·论著(译文)·

Therapeutic Effect of Hyperbaric Oxygen on Experimental Piglet Hypoxic-Ischemic Brain Damage

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Abstract: Objective To investigate the therapeutic effect of hyperbaric oxygen (HBO) on experimental piglet hypoxic-ischemic brain damage (HIBD) and its protective mechanism by using piglets as the animal model for HIBD in newborns. **Methods** Using Levine's method, thirty 7-day-old HIBD piglets were randomly divided into the control group and the HBO-treated group in which the piglets were treated with HBO for 3 hours daily. Half of the piglets in each group were sacrificed on the 1st and 7th day after hypoxic-ischemic (HI) injury respectively. For these two groups, we observed (1) the mortality and neurological symptoms; (2) the velocity of blood flow in the right carotid artery (rVCA); (3) the brain weight; (4) the quantitative analysis of subdiploid cells in the brain by flow cytometry. **Results** The mortality and the incidence of neurological symptoms in the HBO-treated group were less than those in the control group ($P < 0.05$). The brain weights in the two groups were not different significantly ($P > 0.05$). The rVCA in the HBO-treated group was faster significantly than that in the control group when the first HBO therapy ended [(146.8 ±16.8) ml/min vs (123.9 ±27.6) ml/min] ($P < 0.01$). The ratio of subdiploid cells to all cells in the HBO group was less than that in the control group in the bilateral hippocampus on the 1st and 7th day after HI, and in the left frontoparietal cortex on the 1st day after HI. **Conclusions** HBO therapy is effective on HIBD, and should be recommended as a short-term and early treatment.

Key words: Hyperbaric oxygen; Brain hypoxia; Brain ischemia; Newborn; Piglet

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Hypoxic-ischemic brain damage (HIBD) in newborns is an important cause of permanent brain damage or neonatal death or the child may survive with cerebral palsy or mental deficiency. Hyperbaric oxygen (HBO) therapy has shown therapeutic effectiveness in cerebrovascular diseases in adutls and was proved to be effective in animal experiments[1~5]. We lack data about HBO therapy in HIBD. Therefore using piglet models, we studied the therapeutic effect of HBO on experimental HIBD piglets and its protective mechanism in the brain.

1 Materials and Methods

1.1 Instruments and agents

Type YLC 0.5/I infantile HBO vessel (the 701 Institution, China Ship Industry Corporation); Toshiba flow cytometer; RNase (Hua-Mei Bioengineer Coorperation), PI (Sigma Corporation), Protease K (Merck Corporation).

1.2 Replication of animal models

HIBD was produced with modified Levine's method[6]. Thirty 7-day-old healthy piglets were provided by the Laboratory Animal Center, China Medical University. The average body weight was (2.5 ± 0.1) kg. Anesthesia was performed with 10% chloral hydrate (2 ml/kg, intraperitoneal injection). The right common carotid artery blood was interrupted with an artery clamp, and the piglet then inhaled (8.0 ±0.5)% oxygen (temperature 36.5 ±0.5 ℃). The duration lasted 2 hours, and then the cerebral blood flow was recommended and room air was breathed.

The 30 piglets were divided randomly into the control group (14 piglets) and the HBO group (16 piglets). No treatment was attempted in the control

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group, but HBO therapy was given once daily in the HBO group. Each course of treatment lasted 3 hours. The concentration of oxygen was 100 %, and the pressure was 170 kpa. The first HBO therapy was applied as the course of hypoxia-ischemia (HI) was ended. Half of the piglets in each group were sacrificed by decapitation on the 1st and 7th day after the replication of models. The brain was immediately removed and stored in liquid nitrogen.

1.3 Observation index

(1) The mortality and neurological symptoms; (2) the blood velocity in the right carotid artery (rVCA): the comparison of rVCA before HI, in 0-hour after reperfusion and 3-hour after reperfusion between the two groups; (3) the brain weight; (4) the quantitative analysis of subdiploid cells in the brain by flow cytometry: after the bilateral frontoparietal cortex and hippocampus were digested with protease K, the neurocytes were fixed in 70 % alcohol, then digested with RNase A, and stained with PI. By flow cytometry and cellquest software, we calculated the ratio of subdiploid cells to all cells.

1.4 Statistical consideration

Continuous variables were compared with t tests, and discrete variables were compared by chi-squared analysis. A P value < 0.05 was considered significant.

2 Results

2.1 Morbidity and neurological symptoms of the piglets

The mortality in the control group (4/14) was significantly higher than that in the HBO group (0/16) ($P < 0.01$). Death occurred within 24 hours after the reperfusion. Neurological symptoms included seizure, spastic paralysis, right ptosis and hemiglossoplegia; the incidence of those symptoms in the control group was significantly higher than that in the HBO group, too. ($P < 0.05$) (Table 1)

2.2 Brain weight of the piglets

In the same group, the average brain weights of the piglets between the bilateral cerebra were similar on the 1st or 7th day after the reperfusion ($P > 0.05$). At the same time, the ipsilateral average brain weight between the control group and the HBO group were similar, too ($P > 0.05$) (Table 1).

Table 1 Neurological symptoms and brain weight in the control group and HBO group

	Seizure	Spastic Paralysis	Ptosis	Brain Weight		
				Left (g)	Right (g)	Ratio (%)
1st day						
Control Group	8/10	- - - -	- - - - -	19.20 ±1.92	18.40 ±1.52	4.21 ±2.36
HBO Group	5/16[a]	- - - -	- - - -	18.88 ±1.13	17.75 ±1.04	6.38 ±3.15[a]
7th day						
Control Group	- - - -	3/5	4/5	17.00 ±3.56	18.00 ±2.58	7.57 ±16.00
HBO Group	- - - -	1/8[b]	1/8[b]	18.00 ±0.93	17.25 ±1.17	4.52 ±4.36[a]

a vs Control, $P < 0.01$; b vs Control, $P < 0.05$

2.3 Comparison of the blood velocity in the right common carotid artery (rVCA)

When the replication of models ended, the average rVCA was similar between the two groups ($P > 0.05$). But rVCA in the HBO group was significantly faster than that in the control group when the first HBO therapy ended ($P < 0.01$) (Table 2).

Table 2 Comparison of the blood velocity in the right common carotid artery

	n	Before HI	0-h after Reperfusion	3-h after Reperfusion
Control Group	14	138.6 ±2.7	137.9 ±15.8	123.9 ±27.6
HBO Group	16	140.6 ±11.0	135.6 ±14.9	146.8 ±16.8[a]

a vs Control, $P < 0.01$

2.4 Ratio of subdiploid cells

Two cell populations, including the diploid cell population and subdiploid cell population, could be differentiated in the bilateral frontoparietal cortex and hippocampus with flow cytometry. The ratio of subdiploid cells to all cells in the HBO group was lower than that in the control group in the bilateral hippocampus on the 1st and 7th day after the reperfusion, and in the left frontoparietal cortex on the 1st day after the reperfusion ($P < 0.05$) (Table 3).

Table 3 Ratio of subdiploid cells in the HBO group and control group (%)

	n	RFP	RH	LFP	LH
1st day					
Control group	5	20.79 ±6.86	21.84 ±7.58	22.65 ±6.55	21.65 ±8.38
HBO group	8	13.97 ±8.11	13.92 ±6.04[a]	14.66 ±6.16[a]	12.16 ±7.07[a]
7th day					
Control group	5	16.95 ±3.87	25.04 ±6.37	20.28 ±6.72	17.06 ±7.38
HBO group	8	11.32 ±8.26	14.32 ±6.46[a]	16.68 ±11.81	9.41 ±6.00[a]

a vs Control, $P < 0.01$

3 Discussion

It has been reported that HBO therapy could enhance recovery of neurological systems and shorten the course in HIBD[7]. HBO therapy is not accepted in HIBD because it is unethical to use humans as controls. The other treatment effects were not accepted in the investigantion of therapeutic effects of HBO on HIBD. In our experiment, we produced HIBD animal models and built the blank control group to which no treatment was given. The mortality and incidence of neurological symptoms in the HBO group were significantly lower than those in the control group, suggesting that HBO therapy showed some clinical therapeutic effects on HIBD.

Mink et al had found that HBO could deminish the vascular permeability in the cerebral cortex, maintain the blood brain barrier, and decrease the brain edema by maintaining cerebral energy metabolism and the functions of the ionic pump in the cellular membrane[8]. In our experiment, the average brain weights were similar between the bilateral cerebrum in the HBO group or the control group on the 1st day after the reperfusion. It was shown that HBO therapy did not decrease brain edema. But the selected time points were less in the experiment, so the dehydration of HBO may not occur on the 1st day after the reperfusion. And HBO therapy may not decrease brain edema.

In HIBD, the regulatory function of cerebral vessels is impaired. While the blood pressure declines, the cerebral blood flow decreases, perivascular astrocytes and vascular endothelial cells swell, and the vascular space becomes narrow or closed. When the cerebral blood flow recovers, the blood supply does not recover, so the brain tissues in those regions were injured irreversibly. In the experiment, rVCA in the control group was significantly lower than that in the HBO group in 3 hours after the reperfusion, suggesting that the HBO therapy can decrease the resistance of the cerebral vascular bed, and maintain the normal blood supply after the reperfusion. So the cerebral energy metabolism was recovered quickly, which was similar to what Neubauer et al[7] had found.

Neuronal death includes apotosis and necrosis. Fracture of DNA occurs following apotosis and necrosis and the subdiploid cells are considered dead cells. In the experiment, it was shown that the ratio of subdiploid cells diminished after HBO therapy, implying that HBO could decrease neuronal death. But the protective effects of HBO therapy have region selectivity, and are obvious in a short time. Konda et al had found that HBO therapy could inhibit apotosis induced by hypoxia-ischemia in CA1 of the hippocampus, and therapeutic effects of HBO within 6 hours after ischemia were better than the therapy 24 hours after ischemia[9]. Therefore it is suggested that HBO

therapy should be used for short duration time and instituted early.

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