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Forum Review

Complex Role of Heme Oxygenase-1 in Angiogenesis

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ABSTRACT

Angiogenesis occurring during reparative or pathological processes is driven by various inflammatory mediators that influence the synthesis of growth factors. It has been recognized recently that reactive oxygen species (ROS) and nitric oxide (NO) are important modulators of the synthesis and activity of vascular endothelial growth factor (VEGF), a major angiogenic molecule. Moreover, heme oxygenase-1 (HO-1), a ubiquitous stress-inducible enzyme that is induced by ROS and NO, was recently discovered to be involved in angiogenesis. Genetic overexpression of HO-1 enhanced VEGF synthesis and augmented formation of vascular capillaries, improving the blood flow in ischemic tissues. In addition, by-products of HO-1 exert numerous effects that can also influence angiogenesis in both positive and negative ways. Therefore, the antiinflammatory effects of HO-1 can attenuate the excess formation of blood vessels in inflammatory angiogenesis. In this review, the recent data on the role of HO-1 in angiogenesis are critically discussed. It is suggested that further studies using potent and specific augmentation of HO-1 gene expression by viral vectors, as well as targeted, specific inhibition of HO-1 expression, are required to elucidate fully the complex role of this enzymatic pathway in angiogenesis. *Antioxid. Redox Signal.* 6, 858–866.

INTRODUCTION

Formation of new blood vessels is strictly regulated during development and depends on the concerted activities of various growth factors and their receptors expressed on the surface of endothelial and accessory cells (for a review, see 8). Initiation of blood vessel growth also requires the presence of angiogenic stem cells, whose progeny develop into endothelial cells that form a network of capillaries. Such a process of *de novo* formation of blood vessels is termed vasculogenesis. Later in life, new vessels are derived mostly from preexisting ones in a process of angiogenesis. Physiological angiogenesis in adults occurs during the female reproductive cycle, is necessary for maturation of oocytes, and is a major mediator of hair follicle growth (for reviews, see 8, 20). Besides those internally regulated processes, angiogenesis is an inherent feature of numerous reparative and pathological mechanisms. It is a prerequisite for proper healing of injured tissues, as well as the cause or consequence of a number of diseases, such as hemangiomas, psoriasis, cancer, atherosclerosis, and rheumatoid arthritis, to name only a few. Overall, angiogenesis that occurs in reparative processes or pathological states is associated with inflammation and involves inflammatory mediators.

VEGF—A MAJOR MEDIATOR OF BLOOD VESSEL FORMATION

The major mediator of angiogenesis is vascular endothelial growth factor (VEGF) (for reviews, see 22, 65). During development, the level of VEGF is crucial for healthy growth, and the lack of even one functional allele of VEGF (in heterozygotes of VEGF gene knockouts) results in early embryonic lethality (9, 21). Moreover, in adult life, the synthesis of VEGF is potently induced by numerous stimuli, the major ones being hypoxia, cytokines, growth factors, nitric oxide (NO), and reactive oxygen species (ROS) (for a review, see 13).

VEGF binds to two types of VEGR receptors present mostly on endothelial cells (for reviews, see 22, 65, 85) (Fig. 1).

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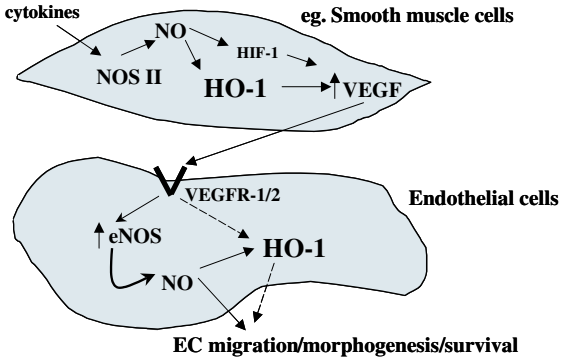

FIG. 1. Role of NO and HO-1 in VEGF synthesis and angiogenic activity of endothelial cells (EC).

VEGF receptor-1 (VEGFR-1; flt-1), which exists in both membrane-bound and soluble form, is regarded as playing a modulatory role, particularly inhibiting excess angiogenesis early in development, but also as having some important role in pathological angiogenesis (64, 79). In turn, VEGF receptor-2 (VEGFR-2; flk-1) is crucial in VEGF-induced angiogenesis (for references see 22, 65, 85).

The immediate effect of VEGFR-2 activation is induction of phospolipase Cγ, resulting in synthesis of inositol 1,4,5-trisphosphate followed by influx of Ca^{2+}, which augments endothelial nitric oxide synthase (eNOS, NOS III) activity and leads to enhanced release of NO (85). Phosphorylation of VEGFR-2 (flk-1) also results in phosphorylation of phosphatidylinositol 3-kinase, which activates the protein kinase B (Akt kinase). Akt phosphorylates the Ser1117 of eNOS, providing the mechanism for sustained Ca^{2+}-independent NO synthesis. Moreover, the late effect of VEGFR-2 activation is the stimulation of protein kinase C, strongly implicated in VEGF induced extracellular kinase activation, which can lead to long-term NO generation (69, 85).

eNOS is necessary for the angiogenic activity of VEGF. Formation of new blood vessels was impaired in eNOS knockout mice (57), and the inhibition of NO synthase (NOS) abolished the stimulatory effect of VEGF on endothelial cells (62). Angiogenic effects of NO may be mediated by activation of soluble guanylyl cyclase (sGC), elevation of 3′,5′-cyclic guanosine monophosphate (cGMP), and phosphorylation of p42/44 kinase (53, 87). NO also enhances the expression of urokinase plasminogen activator, and the lack of urokinase plasminogen activator abolishes the angiogenic effect of VEGF (29).

Interestingly, NO is also active as an upstream mediator of VEGF synthesis. It has been demonstrated that VEGF production in numerous cell types, including vascular smooth muscle cells (14, 15, 36), macrophages (46, 67, 84), keratinocytes (24–26, 71), and tumor cells (40–43), is enhanced by NO. Interestingly, the stimulatory effect of NO on VEGF synthesis involves the activation of hypoxia-inducible factor-1 (HIF-1) (for a review, see 40), the major transcription factor responsible for augmentation of VEGF synthesis under diminished oxygen tension (for a review, see 68). The effect of NO may involve inhibition of HIF-1 degradation or enhancement of its synthesis (52, 73).

NO is also a potent inducer of the expression of heme oxygenase-1 (HO-1) (for a review, see 55). The activity of HO-1, a ubiquitous stress-inducible enzyme, leads to degradation of heme to carbon monoxide (CO), iron, and biliverdin (for a review, see 48). Both NO production, derived from induced NOS II, and HO-1 expression are inherently connected with the inflammatory processes. Therefore, it is reasonable to hypothesize that, as with NO, products of HO activity can be involved in the angiogenic processes that develop in the course of inflammation.

ROLE OF HO-1 IN ANGIOGENESIS

HO-1 expression is induced by numerous stimuli, including hemin, cytokines, NO, growth factors, prostaglandin J_2 (PGJ_2), and various forms of oxidative stimuli, the effect of which involves the augmentation of the synthesis of ROS (for reviews, see 30, 48, 61). Hypoxia is also a potent inducer of HO-1 expression in rodent (74) and bovine cells (54), but surprisingly its effect in human cells as studied to date appears to be the reverse, leading to attenuation of HO-1 synthesis (58, 70, 74).

Involvement of HO-1 in formation of blood vessels has been demonstrated in tumors, wounds, and various experimental models of angiogenesis. Accordingly, Nishie *et al.* (59) demonstrated that the number of blood vessels in rat glioma correlated with the number of infiltrating macrophages and with the immunocytochemically detected expression of HO-1 in those cells. However, as macrophages generate other inducers of angiogenesis, the involvement of HO-1 in angiogenesis on the basis of those observations is not proven.

A direct effect of HO-1 in angiogenesis has been demonstrated by Deramaudt and co-workers (11). Transfection of coronary microvascular endothelial cells with a plasmid vector harboring the human HO-1 gene resulted in enhanced cell proliferation. Moreover, the migratory properties of such engineered endothelial cells were enhanced as demonstrated by increased propensity of HO-1-overexpressing cells to form vascular tubes in Matrigel.

EFFECT OF HO-1 ON VEGF SYNTHESIS

Augmentation of the activity of HO-1, attained both by chemical stimulators and by genetic overexpression of HO-1, revealed involvement of HO-1 both in VEGF synthesis and in the angiogenic effect of VEGF on endothelial cells. In keeping with these results, we have shown that stimuli that strongly induced HO-1 expression also very potently enhanced the synthesis of VEGF. This occurred in vascular smooth muscle cells, in which the VEGF synthesis augmented by cytokines, and dependent on generation of NO, is paralleled by the induction of HO-1 expression (16). On the contrary, the inhibition of HO activity by tin protoporphyrin IX (SnPPIX) resulted in a dose-dependent attenuation of VEGF production. Similarly, hypoxia-induced VEGF generation in rat vascular smooth muscle cells was inhibited by

SnPPIX, but not by the NOS inhibitors N^{ω}-nitro-L-arginine methyl ester (L-NAME) or isothiourea, indicating a role of HO-1, but not NO, in hypoxia-induced VEGF synthesis (16).

PGJ_2, a cyclopentane prostaglandin abundantly generated in the vascular system, is a very potent inducer of VEGF synthesis in smooth muscle cells and macrophages (35), as well as in microvascular endothelial cells (37, 38). The effect of PGJ_2 parallels the induction of HO-1 expression. PGJ_2-induced VEGF synthesis was abolished in the presence of protoporphyrin inhibitors of HO activity. As PGJ_2 is a natural ligand for peroxisome proliferator activating receptor-γ (PPARγ) transcription factor, we also investigated the effect of other PPARγ activators. Ciliglitazone and troglitazone, synthetic PPARγ activators, also enhanced VEGF synthesis (35).

PPARγ has been reported to be involved in oxidized low-density lipoprotein (LDL)-induced VEGF generation in endothelial cells (32). Interestingly, HO-1 expression is also strongly induced by modified LDL (81) and its components, such as hydroperoxides [13-hydroperoxyoctadecadienoic acid (13-HPODE)] (2, 31). Hence, it can be hypothesized that HO-1 is a mediator of oxidized LDL-induced VEGF synthesis.

Hemin, a natural inducer of HO-1 expression and a substrate for HO, appears to be also stimulatory for VEGF synthesis, although its effect is transient and generally much weaker than that of other HO-1 inducers. We have observed enhancement of VEGF synthesis by hemin in human and rat vascular smooth muscle cells (16) and human microvascular endothelial cells (38). The strongest induction of VEGF synthesis by hemin has been observed in human keratinocytes, suggesting that in those cell types heme might be involved in wound healing-related angiogenesis (unpublished observations). On the other hand, Eyssen-Hernandez *et al.* (18) did not observe an effect of hemin on VEGF synthesis in rat neonatal myocytes despite a strong induction of HO-1 expression. In contrast, hemin inhibited hypoxia-induced VEGF gene transcription in rat vascular smooth muscle cells (47). Thus, the effect of hemin on VEGF production may be cell type-dependent and may vary in normoxia and hypoxia.

Gene transfer of HO-1 into human or rat vascular smooth muscle cells and in human endothelial cells resulted in the enhancement of VEGF synthesis (1, 16, 37, 38). Importantly, such an effect has been also observed *in vivo*. Kreiser and coworkers transduced rat placenta with an adenoviral vector containing the human HO-1 gene (44). As a result, VEGF synthesis was strongly augmented. Similarly, in a rat hindlimb ischemia model, transfer of HO-1 to skeletal muscle resulted in the significant elevation of VEGF expression, improvement of the formation of blood vessels, and the concomitant enhancement of the blood flow (76). These effects did not occur after transfer of an empty adenoviral vector and were reversed by treatment of animals with zinc protoporphyrin IX (ZnPPIX), indicating the involvement of HO-1 in the processes (76).

Thus, at least under certain experimental settings, both pharmacological and genetically induced augmentation of HO-1 expression is paralleled by the enhancement of VEGF synthesis.

ROLE OF HO-1 IN ANGIOGENIC ACTIVITY OF ENDOTHELIAL CELLS

In analogy with the role of NOS in angiogenesis, HO-1 appears to be involved both upstream of VEGF synthesis and downstream of the VEGF-mediated activation of endothelial cells (Fig. 1). When human umbilical vein endothelial cells (HUVEC) were stimulated with VEGF, their proliferation was significantly attenuated in the presence of SnPPIX (38). A similar, although weaker, effect has been observed when VEGF-treated cells were growing in the presence of L-NAME. SnPPIX also inhibited the VEGF-induced migration of HUVEC; this effect was even more potent than the influence on proliferation. Again, L-NAME significantly attenuated VEGF-induced cell migration, proving the role of NO. When applied together, L-NAME and SnPPIX completely abolished VEGF-induced migration, indicating the role of both enzymatic pathways in this angiogenic activity (38).

Angiogenic assays performed *in vitro* demonstrated that VEGF-induced tube formation or capillary sprouting was dependent on HO activity (38). Accordingly, the effect was reversed by SnPPIX, but not by copper protoporphyrin IX, which is not an inhibitor of HO. Importantly, gene transfer of HO-1 to HUVEC augmented their angiogenic capabilities. Cells overexpressing HO-1, but not those expressing the control β-galactosidase gene, produced significantly more capillaries in the spheroid angiogenic assays.

Recent experiments revealed that VEGF may induce the expression of HO-1 both *in vitro* (Fig. 2) and *in vivo* (19). In the latter studies, performed in the chicken chorioallantoic membrane model of angiogenesis, stimulation with VEGF led to enhanced expression of HO-1 as demonstrated by western blot. The effect was dependent on protein kinase C activation and was inhibited by chelation of calcium. Importantly, ZnPPIX attenuated the angiogenic response in the developing embryo, suggesting the involvement of HO-1 (19).

PRO- AND ANTIANGIOGENIC EFFECTS OF THE PRODUCTS OF HO ACTIVITY

The fact that the cleavage of heme by HO-1 leads to three different products poses the question of which of them is in-

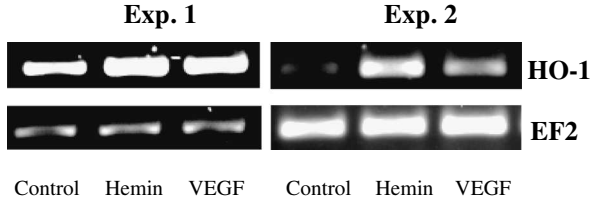

FIG. 2. VEGF induces HO-1 expression in HUVEC. Cells were treated with 10 μ*M* hemin or 30 ng/ml VEGF. RNA was isolated, and RT-PCR for HO-1 and constitutive EF2 gene expression was performed as described (15). Results of two out of four separate experiments are shown.

volved in the angiogenic activity of HO-1. The results are not equivocal or even partially discrepant.

Effect of CO

Similarly to NO, CO has been demonstrated to be both a positive and a negative modulator of VEGF synthesis. Like NO, CO augments the activity of sGC, although its effect on cGMP production is ~20 times weaker than that of NO (for reviews and references, see 12, 63). In our hands, stimulation of HO-1 expression with PGJ_2 resulted in a severalfold increase in CO generation, as determined by spectrophotometric measurement of carboxyhemoglobin formation. The synthesis of VEGF was increased concomitantly with CO release, and the inhibition of sGC with oxadiazoloquinoxalinone diminished VEGF synthesis. Also, in the presence of oxyhemoglobin, a scavenger of CO, the generation of VEGF was diminished, suggesting a role for CO as an inducer of VEGF expression (38).

Culturing of vascular smooth muscle cells in the presence of 1% CO (10,000 ppm) resulted in the strong induction of VEGF synthesis, although this relatively high concentration of CO was already slightly toxic for the cells (16). In another study, a 5% CO concentration inhibited VEGF synthesis (47). On the other hand, treatment of cells with CORM-2 (CO-releasing molecule), a representative of a new class of compounds spontaneously releasing small amounts of CO (56), was only weakly effective in HMEC-1 cells (38), whereas it did not enhance VEGF synthesis in other cell types, such as NIH 3T3 fibroblasts or vascular smooth muscle cells (data not shown). Similarly, dichloromethane, a compound that is metabolized in the liver into CO and CO_2 by cytochrome P450 enzymes and has been demonstrated to exert therapeutic effects, such as prevention of ischemia–reperfusion and liver graft rejection (39), had no effect on VEGF expression in HepG2 hepatocytes (unpublished observations).

Consideration of the effect of CO is complicated by discrepant data. Thus, it has been demonstrated that even the same amounts of CO can exert varying effects. In some experiments, 250 ppm of CO protected endothelial cells from stress- or cytokine-induced apoptosis (5), whereas a similar dosage of CO in another study was toxic for the endothelial cells (78). Therefore, experiments using high concentrations of CO for testing its effect on growth factor synthesis (47) have to be interpreted with caution as the inhibitory effect of CO can result from its overall toxicity.

CO is an inhibitor of electron transport complex IV. Thus, it can be hypothesized that CO generates local hypoxia. It has been shown that cyanides, another complex IV inhibitor, enhanced the stability of HIF-1 in normoxic conditions, leading to the augmentation of VEGF synthesis (83). Thus, it may be hypothesized that similar effects on HIF-1 can be exerted by CO.

CO can also generate oxidative stress and synthesis of ROS (for a review, see 63). Several mechanisms might be involved, including the induction of manganese superoxide dismutase (SOD) expression (27). Although the activity of SODs diminishes the level of superoxide radicals, the final effect on oxidative stress is dependent on the concomitant activity of catalase and glutathione peroxidase, the enzymes that remove hydrogen peroxide. Indeed, under certain conditions, overexpression of SOD may lead to increased ROS production (86). In line with such a hypothesis, we have shown that overexpression of SOD1 increased intracellular ROS content and enhanced VEGF synthesis (28). Thus, it may also be possible that oxidative stress augmented by CO can stimulate the production of VEGF.

Undoubtedly, the effect of CO *in vivo* is connected with the induction of hypoxia. Marti and Risau have shown that animals kept for 6 h in an atmosphere containing 0.1% CO exhibited significant induction of VEGF and VEGF receptors in various organs (50). Thus, the up-regulation of VEGF by CO *in vivo* is likely related to hypoxia-mediated activation of HIF-1 rather than with a direct influence of CO.

Effect of iron on VEGF synthesis

Iron can influence angiogenesis by acting as a cofactor for enzymes involved in the regulation of VEGF synthesis or by enhancing oxidative stress due to the Fenton reaction and formation of noxious hydroxyl radicals (for reviews, see 66, 82). On the other hand, iron induces the synthesis of ferritin, which acts as an antioxidant. Thus, the final outcome of heme degradation and iron release may be beneficial. However, the release of high amounts of reactive iron under conditions of strongly enhanced HO activity and a high supply of heme may do more harm than good for the cells (10, 75).

Iron is also a cofactor for prolyl hydroxylases (PH), the newly described class of enzymes crucial for normoxic destabilization of HIF-1α protein (6, 17, 34, 51). Under normoxic conditions, PHs hydroxylate two critical proline residues (P402 and P564) in constitutively generated HIF-1α. This modification is a signal for von Hippel–Lindau ubiquitin ligase, which by ubiquitination of HIF-1α directs it to degrade in the proteasome. PHs are oxygen-dependent enzymes and require iron and 2-oxoglutarate as cofactors. Thus, when the oxygen level drops, the activity of PHs diminishes. A similar situation occurs when the iron concentration in the cells decreases. Accordingly, treatment of cells with desferrioxamine, a nonpermeable iron chelator that extrudes iron from cells, results in inhibition of PH activity and, consequently, leads to the stabilization of HIF-1 even under normoxic conditions (80; for a review, see 77).

We have observed that desferrioxamine enhanced, whereas iron diminished, VEGF synthesis, the effects being mediated by modulation of hypoxia-responsive element activity (16). Thus, it can be hypothesized that increased release of iron from heme can, under certain conditions, attenuate VEGF-dependent angiogenesis.

On the other hand, it can be also hypothesized that the effect of HO-1 on VEGF synthesis in relation to iron can be related to the iron-pump activity of this enzyme (3, 23). It has been demonstrated that the extrusion of iron from the cells requires active HO-1. The release of iron from the cells can be augmented by the genetic overexpression of HO-1, which may result in similar effects on VEGF synthesis as treatment with iron chelators.

Role of biliverdin and bilirubin in VEGF synthesis

So far, no data on the effect of biliverdin and bilirubin on angiogenesis are available. However, it can be hypothesized that both substances are involved in ROS-dependent regulation of VEGF synthesis. This assumption is based on a recent study demonstrating that biliverdin and bilirubin play a role in the protection of cells against oxidative stress (4). This activity involves the peroxyl radical scavenging properties of bilirubin (72) and requires the presence of biliverdin reductase (BVR) (for a review, see 49). Biliverdin, generated during the process of bilirubin oxidation, is reduced by BVR to bilirubin, and this cycle is thought to be strongly protective against membrane oxidative stress.

If such an activity of biliverdin and bilirubin occurs under physiological conditions, one can consider the potential involvement of those HO-1 products in the regulation of VEGF synthesis induced by ROS. Accordingly, it can be hypothesized that both compounds can diminish VEGF synthesis by reducing oxidative stress.

The data discussed above indicate that the final outcome of HO activity on VEGF synthesis and angiogenesis is complex. At the initiation phase of inflammatory reactions, when HO-1 expression is induced, the products of HO-1 activity, most probably CO, can enhance VEGF synthesis. The stimulatory effect can be also caused by extrusion of iron from the cells, which can potentially lead to diminished PH activity. Then mild oxidative stress, generated due to the release of CO and iron, can sustain the induction of VEGF synthesis. Finally, however, released iron, biliverdin, and bilirubin may attenuate the stimulatory effect on VEGF synthesis.

It can be also presumed that the effect of HO activity on VEGF synthesis is dependent on the stimulus. When HO-1 is induced by non-heme inducers, the amount of products generated by HO activity depends only on intracellular sources of heme, which may be limited, and thus the degradation of heme may not result in the generation of high amounts of reactive iron. On the other hand, when HO-1 activity is induced by heme, the prolonged expression of HO-1 may lead to the formation of an abundance of reactive iron, which can inhibit VEGF synthesis.

MODULATORY EFFECT OF HO-1 IN ANGIOGENESIS

Recent data indicate the crucial role of HO-1 in the modulation of inflammatory reactions. HO-1 activity prevents endothelial cell apoptosis, inhibits the formation of proinflammatory cytokines, and attenuates inflammation occurring in the course of graft rejection, atherosclerosis, or ischemia–reperfusion injury (for a review, see 61).

This activity of HO-1 generates CO, which is a potent antiinflammatory compound at low, physiological concentrations. Its effect is, to a large extent, dependent on the activation of p38 mitogen-activated protein kinase and the synthesis of interleukin-10, the antiinflammatory cytokine with reported antiangiogenic properties (33, 45, 60). Whether those effects of HO and CO are also involved in the modulation of VEGF synthesis and VEGF-induced angiogenesis requires further investigation.

Interestingly, in a recent study, Bussolati and co-workers have shown the bifunctional role of HO-1 in VEGF-induced angiogenesis (7). On the one hand, treatment with VEGF induced prolonged HO-1 expression and activity in HUVEC, and HO-1 inhibition abrogated VEGF-driven angiogenesis. Thus, this effect is similar to that observed in our studies (38; Fig. 2) and reported by others (11). On the other hand, the role of HO-1 in angiogenesis may be either stimulatory or inhibitory, depending on underlying circumstances. Thus, pharmacological inhibition of HO-1 induced marked leukocytic infiltration that enhanced VEGF-induced angiogenesis. However, in the presence of an anti-CD18 monoclonal antibody (applied to block leukocyte migration), VEGF-induced angiogenesis was significantly inhibited when HO activity was attenuated (7). Furthermore, in another model of inflammatory angiogenesis, induced by lipopolysaccharide, potentiation of HO-1 activity by preceding stimulation with cobalt protoporphyrin IX significantly inhibited leukocyte infiltration and prevented angiogenesis. Based on their observations, Bussolati and co-workers propose that during chronic inflammation HO-1 has two roles: (a) by inhibiting leukocyte infiltration, it acts as antiinflammatory agent; and (b), by promotion of VEGF-driven angiogenesis, it facilitates tissue repair (7).

CONCLUSIONS

The role of HO-1 in angiogenesis appears to be complex (Fig. 3). On the one side, it has been shown that HO-1 overexpression is proangiogenic. Increased HO-1 activity enhanced synthesis of VEGF, and endothelial cells overexpressing HO-1 showed enhanced angiogenic activity. Importantly, that effect occurred both *in vitro* and *in vivo*, suggesting a physiological role of HO-1 in angiogenesis. Similarly, the effect of inducers of HO-1 expression, such as cytokines or NO, is also proangiogenic. However, it cannot be excluded that some angiogenic effects caused by non-heme inducers of HO-1 are HO-1-independent.

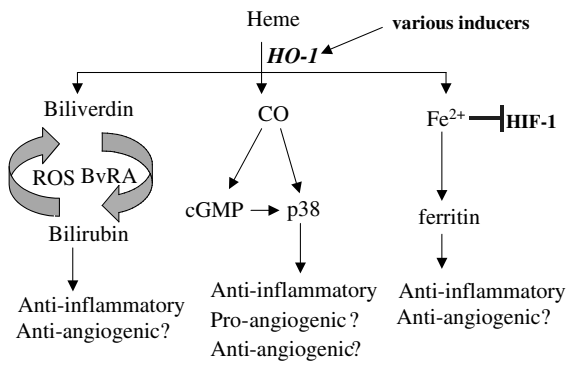

FIG. 3. Potential pro- and antiangiogenic effects of HO-1.

The role of hemin appears to be more complex, as its stimulatory effect on VEGF is much weaker in comparison with those of other mediators. Heme is a potent prooxidant, and its presence, particularly by the release of iron, may generate oxidative stress that, up to a certain threshold can be proangiogenic. However, most often the presence of heme will be antiangiogenic. The effect of bilirubin and biliverdin can be related to their involvement in the scavenging of peroxyl radicals. Iron can be antiangiogenic when Fenton reaction hydroxyl radicals are generated. It is also possible that the release of iron can enhance the activity of PHs, the enzymes involved in HIF-1α degradation.

Finally, it has to be taken into consideration that products of HO activity are antiinflammatory. By decreasing the generation of inflammatory cytokines, influencing the synthesis of NO, and attenuating the production of ROS, the outcome of HO activity can be antiangiogenic. Further studies, using potent and specific augmentation of HO-1 gene expression by viral vectors, as well as targeted inhibition of HO-1 expression by RNA interference, may reveal novel mechanisms of potential important biological implications and therapeutic application.

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ABBREVIATIONS

BVR, biliverdin reductase; cGMP, 3′,5′-cyclic guanosine monophosphate; CO, carbon monoxide; eNOS, endothelial nitric oxide synthase; HIF-1, hypoxia-inducible factor; HO-1, heme oxygenase-1; HUVEC, human umbilical vein endothelial cells; LDL, low-density lipoprotein; L-NAME, *N*ω-nitro-L-arginine methyl ester; NO, nitric oxide; NOS, NO synthase; PGJ_2, prostaglandin J_2; PH, prolyl hydroxylase; PPARγ, peroxisome proliferator activiting receptor-γ; ROS, reactive oxygen species; sGC, soluble guanylyl cyclase; SnPPIX, tin protoporphyrin IX; SOD, superoxide dismutase; VEGF, vascular endothelial growth factor; VEGFR-1, VEGF receptor-1; VEGFR-2, VEGF receptor-2; ZnPPIX, zinc protoporphyrin IX.

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