Protective roles of endogenous carbon monoxide in neointimal development elicited by arterial injury

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Am. J. Physiol. Heart Circ. Physiol. 278: H623–H632, 2000.—We reported that carbon monoxide (CO) generated through heme oxygenase (HO) inhibits mitogen-induced proliferation of vascular smooth muscle cells (VSMCs). We report that balloon injury induces HO-1, the stress-inducible isozyme of HO, in VSMCs and inhibits neointimal formation through the action of endogenous CO. Northern blot analysis and immunohistochemistry revealed that HO-1 is markedly induced in the media as early as 1 day after injury, whereas only a little expression was detected in the intact carotid artery. The neointimal proliferative changes were augmented or inhibited by the HO inhibitors or inducer, respectively, and effects of these interventions were not altered by suppression of endogenous nitric oxide (NO), if any. To elucidate the mechanisms by which HO controls the proliferative changes, effects of alterations in the HO reaction were examined by determining angiotensin II-elicited VSMC proliferation in vitro: the HO inducer attenuated and its inhibitor restored the proliferative response to angiotensin II (1 nM and 100 nM). Hemoglobin, a reagent trapping both NO and CO, but not met-hemoglobin, which can capture NO but not CO, augmented the proliferative response. These data suggest that endogenous CO serves as a protective factor that limits the excessive VSMC proliferation associated with vascular diseases.

heme oxygenase; balloon injury; vascular smooth muscle cell; proliferation; atherosclerosis

The accumulation of vascular smooth muscle cells (VSMCs) in neointima resulting from the migration and proliferation of medial VSMCs in response to endothelial damage is believed to be one of the main events involved in the initiation of atherosclerosis. Although various types of growth factor and cytokines, including endothelin-1 (ET-1), platelet-derived growth factor-B, and angiotensin II (ANG II) have been acknowledged to contribute generally to the development of atherosclerosis, recent studies have indicated that many species of oxidants can be considered to be early growth signals (1, 6, 30).

Heme oxygenase (HO) is the rate-limiting enzyme for heme degradation in mammals (17, 35). It decomposes heme into biliverdin and releases free iron and carbon monoxide (CO). Three isoforms of HO have been identified (17, 20, 34). HO-1 is an inducible form that is transcriptionally upregulated by a variety of chemical and physiological stress-inducing factors such as heavy metals (19, 34), hydrogen peroxide (12), heat shock (32), cytokines (2), hypoxia (24), and its substrate heme (39). On the other hand, HO-2 is constitutively expressed, but its distribution is more widespread. HO-3 has recently been identified and codes a protein of ~33 kDa and exist in the heart, kidney, brain, and liver, whereas its heme-catalyzing activity is reported to be smaller than that of other isozymes (20).

Recently, we demonstrated that cultured VSMCs expressed both HO-1 and HO-2 and released CO into their conditioned media (24). Furthermore, VSMC-derived CO was found to suppress VSMC proliferation through the inhibition of ET-1, platelet-derived growth factor-B, and the E2F-1 gene, in a manner similar to nitric oxide (NO) (22, 23). It is thus not unreasonable to hypothesize that induction of HO could lead to inhibition of excessive VSMCs proliferation in atherosclerotic lesions through the biological action of CO as a reaction product.

In view of potential roles of HO in pathophysiological conditions, this study was aimed to examine perturbation of HO expression in the carotid artery and its functional consequence on VSMC proliferative responses after denudation by balloon injury. We have also attempted to address whether such inhibitory effects of the HO-1 induction on VSMC proliferation are ascribable to the biological action of endogenous CO.

METHODS

Animals. Male Sprague-Dawley rats (400–450 g) were obtained from Charles River, housed in a temperature-controlled room at 26°C with automatic lighting that provided a 12:12-h on-off cycle, and allowed free access to rat chow and tap water.

Balloon injury. Each rat was anesthetized with an intraperitoneal injection of 40 mg/kg pentobarbital sodium, and endothelial denudation of the carotid artery was performed by three passages of a Fogarty 2-Fr balloon catheter inflated to 2 atm (measured by manometer) as described elsewhere (3).
After removal of the catheter, the left external carotid artery was ligated completely with no flow and the wound closed. At the required time after balloon injury, rats were euthanized with a lethal dose of pentobarbital sodium, and their vascular systems were perfused via the left ventricle with phosphate-buffered saline (PBS) for 5 min at 100 mmHg and then fixed with neutral formaldehyde for 10 min at 100 mmHg. Histological examinations. The left common carotid arteries were removed, and three vessel rings (5 mm long) were cut and embedded in paraffin. Sections of each 5-mm ring were cut and stained with hematoxylin and eosin, as well as Azan-Mallory stain. Histological micrographs were captured and processed digitally by a computer-assisted eight-bit image analyzer (Power Macintosh 8800/NIH Image 1.58), and cross-sectional areas of medial and neointimal areas were quantified by the software at three times, and the mean values of the area of interests were calculated for each section.

HO-1 and HO-2 were detected in tissues using monoclonal antibodies against rat HO-1 and HO-2 (8). Briefly, sections were trypsinized with 1% vol/vol trypsin in PBS at 37°C for 60 min and washed with PBS. Nonspecific protein binding was blocked with 0.1% vol/vol horse normal serum at room temperature for 30 min. The sections were then incubated overnight at 4°C with the required monoclonal antibody, and the bound primary antibody was detected using an avidin-biotinylated horseradish peroxidase complex (Vectastain ABC kits, Vector Laboratories, Burlingame, CA). Finally, the tissues were lightly counterstained with hematoxylin. Positive staining with 3,3′-diaminobenzidine tetrahydrochloride appeared as a brownish-black color. The staining of uninjured portions of the external carotid artery of each rat was used as a control.

Treatment of rats. Hemin, an inducer of HO-1, was dissolved in DMSO as described previously (13). ZnPP was dissolved in 50 mM Na2CO3 solution as described previously (19). N‘-nitro-L-arginine (L-NNA) was used to block production of NO in vivo. To this end, the reagent was dissolved in drinking water to give a final dose of 60 mg kg−1·day−1 and was given for 50 days before and until 14 days after balloon injury (11). L-NNA-un-treated or -treated rats were treated with intraperitoneal injection of hemin (15 mg/kg), ZnPP (40 µmol/kg), or SnPP (50 µmol/kg) every other day from 3 days before until 14 days after balloon injury (n = 8–10 rats per group). Control rats were treated with equivalent volumes of vehicles.

Cell culture. Primary cultures of rat aortic VSMCs were grown in Dulbecco’s modified Eagle’s medium (GIBCO, Grand Island, NY) with 10% vol/vol newborn calf serum, passed every 3–4 days as described previously (20), and used between five and nine passages. When the culture reached a 70% confluence, the medium was changed to Dulbecco’s modified Eagle’s medium with supplemented 0.2% vol/vol newborn calf serum, and the cells were cultured for an additional 48 h before the experiment.

Cell proliferation. Cell proliferation was assessed by counting cells 48 h after exposure to ANG II (1 and 100 nM), and in the presence or absence of reagents, the cells were washed twice with ice-cold PBS, harvested, and centrifuged. The cells pellets were resuspended in ice-cold PBS, and the cells were counted with a Coulter counter (Coulter, Hialeah, FL). The values are shown as percentages of the number versus that of control VSMC at the start of culture (n = 8).

RNA analysis. Total tissue RNA was prepared from carotid arteries by guanidinium isothiocyanate extraction from carotid artery, and 15 µg lane were separated by electrophoresis on 1% wt/vol agarose gels containing formaldehyde and transferred to nitrocellulose membranes by blotting. The filters were subsequently hybridized with cDNA probes specific for rat HO-1 and HO-2 (24). The cDNA fragments were labeled with (α-32P)dCTP using a standard random-primed reaction to a specific activity of 1–2 × 106 cpm/µg. The membranes were hybridized for 2 h at 68°C in QuickHyb solution (Stratagene, La Jolla, CA) with 2 × 106 cpm/ml of probe, washed twice in 2× saline sodium citrate containing 0.1% SDS at 60°C for 30 min, and then exposed to film (X-OMat AR; Eastman Kodak, Rochester, NY) with intensifying screens at −80°C. The membranes were subsequently stripped and rehybridized with 32P-labeled mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. For quantitation, we scanned autoradiographs with a laser densitometer (Ultrascan XL; LKB Instruments, Bromma, Sweden) using the Gel Scan XL software package (Pharmacia LKB Biotechnology, Piscataway, NJ).

Determination of HO activity. HO activity in microsomes was determined in rat carotid artery at different time points after balloon injury and compared with the activity in control rats (n = 8–12 for each group). Briefly, microsomes were prepared by ultracentrifugation. The microsomal fraction was resuspended in 1 ml of 0.1 mM potassium phosphate buffer, pH 7.4, containing 2 mM MgCl2 and analyzed for HO activity spectrophotometrically, as judged by the formation of bilirubin as described elsewhere (8, 18, 25). The activity was expressed as picomoles (pmol) of bilirubin formed per milligram of protein per hour. The protein concentration was determined by a dye-binding assay (Bio-Rad).

Determination of cGMP levels. Levels of cGMP in extracts from a carotid artery were measured with the use of a commercial ELISA kit (Amersham). Fourteen days after balloon injury, the carotid arteries were harvested from rats. At the end of experiments, samples were immediately frozen in liquid nitrogen and stored at −80°C until the cGMP assay was carried out as described previously (24). Duplicate measurements were performed on all samples (n = 6–8 each group).

Determination of concentrations of NOx in urine. Concentrations of NOx were measured in urine samples collected before and 14 and 28 days after L-NNA treatment in the presence or absence of ZnPP, SnPP, or hemin with a commercial kit (Nitrate/Nitrite assay kit, cat no. 780001, Cayman Chemical). In these experiments, rats were placed in individual metabolic cages for 24 h, and urine samples were collected for assay. The values were normalized by the levels of creatinine in the urine (Creatinine-Testwako assay kit 275–10502, Wako Pure Chemical, Osaka, Japan).

Drug preparation. SnPP and ZnPP were purchased from Porphyrin Products (Logan, UT). All other reagents used were obtained from Sigma, unless otherwise specified. Pure hemoglobin (Hb) was prepared by treatment with excess reducing agent as described previously (8). MethHb was prepared with oxidization of purified Hb as described previously (24). The purity of MethHb used for the current experiment was checked spectrophotometrically, being >90%. L-NNA, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxaline-1-one (ODQ), 8-bromo-cGMP, and clotrimazole were prepared for cell proliferation assay as described elsewhere (23, 27, 33). All of these reagents were dissolved at desired concentrations on the day of the experiment.

Data analysis. Significant differences were determined by one-way ANOVA, and P < 0.05 was considered statistically significant.
RESULTS

Expression of HO in normal carotid artery. Expression of HO-1 or HO-2 in normal carotid arteries was examined by Northern blot analysis as well as by immunohistochemistry, using specific monoclonal antibodies against these isozymes. Northern blot analysis revealed low levels of HO-1 transcripts, whereas HO-2 mRNA expression was demonstrated clearly, in the normal carotid artery (Fig. 1, left). Immunohistochemistry showed distribution of the HO isozyme proteins in the vascular wall. As shown in Fig. 1, right, the HO-1 expression was detectable only slightly in the media of the rat normal carotid artery, whereas HO-2 expression in the normal tissue was evident in endothelial, medial, and adventitial layers. Distribution of HO-1- and HO-2-positive cells in the media appeared to correspond to that of VSMCs, which were able to be identified immunohistochemically by α-smooth muscle actin (data not shown).

Balloon injury induced HO-1 expression in carotid artery with increase in HO activity. Histological examination of hematoxylin-eosin-stained sections prepared at 14 days after balloon injury revealed marked neointimal formation, which was localized in the site of the injury, validating our procedures (data not shown). Figure 2A shows representative changes in HO gene expression demonstrated with Northern blot analysis after balloon injury. Marked HO-1 mRNA expression was induced as early as 1 day after balloon injury and declined thereafter. In contrast, the levels of HO-2 gene expression did not change as clearly as those of HO-1 during the 14 days after injury. As seen in Fig. 2B, relative mRNA levels of HO-1 and HO-2 normalized to those of GAPDH at the indicated time periods illustrated that the HO-1 gene was increased markedly, whereas the HO-2 gene is not induced significantly by balloon injury. Typical histological assessments of HO isozymes at 1, 4, 7, and 14 days after balloon injury are shown in Fig. 2C. As seen in the top panels of Fig. 2C, the HO-1 expression was very low in the normal artery in medial VSMCs and increased markedly as early as 1 day after injury. By 14 days after injury, the HO-1-associated immunoreactivities were decreased, a similar time history to that of Northern blot analysis, and confined to the neointima. In contrast, the HO-2 immunoreactivities shown in the bottom panels of Fig. 2C in medial VSMCs and adventitial cells did not change after injury, and HO-2 was detected in the intima, its expression increasing in parallel with neointimal development.

To determine whether the injury-induced HO gene could result in an increase in the HO activity, we determined the HO activity in carotid artery from rats subjected to balloon injury. Figure 2D shows the time course of the HO activity in denuded artery at the indicated time periods after balloon injury. Upon balloon injury, the HO activity in the denuded artery significantly increased compared with the control (610 ± 45.2 pmol bilirubin·mg protein⁻¹·h⁻¹); the activity became maximal at 1 day after injury (6,219 ± 75.5 pmol bilirubin·mg protein⁻¹·h⁻¹, P < 0.001) and declined subsequently (14 days; 1,078 ± 89.1 pmol bilirubin·mg protein⁻¹·h⁻¹, P < 0.05). The changes in the HO activity in the denuded artery are essentially similar to those in the HO-1 expression.
Induction of HO suppressed neointimal development. We examined the effects of alterations in the HO activity in the denuded carotid artery by treating rats with ZnPP or SnPP to block or with hemin to induce HO activity further. Figure 3 shows the HO activity in the denuded artery collected at 14 days after balloon injury. The HO activity in the denuded artery of the HO inhibitor-treated rats was significantly suppressed (ZnPP-treated rats, 108 ± 20.2 pmol bilirubin·mg protein⁻¹·h⁻¹, P ≤ 0.001; SnPP-treated rats, 122 ± 31.1 pmol bilirubin·mg protein⁻¹·h⁻¹, P ≤ 0.001), whereas hemin-treated rats showed markedly elevated HO activity (7,531 ± 119.0 pmol bilirubin·mg protein⁻¹·h⁻¹, P = 0.001) compared with control rats (1,078 ± 89.1 pmol bilirubin·mg protein⁻¹·h⁻¹). The hemin treatment elicited a sustained level of the HO-1 mRNA expression for 14 days after balloon injury (Fig. 3B). Immunohistochemistry (Fig. 4) revealed that HO-1 was expressed predominantly in medial VSMCs and neointima (Fig. 4C), and the expression level was greater than that observed in the normal (Fig. 4A) and hemin-untreated rats (Fig. 4B). Figure 5, top, shows the typical neointimal development 14 days after balloon injury in the normal and HO-modulated rat carotid...
synthase activity (16, 21). To eliminate the role of NO on neointimal formation, we therefore performed parallel experiments using rats undergoing the 4-wk L-NNA treatment. L-NNA-treated rats showed a significantly elevated systolic blood pressure (from 99.6 ± 6.7 to 119.2 ± 9.9 mmHg, n = 18, P ≤ 0.05) and lowered concentrations of NOx (from 10.7 ± 0.1 µmol/mg creatinine to 0.3 ± 0.1 µmol/mg creatinine, n = 48, P ≤ 0.05), which means that NO production was successfully blocked. After confirming that NO production was blocked, we subjected the rats to balloon injury in the same procedure as L-NNA-untreated rats in the presence or absence of ZnPP, SnPP, or hemin. Two weeks after balloon injury, we determined concentrations of NOx in the urine of these rats. Treatments with these reagents did not affect concentrations of NOx significantly (L-NNA: 0.4 ± 0.2 µmol/mg creatinine, n = 10; L-NNA ± ZnPP: 0.5 ± 0.2 µmol/mg creatinine, n = 12; L-NNA ± SnPP: 0.3 ± 0.1 µmol/mg creatinine, n = 10; L-NNA ± hemin: 0.4 ± 0.2 µmol/mg creatinine, n = 12). Figure 6A shows the ratio of intima to media in the development of neointima 14 days after balloon injury in L-NNA-treated rats. The HO modulators changed intima-to-media ratio in a similar manner to those observed in L-NNA-untreated rats. The inhibitors of HO significantly increased neointimal formation. On the other hand, the HO inducer decreased the ratio, indicating that effects of the HO modulators are not influenced by endogenous NO in the current experimental conditions. We then investigated whether cGMP levels in a denuded artery are changed by the treatment of HO modulators. Figure 6B shows the changes in cGMP levels in a denuded artery from 4-wk L-NNA-treated rats in the presence or absence of the HO modulators. The HO modulators regulated cGMP levels in a similar manner to those of HO activity; that is, hemin increased but ZnPP and SnPP decreased cGMP levels, respectively. These findings indicate that the induction of HO expression leads to an actual increase in the HO activity and results in cGMP elevation in a denuded artery independently of NO.

To establish further whether HO activity regulates the proliferative response of VSMCs to ANG II, we performed cell proliferation assay, because ANG II production has been reported to be induced in the vascular wall by balloon injury (9, 29). Figure 7A shows the proliferative response of serum-deprived VSMCs to ANG II in the presence of L-NNA at the concentrations previously reported (24), because NO is expected to have a suppressive effect on VSMC proliferation. Incubation with ZnPP or SnPP, which blocks HO activity in VSMCs, enhanced the ANG II-induced increase in VSMCs proliferation significantly. Hb, which captures CO from cultures, also augmented this response in a similar manner to the HO blockers. In contrast, the addition of methHb, which cannot bind to CO, did not stimulate an ANG II-induced increase in VSMCs proliferation significantly. Furthermore, addition of hemin, a potent inducer of HO-1 activity, to the cultures significantly reduced the proliferative response of VSMCs.
Fig. 4. Immunohistological examination of denuded carotid artery 14 days after balloon injury revealed HO-1 protein expression was considerably higher in medial VSMCs and neointima of hemin-treated rat (C) than in those of untreated rat (B) (×400). Hemin sustained elevated HO-1 expression in media 14 days after balloon injury. A: rat normal carotid artery (×400). These data are representative of 6–10 independent experiments.
These findings suggest that the enzymatic product, that is, CO, regulates VSMC proliferation. The observation suggesting that suppression or elevation of CO alters proliferative responses of VSMCs in parallel with cellular cGMP levels led us to examine whether supplements of cGMP could mimic the inhibitory action of CO on the cell proliferation in vitro. As seen in Fig. 7, administration of Hb canceled out and further enhanced the proliferative responses. Application of ODQ mimicked the Hb-induced enhancement of the response, but its effect appeared to be limited. The Hb-elicited changes were suppressed by supplemented 8-bromo-cGMP, but its effect were also limited. These results raised a possibility that the stimulatory effect of the CO scavenger such as Hb involves both cGMP-dependent and -independent mechanisms. Among such cGMP-independent mechanisms for the CO-mediated signaling events, cytochrome P-450 monoxygenase constitutes a putative candidate receptor besides soluble guanylate cyclase, because CO could bind to heme enzymes, which possess ferrous heme as a prosthetic molecule for the enzyme reaction under steady-state conditions (33). We have thus tested the effects of clotrimazole on the Hb-induced changes. This reagent is known to bind the prosthetic heme of cytochrome P-450 monoxygenases and shares the inhibitory action on the enzyme with CO. As seen, pretreatment with the reagent significantly repressed the Hb-induced enhancement of the proliferation. Furthermore, coapplication of this reagent with 8-bromo-cGMP additively suppressed the Hb-induced enhancement of the proliferation, suggesting the presence of cGMP-dependent and -independent mechanisms.

DISCUSSION

In this study, we found that the HO expression patterns of normal and denuded carotid arteries differed. Only little expression of HO-1, an inducible form, was detected, if any, except for a small extent of the expression in the medial VSMC layer, whereas HO-2, a constitutive isozyme of HO, was expressed prominently in the endothelium, medial VSMCs, and adventitial cells of the rat normal carotid artery. As early as 1 day after the balloon injury, the HO-1 expression increased rapidly and site specifically in medial VSMC layers, and then HO-1 relocalized to the neointima in parallel with the development of intimal thickening. On the other hand, the levels of HO-2 gene expression in a
denuded artery determined as a whole tissue did not change significantly throughout 14 days after the injury, suggesting that the transient elevation of the HO activity apparently occurs mainly through upregulation of HO-1. However, careful examination of the regional protein expression by immunohistochemistry revealed that HO-2 became detectable in the neointima formed after the vascular insult, to a similar extent to that of HO-1. Upregulation of protein expression of these HO isozymes is accompanied by the enzyme activity in the same tissue. Considering that CO generated through HO in the extravascular space could lead to alterations in vascular functions such as a reduction of vascular tone (8, 40), it is not unreasonable to
suggest that these newly upregulating HO isozymes contribute to the modulation of proliferative responses in VSMCs. At present, we did not fully assess the role of HO-3 in regulating the local cellular proliferation. However, considering a relative paucity of the heme-catalyzing activity compared with HO-1 and HO-2 (20), the current study suggests that endogenous CO derived mainly from HO-1 and partly from HO-2 functions as an inhibitory regulator preventing excessive VSMC proliferation in the denuded carotid artery.

Balloon injury has been reported to induce the production of many vasoactive factors, including ET-1 (26), renin-angiotensin system (9, 29), and reactive oxygen species (6), resulting in changes in the phenotype of VSMCs. Furthermore, after the denudation of endothelial cells, the VSMC layer is exposed directly to red blood cells in the blood stream, and this may change the shear stress and redox state in the vascular wall. Because HO-1 expression is known to be upregulated by mechanical stress (36), as well as by chemical factors, the rapid induction of HO-1 expression in VSMCs may be mediated by these changes in the vascular wall. We observed that HO-1 expression translocated from the media to the intima during the first week postinjury, and during 14 days it was virtually restricted to the neointima, although expression levels immediately after balloon injury were high in the medial. The time history of the relocalization of HO-1 immunostaining after balloon injury is very similar to that of VSMCs: neointimal formation following balloon injury of the rat carotid artery consists of four steps (7, 31). The first step is replication of VSMCs in the media, which occurs 0–3 days after balloon injury. In the second step, VSMCs exhibit migration from the media to the intima, which occurs 3–14 days after balloon injury. The third and fourth steps are VSMC proliferation and extracellular matrix deposition in the neointima, respectively, which begin 7 days after balloon injury. HO-1 was reported to be expressed by VSMCs in human atherosclerotic lesions but not in normal vessels (37). The phenotype of VSMCs in the intima and atherosclerotic lesions is known to differ from that of those in the media. Taken together, these findings suggest that HO-1 expression and VSMC differentiation are linked to each other. In fact, the levels of HO-1 in cultured VSMCs after passaging are increased, compared with those observed in primary cultures of VSMCs (unpublished observation).

Taken together with our previous studies, the current results suggest that CO derived from the HO reaction serves as a potentially inhibitory mediator for stimulus-elicted cell proliferation in vitro and in vivo. As was suggested previously, activation of soluble guanylate cyclase appears to be involved in the CO-mediated signaling mechanisms in the current study, inasmuch as ODQ restored and 8-bromo-cGMP mimicked the heme-mediated reduction of ANG II-induced cell proliferation at least in part. At the same time, the current result raised an important possibility that the guanylate cyclase-independent mechanism could be involved in a mechanism for CO-mediated regulation of cell proliferation, because sufficient amounts of the membrane-permeable cGMP analog did not fully mimic the effect of the HO-1 induction by hemin. Such a notion against the involvement of soluble guanylate cyclase in the CO-mediated signaling events has recently been emphasized in that CO is not as potent as NO to activate soluble guanylate cyclase (5). We therefore examined whether inhibition of cytochrome P-450 monooxygenase could mimic the effects of CO on VSMC proliferation, because the blockade of this enzyme reaction is one of the well-known biological actions of CO (4). As expected, the enzyme inhibitor clotrimazole exhibited additive suppressive effects with 8-bromo-cGMP on ANG II-induced proliferative responses, suggesting the involvement of cGMP-independent mechanisms. Detailed mechanisms for cytochrome P-450-mediated regulation of cell proliferation have not fully been addressed in the current study. However, the CO-mediated signal reception by this enzyme system has recently had much attention in that a variety of cytochrome P-450-derived products are biologically active to modulate potassium and calcium concentrations (14, 15, 38). Further investigation is obviously required to fully address whether endogenous CO could actually modulate the generation of specific cytochrome P-450-derived products and thereby cause alterations in cell function as a result of HO-1.

In this study, we demonstrated clearly that HO-1 expression is markedly increased in the denuded carotid artery and that the increased levels of CO via HO in the vascular wall results in inhibition of balloon injury-induced neointimal development. Therefore, the HO-CO system in the vascular wall may protect against the excessive VSMCs proliferation associated with vascular diseases.

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