdominal anesthesia with 10% chloral hydrate (0.3 mL/100 g) was prepared for bilateral frontal craniotomy surgery. Craniotomies were performed 3 mm from the center line between the anterior fontanelle and lambdoid suture. The sham-operation group underwent craniotomy surgery without being hit.

HBO therapy

The rats in the intervention group were put into a small high-pressure oxygen cabin for animals (Hong Yuan GY3200, Yantai, China). The cabin was washed with pure oxygen for 10 min, then a uniform pressurization was initiated to achieve a 0.2 MPa cabin pressure in 10 min and maintained for 30 min. The oxygen was supplied discontinuously to maintain a pure oxygen status. The stress decreased to an atmospheric pressure for 20 min at a constant rate. Finally, the rats were exposed to atmospheric pressure for 10 min. The above-mentioned process was considered as single therapy. HBO therapy was performed four times during the day, each 30 min apart for 7 days continuously [6].

Water maze test

The water temperature was controlled at 28±1°C. Melan was added to the water to shield the platform underwater, and to reduce the influence of positioning [7]. Two experiments were conducted. (1) Navigation experiment: The platform was put in 1 quadrant 2 cm beneath the surface. When heading towards the wall, the rats were randomly put into the other three quadrants without a platform in daily experiments. If the rat could not locate the hidden platform in 60 s, the rat would be guided to the platform and remain for 10 s continuously for 3 days. Four sessions of training were necessary every day for each rat with intervals of 15–20 min [8]. (2) Space exploration experiment: The day after the navigation experiment, the rats were put into the water from the contralateral quadrant of platform one. The percentage of time spent locating the quadrant with the platform in the total time would be recorded in 60 s. The water maze baseline was measured in all rats 1 day before modeling. The percentage of residence time during escape latency, in the target quadrant, and the total time were recorded for the water maze test 8 weeks after controlled cortical impact (CCI).

Growth-associated protein-43 (GAP-43) and synaptophysin (Syn) expression

After the behavioral test, the rats were sacrificed and the brain tissues were removed. The brain tissues were placed on ice;

Table 1. Escape latency changes in the water maze test in different groups.

Group	Case (n)	Escape latency		P value	
		Baseline	8 weeks later	Compared with self baseline	Compared with control 8 weeks later
Control	10	26.55±3.11	70.86±7.88	<0.01	–
1 day	10	27.74±3.26	67.89±10.14	<0.01	0.147
3 days	10	28.54±4.88	38.25±9.33	0.142	0.021
5 days	10	25.29±4.69	39.44±7.87	0.086	0.033
1 week	10	26.32±3.76	46.65±8.11	0.043	0.039
2 weeks	10	27.05±3.24	49.06±11.24	0.040	0.042
4 weeks	10	26.58±3.43	56.27±11.03	0.028	0.049

the hippocampuses were isolated and frozen in liquid nitrogen. GAP-43 and Syn expression were detected by Western blot analysis. Immunohistochemical images were captured and analyzed by Image-Pro Plus 6.0 software. Three photographs magnified 200 times were randomly selected from each slice and from each group. Brain tissues should fill the entire field of vision when taking photos to ensure the background light is consistent in each photo. Image-Pro Plus 6.0 software was used to select the same degree of tan as a uniform standard to assign positive results in all photos, and the integrated optical density (IOD) was analyzed and concluded in all photos.

Statistical analysis

Data were analyzed by SPSS 16.0 software. Count data are denoted by absolute values and percentages, while measurement data are recorded as the mean ± standard deviation. The results of the water maze test were determined with repetitive measurement deviation analysis, and the measurement data were compared with single factor analysis of variance by the least significant difference (LSD) test post hoc. Single factor variance analysis was applied in analysis of time spent in the targeted quadrant in the spatial probe test between each group, and the LSD test was applied in comparisons between each group. A value of $P < 0.05$ was considered statistically significant.

Results

Spatial learning ability was reflected by changes of escape latency in the water maze test. The shorter the incubation period, the more prompt the spatial learning ability. The results are shown in Table 1.

No significant difference was found between each group before the test. Eight weeks later, no statistical differences ($P > 0.05$)

existed in the 3- and 5-day groups when compared with baseline. The other groups were statistically different based on auto-comparison ($P < 0.05$). Compared with the control group, significant differences ($P < 0.05$) existed in all of the groups with the exception of the 1-day group after 8 weeks.

Changes in residence time in the targeted quadrant were tested by a water maze. The higher the percentage of residence time in the target quadrant during escape latency in the total time, the stronger the spatial memory ability; the results are shown in Table 2.

No significant difference existed between each group before testing; 8 weeks later, no statistical difference ($P > 0.05$) existed in the 5-day and 1-week groups when compared with baseline. The other groups were statistically different based on auto-comparison ($P < 0.05$); a significant difference ($P < 0.05$) existed in all of the groups with the exception of the 1-day group after 8 weeks when compared with the control group.

The effect of HBO on expression of GAP43 and Syn in different groups is shown in Table 3 and Figures 1, 2.

Eight weeks later, no statistical difference ($P > 0.05$) was observed in the level of expression of GAP-43 and Syn in the 1-day group compared with the control group. When comparing the remaining groups and the control group, there were statistically significant differences ($P < 0.05$). The level of expression of GAP-43 and Syn was significantly different compared with that in the control group ($P < 0.01$) in the 5-day, 1-week, and 2-week groups.

Discussion

Over the past 30 years, irreversible cellular damage after traumatic brain injury has been attributed to the following four

Table 2. Changes in residence time in the targeted quadrant during the water maze test.

Group	Case (n)	Percentage of residence time in the target quadrant during escape latency in total time (%)		P value	
		Baseline	8 weeks later	Compared with self baseline	Compared with control 8 weeks later
Control	10	56.5±4.0	17.2±6.8	<0.01	–
1 day	10	54.3±4.3	20.4±5.6	<0.01	0.224
3 days	10	54.8±6.2	30.8±7.7	0.041	0.036
5 days	10	58.9±7.7	44.2±9.4	0.077	<0.01
1 week	10	57.7±4.9	50.4±8.6	0.102	<0.01
2 weeks	10	57.5±6.6	36.5±8.0	0.056	<0.01
4 weeks	10	56.4±6.5	25.9±4.8	<0.01	0.045

Table 3. IOD value of GAP-43- and Syn-positive cells in the hippocampuses of rats compared with control.

Group	n	GAP43	Syn
Control	10	23437±1566	6136±1566
1 day	10	22829±2036	7357±1079
3 days	10	29337±5329 [#]	18767±5538 [#]
5 days	10	43209±8874 [*]	29778±6324 [*]
1 week	10	53117±8766 [*]	38224±6096 [*]
2 weeks	10	38847±2358 [*]	37453±3870 [*]
4 weeks	10	30564±5378 [#]	30372±6433 [*]

[#] Represents the comparison with control, P<0.05; ^{*} represents the comparison with control, P<0.01.

main mechanisms: (1) excitatory poisoning; (2) oxygen/nitric oxide overload; (3) inflammatory reactions; and (4) apoptosis. Anoxia is an important cause of irreversible cellular damage after traumatic brain injury; thus, a protective effect is identified in ischemic tissue by increasing body oxygen levels. Based on the principle that HBO therapy can improve the concentration of free oxygen in plasma by dozens of times, oxygen diffuses to ischemic tissue and reduces dependence on oxyhemoglobin. In recent years, it has been reported that HBO functions at biochemical, molecular, and hemodynamic levels [9–16]. The detailed mechanism by which HBO functions is as follows: when the body suffers hypoxic-ischemic brain damage (HIBD), ATP synthesis is reduced, which increases glutamate release and calcium overload; over-production of free radicals and inflammation aggravates the mitochondrial damage, which is the organelle that produces ATP. With respect to the time window of HBO therapy, it has been reported that cerebral cellular edema exists to different degrees following local anoxia. Indeed, the only way to correct local hypoxia is HBO treatment, which is conducive to help save and

preserve the dying brain cells. Thus, the curative effect is superior when edema is present. Another study showed that delayed HBO treatment still has an effect [17]. Is earlier treatment better? How long should treatment be extended before the effect declines? Does the same mechanism underlie the curative effect in acute and chronic phases of cerebral damage treated by HBO? It has been shown that when given the same course of HBO therapy (1 week), the rats that began treatment 1 hour or 2 weeks after modeling did not differ significantly in the water maze test (escape latency and percentage of target quadrant residence time in total time) at the end 8 weeks, which indicated that the long-term curative effect of partial learning and memory was not affected by HBO treatment. When compared with the results after 4 weeks of treatment, the recovery of spatial learning and memory is less satisfactory after 8 weeks of treatment; thus, HBO therapy has an impact on the long-term curative effect of cognitive function; however, this finding does not suggest that the sooner treatment is begun, the better. Nevertheless, earlier and continuous HBO treatment will yield the best effect. During the

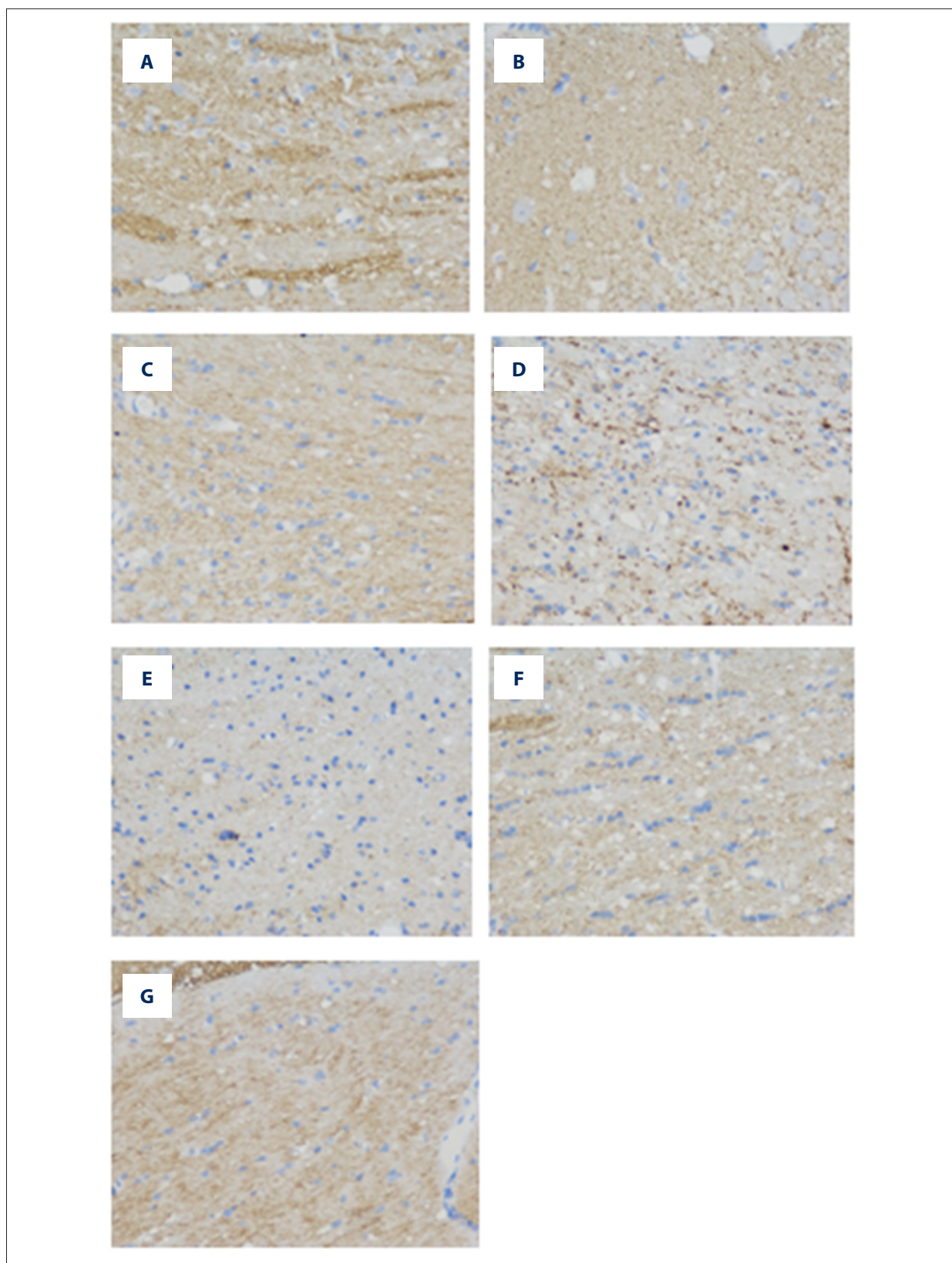


Figure 1. Expression of GAP43 in the hippocampus (*400 \times , GAP43-positive cells stained blue). (A) Control group. (B) One-day group. (C) Three-day group. (D) Five-day group. (E) One-week group. (F) Two-week group. (G) Four-week group.

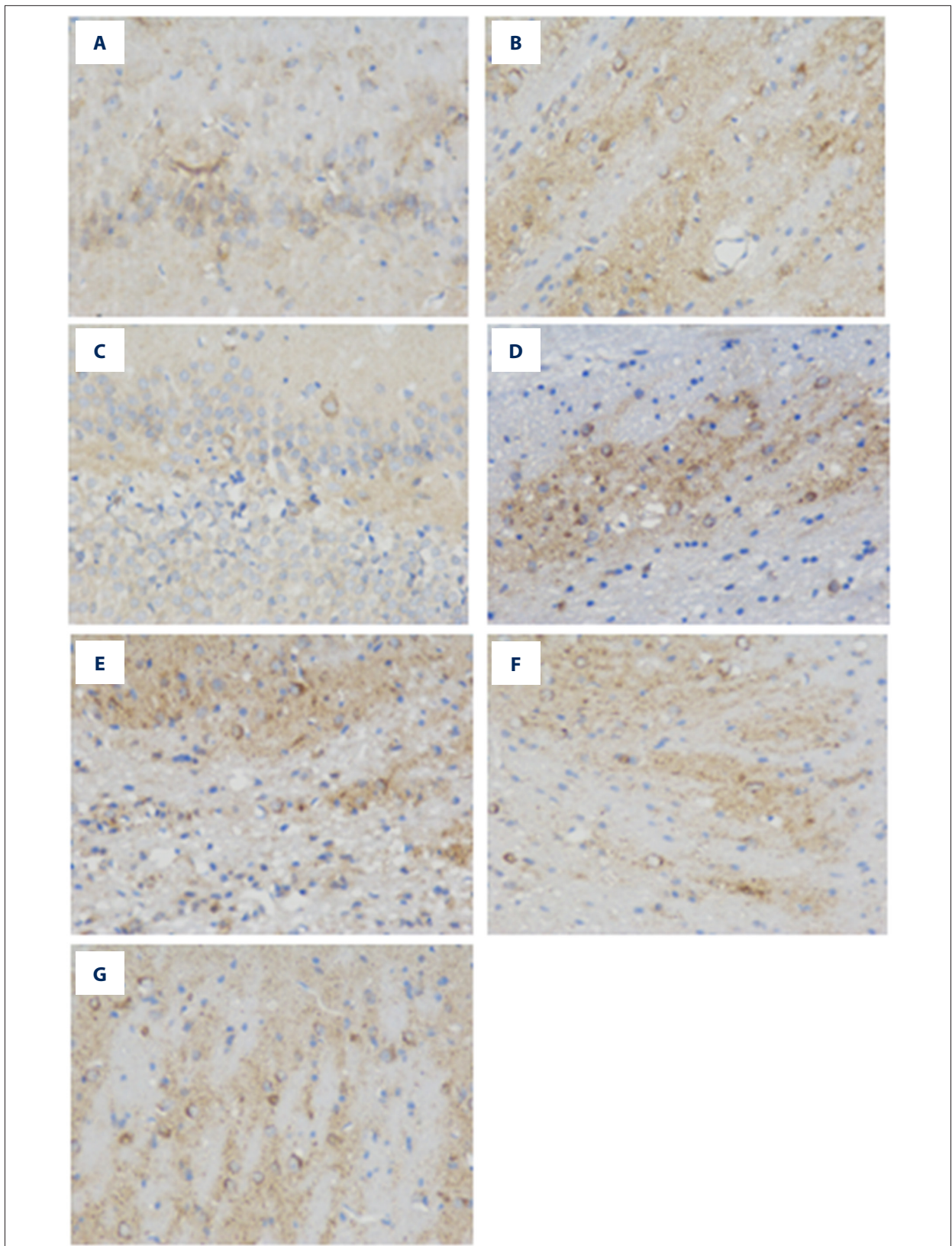


Figure 2. Syn expression in the hippocampus (*400x, Syn-positive cells stained blue). (A) Control group. (B) One-day group. (C) Three-day group. (D) Five-day group. (E) One-week group. (F) Two-week group. (G) Four-week group.

acute phase of brain injury, the mechanism of HBO treatment has been further studied. HBO largely functions by inhibiting the inflammatory reaction and anti-apoptosis resistance to promote restoration of neural cells. Zhang et al. [12] showed that HBO can protect the blood-brain barrier, relieve cerebral edema, preserve the content of glutamate, glucose, and pyruvic acid salt in a relatively stable state in the body, inhibit inflammatory reactions and apoptosis, adjust superoxide dismutase, enhance the expression of Bcl-2, which is a key gene that promotes cell survival in the body, and increase the deformation ability of erythrocytes to improve the microcirculation and reduce the hypoxic-ischemic state. Cheng [9] reported that in a rat stroke model, HBO reduced the mRNA and protein levels of cyclooxygenase-2 and reduced neutrophil invasion, thus inhibiting the inflammatory reaction. Furthermore, interleukin-10 and angiogenesis contribute to the neuroprotection induced by HBO pre-conditioning against brain injury in rats [18].

The studies that have focused on a curative effect and the mechanism underlying the chronic phase of brain damage treated by HBO are fewer. Kraitsy et al. [19] reported that the protective long-term HBO treatment effects following brain injury are mediated by a pronounced remyelination in the ipsilateral injured cortex, as substantiated by the associated recovery of sensorimotor function. Mu et al. [17] reported that delayed daily HBO treatment presents as a potent neuroprotectant in pMCAo rats, which increases CREB expression and signaling activity and bolsters proliferation of regenerative cells in the injured brain. As shown in the acute experiment, the effects of HBO are likely mediated by reducing ubiquitin-dependent CREB degradation owing to HBO-induced activation of PP1 gamma [17].

GAP-43 is also known as neuromodulin, which is an axonal membrane protein and a type of neural-specific protein. GAP-43 participates in neural cell growth and regeneration and the development of synapse formation. GAP-43 is highly expressed in the process of development and regeneration of neurons; and it regulates axonal extension and changes cell morphology. As an intracellular signal, GPA-43 can significantly enhance and transfer function of G protein coupled receptor. Syn exists in nearly all of the neural endings in the central and peripheral nervous systems. Simultaneously, synapses also express Syn in neuroendocrinal cells [20,21], which participate in importing and transporting synaptic vesicles, neurotransmitter release, synaptic vesicle recycling, and synaptogenesis [22].

References:

1. Chen LF, Tian YF, Lin CH et al: Repetitive hyperbaric oxygen therapy provides better effects on brain inflammation and oxidative damage in rats with focal cerebral ischemia. *J Formos Med Assoc*, 2014; 113: 620-28
2. Duan S, Shao G, Yu L, Ren C: Angiogenesis contributes to the neuroprotection induced by hyperbaric oxygen preconditioning against focal cerebral ischemia in rats. *Int J Neurosci*, 2015; 125: 625-34

Syn can serve as a specific marker of the presynaptic terminal to detect the density and distribution of synapses [23] and has been widely applied in the study of synaptogenesis in animal models and human disease, and is considered to be an important marker of neural plasticity.

In the current study we investigated the influence of neural plasticity in the acute and chronic phases of ischemic-hypoxic brain injury in rats treated with HBO through determination of the neural plasticity index (GAP-43 and Syn) at 8 weeks in the hippocampus and striatum. The experimental results are shown below, but when given the same course of HBO therapy (1 week), the expression of GAP-43 and Syn in the 5-day, 1-week, and 2-week groups was significantly higher than that in the other groups in the hippocampus and striatum, especially in the 1-week group. The level of expression of GAP-43 and Syn was not completely consistent with the results of the water maze test, which may indicate that if the start time of HBO is different, the mechanism of improving cognitive function will be different. The mechanism of HBO therapy in the acute phase of hypoxic-ischemic brain injury is mainly by inhibiting inflammatory reactions and anti-apoptosis effects to promote restoring neural cells. The later the initiation of HBO, the more significant the effect on promoting neural plasticity. The effect on the improvement of cognitive function is not apparent with early treatment; thus, a long-term study is warranted.

Conclusions

Initiation of HBO treatment has an influence on long-term cognitive function, but this finding does not mean that the sooner the treatment, the better. In the current study, neither cognitive function nor neural plasticity was significantly improved in rats 1 day immediately after craniocerebral trauma. The most significant improvements in cognitive function and neural plasticity appeared when HBO therapy was provided 5-7 days after brain trauma. If HBO therapy was initiated >2 weeks after hypoxic-ischemic brain injury, the improvement in cognitive function was less than that observed with earlier treatment, while the effect on neural remodeling was still evident. Indeed, the best prognosis is achieved by earlier and continuous HBO treatment.

Conflict of interest

There are no conflicts of interests associated with this study.

3. Xue F, Huang JW, Ding PY et al: Nrf2/antioxidant defense pathway is involved in the neuroprotective effects of Sirt1 against focal cerebral ischemia in rats after hyperbaric oxygen preconditioning. *Behav Brain Res*, 2016; 309: 1–8
4. Dong W, Qi Z, Liang J et al: Reduction of zinc accumulation in mitochondria contributes to decreased cerebral ischemic injury by normobaric hyperoxia treatment in an experimental stroke model. *Exp Neurol*, 2015; 272: 181–89
5. Dixon CE, Lyeth BG, Povlishock JT et al: A fluid percussion model of experimental brain injury in the rat. *J Neurosurg*, 1987; 67(1): 110–19
6. Guseva MV, Kamenskii AA, Gusev VB: Optimization of choline administration regimen for correction of cognitive functions in rats after brain injury. *Bull Exp Biol Med*, 2013; 155: 197–99
7. Duda W, Wesierska M, Ostaszewski P et al: MK-801 and memantine act differently on short-term memory tested with different time-intervals in the Morris water maze test. *Behav Brain Res*, 2016; 311: 15–23
8. Kim SJ, Kwak HH, Cho SY et al: Pharmacokinetics, pharmacodynamics, and efficacy of a novel long-acting human growth hormone: Fc fusion protein. *Mol Pharm*, 2015; 12: 3759–65
9. Cheng O, Ostrowski RP, Wu B et al: Cyclooxygenase-2 mediates hyperbaric oxygen preconditioning in the rat model of transient global cerebral ischemia. *Stroke*, 2011; 42(2): 484–90
10. Kayano AC, Dos-Santos JC, Bastos MF et al: Pathophysiological mechanisms in gaseous therapies for severe malaria. *Infect Immun*, 2016; 84: 874–82
11. Rockswold SB, Rockswold GL, Zaun DA, Liu J: A prospective, randomized Phase II clinical trial to evaluate the effect of combined hyperbaric and normobaric hyperoxia on cerebral metabolism, intracranial pressure, oxygen toxicity, and clinical outcome in severe traumatic brain injury. *J Neurosurg*, 2013; 118: 1317–28
12. Zhang Y, Yang Y, Tang H et al: Hyperbaric oxygen therapy ameliorates local brain metabolism, brain edema and inflammatory response in a blast-induced traumatic brain injury model in rabbits. *Neurochem Res*, 2014; 39: 950–60
13. Duan S, Shao G, Yu L, Ren C: Angiogenesis contributes to the neuroprotection induced by hyperbaric oxygen preconditioning against focal cerebral ischemia in rats. *Int J Neurosci*, 2015; 125: 625–34
14. Lim SW, Wang CC, Wang YH et al: Microglial activation induced by traumatic brain injury is suppressed by post injury treatment with hyperbaric oxygen therapy. *J Surg Res*, 2013; 184: 1076–84
15. Liu XH, Yan H, Xu M et al: Hyperbaric oxygenation reduces long-term brain injury and ameliorates behavioral function by suppression of apoptosis in a rat model of neonatal hypoxia-ischemia. *Neurochem Int*, 2013; 62: 922–30
16. Sahni T, Jain M, Prasad R et al: Use of hyperbaric oxygen in traumatic brain injury: retrospective analysis of data of 20 patients treated at a tertiary care centre. *Br J Neurosurg*, 2012; 26: 202–7
17. Mu J, Ostrowski RP, Soejima Y et al: Delayed hyperbaric oxygen therapy induces cell proliferation through stabilization of cAMP responsive element binding protein in the rat model of MCAo-induced ischemic brain injury. *Neurobiol Dis*, 2013; 51: 133–43
18. Chen X, Duan XS, Xu LJ et al: Interleukin-10 mediates the neuroprotection of hyperbaric oxygen therapy against traumatic brain injury in mice. *Neuroscience*, 2014; 266: 235–43
19. Kraitsy K, Uecal M, Grossauer S et al: Repetitive long-term hyperbaric oxygen treatment (HBOT) administered after experimental traumatic brain injury in rats induces significant remyelination and a recovery of sensorimotor function. *PLoS One*, 2014; 9(5): e97750
20. Kleber J, Chen YC, Michels B et al: Synapsin is required to «boost» memory strength for highly salient events. *Learn Mem*, 2015; 23: 9–20
21. Yin Y, Huang P, Han Z et al: Collagen nanofibers facilitated presynaptic maturation in differentiated neurons from spinal-cord-derived neural stem cells through MAPK/ERK1/2-Synapsin I signaling pathway. *Biomacromolecules*, 2014; 15: 2449–60
22. Dai J, Chen P, Tian H, Sun J: Spontaneous vesicle release is not tightly coupled to voltage-gated calcium channel-mediated Ca²⁺ influx and is triggered by a Ca²⁺ sensor other than synaptotagmin-2 at the juvenile mice calyx of held synapses. *J Neurosci*, 2015; 35: 9632–37
23. Gimber N, Tadeus G, Maritzen T et al: Diffusional spread and confinement of newly exocytosed synaptic vesicle proteins. *Nat Commun*, 2015; 6: 83–92