Stem Cells

Stem cell mobilization by hyperbaric oxygen.

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We hypothesized that exposure to hyperbaric oxygen (HBO(2)) would mobilize stem/progenitor cells from the bone marrow by a nitric oxide (*NO) -dependent mechanism. The population of CD34(+) cells in the peripheral circulation of humans doubled in response to a single exposure to 2.0 atmospheres absolute (ATA) O(2) for 2 h. Over a course of 20 treatments, circulating CD34(+) cells increased eightfold, although the overall circulating white cell count was not significantly increased. The number of colony-forming cells (CFCs) increased from 16 +/- 2 to 26 +/- 3 CFCs/100,000 monocytes plated. Elevations in CFCs were entirely due to the CD34(+) subpopulation, but increased cell growth only occurred in samples obtained immediately post treatment. A high proportion of progeny cells express receptors for vascular endothelial growth factor-2 and for stromal-derived growth factor. In mice, HBO(2) increased circulating stem cell factor by 50%, increased the number of circulating cells expressing stem cell antigen-1 and CD34 by 3.4-fold, and doubled the number of CFCs. Bone marrow *NO concentration increased by 1,008 +/- 255 nM in association with HBO(2). Stem cell mobilization did not occur in knockout mice lacking genes for endothelial *NO synthase. Moreover, pretreatment of wild-type mice with a *NO synthase inhibitor prevented the HBO(2)-induced elevation in stem cell factor and circulating stem cells. We conclude that HBO(2) mobilizes stem/progenitor cells by stimulating *NO synthesis.

Effect of hyperbaric oxygenation on neural stem cells and myelin in neonatal rats with hypoxic-ischemic brain damage

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OBJECTIVE: This study investigated the effect of hyperbaric oxygenation (HBO) on neural stem cells (NSCs) and myelin in neonatal rats following hypoxic-ischemic brain damage (HIBD) and aimed to explore the possible mechanism of the protective effect of HBO on HIBD. METHODS: Seven-day-old Sprague-Dawley rat pups were randomly assigned into 4 groups: Normal control, HIBD, hyperbaric air (HBA), and HBO groups (n=30 each). The HIBD model was produced by permanent occlusion of the left common carotid artery and 2 hrs hypoxemia exposure (8% O2 at 37 degrees C). HBA and HBO treatment was administered (2 ATA, once daily for 7 days) in the HBA and HBO groups respectively 1 hr after HIBD. BrdU immunohistochemistry was used to detect the NSCs in the sub-ventricle zone (SVZ) of the lateral ventricle and the dentate gyrus (DG) of the hippocampus. The myelin damage was assessed by myelin basic protein (MBP) immunostaining. RESULTS: The BrdU-positive cells in the SVZ and the DG of the ischemic hemisphere in the HIBD group were dramatically decreased compared with those of the Normal control group at 3 weeks post-HIBD ($P < 0.01$). The HBO treatment resulted in an increase of BrdU-positive cells in the DG from 153.7 +/- 37.0 to 193.7 +/- 38.8 ($P < 0.05$). The nestin expression in the HIBD and HBA groups was reduced compared with that in the Normal control group. There was no difference in the nestin expression between the HBO and the Normal control groups. Hypoxia-ischemia (HI) led to marked myelin damage at 1 week post-HIBD. HBO or HBA treatment alleviated the damage. CONCLUSIONS: The HBO treatment can result in the proliferation of BrdU-positive cells and alleviate the myelin damage following HIBD in neonatal rats, thereby offering neuroprotectivity against HI insults.

Effect of hyperbaric oxygenation on the differentiation of implanted human neural stem cells into neurons in vivo

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OBJECTIVE: To study the effect of hyperbaric oxygenation (HBO) on the differentiation of the implanted human neural stem cells (hNSCs) into neurons in neonatal rats following hypoxic-ischemic brain damage (HIBD). METHODS: HIBD model was prepared by ligation of the left common carotid artery, followed by 8% hypoxia exposure in 7-day-old Sprague-Dawley rat pups. Three days later, the rats received implantation of hNSCs into the left cerebral ventricles. Then the survived rats were randomly divided into two groups: transplantation alone and transplantation+HBO (n=8 each). HBO treatment was administered (1.8 ATA, 1 hr once daily for 10 days) in the transplantation+HBO group 1 hr after hNSCs transplantation. Brains were removed 10 days after transplantation. Frozen coronal sections were prepared for immunofluorescence analysis to detect the neural differentiation of the transplanted cells in the cerebral cortex and hippocampus. RESULTS: Differentiated neurons of implanted cells distributed mainly in the cortex and the hippocampus of the injured side. There was no difference in the number of neurons in the cortex between the two groups, while the number of neurons in the hippocampus significantly increased in the transplantation+HBO group compared with that in the transplantation alone group (231.4+15.1 vs 162.6+5.6; $P<0.05$). CONCLUSIONS: HBO treatment may promote the differentiation of implanted hNSCs into neurons in the hippocampus of neonatal rats following HIBD.

Therapeutic window of hyperbaric oxygen therapy for hypoxic-ischemic brain damage in newborn rats.

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Previous studies showed that hyperbaric oxygen (HBO) promoted cell proliferation in hypoxic-ischemic (HI) neonate rats. Neural stem cells (NSC) existed in the brain lifelong and can be activated. This study was undertaken to assess whether HBO treatment promoted the proliferation of NSC and repaired the brain damage regardless of when it is started, thus to explore the therapeutic window of HBO treatment. Seven-day-old Sprague-Dawley rats underwent left carotid ligation followed by 2 h of hypoxic stress (8% O(2) at 37 degrees C). Hyperbaric oxygen therapy was administered 3, 6, 12, 24, and 72 h after HI. 5-bromo-2'-deoxyurindine and 5-bromo-2'-deoxyuridine/nestin were detected by immunofluorescence and nestin was examined by western blot analysis 10 days after HI. T-maze forced alternation, the foot-fault test, and the radial arm maze were conducted at P 22 days (14 days after HI), P 30 days, and P 34 days. Thereafter, cerebral morphology was examined by Nissl-staining 28 days after HI. There were remarkable increases in the proliferation of neural stem cells in the HBO-treated group, 3, 6, 12, and 24 h after HI, as compared with the HIBD group. The HBO-treated group, 3, 6, and 12 h after HI, performed better in the behavioral test and had less neural loss in the hippocampal CA1 region as compared with the HIBD group. The therapeutic window for effective HBO treatment could be delayed up to 12 h after HIBD, while the effect decreased 24 h after HI.

Hyperbaric oxygen induces placental growth factor expression in bone marrow-derived mesenchymal stem cells.

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The bone marrow is home to mesenchymal stem cells (MSCs) that are able to differentiate into many different cell types. The effect of hyperbaric oxygen (HBO) on MSCs is poorly understood. Placental growth factor (PlGF) is an attractive therapeutic agent for stimulating revascularization of ischemic tissue. HBO has been shown to improve diabetic wound healing by increase circulating stem cells. We hypothesized that HBO induces PlGF expression in bone marrow-derived MSCs. The MSCs were obtained from adult human bone marrow and expanded in vitro. The purity and characteristics of MSCs were identified by flow cytometry and immunophenotyping. HBO at 2.5 ATA (atmosphere absolute) significantly increased PlGF protein and mRNA expression. The induction of PlGF protein by HBO was significantly blocked by the addition of N-acetylcysteine, while wortmannin, PD98059, SP600125 and SB203580 had no effect on PlGF protein expression. However, the specific inhibitor of nitric oxide synthase, L-NAME did not alter the PlGF protein expression induced by HBO. HBO significantly increased the reactive oxygen species production and pretreatment with N-acetylcysteine significantly blocked the induction of reactive oxygen species by HBO. HBO significantly increased the migration and tube formation of MSCs and pretreatment with N-acetylcysteine and PlGF siRNA significantly blocked the induction of migration and tube formation by HBO. In conclusion, HBO induced the expression of PlGF in human bone marrow-derived MSCs at least through the oxidative stress-related pathways, which may play an important role in HBO-induced vasculogenesis.

Proliferation of neural stem cells correlates with Wnt-3 protein in hypoxic-ischemic neonate rats after hyperbaric oxygen therapy.

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Hyperbaric oxygen therapy promoted brain cell proliferation. Wnt-3 is closely associated with the proliferation of neural stem cells. We examined whether hyperbaric oxygen promoted neural stem cells to proliferate and its correlation with Wnt-3 protein in hypoxic-ischemic neonate rats. Hyperbaric oxygen therapy was administered 3 h after hypoxia ischemia daily for 7 days. The proliferating stem cells and Wnt-3 protein were examined dynamically in the subventricular zone. Results showed that stem cells proliferated and peaked 7 days after hyperbaric oxygen therapy. Wnt-3 protein increased to the higher levels 3 days after therapy. Linear regression analysis showed that nestin protein correlated with Wnt-3 protein. We propose that hyperbaric oxygen treatment promote stem cells to proliferate, which is correlated with Wnt-3 protein.

Angiogenesis and vasculogenesis: inducing the growth of new blood vessels and wound healing by stimulation of bone marrow-derived progenitor cell mobilization and homing.

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During embryonic development, the vasculature is among the first organs to form and is in charge of maintaining metabolic homeostasis by supplying oxygen and nutrients and removing waste products. As one would expect, blood vessels are critical not only for organ growth in the embryo but also for repair of wounded tissue in the adult. An imbalance in angiogenesis (a time-honored term that globally refers to the growth of new blood vessels) contributes to the pathogenesis of numerous malignant, inflammatory, ischemic, infectious, immune, and wound-healing disorders. This review focuses on the central role of the growth of new blood vessels in ischemic and diabetic wound healing and defines the most current nomenclature that describes the neovascularization process in wounds. There are now two well-defined, distinct, yet interrelated processes for the formation of postnatal new blood vessels, angiogenesis, and vasculogenesis. Reviewed are recent new data on vasculogenesis that promise to advance the field of wound healing.

Hyperbaric oxygen therapy might improve certain pathophysiological findings in autism.

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Autism is a neurodevelopmental disorder currently affecting as many as 1 out of 166 children in the United States. Numerous studies of autistic individuals have revealed evidence of cerebral hypoperfusion, neuroinflammation and gastrointestinal inflammation, immune dysregulation, oxidative stress, relative mitochondrial dysfunction, neurotransmitter abnormalities, impaired detoxification of toxins, dysbiosis, and impaired production of porphyrins. Many of these findings have been correlated with core autistic symptoms. For example, cerebral hypoperfusion in autistic children has been correlated with repetitive, self-stimulatory and stereotypical behaviors, and impairments in communication, sensory perception, and social interaction. Hyperbaric oxygen therapy (HBOT) might be able to improve each of these problems in autistic individuals. Specifically, HBOT has been used with clinical success in several cerebral hypoperfusion conditions and can compensate for decreased blood flow by increasing the oxygen content of plasma and body tissues. HBOT has been reported to possess strong anti-inflammatory properties and has been shown to improve immune function. There is evidence that oxidative stress can be reduced with HBOT through the upregulation of antioxidant enzymes. HBOT can also increase the function and production of mitochondria and improve neurotransmitter abnormalities. In addition, HBOT upregulates enzymes that can help with detoxification problems specifically found in autistic children. Dysbiosis is common in autistic children and HBOT can improve this. Impaired production of porphyrins in autistic children might affect the production of heme, and HBOT might help overcome the effects of this problem. Finally, HBOT has been shown to mobilize stem cells from the bone marrow to the systemic circulation. Recent studies in humans have shown that stem cells can enter the brain and form new neurons, astrocytes, and microglia. It is expected that amelioration of these underlying pathophysiological problems through the use of HBOT will lead to improvements in autistic symptoms. Several studies on the use of HBOT in autistic children are currently underway and early results are promising.

Endothelial progenitor cell release into circulation is triggered by hyperoxia-induced increases in bone marrow nitric oxide.

Goldstein LJ, Gallagher KA, Bauer SM, Bauer RJ, Baireddy V, Liu ZJ, Buerk DG, Thom SR, Velazquez OC.

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Endothelial progenitor cells (EPC) are known to contribute to wound healing, but the physiologic triggers for their mobilization are often insufficient to induce complete wound healing in the presence of severe ischemia. EPC trafficking is known to be regulated by hypoxic gradients and induced by vascular endothelial growth factor-mediated increases in bone marrow nitric oxide (NO). Hyperbaric oxygen (HBO) enhances wound healing, although the mechanisms for its therapeutic effects are incompletely understood. It is known that HBO increases nitric oxide levels in perivascular tissues via stimulation of nitric oxide synthase (NOS). Here we show that HBO increases bone marrow NO in vivo thereby increasing release of EPC into circulation. These effects are inhibited by pretreatment with the NOS inhibitor l-nitroarginine methyl ester (l-NAME). HBO-mediated mobilization of EPC is associated with increased lower limb spontaneous circulatory recovery after femoral ligation and enhanced closure of ischemic wounds, and these effects on limb perfusion and wound healing are also inhibited by l-NAME pretreatment. These data show that EPC mobilization into circulation is triggered by hyperoxia through

induction of bone marrow NO with resulting enhancement in ischemic limb perfusion and wound healing.