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Forum Review

Redox Signaling of Angiogenesis

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ABSTRACT

Reactive oxygen species (ROS) play a crucial role in vascular angiogenesis. Both *in vitro* and *in vivo* studies indicate that angiogenic response in vascular tissue is triggered by ROS signaling in a highly coordinated manner. It appears that massive amounts of ROS produced during ischemia and reperfusion in the vascular tissue, especially in heart, cause significant injury to the cardiomyocyte and endothelial cells. However, during the reperfusion, the same ROS potentiates a repair process and triggers a signal transduction cascade leading to angiogenesis. Although several other factors are likely to be involved for such angiogenic response, ROS certainly plays a crucial role as evident from its direct role as mediator of angiogenesis and inhibition of angiogenesis with free radical scavengers and/or antioxidants. Angiogenesis is regulated by redox-sensing transcription factors such as nuclear factor-κB, and oxidants such as hydrogen peroxide and free radicals, such as nitric oxide may function as second messengers in this highly coordinated process. Furthermore, expression of many angiogenic genes including those for vascular endothelial growth factor, fibroblast growth factor, platelet-derived growth factor, and receptors such as Flt-1, Flk-1, Ang-1, and Ang-2 are likely to be regulated by redox signaling. It is tempting to speculate that the angiogenic response is under the autocrine and/or paracrine control of one or more cytokines, which in turn is redox-regulated. Through angiogenesis, ROS appear to pave the way of repairing the vascular tissues that have been damaged during ischemia and reperfusion. *Antioxid. Redox Signal.* 4: 805–815.

INTRODUCTION

IN ORDER TO DEVELOP better and more effective therapeutic strategies using the powerful concept of inducing new vessel growth by employing vascular growth factors, it is essential to further our understanding of the molecular mechanisms and chain of events underlying the fascinating process of angiogenesis. Among the various triggers of angiogenesis, tissue hypoxia has been identified as being a particularly important stimulus for the induction of new vessel growth (28, 60). Tissue hypoxia exerts such a proangiogenic action through various angiogenic factors, the most notable being vascular endothelial growth factor (VEGF), which has been associated chiefly with initiating the process of angiogenesis through the recruitment and proliferation of endothelial cells. Various mechanisms have been shown to be responsible in the regulation of VEGF expression. Oxygen tension appears to play a significant role, both *in vitro* and *in vivo*. VEGF mRNA expression is rapidly induced by exposure to low Po_2 in a variety of cultured cells, such as retinal pigmented epithelial cells (69), myoblast (71), cardiomyocyte (6), and tumor cells (71). Furthermore, occlusion of the left anterior descending coronary artery results in VEGF protein expression (66), suggesting that VEGF is a mediator of the revascularization (spontaneous) that occurs in low oxygen condition. There exist similarities between the mechanisms leading to hypoxic regulation of VEGF and erythropoietin (EPO). Cobalt chloride was found to induce both VEGF and EPO mRNA expression. Hypoxic induction of both the genes was inhibited by carbon monoxide, suggesting the involvement of a heme protein in the process of sensing oxygen levels (22). EPO increases the oxygen-carrying capacity of the blood by stimulating red cell formation, whereas VEGF-induced angiogenesis allows the delivery of oxygen to ischemic tissues.

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Flt-1 (VEGFR-1) and Flk-1/KDR (VEGFR-2) are the endothelial specific tyrosine kinase receptors of VEGF through which its effects are primarily mediated (14, 48). These two VEGF receptors have been found to have two different signal transduction properties (83). Two other angiogenic factors, the angiopoietins 1 and 2 (Ang-1 and Ang-2), have been found to regulate the maturation of new blood vessels from the proliferated endothelial cells (85). Tie-1 and Tie-2 comprise another family of endothelial specific receptor tyrosine kinases, Ang-1 and Ang-2 being the specific ligands for Tie-2.

The fact that VEGF, Flt-1, and Flk-1 expression is up-regulated in response to hypoxia *in vitro* and *in vivo* (5, 37, 38, 49) and to ischemia *in vivo* (23, 42, 81) is well established, although there are conflicting reports with regard to Flk-1 *in vitro,* suggesting the involvement of adenosine acting as a paracrine mediator through the A2 receptor (9, 77). The observed *in vivo* effects of ischemia on the above factors may logically be attributed primarily to its hypoxic component. The existence of a discrete factor with proangiogenic activity was first reported three decades ago (17). Since then, many angiogenic growth factors have been identified and their importance demonstrated in various experimental models of angiogenesis, but a detailed understanding of the interplay among inducing stimuli, growth factors, and their respective molecular targets remains to be elucidated. This review will focus on some important molecular components and events responsible for angiogenic responses in various organs.

ROLE OF REACTIVE OXYGEN SPECIES (ROS)

Oxygen homeostasis is of critical importance for maintaining the viability of all tissues. Lack of sufficient tissue oxygenation is predominantly caused by impaired blood flow. Attempts to restore normal oxygen levels after episodes of hypoxia or ischemia result in the generation of various types of free radicals, such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\cdot}OH$), collectively known as ROS. Although lack of adequate oxygen (*e.g.,* hypoxia) is an initiator of various diseases, it also can trigger a unique "repair" mechanism, and acts as an important inducer of angiogenesis.

ROS play a very important role in signaling pathways stimulated by growth factors in vascular cells. Recent reports suggest that ROS, such as superoxide anions, play an important role in mediating signals initiated by growth factors and inflammatory cytokines (12). In this regard, we have previously shown that hypoxic preconditioning (hypoxia/reoxygenation) mediates the activation of nuclear factor-κB (NFκB) in rat myocardium and human coronary arteriolar endothelial cells (25, 92). Our study provides evidence that hypoxic preconditioning accelerates tubular morphogenesis along with the activation of ROS-inducible nuclear transcription factor, NFκB, phosphatidylinositol 3-kinase (PI3-kinase), and broad-spectrum antiapoptotic protein survivin in the human coronary endothelial cells (HCAEC). The formation of tubular morphogenesis was inhibited by using PI3-kinase and NFκB antagonist, LY294002 and SN50, respectively (Fig. 1). Recently, it was demonstrated in a mouse model that NFκB activation is obligatory for retinal angiogenesis: the administration of pyrrolidine dithiocarbamate (PDTC), a NFκB inhibitor, suppressed retinal neovascularization (90). Hypoxia is characterized by inadequate oxygen delivery to the myocardium with a resulting imbalance between oxygen demand and energy supply. Studies regarding the effects of hypoxia on blood pressure, and autonomic cardiovascular responses such as carotid baroreflex control, have shown that hypoxic exposure significantly influences these parameters (24, 50, 65). A strong resemblance exists between the patterns of acute stress response induced by hypoxia/reoxygenation, ischemia/reperfusion, or any means of generating ROS (Fig. 2). Whereas prolonged ischemia and hypoxia (which cause irreversible cell injury) cause suppression of mRNA and protein synthesis, short durations of ischemia (ischemic preconditioning) or hypoxia (hypoxic preconditioning) can stimulate, rather than inhibit, mRNA and protein synthesis.

First described by Neely and Grotyohann in 1984 (52), hypoxic preconditioning, like ischemic preconditioning, can attenuate stunning caused by repeated coronary artery occlusions (4), and enhance postischemic recovery of myocardial function (39). Hypoxia has been found to be the strongest inducer both *in vitro* and *in vivo,* of VEGF. ROS often utilize H_2O_2 as a second messenger to activate transcription factors. NFκB (67), activator protein-1 (AP-1) (39), T-cell, and serum response factor (2) are all involved in ROS signaling mediated by H_2O_2 (47). The H_2O_2-induced angiogenesis was completely blocked in human microvascular endothelial cells by the coadministration of NFκB antisense oligonucleotide (55). Expression of AP-1 (c-fos and c-jun) was found to increase significantly in response to H_2O_2, where c-jun was partly involved in H_2O_2-mediated angiogenesis in human microvascular endothelial cells. H_2O_2 can also stimulate interleukin-8 (IL-8) production in cultured endothelial cells to a level that can induce angiogenesis (55). The administration of IL-8 antibody completely impairs the angiogenic process in the same system.

Several *in vitro* studies directly established the role of nitric oxide (NO) in angiogenesis (70, 93). Treatment of HepG2 cells with NO donor SNAP was found to increase VEGF mRNA expression. Guanylate cyclase is likely to be important for NO-mediated VEGF activation (94). There is also considerable evidence that NO down-regulates the expression of VEGF gene (11, 80). In spite of several negative observations, activation of angiogenesis in mammalian (human) monocytes is believed to be NO-dependent (74). Indeed, several studies have documented that NO-generating compounds stimulate angiogenesis in human glastoma and hepatoma cells (40). A positive correlation was found between NO synthase, cyclic GMP levels, and tumor angiogenesis in head and neck cancer (19). However, the role of NO in angiogenesis is still controversial.

INVOLVEMENT OF TRANSCRIPTION FACTORS IN THE PROCESS OF ANGIOGENESIS

Role of hypoxia-inducible factor-1 (HIF-1)

HIF-1 is a major mediator of hypoxic signaling. A significant amount of data has highlighted transcription factor HIF-1α as a central hypoxia signaling molecule (91). Genes

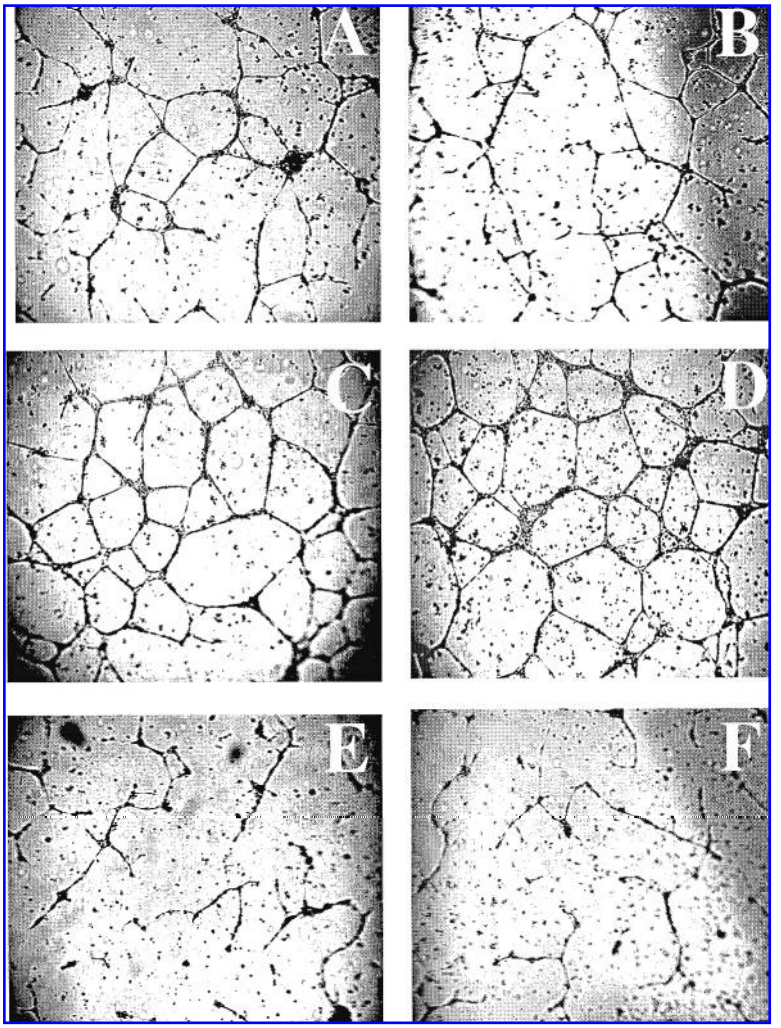

FIG. 1. Development of tube-like structures by HCAEC. Cells were plated onto matrigel when they were confluent. (**A**) Baseline control, normoxic. (**B**) Cells exposed to 4 h of hypoxia followed by 12 h of reoxygenation. (**C**) Cells exposed to 6 h of hypoxia followed by 12 h of reoxygenation. (**D**) Cells exposed to 8 h of hypoxia followed by 12 h of hypoxia. (**E**) Cells pretreated with SN50, NFκB inhibitor, followed by 8 h of hypoxia and 12 h of reoxygenation. (**F**) Cells pretreated with LY294002, PI3-kinase inhibitor, followed by 8 h of hypoxia and 12 h of reoxygenation. Note the inhibition of tube formation by SN50 and LY294002. The tube formation is very prominent when the cells were exposed to 6–8 h of hypoxia followed by 12 h of reoxygenation.

that are transcriptionally activated by HIF-1 in hypoxic cells encode proteins that increase O_2 delivery or allow metabolic adaptation to limited O_2 availability. Deletion of the gene (homozygous) that encodes the HIF-1 results in embryonic lethality with profound abnormalities in angiogenesis and cardiovascular development (31, 63). HIF-1 is a heterodimer consisting of the constitutively expressed ARNT (the aryl hydrocarbon receptor nuclear transporter; HIF-1β) protein and the hypoxia-inducible HIF-1α protein. The molecular mechanism by which a cell senses decreased oxygen levels and transmits the signal to HIF-1α is not known. There are two theories behind this mechanism: (a) heme protein is the oxygen sensor and reactive oxygen intermediates are propagators of the signal (10, 26, 27); and (b) oxygen sensing does not involve a heme protein, but instead HIF-1α itself binds iron and thereafter this complex is capable of sensing oxygen concentrations via this bound iron (75). HIF-1 responsive genes include VEGF, EPO, Flt-1, lactate dehydrogenase, heme oxygenase, inducible NO synthase, aldolase, and endothelin-1.

In HIF-1α$^{-/-}$ cells, VEGF mRNA expression was not induced in response to hypoxia, whereas an induction was observed in response to glucose deprivation, suggesting that HIF-1 mediated increased VEGF expression specifically in response to hypoxia (31). VEGF is not expressed under normoxic conditions, but on exposure to hypoxia, VEGF mRNA levels are found to increase dramatically within a few hours. This increase in transcription was mediated by a 28-bp element in the 5′ region of the gene that contains the HIF-1 binding site (43). The HIF-1 site of the VEGF gene acts as an enhancer by stimulating expression of a reporter gene under hypoxia.

Aortic smooth muscle cells from old rabbits manifest impaired hypoxia-induced VEGF expression due to a reduction in HIF-1 DNA binding protein (61). HIF-1α gene therapy has the potential advantage of inducing the expression not only of VEGF, but also of other hypoxia-induced angiogenic or survival factors (*e.g.,* Ang-2, insulin growth factor-2) and their receptors on endothelial cells such as Flt-1. In a recent study, HIF-1α gene therapy was found to be as effective as VEGF in stimulating therapeutic angiogenesis in a rabbit hindlimb ischemia model (82). Moreover, other studies have documented the essential role of HIF-1 in hypoxia-induced, VEGF-mediated angiogenesis and suggested that HIF-1 mediates hypoxia-induced preconditioning in the brain, heart, and other organs.

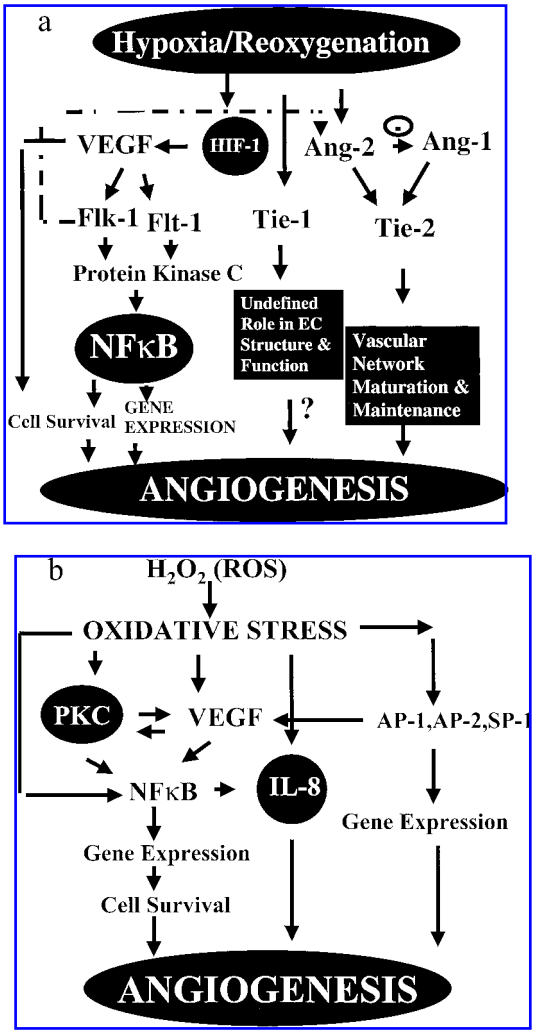

FIG. 2. (**a**) Possible signaling pathway during hypoxia/reoxygenation in angiogenesis. (**b**) Possible signaling mechanism during oxidative stress in angiogenesis.

Role of NFκB

The activated form of NFκB is a heterodimer, which consists of two proteins, a p65 subunit and a p50 subunit. In normal cells, NFκB is maintained in the cytoplasm by protein–protein interaction with inhibitor IκB. Recently, it was demonstrated in a mouse model that NFκB activation is obligatory for retinal angiogenesis, and it was also documented that the administration of PDTC suppressed retinal neovascularization (90). In another study, it was documented that hypoxia/reoxygenation, and not hypoxia alone, can cause formation of ROS and the activation of the NFκB, both of which were inhibited by ROS scavengers, and was accompanied by inhibition of tube formation in angiogenesis (41). Therefore, in the clinical setting of hypoxia/reoxygenation and during ischemic preconditioning, the activation of ROS-dependent intracellular signaling may accelerate the rate of neovascularization also *in vivo*. Hypoxia has been shown to induce NFκB activation and increased IL-8, as well as VEGF gene expression, in glial cells *in vitro*. Furthermore, PDTC, a very specific inhibitor of NFκB, prevented the induction of IL-8 gene expression, but had no effect on the VEGF gene in the *in vitro* study. This finding suggested that IL-8 gene induced by hypoxia and mediated by NFκB may contribute to the pathogenesis of intraocular neovascularization (89).

Hypoxia /reoxygenation-induced myocardial angiogenesis as measured by CD-31 labeling was inhibited by the intraperitoneal injection of PDTC prior to hypoxia (25). It is already documented that hypoxia/reoxygenation, but not hypoxia alone, caused the production of ROS and thereby activated NFκB (41). Consistent with these previous reports, DNA binding activity of NFκB remained almost at the baseline level when rats were subjected to hypoxia only. In contrast, when the rats were subjected to hypoxia followed by reoxygenation, a significant amount of DNA binding activity was observed in the myocardium (25). This indicates that the angiogenesis is accompanied by, and also requires the formation of, ROS. This study also provides evidence for direct involvement of ROS and ROS-mediated signaling via NFκB *in vivo* in myocardial angiogenesis. In another recent study, we have documented that hypoxic preconditioning accelerates tubular morphogenesis along with the activation of ROS-inducible nuclear transcription factor, NFκB, PI3-kinase, and broad-spectrum antiapoptotic protein survivin in the HCAEC. The formation of tubular morphogenesis was inhibited by using PI3-kinase and NFκB antagonist, LY294002 and SN50, respectively (92). LY294002 and SN50 also inhibited the activation of survivin by hypoxic preconditioning along with increased apoptosis in HCAEC (Fig. 3). These data demonstrate a crucial role of PI3-kinase/Akt/NFκB/survivin signaling in tubular morphogenesis of HCAEC triggered by hypoxic preconditioning.

Role of AP-1

The AP-1 binding complex consists of either Jun–Fos heterodimers or Jun–Jun homodimers (73). Several studies have shown that AP-1 and NFκB are differentially activated by oxygen tension. Several potential binding sites for the transcription factors AP-1, AP-2, and SP-1 are localized in the VEGF gene promoter (78). Tumor necrosis factor-α (TNFα) or basic fibroblast growth factor (bFGF) appears to stimulate expression of the VEGF gene through SP-1 on its promoter (71). Among eight human glioma cell lines, cellular mRNA levels of transcription factors SP-1 and AP-1 were found to be closely correlated with those of VEGF (64).

ROLE OF ANGIOGENIC GROWTH FACTORS

Fibroblast Growth Factors (FGFs)

FGFs are members of a family of polypeptides synthesized by a variety of cell types during the process of embryonic development and in adult tissues. These growth factors have been detected in normal and malignant cells and show a biological profile that includes mitogenic and angiogenic activity. FGF and FGF receptors play significant roles in many biological systems. The FGF receptors are a family of trans-

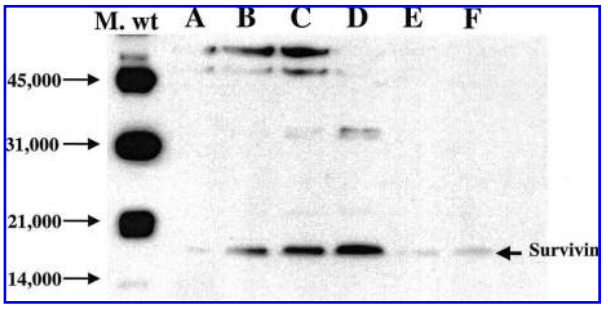

FIG. 3. Western blot analysis for survivin expression. Lane A, control baseline. Lane B, cells exposed to 4 h of hypoxia followed by 24 h of reoxygenation. Lane C, 6 h of hypoxia followed by 24 h of reoxygenation. Lane D, 8 h of hypoxia followed by 24 h of reoxygenation. Lane E, cells pretreated with SN50 followed by 8 h of hypoxia and 24 h of reoxygenation. Lane F, cells pretreated with LY294002 followed by 8 h of hypoxia and 24 h of reoxygenation.

membrane tyrosine kinase involved in signaling via interactions with the family of FGFs. The first agent to be used in an attempt to stimulate myocardial neovascularization was FGF-1 (6). In animal models, FGF has been shown to stimulate angiogenesis, granulation, tissue formation, epithelial growth, and wound tonsil strength (32).

Recently, several animal studies indicate that both bFGF and acidic FGF enhance the regeneration of peripheral nerve (1).

VEGF system

Hypoxia has been found to be the one of the strongest inducers of VEGF, both *in vitro* and *in vivo* (49). VEGF, a protein coded by a 7-exon gene localized on chromosome 6, serves as a major angiogen in normal cardiac development (16). A modern experimental strategy for treating myocardial ischemia is to induce neovascularization of the heart by use of "angiogens," mediators that induce the formation of blood vessels, or angiogenesis (62).

The process of angiogenesis is regulated by the signals obtained from the transmembrane receptor tyrosine kinases (RTKs) and non-RTKs (Src family) of endothelial cells. Flk-1 and Flt-1 are two such RTKs, which together with their ligand VEGF have been shown to control blood vessel development during embryogenesis (18). This receptor/ligand system has been shown to augment neovascularization (30). VEGF is not only an endothelial cell-specific angiogenic factor but also a critical regulator of angiogenesis that stimulates proliferation, migration, and proteolytic activity of endothelial cells (46). Yet the signaling pathways that modulate the mitogenic effects of VEGF in vascular endothelial cells are still ill defined (52). A recent study demonstrated that VEGF was localized and expressed in the embryonic/fetal heart and that its level remained high during the early postnatal period when capillary proliferation is high (79). Flt-1 (VEGFR-1) and Flk-1/KDR (VEGFR-2) are endothelial specific tyrosine kinase receptors of VEGF through which its effects are primarily mediated (14). It is well established that VEGF, Flk-1, and Flt-1 expressions are up-regulated in response to hypoxia *in vitro* and *in vivo* (42) and to ischemia *in vivo* (77). Some studies with regard to Flk-1 *in vitro* suggest the involvement of adenosine acting as a paracrine mediator through the A_2 receptor (29). The biological functions of VEGF, triggered by external stimuli, are initiated through the activation of intracellular signal transduction cascades involving specific kinases. It is reported that a rapid increase in VEGF expression under hypoxic challenge is due to the presence of HIF-sensitive elements located in the VEGF promoter, which up-regulated the transcription factor of VEGF (59). Furthermore, endothelial cells detect external angiogenic stimuli via oncogenes (87). Very recently we have demonstrated that hypoxic preconditioning (HMI) mediated a decrease in the percentage of endothelial cell apoptosis in an infarcted rat model. A decrease in the level of apoptosis may be due to the survival signal obtained from the increase in VEGF level in the HMI group (33% after 1 week and 64% after 3 weeks of hypoxic preconditioning) (Fig. 4) when compared with the nonpreconditioned CMI group of animals (66).

Ang-Tie system

The Tie receptors, Tie-1 and Tie-2, are among many RTKs expressed on endothelial cells (87). These unique RTKs have received great attention for their possible involvement in angiogenic response (51). The multiple gene family motifs that comprise the Tie RTKs have led to the notion that Tie-1 and Tie-2 may play a role in hematopoietic cell differentiation and/or in blood endothelial cell interaction (57). Recently, the ligands of the Tie-2 receptor have been identified as Ang-1 and Ang-2, also known as angiopoietins. The name angiopoietin reflects the role of this protein in angiogenesis (76) and its potential role in hematopoiesis. Ang-1 is the major physiological ligand for Tie-2, which is responsible for recruiting and sustaining periendothelial support cells (13). Ang-2 has been found to be responsible in disrupting vessel formation in the developing embryo by antagonizing the effects of Ang-1 and Tie-2. Therefore, Ang-2 represents a natural Ang-1/Tie-2 inhibitor. Several reports have already established the involvement of Ang-1 in the maturation and stabilization of developing neovasculature (76), whereas Ang-2 may cause destabilization required for additional sprout formation (44). Tie-1 and Tie-2 are homologous to each other, but unlike the VEGF receptors, they contain matrix association motifs in their extracellular domains. Both are expressed very early in development (15). Tie-2 is expressed in the blood islands and in intraembryonic angioblasts, where it appears earlier than von Willebrand factor.

REGULATION OF ANGIOGENIC FACTORS BY KINASES: PROTEIN KINASE, MITOGEN ACTIVATED PROTEIN KINASE (MAPK)

MAPK activation is found in cells exposed to mitogens, including bFGF (88). In an another study, it was shown that the reduced activation of MAPK by antisense expression blocks the proliferative action of bFGF in fibroblasts (54). Recently, it was reported that VEGF stimulated phosphorylation of MAPK in rat liver sinusoidal endothelial cells (68). Another study demonstrated the ability of VEGF to up-

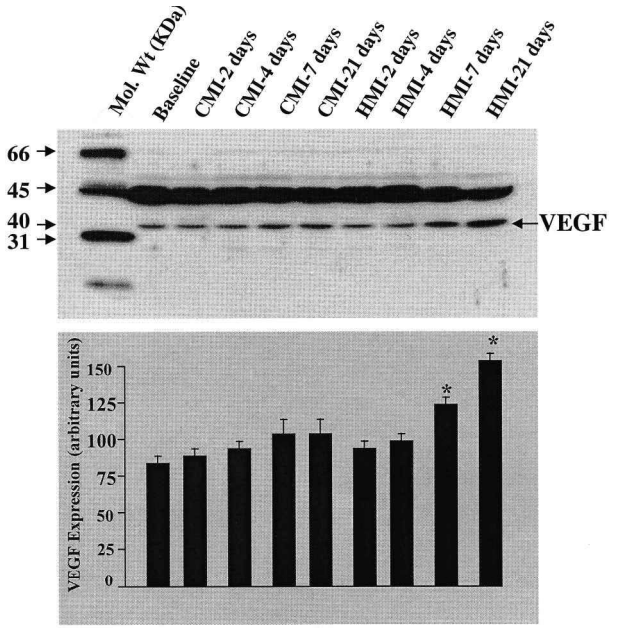

FIG. 4. Representative western blots showing the effects of systemic hypoxia and LAD occlusion on the expression of VEGF in rat myocardium in vivo after 2, 4, 7, and 21 days. VEGF proteins were expressed as 40 kDa. Similar results were obtained in six independent experiments performed in triplicate. Densitometric scanning of blots was used to determine levels of proteins relative to baseline control (sham). $*p < 0.01$ compared with baseline. Baseline, control sham; CMI, normoxia + LAD occlusion; HMI, hypoxia/reoxygenation + LAD occlusion.

regulate Ang-2 through its Flk-1 receptor via the protein kinase C (PKC) and MAPK pathway (45). There are several members of the PKC protein family. However, PKC-α and -β are not observed in adult myocytes, but PKC-ε, -δ, -ζ are present in detectable amounts (58). Recently, it was documented that ischemic preconditioning induced translocation of the PKC-ε isoform to the nucleus and enhanced expression of VEGF mRNA in the infarcted cardiac myocytes, thereby inducing capillary angiogenesis. Chelerythrine, an inhibitor of PKC, inhibited all the effects of ischemic preconditioning, supporting the close association between PKC activation, VEGF mRNA up-regulation, and enhanced angiogenesis (34). It is quite likely that the nuclear translocation of PKC-ε plays a significant role in the induction of VEGF mRNA in the preconditioned rat heart.

ROLE OF CELL ADHESION MOLECULES/CYTOKINES AND INTEGRINS

Cell adhesion molecules are surface proteins involved in intercellular communication among a wide variety of different cell types. Several families of adhesion molecule receptors have been identified, and they include integrins, cadherins, selectins, membrane-associated proteoglycans, and the immunoglobulins. Expression of several endothelial cell integrins in association with components of extracellular matrix are important for the attachment, alignment, and subsequent migration of endothelial cells during angiogenesis (33). Endothelial cells utilize the α1β1 and α2β1 integrins to bind to collagens I, IV and laminin. Using the rabbit cornea and the chick chorioallantoic membrane, it was documented that angiogenesis induced by FGF or by TNFα is dependent on αvβ3. However, angiogenesis initiated by vascular endothelial cell growth, transforming growth factor-α (TGFα), or phorbol ester is dependent on αvβ5. Cadherins are a family of adhesive molecules, present at adherens junctions, which mediate cell–cell contacts. Vascular endothelial cells express two different cadherins: N-cadherin and VE-cadherin. VE-cadherin plays a fundamental role in fibrin-induced or collagen- induced capillary tube formation and is localized at areas of intracellular contact.

Cytokines play a pivotal role in many biological processes, including immune response, recruitment of inflammatory cells, cytotoxicity, antiviral activity, wound repair, angiogenesis, and apoptosis. Neovascularization is a complex process that involves a sequence of events. The entire sequence of events is mediated by various types of cells, including macrophages, lymphocytes, mast cells, platelets, and polymorphonuclear leukocytes (35). Moreover, VEGF, bFGF, insulin-like growth factors, TGF-β and other cytokines such as IL-1, IL-8, and TNFα are thought to be involved in various stages of angiogenesis. IL-1 and IL-8 activate vascular endothelial cells, whereas IL-1 is known to induce corneal collagenase and metalloproteinase expression (21, 84). This eventually plays an important role in extracellular matrix degradation, allowing the formation of new vessesls (35). IL-1 was found to be a potent inducer of corneal angiogenesis (8). Others have shown that IL-8 can also induce neovascularization in the cornea (36). IL-8 was found to contribute to the angiogenic activity of non–small lung cancer cells (7). Recombinant IL-8 mediates endothelial cell chemotactic and proliferative activity *in vitro* and angiogenic activity *in vivo* (36). IL-8 possesses similar angiogenic activity to TNFα, bFGF, angiogenin, angiotropin, and VEGF (36). Adenocarcinoma (A549) and Calu 1 (squamous cell carcinoma) constitutively produce IL-8 *in vitro*, and *in vivo* produce IL-8 in a time-dependent manner that directly correlates with the rate of tumor growth (3). The IL-8 transgenic mice demonstrated a defect in neutrophil recruitment to the peritoneal cavity after intraperitoneal injection of either exogenous IL-8 or thioglycollate (72). The release of IL-1 cytokine after injury was found to induce redox-regulated transcription factors AP-1 and NFκB, which eventually promote expression of genes involved in cell survival, proliferation, and angiogenesis. IL-1α was found to be expressed autonomously by head and neck squamous cell carcinomas (86). IL-1 also induced coexpression of IL-8 through the activation of NFκB and AP-1 and increased cell survival and the growth of head and neck squamous cell carcinomas (86).

ROLE OF ANGIOGENIC COMPONENTS TO ENHANCE CELL SURVIVAL

A recent report suggested VEGF induced expression of Bcl-2, which eventually functions to enhance the survival of

endothelial cells in the toxic, oxygen-deficient environment (53). This report points out that enhanced level of VEGF may have some role in the inhibition of endothelial cell apoptosis. Another very recent investigation demonstrated that VEGF, a potent promoter of angiogenesis, up-regulates the expression of the intracellular adhesion molecule-1 (ICAM-1) through a novel pathway that includes PI3-kinase and AKT resulting in the migration of brain microvascular endothelial cells. It was found that *in vitro* VEGF treatment phosphorylates AKT in a PI3-kinase-dependent manner (20). The PI3-kinase/AKT pathway appears to be a general mediator of cytokine-induced survival and antiapoptotic signals. Recently, proapoptotic factor, BAD, was reported to be phosphorylated by activated AKT on a serine residue causing BAD to dissociate from BCL-X_L. No *in vivo* study has been done so far to investigate the involvement of the PI3-kinase/AKT pathway and endothelial cell survival. Another recent study reported inhibition of endothelial cell apoptosis by Ang-1 via the Akt/survivin pathway, which contributed Ang-1-mediated stabilization of vascular structures during angiogenesis (56). It is also reported that activation of the MAPK pathway together with inhibition of stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) activity by VEGF appears to be a key event in determining whether an endothelial cell is going to survive or undergo programmed cell death. In a very recent study (25), we have documented the effects of hypoxic preconditioning on myocardial angiogenesis, including blood flow and cardiac performance in the myocardial infarction model. The results documented that hypoxic preconditioning improved myocardial performance and angiogenesis at the level of capillary and arteriolar density after 1 week of left anterior descending coronary artery (LAD) occlusion; the effect lasted even after 3 weeks (Figs. 5 and 6). Such angiogenic effects of preconditioning were found to be due to its ability to augment VEGF protein expression, which presumably played a crucial role in reducing endothelial cell apoptosis, a proven determinant for congestive heart failure. Thus, apoptosis may represent a major aspect of the regulatory activity of VEGF on the vascular endothelium for angiogenesis.

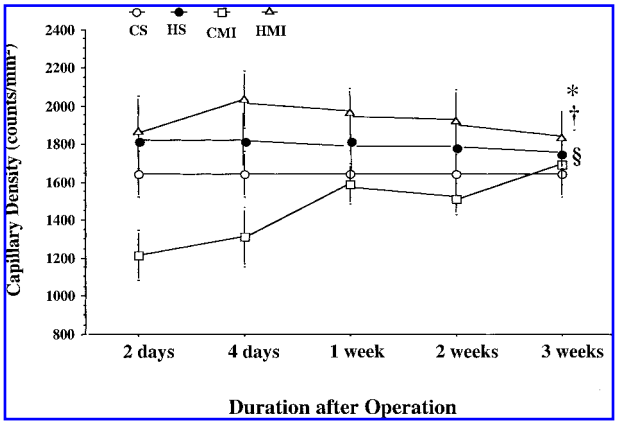

FIG. 5. Left ventricular endocardial capillary density. Tissue sections were processed for CD31 staining. Eight nonoverlapping random fields were selected from endocardial regions of the left ventricle from each heart (magnification $\times 400$, $n = 6$). Images were captured and stored in digital tiff file format for image analysis. $^*p < 0.01$, compared with sham operation; $^\dagger p < 0.01$, compared with CMI; $^\S p < 0.05$, compared with CS. CMI, normoxia + LAD occlusion; HMI, hypoxia/reoxygenation + LAD occlusion; CS, normoxia + sham surgery; HS, hypoxia/reoxygenation + sham surgery. Rats were randomly assigned to the CS and HS served as the sham-operated controls for the CMI and HMI groups and groups, respectively.

SUMMARY AND CONCLUSION

Angiogenesis seems to be induced when the metabolic requirements of the tissue exceed the perfusion capability of existing vessels. It is clear from the above discussion that angiogenesis is a sequence of events and appears to be a tightly regulated process. Both *in vitro* and *in vivo* studies indicate that angiogenic response in vascular tissues is triggered by ROS signaling in a highly coordinated manner. ROS, such as superoxide anions, play an important role in mediating signals initiated by growth factors and inflammatory cytokines. NO affects angiogenesis both directly and indirectly. Redox-sensing transcription factors, such as NFκB, AP-1, and HIF-1, play a pivotal role in modulating angiogenesis. Vascular-derived growth factors, VEGFs, bFGF, TNFs, TNF, and cytokines are general stimulators of angiogenesis, activating many endothelial cell types, and they are quite capable of acting either alone or in concert with other growth factors to ensure various stages of angiogenesis. Inhibition of endothelial cell apoptosis is also an obligatory prerequisite of angiogenesis. Manipulation of this pathway may increase endothelial cell viability in compensatory angiogenesis or facilitate endothelial cell apoptosis and promote vascular regression during tumor angiogenesis.

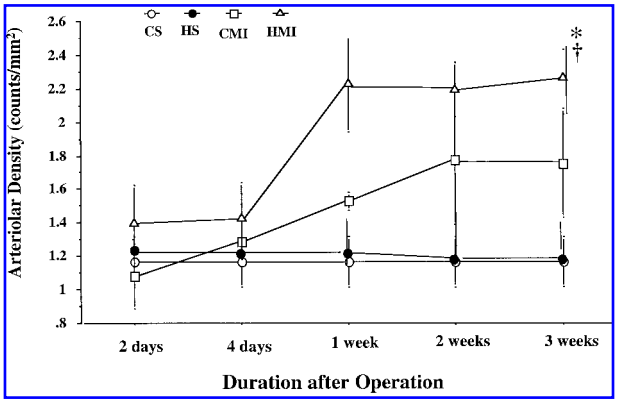

FIG. 6. Left ventricular endocardial arteriolar density. Left ventricular tissue sections were labeled using monoclonal anti-smooth muscle actin, and eight nonoverlapping random fields were selected from endocardial regions of the left ventricle. $^*p < 0.01$, compared with sham operation; $^\dagger p < 0.01$, compared with CMI. CMI, normoxia + LAD occlusion; HMI, hypoxia/reoxygenation + LAD occlusion; CS, normoxia + sham surgery; HS, hypoxia/reoxygenation + sham surgery. Rats were randomly assigned to the CS and HS groups and served as the sham-operated controls for the CMI and HMI groups, respectively.

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ABBREVIATIONS

Ang-1, angiopoetin-1; Ang-2, angiopoetin-2; AP-1, activator protein-1; bFGF, basic fibroblast growth factor; EPO, erythropoietin; FGF, fibroblast growth factor; HCAEC, human coronary arteriolar endothelial cell; HIF, hypoxia-inducible factor; H_2O_2, hydrogen peroxide; IL, interleukin; LAD, left anterior descending coronary artery; MAPK, mitogen-activated protein kinase; NFκB, nuclear factor-κB; NO, nitric oxide; PDTC, Pyrrolidine dithiocarbamate; PI3- kinase, phosphatidylinositol 3-kinase; PKC, protein kinase; ROS, reactive oxygen species; RTKs, receptor tyrosine kinases; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

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