Life Sciences 83 (2008) 236–241

Contents lists available at ScienceDirect

Life Sciences

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

Hyperbaric oxygen preconditioning promotes angiogenesis in rat liver after partial hepatectomy

Ping Ren [a,1], ZhiMing Kang [b,1], GuoJun Gu [b], Yun Liu [b], WeiGang Xu [b], HengYi Tao [b], John H. Zhang [c], XueJun Sun [b,*], Hui Ji [a,*]

[a] Department of Pharmacology, China Pharmaceutical University, Nanjing, 210009, PR China
[b] Department of Diving Medicine, Faculty of Naval Medicine, Secondary Military Medical University, Shanghai, 200433, PR China
[c] Department of Neurosurgery, Loma Linda University, Loma Linda, California, USA

ARTICLE INFO

Article history:
Received 25 October 2007
Accepted 5 June 2008

Keywords:
Angiogenesis
Hyperbaric oxygen preconditioning
Hypoxia inducible factor-1
Vascular endothelial growth factor

ABSTRACT

Hyperbaric oxygen preconditioning (HBO-PC) increases the level of HIF-1α (hypoxia inducible factor-1α) and its target gene VEGF (vascular endothelial growth factor) which is involved in angiogenesis. Liver regeneration is an angiogenesis-dependent process. We hypothesized that HIF-1α and VEGF mediated the angiogenesis effect of HBO-PC on regenerating rat liver. Male Sprague Dawley rats received HBO-PC followed by 70% partial hepatectomy. Proliferation of hepatocytes and endothelial cells was evaluated by BrdU (bromodeoxyuridine) staining. Microvascular density was assessed by immunohistochemistry. mRNA expression of HIF-1α was assessed by quantitative RT-PCR and protein levels of HIF-1α and VEGF were assessed by western blot. HIF-1α DNA-binding activity was determined with an ELISA-based kit. HBO-PC increased the proliferation index of endothelial cells and microvascular density at 48 h after partial hepatectomy. The protein level and DNA-binding activity of HIF-1α and the protein level of VEGF were increased by HBO-PC before and after partial hepatectomy. Partial hepatectomy alone also increased proliferation index and the expressions of HIF-1α and VEGF. Our results indicated that the angiogenesis effect of HBO-PC on liver after partial hepatectomy could be achieved by increased HIF-1α activity and VEGF expression. However, the angiogenic effect of HBO-PC is moderate and HBO-PC failed to produce additional effect on the enhancement of HIF-1α and VEGF induced by partial hepatectomy alone.

Introduction

Liver is the only mammalian organ that regenerates its biologically functional parenchymal mass after resection or injury, instead of healing with biologically nonfunctional scar. Thus, a massive hepatectomy has been aggressively performed for hepatocellular malignancies (Al-Hadeedi et al., 1990). The clinical outcome, morbidity, and mortality of patients depend on an efficient regeneration of the liver after partial hepatectomy (PH) and liver failure is the cause of major complications and the high mortality rate. Therefore, reducing liver injury and promoting hepatocyte growth and viability, especially after PH are important clinical issues.

Previous studies have shown that ischemic preconditioning (IPC) enhanced hepatic function by improving hepatic tissue growth and by reducing cellular disturbances (Yoshizumi et al., 1998; Fernandez et al., 2004). IPC has also been proven an effective strategy against hepatic ischemia–reperfusion injury in both animal and human studies (Clavien et al., 2003). However, IPC has not been widely employed clinically because of safety concerns. On the contrary, hyperbaric oxygen (HBO) has been shown to exhibit protective effects in brain ischemia, spinal cord injury, and myocardial infarction in animals. HBO ameliorated the ischemia–reperfusion injury of the liver and induced compensatory hypertrophy of the predicted remnant liver in rats after portal vein ligation (Uwagawa et al., 2001). HBO augmented liver regeneration by stabilizing the energy metabolism and decreasing oxidative stress after hepatectomy in rats (Ozden et al., 2004). In addition, HBO preconditioning (HBO-PC) has been shown to have neuroprotective effects against focal and global cerebral ischemia (Xiong et al., 2000; Wada et al., 2001). It has also been proposed as a preconditioning treatment to prevent myocardial ischemic reperfusion injury (Kaljusto et al., 2008). HBO-PC provided neuroprotection in neonatal rats which is equivalent to hypoxia preconditioning (HPC) (Freiberger et al., 2006). Because HBO-PC is proven to protect against ischemic injury in several organs and, as a preconditioning stimulus, it is practical and safer, it would have great appeal for injury prevention in high-risk clinical conditions.

Liver regeneration requires the formation of new blood vessels to provide nutrition and oxygen to growing tissues. Inhibition of angiogenesis with angiostatin impairs liver regeneration, indicating that liver regeneration is dependent on angiogenesis (Drixler et al., 2002). Recent studies have shown that VEGF (vascular endothelial growth factor) is

* Corresponding authors. Ji is to be contacted at Department of Pharmacology, China Pharmaceutical University, 24 TongJiaXiang, Nanjing 210009, PR China. Tel.: +86 130 3254 8063; fax: +86 25 8330 1606. Sun, Department of Diving Medicine, Second Military Medical University, 800 Xiangyin Rd, Shanghai 200433, PR China. Tel.: +86 21 2507 0349; fax: +86 21 6549 2382.
E-mail addresses: sunxjk@hotmail.com (X. Sun), huijicpu@163.com (H. Ji).

0024-3205/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2008.06.011

upregulated after PH (Sato et al., 2001) and exogenous VEGF increased hepatocyte proliferation after PH (Ross et al., 2001). In addition, HBO induces VEGF expression and reduces liver injury in regenerating rat (Ijichi et al., 2006). In the present study, we examined the roles of HIF-1α (hypoxia inducible factor-1α) and VEGF in the potential effect of HBO-PC on regenerating liver following 70% PH.

Materials and methods

Animals and study protocols

All experimental protocols were conducted according to the Guide for the Care and Use of Laboratory Animals prepared by the National Institutes of Health and approved by the Ethics Committee for Animal Experiment of the Second Military Medical University.

Fifty-six male Sprague Dawley rats weighing between 200 and 220 g were used in all experiments. The animals were housed in individual cages in a temperature-controlled room with a 12 h light/dark cycle. Rats were randomly assigned to four groups (fourteen in each group). Rats served as control group (Con) breathed room air; rats in HBO group (HBO) were exposed to hyperbaric oxygen (100% O_2 at 2.5 ATA, once per day for three consecutive days), and were sacrificed 12 h after the last HBO exposure; rats in PH group (PH) received a 70% PH according to the method of Higgins and Anderson (1934), and the mass of tissue removed was weighed. Briefly, after a midline laparotomy, the liver was exposed and the left lateral and median lobes were ligated and resected, and were sacrificed 48 h after PH; the last group of rats (HBO+PH) were exposed to hyperbaric oxygen (100% O_2 at 2.5 ATA, once per day for three consecutive days) followed by 70% PH, and were sacrificed 48 h after PH. After sacrifice, the livers were removed, weighed and frozen in liquid nitrogen until use. Eight rats of each group were administered bromodeoxyuridine (BrdU, Sigma) intraperitoneally at a dose of 50 mg/g body weight twice daily for 2 days before sacrificed, and the livers were perfusion-fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for immunohistochemical studies.

Liver regeneration

The total liver weight was estimated by the excised liver weight/(66%). The regeneration ratio was determined as the percentage of regenerated liver mass using this formula: (remnant liver weight at sacrifice/the estimated total liver weight at surgery)×100%.

HBO exposure

Animals were placed (two animals each time) into a custom-made pressure chamber of transparent acrylic plastic (inner diameter 25 cm, length 50 cm). Compression was performed at a rate of 1 kg/cm^2/min to 2.5 ATA/100% oxygen and maintained for 60 min. In order to eliminate carbon dioxide accumulation, a small container with soda lime was put in the chamber to absorb CO_2 and the chamber was flushed with 100% oxygen for 1 min every 20 min during exposure. Decompression was performed at 0.2 kg/cm^2/min. All exposures were started at 8:00am to minimize the effects of diurnal variation. Controls were also transferred into the chamber with room air at normal atmosphere.

Immunohistochemistry

Perfusion-fixed liver tissues were further fixed overnight in the same solution (4% paraformaldehyde in PBS), processed for embedding in paraffin and cut into 5-μm-thick serial sections. Immunohistochemical staining was performed by the avidin–biotin complex method using antibodies against CD31 (Santa Cruz Biotechnology). The antibody binding was visualized using an ABC Kit (Vector Laboratories). For BrdU staining, a BrdU Immunohistochemistry Kit (Chemicon International) was used. The number of BrdU positive cells was counted in 10 random high-power fields. Data were expressed as the BrdU positive cells per field.

Western blot analysis

Nuclear extracts (for HIF-1α) and total cell lysate (for VEGF) were obtained using a nuclear extraction kit (Active Motif) and following the manufacturer's protocol. Equal amounts of the samples were loaded per lane, separated on 10% dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred electrophoretically to nitrocellulose membranes. Membranes were blocked with 2% BSA in Tris-buffered saline containing 0.1% Tween20 (TBST) at room temperature for 1 h and then incubated overnight at 4 °C with anti-VEGF, anti-HIF-1α (Santa Cruz Biotechnology), and β-actin (Sigma) diluted 1:1000 in 2% BSA in TTBS. Horseradish peroxidase-conjugated antimouse or antirabbit serum (Santa Cruz Biotechnology) was used as a secondary antibody (1:2000 dilution in 2% BSA in TBST, 1 h incubation) and the antigen antibody complexes were visualized using an enhanced chemiluminescence detection reagent (Amersham). Bands were scanned using a densitometer (GS-700; Bio-Rad Laboratories), and quantification was performed using Multi-Analyst 1.0.2 software (Bio-Rad).

HIF-1α DNA-binding assay

HIF-1α DNA-binding activity of the nuclear fraction was determined by using an ELISA-based kit (Active Motif) according to the manufacturer's specifications. DNA-binding activity was expressed as optical density at 450–655 nm relative to that of control and represents triplicate samples from two independent experiments.

RNA isolation and quantitative real-time PCR

RNA was isolated from frozen liver tissue maintained in RNAlater (Qiagen) using Trizol reagents (Invitrogen). RNA samples were reverse-transcribed using a Superscript II kit (Invitrogen) and random primers as suggested by the manufacturer. The PCR reactions were carried out using 20 ng of cDNA, 67 nM of each primer, and SYBR Green master mix (Applied Biosystems, Foster City CA) in 10 Al reactions. Levels of Q-RT-PCR products were measured using SYBR Green fluorescence collected during real-time PCR on an Applied Biosystems 7900HT system. A control cDNA dilution series was created for each gene to establish a standard curve. Each reaction was subjected to melting point analysis to confirm single amplified products. Reactions were run in triplicate, and results were averaged. Each value was normalized to β-actin to control for variations in the amount of input cDNA. Fold-change values represent a mean of six samples divided by the mean of the six controls. The primers were AGTCGGACAGCCTCACCAGA and TTCTGCTGCCTTGTATG GGA for mouse HIF-1α, and CCTCTATGCCAACACAGTGC and GTACTCCT GCTTGCTGATCC for β-actin.

Data analysis

Results are presented as means ± SD. A parametric one-way ANOVA using SPSS 10.0 was used to test for any differences among the groups. If the result was found significant, the Tukey's Multiple Comparison Test was then used to determine the specific differences between group means. $p<0.05$ was considered to be statistically significant.

Results

Wet regeneration

PH and HBO+PH caused remarkable increase of wet liver weight compared to those of control and HBO alone groups. However, no difference was observed between PH and HBO+PH groups (Fig. 1).

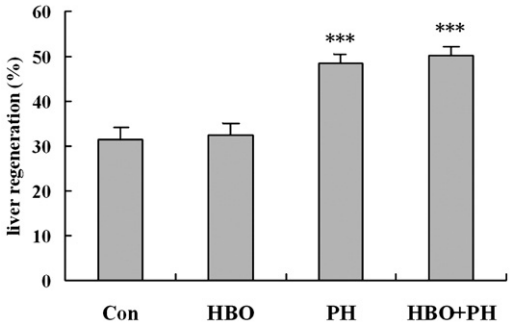

Fig. 1. The effect of HBO-PC on the wet liver weight of rats at 48 h after PH. PH and HBO+PH caused remarkable increase of wet liver weight when compared to those of control and HBO alone groups. Values are means±SD, ***$p<0.001$ vs Con, $n=8$.

Proliferation index BrdU

Regenerating livers were stained with BrdU to identify cells undergoing proliferation. A strong BrdU stain was noticed at 48 h following hepatectomy, and the proliferating hepatocytes were more evident in the periportal area than in the pericentral area (Fig. 2A). The round dark-stained nuclei represented hepatocytes, and the sinusoidal endothelial cells (SECs) showed long-shuttled nuclei and peri-hepatocyte localization characteristics (Fig. 2B). HBO alone had no effect on hepatocyte proliferation. Forty-eight hours after PH, the number of proliferating hepatocytes significantly increased to 32±9 in PH group and 40±12 in HBO+PH group ($p<0.01$ vs Con, Fig. 2C). Meanwhile, both HBO and PH significantly increased the proliferation index of SECs following PH (7±1 in HBO group and 5±1 in PH group, Fig. 2D, $p<0.01$ vs Con). An additive effect was observed in HBO+PH group and the proliferating endothelial cell was increased to 11±4 ($p<0.05$ vs PH, Fig. 2D).

Microvascular density

In livers of PH alone rats, 9±4 blood vessels per high-power field were counted (Fig. 3B and C). In rats subjected to HBO-PC followed by PH, the hepatic MVD increased significantly to 12±3 blood vessels per high-power field (Fig. 3C, $p<0.05$ vs PH, $p<0.01$ vs Con) at 48 h following PH.

HIF-1α

Quantitative real-time PCR showed no significant differences of the HIF-1α mRNA among the four groups (Fig. 4A). The HIF-1α protein band (120kD) in the control group was fairly faint but detectable (Fig. 4C). Both HBO and PH stabilized the protein of HIF-1α, and an almost 2-fold increase of nuclear HIF-1α was noticed at 12 h after the last HBO exposure and at 48 h after PH. No additive effect was observed in HBO+PH group (Fig. 4D). The change trend of HIF-1α DNA-binding activity among the four groups was in accordance with that of HIF-1α protein level (Fig. 4B).

VEGF

Twelve hours after the last HBO exposure, HBO-PC increased the protein level of VEGF by almost 1.8-fold. PH alone caused a 1.6-fold increase of VEGF protein compared with that of control. And similar to the change of HIF-1α, no additive effect of the two stimuli was observed in HBO+PH group (Fig. 5).

Discussion

In the present study we observed that HBO-PC induced the sinusoidal endothelial cells proliferation (BrdU) and increased the microvascular density after PH. HIF-1α and VEGF expression and activity were

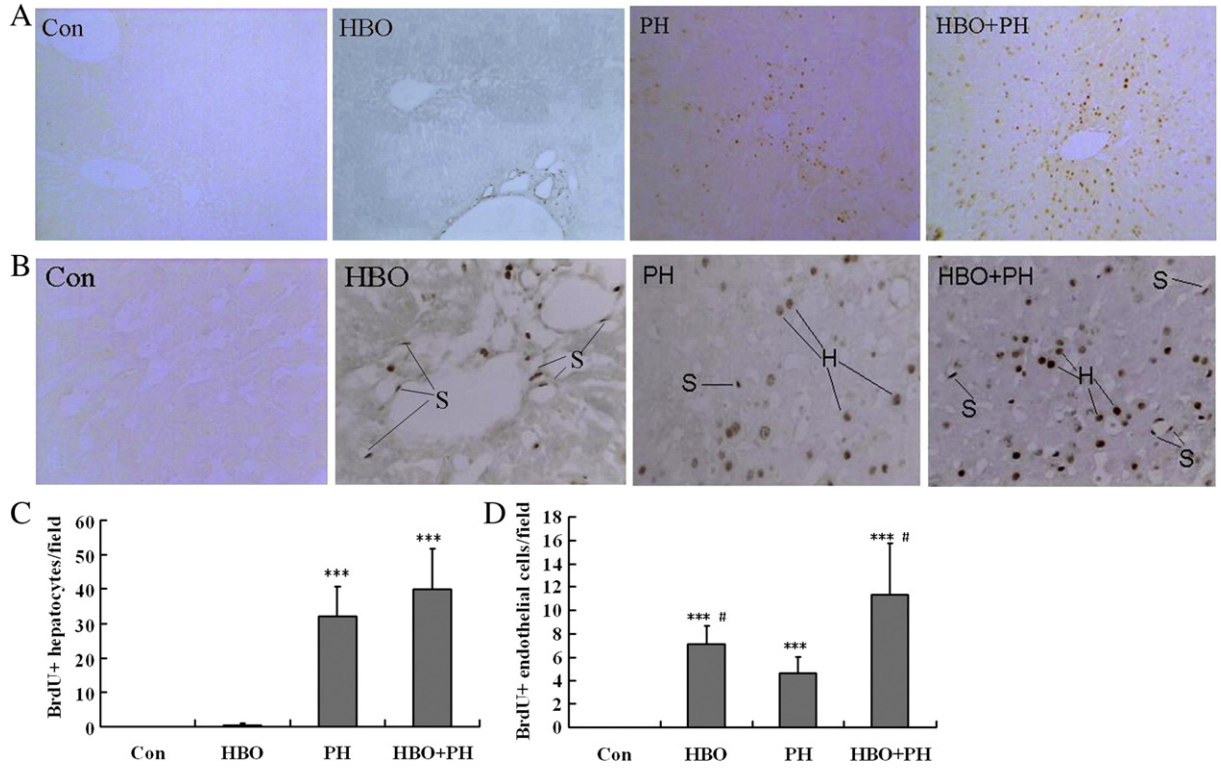

Fig. 2. BrdU staining of regenerating liver (A×100, B×400). H, hepatocyte; S: sinusoidal endothelial cell. C showed the number of BrdU positive hepatocyte and D showed the number of BrdU positive sinusoidal endothelial cells. HBO preconditioning followed by PH significantly increased the SECs undergoing proliferation compared with that of PH alone group. Cells were counted in 10 random high-power fields. Data were expressed as the number of BrdU positive cells per field. Values are means±SD ($n=8$). ***$p<0.01$ vs Con group, #$p<0.05$ vs PH group.

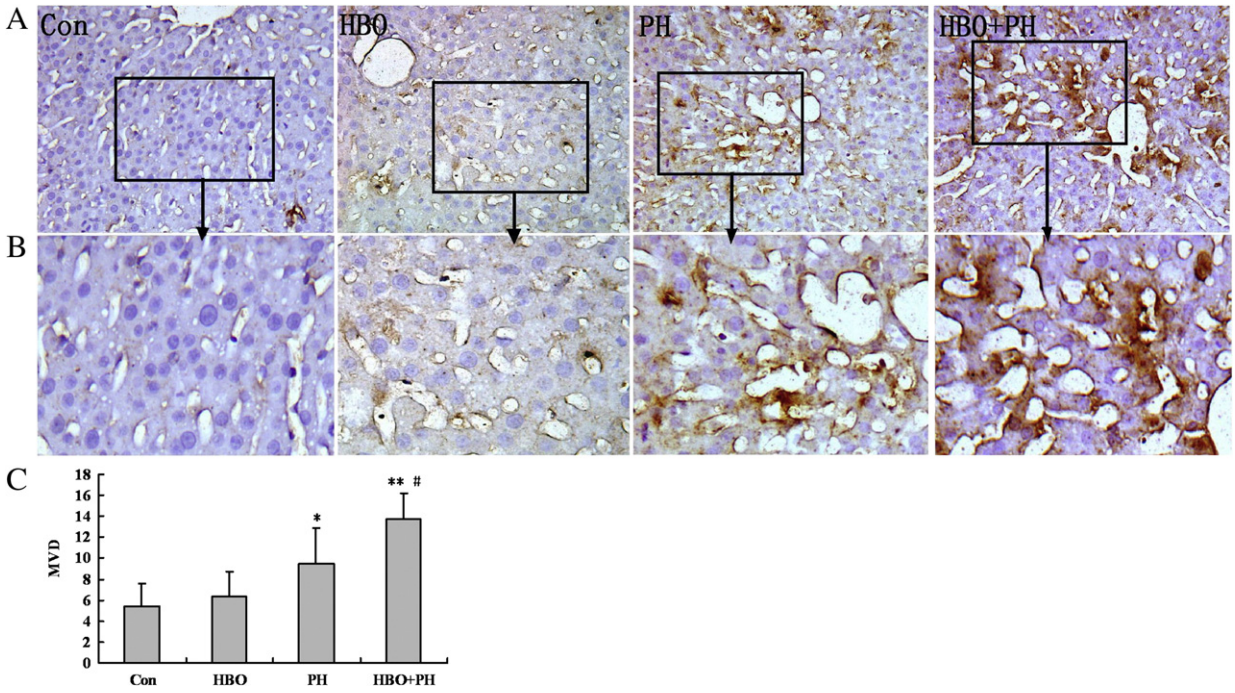

Fig. 3. Effects of HBO preconditioning on microvascular density (MVD) in regenerating liver. The identification of sinus endothelial cells was performed by immunohistochemistry with antibody against CD-31, (A ×200, B ×1000). C showed the MVD quantified from 10 random ×1000 magnification fields. Data were expressed as the number of MVD per field. Values are means ± SD (n=8). *p<0.05 vs Con group, **p<0.01 vs Con group, #p<0.05 vs PH group.

increased by PH and by HBO-PC. It is likely that PH produced hypoxia in the liver and hypoxia may potentiate HIF-1α and VEGF. Rather than an additional increase of HIF-1α and VEGF expression after PH, the additional effect of HBO-PC on increased microvascular density was achieved by an accumulation of proliferating endothelial cells.

Liver is the organ with the most profound ability of regeneration. The rodent liver will regenerate 100% of its lost mass within 8 days after surgical resection (O'Reilly et al., 1994). The regeneration of liver is known to require angiogenesis. Previous studies have shown that after PH the normal liver architecture was restored by a robust endothelial cell proliferation and remodeling of the sinusoidal network (Martinez-Hernandez and Amenta, 1995), and the growing liver after PH required the synthesis of new blood vessels to support the rapidly increasing mass with nutrition and oxygen. Furthermore, a complex

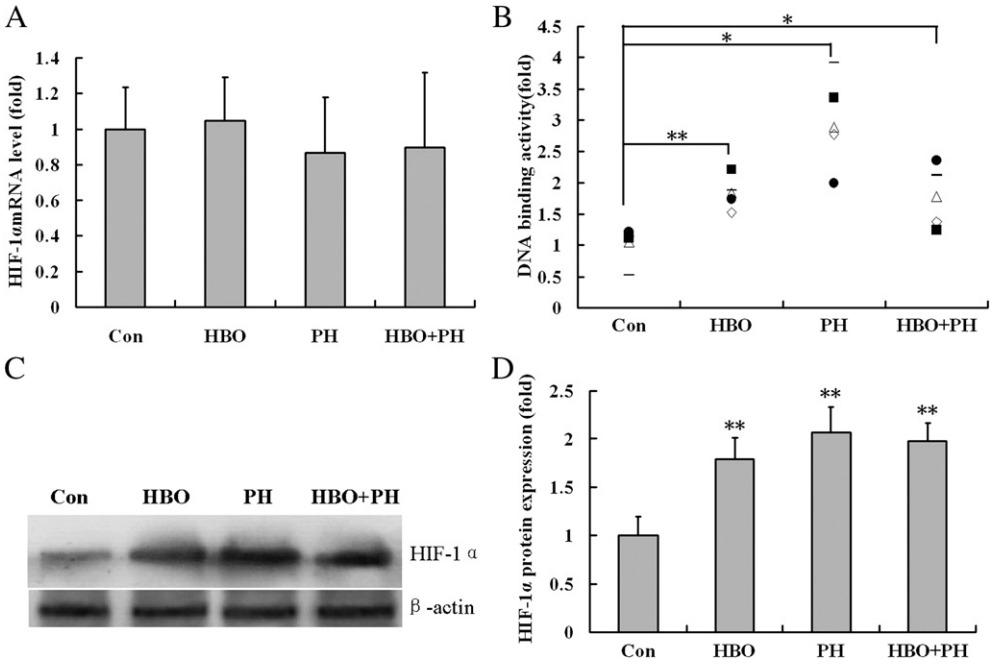

Fig. 4. A showed the HIF-1α mRNA expression assessed by quantitative real-time PCR. Each value was normalized to β-actin to control for variations in the amount of input cDNA. All normalized values divided by one of the control samples (n=6). B showed the nuclear HIF-1α DNA-binding activity analyzed by an ELISA-based kit. Fold-change values represent the OD of each sample divided by one of the five controls (n=5). C and D showed the HIF-1α protein content analyzed by western blot. Fold-change represent the value of each sample divided by one of the controls (n=6). Con, rats served as control; HBO, 12 h after the last HBO exposure; PH, 48 h after PH; HBO+PH, 48 h after PH following HBO pretreatment. Data are presented as mean ± SD, *p<0.05 vs Con; **p<0.01 vs Con.

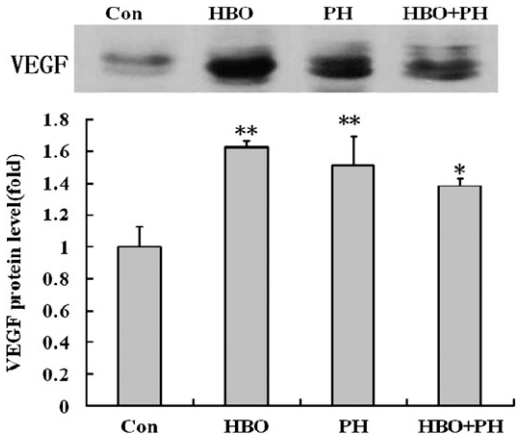

Fig. 5. VEGF protein content assessed by western blot. Con, rats served as control; HBO, 12 h after the last HBO exposure; PH, 48 h after PH; HBO+PH, 48 h after PH following HBO pretreatment. Fold-change represent the value of each sample divided by one of the controls ($n=6$). Data are presented as mean±SD, *$p<0.05$ vs Con; **$p<0.01$ vs Con.

paracrine interaction between hepatocytes and endothelial cells existed during liver regeneration. Endothelial cells proliferate, migrate and release potent hepatocyte mitogens such as IL-6 and Hepatocyte Growth factor (HGF), inducing the liver regeneration. In the present study, we found that HBO-PC significantly increased the SEC proliferation in rat liver after PH. As shown in Fig. 1, the BrdU positive SECs of HBO-PC group doubled at 48 h compared with that of PH alone group. Together with our observation of increased MVD, it suggests the formation of new blood vessels after PH.

VEGF belongs to the most potent angiogenic factors (Dvorak et al., 1999; Ferrara et al., 2003). It is well recognized that VEGF is important during liver regeneration (Redaelli et al., 2004). Injury such as resection leads to the secretion of VEGF, which binds to its receptors flt1 and flk1, triggering the proliferation of endothelial cells. The increased MVD observed 48 h after PH may be induced by the high level of VEGF protein content after PH and after HBO-PC. VEGF is a target gene of HIF-1α. HIF-1α is a transcription factor that is found to be a crucial regulator in hypoxia and regulates cell proliferation, apoptosis, energy preservation and angiogenesis (Caro, 2001; Semenza, 2001a,b). Gopfert et al. (1996) have shown that moderate hypoxia enhances HIF-1α expression in rat hepatocytes. Vectors coding for stabilized HIF-1α mutants were used to demonstrate that HIF-1α can induce angiogenesis in different tissues (Vincent et al., 2000; Elson et al., 2001). Expression of such a mutant under the control of the keratin-14 promoter in transgenic mice induced a regular network of dense dermal capillaries without vascular leakage. Indeed, a recent report described the temporal upregulation of HIF-1α prior to the onset of VEGF synthesis in a rat model of PH and postulated that HIF-1α may have a role in the regeneration of the sinusoidal endothelium after liver injury (Maeno et al., 2005).

In the present study, PH increased the protein and the DNA-binding activity of nuclear HIF-1α subunit, while the mRNA level kept unchanged, indicating that the accumulation effect of PH on HIF-1α is post-transcriptional. In normoxic condition, HIF-1α expression at the protein level is kept to a minimum level. In the presence of oxygen, the prolyl residues within the oxygen-dependent degradation domain of HIF-1α are hydroxylated by prolyl hydroxylase domain containing protein (PHD) 1, 2, and 3. Hydroxylation of these residues the binding of von Hippel–Lindau protein (pVHL) which polyubiquitylate HIF-1α and tag it for proteosomal degradation. Under oxygen-deprived conditions, the protein of HIF-1α is stabled because the prolines remain unmodified. After PH, single cell wide plates of hepatocytes have grown to avascular clusters due to the exponential growth of hepatocytes. The proliferating hepatocytes are devoid of clear sinusoid structures until reconstruction of normal vascular architecture. The liver thus appears to be under hypoxic state. The nuclear HIF-1α accumulation following PH might be the result of transient hypoxia caused by exponential hepatocyte proliferation. Besides hypoxia, growth factor-stimulated signal transduction pathways and oxidative stress have been reported to cause induction of HIF-1α (Semenza, 2001a,b; Haddad, 2002a,b). Nitrosylation by reactive nitrogen leading to HIF-1α stabilization has also been implicated as a critical mechanism of induction (Wellman et al., 2004; Metzen et al., 2003). In vitro studies have shown that oxygen radicals increase HIF-1α expression. We postulate that the HBO induced HIF-1α accumulation may involve formation of oxygen radicals that are produced during HBO therapy (Golembe, 1997). However, increases of both VEGF and HIF-1 were observed after HBO-PC and after PH alone. The results from the present study supported our initial hypothesis that proliferation and microvascular density were increased by HBO-PC but not entirely mediated by HIF-1α or VEGF since both HIF-1α and VEGF were enhanced by PH alone.

Forty-eight hours after PH, although HBO-PC did not further increase HIF-1α and VEGF level compared with PH alone group, more proliferating endothelial cells and higher MVD were observed in rats that had received HBO-PC. It could be an accumulation of the proliferating endothelial cells. Our results showed that HBO and PH increased HIF-1α and VEGF to a similar degree. BrdU immunohistochemistry showed that HBO alone caused proliferation of endothelial cells located at the periportal area, indicating that SECs primed by HBO-PC entered the cell cycle in advance. Thom et al. (2006) reported that exposure to HBO mobilized stem/progenitor cells from the bone marrow by a nitric oxide dependent mechanism. It is possible that the mobilized endothelial progenitors could be one source of BrdU positive cells observed in this study.

HIF-1α accumulates under hypoxia conditions. HBO-PC increases tissue oxygen levels by giving 100% oxygen at higher pressures. After HBO-PC, liver tissue will experience a relative hypoxia because the oxygen level is reduced to normal level at 21%. Therefore, repeating HBO-PC may produce HIF-1α accumulation. In addition to hypoxia, accumulating evidence suggests that a variety of non-hypoxic stimuli including growth factors, hormones, vasoactive peptides and metal ions can induce HIF-1α under normoxia (BelAiba et al., 2004; Park et al., 2003). Another mechanism for HIF-1α accumulation is the generation of reactive oxygen species (ROS) by HBO-PC. ROS may regulate HIF-1α expression via two major pathways. Firstly, ROS could control α as a signaling molecule through participating in some signal transduction ways, such as PI3-K/PKB (Gao et al., 2004) and MAPK (Wang et al., 2004). Secondly, ROS may also have the potential to interfere with hydroxylase activity (Metzen et al., 2003). In addition, ROS have been found to modulate the levels of HIF-1α not only under hypoxia, but also in response to other factors and under different stress conditions. It was demonstrated that under normoxic conditions ROS generated by cytokines mediate HIF-1α upregulation (Richard et al., 2000; Haddad, 2002a,b). Some reports suggested that ROS generated at mitochondrial complex III participate in HIF-1α stabilization (Chandel et al., 1998, 2000; Agani et al., 2000; Schroedl et al., 2002). These potential mechanisms may contribute to several recent reports. Gu et al. (2008) reported that HBO induced a marked increase in the protein expression of HIF-1α in rat brain and the neuroprotection induced by HBO preconditioning may be mediated by an upregulation of HIF-1α and its target gene EPO. Salhanick et al. (2006) also found that HIF-1α in liver of rats was increased by HBO exposure.

Our results indicated that even though HBO-PC increased the number of new cells and the density of microcirculation in the regenerating liver, its effect on the expression of HIF-1α and VEGF was limited and its action did not translate into wet liver weight. Our observation seems consistent with a recent publication indicating a role of VEGF only in the early stage of angiogenesis. Bockhorn et al. (2007) found that blocking of endogenous VEGF could delay regeneration almost completely for about 24 h. However, the proliferation index reached normal levels thereafter despite the readministration of anti-VEGF. The report suggested an important role for VEGF in the early stage,

while it was not essential in the second phase of liver regeneration after partial hepatectomy. The beneficial effect of HBO-PC on new cells and on microcirculation may be speculated to the prime of endothelial cells and make them readily responsive to stimuli and enter the cell cycle more rapidly.

Acknowledgement

The present study was funded by National Natural Science Foundation of China (No. 30500579).

References

Agani, F.H., Pichiule, P., Chavez, J.C., LaManna, J.C., 2000. The role of mitochondria in the regulation of hypoxia-inducible factor 1 expression during hypoxia. Journal of Biological Chemistry 275 (46), 35863–35867.

Al-Hadeedi, S., Chori, T.K., Wong, J., 1990. Extended hepatectomy for hepatocellular carcinoma. British Journal of Surgery 77 (11), 1247–1250.

BelAiba, R.S., Djordjevic, T., Bonello, S., Flugel, D., Hess, J., Kietzmann, T., Gorlach, A., 2004. Redox-sensitive regulation of the HIF pathway under non-hypoxic conditions in pulmonary artery smooth muscle cells. Biological Chemistry 385 (3–4), 249–257.

Bockhorn, M., Goralski, M., Prokofiev, D., Dammann, P., Grünewald, P., Trippler, M., Biglarnia, A., Kamler, M., Niehues, E.M., Frilling, A., Broelsch, C.E., Schlaak, J.F., 2007. VEGF is important for early liver regeneration after partial hepatectomy. Journal of Surgical Research 138 (2), 291–299.

Caro, J., 2001. Hypoxia regulation of gene transcription. High Altitude Medicine & Biology 2 (2), 145–154.

Chandel, N.S., Maltepe, E., Goldwasser, E., Mathieu, C.E., Simon, M.C., Schumacker, P.T., 1998. Mitochondrial reactive oxygen species trigger hypoxia induced transcription. Proceedings of the National Academy of Sciences 95 (20), 11715–11720.

Chandel, N.S., McClintock, D.S., Feliciano, C.E., Wood, T.M., Melendez, J.A., Rodriguez, A.M., Schumacker, P.T., 2000. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O_2 sensing. Journal of Biological Chemistry 275 (33), 25130–25138.

Clavien, P.A., Selzner, M., Rudiger, H.A., Graf, R., Kadry, Z., Rousson, V., Jochum, W., 2003. A prospective randomized study in 100 consecutive patients undergoing major liver resection with versus without ischemic preconditioning. Annals of Surgery 238 (6), 843–850.

Drixler, T.A., Vogten, M.J., Ritchie, E.D., Van Vroonhoven, T.J., Gebbink, M.F., Voest, E.E., Borel Rinkes, I.H., 2002. Liver regeneration is an angiogenesis-associated phenomenon. Annals of Surgery 236 (6), 703–712.

Dvorak, H.F., Nagy, J.A., Feng, D., Brown, L.F., Dvorak, A.M., 1999. Vascular permeability factor/vascular endothelial growth factor and the significance of microvascular hyperpermeability in angiogenesis. Current Topics in Microbiology and Immunology 237, 97–132.

Elson, D.A., Thurston, G., Huang, L.E., Ginzinger, D.G., McDonald, D.M., Johnson, R.S., Arbeit, J.M., 2001. Induction of hypervascularity without leakage or inflammation in transgenic mice overexpressing hypoxia-inducible factor-1α. Genes and Development 15 (19), 2520–2532.

Fernandez, L., Carrasco-Chaumel, E., Serafin, A., Xaus, C., Grande, L., Rimola, A., Rosello-Catafau, J., Peralta, C., 2004. Is ischemic preconditioning a useful strategy in steatotic liver transplantation? American Journal of Transplantation 4 (6), 888–899.

Ferrara, N., Gerber, H.P., LeCouter, J., 2003. The biology of VEGF and its receptors. Nature Medicine 9 (6), 669–766.

Freiberger, J.J., Suliman, H.B., Sheng, H., McAdoo, J., Piantadosi, C.A., Warner, D.S., 2006. A comparison of hyperbaric oxygen versus hypoxic cerebral preconditioning in neonatal rats. Brain Research 1075 (1), 213–222.

Gao, N., Shen, L., Zhang, Z., Leonard, S.S., He, H., Zhang, X.G., Shi, X., Jiang, B.H., 2004. Arsenite induces HIF-1alpha and VEGF through PI3K, Akt and reactive oxygen species in DU145 human prostate carcinoma cells. Molecular and Cellular Biochemistry 255 (1–2), 33–45.

Golembe, E., 1997. Re: hyperbaric oxygen therapy and free radical production: an experimental study in doxorubicin (Adriamycin) extravasation injuries. Annals of Plastic Surgery 39 (1), 105.

Gopfert, T., Gess, G., Eckardt, K., Kurtz, A., 1996. Hypoxia signaling in the control of erythropoietin gene expression in rat hepatocytes. Journal of Cellular Physiology 168 (2), 354–361.

Gu, G.J., Li, Y.P., Peng, Z.Y., Kang, Z.M., Xu, W.G., Tao, H.Y., Ostrowski, R.P., Zhang, J.H., Sun, X.J., 2008. Mechanism of Ischemic tolerance induced by hyperbaric oxygen preconditioning involves up-regulation of hypoxia-inducible factor-1{α} and erythropoietin in rats. Journal of Applied Physiology 104 (4), 1185–1191.

Haddad, J.J., 2002a. Antioxidant and prooxidant mechanisms in the regulation of redox(y)-sensitive transcription factors. Cell Signalling 14 (11), 879–897.

Haddad, J.J., 2002b. Recombinant human interleukin (IL)-1 beta-mediated regulation of hypoxia-inducible factor-1 alpha (HIF-1 alpha) stabilization, nuclear translocation and activation requires an antioxidant/reactive oxygen species (ROS)-sensitive mechanism. European Cytokine Network 13 (2), 250–260.

Higgins, G.M., Anderson, R.M., 1934. Experimental pathology of liver: restoration of liver of the white rat following partial surgical removal. Archives of Pathology 12, 186–202.

Kaljusto, M.L., Stensløkken, K.O., Mori, T., Panchenko, A., Frantzen, M.L., Valen, G., Vaage, J., 2008. Preconditioning effects of steroids and hyperoxia on cardiac ischemia–reperfusion injury and vascular reactivity. European Journal of Cardiothoracic Surgery 33 (3), 355–363.

Ijichi, H., Taketomi, A., Yoshizumi, T., Uchiyama, H., Yonemura, Y., Soejima, Y., Shimada, M., Maehara, Y., 2006. Hyperbaric oxygen induces vascular endothelial growth factor and reduces liver injury in regenerating rat liver after partial hepatectomy. Journal of Hepatology 45 (1), 28–34.

Maeno, H., Ono, T., Dhar, D.K., Sato, T., Yamanoi, A., Nagasue, N., 2005. Expression of hypoxia inducible factor-1alpha during liver regeneration induced by partial hepatectomy in rats. Liver International 25 (5), 1002–1009.

Martinez-Hernandez, A., Amenta, P.S., 1995. The extracellular matrix in hepatic regeneration. FASEB Journal 9 (14), 1401–1410.

Metzen, E., Zhou, J., Jelkmann, W., Fandrey, J., Brune, B., 2003. Nitric oxide impairs normoxic degradation of HIF-1α by inhibition of prolyl hydroxylases. Molecular Biology of the Cell 14 (8), 3470–3481.

O'Reilly, M.S., Holmgren, L., Shing, Y., Chen, C., Rosenthal, R.A., Moses, M., Lane, W.S., Cao, Y., Sage, E.H., Folkman, J., 1994. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastasis by a Lewis lung carcinoma. Cell 79 (2), 315–328.

Ozden, T.A., Uzun, H., Bohloli, M., Toklu, A.S., Paksoy, M., Simsek, G., Durak, H., Issever, H., Ipek, T., 2004. The effects of hyperbaric oxygen treatment on oxidant and antioxidants levels during liver regeneration in rats. Tohoku Journal of Experimental Medicine 203 (4), 253–265.

Park, J.H., Kim, T.Y., Jong, H.S., Kim, T.Y., Chun, Y.S., Park, J.W., Lee, C.T., Jung, H.C., Kim, N.K., Bang, Y.J., 2003. Gastric epithelial reactive oxygen species prevent normoxic degradation of hypoxia inducible factor-1alpha in gastric cancer cells. Clinical Cancer Research 9 (1), 433–440.

Redaelli, C.A., Semela, D., Carrick, F.E., Ledermann, M., Candinas, D., Sauter, B., Dufour, J.F., 2004. Effect of vascular endothelial growth factor on functional recovery after hepatectomy in lean and obese mice. Journal of Hepatology 40 (2), 305–312.

Richard, D.E., Berra, E., Pouyssegur, J., 2000. Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1alpha in vascular smooth muscle cells. Journal of Biological Chemistry 275 (35), 26765–26771.

Ross, M.A., Sander, C.M., Kleeb, T.B., Watkins, S.C., Stolz, D.B., 2001. Spatiotemporal expression of angiogenesis growth factor receptors during the revascularization of regenerating rat liver. Hepatology 34 (6), 1135–1148.

Salhanick, S.D., Belikoff, B., Orlow, D., Holt, D., Reenstra, W., Buras, J.A., 2006. Hyperbaric oxygen reduces acetaminophen toxicity and increases HIF-1alpha expression. Academic Emergency Medicine 13 (7), 707–714.

Sato, T., El-Assal, O.N., Ono, T., Yamanoi, A., Dhar, D.K., Nagasue, N., 2001. Sinusoidal endothelial cell proliferation and expression of angiopoietin/Tie family in regenerating rat liver. Journal of Hepatology 34 (5), 690-198.

Schroedl, C., McClintock, D.S., Budinger, G.R., Chandel, N.S., 2002. Hypoxic but not anoxic stabilization of HIF-1alpha requires mitochondrial reactive oxygen species. American Journal of Physiology. Lung Cell Molecular Physiology 283 (5), L922–L931.

Semenza, G.L., 2001a. HIF-1 and mechanisms of hypoxia sensing. Current Opinion in Cell Biology 13 (2), 167–171.

Semenza, G.L., 2001b. HIF-1, O_2, and the 3 pdhs: how animal cells signal hypoxia to the nucleus. Cell 107 (1), 1–3.

Thom, S.R., Bhopale, V.M., Velazquez, O.C., Goldstein, L.J., Thom, L.H., Buerk, D.G., 2006. Stem cell mobilization by hyperbaric oxygen. American Journal of Physiology. Heart and Circulatory Physiology 290 (4), H1378–1386.

Uwagawa, T., Unemura, Y., Yamazaki, Y., 2001. Hyperbaric oxygenation after portal vein emobilization for regeneration of the predicted remnant liver. Journal of Surgical Research 100 (1), 63–68.

Vincent, K.A., Shyu, K.G., Luo, Y., Magner, M., Tio, R.A., Jiang, C., Goldberg, M.A., Akita, G.Y., Gregory, R.J., Isner, J.M., 2000. Angiogenesis is induced in a rabbit model of hindlimb ischemia by naked DNA encoding an HIF-1α/VP16 hybrid transcription factor. Circulation 102 (18), 2255–2261.

Wada, K., Miyazawa, T., Nomura, N., Tsuzuki, N., Nawashiro, H., Shima, K., 2001. Preferential conditions for and possible mechanisms of induction of ischemic tolerance by repeated hyperbaric oxygenation in gerbil hippocampus. Neurosurgery 49 (1), 160–167.

Wang, F.S., Wang, C.J., Chen, Y.J., Chang, P.R., Huang, Y.T., Sun, Y.C., Huang, H.C., Yang, Y.J., Yang, K.D., 2004. Ras induction of superoxide activates ERK-dependent angiogenic transcription factor HIF-1αlpha and VEGF-A expression in shock wave-stimulated osteoblasts. Journal of Biological Chemistry. 279 (11), 10331–10337.

Wellman, T.L., Jenkins, J., Penar, P.L., Tranmer, B., Zahr, R., Lounsbury, K.M., 2004. Nitric oxide and reactive oxygen species exert opposing effects on the stability of hypoxia-inducible factor-1alpha (HIF-1alpha) in explants of human pial arteries. FASEB Journal 18 (2), 379–381.

Xiong, L., Zhu, Z., Dong, H., Hu, W., Hou, L., Chen, S., 2000. Hyperbaric oxygen preconditioning induces neuroprotection against ischemia in transient not permanent middle cerebral artery occlusion rat model. Chinese Medical Journal (Engl) 113 (9), 836–839.

Yoshizumi, T., Yanaga, K., Soejima, Y., Maeda, T., Uchiyama, H., Sugimachi, K., 1998. Amelioration of liver injury by ischaemic preconditioning. British Journal of Surgery 85 (12), 1636–1640.