Cyclo-Oxygenase-2 Mediates Hyperbaric Oxygen Preconditioning-Induced Neuroprotection in the Mouse Model of Surgical Brain Injury

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Background and Purpose—We investigated the role of cyclo-oxygenase-2 (COX-2) in mechanisms of hyperbaric oxygen preconditioning (HBO-PC) in the mouse model of surgical brain injury (SBI).

Methods—C57BL mice were administered 100% oxygen for 1 hour at 2.5 atmosphere absolute for 5 consecutive days and subjected to SBI. Neurological status and brain edema were evaluated at 24 hours and 72 hours after the brain insult. Fluorescent immunostaining and Western blotting were performed to study hypoxia-inducible factor-1α and COX-2, respectively. Two doses of COX-2 inhibitor, NS398 (3 mg/kg and 10 mg/kg) were used to verify the role of COX-2 signaling pathway in the mechanism of HBO-PC.

Results—HBO-PC improved neurological status and decreased brain edema at 24 hours and 72 hours after SBI. HBO-PC by itself and SBI independently increased COX-2 levels by 2-fold and 4-fold, respectively. HBO-PC, however, reduced increase in hypoxia-inducible factor-1α and COX-2 expression after SBI. The HBO-PC-induced improvement in neurological status and brain edema was reversed by a suboptimal dose of the COX-2 inhibitor, NS398 (10 mg/kg intraperitoneally; 1/4th of dose shown to provide neuroprotection), which itself had no effect on investigated end points.

Conclusions—HBO-PC attenuates postoperative brain edema and improves neurological outcomes after SBI. The HBO-PC-induced neuroprotection is mediated through COX-2 signaling pathways. (Stroke. 2009;40:3139-3142.)

Key Words: brain edema ■ brain injury ■ cyclo-oxygenase-2 ■ hyperbaric oxygen ■ preconditioning ■ surgical neuroprotection

Hyperbaric oxygen preconditioning (HBO-PC) has been shown to provide neuroprotection in rodent models of stroke.1–4 The efficacy and mechanisms of HBO-PC in surgical brain injury, however, have not been established. Cyclo-oxygenase-2 (COX-2) is known to play a critical role in a variety of brain injuries and was recently suggested to mediate ischemic preconditioning.5 Using a mouse surgical brain injury (SBI) model, we investigated the role of COX-2 in the mechanism of HBO-PC.

Methods

All procedures were approved by Loma Linda University Animal Care Committee. A total of 99 adult male C57BL mice (Charles River Laboratories International, Inc; Wilmington, Mass) were used. The SBI procedure involved partial resection of the right frontal lobe under volatile anesthesia (induction with 4% isoflurane and 1.5% isoflurane used to maintain anesthesia).6 Sham animals received only craniotomy. Animals preconditioned with hyperbaric oxygen (100% oxygen for 1 hour at 2.5 atmosphere absolute for 5 consecutive days; the last treatment was administered 24 hours before induction of SBI) and subjected to SBI and were compared with a normoxia/normobaric-SBI group (room air exposure) and a sham group at 24 hours and 72 hours for neurological evaluation and brain water content measurements in a blinded fashion. Some mice underwent HBO-PC alone and were euthanized at 24 hours after the last oxygenation. Additional groups of mice received the COX-2 inhibitor, NS398 combined with HBO-PC (3 or 10 mg/kg, administered intraperitoneally 1 hour before each HBO-PC treatment) or alone (but after the same injection regimen) and were euthanized at 24 hours after SBI.

Neurological evaluation included a 21-point sensorimotor scoring and wire-hang and beam-balance tests.6 Brain water content was calculated in the right (ipsilateral) and left frontal and parietal lobes, cerebellum, and brainstem as reported previously.6,7 Western blotting protocol was performed with anti-COX-2 and anti-β-actin goat polyclonal antibodies (Santa Cruz Biotechnology) on brain tissue from the right frontal lobe at 24 hours.7 A standard fluorescent immunostaining protocol6 was adopted for studying hypoxia-inducible factor-1α (HIF-1α) expression using goat anti-HIF-1α antibody (Santa Cruz Biotechnology). Mouse monoclonal antineuronal marker antibody (Chemicon), and rabbit polyclonal antineuronal fibrillary acidic protein antibody (Dako). Negative control stainings were performed with omission of primary antibodies.

The data are expressed as mean±SEM. Differences between groups were assessed with a one-way analysis of variance and Holm-Sidak post hoc test. A value of P<0.05 was considered statistically significant.
Results

Overall low mortality rates (<5%) were not significantly different ($\chi^2$ test) between the normoxia+SBI and HBO-PC+SBI groups.

All animals subjected to SBI showed significant worsening of functional performance on the 21-point neurological score and wire-hang and beam-balance scores at 24 hours and 72 hours compared with the sham group (Figure 1A). The HBO-PC+SBI group showed significant improvement in neurological score at 72 hours, whereas in other tests, the improvement was found at 24 hours and 72 hours. Similarly, the HBO-PC+SBI group showed a significant decrease in right frontal lobe water content at 24 hours and 72 hours compared with the normoxia+SBI group (Figure 1B). HBO-PC reduced water content also in the right parietal lobe at 72 hours after SBI.

The HIF-1α (Figure 2A) and COX-2 (Figure 2B) expression in the right frontal lobe increased after SBI. This increase was reduced with HBO-PC. HBO-PC alone significantly increased (2-fold) COX-2 levels. HIF-1α expression was observed in neurons (colocalized with neuronal marker; Figure 2B) but not in astrocytes (glial fibrillary acidic protein; Figure 2C).

A suboptimal dose of COX-2 inhibitor (1/4th of dose shown to provide neuroprotection) reversed the HBO-PC-induced lowering of brain edema ($P<0.05$) and improvement of wire-hang performance ($P<0.05$) at 24 hours (Figure 3). It also tended to reduce improvement in beam-balance scores ($P=0.07$). This suboptimal dose of NS398, however, had itself no effect on brain water content or functional performance.

Discussion

Our present study showed for the first time that HBO-PC provided protection against SBI by improving neurological outcomes and reducing postoperative brain edema. HBO-PC effectively attenuated COX-2 increase after SBI, possibly through neuronal suppression of HIF-1α, a COX-2 upstream regulator. Less COX-2 could result in lessened brain injury through reduced inflammation, oxidative cell death, and apoptosis. COX-2 is well implicated in these components of brain injury.

The present data demonstrate that HBO-PC by itself increases COX-2 protein level by 2-fold, which may suggest that HBO preconditions the brain by increasing COX-2 expression/activation to subinjurious levels. Consistently, the COX-2 inhibitor effectively blocked neuroprotection induced by HBO-PC.

In line with our previous studies indicating relatively high basal brain water content in C57BL mice, brain water content >80% was found in sham-operated mice. These animals,
however, did not show any abnormalities in brain structure or functional performance.

There are several clinical implications of this study. Because HBO-PC reduces COX-2 upregulation after SBI, HBO-PC may be especially useful for patients with conditions known to enhance expression of COX-2 in response to brain injury (e.g., diabetes). The preconditioning mechanism, however, itself requires COX-2 activation. Consequently, patients treated with COX-2 inhibitors are less likely to benefit from HBO-PC. These inhibitors should be stopped or replaced with alternative agents before HBO-PC.

Conclusions
HBO-PC improved neurological outcomes and reduced postoperative brain edema after SBI. HBO-PC-induced neuroprotection is mediated through the COX-2 signaling pathway.

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Disclosures
None.

Figure 2. A, Western blotting data (n=4) demonstrate that the increase in COX-2 levels in the normoxia+SBI group at 24 hours was reduced with HBO-PC. Symbols *, #, and “a” indicate significance versus control, HBO-PC, and HBO-PC+SBI groups, respectively. B–C, HBO-PC attenuated neuronal tissue expression of HIF-1α in the frontal lobe at 24 hours after SBI. Neg. Ctrl, negative control.

References
Figure 3. NS398 (10 mg/kg) reversed the beneficial effects of HBO-PC on brain edema and functional performance at 24 hours after SBI. Symbols *, #, “a,” and “b” indicate significance versus respective sham, normoxia+SBI, HBO-PC+SBI, and NS398 (3 mg/kg)+HBO-PC+SBI groups. Animal numbers are indicated in the figure bars.
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