

# The Pressure Point

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## Medical Humor Row



### A Look at Research Hypoxia Induced Brain Injuries

The following research papers have been compiled to show members a selection of data with important implications for hypoxia related injuries.

It is worth noting that while many of these papers discuss non-human research, the results of these formal research works has, to date, been seen as equally effective in humans by the work of doctors currently practicing hyperbaric medicine in children and adults with hypoxia induced brain injuries.

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## Testimonials from Provider Centers Cerebral Palsy

### Alex’s Story 5 years old Cerebral Palsy

I have a son who is 5 years old and has cerebral palsy. When I first heard about HBOT a year ago, I was skeptical. I didn’t doubt that other parents were seeing things happen with their children who were undergoing treatment, but I didn’t believe that it could make a huge difference for my son, Alex.

About a year and a half ago, my son had a major seizure that left him with reduced central vision and almost no function in his right arm. He also did not have much cognitive

awareness of his surroundings. When an HBOT facility opened near our home last August, my husband and I began to give it serious thought.

Of course, all of the doctors who treat my son did not believe in the therapy. However, my son will start kindergarten in September, and we decided that the potential gains he could make with this therapy, particularly in speech, cognition and vision, would help him get the most out of his schooling.

To date, we have only had 17 treatments of HBOT, but we are already starting to see subtle changes in all three areas. At the start of treatments, Alex would not pronounce the “g” sound, and his

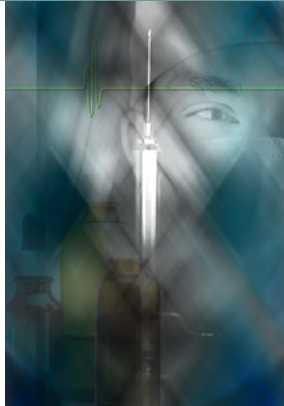


“Alex seems more “awake” now, like a sponge waiting to be filled with knowledge.”

“p” was not very strong either. After just four weeks of treatments, Alex can clearly imitate the word “purple” and he said “papa” when he heard my father-in-law was on the phone.

(Continued on page 19)

## Research Corner: A Look Back



: *J Thorac Cardiovasc Surg.* 2005 Dec;130(6): 1623-30  
: Epub 2005 Oct 26.

### Pretreatment with HBOT (hyperbaric oxygen)

Its effect on neuro-psychometric dysfunction and systemic inflammatory response after cardio-pulmonary bypass: [A prospective randomized double-blind trial]

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**OBJECTIVE:** Animal studies have shown that pretreatment with hyperbaric oxygen can induce central nervous system ischemic tolerance and also modulate the inflammatory response. We evaluated this therapy in patients undergoing cardiopulmonary bypass.

**METHODS:** Sixty-four patients were prospectively randomized to group A (n = 31; atmospheric air, 1.5 atmospheres absolute) or group B (n = 33; hyperbaric oxygen, 2.4 atmospheres absolute) before on-pump coronary artery bypass grafting.

Age, sex, body mass index, diabetes, hypertension, smoking, coronary disease severity, left ventricular function, Parsonnet score, Euro-score, bypass time, myocardial ischemia time, and number of grafts were comparable in both groups.

Canadian Cardiovascular Society angina, New York Heart Association dyspnea, and previous myocardial infarction were significantly higher in group B. Inflammatory markers were analyzed before surgery and 2 and 24 hours after bypass.

Neuropsychometric testing was performed 48 hours before surgery and

4 months after surgery and included trail making A and B, the Rey auditory verbal learning test, grooved peg board, information processing table A, and digit span forward and backward. Neuropsychometric dysfunction was defined as more than 1 SD deterioration in more than 2 neuropsychometric tests.

Chi-square tests, Fisher tests, t tests, and analysis of variance were used as appropriate for statistical analysis.

**RESULTS:** Group A had a significant postoperative increase in the inflammatory markers soluble E-selectin, CD18, and heat shock protein 70. This was not observed in group B.

Neuropsychometric dysfunction was also significantly higher in group A compared with group B. There was no difference in any other early postoperative clinical outcome.

**CONCLUSIONS:** Our results seem to indicate that pretreatment with hyperbaric oxygen can reduce neuropsychometric dysfunction and also modulate the inflammatory response after cardiopulmonary bypass. However, further multicenter randomized trials are needed to clinically evaluate this form of therapy.



*J Neurosurg.* 2001 Mar;94(3):403-11.



### Effects of hyperbaric oxygenation therapy

on cerebral metabolism and intracranial pressure in severely brain injured patients

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**OBJECT:** Hyperbaric oxygenation (HBO) therapy has been shown to reduce mortality by 50% in a prospective randomized trial of severely brain injured patients conducted at the authors' institution. The purpose of the present study was to determine the effects of HBO on cerebral blood flow (CBF), cerebral metabolism, and intracranial

pressure (ICP), and to determine the optimal HBO treatment paradigm.

**METHODS:** Oxygen (100% O<sub>2</sub>, 1.5 atm absolute) was delivered to 37 patients in a hyperbaric chamber for 60 minutes every 24 hours (maximum of seven treatments/patient). Cerebral blood flow, arteriovenous oxygen difference (AVDO<sub>2</sub>), cerebral metabolic rate of oxygen (CMRO<sub>2</sub>), ventricular cerebrospinal fluid (CSF) lactate, and ICP values were obtained 1 hour before and 1 hour and 6 hours after a session in an HBO chamber.

Patients were assigned to one of three categories according to whether they had reduced,

normal, or raised CBF before HBO. In patients in whom CBF levels were reduced before HBO sessions, both CBF and CMRO<sub>2</sub> levels were raised 1 hour and 6 hours after HBO (p < 0.05). In patients in whom CBF levels were normal before HBO sessions, both CBF and CMRO<sub>2</sub> levels were increased at 1 hour (p < 0.05), but were decreased by 6 hours after HBO.

Cerebral blood flow was reduced 1 hour and 6 hours after HBO (p < 0.05), but CMRO<sub>2</sub> was unchanged in patients who had exhibited a raised CBF before an HBO session.

In all patients AVDO<sub>2</sub> remained constant both before and after HBO. Levels of CSF lactate were consistently decreased 1

(Continued on page 12)

## Current Research from China

### Clinical effectiveness of treatment with hyperbaric oxygen for neonatal hypoxic-ischaemic encephalopathy: systematic review of Chinese literature

BMJ 2006;333:374 (19 August),  
doi:10.1136/bmj.38776.731655.2F (published 11 May 2006)

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#### Abstract

**Objectives** To investigate the clinical effectiveness of treatment with hyperbaric oxygen for neonates with hypoxic-ischaemic encephalopathy. This treatment is frequently used in China but much less often in the West.

**Data sources** Western (Cochrane controlled trials register and database of systematic reviews, Medline, Embase, CINAHL, and HealthSTAR) and Chinese (China Hospital Digital Library, Chinese Medical Journal Network) databases and hand search of Chinese journals. No language restrictions.

**Review methods** Randomized or quasi-randomized controlled trials of treatment with hyperbaric oxygen compared with "usual care" in term neonates with hypoxic-ischaemic encephalopathy. Outcomes included mortality and long term neurological sequelae. Standardized forms were used to extract and compare data. Criteria of York Centre for Reviews and Dissemination were used to assess quality. Analysis was mainly qualitative but included meta-analysis.

**Results** 20 trials were found, mainly from Chinese sources. The reporting quality of trials was poor by Western (CONSORT) standards. Treatment with hyperbaric oxygen had better outcomes than the comparator in almost all trials. The odds ratios of the meta-analyses were 0.26 (95% confidence interval 0.14 to 0.46) for mortality and 0.41 (0.27 to 0.61) for neurological sequelae.

**Conclusion** Treatment with hyperbaric oxygen possibly reduces mortality and neurological sequelae in term neonates with hypoxic-ischaemic encephalopathy. Because of the poor quality of reporting in all trials and the possibility of publication bias, an adequately powered, high quality randomized controlled trial is needed to investigate these findings. The Chinese medical literature may be a rich source of evidence to inform clinical practice and other systematic reviews.

#### Introduction

Hypoxic-ischaemic encephalopathy is a severe complication of asphyxia that occurs before, during, or after birth.

It can result in death or neurological damage, which can manifest in the short term (within 12-24 hours) as seizures, altered reflexes, or altered level of consciousness (or a combination), and in the longer term by developmental delay, epilepsy, mental retardation, or cerebral palsy (or a combination). Diagnosis is by a history of asphyxia that has caused acidemia, a low Apgar score, neurological damage, and the involvement of many organs. The condition is commonly graded as mild, moderate, or severe. Sarnat stages can be used to classify the neurological damage—stage I is least severe and stage III most severe.<sup>1</sup> The condition occurs in 3.5-6/1000 live births, and the outcome is worse for more severely affected neonates.<sup>2</sup> One case series of 38 births reported 14 deaths and 13 patients with a poor outcome.<sup>3</sup> In another series of 42 survivors with moderate hypoxic-ischaemic encephalopathy followed up at one year, two were dead, 13 had cerebral palsy, one had another severe disability, four had mild developmental delay, and 22 had developed normally.<sup>1</sup> Treatments evaluated for this condition include hypothermia, magnesium sulphate, anticonvulsants, mannitol, dexamethasone, nicardipine, and caffeic acid phenethyl ester, but none has been effective.<sup>4-7</sup> Management usually consists of supportive care and keeping oxygen saturation at 95%.<sup>2</sup>

Patients treated with hyperbaric oxygen inhale 100% oxygen inside a hyperbaric chamber that is pressurized to > 0.1 MPa (megapascals). This treatment has been evaluated in the West for a wide range of conditions, including cerebral oedema, brain injuries, and cerebral palsy, but not for hypoxic-ischaemic encephalopathy.<sup>8-10</sup> In Russia, hyperbaric oxygen has been used to treat neonatal injuries (fetal asphyxia), and this treatment is used for hypoxic-ischaemic encephalopathy in China, but apparently not in Hong Kong.<sup>11-13</sup> Hyperbaric oxygen is usually given one to three times per day at 0.15-0.17 MPa for 60-120 minutes, with the aim of increasing oxygen in the tissues.<sup>14</sup> The rationale for this treatment is that it may reverse local hypoxia, inhibit post-ischaemic vasoconstriction, and promote the formation of collagen matrix, which is essential for angiogenesis and restoration of blood flow to injured tissue.<sup>14</sup> This systematic review investigates whether hyperbaric oxygen is clinically effective for the treatment of neonates born at term with hypoxic-ischaemic encephalopathy.

#### Methods

The protocol for this systematic review was developed as part of a masters degree in health technology assessment.

The search strategy comprised a search of Western electronic databases and a search of Chinese databases and other sources. We searched the Cochrane controlled trials register and database of systematic reviews, Medline, Embase, CINAHL, and HealthSTAR up to November 2004 using search terms "hyperbaric oxygen", "hyperbaric oxygenation", "neonate(s)", "newborn(s)", "infant newborn(s)", "hypoxic-ischemic", "encephalopathy", "encephalopathies", "brain injury", "brain injuries", "brain damage", "brain ischemia", "hypoxia brain", and "birth asphyxia". We also searched a

variety of Chinese electronic databases and hand searched selected Chinese journals (see box) to July 2004.

We identified relevant studies by searching electronic databases, scanning reference lists, and consulting experts in the specialty. Publications in any language were eligible. Reference lists were hand searched for further references. We examined titles, abstracts, and keywords of citations as given on the databases for the terms for "hyperbaric oxygen therapy for neonatal hypoxic-ischaemic encephalopathy". Where possible, we obtained the full text of all potentially relevant citations.

The predetermined inclusion criteria were fully published randomized or quasi-randomized controlled trials of treatment with hyperbaric oxygen compared with "usual care" in full term neonates (more than 36 weeks' gestation) with hypoxicischaemic encephalopathy and a history of perinatal asphyxia. We accepted alternate allocation as quasi-randomized. Outcomes were mortality and incidence of long term neurological sequelae (developmental delay, epilepsy, mental retardation, or cerebral palsy, or a combination). One reviewer (ZL) assessed studies for inclusion and this was checked independently by a second reviewer (TX). Both reviewers independently extracted data from the papers using a standardized, pre-designed data extraction form, and no disagreements were encountered. We assessed the quality of the included trials by using criteria of the York Centre for Reviews and Dissemination; we focused on randomization, allocation concealment, presence of blinding, explanation of withdrawals, and presence or absence of intention to treat analysis.<sup>15</sup>

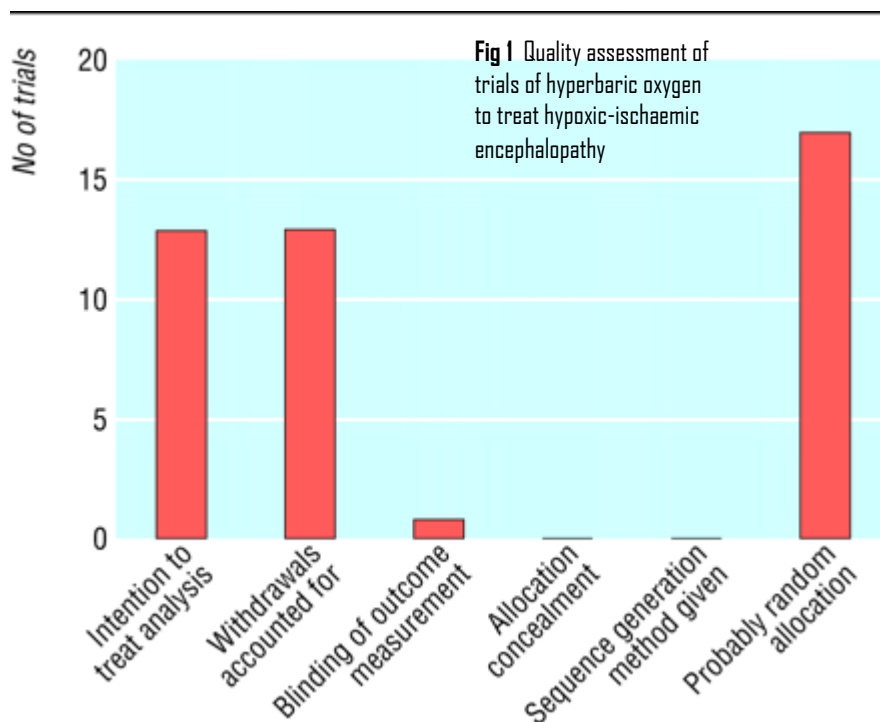
We tabulated the characteristics and results of all the included studies; analysis was mainly qualitative. We

carried out meta-analysis using Metaview 4.1 (Cochrane Collaboration Review Manager 4.1 software). We used fixed effects models when statistical heterogeneity was absent and random effects models when heterogeneity was present. Statistical heterogeneity was present if 2 values were greater than the degrees of freedom.<sup>16</sup>

### Results

We found six citations in Western databases, but none met the inclusion criteria. We identified 126 citations from the Chinese searches. Twenty trials met the inclusion criteria and 106 were excluded (59 had the wrong study design, 37 did not specify the term neonate, two had different interventions, and eight had different outcomes).<sup>17-36</sup> All of the included trials were conducted in China and published in Chinese language medical journals. The trials studied between 40 and 198 patients. Four different sets of criteria were used to diagnose hypoxic-ischaemic encephalopathy (see table 1).<sup>37-40</sup> These sets are similar and the criteria used are an abnormal obstetric history of fetal anoxia and distress; asphyxia after birth resulting in a low Apgar score and disturbance of consciousness; change in muscle tone; and abnormal reflexes within 12 hours of birth. The severity of hypoxic-ischaemic encephalopathy varied and grading of severity was probably not applied uniformly across the trials. Trials used various doses of hyperbaric oxygen and some had additional treatments, such as antioxidants and neurotrophic agents in each arm. Populations and the delivery of hyperbaric oxygen varied greatly. Table 1 (page 6/7) shows the characteristics of the studies.

Seventeen of the 20 studies mentioned "random" in the methods section, but few other trial details were given. None of the randomized trials mentioned the method of



**Chinese data sources**

**Electronic databases**

*China Hospital Digital Library, administered by the Department of Education of China and sponsored by Tsinghua University and the Chinese Medical Association. This database provides full text data from published periodicals and newspapers in China on medical specialties, bioscience, government of hospitals, library science, and information science. The main component is the China hospital knowledge database, which contains more than 1300 professional periodicals and 3000 related periodicals; it covers 96% of articles on medicine published in China. Relevant keywords were used for searching.*

*An online bibliographical database from the China Hyperbaric Oxygen Medicine Information Centre, which contains data on relevant literature published in Chinese medical journals.*

*Chinese Medical Journal Network for full text medical literature*

**Hand searches**

*Selected journals in Chinese: Journal of Clinical Pediatrics and Chinese Journal of Practical Pediatrics*

sequence allocation or whether allocation was concealed. In the other three studies treatment was allocated on an alternate basis. They were included because order of birth was considered to be a random event.<sup>22 25 27</sup> Selection bias may have been more prominent in these studies, but because of the uniformly poor methodological quality of reporting this could not be determined. Only one trial mentioned blinding (of outcome assessment).<sup>22</sup> No trials with losses to follow-up described reasons for the losses. The only trials with intention to treat analysis were those without losses to follow-up. Figure

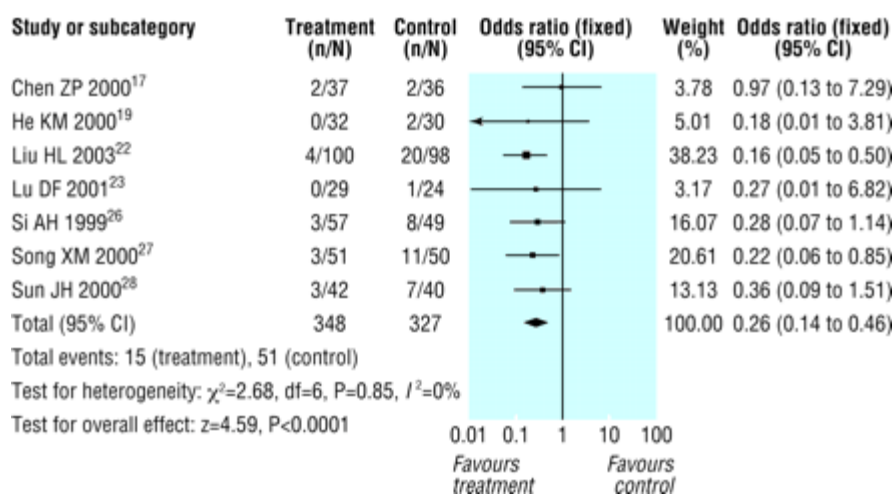
1 (page4) shows quality factors.

Table 2 (page 8/9) shows the results of the studies. Not all outcomes were reported in each trial, but overall, treatment with hyperbaric oxygen had a better outcome than the comparator. Only seven trials reported mortality (fig 2). Hyperbaric oxygen significantly reduced mortality in hypoxic-ischaemic encephalopathy (odds ratio 0.26, 95% confidence interval 0.14 to 0.46). Seven trials measured neurological sequelae (fig 3). Neurological sequelae were significantly reduced in neonates

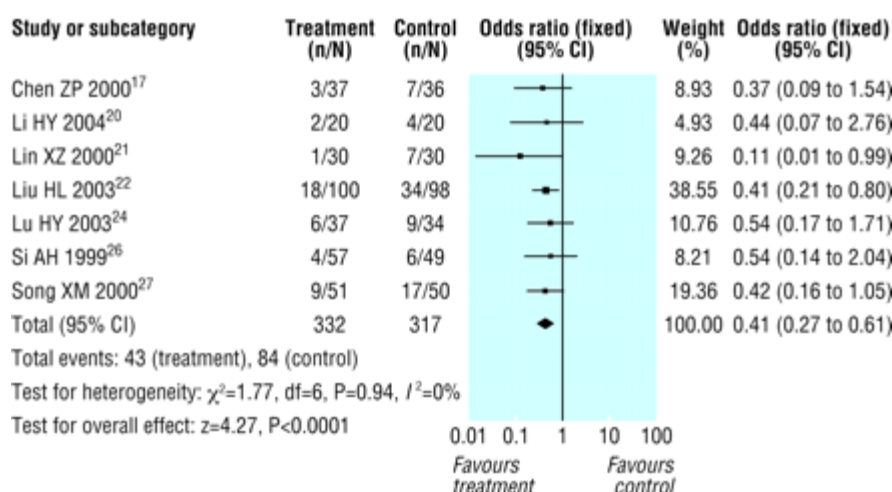
treated with hyperbaric oxygen compared with controls (0.41, 0.27 to 0.61). Little heterogeneity was seen between the trials for both comparisons.

Adverse events were reported in only one trial—retrolental fibroplasia occurred in one case each in the intervention group and the control group at follow-up.<sup>36</sup> Seven trials reported no adverse events, and the remainder did not mention adverse events (table 2, pg 8).

**Fig 2** Effect of treatment with hyperbaric oxygen on mortality in hypoxic-ischaemic encephalopathy



**Fig 3** Effect of treatment with hyperbaric oxygen on neurological sequelae in hypoxic-ischaemic encephalopathy



### Discussion

The results of this systematic review suggest that treatment with hyperbaric oxygen may reduce mortality and neurological sequelae in term neonates with hypoxic-ischaemic encephalopathy. Hyperbaric oxygen has been used to treat various conditions for several decades and has been used in neonates. Although this form of treatment is controversial, it has developed rapidly in China over the past decade and is widely used there.

#### Limitations

Trial reports were of poor quality according to the criteria of the York Centre for Reviews and Dissemination, were not written to CONSORT standards, and lacked many details. Publication bias is a possibility as studies with negative results may not have been published. The 20 trials differed greatly in terms of the severity and status of the condition, exposure to hyperbaric oxygen, time to treatment and other baseline characteristics, and the measurement of outcomes. In addition, little information was given on side effects such as retrolental fibroplasia.

#### Implications

An adequately powered, high quality, randomized controlled trial is needed to investigate the effectiveness of hyperbaric oxygen in term neonates with hypoxic-ischaemic encephalo-

Table 1

Table 1 Characteristics of 20 trials investigating treatment with hyperbaric oxygen in hypoxic-ischaemic encephalopathy

Study	No of patients*		Diagnostic criteria (severity*)	Baseline comparisons		Age at start of treatment		Treatment †	Usual care
	Intervention	Control		Treatment	Usual care †				
Chen 2000 <sup>17</sup>	37	36	Ji Nan conference (I, II, and III)	No statistically significant difference in status of consciousness, convulsion, muscle tone, reflexes, or computed tomography results	2-5 days	NA	0.14-0.16 MPa (concentration=80%); 20 min/30 min/20 min; once a day x 10 days; 1-5 courses; not clear	General	
Ding 2003 <sup>18</sup>	92	86	Han Zhou conference (NA)	No statistically significant difference in sex, age, status of asphyxia, or clinical symptoms	<48 hours	<24 hours	0.15-0.17 MPa; 15 min/30 min/20 min; once a day x10 days; not clear; not clear	General and drug that promotes cerebral metabolism	
He 2000 <sup>19</sup>	I: 4; II: 18; III: 10	I: 4; II: 17; III: 9	Han Zhou conference (I, II, and III)	No statistically significant difference in birth weight, degree of asphyxia, or main clinical symptoms	<72 hours	<24 hours	0.15 MPa; 15 min/30 min/15 to 30 min; once a day x10 days; not clear; not clear	General and drug that promotes cerebral metabolism	
Li 2004 <sup>20</sup>	20	20	Hu and Jiang (I, II, and III)	No statistically significant difference in ways of delivery, sex, gestational age, birth weight, age, or clinical grade	I and II: 15 hours to 7 days III: >7 days	NA	Not clear; not clear; 10 times; I and II=1-2 courses; III=3-5 courses; 10-15 days	General	
Lin 2000 <sup>21</sup>	30	30	NA (I, II, and III)	No statistically significant difference in sex, birth weight, illness status, maternity age, parents' social status, or family status	NA	≤ 1 day	Not clear; not clear; once a day x10 days; not clear; not clear	General	
Lu 2003 <sup>22</sup>	I: 61; III: 39	I: 60; III: 38	Han Zhou conference (II and III)	No statistically significant difference in sex, birth weight, illness status, maternity age, parents' social status, or family status	Within 24 days	Within 24 days	0.15-0.17 MPa; 15 min/30 min/20 min; once a day x10 days; not clear; not clear	General	
Lu 2001 <sup>23</sup>	I: 19; III: 10	I: 16; III: 8	Han Zhou conference (I and II; excluded cerebral haemorrhage)	No statistically significant difference in birth weight, gestational age, time starting treatment, or clinical grade	2-8 days	NA	0.12 MPa. 20 min/30 min/20 min; once; not clear; not clear	General	
Lu 2003 <sup>24</sup>	37	34	Han Zhou conference (II and III; included cerebral haemorrhage)	No statistically significant difference in sex, birth weight, or degree of asphyxia and anoxia	<48 hours after admission; stable if with cerebral haemorrhage	NA	0.16 MPa; 20 min/30 min/20 min; once a day x10 days; II=2 courses, III=3 courses; not clear	General and drugs that promote cerebral metabolism and eliminate free radicals	
Lu 1999 <sup>25</sup>	I: 15; II: 11; III: 6	I: 12; II: 13; III: 5	Han Zhou conference (I, II, and III)	No statistically significant difference in sex, birth weight, status of asphyxia and anoxia, or clinical symptoms	<48 hours	<24 hours	0.15-0.17 MPa; 15 min/30 min/20 min; once a day x10 days; not clear; not clear	General and drugs that promote cerebral metabolism and eliminate free radicals	

Si 1999 <sup>26</sup>	I: 11; II: 27; III: 19 I: 10; II: 22; III: 17	Han Zhou conference (I, II, and III: excluded those with brain malformation and severe decomposition)	No statistically significant difference in sex, birth weight and status of asphyxia.	NA	0.2 MPa; 20 min/60 min/20 min; once a day x7 days; not clear; not clear	General
Song 2000 <sup>27</sup>	51	Han Zhou conference (II and III)	No statistically significant difference in sex, birth weight, clinical grade, <b>maternity</b> age, and family status	<48 hours	0.15-0.17 MPa; 15 min/30 min/20 min; once a day x10 days; not clear; not clear	General
Sun 2000 <sup>28</sup>	I: 21; III: 21 I: 19; III: 21	Ji Nan conference (I and III)	NA	NA	<3 days	General (3rd arm not included)
Sun 1998 <sup>29</sup>	I: 10; II: 18; III: 4 I: 7; II: 13; III: 2	Ji Nan conference (I, II, and III: included complications of cerebral haemorrhage)	No statistically significant difference in various aspects including symptoms	NA	0.14 MPa (1.4 atm): 15 min/40 min/20 min; once a day x7-10 days; not clear; not clear	General
Wang 2002 <sup>30</sup>	I: 15; II: 22; III: 11 I: 16; II: 20; III: 10	Ji Nan conference (all)	No statistically significant difference in sex, birth weight, or degree of asphyxia and anoxia	NA	0.14 MPa; 20 min/50 min/25 min; 7 days; I=1 course, II=2-3 courses, III=3-4 courses; 3-5 days	General, antioxidants, and cerebrolysin
Wang 1999 <sup>31</sup>	II: 14; III: 9 II: 15; III: 7	Han Zhou conference (II and III: excluded cerebral haemorrhage from birth injury and malformation)	No statistically significant difference in sex, birth weight, gestational age, or Apgar score	72 hours	0.15-0.16 MPa; (concentration=60%); 20 min/30 min/20-30 min; once x5-8 days; not clear; not clear	General and nicholin
Wang 2001 <sup>32</sup>	80 <sup>†</sup> I: 24; II: 36; III: 20	Defined in text (I, II, and III)	No statistically significant difference in illness status	As early as possible	Not clear; 20 min/60 min/20 min; once a day x10 days; not clear; not clear	General
Wang 2001 <sup>33</sup>	I: 26; II: 46; III: 12 I: 24; II: 45; III: 11	Jin and Huang (I, II, and III: except congenital disease)	No significant difference in age, gestational age, illness status, or family status	NA	0.15 MPa; total time=60-70 min each time; once a day x5-7 days; not clear; not clear	General
Wen 2001 <sup>34</sup>	38 38	Han Zhou conference (II and III)	No significant difference in sex or age	<3 days	0.13 MPa; 15 min/30 min/15 min; once a day x10 days; not clear; not clear	General
Yuan 1999 <sup>35</sup>	I: 16; II: 9; III: 5 I: 17; II: 8; III: 5	Ji Nan conference (I, II, and III)	Listed symptoms but did not compare	Within 2 days of admission	0.14-0.16 MPa; 20 min/30 min/20-30 min; I=2-3 times, II=5 times, III=10 times; not clear	General
Zhang 2000 <sup>36</sup>	60 56	Jinan conference (II and III)	No statistically significant difference in sex, gestational age, birth weight, or Apgar score	NA	0.16 MPa, 10-15 min/30 min/15-20 min; once a day x10 days; II=2 courses, III=3 courses; 1 week	General

MPa, megapascals; NA=not available.  
 \* Severity grade: I=mild, II=moderate, III=severe.  
 † The age starting usual care was assumed as the time of admission.  
 ‡ Pressure: time of increasing/stable/decreasing pressure; 1 course; length; interval. Time is the duration increase  
 † ing/stable/decreasing pressure of a single treatment, length is the number of courses of treatment, and interval is the time between two courses of treatment.  
 ¶ Alternate patient allocation.  
 ¶ The numbers given for severity grades I (26) II (37) and III (21) and up to 84 but the total number in the intervention group was given as 80.

# Table 2

## Table 2 Results of 20 trials investigating treatment with hyperbaric oxygen in

Study	Total effectiveness rate (measurement index)	Mean (SD) days to recovery	Length of follow-up; losses to follow-up	Long term neurological sequelae	Mortality	Adverse events
Chen 2000 <sup>17</sup>	NA	Seizures controlled, consciousness, and muscle tone. 2-5 fewer days in I than C	12-24 months; none	I: 8.1% (3/37), C: 19.4% (7/36) (numbers in text do not add up)	I: 5.4% (2/37), C: 5.5% (2/36)	NA
Ding 2003 <sup>18</sup>	I did significantly better than C; P<0.005 (clinical symptoms disappeared in 10 days)	NA	>10 days; NA	NA	NA	None
He 2000 <sup>19</sup>	I: 94% (30/32), C: 67% (20/30); P<0.01 (clinical symptoms disappeared in 10 days)	I: 12.5 (2.8), C: 15.5 (3.1)	NA; none	NA	I: 0% (0/32), C: 6.7% (2/30)	None
Li 2004 <sup>20</sup>	I: 90% (18/20), C: 60% (12/20); P<0.05 (clinical symptoms—cerebral oedema by ultrasound disappeared in 5 days)	NA	3 years; probably none	I: 10.0% (2/20), C: 20.0% (4/20)	NA	None
Lin 2000 <sup>21</sup>	I: 97% (29/30), C: 73% (22/30); P<0.05 (clinical conditions improved in 1 week)	NA	5 months to 2 years; none for total effectiveness rate, 7 for neurological sequelae	I: 3.3% (1/30), C: 23.3% (7/30)	NA	NA
Liu 2003 <sup>22*</sup>	I: 90% (90/100), C: 67% (66/98); P<0.01 (clinical symptoms disappeared in 10 days)	NA	6-14 months; 34	I: 20.4% (18/100), C: 44.7% (34/98)	I: 4.0% (4/100), C: 20.4% (20/98); P<0.05	NA
Lu 2001 <sup>23</sup>	NA	Symptoms: I: 4.3 (2.3), C: 5.8 (2.0); P<0.001	NA; none	NA	I: 0% (0/29), C: 4.2% (1/24)	None
Lu 2003 <sup>24</sup>	I: 92% (34/37), C: 74% (25/34); P<0.01 (clinical symptoms—cerebral oedema—disappeared in 10 days; how cerebral oedema was detected not given)	Muscle tone. Grade II: I: 6.94 (2.06), C: 7.48 (2.20); P<0.05; grade III: I: 11.17 (2.57), C: 12.44 (3.32); P<0.01. Reflexes. Grade II: I: 4.54 (1.53), C: 5.47 (1.96); P<0.01; grade III: I: 9.29 (2.57), C: 10.33 (2.74); P<0.01. Consciousness. Grade II: I: 3.19 (1.04), C: 3.88 (1.49); P<0.05; grade III: I: 5.14 (1.81), C: 6.44 (1.87); P<0.01	NA; none	I: 16.2% (6/37), C: 26.5% (9/34); P<0.01	NA	None
Lu 1999 <sup>25*</sup>	I: 97% (31/32), C: 70% (21/30); P<0.05 (clinical symptoms disappeared in 10 days)	NA	>10 days; none	NA	NA	None

Si 1999 <sup>26</sup>	I: 93% (53/57), C: 76% (37/49); P<0.01 (clinical symptoms and convulsions disappeared in 10 days)	Days in hospital. I: 12.8, C: 17.6	3 months to 4 years; none for total effectiveness, not clear for long term sequelae	I: 5.3% (3/57), C: 16.3% (8/49)	NA
Song 2000 <sup>27*</sup>	I: 90% (46/51), C: 66% (33/50); P<0.01 (clinical symptoms disappeared in 14 days)	NA	6-14 months; I: 3, C: 11	I: 18.8% (9/50), C: 43.6% (17/50); P<0.05	I: 5.9% (3/51), C: 22.0% (11/50)
Sun 2000 <sup>32</sup>	I: 93% (39/42), C: 58% (33/40); cured, improved, alive; time to outcomes not given	NA	Not clear; none	NA	I: 7.1% (3/42), C: 17.5% (7/40)
Sun 1998 <sup>33</sup>	I: 100%, C: 76% (numbers not given); P<0.01 (measurement index not mentioned)	NA	NA; NA	NA	None
Wang 2002 <sup>34</sup>	NA	Seizure controlled; recovery of muscle tone, reflexes, and consciousness. 4-6 days fewer in I than C	NA; none	NA	NA
Wang 1999 <sup>35</sup>	I: 97% (22/23), C: 68% (15/22); P<0.05 (clinical symptoms disappeared in 10 days)	NA	>10 days; none	NA	NA
Wang 2001 <sup>36</sup>	I: 100% (80/80), C: 80% (64/80); P<0.05 (cerebral oedema—by computed tomography—disappeared in 10 days)	Symptoms. At least 3 days fewer in I than C	10 days; none	NA	NA
Wang 2001 <sup>37</sup>	I: 90% (76/84), C: 76% (61/80); P<0.05 (clinical symptoms disappeared in 1 week)	NA	Not clear; none	NA	NA
Wen 2001 <sup>38</sup>	NA	Muscle tone. I: 9.13, C: 11.80; consciousness: I: 4.37, C: 5.60	Not clear; NA	NA	NA
Yuan 1999 <sup>39</sup>	I: 93% (28/30), C: 70% (21/30); P<0.05 (clinical symptoms and cerebral oedema disappeared in 10 days. How cerebral oedema was detected not given)	NA	>10 days; none	NA	NA
Zhang 2000 <sup>40</sup>	NA	Symptoms. I: 5 (2/9), C: 7.8 (1.2); P<0.001	Not clear; none	NA	Retrolental fibroplasia I: 1.6% (1/60), C: 1.8% (1/56)

C=control group, I=intervention group, NA=not available.

\* Alternate patient allocation.

† Severity grade: I=mild, II=moderate, III=severe.

pathy. If the effectiveness of this treatment is confirmed, this will have two main implications. Firstly, the treatment of hypoxic-ischaemic encephalopathy will change radically in the West and hyperbaric oxygen chambers will be required in all special care baby units. The costs of providing this treatment could be high, but they might be outweighed by fewer neonatal deaths and reduced requirements for specialist paediatric medical and nursing care.

Secondly, evidence of the effectiveness of this treatment came from Chinese sources that are not routinely searched when systematic reviews are carried out in the West. It is not known at present how much useful evidence will be found once researchers start to look. This may also be true for evidence collected in Russia. To determine whether the inclusion of Chinese and Russian trials would reinforce or change the conclusions of systematic reviews, Chi-

nese and Russian trials of interventions should be reviewed and the results compared with currently available systematic reviews. In the future, it may become general policy to check these databases, so systematic review groups would need reviewers skilled in these languages who also have access to the relevant databases and journals.

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**Contributors:** ZL conducted the original systematic review submitted for the masters degree in full. TX checked the inclusions and duplicate data extraction. CM supervised the original systematic review, wrote the journal article from it, and is guarantor.

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**Competing interests:** None declared.

**Ethical approval:** Not required.

### What is already known on this topic

- Hypoxic-ischaemic encephalopathy is a severe complication of asphyxia before, during, or after birth and occurs in 3.5-6/1000 live births.
- Current treatment in the West consists mainly of best supportive care.
- Hyperbaric oxygen is commonly used in China to treat this condition.



### What this study adds

- This systematic review of 20 Chinese trials found that treatment with hyperbaric oxygen reduced mortality and neurological sequelae such as epilepsy, mental retardation, and cerebral palsy, but in all trials reporting of methods was poor and publication bias is a possibility.
- A high quality randomized controlled trial is needed to investigate and confirm the effectiveness of this treatment.

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**Related Articles**

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**SPEND A GLORIOUS DAY AT THE WESTIN MISSION HILLS RESORT AND GIVE FAMILIES H.O.P.E.!**

The medical world has only begun to uncover the vast applications of hyperbaric technology. To date, thousands—many, sick children—have discovered the amazing benefits of Hyperbaric Oxygen Therapy (HBOT).

However, there are still families who lack the financial support to afford the treatment their children desperately need. And, because debilitating conditions such as cerebral palsy, multiple sclerosis, autism, as well as mitochondrial deficiency disorders and diabetes are now being successfully treated with hyperbarics, your involvement is even more important.

In response, the Hyperbaric Oxygen Pediatric Endowment (HOPE) will be “teeing off” to raise funds for this very worthy drive. Players will compete in a game of 2-man scramble and vie for first, second and third place trophies. In addition, special awards will be given for the longest drive and “closest to the pin” shots on designated holes. All players will receive a box lunch, hat, t-shirt, goody bag and an invitation to a gala event that evening. Shotgun starts promptly at 11:30 a.m. and 12 noon.

Barry Bostwick will be joining us for a great day of gold and sun—all in the pursuit of a great cause, HOPE!



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## Research Corner: A Look Back (continued)

*(Continued from page 2)*

hour and 6 hours after HBO, regardless of the patient's CBF category before undergoing HBO ( $p < 0.05$ ).

Intracranial pressure values higher than 15 mm Hg before HBO were decreased 1 hour and 6 hours after

HBO ( $p < 0.05$ ). The effects of each HBO treatment did not last until the next session in the hyperbaric chamber.

**CONCLUSIONS:** The increased CMRO2 and decreased CSF lactate levels after treatment indicate that HBO may improve aerobic metabo-

lism in severely brain injured patients.

This is the first study to demonstrate a prolonged effect of HBO treatment on CBF and cerebral metabolism.

On the basis of their data the authors assert that shorter, more frequent exposure to HBO may optimize treatment.



*Brain Res. 2002 Sep 27;951(1):1-8.*

Hyperbaric oxygenation prevented brain injury induced by hypoxia-ischemia in a neonatal rat model.



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The occurrence of hypoxia-ischemia (HI) during early fetal or neonatal stages of an individual leads to the damaging of immature neurons resulting in behavioral and psychological dysfunctions, such as motor or learning disabilities, cerebral palsy, epilepsy or even death. No effective treatment is currently available and this study is the first to use hyperbaric oxygen (HBO) as a treatment for neonatal HI.

Herein, we sought out to determine if HBO is able to offer neuroprotectivity against an HI insult. Seven-day-old rat pups were subjected to unilateral carotid artery ligation followed by 2.5 h of hypoxia (8% O<sub>2</sub>) at 37 degrees C. HBO treatment was administered by placing pups in a chamber (3 ATA for 1 h) 1 h after hypoxia exposure. Brain injury was assessed based on ipsilateral hemispheric weight divided by contralateral hemispheric weight, light microscopy, and EM. Sensorimotor functional tests were administered at 5 weeks after hypoxia exposure. After HI, the ipsilateral hemisphere was 52.65 and 57.64% ( $P < 0.001$ ) of the contralateral hemisphere at 2 and 6 weeks, respectively.

In HBO treated groups, the ipsilateral hemisphere was 77.77 and 84.19% ( $P < 0.001$ ) at 2 and 6 weeks. There was much less atrophy and apoptosis in HBO treated animals under light or electron microscopy. Sensorimotor function was also improved by HBO at 5 weeks after hypoxia exposure (Chi-square,  $P < 0.050$ ).

The results suggest that HBO is able to attenuate the effects of HI on the neonatal brain by reducing the progression of neuronal injury and increasing sensorimotor function.

### Testimonials from Provider Centers

Meconium Aspirated Hypoxia

#### Jocosa's Story

6 years old  
Meconium Aspirated Hypoxia

I My daughter Jocosa was born with Meconium Aspirated Hypoxia, which resulted in loss of oxygen and blood flow to her brain. Jocosa was sent home from the hospital to be placed in hospice care to die and was considered a "failure to thrive" case.

Jocosa is non-ambulatory, non-verbal, and has cortical visual impairment.

Jocosa began taking Hyperbaric Oxygen Therapy (HBOT) treatments in January 2006. She has completed 57 HBOT treatments thus far and I have noticed improvements with Jocosa's head control, and drooling. I've also seen some improvements with Jocosa's eyes; they seem to move together instead of wandering in different directions.

I am hopeful that Jocosa will continue to progress from Hyperbaric Oxygen Therapy.

Sincerely, Brandi

- Mother of Jocosa



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10.1152/jappphysiol.00630.2003.

## Effect of hyperbaric oxygen on apoptosis in neonatal hypoxia-ischemia rat model

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[Submitted 17 June 2003; accepted in final form 21 July 2003]

We have previously demonstrated that a transient exposure to hyperbaric oxygen (HBO) attenuated the neuronal injury after neonatal hypoxia-ischemia. This study was undertaken to determine whether HBO offers this neuroprotection by reducing apoptosis in injured brain tissue. Seven-day-old rat pups were subjected to unilateral carotid artery ligation followed by 2 h of hypoxia (8% oxygen). Apoptotic cell death was examined in the injured cortex and hippocampus tissue. Caspase-3 expression and activity increased at 18 and 24 h after the hypoxia-ischemia insult. At 18–48 h, poly(ADPribose) polymerase (PARP) cleavage occurred, which reduced the band at 116 kDa and enhanced the band at 85 kDa. There was a time-dependent increase in the number of terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL)-positive cells. A single HBO treatment (100% oxygen, 3 ATA for 1 h) 1 h after hypoxia reduced the enhanced caspase-3 expression and activity, attenuated the PARP cleavage, and decreased the number of TUNEL-positive cells observed in the cortex and hippocampus. These results suggest that the neuroprotective effect of HBO is at least partially mediated by the reduction of apoptosis.

**HYPOXIA-ISCHEMIA IS A COMMON** cause of brain injury in the perinatal period. It is thought to be the single largest contributor to static encephalopathies in children and can result in mental impairment, seizures, and permanent motor deficits, such as cerebral palsy (2, 6, 17, 18, 24, 26). Statistics (45, 46) show that 20 of every 1,000 full-term infants experience systemic asphyxia, and 20–50% of asphyxiated neonates who experience a hypoxia-ischemia insult expire during the newborn period. Of the ones that survive, 25% exhibit some sort of permanent neuropsychological handicap.

One of the primary setbacks to the brain after a hypoxia-ischemia insult is the reduction in oxygen delivery to the tissue. Administration of 100% oxygen under increased ambient pressure is a potent means of increasing the amount of oxygen dissolved in blood plasma, thereby increasing oxygen delivery to the brain (33). Studies have shown that hyperbaric oxygen (HBO) treatment has improved single-photon-emission computed tomography imaging, increased cerebral oxygenation, improved patient condition (20, 32), and prevented recurrent cerebral stroke in patients (36).

Currently, in newborns and children, HBO has been a successful treatment for radiation-induced bone and soft

tissue complications, cyanotic congenital heart disease, and carbon monoxide poisoning (3, 5, 14, 15, 22, 40). We have shown previously that HBO is able to attenuate the effects of hypoxia-ischemia on the neonatal rat brain by reducing the progression of neuronal injury (12). The exact mechanism by which HBO offers this neuroprotection has yet to be elucidated. The purpose of the present study was to determine whether HBO offers this neuroprotection by reducing the hypoxia-ischemia-induced apoptosis that is known to accompany a hypoxia-ischemia insult.

## MATERIALS AND METHODS

*Hypoxia-ischemia model and HBO treatment.* The Animal and Ethics Review Committee at the Louisiana State University Health Sciences Center-Shreveport evaluated and approved the protocol used in this study. The model used in this study is based on the Rice-Vannucci model (38, 45), as previously described (12). Pups were housed with the dam under a 12:12-h light-dark cycle, with food and water available ad libitum throughout the study. Unsexed 7-day-old (day 0 = day of birth) Sprague-Dawley (Harlan) rats were anesthetized by inhalation with isoflurane (0.1%) in oxygen. The rats were kept at a temperature of 37°C as the right common carotid artery of each pup was exposed and ligated with 5-0 surgical sutures.

The duration of the anesthesia did not exceed 20 min, and the pups were allowed to recover with their dams for 2 h. They were then placed in a jar perfused with a humidified gas mixture (8% oxygen-balance nitrogen) for 2 h. Both the jar and the gas mixture were kept at 37°C. The pups were returned to their dams after the hypoxic exposure. The pups that underwent HBO treatment were allowed to recover from the hypoxic exposure for 1 h before being placed in the HBO chamber. The HBO treatment of 100% oxygen was administered at a pressure of 3 atmospheres absolute (ATA) for 1 h, and the pups were then returned to their cages after the treatment. Only one HBO treatment was conducted for each pup.

*Experimental groups.* The pups were divided into the following three groups: 1) control (no anesthesia, carotid ligation, hypoxia, or HBO exposure), 2) hypoxia-ischemia, and 3) hypoxia-ischemia + HBO. Each group was composed of pups from each litter to obtain parity within the groups. The brains were removed at various times after the hypoxia-ischemia insult: 12 h, 18 h, 24 h, 48 h, 2 wk, and 6 wk.

*Paraffin embedding and TUNEL staining.* Paraffin embedding was performed as described previously (31). In short, 12, 18, and 24 h after the hypoxia-ischemia insult, pups ( $n = 3$  for each group at each time point) were perfused with PBS under deep anesthesia, followed by 4% PAF in 0.1 M PBS. The brains were removed and placed in the same fixative solution for 1 wk before being embedded in paraffin. Paraffinembedded brains were sectioned and processed for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining. TUNEL staining was performed on paraffin-embedded sections by using the in situ cell death detection kit, Fluorescein (Roche Diagnostics, Mannheim, Germany) as described previously (35, 39). Briefly, the procedure was

carried out according to the manufacturer's instructions.

The slides were dewaxed and rehydrated by heating the slides at 60°C. The slides were then washed in xylene and rehydrated through a graded series of ethanol and double-distilled water. Next, the slides were incubated for 30 min at 37°C in a 20 µg/ml proteinase K working solution. Then the slides were rinsed with PBS, and the area around the sample was dried. The slides were then incubated with 50 µl of the TUNEL reaction mixture containing terminal deoxynucleotidyl transferase for 60 min in a dark, humidified atmosphere at 37°C. After the slides were rinsed three times with PBS, they were analyzed with a fluorescence microscope (515–565 nm). Two investigators who were blinded to the experimental protocol evaluated the TUNEL staining in sections from the cortical and hippocampal brain regions. These regions were selected because it is known that they are susceptible to hypoxia-ischemia injury (31). Regions in the contralateral hemisphere were also analyzed. Multiple fields (6 from each region) were analyzed, and data were represented as the number of TUNEL cells per high-power field previously described (13).

**Caspase activity assay.** Tissues from the cortex and hippocampus were taken from the lesioned hemispheres of P7 rat pups at 12, 18, 24, and 48 h after the hypoxia-ischemia insult ( $n = 4$  for each group at each time point). The tissue was frozen in liquid nitrogen and stored at -80°C until use. Caspase-3 cellular activity was measured with caspase-3 cellular activity assay kit PLUS-AK-703 (BIOMOL Research Laboratories, Plymouth Meeting, PA) as described previously (30). The brain tissue was homogenized in ice-cold cell lysis buffer consisting of 50 mM HEPES, pH 7.4, 0.1% 3-([3-cholamidopropyl] dimethylammonio)-1 propanesulfonate (CHAPS), 5 mM DTT, and 0.1 mM EDTA and centrifuged at 12,000 g for 10 min at 4°C. Protein content was measured by using the DC protein assay (Bio-Rad, Hercules, CA). DEVDpNA cleavage activity was measured from the cell lysate supernatants as described previously (13). Ten microliters of the lysate were incubated in a 96-well plate with 80 µl of the assay buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 10 mM DTT, 1 mM EDTA, 10% glycerol). The plate was incubated at 37°C for 10 min. The reaction was started by adding 10 µl of 30 µM Ac-DEVD-pNA. The plate was then incubated in the dark at 37°C for 30 min before it was read at a wavelength of 405 nm in a microtiter-plate reader. The data were represented as the pmol pNA·min<sup>-1</sup>·µg<sup>-1</sup> of protein.

**Western blot.** Western blot analysis was performed as previously described (30). Tissues from the cortex and hippocampus were taken from the lesioned hemispheres of P7 rat pups at 18, 24, and 48 h after the hypoxia-ischemia insult ( $n = 4$  for each group at each time point). The tissue was frozen in liquid nitrogen and stored at -80°C until use. Then 100–300 mg of brain tissue were homogenized in 1 ml of ice-cold lysis buffer (0.32 M sucrose, 1 mM EDTA, 5 mM Tris HCl, pH 7.4, 0.1 mM PMSF, 10 µM leupeptin, 1 mM β-mercaptoethanol). The homogenate was centrifuged at 1,330 g for 2 min to remove debris. The supernatant was transferred into a new tube and centrifuged at 12,000 g for 10 min at 4°C. Protein content

was measured by use of the DC protein assay (Bio-Rad). Equal amounts of protein (20 µg) were loaded in each lane of polyacrylamide-SDS gels [7% for poly (ADP-ribose) polymerase (PARP), 12% for caspase-3]. The gels were electrophoresed, followed by a transfer of the protein to a nitrocellulose membrane. The membrane was blocked with a blocking solution and then probed with rabbit polyclonal IgG PARP antibody (1:400) and rabbit polyclonal IgG caspase-3 antibody (1:400) (Santa Cruz Biotechnology, Santa Cruz, CA). The membrane was then probed with antirabbit IgG-HRP antibody (1:2,500) (Santa Cruz Biotechnology). The membranes were then probed with the Immun-star horseradish peroxidase substrate kit (Bio-Rad), and densitometry analysis was performed with the ChemiDoc detection system (Bio-Rad) and Quantity One software (Bio-Rad). The membranes were also probed with rabbit polyclonal IgGα-tubulin antibody (1:400) (Santa Cruz Biotechnology) as an internal control.

**Brain weight.** Brain weight was determined as previously described (12). The pups ( $n = 7$  for each group) were killed under deep anesthesia 2 or 6 wk after the hypoxia-ischemia insult. After removal of the brain, the cerebellum and brainstem were removed from the forebrain. The hemispheres were separated by a midline incision and then weighed on a high-precision balance (sensitivity ±0.001 g). Brain damage was expressed as the percent reduction of the ipsilateral (right) hemisphere compared with the contralateral (left) hemisphere.

**Statistical analysis.** The data are represented as means ± SE. Statistical differences were compared by using a one-way ANOVA and then, if a significant difference was found, a Student-Newman-Keuls method for multiple comparisons. A P value of <0.050 was considered to be statistically significant.

## RESULTS

HBO treatment reduced changes in caspase-3 activity after hypoxia-ischemia insult. To confirm that HBO treatment offers its neuroprotective effects by reducing hypoxia-ischemia-induced apoptosis, we measured the amount of caspase-3 activity at 12, 18, 24, and 48 h after the hypoxia-ischemia insult (Fig. 1). The cleavage of Ac-DEVD-pNA, which reflects caspase-3 activity, was measured in both the cortex and hippocampus.

Previously, it has been shown that caspase-3 activity increases 12 h after a hypoxia-ischemia insult, peaks between 24–36 h, and returns to baseline levels by 48 h (13). We found similar results in both the cortex and hippocampus (Fig. 1). After treatment with HBO, we found that the caspase-3 activity induced by hypoxia-ischemia was decreased in the cortex and hippocampus at 24 h after the hypoxia-ischemia insult ( $P < 0.05$ ).

Furthermore, we investigated the expression of caspase-3 after a hypoxia-ischemia insult and subsequent HBO treatment by analyzing the active subunit of caspase-3 via Western blot analysis (Fig. 2). The p17 subunit of caspase-3 was present in both the cortex and hippocampus 18 and 24 h after the hypoxia-ischemia insult. Densitometric analysis of the immunoblots showed that the expression of caspase-3 increased in the cortex and hip-

pocampus at 18 h ( $P < 0.05$ ) and 24 h ( $P < 0.05$ ) after the insult compared with the control levels (age-matched normal brain). Treatment with HBO decreased the hypoxia-ischemia-induced expression of caspase-3 in the cortex and hippocampus at both 18 h ( $P < 0.05$ ) and 24 h ( $P < 0.05$ ) after the insult.

**HBO treatment reduced changes in PARP cleavage after hypoxia-ischemia insult.** We next investigated how HBO affects cleavage of PARP, a caspase-3 substrate. Again, we used Western blotting and densitometric analysis to assess the expression of PARP after hypoxia-ischemia and subsequent HBO treatment (Fig. 3). We found that after a hypoxia-ischemia insult, PARP cleavage increased in both the cortex and hippocampus at 24 h ( $P < 0.05$  for both regions) and 48 h ( $P < 0.05$  for both regions) over control levels (age-matched normal brain). After HBO treatment, the hypoxia-ischemia-induced PARP cleavage in both the cortex and hippocampus was decreased at 24 h ( $P < 0.05$ ) and at 48 h ( $P < 0.05$ ).

**HBO treatment reduced DNA fragmentation after hypoxia-ischemia insult.** Cleavage of PARP leads to DNA fragmentation, and the fragmentation of nuclear DNA in cells has been identified extensively with TUNEL staining (7, 13,

25). We found that there was a time-dependent increase in the number of TUNEL-positive cells in the ipsilateral hemisphere (Fig. 4). Multiple fields (6 fields in each region) were analyzed, and the data were represented as the number of TUNEL-positive cells per high power field. In the cortex (Fig. 5A), there were ~4–5 cells per high power field by 12 h after the hypoxia-ischemia insult, ~20 cells per high power field by 18 h after the insult, and ~44 cells per high power field by 24 h after the insult. After HBO treatment, the number of TUNEL-positive cells observed in the cortex had decreased dramatically at both 18 and 24 h. Similar results were also observed in the hippocampus (Fig. 5B).

Under light microscopy, TUNEL-positive cells were markedly increased especially in hippo-

(Continued on page 16)

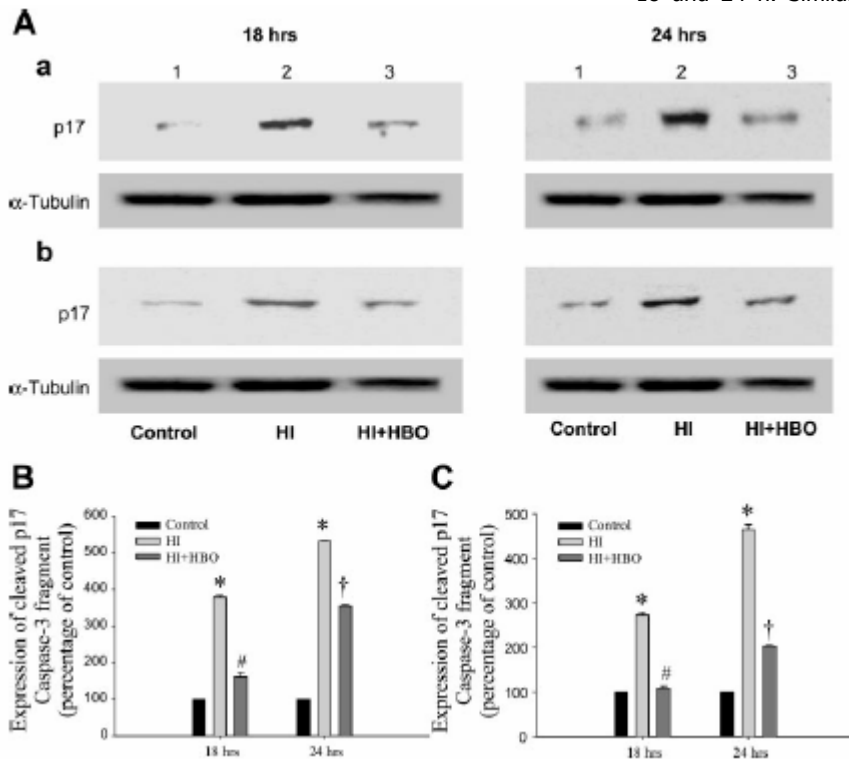


Fig. 2. *A*, representative immunoblot from 3 separate Western blot experiments showing the expression of the active subunit (p17 fragment) of caspase-3 in the cortex (*a*) and hippocampus (*b*). Lane 1 is control, lane 2 is HI, and lane 3 is HI+HBO.  $\alpha$ -Tubulin was blotted to ensure that equal amounts of protein were loaded into each well. Expression of the active subunit (p17 fragment) of caspase-3 in the cortex (*b*) and hippocampus (*c*) 18 and 24 h after a HI insult was summarized. Data are represented as a percentage of the control levels, as measured by densitometry analysis. \* $P < 0.05$  compared with control; # $P < 0.05$  compared with HI; † $P < 0.05$  compared with control and HI (ANOVA).

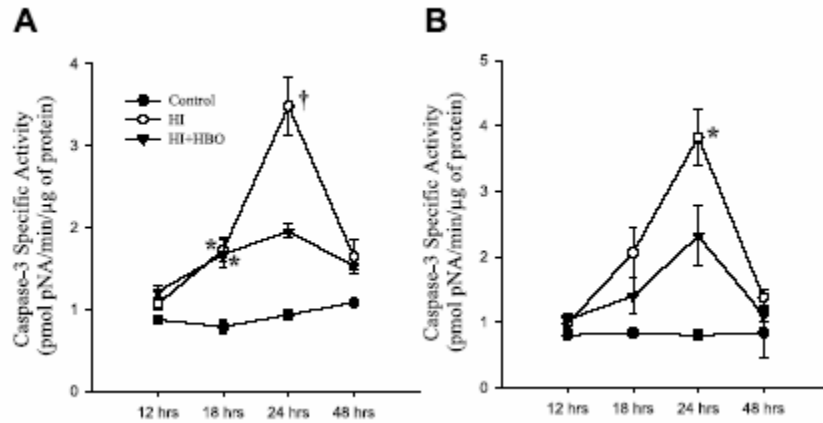


Fig. 1. Ac-DEVD-pNA cleavage activity was used to measure caspase-3 activity in the cortex (*A*) and hippocampus (*B*) at 12, 18, 24, and 48 h after a hypoxia-ischemia (HI) insult. Activity peaked ~24 h after the insult. Hyperbaric oxygen (HBO) treatment reduced the activity of caspase-3 in both the cortex and hippocampus. \* $P < 0.05$  compared with control; † $P < 0.05$  compared with control and HI (ANOVA).

Fig. 3. *A*, representative immunoblot from 3 separate Western blot experiments showing the expression of poly (ADP-ribose) polymerase (PARP) cleavage in the cortex (*a*) and hippocampus (*b*). Lane 1 is control, lane 2 is HI, and lane 3 is HI+HBO.  $\alpha$ -Tubulin was blotted to ensure that equal amounts of protein were loaded into each well. Expression of PARP cleavage in the cortex (*a*) and hippocampus (*b*) 24 and 48 h after a HI insult was summarized. Data are expressed as the ratio of 85-kDa fragment to 116-kDa fragment and then expressed as a percentage of the control levels. \* $P < 0.05$  compared with control; # $P < 0.05$  compared with HI (ANOVA).

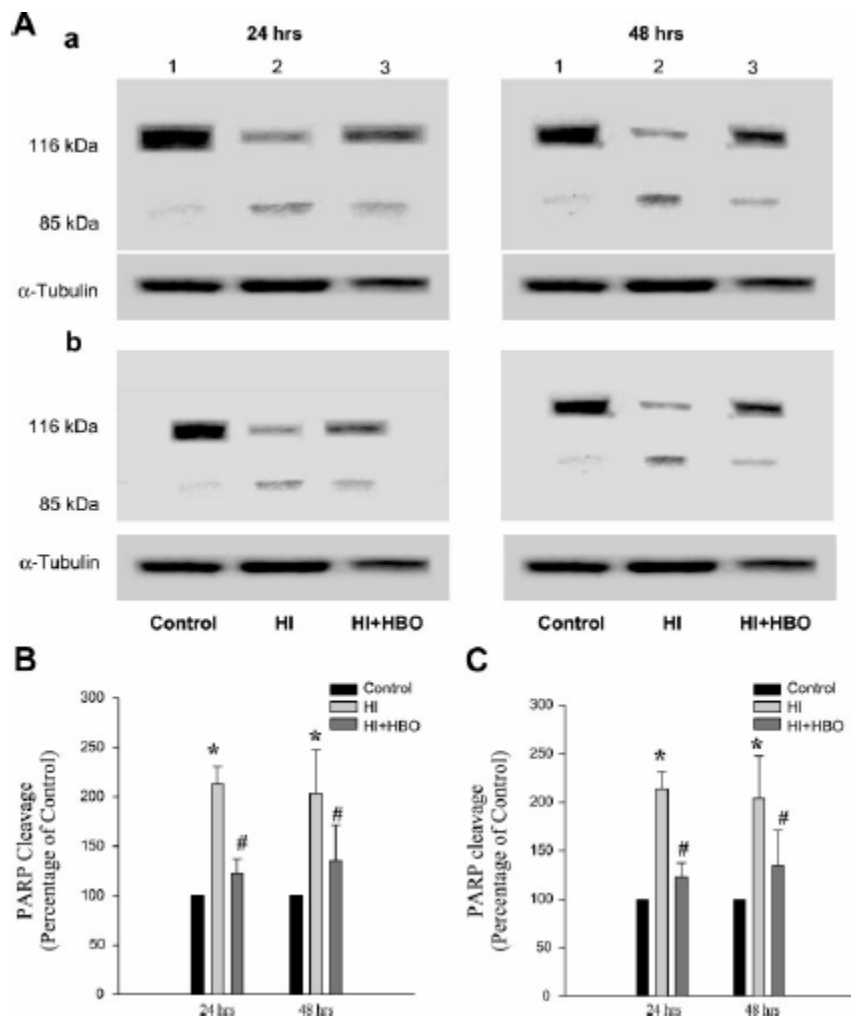
campus (Fig. 6E), and HBO reduced the number of TUNEL-positive cells (Fig. 6F). At higher magnification, the nuclei of cells were clearly stained in both hippocampus and cortex (Fig. 6, H and K). Again, HBO reduced the number of TUNEL-positive cells (Fig. 6, I and L). A few TUNEL-positive cells were identified in control slides (Fig. 6, G and J).

*HBO preserves brain weight.* Several pups in each litter were allowed to grow to 2 or 6 wk of age so that brain injury could be assessed on the basis of brain weight. Animals that were subjected to a hypoxia-ischemia insult showed brain atrophy and brain weight loss at 2 or 6 wk after hypoxia insult.

The ipsilateral hemispheric weights at 2 wk after the insult were as follows:  $0.529 \pm 0.006$  g for control pups,  $0.253 \pm 0.008$  g ( $P < 0.05$ , ANOVA) for hypoxia-ischemia pups, and  $0.396 \pm 0.115$  g ( $P < 0.05$ , ANOVA) for hypoxia-ischemia + HBO pups. The ipsilateral hemispheric weights at 6 wk after the insult were as follows:  $0.608 \pm 0.0065$  g for control pups,  $0.348 \pm 0.412$  g for hypoxia-ischemia pups, and  $0.480 \pm 0.264$  g for hypoxia-ischemia + HBO pups. So a hypoxia-ischemia insult resulted in brain retardation up to 50% at 2 or 6 wk, and HBO treatment preserved brain growth up to 70–80%. Even though the brain weight in HBO-treated groups is significantly smaller ( $P < 0.05$ , ANOVA) than that of normal pups, it is significantly larger than those who suffered a hypoxia-ischemia insult and did not receive treatment ( $P < 0.05$ , ANOVA).

## DISCUSSION

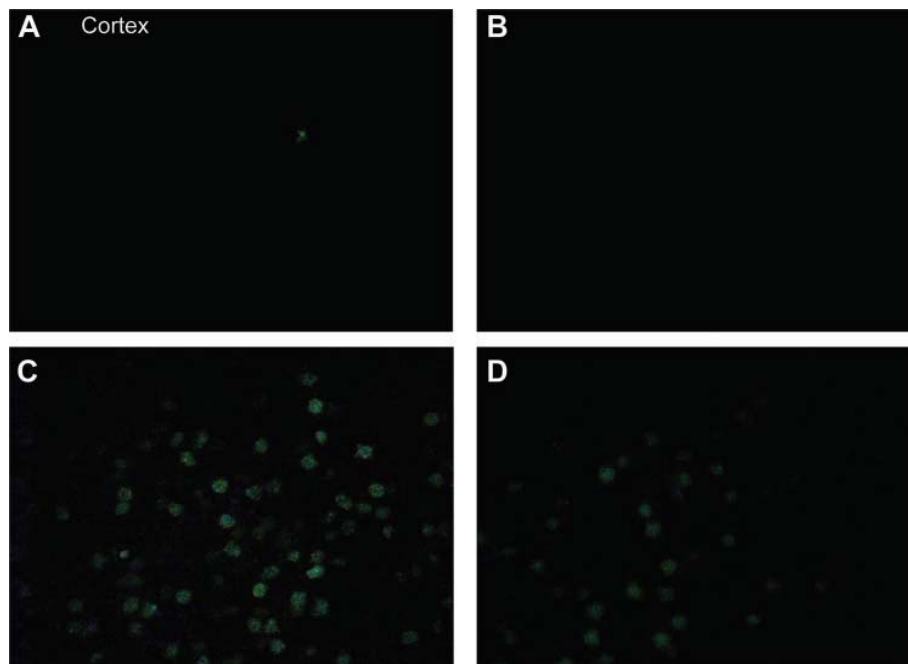
Because early brain injury occurs within 24 h after a hypoxia-ischemia insult (12), which might contribute to the delayed brain atrophy, we decided to study apoptotic changes from 12 to 48 h. We analyzed two different re-



gions of the brain, the hippocampus and the cortex. These regions were selected because it is known that these regions are susceptible to hypoxia-ischemia injury (31), which is also consistent with our own observations (12). The results of the present study, using different detection methods including caspase-3 expression and activity, PARP cleavage, and TUNEL staining, demonstrate that HBO treatment affords neuroprotection partially by reducing apoptosis in the early stage of neonatal hypoxia-ischemia.

*Apoptosis in hypoxia-ischemia.* Patterns of cell loss after hypoxia-ischemia are likely to depend on the severity of the injury. Selective neuronal loss may develop after brief or acute injuries, whereas infarction or tissue necrosis, as well as glial loss, may result from more severe injuries. Different mechanisms of damage are likely to be associated with these distinct patterns of cell loss (7–10, 16, 29, 34). Evidence has suggested that neuronal death, including ischemia-induced death, occurs via apoptosis as well as necrosis (29). Apoptosis has also been shown to play an important role in animal and human models of various diseases, including ischemic brain damage (7). Although several different models of neonatal hypoxia-ischemia

Fig. 4. Terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) staining of cortex 24 h after a HI insult. Staining was performed on paraffin-embedded sections by using the in situ cell death detection kit, fluorescein. *A*: an occasional TUNEL-positive cell was found in the cortex of a normal pup. *B*: the same was true for the hemisphere contralateral of the HI insult. *C*: by 24 h after the insult, the greatest number of TUNEL-positive cells was observed in the hemisphere ipsilateral to the HI insult. *D*: after HBO treatment, there were significantly fewer TUNEL-positive cells

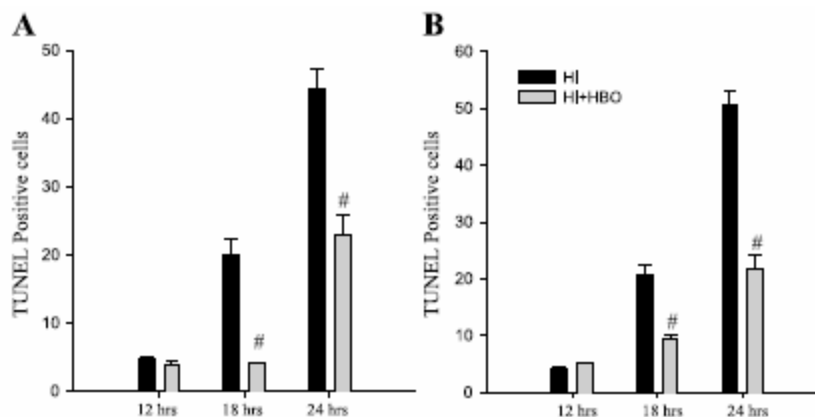


have been developed in the past, the modified Levine preparation (38) in 7-day-old rats is the most commonly used model of cerebral hypoxia-ischemia in immature animals, and it has widely been used to assess the efficiency of putative neuroprotective agents (4). This model produces consistent cell death in the cerebral cortex, hippocampus, striatum, and thalamus, as well as white matter tracts (13, 37). Cell death after hypoxiaischemia in this model is not the result of classic necrosis but may involve one or more components of the apoptotic pathway (21, 23). Now that it is established that apoptosis occurs in the neonatal brain after a hypoxia-ischemia insult, the question remains how to prevent its occurrence. Over the past several years, studies have been conducted with the idea of preventing or reducing apoptosis after a hypoxia-ischemia insult. Dexamethasone (13, 28), N-methyl-D-aspartate receptor open-channel blocker (43), group II metabotropic glutamate receptor agonist (11), N-tosyl-L-phenylalanyl-chloromethylketone (27), protein-disulfide isomerase (44), brain-derived neurotrophic factor (21), and hypothermia (1) have all been used with various de-

grees of success. These studies have provided an initial insight and understanding into the components that are involved in the apoptotic pathway. However, many questions remain unanswered.

*Effect of HBO on hypoxia-ischemia-induced apoptosis.* Present evidence seems to suggest that hypoxia in and of itself does not induce apoptosis, but rather it does so indirectly through energy depletion, altered ionic homeostasis, or oxygen-sensing molecules, which in turn activate the apoptotic pathway (7). Of the apoptotic activators, the cysteine proteases or caspases are of particular interest. Caspases are expressed as proenzymes containing three subunits that are activated after proteolytic processing and association of the large and small subunits (7, 9, 19). Once activated, caspases cleave proteins in a relatively substrate-specific manner, which provides for the morphological changes observed in cells during apoptosis (7, 9). Of the caspases, caspase-3 is the most widely studied in the neonatal hypoxia-ischemia model. Caspase-3 plays an effector's role in neuronal cell death during

Fig. 5. TUNEL-positive cells observed in the cortex (*A*) and hippocampus (*B*) of HI and HI+HBO pups 12, 18, and 24 h after the insult. Staining was performed on paraffin-embedded sections by using the in situ cell death detection kit, fluorescein. This kit labels DNA strand breaks that are generated during apoptosis, allowing for the differentiation of apoptosis from necrosis. #*P* < 0.05 compared with HI (ANOVA).



normal brain development as well as after a hypoxia-ischemia insult. Caspase-3 and its downstream events were a main focus in the present study because caspase-3 is implicated in the apoptotic changes after neonatal hypoxia (19). Cheng et al. (13) showed that, in the ipsilateral hippocampus, caspase-3-like activity detected by Ac-DEVD-AMC increased 12 h after hypoxia-ischemia, peaked at ~24–36 h, and then returned to basal levels by 48 h. Han et al. (21) found that caspase-3 activation occurs predominantly in neurons and their processes in a specific and delayed time course after hypoxia-ischemia. Furthermore, Han et al. showed that caspase-dependent components of cell death do not peak until 12–24 h after the injury. Considering these studies, we looked at caspase-3 activity and the expression of caspase-3 around the peak times observed. We found that in both the cortex and hippocampus at 18 and 24 h after a hypoxia-ischemia insult, caspase-3 activity and expression were increased. HBO reduced the enhanced caspase-3 activity and expression.

Furthermore, we looked at PARP cleavage, a caspase-3 substrate. PARP has been shown to be synthesized after the activation of the apoptotic pathway by being degraded by caspases (42). The 116-kDa fragment of PARP cleaves to form fragments of 85 and 24 kDa. Cheng et al. (13) found that the 116-kDa fragment of PARP becomes cleaved after a hypoxia-ischemia insult. We detected the expression of the 116- and 85-kDa fragments of PARP after a hypoxia-ischemia insult and subsequent HBO treatment. At 24 and 48 h after a hypoxia-ischemia insult, there was a significant amount of PARP cleavage in both the cortex and hippocampus. However, after HBO treatment, PARP cleavage decreased in both regions at both time points. Cheng et al. (13) found that cell injury is delayed 6–24 h after insult detected with both biochemical and anatomic methods designed to detect DNA damage. They also found that there was a time-dependent increase in the number of TUNEL-positive cells in the ipsilateral hemisphere, which peaked between 18 and 24 h after the insult. We also found similar results suggesting that the number of TUNEL-positive cells peaked ~18–24 h after the insult in both the cortex and hippocampus. Previously, our laboratory has confirmed apoptotic cells by transmission electron microscopy (12), a gold standard for apoptosis (41) in the dentate gyrus 24 h after a hypoxia-ischemia insult.

After HBO treatment we observed far less apoptotic cells. Other studies (31, 34) have shown that apoptosis is present in the neonatal brain after hypoxia-ischemia and that it is delayed, which suggests that it could be an important target for treatment. We found that a single treatment of HBO (100% oxygen at 3 ATA) for 1 h reduced the number of TUNEL-positive cells present in the ipsilateral hemisphere of the cortex and hippocampus at 18 and 24 h after a hypoxia-ischemia insult.

In our laboratory's previous study, we found that HBO not only attenuated the effects of hypoxia-ischemia on the neonatal rat brain by reducing the progression of neuronal injury, but it also preserved tissue function (12). Now, this study shows that a single treatment of HBO

(100% oxygen, 3 ATA) for 1 h reduced the hypoxia-ischemia-induced increase of TUNEL-positive cells, the increase in the activity of caspase-3, the expression of caspase-3, and PARP cleavage. Taken together, the results of this present study suggest that HBO does in fact offer its neuroprotection by reducing hypoxia-ischemia-induced apoptosis. The rationale of using HBO as a treatment of hypoxia-ischemia comes from the evidence that HBO increases tissue oxygen delivery, especially to areas of diminished flow, enhances neuronal viability, reduces brain edema, improves the integrity of the blood-brain barrier, and regulates post-ischemia metabolism (33).

Whether HBO affords its neuroprotective effects by directly intervening in the apoptotic pathway somewhere upstream of caspase-3, by restoring the depletion of energy and the altered ionic homeostasis, or by changing the expression of oxygen-sensing molecules remains to be determined.

*We thank Joe Jones from the Department of Anatomy for assisting in making the paraffin slides.*

## DISCLOSURES

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## Testimonials Continued

(Continued from page 1)

More importantly, yesterday he referred to his father as “Daddy”, instead of his usual “ada.” He now tries to say everything he can. Today he made a good attempt at “dog” and he clearly said “elephant!”

Last week, I taught him the word “again” while we were in the chamber together. He usually says “mo” to mean “more.” Two days later, he said “again” after his favorite CD finished playing! It was spontaneous and clear, with a strong “g” sound. Not only did he say it well, but he understood when to say it.

In a touching moment in the car a few weeks ago, I was listening to a religious rock son on the radio. Alex said “amen” which is what he says during bedtime prayers. I told him that he was right, the song was about God, and he clearly started saying “God” over and over. He said it again on our way to church last Sunday.

Regarding his vision, he wasn’t very interested in watching DVDs in the chamber during our initial treatments. Now, however, he is very focused on them, making appropriate exclamations and anticipating the next song or character to appear. One day last week as we turned into the parking lot of the HBOT facility,

Alex looked at the building and said “Dave!”— the name of the man who operates the chambers. I couldn’t believe he saw the building and made the cognitive connection between the place and the name.

Being in the HBOT chamber with Alex is fascinating. Alex seems more “awake” now, like a sponge waiting to be filled with knowledge. If we can give him the gift of speech and help him to be more aware of his environment, he will get so much more out of life. It will be the greatest thing we can do for him. We look forward to helping him explore his newly-emerging world.



*"Mundo vitam dare"*



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