Effects of stromal cell derived factor-1 and CXCR4 on the promotion of neovascularization by hyperbaric oxygen treatment in skin flaps

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Abstract. Hyperbaric oxygen (HBO) is known to increase the survival of skin flaps by promoting neovascularization; however, the detailed mechanisms involved are not fully understood. In the present study, we aimed to characterize the effects of HBO treatment on neovascularization and skin flap survival. We also analyzed the mechanisms associated with the expression of angiogenic molecules, such as stromal cell derived factor-1 (SDF-1) and its specific receptor CXCR4, to assess the effects of SDF-1 and CXCR4 on the promotion of neovascularization by HBO treatment in skin flaps. The epigastric pedicle skin flap model was established in rats that were randomly divided into the following groups: i) sham-operated (SH group); ii) ischemia followed by reperfusion and analysis on the third and fifth day (IR3d and IR5d groups, respectively) postoperatively; iii) ischemia followed by reperfusion, HBO treatment and analysis on the third and fifth day (HBO3d and HBO5d groups, respectively) postoperatively. In the two HBO groups, animals received 1 h of HBO treatment in a 2.0 ATA chamber with 100% O_2 twice per day for 3 days and then daily for 2 consecutive days following surgery. On the postoperative third and fifth day, skin flap survival measurement, histological analysis, immunohistochemical staining and western blotting for SDF-1 and CXCR4 expression, were performed. Compared with those of the IR groups, skin flap survival, microvessel density (MVD) and expression of SDF-1 and CXCR4 proteins were significantly increased in the HBO groups. Pearson's correlation analysis demonstrated a positive correlation between MVD and the high expression of SDF-1 and CXCR4 following HBO treatment. Results of this study suggested that the effects of HBO treatment in promoting neovascularization may be explained by the upregulation of SDF-1 and CXCR4 expression in the skin flaps of rats.

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Introduction

Surgical skin flaps are often used in plastic and reconstructive surgery to repair defects resulting from trauma, congenital defects, cancer excision or additional mechanisms. Partial or complete skin flap necrosis is a common problem encountered postoperatively. Inadequate blood perfusion is thought to be the main factor resulting in flap necrosis. Several methods have been used in an attempt to increase blood supply and tissue perfusion in compromised tissues, including therapeutic angiogenesis using exogenous growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (1,2), as well as ischemic preconditioning (3) and surgical delay (4). However, these methods are not widely used in the clinic due to lack of validation of efficacy and side effects.

The density of vascular structures affects blood circulation and perfusion to the skin flap. The skin flap transplantation process is associated with tissue injury, and the regeneration of blood vessels (neovascularization) is required to revascularize the injured tissue. Stromal cell derived factor-1 (SDF-1) is a constitutively expressed and inducible chemokine that regulates multiple physiological processes including organ repair and tumor development. The biological effects of SDF-1 are mediated by the specific receptor CXCR4, which belongs to a family of G-protein-coupled receptors, and is widely and constitutively expressed by numerous hematopoietic and endothelial cells (5). It has been demonstrated that SDF-1 and CXCR4 are constitutively expressed in a wide range of tissues, including brain, lung, liver, skin and bone marrow (6). In addition, recent studies have shown that the expression of SDF-1 in a large number of tumors and injured tissues strongly suggests that the activation of CXCR4 is involved in promoting neovascularization (7,8).

Numerous studies have demonstrated the efficacy of hyperbaric oxygen (HBO) treatment on enhancing skin flap and skin graft survival by increasing arterial oxygen tension,

reducing ischemia-reperfusion injury, enhancing host response to local infections, and stimulating neovascularization and tissue growth (4,9-11). The present study aimed to assess whether HBO treatment is able to improve skin flap survival and neovascularization, whether the expression of SDF-1 and CXCR4 in skin flaps may be induced by HBO treatment and whether neovascularization is affected by the expression of SDF-1 and CXCR4.

Materials and methods

Experimental animals. All the experiments were performed in accordance with the ethical guidelines determined by the Committee for the Purpose of Control and Supervision of Experiments on Animals at the Capital Medical University (Beijing, China). Forty healthy adult male Sprague Dawley rats (weighing, 250-300 g at the beginning of the study) were used. Rats were maintained at 25±1.0˚C with a 12/12-h light/dark cycle and received food and water *ad libitum*.

Experimental groups. Forty rats were randomly assigned to one of the following groups (n=8 per group): i) sham-operated (SH group); ii) ischemia followed by reperfusion and analysis on the third and fifth day postoperatively (IR3d and IR5d groups, respectively); and iii) ischemia followed by reperfusion and HBO treatment and analysis on the third and fifth day postoperatively (HBO3d and HBO5d groups, respectively).

Epigastric pedicle skin flap model. The epigastric pedicle skin flap model used in this study has been described previously (12), with a modification in flap design. Procedures were performed aseptically under anesthesia using intraperitoneal injections of 350 mg/kg 10% chloral hydrate. Rats were fixed on the operating table, the abdomen was shaved and washed before the single inferior epigastric vessel pedicle skin flaps (9x6 cm) were obtained. Skeletonization of the right inferior epigastric artery and vein pedicle was performed, while the contralateral inferior epigastric vessel was ligated with sutures and the feeding vessel was clamped using a microvascular clamp to achieve ischemia. For reperfusion, the microvascular clamp was removed 3 h later and blood flow was restored. The skin flaps were repositioned above a silicone sheet (the same area as the flap) with continuous 5-0 monofilament nylon sutures to prevent vascular supply other than that of the pedicle. The sham-operated group underwent the same surgery but the rats were not exposed to ischemia. All the rats received a single dose of 0.8 mg/g intramuscular penicillin sodium postoperatively.

HBO treatment. In the HBO3d and HBO5d groups, the rats were placed into a custom-made pressure chamber of transparent acrylic plastic (701 Space Research Institute, Beijing, China) immediately following surgery and received 1 h of HBO treatment in a 2.0 ATA chamber in 100% O_2 twice per day (8-h intervals) for 3 days and then daily for 2 consecutive days. Compressed air was administered at a rate of 1 kg/cm^2/min into the 2.0 ATA chamber including 100% O_2 and maintained for 60 min. The chamber was flushed with 100% O_2 at a rate of 5 l/min to avoid CO_2 accumulation and decompression was performed at 0.2 kg/cm^2/min. During HBO exposure, O_2 and CO_2 content were continuously monitored and maintained at ≥98% and ≤0.03%, respectively, while the chamber temperature was maintained between 22 and 25˚C. To minimize the effects of diurnal variation, HBO exposure was initiated at approximately 8 am and 4 pm. Additionally, in the SH, IR3d and IR5d groups, rats were treated with normobaric air in a 1.0 ATA chamber with 21% O_2 at an ambient temperature of 22-25˚C, postoperatively.

Flap measurements. Evaluation of the skin flaps occurred on the third and fifth day postoperatively. The survival area of the skin flaps was determined based on appearance, color and texture. Viability of the flaps was calculated using Image-Pro Plus software (version 6.0, Media Cybernetics LP, Silver Spring, MD, USA). The results were expressed as percentages relative to the total flap surface area.

Histological analysis. The skin flaps were evaluated on the third and fifth day following surgery. For each skin flap, 3-4 mm-sectioned tissue blocks from viable regions were fixed in 10% formalin and embedded in paraffin for hematoxylin and eosin staining. Microvessel density (MVD) was measured by two double-blinded pathologists using the 'hot spot' method. Briefly, five areas with the highest accumulation of small vessels were identified under a low magnification (x100) in each tissue section. These areas were then examined under a high magnification (x400) and microvessels were counted in five high-power fields. Blood vessels with muscular layers were excluded.

Immunohistochemical staining. Monoclonal antibody analysis was performed on the third and fifth day following surgery. For each skin flap, sectioned tissue blocks (3-4 mm) obtained from the viable portion of the flap were preserved in 10% formalin. Tissue sections (4 μm) were obtained and embedded in paraffin for immunohistological analysis. The monoclonal antibodies anti-SDF-1 (1:100) and anti-CXCR4 (1:500) (both from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), were used for immunohistochemical staining. The proportion of positive cells in at least 10 areas in each section were analyzed under a high magnification (x400) by two double-blinded pathologists.

Protein preparation. Skin flap tissues were frozen in liquid nitrogen and then stored at -80˚C until analysis. The tissues were homogenized in ice-cold isolation solution containing 250 mmol/l sucrose, 10 mmol/l triethanolamine, 1 μg/ml leupeptin and 0.1 mg/ml phenylmethylsulfonyl fluoride. Homogenates were centrifuged at 15,000 x g for 10 min at 4˚C to separate the incomplete homogenized tissue. The supernatants were obtained and the protein concentrations were measured using a protein assay kit (Beijing Sunbio Biotech Co., Ltd., Haidian, China). For deglycosylation of proteins, an N-glycosidase F Deglycosylation Kit (Roche Diagnostics GmbH, Mannheim, Germany) was used.

Western blot analysis. Total proteins (50 μg/sample) were diluted in 5X loading buffer (0.25 mol/l Tris HCl, pH 6.8; 10% sodium dodecyl sulfate; 0.5% bromophenol blue; 50% glycerol; and 0.5 mol/l dithiothreitol) and then heated to boiling point for 5 min. Sodium dodecyl sulfate-polyacrylamide

gel electrophoresis was carried out on 12% gradient gels. The proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes that had been previously treated with methanol and blocked for 1 h at room temperature in TBST (Tris-buffered saline containing 0.1% Tween-20) containing 5% non-fat dry milk. PVDF membranes were then incubated overnight at 4˚C with anti-SDF-1 (1:100) and anti-CXCR4 antibodies (1:500) (Santa Cruz Biotechnology, Inc.) in TBST containing 5% nonfat dry milk. Membranes were washed in TBST and incubated with horseradish peroxidase-labeled anti-rabbit antibody (1:3,000, Santa Cruz Biotechnology, Inc.) for 2-3 h at room temperature. Blots were developed with enhanced chemiluminescence agents (ECL Plus, Beijing Sunbio Biotech Co. Ltd.) prior to being exposed to X-ray. To confirm equivalent loading of samples, the same membranes were incubated with anti-β-actin antibody (1:300, Santa Cruz Biotechnology, Inc.) and visualized using ECL, as mentioned earlier. For quantification, western blotting films were scanned using a Minolta scanner (Konica Minolta, Inc.) and Adobe Photoshop software. The labeling density was quantitated using LabWorks software (UVP Inc., Upland, CA, USA). The value of the relative density of SDF-1 and CXCR4 bands was normalized to the density of actin to represent the expression of SDF-1 and CXCR4 proteins.

Statistical analysis. Statistical analysis was performed using SPSS software, version 15.0 (SPSS, Chicago, IL, USA). Quantitative data were expressed as the mean ± standard deviation. One-way analysis of variance procedures were used to test the differences in MVD, SDF-1, CXCR4 and the skin flap survival area. The t-test was used to determine the differences in skin flap survival between the HBO3d and HBO5d groups. Relationships between MVD and expression of SDF-1 and CXCR4 were analyzed by calculating the Pearson product-moment correlation coefficient. P<0.05 was considered to indicate a statistically significant difference.

Results

Flap measurements. The regions of survival and necrosis of skin flaps in different groups were clearly demarcated. The surviving skin appeared pink to white, was tender, normal in texture and bled when cut with a scalpel. By contrast, the necrotic skin was black, rigid, abnormal in texture and did not bleed when cut. Rats in the SH group showed an average viable area of 77.5%, compared with that of the IR3d (43.0%) and IR5d (32.2%) groups. The average viable area was significantly increased in the HBO3d (54.7%) and HBO5d (64.7%) groups compared with that of the IR groups (P<0.01). These results indicate that HBO treatment significantly improved skin flap survival. Additionally, when the number of HBO treatments increased from 3 to 5 days, the survival area of skin flaps also significantly increased (P<0.05) (Fig. 1).

Flap neovascularization measurements. To evaluate whether neovascularization varied among the different groups, we measured MVD in the skin flaps and found that MVD was significantly increased in the groups treated with HBO (HBO3d, 8.5±2.9 and HBO5d, 10.0±2.9), compared with that of the IR groups (IR3d, 5.5±1.8 and IR5d, 3.8±1.3) (Fig. 2).

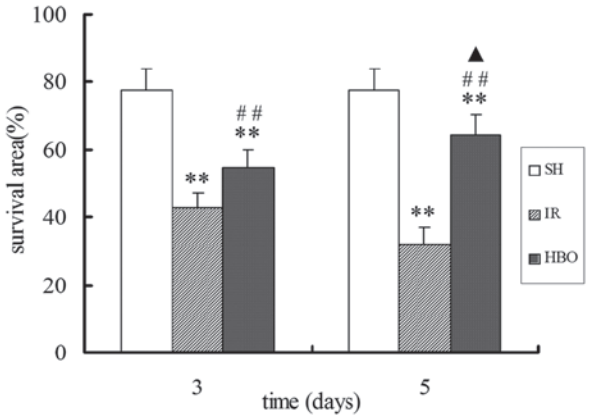

Figure 1. Viable area of the skin flaps was compared in each group. Flap survival in the IR and HBO groups was significantly lower than that in the SH group (**P<0.01). Regardless of the number of HBO treatments, flap survival was significantly lower in the IR groups compared with the HBO groups (##P<0.01). Significant flap survival was demonstrated in the HBO5d group when compared with HBO3d group (▲P<0.05). IR, ischemia reperfusion; HBO, hyperbaric oxygen; SH, sham-operated.

These results demonstrate that HBO treatment increased neovascularization in the skin flaps of rats.

Immunohistochemical staining of SDF-1 and CXCR4. Immunohistochemical staining showed that cells were positive for brown particles in the cell membrane, cytoplasm and nucleus. The expression of SDF-1 and CXCR4 in the IR groups was higher than that in the SH group (P<0.05 and P<0.01, respectively). Moreover, the expression of SDF-1 and CXCR4 in the HBO treatment groups was significantly higher than that in the IR groups (P<0.01) and the SH group (P<0.01) (Figs. 3 and 4). These results suggest that HBO treatment significantly increased the expression of SDF-1 and CXCR4 in the skin flaps of rats.

Western blot analysis of SDF-1 and CXCR4. Western blot analysis identified that the expression of SDF-1 proteins in the HBO3d (0.69±0.17) and HBO5d groups (0.81±0.10) was significantly increased compared with that in the IR3d (0.47±0.12) and IR5d groups (0.41±0.11) (P<0.05 and P<0.01, respectively). The expression of CXCR4 proteins in the HBO3d (0.67±0.16) and HBO5d groups (0.84±0.17) was also significantly increased compared with that in the IR3d (0.41±0.13) and IR5d groups (0.38±0.09) (P<0.01). The expression of SDF-1 and CXCR4 in the HBO groups was significantly higher than that in the SH group (SDF-1, 0.33±0.11; CXCR4, 0.31±0.08) (P<0.01) (Fig. 5). These results support the previous findings that HBO treatment significantly increased the expression of SDF-1 and CXCR4 proteins in the skin flaps of rats.

Positive correlation between neovascularization and SDF-1 and CXCR4 expression. Pearson's correlation analysis demonstrated that at the protein level, there was a positive correlation between neovascularization (MVD) and the expression of SDF-1 and CXCR4 in the skin flaps of rats treated with HBO (Table I).

Table I. Pearson's correlation analysis between neovascularization (MVD) and SDF-1 and CXCR4 expression in the skin flap of rats (n=6 per group) treated with HBO.

Factors	Immunohistochemical staining				Immunoblot analysis			
	3 days		5 days		3 days		5 days	
MVD	SDF-1	CXCR4	SDF-1	CXCR4	SDF-1	CXCR4	SDF-1	CXCR4
R	0.851[a]	0.838[a]	0.914[a]	0.835[a]	0.879[a]	0.821[a]	0.829[a]	0.866[a]
P	0.032	0.037	0.011	0.039	0.021	0.045	0.041	0.026

[a]P<0.05, compared with P. MVD, microvessel density

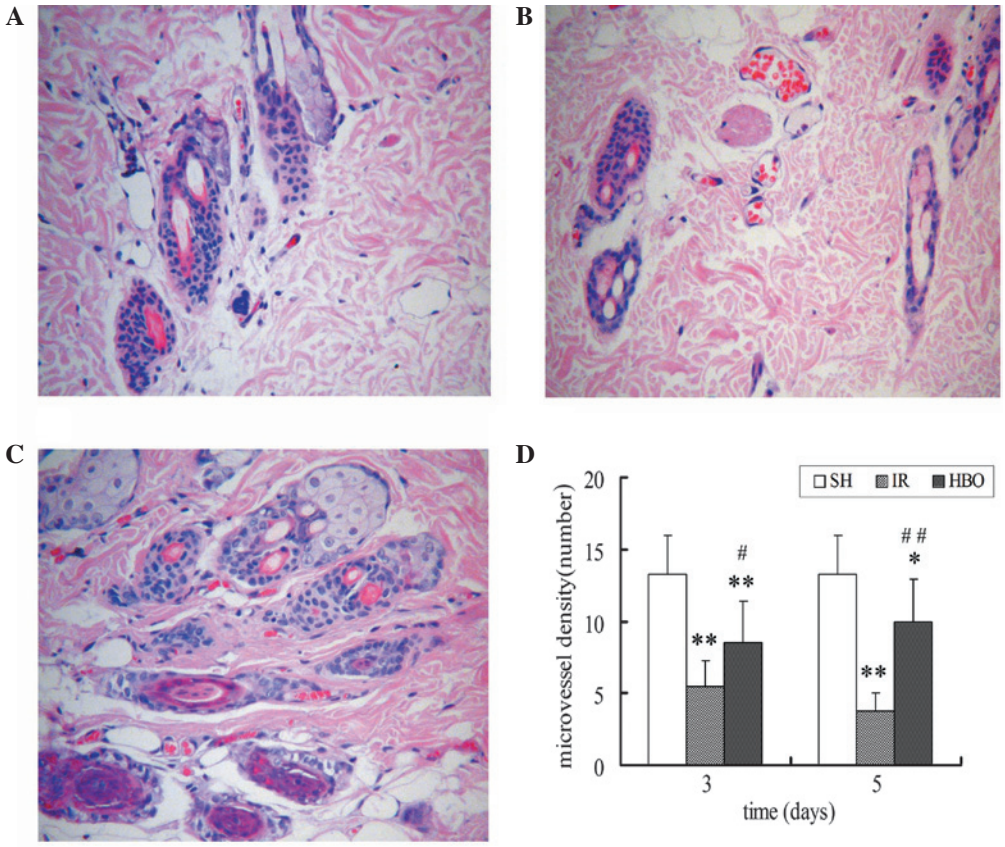

Figure 2. Vascularization in the skin flaps of rats. MVD was defined as the total number of microvessels divided by the number of microvessels observed under a high-power field microscope (magnification, x400) in the (A) IR5d, (B) HBO5d and (C) SH groups. (D) MVD in the IR and HBO groups was significantly lower than that in the SH group (**P<0.01 and *P<0.05). Compared with the same day IR groups, MVD was significantly increased in the HBO groups ($^{\#\#}$P<0.01 and $^{\#}$P<0.05). MVD, microvessel density; IR, ischemia reperfusion; HBO, hyperbaric oxygen; SH, sham-operated.

Discussion

Improving the survival rate of skin flaps is one of the main aims in plastic surgery. A number of studies have demonstrated the beneficial effects of HBO treatment on improving survival of compromised skin grafts and flaps, including the venous flap, the tubed pedicle flap, the composite graft and the random pattern skin flap (10,11,13-15). However, the detailed mechanisms involved are not fully understood. In the present study, we aimed to characterize the effects of HBO treatment on neovascularization and skin flap survival, as well as investigate its mechanism at a molecular level by analyzing the expression of angiogenic mediators, such as SDF-1 and CXCR4 in the skin flaps of rats.

Observations by Ju *et al* (16) showed that HBO treatment may increase angiogenesis of the expanded skin and subsequently increase skin flap survival. HBO treatment increases neovascularization through angiogenic stimulation, resulting in blood vessel formation from local endothelial cells, and by stimulating systemic stem/progenitor cells to differentiate into blood vessels (17). At present, MVD assessment is one of the most reliable methods of measuring angiogenic activity (18). Consistent with previous studies, the present study measured MVD in skin flap biopsies and findings indicated that MVD

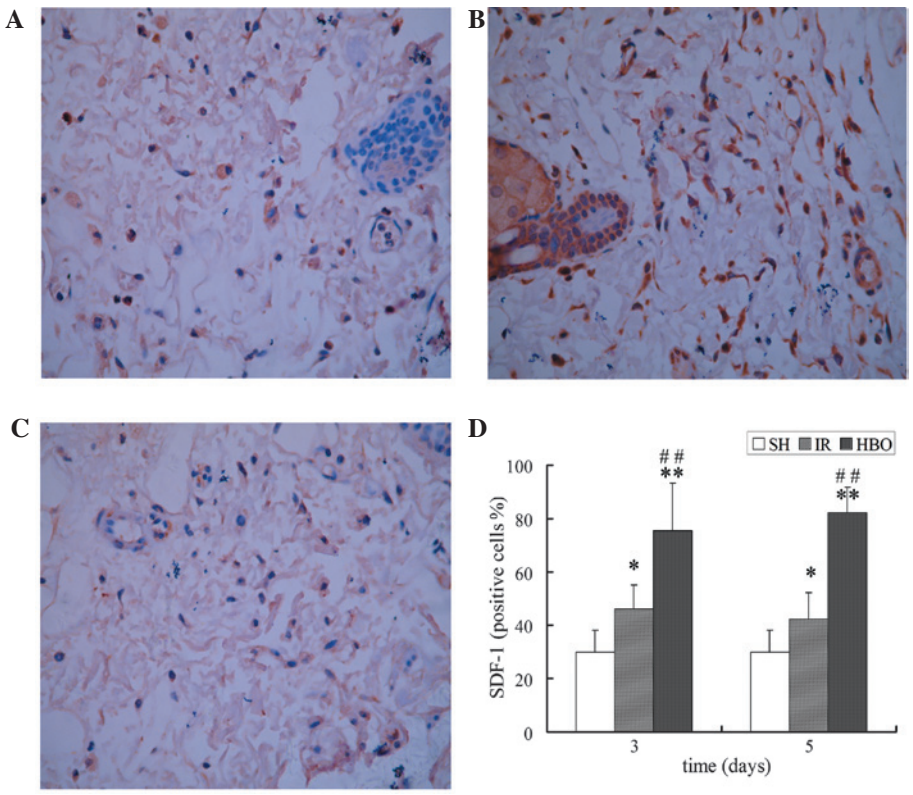

Figure 3. Immunohistochemical staining of SDF-1 in each group. The proportion of positive cells was analyzed under a high magnification (x400) in the (A) IR5d, (B) HBO5d and (C) SH groups. (D) SDF-1-positive cells in the IR and HBO groups were significantly higher than those in the SH group (*P<0.05 and **P<0.01). Compared with the same day IR groups, SDF-1-positive cells were significantly increased in the HBO groups ($^{\#\#}$P<0.01). IR, ischemia reperfusion; HBO, hyperbaric oxygen; SH, sham-operated.

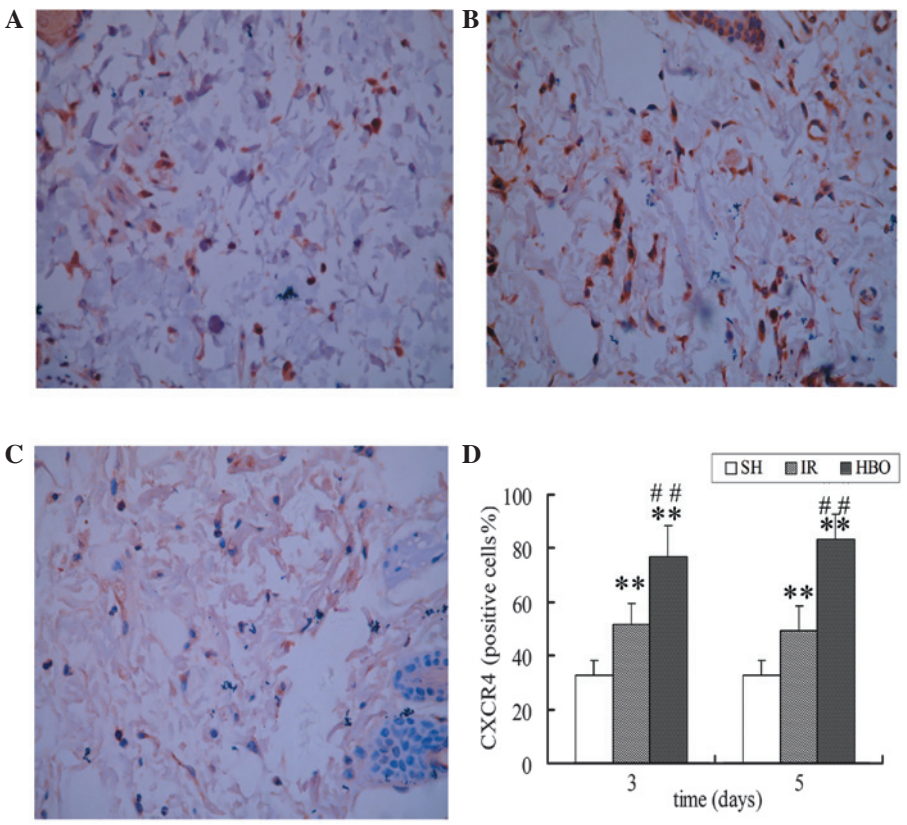

Figure 4. Immunohistochemical staining of CXCR4 in each group. The proportion of positive cells were analyzed under a high magnification (x400) in the (A) IR5d, (B) HBO5d and (C) SH groups. (D) CXCR4-positive cells in the IR and HBO groups were significantly higher than those in the SH group (**P<0.01). Compared with the same day IR groups, CXCR4-positive cells were significantly increased in the HBO groups ($^{\#\#}$P<0.01). IR, ischemia reperfusion; HBO, hyperbaric oxygen; SH, sham-operated.

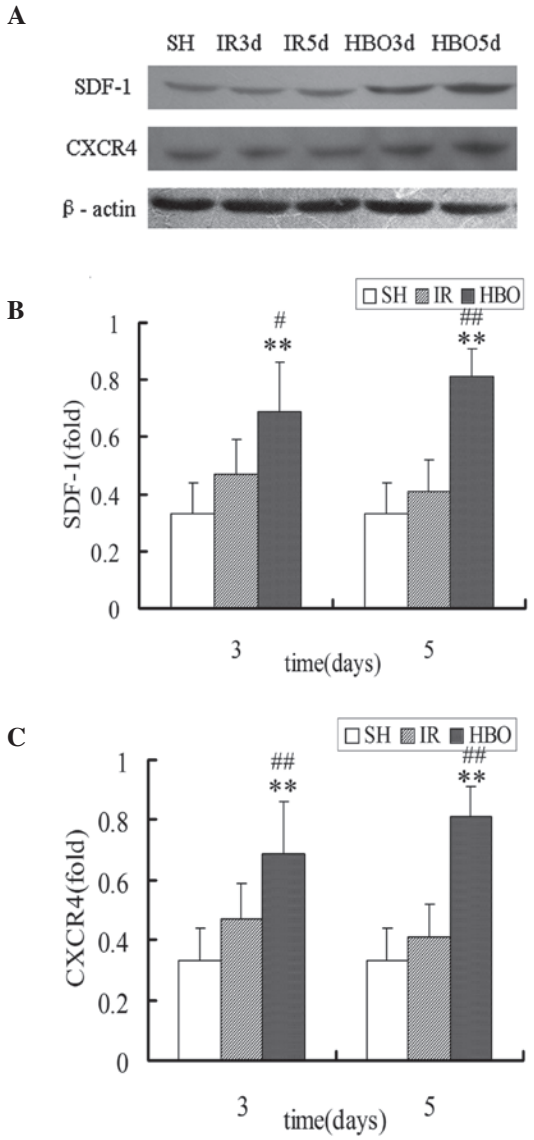

Figure 5. (A) Immunoblot analysis of SDF-1 and CXCR4 in each group. Western blot analysis of (B) SDF-1 and (C) CXCR4 in the skin flap tissue of each group. Data are presented as the mean ± standard deviation. **P<0.01, HBO groups versus SH group; compared with the same day IR groups, SDF-1 and CXCR4 were significantly increased in the HBO groups ($^{\#\#}$P<0.01 and $^{\#}$P<0.05). HBO, hyperbaric oxygen; SH, sham-operated; IR, ischemia reperfusion.

was significantly increased in the HBO treatment groups compared with that of the IR groups. These findings demonstrate that HBO treatment increased neovascularization in the skin flaps. To the best of our knowledge, this study analyzed for the first time the concentration of SDF-1 and CXCR4 in the skin flaps of rats, as well as the effects of HBO treatment on the expression of SDF-1 and CXCR4. We found that postoperative administration of HBO resulted in a high expression of SDF-1 and CXCR4 in skin flaps. In addition, Pearson's correlation analysis indicated a positive correlation between neovascularization and the expression of SDF-1 and CXCR4 in the skin flaps of rats treated with HBO. The results of this study provide a theoretical basis for broadening the clinical application of HBO treatment.

In the present study, the expression of SDF-1 and CXCR4 in the IR groups was higher than that in the SH group, and compared with that of the IR groups, the expression of SDF-1 and CXCR4 was much higher in the HBO groups. Hypoxia initiates neovascularization by inducing angiogenic factor expression, but cannot sustain it. A threshold level of oxygenation is required to support the metabolic needs of tissue remodeling. Acute hypoxia facilitates the angiogenic process (19), while chronic hypoxia impairs wound angiogenesis (20). Sustained hypoxia results in the dysfunction of tissues and cell death. SDF-1 is upregulated in hypoxic or ischemic tissues, such as arteries (21) and brain (22). Moreover, hyperoxia induces the expression of SDF-1 and CXCR4 via the upregulation of VEGF expression (23,24). In addition, Salvucci *et al* (25) demonstrated that endothelial expression of SDF-1 and CXCR4 may be reduced by inflammatory cytokines, such as tumor necrosis factor-α and interferon-γ. As HBO treatment inhibits these inflammatory cytokines, the expression of SDF-1 and CXCR4 may be upregulated by HBO through the inhibition of inflammatory cytokines. It is well known that bone marrow (BM)-derived endothelial progenitor cell (EPC) is a key cell involved in neovascularization and homes to peripheral tissue in response to ischemia (26). Endothelial nitric oxide synthase (eNOS) is essential in the BM microenvironment and increased BM NO levels results in the mobilization of EPCs from BM niches into circulation, ultimately allowing their participation in tissue-level vasculogenesis and wound healing (27,28). Induction of hyperoxia via HBO treatment has been shown to increase NO levels in perivascular tissues and BM via a NOS-mediated mechanism, resulting in EPC release from the BM and an increase in EPCs in tissues (29-31). EPCs themselves express and secrete SDF-1 and CXCR4 (32), thus following HBO treatment, the expression of SDF-1 and CXCR4 may be increased. The effects of HBO treatment on the expression of SDF-1 and CXCR4 were supported in the present study.

Ghadge *et al* (33) indicated that manipulating SDF-1 and its receptor CXCR4 in ischemic cardiac disease is beneficial for neovascularization and tissue repair. Li *et al* (34) indicated that SDF-1 may induce neovascularization potentially via the SDF-1/CXCR4 axis following traumatic brain injury. Blocking the SDF-1/CXCR4 axis resulted in reduced homing of cells to lesions and decreased MVD after ischemic heart disease (35). These findings suggest that the SDF-1/CXCR4 axis is important in blood vessel growth and development. In the present study, the Pearson's correlation analysis demonstrated a positive correlation between neovascularization and the expression of SDF-1 and CXCR4 in the skin flaps of rats. There may be various mechanisms involved in the SDF-1/CXCR4 axis governing neovascularization. In addition to its role in recruiting EPCs to ischemic sites (36), SDF-1 directly participates in blood vessel formation. SDF-1/CXCR4 has an angiogenic effect on endothelial cells by inducing cell proliferation, differentiation, sprouting and tube formation *in vitro* and by preventing apoptosis of EPCs (37). SDF-1 also modulates vascularization of ischemic tissues and tumors by improving the secretion of other angiogenic factors, such as VEGF-A, interleukin-6 (IL-6), IL-8, tissue inhibitor of metalloproteinase-2, and decreasing the production of the anti-angiogenic molecules such as angiostatin (38,39).

In conclusion, the present study established a modified epigastric pedicle skin flap model in rats. Data of this study

indicate that HBO treatment promoted neovascularization and increased the survival of skin flaps. In addition, the expression of SDF-1 and CXCR4 was upregulated in the skin flaps of rats treated with HBO. Pearson's correlation analysis demonstrated a positive correlation between neovascularization and the high expression of SDF-1 and CXCR4. These results suggest that the beneficial effects of HBO treatment in promoting neovascularization may be explained by the upregulation of SDF-1 and CXCR4 expression in the skin flaps of rats.

References

1. Kryger Z, Zhang F, Dogan T, Cheng C, Lineaweaver WC and Buncke HJ: The effects of VEGF on survival of a random flap in the rat: examination of various routes of administration. Br J Plast Surg 53: 234-239, 2000.
2. Lu WW, Ip WY, Jing WM, Holmes AD and Chow SP: Biomechanical properties of thin skin flap after basic fibroblast growth factor (bFGF) administration. Br J Plast Surg 53: 225-229, 2000.
3. de Souza Filho MV, Loiola RT, Rocha EL, Simão AFL and Ribeiro RA: Remote ischemic preconditioning improves the survival of rat random-pattern skin flaps. Eur J Plast Surg 33:147-152, 2010 (In Spanish).
4. Ulkür E, Karagoz H, Ergun O, Celikoz B, Yildiz S and Yildirim S: The effect of hyperbaric oxygen therapy on the delay procedure. Plast Reconstr Surg 119: 86-94, 2007.
5. Hamon M, Mbemba E, Charnaux N, et al: A syndecan-4/CXCR4 complex expressed on human primary lymphocytes and macrophages and HeLa cell line binds the CXC chemokine stromal cell-derived factor-1 (SDF-1). Glycobiology 14: 311-323, 2004.
6. Ratajczak MZ, Zuba-Surma E, Kucia M, Reca R, Wojakowski W and Ratajczak J: The pleiotropic effects of the SDF-1-CXCR4 axis in organogenesis, regeneration and tumorigenesis. Leukemia 20: 1915-1924, 2006.
7. Hassan S, Ferrario C, Saragovi U, et al: The influence of tumor-host interactions in the stromal cell-derived factor-1/CXCR4 ligand/receptor axis in determining metastatic risk in breast cancer. Am J Pathol 175: 66-73, 2009.
8. Tysseling VM, Mithal D, Sahni V, et al: SDF1 in the dorsal corticospinal tract promotes CXCR4+ cell migration after spinal cord injury. J Neuroinflammation 8: 16, 2011.
9. Jones SR, Carpin KM, Woodward SM, Khiabani KT, Stephenson LL, Wang WZ and Zamboni WA: Hyperbaric oxygen inhibits ischemia-reperfusion-induced neutrophil CD18 polarization by a nitric oxide mechanism. Plast Reconstr Surg 126: 403-411, 2010.
10. Richards L, Lineaweaver WC, Stile F, Zhang J and Zhang F: Effect of hyperbaric oxygen therapy on the tubed pedicle flap survival in a rat model. Ann Plast Surg 50: 51-56, 2003.
11. Zhang T, Gong W, Li Z, et al: Efficacy of hyperbaric oxygen on survival of random pattern skin flap in diabetic rats. Undersea Hyperb Med 34: 335-339, 2007.
12. Lubiatowski P, Goldman CK, Gurunluoglu R, Carnevale K and Siemionow M: Enhancement of epigastric skin flap survival by adenovirus-mediated VEGF gene therapy. Plast Reconstr Surg 109: 1986-1993, 2002.
13. Yücel A and Bayramiçli M: Effects of hyperbaric oxygen treatment and heparin on the survival of unipedicled venous flaps: an experimental study in rats. Ann Plast Surg 44: 295-303, 2000.
14. Fodor L, Ramon Y, Meilik B, Carmi N, Shoshani O and Ullmann Y: Effect of hyperbaric oxygen on survival of composite grafts in rats. Scand J Plast Reconstr Surg Hand Surg 40: 257-260, 2006.
15. Metselaar M, Dumans AG, van der Huls MP, Sterk W and Feenstra L: Osteoradionecrosis of tympanic bone: reconstruction of outer ear canal with pedicled skin flap, combined with hyperbaric oxygen therapy, in five patients. J Laryngol Otol 123: 1114-1119, 2009.
16. Ju Z, Wei J, Guan H, Zhang J, Liu Y and Feng X: Effects of hyperbaric oxygen therapy on rapid tissue expansion in rabbits. J Plast Reconstr Aesthet Surg 65: 1252-1258, 2012.
17. Thom SR: Hyperbaric oxygen: its mechanisms and efficacy. Plast Reconstr Surg 127: S131-S141, 2011.
18. Zhang Y, Zhao H, Zhao D, et al: SDF-1/CXCR4 axis in myelodysplastic syndromes: correlation with angiogenesis and apoptosis. Leuk Res 36: 281-286, 2012.
19. Semenza GL: HIF-1: using two hands to flip the angiogenic switch. Cancer Metastasis Rev 19: 59-65, 2000.
20. Allen DB, Maguire JJ, Mahdavian M, et al: Wound hypoxia and acidosis limit neutrophil bacterial killing mechanisms. Arch Surg 132: 991-996, 1997.
21. Shiba Y, Takahashi M, Yoshioka T, et al: M-CSF accelerates neointimal formation in the early phase after vascular injury in mice: the critical role of the SDF-1-CXCR4 system. Arterioscler Thromb Vasc Biol 27: 283-289, 2007.
22. Schönemeier B, Schulz S, Hoellt V and Stumm R: Enhanced expression of the CXCI12/SDF-1 chemokine receptor CXCR7 after cerebral ischemia in the rat brain. J Neuroimmunol 198: 39-45, 2008.
23. Sheikh AY, Gibson JJ, Rollins MD, Hopf HW, Hussain Z and Hunt TK: Effect of hyperoxia on vascular endothelial growth factor levels in a wound model. Arch Surg 135: 1293-1297, 2000.
24. Hong X, Jiang F, Kalkanis SN, et al: SDF-1 and CXCR4 are up-regulated by VEGF and contribute to glioma cell invasion. Cancer Lett 236: 39-45, 2006.
25. Salvucci O, Basik M, Yao L, Bianchi R and Tosato G: Evidence for the involvement of SDF-1 and CXCR4 in the disruption of endothelial cell-branching morphogenesis and angiogenesis by TNF-alpha and IFN-gamma. J Leukoc Biol 76: 217-226, 2004.
26. Yamaguchi J, Kusano KF, Masuo O, et al: Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization. Circulation 107: 1322-1328, 2003.
27. Aicher A, Heeschen C, Mildner-Rihm C, et al: Essential role of endothelial nitric oxide synthasefor mobilization of stem and progenitor cells. Nat Med 9: 1370-1376, 2003.
28. Murohara T, Asahara T, Silver M, et al: Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. J Clin Invest 101: 2567- 2578, 1998.
29. Thom SR, Fisher D, Zhang J, et al: Stimulation of perivascular nitric oxide synthesis by oxygen. Am J Physiol Heart Circ Physiol 284: H1230-H1239, 2003.
30. Goldstein LJ, Gallagher KA, Bauer SM, et al: Endothelial progenitor cell release into circulation is triggered by hyperoxia-induced increases in bone marrow nitric oxide. Stem Cells 24: 2309-2318, 2006.
31. Gallagher KA, Liu ZJ, Xiao M, et al: Diabetic impairments in NO-mediated endothelial progenitor cell mobilization and homing are reversed by hyperoxia and SDF-1 alpha. J Clin Invest 117: 1249-1259, 2007.
32. Urbich C, Aicher A, Heeschen C, Dernbach E, Hofmann WK, Zeiher AM and Dimmeler S: Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. J Mol Cell Cardiol 39: 733-742, 2005.
33. Ghadge SK, Mühlstedt S, Ozcelik C and Bader M: SDF-1α as a therapeutic stem cell homing factor in myocardial infarction. Pharmacol Ther 129: 97-108, 2011.
34. Li S, Wei M, Zhou Z, Wang B, Zhao X and Zhang J: SDF-1α induces angiogenesis after traumatic brain injury. Brain Res 1444: 76-86, 2012.
35. Dai S, Yuan F, Mu J, et al: Chronic AMD3100 antagonism of SDF-1alpha-CXCR4 exacerbates cardiac dysfunction and remodeling after myocardial infarction. J Mol Cell Cardiol 49: 587-597, 2010.
36. Rosenkranz K, Kumbruch S, Lebermann K, Marschner K, Jensen A, Dermietzel R and Meier C: The chemokine SDF-1/CXCL12 contributes to the 'homing' of umbilical cord blood cells to a hypoxic-ischemic lesion in the rat brain. J Neurosci Res 88: 1223-1233, 2010.
37. Zheng H, Dai T, Zhou B, Zhu J, Huang H, Wang M and Fu G: SDF-1alpha/CXCR4 decreases endothelial progenitor cells apoptosis under serum deprivation by PI3K/Akt/eNOS pathway. Atherosclerosis 201: 36-42, 2008.
38. Wang J, Wang J, Sun Y, Song W, Nor JE, Wang CY and Taichman RS: Diverse signaling pathways through the SDF-1/CXCR4 chemokine axis in prostate cancer cell lines leads to altered patterns of cytokine secretion and angiogenesis. Cell Signal 17: 1578-1592, 2005.
39. Wang J, Wang J, Dai J, et al: A glycolytic mechanism regulating an angiogenic switch in prostate cancer. Cancer Res 67: 149-159, 2007.