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

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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 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% O₂, 2.5 atmospheres absolute) 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-1 α (HIF-1 α) inhibitor 2-methoxyestradiol (2ME2) and activator cobalt chloride (CoCl₂), and MMPs inhibitor SB-3CT were administrated for mechanism study. The activity of MMP-2 and MMP-9, and the expression HIF-1 α were measured. HBO-PC improved neurological deficits, and reduced hemorrhagic volume; the expression of HIF-1 α 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-1 α and its downstream MMP-2 and MMP-9 in hyperglycemic MCAO rats.

Keywords

hyperbaric oxygen preconditioning; MMP-2 and MMP-9; hemorrhagic transformation; MCAO

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

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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 MMPs-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), 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-1 α (HIF-1 α) (Peng, et al., 2008). HIF-1 α 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-1 α and its downstream genes MMP-2 and MMP-9, and enhance the tolerance to BBB destruction in brain ischemia/reperfusion injury.

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-1 α 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 and fifteen male Sprague-Dawley rats were purchased from Harlan Laboratories (Indianapolis, IN). Interventions targeting ROS and HIF-1 α were performed to investigate their roles in the pathway of HBO-PC in protection of BBB. HIF-1 α inhibitor 2-methoxyestradiol (2ME2, St. Louis, MO), reactive oxygen species scavenger n-acetyl cysteine (NAC, St. Louis, MO), HIF-1 α activator cobalt chloride (CoCl₂, St. Louis, MO) and MMPs inhibitor SB-3CT (Dallas, TX) were used. Animals were randomly divided into eight 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+CoCl₂ (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-1 α , 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-1 α , 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+CoCl₂ (n=5).

Hyperglycemia Induction and MCAO

All rats received 50% dextrose (6 ml/kg) intraperitoneally 30 minutes 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 mechanisms study, NAC (150 mg/kg, i.p.) was injected 30 min before each HBO session, 2ME2 (5 mg/kg, i.p.), and CoCl₂ (60 mg/kg, s.c.) were injected 24 h before MCAO, SB-3CT (25 mg/kg, i.p.) was injected 1 h before MCAO. NAC, 2ME2, CoCl₂ 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 hours), 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 1h.

Rats were pressurized in a research hyperbaric chamber (1300B; Sechrist) at 2.5 atmospheres 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 infarcted 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 was 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 minutes, and were then transferred to pure nitrocellulose membrane. The membranes were blocked in nonfat milk and probed with the primary antibody (polyclonal rabbit HIF-1 α antibody, 1:500 dilution, Santa Cruz Biotechnology Inc), and then immunoprobed by a secondary antibody (peroxidase-conjugated goat–antirabbit antibody, Bio-Rad). The antigen–antibody complexes were studied using a chemiluminescence system (ECL plus Western blotting detection kit; GE Healthcare) and recorded on X-ray film (Kodak). Bands were quantified by optical density method using Image J software, and densities were expressed relative to actin or sham.

MMP Zymography

Similarly prepared protein samples as for Western blot were subjected to gelatin zymography. Approximately 50 μg of each sample was loaded per lane into the well of precast gels (10% polyacrylamide minigels containing 0.1% gelatin; Invetrogen) with SDS running buffer (1:1; Novex). Electrophoresis was performed with a Tris-glycine running buffer at 125 V constant voltage for 1.5–2 h. The gel was removed and incubated for 1 h at room temperature in 100 ml of 2.7% Triton X-100 (renature buffer, Invetrogen) on a rotary shaker. Then washed with distilled water for 10 times. Each gel was incubated with 100 ml of development buffer (50 mM Tris base, 40 mM HCl, 200 mM NaCl, 5 mM CaCl_2 , and 0.2% Brij 35; Invetrogen) at 37°C for 14 h on a rotary shaker. Staining was performed with 100 ml of 0.5% Coomassie blue G-250 in 30% methanol and 10% acetic acid for at least 1 h, and gels were then destained with three changes of solutions. Gelatinolytic activity was demonstrated as clear zones or bands at the appropriate molecular weights. Human MMP-9 and human MMP-2 (from Chemicon Temecula, CA) were used as standards. The activity of MMP-2 and MMP-9 was quantified in Image J software.

Statistical Analyses

Data were expressed as the mean $\pm$ SEM. Statistical differences among groups were analyzed by using ANOVA followed by the Turkey method. Mortality was evaluated by exact Fisher test. $p < 0.05$ was considered statistically significant.

Results

Blood Glucose Level

The blood glucose levels at 2 h after injection of dextrose in all groups were significantly higher than the baseline and high glucose level lasted until 6 hours after injection. HBO-PC had no effects on the blood glucose level (Figure 1A).

HBO-PC attenuated hyperglycemia induced HT after MCAO mediated by ROS and HIF-1 α

Hyperglycemia induced extensive HT in ischemic territories in MCAO rats at 24 h after ischemia (Figure 1B). Compared with the control, HBO-PC reduced hemorrhage volume significantly at 24 h and 7 days ($p < 0.05$ vs. MCAO, Figure 1D), but had no effects on infarct volume ($p > 0.05$ vs. MCAO, Figure 1B, 1C). At 24 h after MCAO, Neurological scores were higher (better) in the HBO-PC group compared with MCAO group ($p < 0.05$ vs. MCAO, Figure 1E); there is no significant difference of neurological scores between MCAO and HBO+MCAO groups at 7 days, (Figure 1E). NAC and 2ME2 potentially reversed the effects of HBO-PC and increased the hemorrhage volume ($p < 0.05$ vs. HBO+MCAO, Figure 2B); while, pretreatment with HIF-1 α activator CoCl₂ abolished the effects of NAC ($p < 0.05$ vs. HBO+MCAO+NAC, figure 2B). HBO-PC, as well as NAC, 2ME2 and CoCl₂ showed no influence on infarct volume after MCAO ($p > 0.05$ vs. MCAO, Figure 2A). Pretreatment with HIF-1 α activator CoCl₂ effectively improved the neurobehavioral functions after MCAO ($p < 0.05$ vs. MCAO, figure 2C).

The mortality of 24 hours in MCAO group was 34.38% (11/32), which is significantly increased compared with the sham group (0/12). HBO-PC decreased the mortality to 23.33% (7/23), but showed no significance. The mortality in HBO+NAC, HBO+2ME2 and HBO+NAC+CoCl₂ groups was 33.33% (10/30), 19.36% (6/31) and 21.43% (6/28) respectively ($p > 0.05$ vs. MCAO, Figure 2D).

HBO-PC showed a tendency to increase the activity of MMP-2 and MMP-9 in naïve rats, and decreased their activity 24 hours after MCAO in hyperglycemic rats

To study how HBO-PC decreased HT after MCAO in hyperglycemic rats, we evaluated the activity of MMP-2 and MMP-9. In naïve rats, densitometric analysis showed HBO-PC had a remarkable tendency to increase the activity of MMP-2 and MMP-9 at 24 hours, but without statistical significance ($p > 0.05$ vs. Naïve, Figure 3A, 3B).

In hyperglycemic rats, the activity of MMP-2 and MMP-9 were greatly increased at 24 hours after MCAO, HBO-PC remarkably decreased the activity of MMP-2 and MMP-9 ($p < 0.05$ vs. MCAO, Figure 4A, 4B, 4C); MMPs inhibitor SB-3CT enhanced the effect of HBO-PC ($p < 0.05$ vs. HBO+MCAO, Figure 4A, 4B, 4C). These data suggested that HBO-PC attenuated the hyperglycemia enhanced HT may be associated with the inhibition of MMP-2 and MMP-9 after MCAO.

HBO-PC decreased HIF-1 α expression in hyperglycemic MCAO rats

Numerous studies have demonstrated that HIF-1 α is the pivotal transcription factor of MMP-2 and MMP-9. To examine whether HIF-1 α was the mediator of HBO-PC induced suppression of the activity of MMP-2 and MMP-9 after MCAO, we evaluated the expression of HIF-1 α by Western blot. In naïve rats, HBO showed a remarkable tendency to up-regulate HIF-1 α at 24 hours, but no statistically significant difference ($p > 0.05$ vs. MCAO, Figure 5A). The interventions of ROS and HIF-1 α by NAC and 2ME2 had no significant change on the expression of HIF-1 α after HBO treatment in naïve rats ($p > 0.05$ vs. HBO, figure 5A). In hyperglycemic rats, HIF-1 α was greatly increased 24 h after MCAO. HBO-PC apparently reduced HIF-1 α compared with the MCAO group ($p < 0.05$ vs.

MCAO, figure 5B). We deduced that the pre-increased HIF-1 α by HBO-PC might be a stimulus to prepare the rats to be more resilient against the following MCAO injury and result in decreased expression of HIF-1 α after MCAO.

Discussion

In the present study, we observed that HBO-PC ameliorated the hyperglycemia-enhanced HT and improved neurologic deficits in rats subjected to MCAO. HBO-PC induced a preischemic increasing of ROS/HIF-1 α , which subsequently suppressed the expression of HIF-1 α and its downstream MMP-2 and MMP-9. ROS scavenger NAC and HIF-1 α inhibitor 2ME2 abolished the protective effect of HBO-PC. Pretreatment with HIF-1 α activator CoCl₂ showed similar results as HBO-PC. In naïve rats, a remarkable tendency of increased HIF-1 α was observed after HBO treatment, as well as increased activity of MMP-2 and MMP-9.

Our results strongly suggested that HBO-PC as sub-injury stress stimulation induced ROS generating and enhanced the tolerance against brain injury in the subsequent MCAO/reperfusion injury through inhibiting the HIF-1 α /MMPs pathway. It has been reported that HBO-PC significantly increased the expression of HIF-1 α and prevented the subsequent BBB disturbance and dysfunction in experimental global hypoxia (Peng, et al., 2008). Our results are in accordance with this study that suggest the role of pre-increased HIF-1 α in the neuroprotection of HBO-PC.

How HBO-PC up-regulated the expression of HIF-1 α is an interesting question. ROS seems to play a role in HBO induced effect. ROS has been proven to act as the second messenger for the induction of HIF-1 α in both prokaryotes and eukaryotes (Cash, et al., 2007). It has also been reported that exposure to HBO leads to an increase in the amount of dissolved oxygen and therefore ROS in the blood (Thom, 2009). In adult male rats, acute HBO inhalation (60 min at 3 atmospheres absolute) reduced the activity of catalase which is a key enzyme for removing hydrogen peroxide (Gregorevic, et al., 2001). Since HBO stimulates ROS production, it is reasonable to deduce that HBO-PC increases the level of ROS and stimulates the expression of the HIF-1 α before MCAO, which makes the brain develop resistance to the following ischemia/reperfusion injury and reduced the post-ischemia expression of HIF-1 α and its downstream genes. In our experiment, ROS scavenger NAC and HIF-1 α inhibitor 2ME2 administration abolished the protective effects of HBO-PC on hemorrhagic transformation, and pretreatment with HIF-1 α activator CoCl₂ evoked similar results as HBO-PC. These findings proved that HBO-PC enhanced the tolerance against MCAO injury in hyperglycemic rats through increasing ROS generation and HIF-1 α expression.

The activity of MMP-2 and MMP-9 were increased after MCAO and suppressed by HBO-PC in our experiments, which is consistent with the variation of HT. The MMPs are intercellular proteases that cleave the extracellular matrix, including major components of the basal lamina and tight junctions between endothelial cells. This proteolytic cleavage results in disruption of the BBB (Hu, et al., 2009). After brain ischemia/reperfusion injury, the integrity of the BBB is compromised, vessel permeability increases, which leads to an increase of blood cells in parenchyma, defining hemorrhagic transformation. HIF-1 α is the upstream protein regulating the transcription of MMP-2 and MMP-9 (Greer, et al., 2012). In our study, HIF-1 α was increased after MCAO; HBO-PC decreased the post-ischemia expression of HIF-1 α and MMP-2 and MMP-9; HIF-1 α inhibitor 2ME2 counteracted the effects of HBO-PC on hemorrhagic transformation. Also in naïve rats HBO showed a tendency to induce HIF-1 α and increase the activity of MMP-2 and MMP-9. We suggested the pre-activated MMP-2 and MMP-9 by HBO-PC enhanced the adaption to the following

catastrophic activation of these two proteases and attenuated HT after MCAO/reperfusion injury in hyperglycemic rats.

It is important to point out that there exist some other mechanisms, in addition to ROS/HIF-1 α /MMPs, such as inflammation (Harris, et al., 2005) and apoptosis (Song, et al., 2003), all responsible for HT after stroke. Thus, the induction of ROS and the sub-injury increase of HIF-1 α and MMPs before MCAO may possibly be one of potential mechanisms for HBO-PC-induced enhancement of tolerance to sustain the oxidative cascade in hyperglycemic rats. To explore other related mechanisms demands further studies.

In conclusion, the present study showed that HBO-PC (2.5 ATA, 100% O₂, 1 h/day for 5 days) decreased the expression of HIF-1 α and down-regulated the activity of MMP-2 and MMP-9 in ischemic brain tissue thereby protected BBB against ischemia/reperfusion damage. NAC, a potent free radical scavenger, and 2ME2 a HIF-1 α inhibitor, abolished the brain protective effects of HBO-PC. The results suggest that a sub-injury level of ROS generation by HBO-PC triggers the cascade in cellular events leading to increased HIF-1 α before MCAO, which facilitate adaptation to the subsequent oxidative stress and decrease the activity of MMP-2 and MMP-9, attenuating hyperglycemia enhanced HT after cerebral ischemia. HBO-PC may hold therapeutic value for treating HT in ischemic stroke patients with hyperglycemia.

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Highlights

- HBO-PC attenuated HT and improved neurobehavioral scores in hyperglycemic MCAO rats.
- HBO-PC showed a tendency to increase HIF-1 α and the activity of MMP-2 and MMP-9 in naïve rats.
- HBO-PC decreased HIF-1 α and the activity of MMP-2 and MMP-9 in hyperglycemic MCAO rats.
- HBO-PC may hold therapeutic value for treating HT in hyperglycemic stroke patients through suppressing the activity of MMP-2 and MMP-9.

Figure 1.

HBO-PC attenuated HT and improved neurology scores in hyperglycemic MCAO rats. A: Statistic analysis of blood glucose levels. HBO-PC had no effects on blood glucose at the 4 time points (n=8 for each group). B: Representative photographs of whole brain and slices with TTC staining of each group were demonstrated. HBO-PC reduced hemorrhagic transformation (arrows) after MCAO, while had no effect on infarction volume. C: Bar graph of infarction volume from each sample. MCAO increased the infarction volume compared with Sham group, and HBO-PC had no effects on infarct volume after MCAO. Sham: n=3; MCAO: n=8; HBO+MCAO: n=9. D: Hyperglycemia induced significant HT after MCAO, and HBO-PC potentially reduced the hemorrhagic volume both at 24 h and 7 days. For 24 h, Sham: n = 3; MCAO: n = 7; HBO+MCAO: n=8; For 7 days, n=6 for each group. E: Neurological scores at 24 h and 7 days after MCAO. Hyperglycemia significantly deteriorated neurological deficit after MCAO. HBO-PC significantly improved the neurological deficit at 24 h, but showed no benefits at 7 days. For 24 h, Sham: n = 12; MCAO: n = 22; HBO+MCAO: n = 19; For 7 days, n=6 for each group. * $p < 0.05$ vs. Sham, # $p < 0.05$ vs. MCAO.

Figure 2.

Outcome study of the interventions with NAC, 2ME2, and CoCl₂ after HBO-PC in hyperglycemic MCAO rats. A: NAC, 2ME2, and CoCl₂ had no effect on infarction volume after HBO-PC in hyperglycemic MCAO rats. B: HBO-PC reduced hemorrhagic volume after MCAO, NAC counteracted the effect of HBO-PC, and CoCl₂ decreased the hemorrhagic volume after NAC treatment. C: NAC and 2ME2 abolished the improvement of neurology scores after HBO-PC, and CoCl₂ pretreatment showed similar results as HBO-PC after administration of NAC. D: HBO-PC, NAC, 2ME2, and CoCl₂ had no effect on mortality 24 h after MCAO in hyperglycemic rats. ANOVA, * $p < 0.05$ vs. MCAO, # $p < 0.05$ vs. HBO+MCAO, & $p < 0.05$ vs. HBO+MCAO+NAC. HBO+MCAO+NAC: n=9; HBO+MCAO+2ME2: n=11; HBO+MCAO+NAC+CoCl₂: n=9.

Figure 3.

Representative zymograms and densitometric analyses of MMP-2 (A) and MMP-9 (B) 24 h after HBO treatment in naïve rats. HBO treatment showed a tendency to increase the activity of MMP-2 and MMP-9, but no statistic difference. ANOVA, $p > 0.05$ vs. naïve, $n = 5$ for each group.

Figure 4.

Representative zymograms and densitometric analyses of MMP-2 (A, B) and MMP-9 (A, C) 24 h after HBO-PC in hyperglycemic MCAO rats. HBO-PC significantly reduced the activity of MMP-2 and MMP-9 compared with MCAO group, MMPs inhibitor SB-3CT significantly enhanced the effects of HBO-PC. ANOVA, * $p < 0.05$ vs. Sham, # $p < 0.05$ vs. MCAO, & $p < 0.05$ vs. HBO+MCAO, $n=6$ for each group.

Figure 5.

Western blot of HIF-1α in naïve rats and MCAO rats 24 h after HBO-PC. A: In naïve rats, HBO and HIF-1α activator CoCl₂ showed a tendency to increase the expression of HIF-1α, but no statistic significance. ANOVA, $p > 0.05$, $n = 5$ for each group. B: After MCAO, the expression of HIF-1α was remarkably increased, and HBO-PC decreased it significantly. ANOVA, $p < 0.05$ vs. MCAO, $n = 5$ for each group.