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Preconditioning with hyperbaric oxygen induces tolerance against oxidative injury via increased expression of heme oxygenase-1 in primary cultured spinal cord neurons

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Abstract

Hyperbaric oxygen (HBO) preconditioning can induce ischemic tolerance in the spinal cord. The effect can be attenuated by the administration of an oxygen free radical scavenger or by inhibition of antioxidant enzymes. However, the mechanism underlying HBO preconditioning of neurons against ischemic injury remains enigmatic. Therefore, in the present study primary cultured spinal cord neurons were treated with HBO and then subjected to a hydrogen peroxide (H_2O_2) insult. The results show that H_2O_2 stimulation of the cultured spinal neurons caused severe DNA damage and decreased cell viability, and that these neurons were well protected against damage after a single exposure to HBO preconditioning (0.35 MPa, 98% O_2, 37 °C, 2 h). The protective effect started 4 h after pretreatment and lasted for at least 24 h. The cultured neurons after HBO treatment also exhibited increased heme oxygenase-1 (HO-1) expression at both the protein and mRNA levels, which paralleled the protective effect of HBO. Treatment with tin-mesoporphyrin IX (SnMP), a specific HO-1 inhibitor, before HBO pretreatment abolished the HBO-induced adaptive protection noted in the cultured spinal neurons. In conclusion, HBO preconditioning can protect primary cultured spinal cord neurons against oxidative stress, and the upregulation of HO-1 expression plays an essential role in HBO induced preconditioning effect.

Keywords: Hyperbaric oxygen; Oxidative injury; Heme oxygenase-1; Primary cultured spinal cord neurons

Introduction

Hyperbaric oxygen (HBO) preconditioning can induce ischemic tolerance in both the brain and the spinal cord (Wada et al., 1996; Prass et al., 2000; Kim et al., 2001; Dong et al., 2002; Miljkovic-Lolic et al., 2003). The tolerance can be attenuated by the administration of dimethylthiourea (DMTU), an oxygen free radical scavenger (Xiong et al., 2001) or by the inhibition of catalase, an antioxidant enzyme (Nie et al., 2006). These results imply that a nonlethal level of reactive oxygen species (ROS) produced during HBO preconditioning may be a mediator of the tolerance induction, and that the increased activity of antioxidant enzymes during HBO may be responsible for the protective effect of HBO pretreatment (Ohtsuki et al., 1992; Nie et al., 2006). However, it is still unknown whether ischemic tolerance is a direct effect of HBO preconditioning on the neurons or is an effect secondary to other changes occurring in the rest of the body. Therefore, in the present study an in vitro HBO treatment system was established for primary cultured spinal cord neurons. Hydrogen peroxide (H_2O_2)-mediated oxidative injury was produced in the cultured spinal neurons to simulate the damage induced by ischemia–reperfusion. The aim of the current study was to determine if

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HBO preconditioning could induce tolerance against oxidative injury in primary cultured spinal cord neurons and, if so, to identify the mechanism for the tolerance induction.

Materials and methods

Experimental design

This study consisted of three experiments. Experiment 1 was designed to investigate the effect of hyperbaric oxygen preconditioning on oxidative injury in spinal cord neurons. Experiment 2 was designed to investigate the effect of hyperbaric oxygen preconditioning on antioxidant enzyme activities in spinal cord neurons, and experiment 3 was designed to investigate the effect of SnMP, a specific inhibitor of HO-1 activity, on antioxidant enzyme activities after HBO preconditioning.

In experiment 1, the primary cultured spinal cord neurons were randomly divided into three groups: control, H_2O_2, and HBO+H_2O_2 groups. In order to maintain the physiological power of hydrogen (pH) for 2 h at 37 °C, neurons in the HBO+H_2O_2 group were exposed to HBO at 0.35 MPa with a gas composition consisting of 98% O_2 and 2% carbon dioxide (CO_2). As a sham pretreatment, neurons in the H_2O_2 and control groups were placed in a chamber (21% O_2, 2% CO_2), in which the air was not pressurized, on the same schedule that was used for the HBO+H_2O_2 group. At various time points after HBO exposure (0 h, 3 h, 7 h, 11 h, and 23 h), H_2O_2 (Sigma-Aldrich Co., St. Louis, MO, USA) was added into the culture medium of the HBO+H_2O_2 and H_2O_2 groups at a final concentration of 50 μM and maintained for 1 h at 37 °C. The neurons of the three groups were assayed for methyl thiazoleterazolium (MTT) or harvested to detect DNA damage using the comet assay (single cell gel electrophoresis) at various time points after HBO exposure (1 h, 4 h, 8 h, 12 h, and 24 h). Each experiment was repeated three times (n=3).

In experiment 2, the primary cultured spinal cord neurons were randomly divided into two groups: control and HBO groups. Neurons in the HBO group were exposed to HBO (0.35 MPa, 98% O_2, 2% CO_2) for 2 h at 37 °C, and those in the control group received a sham pretreatment as described in experiment 1. The neurons were harvested after HBO exposure (at 1 h, 4 h, 8 h, 12 h, and 24 h) and examined for changes related to antioxidant enzyme expression using Western blot or reverse transcriptase polymerase chain reaction (RT-PCR). Each experiment was repeated three times (n=3).

In experiment 3, the cultured spinal neurons were randomly divided into three groups: control, H_2O_2, and HBO+H_2O_2 groups as in experiment 1. In the H_2O_2 and HBO+H_2O_2 groups, 23 h after HBO exposure, the neurons were subjected to an H_2O_2 insult as described in experiment 1. In one half of each group's wells, the neurons were given SnMP (Alexis, San Diego, CA, USA) 10 min before HBO exposure at a final concentration of 10 μM. Ten micromolar SnMP was chosen for the study since this concentration has been shown to significantly decrease HO activity (Koeppen et al., 2004; Suttner et al., 1999). The neurons in the other half of each group's wells were assayed without SnMP pretreatment. The three groups' neurons were assayed for MTT or harvested to detect DNA damage using the comet assay 24 h after HBO preconditioning. Each experiment was repeated three times (n=3).

Cell culture

Primary culture of spinal cord neurons was done using the method described by Moreels et al. (2005) with minor modifications. Briefly, spinal cords from 11- to 14-day-old fetal mice were minced and incubated for 30 min at 37 °C in a buffered solution containing papain (0.67 mg/ml). The cell suspension was titrated using 40 μg/ml DNase in MEM supplemented with 10% FBS and 10% horse serum, then seeded in poly-L-lysine-coated 6-well plates at a density of 8×10^5 cells per well for the comet assay, Western blot analysis, and RT-PCR protocol, at a density of 4×10^5 per well in 24-well plates for immunocytochemical staining, and at a density of 1×10^5 per well in 96-well plates for MTT assay.

The identity of cultured cells

Cells cultured in complete medium for six days were plated out on collagen-coated coverslips and incubated at 37 °C with 5% CO_2 until they were approximately 20% confluent. Immunocytochemical staining for β-tubulin was done to determine the identity of the cultured cells.

Hyperbaric oxygen preconditioning

The plated primary cultured spinal cord neurons were put into a small, temperature-controlled (37 °C), hyperbaric chamber (DWC450-1150, Qilingyi Institute, Shanghai, China) according to the method described by Messier and Fisher (1990). In order to maintain a physiological pH, the chamber's gas composition was 98% O_2 and 2% CO_2. At a compression rate of 0.02 MPa/min, a pressure of 0.35 MPa was achieved within 20 min and maintained for 2 h. Then, the chamber was gradually decompressed to atmospheric pressure at the same rate.

H_2O_2-induced oxidative injury

At various time points after HBO exposure (0 h, 3 h, 7 h, 11 h, and 23 h), H_2O_2 (Sigma-Aldrich Co.) diluted in distilled water was added into the culture medium at a final concentration of 50 μM and maintained for 1 h at 37 °C. Then, the cells were assayed for MTT or harvested to detect DNA damage with the comet assay. To examine the changes in enzyme expression using Western blot or RT-PCR, the cells were harvested at various time points after HBO exposure (1 h, 4 h, 8 h, 12 h, and 24 h) without subsequent H_2O_2 stimulation. The H_2O_2-mediated oxidative injury was produced in the present study to simulate the damage induced by ischemia–reperfusion. The protocol chosen in this study is due to the findings that ROS is one of main compositions and plays an

important role in ischemia–reperfusion injury (Cuzzocrea et al., 2001; Li and Jackson, 2002).

MTT assay to determine cell viability

Briefly, the culture medium was changed to a medium containing 0.5 g/l MTT and incubated for 4 h at 37 °C. Then the supernatant was discarded, and the cells were mixed thoroughly with dimethylsulfoxide (DMSO, 100 μl/well). When the crystals were dissolved, the optical density absorbance values of 10 wells in each group were measured with an Elx800 plate reader at 570 nm. Cell viability is directly proportional to the absorbance value.

Comet assay (single cell gel electrophoresis) to determine neuronal DNA damage

Comet assay was done as described by Speit and Bonzheim (2003). Briefly, a 10 μl cell suspension (5×10^5 in number) was mixed with 100 μl low melting agarose (LMA; 0.5% in PBS), added to microscope slides that had been covered with a bottom layer of 1.5% normal melting agarose (NMA), and then dried. After alkali denaturation (pH 13) and electrophoresis (0.86 V/cm), the slides were stained with ethidium bromide and fifty images randomly selected from each group were analyzed. To determine the median tail moment (tail length × percentage of DNA in the tail) per cellular nucleus, measurements were made under a fluorescence microscope (Nikon, TE2000, Tokyo, Japan) using an image analysis system (Comet Assay II, V1.02, Haverhill, UK).

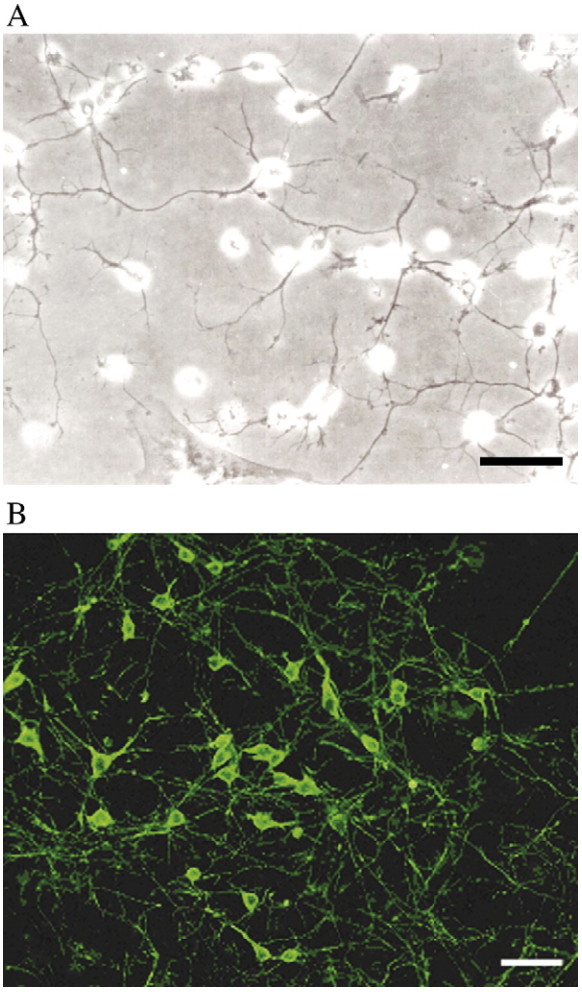

Fig. 1. Photomicrographs showing the morphological features of the primary cultured spinal cord neurons. A: taken under phase-contrast microscope after 3 days of culture. B: taken under fluorescence microscope after 5 days of culture; the cells were immunocytochemically stained for β-tubulin, a neuronal marker. Scale bars = 50 μm.

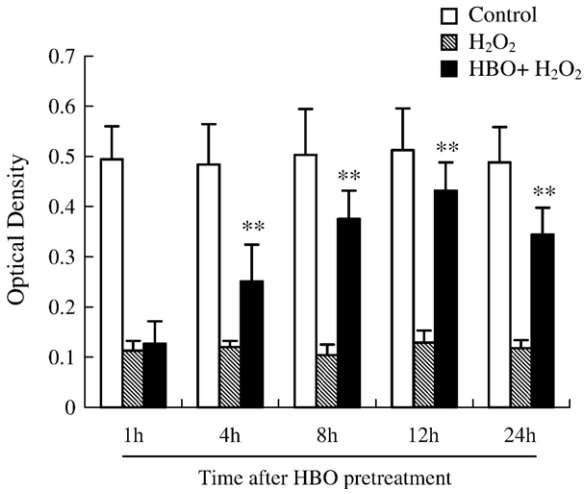

Fig. 2. The effects of H_2O_2 treatment and HBO preconditioning on the cellular viability of primary cultured neurons. The optical density of MTT absorbance was used as a reflection of cell viability. Con, the group in which the neurons received sham pretreatment; H_2O_2, the group in which the neurons received sham pretreatment and H_2O_2 administration (50 μM, 1 h at 37 °C); HBO + H_2O_2, the group in which the neurons received HBO preconditioning and H_2O_2 administration. Data are presented as the mean ± SD from three separately repeated experiments. *: $P<0.05$; **: $P<0.01$, vs. the H_2O_2 group at the corresponding time point.

Western blot analysis of antioxidant enzyme expression

Protein extracts from the primary cultured spinal cord neurons of each group were obtained with a RIPA-buffer. The protein content of the samples was determined as previously described (Selesniemi et al., 2005). An equal amount of protein (20 μg/lane) determined by the BCA method was loaded. Western blot analysis was performed with rabbit anti-mice HO-1 antibody or rabbit anti-mice superoxide dismutase (SOD) antibody (1:500, respectively; StressGen, Victoria, BC, Canada). Following 2 h incubation at 37 °C, the membrane was washed extensively with PBST and incubated for 1 h at 37 °C in a goat anti-rabbit IgG antibody (1:5000, Sigma-Aldrich Co.). The band intensities of HO-1 protein on the PVDF-membrane were analyzed with an image analysis system and are shown as relative intensity values compared to β-actin.

RT-PCR analysis of antioxidant enzyme expression

RT-PCR was used to detect the level of HO-1 mRNA in the HBO-treated primary cultured neurons. β-actin mRNA was detected as well to serve as a loading control. Total RNA was extracted from the spinal cord neurons using TriZOL reagent

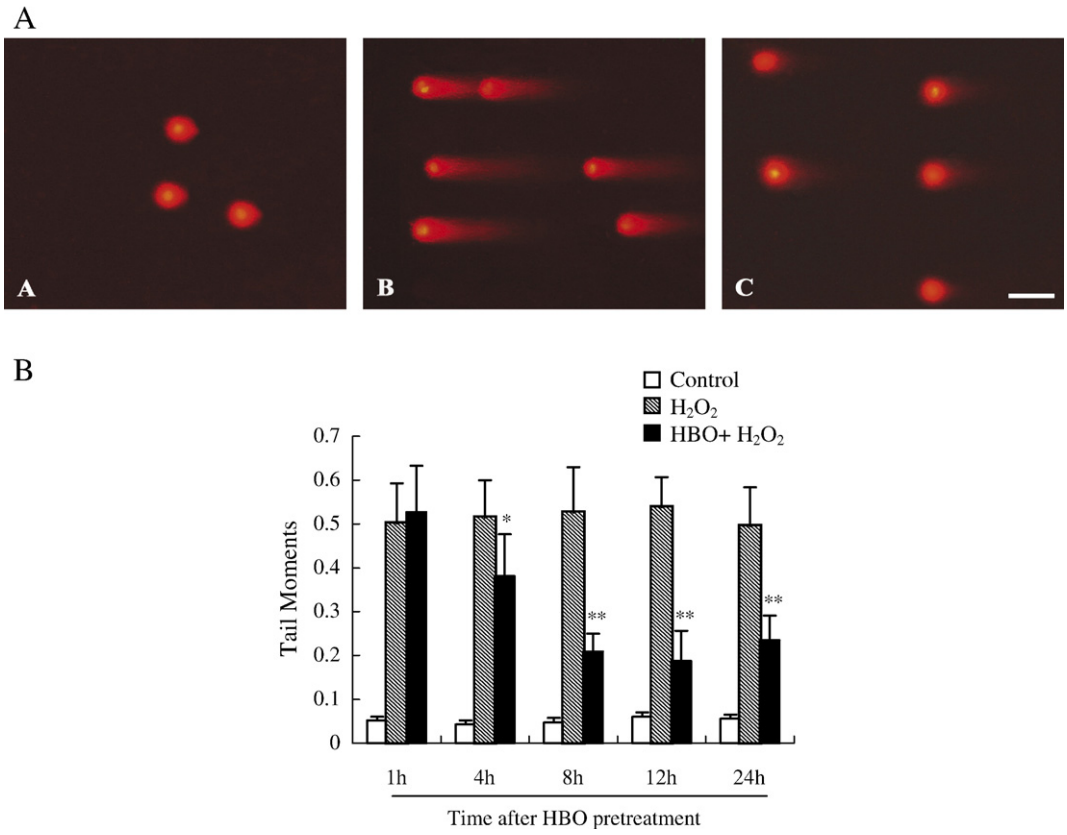

Fig. 3. The protective effect of HBO pretreatment on H_2O_2-induced DNA damage in the primary cultured spinal neurons. A: Photomicrographs showing the nuclei of spinal neurons in the control group 24 h after HBO pretreatment (A), the H_2O_2 group 24 h after HBO pretreatment (B), and the HBO+H_2O_2 group 24 h after HBO pretreatment (C). The magnifications in all three photos are identical, and the scale bar represents 20 μm. B: Histograms showing the effects of H_2O_2 treatment and HBO preconditioning on the DNA damage of the primary cultured neurons shown as the tail moment values of DNA migration. Control, the group in which the neurons received sham pretreatment; H_2O_2, the group in which the neurons received sham pretreatment and H_2O_2 administration (50 μM, 1 h at 37 °C); HBO+H_2O_2, the group in which the neurons received HBO preconditioning and H_2O_2 administration. Data are presented as mean±SD from three separately repeated experiments. *: $P<0.05$; **: $P<0.01$, vs. the H_2O_2 group at the corresponding time point.

(GIBCO-BRL, Gaithersburg, MD, USA) and reverse-transcribed into cDNA according to the manufacturer's protocol. The primers were designed according to published HO-1 and β-actin sequences (Morita et al., 1995). The PCR products were separated by electrophoresis on a 1% agarose gel, stained with ethidium bromide, and photographed under ultraviolet light. The level of HO-1 mRNA was expressed as the integrated optical density of the HO-1 DNA band divided by that of the β-actin.

Statistical analysis

Data are expressed as mean±standard deviation (SD) and compared by using variance (ANOVA), followed by a Student–Newman–Keuls (SNK) test for multiple comparisons. A P-value of less than 0.05 was considered to be statistically significant.

Results

HBO preconditioning protects spinal cord neurons

The identity of cultured cells

After six days of culture in the medium, the cells became spiny, triangular, or pyramidal in form with a strong refraction, which is compatible with the morphological features of neurons in primary culture (Fig. 1A). The cell processes were long, thin, and homogeneously arranged. Over 90% of the cells were stained immunocytochemically for β-tubulin, a marker of neurons, which confirmed their neuronal identity (Fig. 1B). This degree of purity met the demand for subsequent experiments using the primary neurons.

Cell viability

The MTT assay was used to determine the effect of HBO preconditioning on cell viability in H_2O_2-insulted spinal neurons. The results showed that the treatment caused a dramatic decrease in cell viability, whereas after a single preconditioning HBO exposure, cell viability was significantly improved. The protective effect of HBO preconditioning on cell viability began 4 h after the treatment, reached its peak at 12 h, and lasted at least 24 h, which was as long as we observed (Fig. 2).

DNA damage

Fig. 3 illustrates the results of the tail moment of DNA migration detected with a comet assay using the H_2O_2-injured spinal neurons with or without HBO preconditioning. The results revealed that the DNA strand breaks in the spinal cord

neurons subjected to a H_2O_2 insult but not to HBO preconditioning were much more frequent. On the other hand, at various time points after HBO exposure, the same degree of H_2O_2 treatment induced less DNA damage in the neurons (shown as a reduction in the tail moment values). The protective effect of HBO preconditioning started 4 h after a single HBO exposure and lasted at least 24 h.

HBO preconditioning upregulates HO-1

The expression of antioxidant enzyme protein

Western blot results showed that the amount of antioxidant enzyme HO-1 in spinal cord neurons was enhanced 4 h after HBO exposure, achieved a peak at 8 h, and lasted at least 24 h, which was as long as we observed. However, there was no clear difference in the expression of SOD between the groups with and without HBO treatment (Fig. 4).

HO-1 mRNA transcription

A representative photograph of RT-PCR is presented in Fig. 5A, and a histogram of the correlated results from the densitometric analysis of three separate experiments is shown in Fig. 5B. The HO-1 mRNA level in each group is shown as a ratio to that of β-actin. The results demonstrate that the transcription of HO-1 mRNA was barely seen in the group without HBO treatment. In contrast, after HBO preconditioning, the expression of HO-1 mRNA in spinal cord neurons was increased 1 h after HBO exposure, and peaked at 8 h after HBO treatment (about a three-fold increase). Then, the HO-1 mRNA levels gradually declined and reached the level observed in the control group.

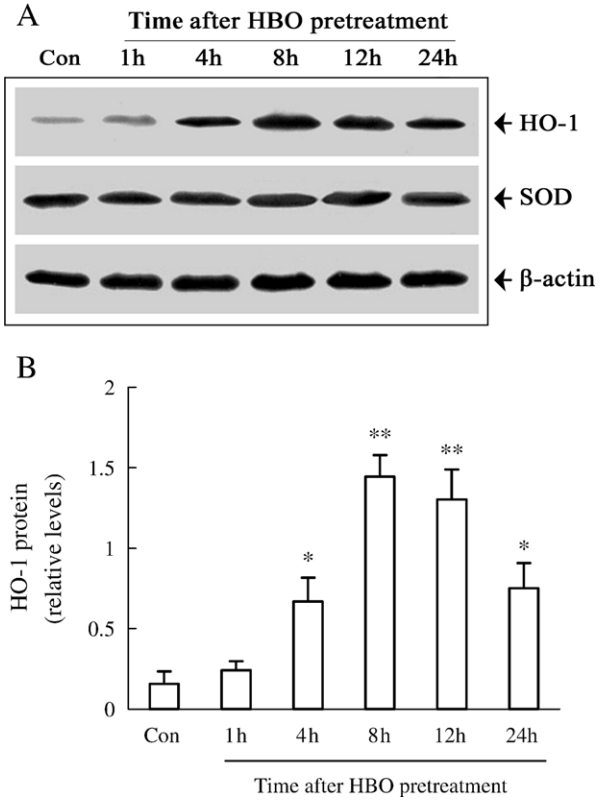

Fig. 4. A: HO-1 and SOD expressions in primary cultured spinal neurons at various time points after HBO exposure. Equal loading was verified by immunoblotting of β-actin. B: Densitometric analysis of HO-1 expression detected by Western blot. Data were averaged from three separate experiments and are shown as the relative intensity values of HO-1 to β-actin. Con: the control group in which the neurons received sham pretreatment. *: $P<0.05$; **: $P<0.01$ vs. control.

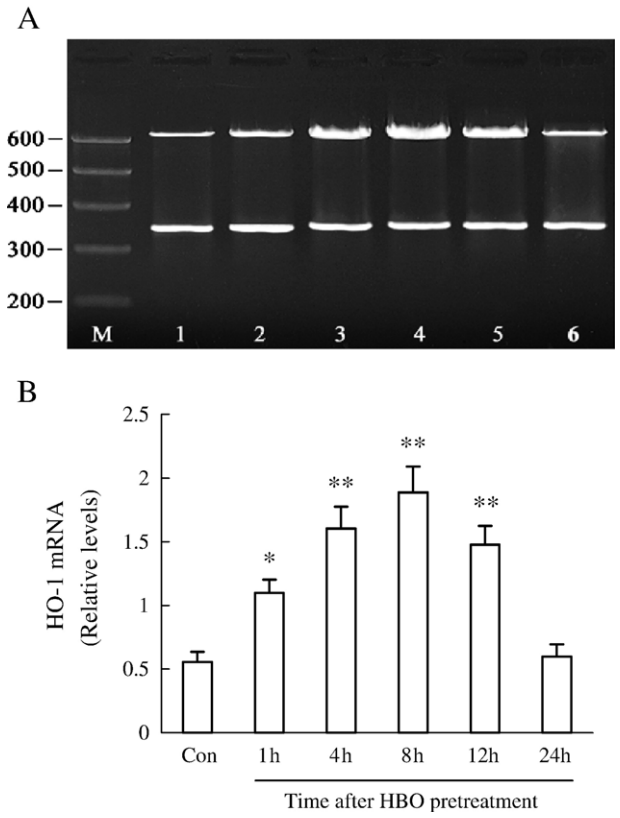

Fig. 5. A: A representative RT-PCR photograph showing HO-1 mRNA (upper line) in primary cultured spinal neurons at various time points after HBO exposure (lanes 2–6) and in the group without HBO exposure as a control (lane 1). Equal loading was verified by immunoblotting of β-actin (lower line). Lanes from left, M: DNA marker (600 bp ladder); 1: the control group; 2: 1 h after HBO; 3: 4 h after HBO; 4: 8 h after HBO; 5: 12 h after HBO; 6: 24 h after HBO. B: Densitometric analysis. Data were averaged from three separate experiments (mean±SD) and are shown as a ratio to β-actin expression. Con: the control group in which the neurons received sham pretreatment. *: $P<0.05$; **: $P<0.01$ vs. control.

SnMP blocks HBO-induced tolerance

Pretreatment of the spinal cord neurons with 10 μM SnMP before HBO treatment abolished the protective effect of HBO preconditioning against a H_2O_2-induced decrease in cell viability (Fig. 6A) and DNA damage (Fig. 6B). In the HBO+H_2O_2 group, cells pretreated with SnMP showed decreased cell viability and increased tail moment values compared to cells without SnMP pretreatment. By itself, SnMP had no effect on

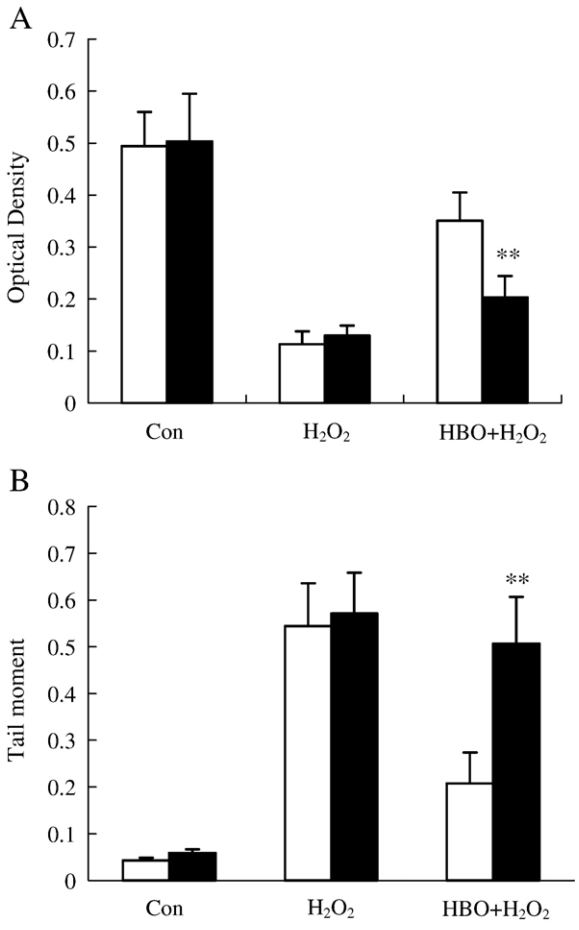

Fig. 6. The effects of SnMP administration on HBO preconditioning-induced protection in cultured spinal neurons. A: The changes in cellular viability B: The changes in DNA damage. Con, the group in which the neurons received sham pretreatment; H_2O_2, the group in which the neurons received sham pretreatment and H_2O_2 administration 23 h after HBO pretreatment; HBO+H_2O_2, the group in which the neurons received HBO preconditioning and H_2O_2 administration 23 h after HBO pretreatment. ■: 10 μM SnMP administered; □: Without SnMP treatment. Data are presented as mean±SD. **: $P<0.01$ vs. the neurons without SnMP treatment.

cell viability and DNA migration, both in control and H_2O_2 groups.

Discussion

The present study demonstrates that a single HBO pretreatment given to primary cultured spinal cord neurons improved cellular viability and reduced DNA damage caused by the H_2O_2 challenge. After HBO treatment, an increase in HO-1 expression at both the protein and mRNA levels was identified in the cultured neurons. Treatment with SnMP, a specific HO-1 inhibitor, prior to HBO pretreatment abolished the HBO-induced adaptive protection that had been noted in cultured spinal neurons. These results indicate that HBO preconditioning can directly act on neurons, enhance endogenous antioxidant enzyme activities, and thereby induce the tolerance against secondary oxidative stress in cultured spinal neurons.

Our previous in vivo studies found that an initial oxidative stress during HBO pretreatment plays an important role in the formation of the tolerance against spinal cord ischemia injury (Dong et al., 2002; Nie et al., 2006). However, it is unknown whether the enhancement of antioxidant enzyme activities plays a role in HBO preconditioning induced tolerance in neurons. In the present study, we measured the levels of HO-1 and superoxide dismutase (SOD), and found that the HO-1 levels in the primary cultured neurons were significantly enhanced after a single exposure to HBO. HO-1 expression, detected with Western blot and RT-PCR, was increased 4 h after HBO treatment, peaked at 8 h, and lasted for at least 24 h, the observation period of this study. This induction pattern paralleled the development of the HBO preconditioning effect on cellular viability and neuronal DNA. There were no significant changes in SOD level after HBO preconditioning in the present study, which is consistent with the result from studies on lymphocytes (Speit et al., 2000; Rothfuss et al., 2001). However, increased SOD expression in spinal cord after repeated HBO preconditioning was found in our previous in vivo study (Nie et al., 2006). The possible explanation for the divergent findings might be that the number of HBO exposures was different in the experiments.

Several studies have shown that upregulation of HO-1 confers increased resistance against oxidative threat (Applegate et al., 1991; Ferris et al., 1999; Lee et al., 2005; Ryter and Choi, 2005). In addition, experiments with HO-1 knock-out mice found a reduction in stress defense of oxidative damage (Chen-Roetling et al., 2005). The present study found that treatment with 10 μM SnMP before HBO pretreatment abolished the HBO-induced adaptive protection in cultured spinal neurons, therefore demonstrating that HO-1 plays a key role in HBO preconditioning against oxidative injury.

The mechanism(s) by which HO-1 exhibits its role in anti-oxidant protection is not yet clear. HO-1 is one of two isoforms of heme oxygenase that catabolize cellular heme to biliverdin, carbon monoxide, and free iron. It is well known that bilirubin, a metabolite of heme degradation, is itself a potent antioxidant (Stocker et al., 1987). The induction of ferritin synthesis, as a result of iron removal from the degradation of heme by HO-1, may also be involved (Meneghini, 1997). Intracellular free iron can react with H_2O_2 and give rise to the toxic hydroxyl radical via the Fenton reaction. Due to the release of free iron during the catalysis of heme by HO-1, ferritin may be released to restrict iron from participating in the Fenton reaction. Thus, accumulation of ferritin is related to iron sequestration and may provide protection against oxidative damage (Meneghini, 1997).

In conclusion, the present study shows that a single episode of HBO preconditioning upregulates HO-1 expression and induces protection against oxidative injury in primary cultured spinal cord neurons. SnMP, a specific HO-1 inhibitor, abolished HBO-induced preconditioning effects, indicating that HO-1 plays an essential role in HBO-induced protection against oxidative injury in the spinal neurons.

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References

Applegate, L., Luscher, P., Tyrrell, R., 1991. Induction of heme oxygenase: a general response to oxidant stress in cultured mammalian cells. Cancer Research 51, 974–978.

Chen-Roetling, J., Benvenisti-Zarom, L., Regan, R.F., 2005. Cultured astrocytes from heme oxygenase-1 knockout mice are more vulnerable to heme-mediated oxidative injury. Journal of Neuroscience Research 82 (6), 802–810.

Cuzzocrea, S., Riley, D.P., Caputi, A.P., Salvemini, D., 2001. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/reperfusion injury. Pharmacological Reviews 53, 135–159.

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.

Ferris, C.D., Jaffrey, S.R., Sawa, A., Takahashi, M., Brady, S.D., Barrow, R.K., Tysoe, S.A., Wolosker, H., Baranano, D.E., Dore, S., Poss, K.D., Snyder, S.H., 1999. Heme oxygenase-1 prevents cell death by regulating cellular iron. Nature Cell Biology 1, 152–157.

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 Archive-European Journal of Physiology 442 (4), 519–525.

Koeppen, A.H., Dickson, A.C., Smith, J., 2004. Heme oxygenase in experimental intracerebral hemorrhage: the benefit of tin-mesoporphyrin. Journal of Neuropathology and Experimental Neurology 63 (6), 587–597.

Lee, P.C., Ho, I.C., Lee, T.C., 2005. Oxidative stress mediates sodium arsenite-induced expression of heme oxygenase-1, monocyte chemoattractant protein-1, and interleukin-6 in vascular smooth muscle cells. Toxicological Sciences 85 (1), 541–550.

Li, C., Jackson, R.M., 2002. Reactive species mechanisms of cellular hypoxia-reoxygenation injury. American Journal of Physiology. Cell Physiology 282, 227–241.

Meneghini, R., 1997. Iron homeostasis, oxidative stress, and DNA damage. Free Radical Biology & Medicine 23, 783–792.

Messier, A.A., Fisher, H.W., 1990. Sensitivity of cultured mammalian cells to oxidative stress: adaptation to repeated exposures of hyperbaric oxygen. Undersea Biomedical Research 17, 305–322.

Miljkovic-Lolic, M., Silbergleit, R., Fiskum, G., Rosenthal, R.E., 2003. Neuroprotective effects of hyperbaric oxygen treatment in experimental focal cerebral ischemia are associated with reduced brain leukocyte myeloperoxidase activity. Brain Research 971 (1), 90–94.

Moreels, M., Vandenabeele, F., Deryck, L., Lambrichts, I., 2005. Radial glial cells derived from the neonatal rat spinal cord: morphological and immunocytochemical characterization. Archives of Histology and Cytology 68 (5), 361–369.

Morita, T., Perrella, M.A., Lee, M.E., Kourembanas, S., 1995. Smooth muscle cell-derived carbon monoxide is a regulator of vascular cGMP. Proceedings of the National Academy of Sciences 92, 1475–1479.

Nie, H., Xiong, L., Lao, N., Chen, S., Xu, N., Zhu, Z., 2006. Hyperbaric oxygen preconditioning induces tolerance against spinal cord ischemia by upregulation of antioxidant enzymes in rabbits. Journal of Cerebral Blood Flow and Metabolism 26 (5), 666–674.

Ohtsuki, T., Matsumoto, M., Kuwabara, K., Kitagawa, K., Suzuki, K., Taniguchi, N., Kamada, T., 1992. Influence of oxidative stress on induced tolerance to ischemia in gerbil hippocampus neurons. Brain Research 599, 246–252.

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 Research 871 (1), 146–150.

Rothfuss, A., Radermacher, P., Speit, G., 2001. Involvement of heme oxygenase-1 (HO-1) in the adaptive protection of human lymphocytes after hyperbaric oxygen (HBO) treatment. Carcinogenesis 22, 1979–1985.

Ryter, S.W., Choi, A.M., 2005. Heme oxygenase-1: redox regulation of a stress protein in lung and cell culture models. Antioxidants and Redox Signalling 7 (1–2), 80–91.

Selesniemi, K., Reedy, M., Gultice, A., Guilbert, L.J., Brown, T.L., 2005. Transforming growth factor-beta induces differentiation of the labyrinthine trophoblast stem cell line SM10. Stem Cells and Development 14 (6), 697–711.

Speit, G., Bonzheim, I., 2003. Genotoxic and protective effects of hyperbaric oxygen in A549 lung cells. Mutagenesis 18 (6), 545–548.

Speit, G., Dennog, C., Eichorn, U., Rothfuss, A., Kaina, B., 2000. Induction of heme oxygenase-1 and adaptive protection against the induction of DNA damage after hyperbaric oxygen treatment. Carcinogenesis 21, 1795–1799.

Stocker, R., Yamamoto, Y., McDonagh, A.F., Glazer, A.N., Ames, B.N., 1987. Bilirubin is an antioxidant of possible physiological importance. Science 235, 1043–1045.

Suttner, D., Sridhar, K., Lee, C., Tomura, T., Hansen, T., Dennery, P., 1999. Protective effects of transient HO-1 overexpression on susceptibility to oxygen toxicity in lung cells. American Journal of Physiology 276, L443–L451.

Wada, K., Ito, M., Katoh, H., Shima, K., Chigasaki, H., 1996. Repeated hyperbaric oxygen induces ischemic tolerance in gerbil hippocampus. Brain Research 740, 15–20.

Xiong, L., Dong, H., Zhu, Z., Hou, L., Xiong, D., Lu, Z., Chen, M., 2001. The mechanism of hyperbaric oxygen preconditioning-induced ischemic tolerance in the spinal cord. Journal of Fourth Military Medical University 22, 2166–2170.