Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 302 (2003) 138–143
BBRC
www.elsevier.com/locate/ybbrc

Facilitated angiogenesis induced by heme oxygenase-1 gene transfer in a rat model of hindlimb ischemia

Masatoshi Suzuki,[a] Naoyuki Iso-o,[b] Satoshi Takeshita,[c] Kazuhisa Tsukamoto,[b] Ichiro Mori,[d] Tomohide Sato,[c] Minoru Ohno,[a] Ryozo Nagai,[a] and Nobukazu Ishizaka[a,*]

[a] *Department of Cardiovascular Medicine, University of Tokyo Graduate School of Medicine, Bunkyo-ku Hongo 7-3-1, Tokyo 113-8655, Japan*
[b] *Department of Diabetes and Metabolic Disease, University of Tokyo Graduate School of Medicine, Bunkyo-ku Hongo 7-3-1, Tokyo 113-8655, Japan*
[c] *Department of Medicine, Teikyo University School of Medicine, Itabashi-ku Kaga 2-11-1, Tokyo 173-8606, Japan*
[d] *Department of Pathology, Wakayama Medical University, Wakayama-city Kimiidera 811-1, Wakayama 641-8509, Japan*

Received 15 January 2003

Abstract

Heme oxygenase-1 (HO-1) is an inducible form of heme oxygenase that catabolizes heme to carbon monoxide, biliverdin, and ferrous iron. We have investigated whether HO-1 can induce angiogenic effects in vivo. Rats were subjected to a bolus injection of either wild type adenovirus (ad-wt) or adenovirus encoding HO-1 (ad-HO-1) through the right femoral artery, which was then removed immediately. HO-1 gene transfer resulted in about a sixfold increase in HO-1 protein levels as compared to the non-treated animals. The increase in both blood flow and capillary density was significantly greater in the ischemic hindlimbs that had been injected with ad-HO-1 than in those injected with ad-wt. These angiogenic effects of ad-HO-1 infection could be completely abolished by treating the animals with the HO inhibitor, zinc protoporphyrin, indicating that they were specifically due to the expression of HO-1. Thus, HO-1 gene transfer improves the blood flow in ischemic hindlimb, at least in part, via angiogenesis facilitated by the induction of this molecule.

Keywords: Angiogenesis; Ischemia; Carbon monoxide; Ferritin; Adenovirus; Gene transfer

The enzyme heme oxygenase catabolizes heme to carbon monoxide, biliverdin, and ferrous iron. Heme oxygenase-1 (HO-1) is an inducible form of heme oxygenase that is known to be induced by various types of oxidants and by physiological stress. Carbon monoxide shares several similar characteristics with another gaseous molecule, nitric oxide [1–7]. Recent studies have shown that nitric oxide may modulate angiogenesis in response to tissue ischemia [8] and that nitric oxide may upregulate the angiogenic factor, vascular endothelial growth factor (VEGF) in vascular cells [9]. Interestingly, carbon monoxide may also have a role in the expression of VEGF [10] and an in vitro study has shown that transferring the HO-1 gene into endothelial cells promotes the formation of blood vessels [4]. Malaguarnera and colleagues [11] have shown that HO-1 may modulate prolactin-mediated angiogenesis. Furthermore, Nishie and colleagues [12] have reported that the expression of HO-1 correlates with angiogenesis in human gliomas. Collectively, these findings suggest that the HO-1 pathway may play a role in angiogenesis in some pathological conditions. Here we have investigated the effect of HO-1 gene transfer in a rat model of hindlimb ischemia.

Materials and methods

Construction of recombinant adenoviruses. Second generation recombinant adenovirus constructs were constructed and purified as described previously [13]. Wild type adenovirus, adenovirus encoding a rat HO-1 (a gift from Dr. S. Shibahara, Tohoku University School of Medicine, Japan), and adenovirus encoding an *Escherichia coli* lacZ were designated as ad-wt, ad-HO-1, and ad-lacZ, respectively. Viruses were amplified in 293 cells and concentrated by centrifugation through a CsCl gradient.

* Corresponding author. Fax: +81-3-3974-2236.
E-mail address: nobuishizka-tky@umin.ac.jp (N. Ishizaka).

0006-291X/03/$ - see front matter © 2003 Elsevier Science (USA). All rights reserved.
doi:10.1016/S0006-291X(03)00114-1

Rat ischemic hindlimb model and intra-arterial adenovirus gene delivery. A rat model of hindlimb ischemia was induced as described previously [14]. Briefly, male Sprague–Dawley rats (160–180 g) (Saitama Animal Supply, Saitama, Japan) were anesthetized with pentobarbital (50 mg/kg i.p.) and a longitudinal incision, extending inferiorly from the inguinal ligament to a point just proximal to the patella, was made. Through the proximal portion of the femoral artery, 10^{11} particles of ad-HO-1 in 500 μL of phosphate buffered saline were injected over 5–10 s, while simultaneous obstruction of venous outflow was carried out by temporary occlusion of the femoral vein. In control animals, the same titer of ad-lacZ or ad-wt was infused through the femoral artery. Immediately after the injection of virus, the right femoral artery was completely excised. To examine the localization of transgene expression, hindlimb muscles were harvested 3 days after ad-lacZ gene injection and X-Gal staining was done as described previously [15,16].

Analysis of capillary density. Microscopic angiogenesis was evaluated in terms of capillary density by light-microscopy of sections taken from the ischemic hindlimb as described previously [17]. Unless otherwise stated, the animals were killed 28 days after the operation, and tissue specimens were obtained as transverse sections from the gastrocnemius and the anterior tibial muscles of the ischemic hindlimb. Frozen tissue sections (5 μm in thickness) were first stained for alkaline phosphatase using the indoxyl-tetrazolium method to detect capillary endothelial cells and were then counterstained with eosin. A total of 20 randomly selected fields from one muscle section were examined under a 20× objective to determine the capillary density and are expressed as the mean number of capillaries per 1 mm^2.

Protein purification and immunoblot analysis. Protein was isolated by homogenizing samples in the lysis buffer (50 mmol/L Hepes, 5 mmol/L EDTA, and 50 mmol/L NaCl; pH 7.5) containing protease inhibitors (10 μg/mL aprotinin, 1 mmol/L PMSF, and 10 μg/mL leupeptin) [18]. Polyclonal antibodies against rat HO-1 (StressGen, Victoria, BC, Canada) and monoclonal antibodies against VEGF (Santa Cruz Biotechnology, CA, USA) were used at a 1/1000 dilution. Band intensity is expressed as the fold increase over the control value.

Laser Doppler perfusion image. Blood flow in both ischemic (right) and nonischemic (left) hindlimbs was measured using a laser Doppler perfusion imager system (PIM-II; Lisca Development AB, Stockholm, Sweden). The perfusion signal was subdivided into six different intervals, each displayed in a separate color from dark blue (low or no perfusion) to red (very high perfusion). The stored perfusion values behind the color-coded pixels were used for analysis. The blood flow in ischemic limb was normalized to that of the contralateral non-ischemic hindlimb to minimize variables including ambient light and temperature. Perfusion analyses were done sequentially before, immediately after, and 2–28 days after the surgery. Some rats were given a daily intraperitoneal injection of the HO inhibitor zinc-protoporphyrin (ZnPP; Porphyrin Products, Logan, UT, USA) at a concentration of 50 μmol/kg/day, starting two days before and continuing until 7 days after the operation.

Statistical analysis. Data are expressed as the means ± SEM. ANOVA followed by a multiple comparison test was used to compare raw data before they were converted to a percentage of the control value using the statistical analysis software, Statistica ver. 5.1J for Windows (StatSoft Inc., Tulsa, OK, USA). A value of $p < 0.05$ was considered to be statistically significant.

Results and discussion

Assessment of localization and semi-quantification of the expression of transgenes

First, X-gal staining of the hindlimb 3 days after the bolus intra-arterial injection of ad-lacZ was performed to verify the integrity of the gene transfer method. X-gal staining was found to be positive in the myofibers, capillaries (Figs. 1A–C), arterioles/venules, and perivascular cells, presumably fibroblasts (Figs. 1D), which suggested that gene transfer to these cells had been successful. The validity of this assessment was supported by the fact that no positive staining was seen in hindlimb muscles injected with ad-HO-1-injected (Figs. 1E and F). Next, expression of HO-1 protein at various time points after the adenovirus-mediated gene transfer and the simultaneous induction of ischemia were assessed semi-quantitatively by Western blot analysis. Immunoblot analysis showed a robust increase in HO-1 expression that peaked 2 days after surgery in rats infected with ad-HO-1 (Figs. 1G and H). Although HO-1 expression also increased slightly 2 days after surgery in control rats infected with ad-wt, the extent was significantly smaller than that in the ad-HO-1 infected rats.

We employed an intra-arterial approach for delivering the adenovirus vector into hindlimb because we found that this method resulted in a greater efficiency of transgene expression than did direct intramuscular adenovirus injection (see Fig. 5), in agreement with the findings of other investigators [19,20]. We previously found that pressurization of the arterial wall during adenovirus-mediated gene transfer markedly augments the efficacy of gene delivery [21,22]. Thus, in the present study, we simultaneously obstructed venous outflow by the temporary occlusion of the femoral vein at the time of intra-arterial adenovirus injection in an attempt to increase the transgene efficacy. Because HO-1 expression was increased also in the ad-wt-infected muscle, this may indicate that hypoxia itself can increase the expression of HO-1 in some tissues, possibly by a mechanism involving hypoxia-inducible factor-1 [23].

Laser Doppler flow analysis after adenovirus mediated HO-1 gene transfer

Laser Doppler blood flow analysis was performed immediately before and after, and subsequently at 2, 14, and 28 days after the induction of hindlimb ischemia. Both the ad-HO-1-infected and the ad-wt-infected hindlimb muscles showed progressive recovery of blood perfusion (Fig. 2); however, the recovery of blood flow was found to be significantly greater in the hindlimb of ad-HO-1 infected rats than in control infected animals (Fig. 2).

Analysis of capillary density

To determine whether the facilitated recovery in hindlimb perfusion that seen in the ad-HO-1-infected hindlimb was attributable to the angiogenic effects of HO-1, we analyzed the capillary density of the hindlimb muscle at 28 days after the surgery and concomitant

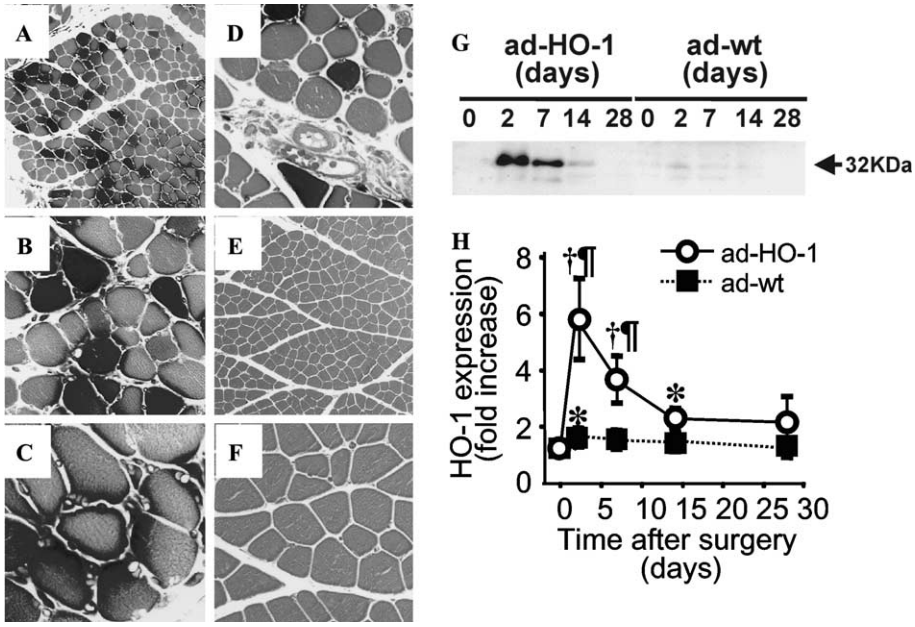

Fig. 1. Gene expression after adenovirus-mediated gene transfer in the ischemic hindlimb. (A–F) X-Gal staining of the rat hindlimb muscle 3 days after adenovirus-mediated gene transfer. Rats were subjected to intra-arterial injection of either ad-lacZ (A–D) or ad-wt (E,F) at a dose of 10^{11} particles per rat, followed by immediate removal of the femoral artery. Positive X-Gal staining is observed in myofibers in ad-lacZ-injected muscle, but not in ad-wt-injected muscle. At higher magnification, positive X-Gal staining can be seen not only in myofibers (B) and capillaries (C), but also in venous veins (D). Original magnifications are 250× (A and E) and 800× (B–D, and F). (G,H) Time course of HO-1 expression in the hindlimb after gene transfer and immediate induction of ischemia. (G) Representative immunoblot. (H) Summary of the data from four to six animals for each time point. Although expression of HO-1 increased slightly in the ad-wt-infected hindlimb muscle slightly increased in some animals, this difference did not reach statistical significance. $^{*}p < 0.05$ and $^{\dagger}p < 0.01$ versus the value at day 0, i.e., before injection. Values are means ± SEM. $^{\P}p < 0.05$ versus values of ad-wt-infected animals at the same time point after adenovirus gene transfer.

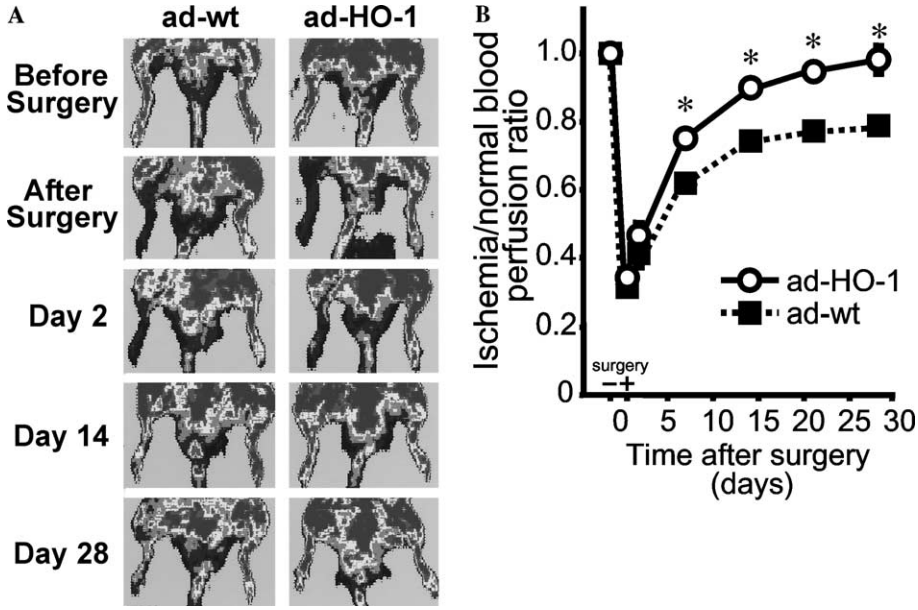

Fig. 2. Time course of hindlimb blood flow recovery after administration of adenovirus followed by immediate removal of the femoral artery. Measurements of hindlimb blood flow were performed with laser Doppler blood-flow analysis and have been normalized to blood flow in the normal hindlimb. (A) Representative laser Doppler perfusion imaging recorded at the indicated days after injection of either ad-wt or ad-HO-1. (B) Line graph summarizing data from 10 to 12 different animals for each time point. Values are means ± SEM. $^{*}p < 0.05$ versus values of ad-wt-infected animals at each time point after adenovirus gene transfer.

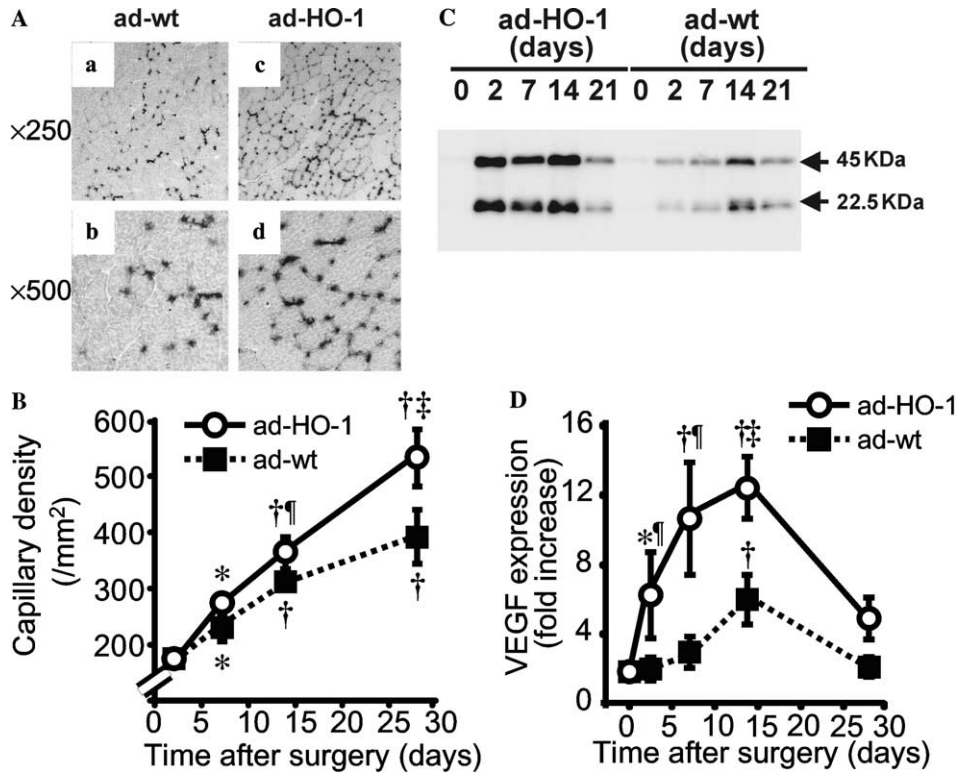

Fig. 3. Capillary density measurement and VEGF expression after adenovirus mediated gene transfer. (A,B) Capillary density analysis. (a) Alkaline phosphatase staining of the frozen section of the hindlimb muscles at 28 days after adenovirus infection followed by immediate induction of ischemia. (a,b) ad-wt-infected muscle. (c,d) ad-HO-1-infected muscle. Original magnifications are 250× (a and c), and 500× (b and d). (B) Bar graph summarizing the data from six animals in each group. Values are means ± SEM. $*p < 0.05$ versus values of ad-wt-infected animals. (C,D) Time course of VEGF expression after gene transfer. A representative immunoblot. (B) Summary of the data from four to six animals for each time point. Although HO-1 expression increased slightly in the ad-wt-infected hindlimb muscle in some animals, this difference did not reach statistical significance as compared with control levels. Values are means ± SEM. $*p < 0.05$ and $^{\ddagger}p < 0.01$ versus the value at day 0, i.e., before injection. $^{\P}p < 0.05$ and $^{\dagger}p < 0.01$ versus values of ad-wt-infected animals at the same time point after adenovirus gene transfer.

gene transfer. The capillary density in the hindlimb of ad-HO-1-injected rats was significantly greater than that in the hindlimb of the ad-wt-injected animals (Figs. 3A and B), which suggests that ad-HO-1 can increase blood flow in the hindlimb, at least in part, by promoting angiogenesis. As HO-1 may induce VEGF in some conditions [24,25], we also investigated the expression of VEGF after gene transfer. VEGF protein was increased in the ad-HO-1-infected ischemic hindlimb and peaked 14 days after the surgery. Although VEGF protein was also increased in the hindlimb of rats infected with ad-wt, the increase was significantly smaller than that in rats infected with ad-HO-1 (Figs. 3C and D). This observation is consistent with the finding that limb ischemia itself can increase local expression of VEGF [26].

Effect of HO inhibitor on ad-HO-1-induced increase in blood flow and capillary formation in the hindlimb

To examine whether the increase in hindlimb blood flow seen in ad-HO-1-infected animals was mediated by the specific actions of HO-1, we investigated the effect of an HO inhibitor, ZnPP, on the ad-HO-1 induced facilitated recovery of blood flow. Administration of ZnPP had abolished the facilitated increase in hindlimb blood flow induced by infection with ad-HO-1 at both 7 days (Fig. 4A) and 21 days (Fig. 4B) after the induction of ischemia and concomitant gene transfer. In addition, ZnPP treatment also abolished the increase in capillary density facilitated by ad-HO-1 infection at both 14 days (Fig. 4C) and 28 days (Fig. 4D). These data indicate that the facilitated increase in blood flow and capillary density observed in ischemic hindlimb infected with ad-HO-1 is mediated specifically by HO-1.

Effect of HO-1 gene transfer on the blood flow of the non-ischemic hindlimb

Finally, we investigated whether HO-1 gene transfer could also increase blood flow in non-ischemic hindlimb. As the femoral artery was not removed in this experiment, either ad-HO-1 or ad-wt was injected intramuscularly into the non-ischemic hindlimb of the rats. Gene transfer of ad-HO-1, but not ad-wt, significantly increased expression of HO-1 protein (about 3.5-fold) at 2 days after gene transfer (Fig. 5A). Laser Doppler blood

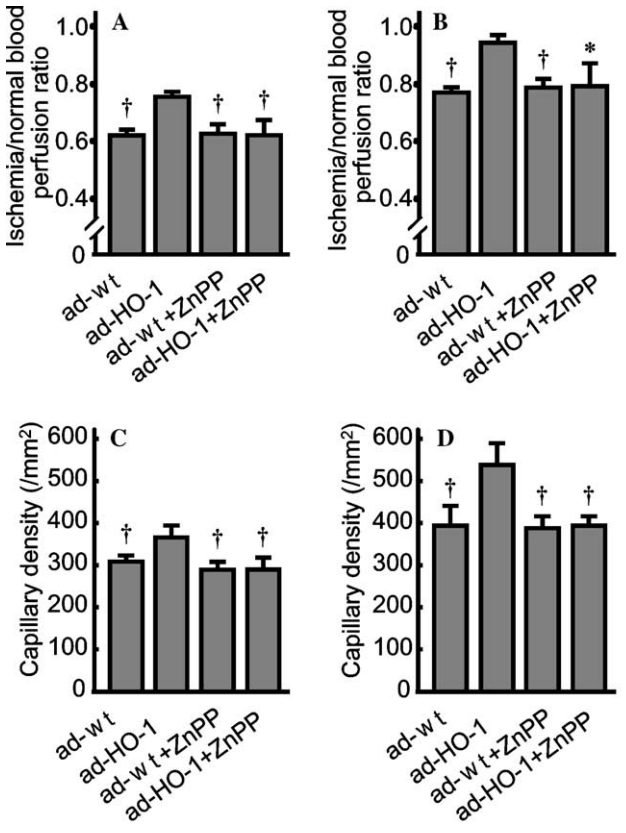

Fig. 4. Effect of the HO inhibitor, zinc protoporphyrin (ZnPP), on the ad-HO-1-induced increase in blood flow and in capillary formation in the ischemic hindlimb. (A,B) Effect of ZnPP on blood flow. Blood flow was measured at 7 days (A) and 21 days (B) after adenovirus gene transfer. Values have been normalized to blood flow in the normal hindlimb and are the mean ± SEM. $*p < 0.05$ and $^{\dagger}p < 0.01$ versus values of ad-HO-1-infected animals that had not received ZnPP treatment. (C,D) Effect of ZnPP on capillary density. Capillary density was measured at 14 days (C) and 28 days (D) after adenovirus gene transfer. Values are means ± SEM. $*p < 0.05$ and $^{\dagger}p < 0.01$ versus values of ad-HO-1-infected animals that had not received ZnPP treatment.

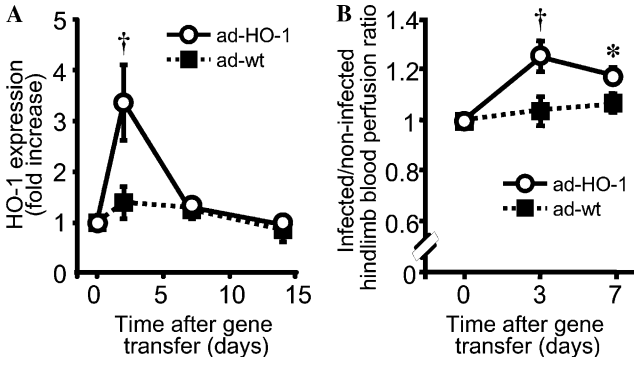

Fig. 5. Effect of ad-HO-1 infection on the non-ischemic hindlimb. (A) Time course of HO-1 expression. Summary of the data from four to six animals for each time point. (B) Result of laser Doppler perfusion imaging recorded at the indicated days after adenovirus gene transfer. (B) Line graph summarizing data from 10 to 12 different animals for each time point. Values have been normalized to blood flow in the non-infected hindlimb and are means ± SEM. In (A) and (B) $*p < 0.05$ and $^{\dagger}p < 0.01$ versus values of ad-wt-infected animals at the same time point.

flow analysis was performed on days 3 and 7 after gene transfer, and blood flow in the hindlimb that had undergone gene transfer was normalized to the blood flow in the contralateral hindlimb. Gene transfer of ad-HO-1, but not ad-wt, significantly increased blood flow in the non-ischemic hindlimb (Fig. 5B). However, capillary density was not increased in the ad-HO-1-infected hindlimb (97% of the ad-wt-infected hindlimb, day 14 after gene transfer, $n = 4$ in each group) as compared to the ad-wt-infected hindlimb; thus, this effect was considered not to be due to the angiogenic effects of HO-1.

In the present study, adenovirus-mediated HO-1 gene transfer into the ischemic hindlimb facilitated a significant recovery of blood flow in the hindlimb; this effect was, at least in part, due to an increase in the capillary density and, thus, to angiogenic effects of HO-1. There are several possible pathways by which activating the HO system may be linked to the process of angiogenesis. First, bilirubin, an HO-mediated degradation product of heme, is a strong scavenger of peroxyl radicals [27], which may have an anti-angiogenic effect [28]. Alternatively, induction of HO-1 may increase expression of the angiogenic growth factor, VEGF, as was indeed observed in the present study (Figs. 3C and D); expression of VEGF can be mediated by another heme-catabolizing product, carbon monoxide [10]. Interestingly, a recent study has shown that carbon monoxide may increase the release of nitric oxide in microvessels [29]; therefore, a third possibility is that ad-HO-1 infection promotes angiogenesis by increasing the release of nitric oxide, although this possibility was not examined in the present study.

In the final set of experiments, we showed that ad-HO-1 gene transfer could also potently increase the blood flow in the non-ischemic hindlimb, and this phenomenon was not accompanied by an increase in the capillary density as compared to the ad-wt-infected hindlimb. This increase in blood flow may be caused by the vasodilatory action of carbon monoxide [30]; however, this possibility remains to be investigated in future studies.

In summary, the intra-arterial administration of an adenovirus encoding HO-1 at the time of the induction of ischemia of hindlimb facilitated an improvement in the blood flow of the ischemic hindlimb. This was at least partly mediated through an increase in capillary density; in other words, expression of HO-1 facilitated angiogenesis. As HO-1 is a member of the heat shock protein family and is readily induced by various stimuli such as oxidative stress, the induction of local HO-1 expression represents a potential therapeutic target for the treatment of limb ischemia.

References

[1] F.M. Ho, S.H. Liu, C.S. Liau, P.J. Huang, S.G. Shiah, S.Y. Lin-Shiau, Nitric oxide prevents apoptosis of human endothelial cells

from high glucose exposure during early stage, J. Cell. Biochem. 75 (1999) 258–263.
[2] S. Brouard, L.E. Otterbein, J. Anrather, E. Tobiasch, F.H. Bach, A.M. Choi, M.P. Soares, Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis, J. Exp. Med. 192 (2000) 1015–1026.
[3] M. Ziche, A. Parenti, F. Ledda, P. Dell'Era, H.J. Granger, C.A. Maggi, M. Presta, Nitric oxide promotes proliferation and plasminogen activator production by coronary venular endothelium through endogenous bFGF, Circ. Res. 80 (1997) 845–852.
[4] B.M. Deramaudt, S. Braunstein, P. Remy, N.G. Abraham, Gene transfer of human heme oxygenase into coronary endothelial cells potentially promotes angiogenesis, J. Cell. Biochem. 68 (1998) 121–127.
[5] L. Xue, G. Farrugia, S.M. Miller, C.D. Ferris, S.H. Snyder, J.H. Szurszewski, Carbon monoxide and nitric oxide as coneurotransmitters in the enteric nervous system: evidence from genomic deletion of biosynthetic enzymes, Proc. Natl. Acad. Sci. USA 97 (2000) 1851–1855.
[6] J.L. Wallace, N. Vergnolle, M.N. Muscara, S. Asfaha, K. Chapman, W. McKnight, P. Del Soldato, A. Morelli, S. Fiorucci, Enhanced anti-inflammatory effects of a nitric oxide-releasing derivative of mesalamine in rats, Gastroenterology 117 (1999) 557–566.
[7] L.E. Otterbein, F.H. Bach, J. Alam, M. Soares, H. Tao Lu, M. Wysk, R.J. Davis, R.A. Flavell, A.M. Choi, Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway, Nat. Med. 6 (2000) 422–428.
[8] T. Murohara, T. Asahara, M. Silver, C. Bauters, H. Masuda, C. Kalka, M. Kearney, D. Chen, J.F. Symes, M.C. Fishman, P.L. Huang, J.M. Isner, Nitric oxide synthase modulates angiogenesis in response to tissue ischemia, J. Clin. Invest. 101 (1998) 2567–2578.
[9] J. Dulak, A. Jozkowicz, A. Dembinska-Kiec, I. Guevara, A. Zdzienicka, D. Zmudzinska-Grochot, I. Florek, A. Wojtowicz, A. Szuba, J.P. Cooke, Nitric oxide induces the synthesis of vascular endothelial growth factor by rat vascular smooth muscle cells, Arterioscler. Thromb. Vasc. Biol. 20 (2000) 659–666.
[10] A. Jozkowicz, I. Huk, A. Nigisch, G. Weigel, F. Weidinger, J. Dulak, Effect of prostaglandin-J(2) on VEGF synthesis depends on the induction of heme oxygenase-1, Antioxid. Redox Signal 4 (2002) 577–585.
[11] L. Malaguarnera, M.R. Pilastro, S. Quan, M.H. Ghattas, L. Yang, A.V. Mezentsev, T. Kushida, N.G. Abraham, A. Kappas, Significance of heme oxygenase in prolactin-mediated cell proliferation and angiogenesis in human endothelial cells, Int. J. Mol. Med. 10 (2002) 433–440.
[12] A. Nishie, M. Ono, T. Shono, J. Fukushi, M. Otsubo, H. Onoue, Y. Ito, T. Inamura, K. Ikezaki, M. Fukui, T. Iwaki, M. Kuwano, Macrophage infiltration and heme oxygenase-1 expression correlate with angiogenesis in human gliomas, Clin. Cancer Res. 5 (1999) 1107–1113.
[13] K. Tsukamoto, P. Smith, J.M. Glick, D.J. Rader, Liver-directed gene transfer and prolonged expression of three major human ApoE isoforms in ApoE-deficient mice, J. Clin. Invest. 100 (1997) 107–114.
[14] S. Takeshita, T. Isshiki, H. Mori, E. Tanaka, K. Eto, Y. Miyazawa, A. Tanaka, Y. Shinozaki, K. Hyodo, M. Ando, M. Kubota, K. Tanioka, K. Umetani, M. Ochiai, T. Sato, H. Miyashita, Use of synchrotron radiation microangiography to assess development of small collateral arteries in a rat model of hindlimb ischemia, Circulation 95 (1997) 805–808.
[15] M. Suzuki, N. Ishizaka, K. Tsukamoto, K. Minami, J. Taguchi, R. Nagai, M. Ohno, Pressurization facilitates adenovirus-mediated gene transfer into vein graft, FEBS Lett. 470 (2000) 370–374.
[16] Y. Yang, F.A. Nunes, K. Berencsi, E.E. Furth, E. Gonczol, J.M. Wilson, Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy, Proc. Natl. Acad. Sci. USA 91 (1994) 4407–4411.
[17] S. Takeshita, L.P. Zheng, E. Brogi, M. Kearney, L.Q. Pu, S. Bunting, N. Ferrara, J.F. Symes, J.M. Isner, Therapeutic angiogenesis. A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model, J. Clin. Invest. 93 (1994) 662–670.
[18] T. Aizawa, N. Ishizaka, J. Taguchi, R. Nagai, I. Mori, S.S. Tang, J.R. Ingelfinger, M. Ohno, Heme oxygenase-1 is upregulated in the kidney of angiotensin II-induced hypertensive rats: possible role in renoprotection, Hypertension 35 (2000) 800–806.
[19] W.G. Feero, J.D. Rosenblatt, J. Huard, S.C. Watkins, M. Epperly, P.R. Clemens, S. Kochanek, J.C. Glorioso, T.A. Partridge, E.P. Hoffman, Viral gene delivery to skeletal muscle: insights on maturation-dependent loss of fiber infectivity for adenovirus and herpes simplex type 1 viral vectors, Hum. Gene Ther. 8 (1997) 371–380.
[20] G. Acsadi, A. Jani, B. Massie, M. Simoneau, P. Holland, K. Blaschuk, G. Karpati, A differential efficiency of adenovirus-mediated in vivo gene transfer into skeletal muscle cells of different maturity, Hum. Mol. Genet. 3 (1994) 579–584.
[21] W.K. Cho, S. Ebihara, J. Nalbantoglu, R. Gilbert, B. Massie, P. Holland, G. Karpati, B.J. Petrof, Modulation of Starling forces and muscle fiber maturity permits adenovirus-mediated gene transfer to adult dystrophic(mdx) mice by the intravascular route, Hum. Gene Ther. 11 (2000) 701–714.
[22] M. Suzuki, N. Ishizaka, W.M. Hwang, K. Tsukamoto, K. Minami, T. Aizawa, I. Mori, J. Taguchi, R. Nagai, M. Ohno, Application of hydrostatic pressure facilitates ex vivo adenovirus gene transfer into rabbit aortas, Biochem. Biophys. Res. Commun. 274 (2000) 422–426.
[23] P.J. Lee, B.H. Jiang, B.Y. Chin, N.V. Iyer, J. Alam, G.L. Semenza, A.M. Choi, Hypoxia-inducible factor-1 mediates transcriptional activation of the heme oxygenase-1 gene in response to hypoxia, J. Biol. Chem. 272 (1997) 5375–5381.
[24] D. Kreiser, X. Nguyen, R. Wong, D. Seidman, D. Stevenson, S. Quan, N. Abraham, P.A. Dennery, Heme oxygenase-1 modulates fetal growth in the rat, Lab. Invest. 82 (2002) 687–692.
[25] J. Dulak, A. Jozkowicz, R. Foresti, A. Kasza, M. Frick, I. Huk, C.J. Green, O. Pachinger, F. Weidinger, R. Motterlini, Heme oxygenase activity modulates vascular endothelial growth factor synthesis in vascular smooth muscle cells, Antioxid. Redox Signal 4 (2002) 229–240.
[26] F. Luo, D. Wariaro, G. Lundberg, H. Blegen, E. Wahlberg, Vascular growth factor expression in a rat model of severe limb ischemia, J. Surg. Res. 108 (2002) 258–267.
[27] R. Stocker, Y. Yamamoto, A.F. McDonagh, A.N. Glazer, B.N. Ames, Bilirubin is an antioxidant of possible physiological importance, Science 235 (1987) 1043–1046.
[28] H. Sauer, J. Gunther, J. Hescheler, M. Wartenberg, Thalidomide inhibits angiogenesis in embryoid bodies by the generation of hydroxyl radicals, Am. J. Pathol. 156 (2000) 151–158.
[29] C. Thorup, C.L. Jones, S.S. Gross, L.C. Moore, M.S. Goligorsky, Carbon monoxide induces vasodilation and nitric oxide release but suppresses endothelial NOS, Am. J. Physiol. 277 (1999) F882–889.
[30] F. Coceani, L. Kelsey, E. Seidlitz, G.S. Marks, B.E. McLaughlin, H.J. Vreman, D.K. Stevenson, M. Rabinovitch, C. Ackerley, Carbon monoxide formation in the ductus arteriosus in the lamb: implications for the regulation of muscle tone, Br. J. Pharmacol. 120 (1997) 599–608.