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Research Report

Up-regulated HIF-1α is involved in the hypoxic tolerance induced by hyperbaric oxygen preconditioning

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ABSTRACT

Hyperbaric oxygen preconditioning (HBO-PC) has been shown to be effective in preventing hypoxic injuries in many animal models. The aim of the present study was to examine the hypoxic tolerance induced by HBO-PC and to explore the role of hypoxia-inducible factor-1α (HIF-1α) in a global hypoxia model. Male mice received HBO-PC before hypoxia exposure and swimming. HBO-PC significantly prolonged the survival time and the tolerance time of swimming under normobaric hypoxia. HBO-PC increased the protein content of HIF-1α and erythropoietin (EPO) in the cerebral cortex and hippocampus and prevented the changes of blood brain barrier (BBB) permeability and brain edema caused by hypoxia exposure. The results suggested that HBO-PC induced hypoxic tolerance in mice via up-regulation of HIF-1α and its downstream genes.

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1. Introduction

Hypoxia is a state of oxygen deficiency in the body which is sufficient to cause impairment of function. It is caused by the reduction in the partial pressure of oxygen, inadequate oxygen transport, or the inability of the tissues to use oxygen, which is a focus of many fields such as altitude medicine, aviation medicine and sports medicine (Sen Gupta et al., 1979; Robach et al., 2000; Casas et al., 2000). The common preventive measure against hypoxia is hypoxic preconditioning. However it has not been employed clinically because of safety concerns. Hyperbaric oxygen (HBO) has been used widely as a primary therapy in patients with carbon monoxide poisoning, decompression sickness, and arterial gas embolism, and as an adjunctive therapy for the treatment of various diseases accompanied by impaired oxygen delivery (Sheridan and Shank, 1999; Tibbles and Edelsberg, 1996). Recent studies have shown that HBO preconditioning (HBO-PC) has neuroprotective effects against focal and global cerebral ischemia (Xiong et al., 2000; Wada et al., 1996, 2001; Ostrowski et al., 2008), and induces ischemic tolerance in other organs including spinal cord (Dong et al., 2002), myocardium (Kim et al., 2001) and liver (Yu et al., 2005).

Hypoxia-inducible factor-1 (HIF-1) is a transcription factor specifically activated by hypoxia (Chavez and LaManna, 2002).

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Abbreviations: ANOVA, analysis of variance; ATA, atmosphere absolute; BBB, blood brain barrier; EPO, erythropoietin; GLUTs, glucose transporters; HIF-1α, hypoxia-inducible factor-1α; HBO, hyperbaric oxygen; HBO-PC, hyperbaric oxygen preconditioning; HPA, high pressure air; IHC, immunohistochemistry; NC, normal control; NPA, normal pressure air; PBS, phosphate-buffered saline; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor

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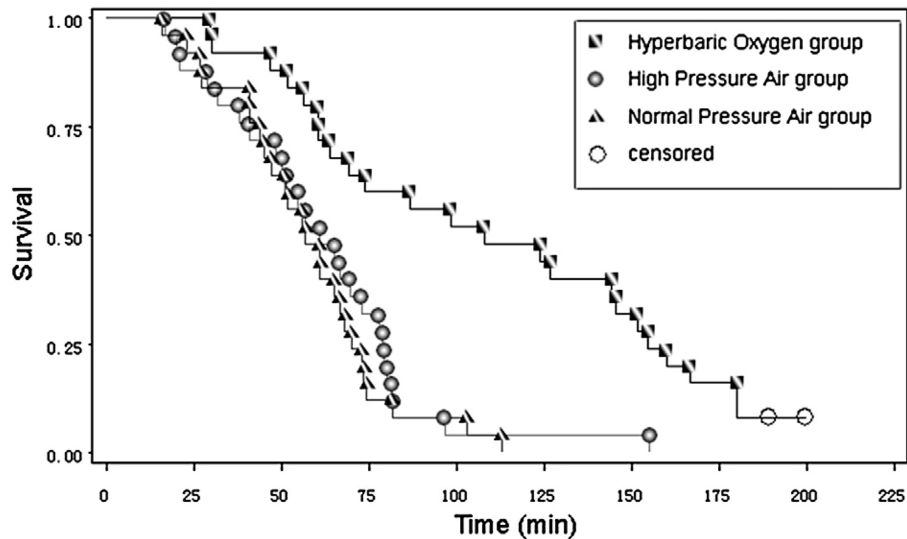


Fig. 1 – Survival time during normobaric hypoxia exposure. Median survival time and 95% confidence interval (CI) were: HBO-PC group 108.00 min, 95% CI (64.00, 152.00) min, HPA group 61.00 min, 95% CI (48.42, 78.12) min, and NPA control group 57.00 min, 95% CI (45.29, 68.00) min respectively. Survival time curve log-rank test demonstrated significant differences between HBO group and the two control groups ($P<0.05$). No significant difference between NPA and HPA group was found ($P>0.05$).

The accumulation of HIF-1α in ischemic or hypoxic tissues might promote adaptive mechanisms for cell survival (Bergeron et al., 1999) and was found to be an important mediator of hypoxia-induced tolerance to ischemia (Bergeron et al., 2000; Jones and Bergeron, 2001; Bernaudin et al., 2002). Recent reports indicated that HIF-1α was also induced by HBO exposure (Salhanick et al., 2006). Erythropoietin (EPO), one of the downstream genes regulated by HIF-1α, is a hematopoietic growth factor. Recently EPO was found to be expressed in the central nervous system and exerted neuroprotective effects (Digicaylioglu et al., 1995; Iwai et al., 2007; Wakida et al., 2007). In addition, EPO transcription and translation were also found in preconditioned brains (Bernaudin et al., 2002; Jones and Bergeron, 2001).

To demonstrate whether HBO-PC induces ischemic tolerance, we studied the survival time and the physical stamina of mice under normobaric hypoxia. The central nervous system, especially the cortex, is sensitive to hypoxia that the pathological changes of the brain are more severe than that of other organs under the same condition. Thus we evaluated the neuroprotective effects of HBO-PC and explored the expression of HIF-1α and EPO, one of the downstream genes regulated by HIF-1, in the cerebral cortex and hippocampus.

2. Results

2.1. *Survival time*

Survival time was measured 12 h after HBO-PC. The data presented were the median survival time (Fig. 1). We found that HBO-PC significantly prolonged the survival time of mice under hypoxia from 57.00 min, 95% CI (45.29, 68.00) min in normal pressure air group to 108.00 min, 95% CI (64.00, 152.00) min ($P<0.05$). There is no difference between high pressure air and normal pressure air groups ($P>0.05$).

2.2. *Tolerance time of swimming under hypoxia*

12 h after HBO-PC, tolerance time of swimming under hypoxia was measured. Tolerance time of swimming under hypoxia of HBO-PC group (45.19±17.27 min) was significantly longer than that of normal pressure air (20.96±11.30 min) ($P<0.05$). No difference between high pressure air (22.37±11.29 min) and normal pressure air groups was found (Fig. 2).

2.3. *Brain-water content*

Brain-water contents of normal pressure air (77.95±0.27) and high pressure air groups (77.81±0.25) were significantly higher than that of normal control group (77.34±0.35) ($P<0.05$). HBO-PC efficiently prevented brain edema (77.32±0.49) (Fig. 3).

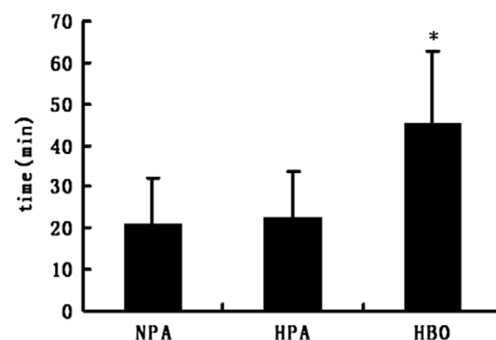


Fig. 2 – Tolerance time of swimming under hypoxia. Data are presented as mean±s.d. ($n=16$). Compared to NPA or HPA group, HBO preconditioning significantly prolonged the tolerance time of swimming under hypoxia ($P<0.05$). No significant difference between NPA and HPA groups was found. *$P<0.05$ vs NPA.

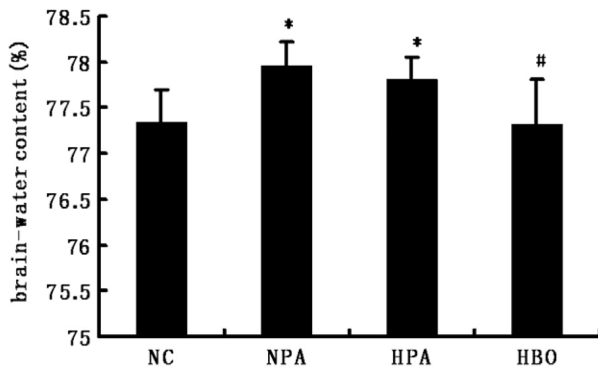


Fig. 3 – Brain-water content after hypoxia exposure. Data are presented as mean ± s.d. (n = 10). Hypoxia exposure caused significant increase of brain-water content in both NPA (77.95 ± 0.27) and HPA (77.81 ± 0.25) groups (P < 0.05), while the brain-water content in HBO-PC (77.32 ± 0.49) group was comparable to that of NC (77.34 ± 0.35). *P < 0.05 vs NC, #P < 0.05 vs NPA.

2.4. Evans Blue extravasation

The Evans Blue content was (1.56 ± 0.22) μg/g tissue in the normal control group. The normal pressure air (3.35 ± 0.67) group and high pressure air groups (3.14 ± 0.80) had significantly higher extravasation of Evans Blue dye than in normal control group. Evans Blue dye extravasation was less in HBO-PC group (2.03 ± 0.36) than in normal pressure air group (P < 0.05) (Fig. 4).

2.5. Western blot analysis for HIF-1α

The immunoreactive band of 120 kDa detected by Western blot analysis stands for HIF-1α protein. We evaluated the HIF-1α protein in mice exposed to HBO for 1 day (HBO1), 3 days (HBO3) and 5 days (HBO5), and exposed to normal pressure air for 1 day (HPA1), 3 days (HPA3) and 5 days (HPA5), respectively. The levels of HIF-1α protein expression in normal pressure air, high pressure air 1 and high pressure air 3 groups were barely detectable. High pressure air 5 group showed a faint HIF-1α band. In mice preconditioned with HBO, the protein of HIF-1α was markedly increased at all 3 time points, among which HBO3 caused the most obvious increase of 3.1 fold (Fig. 5). The change trends of HIF-1α protein expression were the same between cerebral cortex and hippocampus.

2.6. Immunohistochemistry for HIF-1α

We evaluated the expression of HIF-1α in cerebral cortex and hippocampus after HBO-PC using immunohistochemistry. Results showed that HBO exposure for 1 day, 3 days and 5 days all produced HIF-1α accumulation in both cortex and hippocampus. In normal pressure air and high pressure air groups, only a faint nuclear staining for HIF-1α was found (Fig. 6).

2.7. Western blot analysis for EPO

HBO exposure for 3 days significantly increased the EPO protein level by 1.2 fold in the cerebral cortex and by 1.8 fold in the hippocampus (P < 0.05) (Fig. 7). No differences were found between normal pressure air and high pressure air groups.

3. Discussion

In the present study we found that HBO-PC significantly prolonged the survival time and the tolerance time of swimming of mice under hypoxia, indicating that HBO-PC effectively induced hypoxic tolerance and enhanced the physical stamina under hypoxia. HBO-PC increased the protein content of HIF-1α and EPO, one of the downstream genes regulated by HIF, in the cerebral cortex and hippocampus, prevented the increased BBB permeability and brain-water content caused by hypoxia exposure. Our results suggested that the hypoxic tolerance induced by HBO-PC in mice involved up-regulation of HIF-1α and its downstream genes.

Hypoxia or ischemia injury causes complex pathophysiological changes with a number of contributing factors. It is difficult to achieve effective treatment or protection by targeting individual mediators or mechanisms. It has been previously suggested that exposure to sublethal stress can induce protection against subsequent more severe stress, which is known as preconditioning (Zemke et al., 2004). With the increasing availability of HBO chamber, HBO has extended its clinical application into the fields of perioperative care and intensive care (Muth et al., 2002) and HBO-PC strategies have been studied and appear to increase the resistance to hypoxia or ischemia injuries. The majority of studies have examined the protective effects of HBO-PC on a certain organ. Instead, we studied the survival time and the physical stamina of mice under hypoxia exposure to exam the protective effects of HBO-PC on the whole body as an integrated functional unit. We found that HBO-PC significantly prolonged the median survival time of mice under hypoxia from 57.00 min in normal pressure air group to 108.00 min. Studies using other animal model reported that HBO-PC increased the percentage of rat pups surviving hypoxia

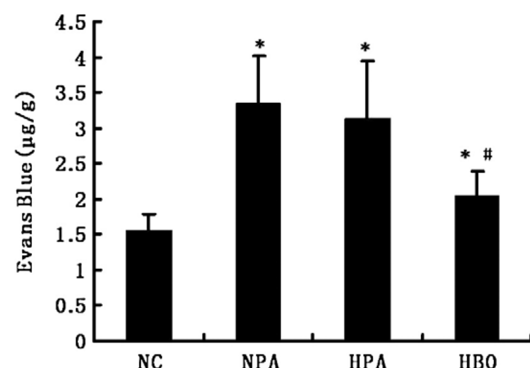


Fig. 4 – Evans Blue content after hypoxia exposure. Data are presented as mean ± s.d. (n = 10). The Evans Blue content was (1.56 ± 0.22) μg/g tissue in the NC group. Hypoxia exposure caused significant increase of Evans Blue content in both NPA (3.35 ± 0.67) and HPA (3.14 ± 0.80) groups (P < 0.05), and in HBO-PC (2.03 ± 0.36) group (P < 0.05). Compared to NPA, HBO preconditioning significantly decreased the Evans Blue content (P < 0.05). *P < 0.05 vs NC, #P < 0.05 vs NPA.

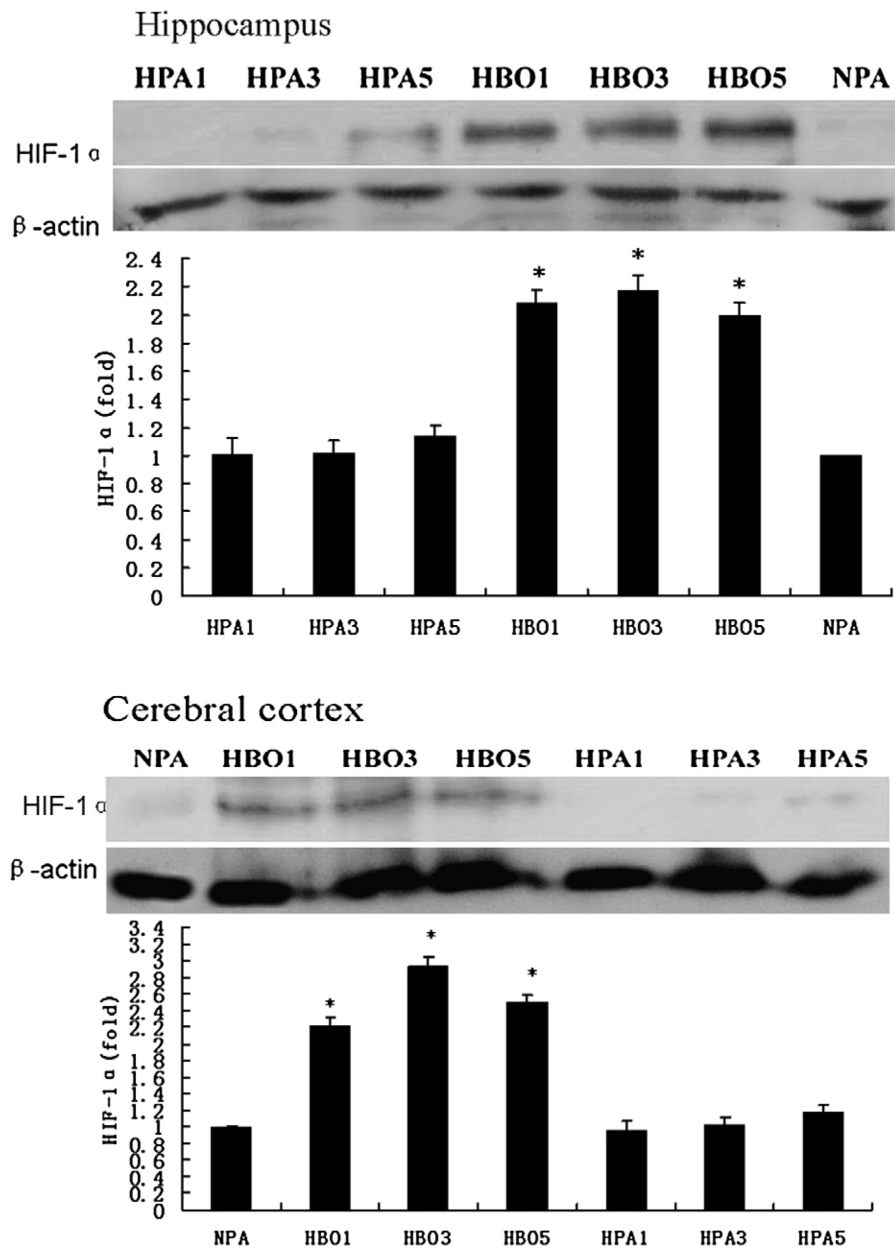
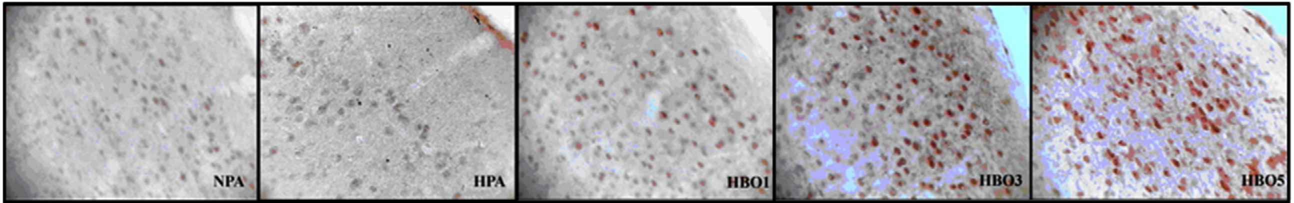


Fig. 5 – Western blot analysis of HIF-1α in cerebral cortex and hippocampus of mice exposed to HBO/HPA for 1 day, 3 days and 5 days. Fold-change values represent a mean of eight samples divided by the mean of the eight controls ($n = 8$). Data are presented as mean ± s.d., *$P < 0.05$ vs NPA.

combined with right carotid cauterization to the same level as hypoxic preconditioning did (Freiberger et al., 2006).

HIF-1α is generally considered to be the most important factor involved in the cellular response to hypoxia (Mazure et al., 2004; Semenza, 2000). By binding to the hypoxia-response element (Paul et al., 2004) in enhancers and promoters, HIF-1α up-regulates many target genes (Bergeron et al., 2000). HIF-1α has been found to play a complicated role in hypoxia and it can regulate the expression of both prosurvival and prodeath genes (Halterman and Federoff, 1999). To date, there are more than one hundred HIF-1α downstream genes identified with various functions (Salhanick et al., 2006) including EPO, glucose transporters (GLUTs), glycolytic enzymes, vascular endothelial growth factor (VEGF), p53, and the inducible isoform of nitric oxide synthase (Bergeron et al., 2000). HBO treatment was found to reduce the elevated expressions of HIF-1α in early transplanted islets, hypoxia–ischemia in neonatal rats, and focal/global cerebral ischemia in rats (Huang et al., 2007; Miao et al., 2006; Calvert et al., 2006; Ostrowski et al., 2005; Li et al., 2005), thereby decreased HIF-1-mediated cell death through a p53-mediated apoptotic cascade (Piret et al., 2002; Calvert et al., 2006). However, studies also demonstrated that the up-regulation of HIF-1α, GLUT-1, EPO, and some glycolytic enzymes after hypoxic preconditioning protected the neonatal rat brain against a subsequent lethal hypoxic ischemic insult (Jones and Bergeron, 2001). Studies have demonstrated that administration of EPO protected the BBB and decreased brain edema, thereby offering significant neuroprotection to the CNS (Grasso et al.,



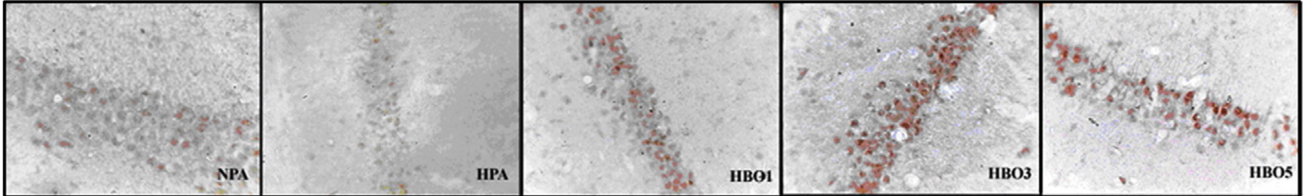


Fig. 6 – Immunohistochemistry of HIF-1α in cerebral cortex and hippocampus of mice exposed to HBO for 1 day, 3 days and 5 days. Positive staining nuclei were observed in neurons and gliacytes (magnification ×200). (*n* = 8).

2007; Verdonck et al., 2007). If HIF-1α is activated by HBO-PC, it could link HBO-PC to known preconditioning pathways via activation of adaptive genes. Recently, Salhanick et al found that HIF-1α in liver of rats was increased by HBO exposure (Salhanick et al., 2006). Using western blot and immunohistochemistry assays, we found that HBO-PC elevated the protein level of HIF-1α and EPO in cerebral cortex and hippocampus in mice. The neuroprotective effects of EPO have been demonstrated in vivo (Sakanaka et al., 1998; Brines et al., 2000; Prass et al., 2003) and in vitro (Morishita et al., 1997; Ruscher et al., 2002). Thus the up-regulated HIF-1α and its downstream gene may play an important role in the adaptive response induced by HBO-PC.

The mechanisms of HBO-PC-induced HIF-1α accumulation remain to be established. HBO-PC increases tissue oxygen levels by giving 100% oxygen at higher pressures. After HBO-PC, brain tissues will experience a relative hypoxia because the oxygen level is reduced to normal level at 21%. Therefore, repeating HBO-PC may produce a cycle of hyperoxia and then hypoxia and lead to HIF-1α accumulation. Furthermore, there are evidence that suggest that some non-hypoxic stimuli including growth factors, hormones, vasoactive peptides and metal ions can induce HIF-1α under normoxia (BelAiba et al., 2004; Park et al., 2003). Another possible mechanism for HIF-1α accumulation is the generation of reactive oxygen species (ROS) by HBO-PC. ROS could regulate HIF-1α by activation of PI3-K/PKB (Gao et al., 2004) and MAPK (Wang et al., 2004). ROS interfere with hydroxylase activity and lead to HIF-1α accumulation (Metzen et al., 2003). Under normoxic conditions ROS up-regulate HIF-1α expression (Richard et al., 2000; Haddad, 2002) and stabilize HIF-1α (Chandel et al., 1998, 2000; Agani et al., 2000; Schroedl et al., 2002). Indeed, Gu et al. (2008) reported that HBO induced a marked increase in the protein expression of HIF-1α and its target gene EPO. Salhanick et al. (2006) also found that HBO-PC increased HIF-1α in liver of rats.

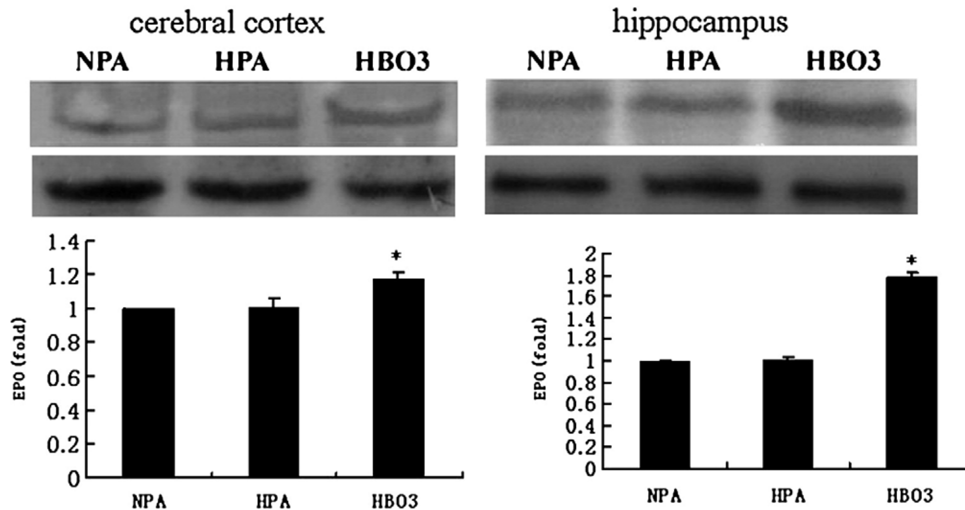


Fig. 7 – Western blot analysis of EPO in cerebral cortex and hippocampus of mice exposed to HBO for 3 days. Fold-change values represent a mean of eight samples divided by the mean of the eight controls (*n* = 8). Data are presented as mean ± s.d., **P* < 0.05 vs NPA.

Although evidence of HBO-PC has been observed in various models, HBO-PC is controversial due to differences of exposure pressure, the duration of HBO sessions, the number of HBO sessions, the timing of administration, and the species (Prass et al., 2000; Zhang et al., 2004). Previous studies reported that one episode of HBO was insufficient to induce ischemic tolerance in the brain. Ostrowski RP, et al. (2008) suggested that longer (e.g. 5 times HBO) preconditioning should be considered because it was more effective than 3 days HBO-PC. However a recent study has demonstrated that a single dose HBO-PC provided an equivalent neuroprotective effect to that with hypoxic preconditioning in neonatal rats (Freiberger et al., 2006). In the present study we evaluated the HIF-1α protein in mice exposed to HBO for 1 day, 3 days and 5 days. We found that the protein of HIF-1α was increased at all 3 time points, and HBO exposure for 3 days (6 HBO sessions) seems more effective at increasing the levels of HIF-1α. Therefore, in spite of the fact that short preconditioning might be effective, a longer preconditioning (such as for 3 days) could be considered if possible to achieve more effective protection.

In summary our results demonstrated that the HBO-PC appears effective to induce hypoxic tolerance in mice, and that the up-regulated HIF-1α and its downstream gene may contribute to the adaptive response induced by HBO-PC.

4. Experimental procedures

4.1. *Animals and groups*

Male Pathogen-free Kun-ming mice (20–22 g, Sipper-BK Company in Shanghai) were used. The animals were maintained on a 12 h light–dark cycle. Mice were divided into three groups: HBO group (received HBO pre-treatment twice a day for five consecutive days), high pressure air group (exposed to high pressure air twice a day for 5 consecutive days) and normal pressure air group (treated with normal pressure air).

4.2. *HBO or HPA exposure*

Mice were placed into a custom-made pressure chamber of transparent acrylic plastic. Compression was performed at a rate of 1 kg/cm^2/min to 2.5 ATA/100% oxygen or normal air and maintained for 60.00 min. The chamber was flushed with 100% oxygen or normal air at a rate of 5 L/min to avoid carbon dioxide accumulation. Decompression was performed at 0.2 kg/cm^2/min. For HBO exposure, oxygen and carbon dioxide contents were continuously monitored and maintained at ≥98% and at ≤0.03% respectively. Chamber temperature was maintained between 22 and 25 °C. To minimize the effects of diurnal variation, all exposures were started at10:00 and 16:00. Normal and high pressure air studies were conducted in room air.

4.3. *Survival time during normobaric hypoxia exposure*

12 h after the last HBO exposure, the mice were placed in the chamber and flushed with a gas mixture of 5% O_2–95% N_2 to measure survival time during normobaric hypoxia exposure. The volume of the chamber is 24.5 L and the gas flux was kept at 5 L/min. The time from onset of exposure to the last gasp (survival time) was measured for each animal.

The animal procedures were approved by the local animal care committee.

4.4. *Tolerance time of swimming under hypoxia*

12 h after the last HBO exposure, the mice bound with load of 5% body weight were kept in the chamber with water, and then flushed with a gas mixture of 10% O_2–90% N_2. Water temperature was maintained 25 °C and the gas flux was controlled at 5 L/min. The time from onset of exposure to the sink (tolerance time) was measured for each animal.

4.5. *Brain-water content*

12 h after the last HBO exposure, the mice were placed in the chamber flushed with a gas mixture of 10% O_2–90% N_2. The gas flux was controlled at 5 L/min to maintain the oxygen content at 10%. The total hypoxia exposure time was 12 h. Mice untreated with hypoxia exposure were served as normal control.

Right after hypoxia exposure, the brain samples were moved, weighed immediately (wet weight) and dried at 105 °C for 24 h (dry weight). The percent of water content was calculated with the formula ([wet weight − dry weight]/wet weight) × 100%.

4.6. *Evans Blue extravasation*

The hypoxia exposure was performed as 2.5. BBB permeability was assessed with Evans Blue extravasation. Briefly, Evans Blue dye (1%; 2 mL/kg) was injected via a femoral vein and allowed to circulate for 2 h. Rats were deeply anesthetized and transcardially perfused with PBS until a colorless perfusion fluid was obtained from the right atrium. The brains were removed and divided into the right and left hemispheres and weighed. Evans Blue dye exuded in the right brain was extracted by incubating in 2 mL/100 mg formamide for 24 h at 65 °C, and dye concentrations were measured by absorption at 620 nm and determined by a standard curve as reported previously (Udaka et al., 1970; Saria and Lundberg, 1983). The amount of Evans Blue dye was expressed as μg of Evans Blue dye per g wet weight of the tissue.

4.7. *Western blot analysis*

At designed time points, after one-day (2 HBO sessions), three-day (6 HBO sessions), and five-day (10 HBO sessions) HBO exposures, mice were decapitated, and their brains were rapidly removed and frozen in liquid nitrogen. Cortex and hippocampus were dissected. Nuclear extracts (for HIF-1α) and cytosolic extracts (for EPO) were obtained using a nuclear extraction kit and following the manufacturer protocol (Active Motif). Equal amounts of the samples were loaded per lane. The primary antibodies were polyclonal anti-HIF-1α (1:200, Santa Cruz Biotechnology, Inc), goat polyclonal antibody EPO (1:200; Santa Cruz Biotechnology), and β-Actin (1:10000; Sigma). Western blots were performed with horseradish peroxidase-conjugated immunoglobulin G (Cell Signaling Technology) with the use of enhanced chemiluminescence detection reagents (Amersham). Bands were scanned using a densitometer (GS-700; Bio-Rad Laboratories), and quantification was performed using Multi-Analyst 1.0.2 software (Bio-Rad).

4.8. Immunohistochemistry

At designed time points, mice were deeply anesthetized with aether and perfused intracardially with ice-cold phosphate-buffered saline (PBS, pH 7.4) followed by 4% phosphate-buffered paraformaldehyde. Brains were removed and fixed in 2% paraformaldehyde for 24 h and embedded in paraffin. Serial sections (6 μm) were cut, mounted on gelatin-coated slides, air-dried, and stored for immunohistochemistry. Briefly, sections were deparaffinized, hydrated, and subjected to antigen retrieval at 90 °C for 25.00 min using Target retrieval solution, according to the manufacturer's instructions. The primary antibody was a rabbit polyclonal antibody against HIF-1α (1:100, Santa Cruz Biotechnology, Inc) and the antibody binding was visualized using an ABC Kit (Vector Laboratories).

4.9. Statistics

Survival time was analyzed with survival time curve log-rank test. All quantitative data were expressed as mean ± standard deviation (SD) and verified by analysis of variance (ANOVA) using SPSS 10.0. If a significant variance was found, the Tukey's Multiple Comparison Test was then used to determine the specific differences between group means. A value of $P < 0.05$ was considered statistically significant.

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REFERENCES

Agani, F.H., Pichiule, P., Chavez, J.C., LaManna, J.C., 2000. The role of mitochondria in the regulation of hypoxia-inducible factor 1 expression during hypoxia. J. Biol. Chem. 275, 35863–35867.

BelAiba, R.S., Djordjevic, T., Bonello, S., Flugel, D., Hess, J., Kietzmann, T., Gorlach, A., 2004. Redox-sensitive regulation of the HIF pathway under non-hypoxic conditions in pulmonary artery smooth muscle cells. Biol. Chem. 385, 249–257.

Bergeron, M., Yu, A.Y., Solway, K.E., Semenza, G.L., Sharp, F.R., 1999. Induction of hypoxia-inducible factor-1 (HIF-1) and its target genes following focal ischaemia in rat brain. Eur. J. Neurosci. 11, 4159–4170.

Bergeron, M., Gidday, J.M., Yu, A.Y., Semenza, G.L., Ferriero, D.M., Sharp, F.R., 2000. Role of hypoxia-inducible factor-1 in hypoxia-induced ischemic tolerance in neonatal rat brain. Ann. Neurol. 48, 285–296.

Bernaudin, M., Nedelec, A.S, Divoux, D., MacKenzie, E.T., Petit, E., Schumann-Bard, P., 2002. Normobaric hypoxia induces tolerance to focal permanent cerebral ischemia in association with an increased expression of hypoxia-inducible factor-1 and its target genes, erythropoietin and VEGF, in the adult mouse brain. J. Cereb. Blood Flow Metab. 22, 393–403.

Brines, M.L., Ghezzi, P., Keenan, S., Agnello, D., de Lanerolle, N.C., Cerami, C., Itri, L.M., Cerami, A., 2000. Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. Proc. Natl. Acad. Sci. U. S. A. 97, 10526–10531.

Calvert, J.W., Cahill, J., Yamaguchi-Okada, M., Zhang, J.H., 2006. Oxygen treatment after experimental hypoxia–ischemia in neonatal rats alters the expression of HIF-1alpha and its downstream target genes. J. Appl. Physiol. 101, 853–865.

Casas, M., Casas, H., Pagés, T., Rama, R., Ricart, A., Ventura, J.L., Ibáñez, J., Rodríguez, F.A., Viscor, G., 2000. Intermittent hypobaric hypoxia induces altitude acclimation and improves the lactate threshold. Aviat. Space Environ. Med. 71, 125–130.

Chandel, N.S., Maltepe, E., Goldwasser, E., Mathieu, C.E., Simon, M.C., Schumacker, P.T., 1998. Mitochondrial reactive oxygen species trigger hypoxia induced transcription. Proceedings of the National Academy of Sciences, 95, pp. 11715–11720.

Chandel, N.S., McClintock, D.S., Feliciano, C.E., Wood, T.M., Melendez, J.A., Rodriguez, A.M., Schumacker, P.T., 2000. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O2 sensing. J. Biol. Chem. 275, 25130–25138.

Chavez, J.C., LaManna, J.C., 2002. Activation of hypoxia-inducible factor-1 in the rat cerebral cortex after transient global ischemia: potential role of insulin-like growth factor-1. J. Neurosci. 22, 8922–8931.

Digicaylioglu, M., Bichet, S., Marti, H.H., Wenger, R.H., Rivas, L.A., Bauer, C., Gassmann, M., 1995. Localization of specific erythropoietin binding sites in defined areas of the mouse brain. Proc. Natl. Acad. Sci. U. S. A. 92, 3717–3720.

Dong, H., Xiong, L., Zhu, Z., Chen, S., Hou, L., Sakabe, T., 2002. Preconditioning with hyperbaric oxygen and hyperoxia induces tolerance against spinal cord ischemia in rabbits. Anesthesiology 96, 907–912.

Freiberger, J.J., Suliman, H.B., Sheng, H., McAdoo, J., Piantadosi, C.A., Warner, D.S., 2006. A comparison of hyperbaric oxygen versus hypoxic cerebral preconditioning in neonatal rats. Brain Res. 1075, 213–222.

Gao, N., Shen, L., Zhang, Z., Leonard, S.S., He, H., Zhang, X.G., Shi, S., Jiang, B.H., 2004. Arsenite induces HIF-1alpha and VEGF through PI3K, Akt and reactive oxygen species in DU145 human prostate carcinoma cells. Mol. Cell. Biochem. 255, 33–45.

Grasso, G., Sfacteria, A., Meli, F., Fodale, V., Buemi, M., Iacopino, D.G., 2007. Neuroprotection by erythropoietin administration after experimental traumatic brain injury. Brain Res. 28 (1182), 99–105.

Gu, G.J., Li, Y.P., Peng, Z.Y., Xu, J.J., Kang, Z.M., Xu, W.G., Tao, H.Y., Ostrowski, R.P., Zhang, J.H., Sun, X.J., 2008. Mechanism of ischemic tolerance induced by hyperbaric oxygen preconditioning involves up-regulation of hypoxia-inducible factor-1{alpha} and erythropoietin in rats. J. Appl. Physiol. 104, 1185–1191.

Haddad, J.J., 2002. Recombinant human interleukin (IL)-1 beta-mediated regulation of hypoxia-inducible factor-1 alpha (HIF-1 alpha) stabilization, nuclear translocation and activation requires an antioxidant/reactive oxygen species (ROS)-sensitive mechanism. European Cytokine Network 13, 250–260.

Halterman, M.W., Federoff, H.J., 1999. HIF-1alpha and p53 promote hypoxia-induced delayed neuronal death in models of CNS ischemia. Exp. Neurol. 159, 65–72.

Huang, Z.X., Kang, Z.M., Gu, G.J., Peng, G.N., Yun, L., Tao, H.Y., Xu, W.G., Sun, X.J., Zhang, J.H., 2007. Therapeutic effects of hyperbaric oxygen in a rat model of endothelin-1-induced focal cerebral ischemia. Brain Res. 1153, 204–213.

Iwai, M., Cao, G., Yin, W., Stetler, R.A., Liu, J., Chen, J., 2007. Erythropoietin promotes neuronal replacement through revascularization and neurogenesis after neonatal hypoxia/ischemia in rats. Stroke. 38, 2795–2803.

Jones, N.M., Bergeron, M., 2001. Hypoxic preconditioning induces changes in HIF-1 target genes in neonatal rat brain. J. Cereb. Blood Flow Metab. 21, 1105–1114.

Kim, C.H., Choi, H., Chun, Y.S., Kim, G.T., Park, J.W., Kim, M.S., 2001. Hyperbaric oxygenation pretreatment induces catalase and reduces infarct size in ischemic rat myocardium. Pflugers Arch. 442, 519–525.

Li, Y., Zhou, C., Calvert, J.W., Colohan, A.R., Zhang, J.H., 2005. Multiple effects of hyperbaric oxygen on the expression of

HIF-1 alpha and apoptotic genes in a global ischemia–hypotension rat model. Exp. Neurol. 191, 198–210.
Mazure, N.M., Brahimi-Horn, M.C., Berta, M.A., Benizri, E., Bilton, R.L., Dayan, F., Ginouves, A., Berra, E., Pouyssegur, J., 2004. HIF-1: master and commander of the hypoxic world. A pharmacological approach to its regulation by siRNAs. Biochem. Pharmacol. 68, 971–980.
Metzen, E., Zhou, J., Jelkmann, W., Fandrey, J., Brüne, B., 2003. Nitric oxide impairs normoxic degradation of HIF-1alpha by inhibition of prolyl hydroxylases. Mol. Biol. Cell. 14, 3470–3481.
Miao, G., Ostrowski, R.P., Mace, J., Hough, J., Hopper, A., Peverini, R., Chinnock, R., Zhang, J., Hathout, E., 2006. Dynamic production of hypoxia-inducible factor-1alpha in early transplanted islets. Am. J. Transplant. 6, 2636–2643.
Morishita, E., Masuda, S., Nagao, M., Yasuda, Y., Sasaki, R., 1997. Erythropoietin receptor is expressed in rat hippocampal and cerebral cortical neurons, and erythropoietin prevents in vitro glutamate-induced neuronal death. Neuroscience 76, 105–116.
Muth, C.M., Radermacher, P., Shank, E.S., 2002. When HBO meets the ICU: intensive care patients in the hyperbaric environment. In: Joiner, J.T., Karles, S. (Eds.), Hyperbaric Surgery: Perioperative Care. Best Publishing Company, Flagstaff, AZ, USA, pp. 111–157.
Ostrowski, R.P., Colohan, A.R., Zhang, J.H., 2005. Mechanisms of hyperbaric oxygen-induced neuroprotection in a rat model of subarachnoid hemorrhage. J. Cereb. Blood Flow Metab. 25, 554–571.
Ostrowski, R.P., Graupner, G., Titova, E., Zhang, J., Chiu, J., Dach, N., Corleone, D., Tang, J., Zhang, J.H., 2008. The hyperbaric oxygen preconditioning-induced brain protection is mediated by a reduction of early apoptosis after transient global cerebral ischemia. Neurobiol. Dis. 29, 1–13.
Park, J.H., Kim, T.Y., Jong, H.S., Kim, T.Y., Chun, Y.S., Park, J.W., Lee, C.T., Jung, H.C., Kim, N.K., Bang, Y.J., 2003. Gastric epithelial reactive oxygen species prevent normoxic degradation of hypoxia inducible factor-1alpha in gastric cancer cells. Clin. Cancer Res. 9, 433–440.
Paul, S.A., Simons, J.W., Mabjeesh, N.J., 2004. HIF at the crossroads between ischemia and carcinogenesis. J. Cell Physiol. 200, 20–30.
Piret, J.P., Mottet, D., Raes, M., Michiels, C., 2002. Is HIF-1alpha a pro- or an anti-apoptotic protein? Biochem. Pharmacol. 64, 889–892.
Prass, K., Wiegand, F., Schumann, P., Ahrens, M., Kapinya, K., Harms, C., Liao, W., Trendelenburg, G., Gertz, K., Moskowitz, M.A., Knapp, F., Victorov, I.V., Megow, D., Dirnagl, U., 2000. Hyperbaric oxygenation induced tolerance against focal cerebral ischemia in mice is strain dependent. Brain Res. 871, 146–150.
Prass, K., Scharff, A., Ruscher, K., Löwl, D., Muselmann, C., Victorov, I., Kapinya, K., Dirnagl, U., Meisel, A., 2003. Hypoxia-induced stroke tolerance in the mouse is mediated by erythropoietin. Stroke 34, 1981–1986.
Richard, D.E., Berra, E., Pouyssegur, J., 2000. Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1alpha in vascular smooth muscle cells. J. Biol. Chem. 275, 26765–26771.
Robach, P., Déchaux, M., Jarrot, S., Vaysse, J., Schneider, J.C., Mason, N.P., Herry, J.P., Gardette, B., Richalet, J.P., 2000. Operation Everest III: role of plasma volume expansion on VO (2)(max) during prolonged high-altitude exposure. J. Appl. Physiol. 89, 29–37.
Ruscher, K., Freyer, D., Karsch, M., Isaev, N., Megow, D., Sawitzki, B., Priller, J., Dirnagl, U., Meisel, A., 2002. Erythropoietin is a paracrine mediator of ischemic tolerance in the brain: evidence from an in vitro model. J. Neurosci. 22, 10291–10301.
Sakanaka, M., Wen, T.C., Matsuda, S., Masuda, S., Morishita, E., Nagao, M., Sasaki, R., 1998. In vivo evidence that erythropoietin protects neurons from ischemic damage. Proc. Natl. Acad. Sci. U. S. A. 95, 4635–4640.
Salhanick, S.D., Belikoff, B., Orlow, D., Holt, D., Reenstra, W., Buras, J.A., 2006. Hyperbaric oxygen reduces acetaminophen toxicity and increases HIF-1alpha expression. Acad. Emerg. Med. 13, 707–714.
Saria, A., Lundberg, J.M., 1983. Evans blue fluorescence: quantitative and morphological evaluation of vascular permeability in animal tissues. J. Neurosci. Methods. 8, 41–49.
Schroedl, C., McClintock, D.S., Budinger, G.R., Chandel, N.S., 2002. Hypoxic but not anoxic stabilization of HIF-1alpha requires mitochondrial reactive oxygen species. Am. J. Physiol. Lung Cell Mol. Physiol. 283, L922–L931.
Semenza, G.L., 2000. Surviving ischemia: adaptive responses mediated by hypoxia-inducible factor 1. J. Clin. Invest. 106, 809–812.
Sen Gupta, J., Mathew, L., Gopinath, P.M., 1979. Effect of physical training at moderate altitude (1850 m) on hypoxic tolerance. Aviat. Space Environ. Med. 50, 714–716.
Sheridan, R.L., Shank, E.S., 1999. Hyperbaric oxygen treatment: a brief overview of a controversial topic. J. Trauma. 47, 426–435.
Tibbles, P.M., Edelsberg, J.S., 1996. Hyperbaric-oxygen therapy. N. Engl. J. Med. 334, 1642–1648.
Udaka, K., Takeuchi, Y., Movat, H.Z., 1970. Simple method for quantitation of enhanced vascular permeability. Proc. Soc. Exp. Biol. Med. 133, 1384–1387.
Verdonck, O., Lahrech, H., Francony, G., Carle, O., Farion, R., Van de Looij, Y., Remy, C., Segebarth, C., Payen, J.F., 2007. Erythropoietin protects from post-traumatic edema in the rat brain. J. Cereb. Blood Flow Metab. 27, 1369–1376.
Wada, K., Ito, M., Miyazawa, T., Katoh, H., Nawashiro, H., Shima, K., Chigasaki, H., 1996. Repeated hyperbaric oxygen induces ischemic tolerance in gerbil hippocampus. Brain Res. 740, 15–20.
Wada, K., Miyazawa, T., Nomura, N., Tsuzuki, N., Nawashiro, H., Shima, K., 2001. Preferential conditions for and possible mechanisms of induction of ischemic tolerance by repeated hyperbaric oxygenation in gerbil hippocampus. Neurosurgery 49, 160–166.
Wakida, K., Shimazawa, M., Hozumi, I., Satoh, M., Nagase, H., Inuzuka, T., Hara, H., 2007. Neuroprotective effect of erythropoietin, and role of metallothionein-1 and -2, in permanent focal cerebral ischemia. Neuroscience 148, 105–114.
Wang, F.S., Wang, C.J., Chen, Y.J., Chang, P.R., Huang, Y.T., Sun, Y.C., Huang, H.C., Yang, Y.J., Yang, K.D., 2004. Ras induction of superoxide activates ERK-dependent angiogenic transcription factor HIF-1alpha and VEGF-A expression in shock wave-stimulated osteoblasts. J. Biol. Chem. 279, 10331–10337.
Xiong, L., Zhu, Z., Dong, H., Hu, W., Hou, L., Chen, S., 2000. Hyperbaric oxygen preconditioning induced neuroprotection against ischemia in transient not permanent middle cerebral artery occlusion rat model. Chin. Med. J. (Engl.) 113, 836–839.
Yu, S.Y., Chiu, J.H., Yang, S.D., Yu, H.Y., Hsieh, C.C., Chen, P.J., Lui, W.Y., Wu, C.W., 2005. Preconditioned hyperbaric oxygenation protects the liver against ischemia–reperfusion injury in rats. J. Surg. Res. 128, 28–36.
Zemke, D., Smith, J.L., Reeves, M.J., Majid, A., 2004. Ischemia and ischemic tolerance in the brain: an overview. Neurotoxicology 25, 895–904.
Zhang, X., Xiong, L., Hu, W., Zheng, Y., Zhu, Z., Liu, Y., Chen, S., Wang, X., 2004. Preconditioning with prolonged oxygen exposure induces ischemic tolerance in the brain via oxygen free radical formation. Can. J. Anaesth. 51, 258–263.