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Hyperbaric oxygen therapy protects rats from hypoxic2ischemic brain damage

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Abstract: Objective To investigate the protective effects of hyperbaric oxygen (HBO) against hypoxic ischemic brain damage (HIBD) of neonatal rats. **Methods** Seven2day2old Sprague2Dawley (SD) rat pups were randomly divided into 4 groups (n = 10 each): Control group, HIBD group, Hyperbaric air (HBA) group, and Hyperbaric oxygen (HBO) group. The HIBD model was produced by permanent occlusion of left common carotid artery and followed by exposure to a mixture of 8 % oxygen and 92 % nitrogen for 2 hrs (at 37 ℃). HBO and HBA treatments [2 atmosphere absolute (ATA) for 1 hr] were administered once daily to rats in the HBO and HBA group respectively after hypoxia for 7 days. Radial arm maze and sensorimotor functional tests were administered from 30 to 35postnatal days. At the end of the behavior trials, the rats were sacrificed and cerebral histology was analyzed. The CA_1 subfield neurons numbers were counted to evaluate the brain damage. **Results** In the behavior test, the HIBD group showed different degrees of neurological damage. HBO treatment resulted in significant protection against hypoxia2ischemia induced behavior impairments (all $P < 0.01$). However, the HBA group did not show any significant improvement. There was a significant reduction of CA_1 neuron density in the left hemisphere of HIBD and HBA groups, compared with that of the Control group and the HBO group (all $P < 0.01$). **Conclusion** HBO therapy can attenuate brain damage and improve learning, memory and sensorimotor functions in neonatal rats after HIBD.

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Key words: Hypoxia2ischemia, brain; Hyperbaric oxygen; Behavior; Rat, neonatal

高压氧治疗对新生大鼠缺氧缺血性脑损伤的保护作用

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[**摘　要**] **目的**　探讨高压氧(HBO)治疗对新生大鼠缺氧缺血性脑损伤(HIBD)的保护作用。**方法**　7 日龄 Sprague2Dawley(SD)新生大鼠随机分为 4 组(n = 10):正常对照组,HIBD 组,高压空气治疗组(HBA)和高压氧治疗组(HBO)。HBA 组和 HBO 组于缺氧缺血后分别行 HBA 和 HBO 治疗[2 个绝对压(ATA),1 h],每日 1 次连续 7 d。至 30 日龄开始对各组动物进行放射形迷宫测试以及感觉运动功能检测。行为学试验验结束后(日龄 35 天),采用尼氏染色检测海马 CA_1 区锥体细胞密度。**结果**　HIBD 组大鼠的学习、记忆和感觉运动功能受损严重,HBO 组行为学损伤明显改善,而 HBA 组改善不明显。HIBD 组和 HBA 组左脑 CA_1 区锥体细胞密度低于 HBO 和对照组($P < 0.01$)。**结论**　HBO 治疗能够减少新生大鼠缺氧缺血后脑损伤,促进学习记忆功能和感觉运动功能的改善。

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[**关　键　词**]　缺氧缺血,脑;高压氧;行为;大鼠,新生

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Despite advances in obstetrics, the morbidity of neonatal hypoxic2ischemic brain damage (HIBD) has not been reduced. In every 1 000 term newborns, 5.5 developed HIBD[1]. HIBD is one of the major

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courses inducing cerebral palsy and mental retardation; but there is presently no specific therapy. Hyperbaric oxygen (HBO) has been broadly used in clinical medicine, and shows affirmative therapeutic effects, but its effectiveness in the treatment of neonatal HIBD has been questioned. Many studies have shown that HBO has a protective effect[2,3]; while others have shown that it has none[4,5]. The reasons for this controversy are versatile, and probably relate to different animal species, inconsistent standard in judging HIBD, different times, duration, pressure of HBO therapy, and various indices of the therapeutic effect. Short-term histopathologic examinations rather than long term behavior functional observations were used in previous investigations. However, immature brain has strong plasticity, so the neuropathologic and biochemical changes several days after HIBD are not the ideal indices for brain damage judgment. Furthermore, morphologic changes are not always positively related to the functional improvement, so the evaluation of neuroprotective effect should combine the histological and functional indices. This study explored whether HBO, with the optimal pressure and duration[6], has a protective effect against HIBD in neonatal rats from both views of histology and functional behavior test.

Materials and methods

Animal modeling and grouping

Seven-day-old Sprague-Dawley (SD) neonatal rats (Laboratory Animal Center of Xiangya School of Medicine, Central South University) were randomly assigned into 4 groups (n = 10 each): Control group, HIBD group, hyperbaric air therapy (HBA) group, and HBO therapy (HBO) group. The rats in the Control group did not receive any treatment. The HIBD model was induced in rats of the HIBD group, HBA group and HBO group by left common carotid artery ligation and hypoxia exposure (8% oxygen, 37 ℃ for 2 hours) according to the Rice method[7]. They were weaned at 21 days old, separated by gender and sacrificed at 35 days old.

HBO and HBA therapy

Rats in the HBO group received HBO therapy once daily for 7 days after hypoxia. The pressure was 2 atmospheres absolute (ATA), and time course of pressure rising and falling was 20 - 30 minutes, and the stabilizing duration was 60 minutes. In the HBA group, the pressure and the time duration were the same as the HBO group, while oxygen was replaced by air.

Behavior test

The behavior test was done by the blind method in a quiet environment at a fixed time and location.

Radial arm maze test[8]

This test was performed with 30-day-old animals. The 8-arm radial maze consisted of a central platform (30 cm diameter) from which 8 arms radiated symmetrically (50 cm long and 12 cm wide). A well was present at the outer end of each arm. Animals were deprived of water for 48 hours before testing. At the end of each daily session, the animals were allowed to drink for 30 minutes. Initially, animals were allowed free exploration sessions on 2 days in a row with all arms baited with water (50 μL per well). For spatial discrimination testing, only 3 arms were always baited, and the sequence of angles between them was 135°, 90°, and 135°. Rats were tested for acquisition over 3 daily sessions composed of 5 trials separated by 12-minute intervals. Each trial began with the placement of the animal on the central platform-facing arm No. 3 and ended when the rat had visited the 3 baited arms. The following data were recorded: 1) time taken to visit the 3 baited arms; 2) number of working memory errors, for example, reentries into already visited baited arms; and 3) number of reference memory errors, for example, each entry into a non-baited arm.

Sensorimotor test[9]

The rats were tested at a postnatal age of 34 days.

Foot-fault test Each rat was placed on a horizontal grid floor (50 ×40 cm, square size 3 ×3 cm, wire diameter 0.4 cm). The foot-fault was defined as when a rat misplaced a fore- or hind-limb and the paw fell through between the grid bars. The number of foot-faults was recorded for 2 minutes. Only the side difference of foot-faults was used for the statistical evaluation to eliminate the influence of the extent of activity.

Limb-placing test Each rat was held by the examiner, and the fore- and hind-limb placement after

different sensory stimuli was recorded as follows: 0 score: immediate and correct paw placing; 1 score: delayed and/or incomplete correction; 2 scores: no placing. Side differences were recorded for each rat. 1) Visual limb placing was tested by lowering the rat toward a table. 2) Forelimb sensory input was tested with the rat's forelimbs touching a table edge. 3) Forelimb placement was tested when the rat was facing the edge of the table. 4) Both forelimb and hindlimb placement was tested when the rat was held by the examiner and slowly moved laterally toward the edge of the table. 5) The rat was placed on the table and gently pushed laterally toward the edge of the table. 6) As 5 above, but the rat was pushed from behind.

Histology

At the end of the experiments (at 35 days old), the rats were anesthetized with ether and perfused intracardially with 0.9% saline followed by 4% paraformaldehyde in PBS. Brain sections were then cut at 10μm on a cryostat. Hippocampal region slices were chosen for Nissl's staining, and CA_1 region positive cell numbers were counted in 3 different planes of dorsal hippocampus under oil immersion lens (Nikon, ×60) (the area of every slice was 5 000 μm^2), and average positive cell density in hippocampal CA_1 region was obtained (cells/mm^2).

Statistical analysis

Data were presented as $x \pm s$. Statistical analysis was performed using SPSS 11.0. Differences among various groups were evaluated by one-way ANOVA, and between two groups were evaluated by t test. The comparison in foot-fault and limb-placing tests were done using Mann-Whitney U test.

Results

Behavior test results

In the radial maze test, significant differences were shown between the groups in the mean time taken to perform the task. The HIBD group (145 ± 23 s) and the HBA group (142 ±20 s) took more time in finding the 3 arms baited with water than the Control group (107 ±11 s) and the HBO group (119 ±28 s) (all $P < 0.05$). Significant group differences were also found in both working memory (14.2 ±2.9, 13.3 ±2.2 vs 10.3 ±1.0, 11.2 ±3.2) and reference memory (1.9 ±0.7, 1.9 ±0.8 vs 0.6 ±0.4, 0.9 ± 0.4) (all $P < 0.05$). The HBO group performed much better than the HIBD and HBA groups.

In the foot-fault test, limb misplacements in control animals were symmetrical. The difference (right-left side) was 0.7 ±1.4 in the Control group, 3.0 ±1.1 in the HIBD group and 2.7 ±1.5 in the HBA group (both $P < 0.01$). In the HBO group, the difference (0.7 ±1.4) was significantly less than the HIBD and HBA groups (both $P < 0.01$).

In the limb-placing test, the side difference of all control rats and HBO rats was 0 - 1, while that of most rats in the HIBD and HBA groups was 2 - 3. The side difference of HBO group was significantly less than that of HIBD group and HBA group ($Z = -3.400$, $P < 0.01$; $Z = -3.436$, $P < 0.01$).

Histology

The pyramidal cell density in the CA_1 region in the left side was significantly lower than that of the right side of the HIBD and HBA groups ($P < 0.01$) and also the left side of the HBO group and the Control group (both $P < 0.01$, See Table 1).

Table 1 Comparison of numbers of CA_1 neuron in different groups ($n = 10$, $x \pm s$)

Group	Left	Right
Control	251 ±31	251 ±34
HIBD	85 ±32[a]	231 ±22[d]
HBA	90 ±24[a]	230 ±26[d]
HBO	175 ±31[b,c]	235 ±29

Note: a Compared with the Control group $P < 0.01$; b Compared with the HIBD group $P < 0.01$; c Compared with the HBA group $P < 0.01$; d Compared with left side of the same group $P < 0.01$

Discussion

The behavior tests showed that the rats in the HIBD group manifested long lasting significantly decreased spatial discrimination, learning and remembering capabilities and sensorimotor functions, which is consistent with that reported in literature[9,10,11,12]. Hippocampus has a close relationship with the functions of spatial discrimination, learning

and remembering, while the sensorimotor function reflects the status of the sensorimotor cortex. Perinatal hypoxia-ischemia induced diffused brain damage, including both hippocampus and sensorimotor cortex. Nissl's staining also showed significantly decreased neuron density in the hippocampus of the ischemic hemisphere.

Our previous studies have shown that the optimal pressure of HBO used in the treatment of HIBD in rats was 2 ATA[6]. The result of this research showed that the histological and behavior impairment caused by HIBD had apparent restoration after HBO therapy. However, the HBA treatment has no therapeutic effect. These results indicate that HBO therapy has a protective effect against HIBD, and this effect relies mainly on oxygen rather than pressure.

The pathophysiological changes occurring in the brain after HIBD include brain edema, excess calcium load, excitatory amino acids toxicity and free radical toxicity. Hyperbaric oxygen not only can induce vasoconstriction, reduce blood flow, decrease intracranial pressure, but can also increase oxygen pressure of tissue. Blood oxygen in high tension can diffuse further than normal. This can improve the brain ischemia and microcirculation, and let the function and structure of brain cells to restore gradually, thereby accelerating the repairing process of brain tissues after damage. In recent years it has been shown that HBO can also promote the formation of nascent capillaries, regeneration of nerve axons and the growth of nerve buds in the damaged area of the brain, thus enhancing the plasticity and reorganization of brain functions. HBO can also accelerate the functional restoration of the neurological system in dogs after brain ischemia[13]. However in this study, the behavior restoration in the HBO therapy group was worse than in the controls, which indicated that HBO therapy can not completely reduce the nerve damage after HIBD. Therefore during HBO therapy other treatments should be used in parallel to gain a better therapeutic effect.

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