Experimental Neurology 247 (2013) 737–743

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Hyperbaric oxygen preconditioning attenuates hyperglycemia-enhanced hemorrhagic transformation by inhibiting matrix metalloproteinases in focal cerebral ischemia in rats

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ARTICLE INFO

Article history:
Received 10 December 2012
Revised 7 March 2013
Accepted 15 March 2013
Available online 26 March 2013

Keywords:
Hyperbaric oxygen preconditioning
MMP-2 and MMP-9
Hemorrhagic transformation
MCAO

ABSTRACT

Hyperglycemia dramatically aggravates brain infarct and hemorrhagic transformation (HT) after ischemic stroke. Oxidative stress and matrix metalloproteinases (MMPs) play an important role in the pathophysiology of HT. Hyperbaric oxygen preconditioning (HBO-PC) has been proved to decrease oxidative stress and has been demonstrated to be neuroprotective in experimental stroke models. The present study determined whether HBO-PC would ameliorate HT by a pre-ischemic increase of reactive oxygen species (ROS) generation, and a suppression of MMP-2 and MMP-9 in hyperglycemic middle cerebral artery occlusion (MCAO) rats. Rats were pretreated with HBO (100% O_2, 2.5 atmosphere absolutes) 1 h daily for 5 days before MCAO. Acute hyperglycemia was induced by an injection of 50% dextrose. Neurological deficits, infarction volume and hemorrhagic volume were assessed 24 h and 7 days after ischemia. ROS scavenger n-acetyl cysteine (NAC), hypoxia-inducible factor-1α (HIF-1α), inhibitor 2-methoxyestradiol (2ME2) and activator cobalt chloride ($CoCl_2$), and MMP inhibitor SB-3CT were administrated for mechanism study. The activity of MMP-2 and MMP-9, and the expression HIF-1α were measured. HBO-PC improved neurological deficits, and reduced hemorrhagic volume; the expression of HIF-1α was significantly decreased, and the activity of MMP-2 and MMP-9 was reduced by HBO-PC compared with vehicle group. Our results suggested that HBO-PC attenuated HT via decreasing HIF-1α and its downstream MMP-2 and MMP-9 in hyperglycemic MCAO rats.

Introduction

Hemorrhagic transformation (HT) following ischemic stroke contributes to the early mortality and poor functional recovery in affected patients. Hyperglycemia has been claimed to be associated with HT and aggravates brain damage after reperfusion (Kagansky et al., 2001; Kumari et al., 2012). It has been demonstrated that hyperglycemia-enhanced HT was linked to increased activity of inflammation and oxidative stress, which cause blood–brain barrier (BBB) disruption and neuronal cell death (Wang and Lo, 2003). Matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) have been suggested as the major perpetrators for BBB degradation and HT formation after ischemia stroke (Elgebaly et al., 2011; Wang et al., 2004). Oxidative stress, which comes from reactive oxygen species (ROS) and reactive nitrogen species (RNS), potentially triggers MMP-induced BBB disruption (Lehner et al., 2011).

Preconditioning (PC) is a phenomenon whereby a sub-injury-inducing stress can cause protection against a subsequent severe injurious event (Selzner et al., 2012). Various types of preconditioning, for example ischemic preconditioning, anesthetic preconditioning and pharmacological preconditioning over the past decades have resulted in various promising therapeutic effects for the treatment of patients with acute brain injury (Dirnagl et al., 2009; Xi, 2010). HBO-PC, as one of the most common and attractive preconditioning strategies has been demonstrated to be neuroprotective in several animal models of neurological diseases, such as focal and global cerebral ischemia (Ostrowski et al., 2008; Soejima et al., 2012), subarachnoid hemorrhage (Ostrowski and Zhang, 2011), spinal cord ischemia (Nie et al., 2006), traumatic and surgical brain injury (Hu et al., 2008; Jadhav et al., 2010). Recently, studies showed that HBO increased ROS generation (Thom, 2009) and that increased ROS levels up-regulated the expression of transcription factor hypoxia-inducible factor-1α (HIF-1α) (Peng et al., 2008). HIF-1α is a key regulator responsible for the induction of MMPs in hypoxic conditions (Finger and Giaccia, 2010). It is possible that HBO-PC through generating ROS, will subsequently decrease HIF-1α and its downstream genes MMP-2 and MMP-9, and enhance the tolerance to BBB destruction in brain ischemia/reperfusion injury.

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http://dx.doi.org/10.1016/j.expneurol.2013.03.019

Therefore, we conceptualized that the activity of MMP-2 and MMP-9 may determine hyperglycemia enhanced HT after cerebral artery occlusion (MCAO). We hypothesized that HBO-PC would ameliorate HT, and the mechanism is mediated by a preischemic increase of ROS generation, and a suppression of HIF-1α and its downstream MMP-2 and MMP-9 after MCAO.

Materials and methods

Animal groups and interventions

All experiments were approved by the Institutional Animal Care and Use Committee of Loma Linda University. Two hundred fifteen male Sprague–Dawley rats were purchased from Harlan Laboratories (Indianapolis, IN). Interventions targeting ROS and HIF-1α were performed to investigate their roles in the pathway of HBO-PC in protection of BBB. HIF-1α inhibitor 2-methoxyestradiol (2ME2, St. Louis, MO), reactive oxygen species scavenger n-acetyl cysteine (NAC, St. Louis, MO), HIF-1α activator cobalt chloride ($CoCl_2$, St. Louis, MO) and MMP inhibitor SB-3CT (Dallas, TX) were used. Animals were randomly divided into seven groups: sham (n = 18), MCAO (Control, n = 40), HBO + MCAO (n = 37), HBO + MCAO + NAC (n = 30), HBO + MCAO + 2ME2 (n = 31), HBO + MCAO + NAC + $CoCl_2$ (n = 28), HBO + MCAO + SB-3CT (n = 6). Infarction volume, hemorrhage volume, neurological scores and mortality were analyzed to study the outcome. The expression of HIF-1α, as well as the activity of MMP-2 and MMP-9 was evaluated. Additional five groups were added to investigate the effects of HBO-PC on the expression of HIF-1α, and activity of MMP-2 and MMP-9 in naïve rats. Animals were randomly divided into five groups: naïve (n = 5), HBO (n = 5), HBO + NAC (n = 5), HBO + 2ME2 (n = 5), and HBO + NAC + $CoCl_2$ (n = 5).

Hyperglycemia induction and MCAO

All rats received 50% dextrose (6 ml/kg) intraperitoneally 30 min before MCAO to induce acute hyperglycemia. Anesthesia was induced with ketamine and xylazine (80 mg/kg and 10 mg/kg respectively, intraperitoneally), followed by atropine at a dose of 0.1 mg/kg subcutaneously. During surgery and postoperative period, rectal temperature was maintained at 37.0 °C by using a feedback-controlled heating pad. MCAO was performed as previously reported (Hu et al., 2011). Briefly, the right external carotid artery was isolated and coagulated. A 4-0 nylon suture with a round tip was inserted into the internal carotid artery through the external carotid artery stump and advanced to occlude the origin of middle cerebral artery. The suture was removed at 1.5 h after occlusion. Sham operated rats underwent the same surgical procedures without insertion of the suture.

In the mechanism study, NAC (150 mg/kg, i.p.) was injected 30 min before each HBO session, 2ME2 (5 mg/kg, i.p.), and $CoCl_2$ (60 mg/kg, s.c.) were injected 24 h before MCAO, and SB-3CT (25 mg/kg, i.p.) was injected 1 h before MCAO. NAC, 2ME2, $CoCl_2$ and SB-3CT were dissolved in 1% DMSO with 0.01 M PBS. Animals in sham, MCAO and HBO + MCAO groups received the same volume of 1% DMSO.

HBO-PC regimen

Due to its potential toxic effects, HBO is currently restricted to short sessions (less than 2 h), at pressures below the threshold of CNS toxicity (0.3 MPa) (Tibbles and Edelsberg, 1996). In our previous studies and preliminary experiments, we tested HBO preconditioning at 1, 1.5, 2, 2.5, and 3 ATA and found 2.5–3 ATA produced more pronounced protective results compared to 1–2 ATA. So in this study, we use HBO at 2.5 ATA for 1 h.

Rats were pressurized in a research hyperbaric chamber (1300B; Sechrist) at 2.5 atmosphere absolutes with 100% oxygen (flow of 22 l/min). Compression and decompression were maintained at a rate of 5 psi/min. A 1 h HBO session was administered daily for 5 consecutive days; the last session was performed 24 h before MCAO.

2,3,5-Triphenyltetrazolium chloride staining and evaluation of infarction volume

2,3,5-Triphenyltetrazolium chloride monohydrate (TTC) staining was performed to determine the infarct volume at 24 h after MCAO as previously reported (Hu et al., 2011). The possible interference of brain edema with infarct volume was corrected by standard methods (whole contralateral hemisphere volume − nonischemic ipsilateral hemisphere volume) and the infracted volume was expressed as a percentage of the whole contralateral hemisphere.

Spectrophotometric assay of hemoglobin

Hemorrhagic volume was quantified with spectrophotometric assay of brain hemoglobin content (Hu et al., 2011). 24 h and 7 days after MCAO, the animals were transcardially perfused with 0.1 mol/l PBS under deep anesthesia until the outflow fluid from the right atrium was colorless. The brain was rapidly removed and dissected into the left hemisphere and the right hemisphere. Cerebral hemorrhage was quantified using a previously described spectrophotometric assay (Hu et al., 2011). A standard curve was obtained using a "virtual" model of hemorrhage. Incremental volumes of homologous blood (0, 2, 4, 8, 16, 32 μl) were added to the perfused brain tissue. The hemispheric brain was then homogenized in distilled water followed by 30-minute centrifugation (13,000 *g*). Drabkin reagent (1.6 ml; Sigma) was added to 0.4 ml supernatant aliquots and optical density was measured at 540 nm via spectrophotometer (Spectronix 3000; Milton-Roy). Hemoglobin measurements were performed and compared with the standard curve to obtain data in terms of hemorrhage volume. The total hemispheric hemoglobin content was expressed as μl of blood per hemisphere.

Neurological scores

A neurological examination was performed by a blinded investigator as previously described with modifications (Garcia et al., 1995) at 24 h and 7 days after MCAO. The scores given to each rat at the completion of the evaluation were the summation of 7 individual test scores (spontaneous activity, symmetry in the movement of four limbs, forepaw outstretching, climbing, body proprioception, response to vibrissae touch, and wire walking). The minimum neurological score (most severe deficit) was 3, and the maximum was 21.

Western blot analysis

Animals were anesthetized and underwent transcardiac perfusion using 0.1 M PBS until colorless perfusion fluid was obtained from the right atrium. Tissue samples of the ipsilateral hemisphere were obtained and immersed in 0.5 ml of Western blot sample buffer and then sonicated for Western blot analysis. Protein concentration of each sample was determined using a Bio-Rad protein assay kit. Western blot analysis was performed as previously described (Hu et al., 2011). 50 μg of protein for each sample was separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis after undergoing denaturation by boiling at 95 °C for 5 min, and was then transferred to pure nitrocellulose membrane. The membranes were blocked in nonfat milk and probed with the primary antibody (polyclonal rabbit HIF-1α antibody, 1:500 dilution, Santa Cruz Biotechnology Inc.), and then immunoprobed by a secondary antibody (peroxidase-conjungated goat–antirabbit antibody, Bio-Rad). The antigen–antibody complexes