·论著(译文)·

Relationship of Dose-Effect and Time-Effect to Hyperbaric Oxygen Therapy on Hypoxic-Ischemic Brain Damage in the Neonatal Rat

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Abstract: Objective To find the best pressure and time of hyperbaric oxygenation (HBO) therapy by comparing different pressures and times in a rat model of neonatal hypoxic-ischemic brain damage (HIBD) and to provide the mechanisms of HBO therapy for neonatal clinical application. **Methods** 7-day-old SD rats were randomly divided into a control(CON) group, HIBD group and HBO group. The HBO group was divided into 7 sub-groups according to the different HBO pressure: 1 ATA(H 1.0), 1.25 ATA(H 1.25), 1.5 ATA(H 1.5), 1.75 ATA(H 1.75), 2 ATA(H 2.0), 2.25 ATA(H 2.25) and 2.5 ATA(H 2.5) group. After choosing the best pressure we divided the rats into two groups according to the different HBO time: 30 min group (H 30 min) and 60 min group (H 60 min). All the rats(14-day-old) were killed at 7 days after HI. Body weight increasing, left/right brain weight ratio, neuropathologic examination and superoxide dismutase (SOD) and malondialdehyde (MDA) content in brain tissue were measured in each group. **Results** ①The weight increasing rate [(72.91 ±21.27) %] and the left/right brain weight ratio (0.6444 ±0.1599) decreased obviously. At the same time the content of SOD [(203.32 ±57.18) NU/ml] in brain tissue decreased and that of MDA [(4.197 ±0.683) nmol/ml] increased in HIBD group. All above data showed a more significant difference in HIBD group compared to the control group [weight increasing rate = (104.97 ±17.34) %, left/right brain weight ratio = 0.9857 ±0.0396, SOD content = (261.53 ±41.38) NU/ml, MDA content = (3.393 ±0.574) nmol/ml] ($P < 0.01$). ②Following the increasing HBO pressure the weight increasing rate and left/right brain weight ratio gradually increased, the content of SOD in brain tissue increased and that of MDA decreased, and to the best in H 2.0 group [left/right brain weight ratio = 0.8583 ±0.1544, SOD content = (259.20 ±45.22) NU/ml, MDA content = (3.462 ±0.498) nmol/ml, compared to HIBD group, $P < 0.01$ or < 0.05]. ③The weight increasing rate, left/right brain weight ratio, the content of SOD and MDA were higher in H 60min group than in H 30min group, but there was no significant difference between the two groups ($P > 0.05$). **Conclusions** HBO of 2.0 ATA pressure and 30～60 min steady time may have the best effect, and reasonable HBO treatment can increase SOD content and decrease MDA content in brain tissue in HIBD neonatal rats.

Key words: Hypoxic-ischemic brain damage; Hyperbaric oxygenation; Therapy; Neonatal rat

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Hypoxic-ischemic encephalopathy (HIE) is one of the commonest and the most critical brain damages in newborns. Mechanisms of HIE remain unclear, and treatment for HIE is controversial. Hyperbaric oxygenation (HBO) can significantly improve the ability of body to intake and utilize oxygen, increasing the oxygen solubility and partial pressures, and diffusion. Some research shows HBO treatment can also relieve or block nerve cell apoptosis and activate the penumbral neuron. Therefore, HBO has been extensively studied for neonatal HIE in recent 10 years. However, HBO therapy con troversial remains because of neonatal specific physiological behavior and structures. There is a lack of data in the study on HBO dose-effect and time-effect relationship of neonatal animals. To try to find out the mechanisms and effects of HBO therapy in neonatal HIE, we studied the effects of different HBO pressures and times, and at the same time assessed the potential contribution of superoxide dismutase (SOD), malondialdehyde (MDA), in order to find out the best dose and time of HBO therapy and provide the mechanisms

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of HBO therapy for neonatal clinical application.

1 Materials and Methods

1.1 Main devices and reagents

①hyperbaric oxygen chamber: the infant type of YLC 0.5/1A, made in the No. 701 Institut of the Head Office of Shipping Industry of China; ②SOD and MDA detection kit: made in Jian Cheng Bioengineering Institute, Nanjing, China.

1.2 Animal groups

We used 137 healthy 7-day-old Sprague-Dawley (SD) rats as research subject and were randomly divided into 3 groups: control (CON) group, HIBD group and HBO group. HBO group was then divided into two sections. ①The analysis of dose-effect relationship, in which 121 rats were randomly divided into 7 groups according to the different HBO pressure: 1 ATA (H 1.0), 1.25 ATA (H 1.25), 1.5 ATA (H 1.5), 1.75 ATA (H 1.75), 2 ATA (H 2.0), 2.25 ATA (H 2.25) and 2.5 ATA (H 2.5) group. ②The time-effect relationship: after selecting the best pressure 16 rats were divided into two groups: 30min group (H 30 min) and 60min group (H 60 min).

1.3 Animal models

The HIBD model was produced in the traditional Rice model[2]. 7-day-old SD rats were anesthetized with diethyl ether, and the left common carotid artery was ligated. After 30 min～2 hour postoperatively, the rats were subjected to hypoxia in a chamber, and a gas mixture of 8 % oxygen in nitrogen was passed through the chamber. The exposure was terminated after 2 hours.

1.4 HBO therapy

Neonatal rats in HBO group were put into the HBO chamber whthin 15～30 min after being made HIBD. Spending 15 minutes in washing the chamber with pure oxygen, and the oxygen flow was 10 L/min. Time of increasing pressure is 15～20 min, the flow of which is 5～8 L/min. Steady pressure had two stages: firstly, fixing the steady pressure time (30 min) we gave the rats 1 ATA, 1.25 ATA, 1.5 ATA, 1.75 ATA, 2.0 ATA, 2.25 ATA and 2.5 ATA HBO therapy, respectively, in order to get the best pressure. Then we gave them different steady time - - - - 30 min and 60 min. The flow of steady pressure is 3～5 L/min. The time of decompression is 20～30 min. HBO therapy is performed once daily for 7 days.

1.5 Statistical analysis

All of the data were processed with the software of Excel 5.0 or SPSS 10.0. Measurement data were expressed as $\bar{x} \pm s$. Comparison of two-exponent-mean used T test, and for poly-exponent-mean was analysis of variance, for constituent ratio was χ^2 test.

2 Results

2.1 Effect of different pressures of HBO therapy

2.1.1 Changes of weight increasing rate and the left/right brain weight ratio in different groups The weight increased obviously slowly in HIBD group. At 7 days after HI, the weight increasing rate was (72.91 ±21.27) %, and the left/right brain weight ratio decreased to 0.6444 ±0.1599. All above data were significantly decreased in HIBD group than that in the CON [(104.97 ±17.34) %] ($P < 0.01$). Following the increasing HBO pressure, the weight increasing rate gradually increased, but there was no significant difference between HBO groups or HIBD group ($P > 0.05$). On the other hand, the left/right brain weight ratio also gradually increased, and to the highest in H 2.0 group (0.8583 ±0.1544), which was significantly higher than that in HIBD group ($P < 0.01$). The left/right brain weight ratio began to decrease in H 2.25 and H 2.5 group, however, which indicated that brain damage was getting worse under higher HBO pressure (Figure 1～2).

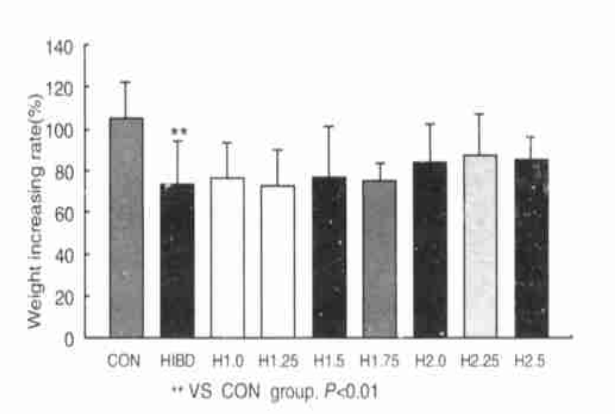

Figure 1 Comparison of weight increasing rate in different groups

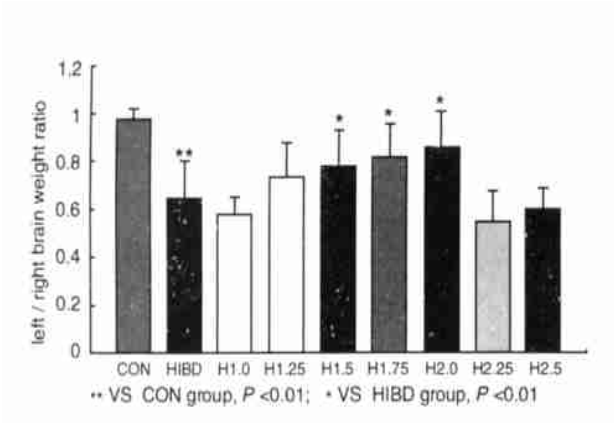

Figure 2 Comparison of left/ reght brain weight ratio in different groups

2.1.2 Morphologic change of left brain tissue in different groups There were three kinds of morphologic change of brain tissue through observation by naked eyes: normal, mild abnormality and serious abnormality. Normal means no change in brain tissue by naked eyes. Mild abnormality means the coverage of atrophy or softening or colliquation in left brain tissue $< 1/2$, and serious abnormality $> 1/2$. Table 1 showed the outcome: the brain abnormal rates in HIBD group was 84.21%. It decreased after HBO therapy, with the lowest value in H 2.0 group (53.85%). Nevertheless, when the HBO pressure increased to 2.25 ATA and 2.5 ATA the brain abnormal rate increased. However, there was no notable significance ($P > 0.05$).

Table 1 Morphologic change of left brain tissue in different groups

	n	Left Brain Abnormality			Brain Abnormal Rate (%)
		Normal	Mild	Serious	
HIBD	19	3	3	13	84.21
HBO 1.0	16	4	5	7	75.00
HBO 1.25	12	4	3	5	66.67
HBO 1.5	18	5	5	8	72.22
HBO 1.75	9	3	4	2	66.67
HBO 2.0	13	6	4	3	53.85
HBO 2.25	8	1	1	6	87.5
HBO 2.5	8	0	0	8	100.0

There were no notable significance among all the groups by χ^2 test ($P > 0.05$)

2.1.3 Changes of SOD and MDA content in brain tissue The content of SOD in brain tissue in HIBD group was (203.32 ±57.18) NU/ml, significantly lower than that in control group [(261.53 ±41.38) NU/ml] ($P < 0.01$). It gradually increased following the increasing HBO pressure, and to the highest in H 2.0 group [(259.20 ±45.22) NU/ml], which was significantly higher than that in HIBD group ($P < 0.05$). SOD content gradually decreased when the pressure was to 2.25 ATA and 2.5 ATA. The content of MDA in brain tissue in HIBD group is (4.197 ±0.683) nmol/ml, significantly higher than that in control group [(3.393 ±0.574) nmol/ml] ($P < 0.01$). It gradually decreased following the increasing HBO pressure, and to the lowest in H 2.0 group [(3.462 ±0.498) nmol/ml], which was significantly lower than that in HIBD group ($P < 0.05$). MDA content returned to increase when the pressure was to 2.25 ATA and 2.5 ATA (Figure 3～4).

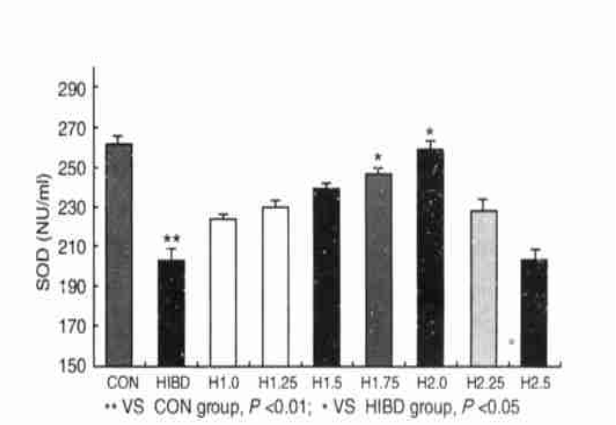

Figure 3 Changes of SOD content in brain tissue in different groups

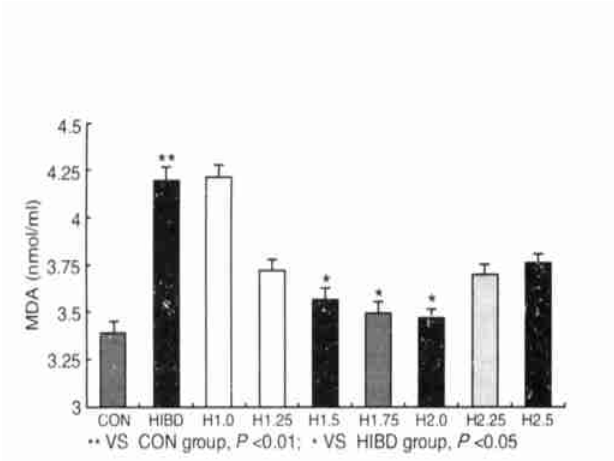

Figure 4 Changes of MDA content in brain tissue in different groups

2.2 Effect of different times of HBO therapy

Selecting the best HBO pressure (2.0 ATA) according to above results. Changing the steady time to 60 min, in order to observe the difference between H 60 min group and H 30 min group.

2.2.1 Comparison of weight increasing rate, left/right brain weight ratio and brain abnormal rate between H 60 min group and H 30 min group The weight increasing rate and left/right brain weight ratio in H 60 min group was (96.75 ±18.71) % and 0.8813 ±0.1250, respective, which was higher than that in H 30 min group (The weight increasing rate was (84.14 ±18.35) %, and left/right brain weight ratio was 0.8583 ±0.1544), but there was no significant difference between the two groups ($P > 0.05$). The brain abnormal rate in H 30 min group was 53.85 %, which includes 30.77 % (4/13) mild abnormality and 23.08 %(3/13) serious abnormality. The brain abnormal rate in H 60 min group was 62.5 %, includes 50.00 % (4/8) mild abnormality and 12.5 %(1/8) serious abnormality. There was no notable significance between the two groups ($P > 0.05$).

2.2.2 Comparison of SOD and MDA content between H 60 min group and H 30 min group The content of SOD and MDA were higher in H 60min group [(296.11 ±53.49) NU/ml, (3.512 ±0.450) nmol/ml] than in H 30 min group[(259.20 ±45.22) NU/ml, (3.462 ±0.498) nmol/ml, respectively], but there was no significant difference between the two groups ($P > 0.05$).

3 Discussion

From the results we found that the effect of HBO therapy was improving steadily following the increasing pressure, and 2.0 ATA pressure was the best. Under 2.0 ATA pressure the left/right brain ratio and the brain abnormal rate was the lowest, SOD content was the highest and MDA content was the lowest, indicating that brain damage was relieved and lipid peroxidation(LPO) caused by HIBD was restrained. At the same time the anti-oxidase system was activated, SOD developed more, in order to more quickly clean oxygen-derived free radicals. Traditional viewpoint thought HBO can cause LPO activated and oxygen-derived free radicals increased[3]. Nowadays more and more studies showed that reasonable HBO can cause decrease of MDA content and increase of SOD content or unchanged[4~6]. Our results agreed to the point. We found the effect of HBO therapy on HIBD in neonatal rats was notable, which provided the theoretical bases of HBO therapy in neonatal clinical application.

When the HBO pressure reached to 2.25 ATA and 2.5 ATA, the left/right brain weight ratio decreased, brain abnormal rate increased, the content of SOD in brain tissue decreased and MDA content increased. It indicated that too high HBO pressure was not only unable to treat brain damage but also worsened brain damage. Reduction of SOD content probably related to the restraint of SOD activation and excessive consumption of SOD and excessive raised MDA.

The weight increasing rate in HIBD rats rose following the increasing HBO pressure regardless of high pressure (such as 2.25 ATA and 2.5 ATA). It showed HBO treatment can promote their physical growth in HIBD rats. This was perhaps because of higher pressure, more sufficient oxygen supply of muscles and bones, more fast cell growth, and higher weight increasing rate even though the brain weight decreased. Above phenomena suggest that the tolerance to HBO was different in different tissues and organs. Too high pressure can worsen brain damage, but we are not sure to all other tissues such as muscles and bones, which require more study.

Study on different time of HBO therapy showed the

weight increasing rate , left/ right brain weight ratio , the content of SOD and MDA were higher in H 60min group than in H 30min group. It suggested that the protective effect of HBO on brain in H 60 min was probably better than that in H 30 min , but there was no significant difference between the two groups perhaps relating to the deficient exponent quantification.

In short , our study indicated that it exists a reasonable "dose" and "time" during the HBO treatment. Reasonable HBO treatment can increase SOD content and decrease MDA content in brain tissue in HIBD neonatal rats. We found that HBO treatment of 2.0 ATA pressure and 30～60min steady time may have the best effect.

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