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Forum Review

Oxidative Stress and Hypoxia: Implications for Plasminogen Activator Inhibitor-1 Expression

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ABSTRACT

Plasminogen activator inhibitor-1 (PAI-1) is the major physiological inhibitor of urokinase-type and tissue-type plasminogen activators. It has gained special interest among clinicians because a number of pathological conditions, such as myocardial infarction, atherosclerosis, thrombosis, several types of cancer, and the metabolic syndrome, as well as type 2 diabetes mellitus, are associated with increased PAI-1 levels. Interestingly, a number of these diseases are also accompanied by oxidative stress and the enhanced production of reactive oxygen species or tissue hypoxia. This article tries to summarize some aspects leading to enhanced PAI-1 production under oxidative stress or hypoxia. *Antioxid. Redox Signal.* 6, 777–791.

INTRODUCTION

After occurrence, a hemostatic plug needs to be removed by the fibrinolytic system, which requires the proteolytic action of plasmin on fibrin and fibrinogen. The fibrinolytic system includes the reactions of plasminogen activation, fibrin degradation, and plasmin inactivation. Plasminogen is converted to plasmin by two different serine proteases: tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). The conversion of plasminogen to plasmin occurs after binding of both plasminogen and tPA to fibrin and the cleavage of a single peptide bond (Arg561–Val562) within plasminogen. Once plasmin is activated, it reacts with its principal substrate fibrin, thus splitting it into several fibrin degradation products (for reviews, see 15, 66).

The action of tPA and uPA is opposed, in part, by plasminogen activator inhibitors (PAIs), which belong to the serine protease inhibitor (serpin) superfamily (68) (Fig. 1). Whereas two types of PAIs, named PAI-1 and PAI-2, can be identified based on biochemical, immunological, and molecular biological properties, it appeared that two other proteins also have PAI activity: protein C inhibitor (PAI-3) and the protease nexin-1. Among the identified inhibitors, PAI-1 is the major PAI and can be secreted from platelets, vascular endothelial cells, vascular smooth muscle cells (VSMC), and several nonvascular cell types, among them hepatocytes (for review, see 66). PAI-1 exists in an active, latent, and inactive conformation and circulates with uPA or tPA in 1:1 complexes that are cleared by hepatocytes. Additionally, PAI-1 can be found within the extracellular matrix bound to vitronectin or other nonprotease ligands, such as heparin or scavenger receptors from the low-density lipoprotein (LDL) receptor family (78). These features may contribute to the importance of PAI-1 not only for the regulation of fibrinolysis.

Although mainly known due to its role in fibrinolysis, the plasminogen/plasmin system is also involved in the conversion of a number of molecules, such as laminin, collagen type IV, hepatocyte growth factor, or transforming growth factor (TGF), which are involved in extracellular matrix turnover, proliferation, and tissue remodeling processes (for review, see 15). Thus, PAI-1 appears to be a key inhibitor not only of fibrinolysis, but also of proteolytic processes, which are associated with neovascularization, tissue remodeling, and regeneration, such as inflammation, cancer metastasis, and fibrosis (Fig. 1).

The importance of the balance between plasminogen activators (PAs) and PAI-1 has been emphasized by several clini-

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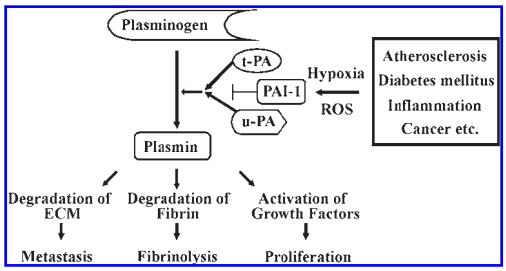

FIG. 1. PAI-1 in health and disease. Plasminogen is activated by tPA and uPA to yield plasmin. The broad-spectrum protease plasmin has a crucial role in the control of fibrinolysis, proliferation, and even metastasis. PAI-1 as a major physiological inhibitor of plasminogen activation has therefore a major regulatory role in these processes. Increased PAI-1 plasma levels are correlated with severe diseases, which are often associated with tissue hypoxia or induced formation of ROS. ECM, extracellular matrix.

cal studies: decreased PAI-1 levels are associated with bleeding diathesis, whereas increased PAI-1 levels have been found in a number of clinical conditions, such as atherosclerosis, coronary heart disease, deep vein thrombosis, acute and chronic inflammatory lung disorders, chronic renal diseases, sepsis, hemorrhage, and cancer metastasis (for review, see 129). Moreover, enhanced PAI-1 levels are also found in patients with insulin-resistant states, such as metabolic syndrome or diabetes type 2 (57).

As clinical conditions such as hemorrhage, prethrombotic events, and thrombus formation are associated with hypoxia, it was assumed that hypoxia might be a direct trigger for PAI-1 expression. In contrast, many other pathological conditions under which PAI-1 levels are enhanced, such as different cardiovascular, pulmonary, and renal diseases or even diabetes mellitus, are associated with oxidative stress and enhanced generation of reactive oxygen species (ROS) (21, 29, 80, 89, 100). Thus, it appears that hypoxia and a reduction in ROS levels, on the one hand, or oxidative stress and an increase in ROS levels, on the other hand, may trigger PAI-1 expression, pointing to a role of ROS as second messengers (Fig. 1). The ability of a cell to cope against varying oxygen tensions or oxidative stress requires a signaling cascade(s) with an elaborate sequence of adaptive mechanisms. Within those ROS-dependent signaling cascades, the regulatory transcription factor(s) become important because they are modified by several chemical modifications, such as phosphorylation, hydroxylation, or oxidation. Within this article, we will try to summarize the signaling mechanisms involved in hypoxia- or oxidative stress-induced PAI-1 gene expression.

ROS AS POISONS AND SIGNALING COMPONENTS

Molecular oxygen is an essential molecule for energy metabolism in all aerobic organisms. Due to its low redox potential, it serves mainly as the final electron acceptor within an electron transfer chain required for building the necessary proton gradient to phosphorylate ADP to ATP. When O_2 is not completely reduced to H_2O, the process of transferring electrons results in the production of either oxygen-containing free radicals or reactive nonradical compounds, which are referred to as ROS. The production of ROS such as superoxide anion radicals ($O_2^{-\bullet}$) may further result in a cascade with generation of other ROS, such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^\bullet$), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl), and singlet oxygen ($^1O_2$) (Fig. 2).

ROS are often generated as by-products of various reactions in several cellular compartments, such as mitochondria, endoplasmic reticulum, cytosol, nuclear and cell membranes, and peroxisomes (for review, see 120). Cellular production of ROS can be enzymatic and nonenzymatic. In mammalian cells, potential enzymatic sources of ROS include not only those involved in cellular respiration, but also enzymes of the arachidonic acid pathway, lipoxygenase and cyclooxygenase, the enzymes of the cytochrome P450 family, glucose oxidase,

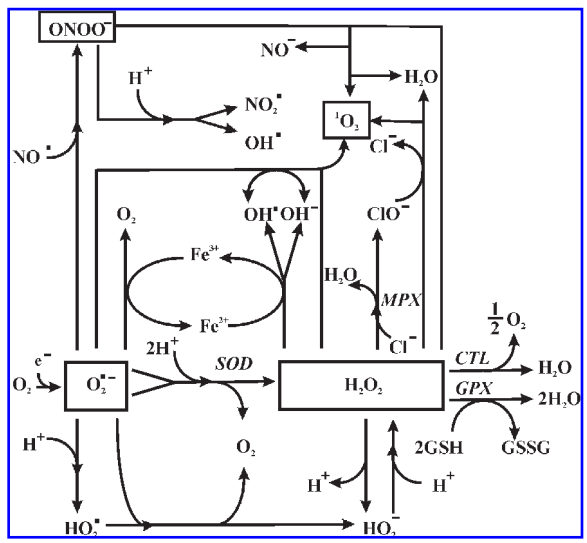

FIG. 2. Generation of ROS. When O_2 accepts an electron, *e.g.,* from semiquinones, flavins, or oxidases of the cytochrome P450 family, superoxide anion radicals ($O_2^{-\bullet}$) are formed. Two molecules of $O_2^{-\bullet}$ are then dismutated either spontaneously or by the action of superoxide dismutase (SOD) to form hydrogen peroxide (H_2O_2). The weak base $O_2^{-\bullet}$ can also be protonated to give the hydroperoxyl radical ($HO_2^\bullet$). Then $O_2^{-\bullet}$ and $HO_2^\bullet$ disproportionate to the basic hydrogen peroxide (HO_2^-), which exists at neutral pH in its protonated form H_2O_2. H_2O_2 can also take part in the Fenton reaction where hydroxyl radicals ($OH^\bullet$) and hydroxyl anions (HO^-) are formed in the presence of metal ions (Fe^{2+}) that can be reduced by $O_2^{-\bullet}$. $O_2^{-\bullet}$ reacts with H_2O_2 or OH^- to produce singlet oxygen ($^1O_2$). $O_2^{-\bullet}$ can also react with nitric oxide ($NO^\bullet$), another radical, to form peroxynitrite anions ($ONOO^-$), which are less stable than H_2O_2 and break into nitrogen dioxide radicals ($NO_2^\bullet$) and $OH^\bullet$. Further, $ONOO^-$ can react with H_2O_2 to form $^1O_2$, nitroxyl anion (NO^-), and water. $^1O_2$ can also be formed by the reaction of H_2O_2 with hypochlorite (ClO^-), which is formed in the myeloperoxidase (MPX) reaction from Cl^- and H_2O_2. As H_2O_2 gives rise to the formation of the highly reactive $OH^\bullet$, ClO^- and $^1O_2$, it is usually detoxified in cells by the action of glutathione peroxidase (GPX) or catalase (CTL).

amino acid oxidase, or xanthine oxidase, NADH/NADPH oxidases, nitric oxide (NO) synthase, and other hemoproteins (for review, see 19). The main nonenzymatic sources of ROS are radiation [*e.g.,* ultraviolet (UV) light], toxic chemicals, and drugs.

Under normal physiological conditions, formation and removal of ROS are balanced, but it appears that a concentration threshold exists above which the production of ROS prevails over the endogenous antioxidant defense system (Fig. 3). This condition is commonly referred to as oxidative stress, which was defined as a disturbance in the prooxidant–antioxidant balance in favor of the former (18). Oxidative stress is accompanied by harmful processes for the cells, such as DNA, protein, and polysaccharide damages, inhibition of enzymes, lipid peroxidation, uncontrolled proliferation, and apoptosis (for review, see 43). To counteract excess ROS formation, cells have an efficient antioxidant defense system consisting of enzymes, such as superoxide dismutases (SODs), glutathione peroxidases, and catalase (Fig. 2), and exogenously taken up micronutrients and vitamins.

Although high levels of ROS may be injurious, ROS are also important determinants for normal growth and metabolism in a variety of cells. Over the last decade, it has been shown that quite frequently the action of a hormone, cytokine, and growth or coagulation factor is mediated not only by its own redox-insensitive receptor-signaling chain, but also by ROS. A number of studies have shown that the action of epidermal growth factor (11), platelet-derived growth factor (PDGF), tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1) (115), or thrombin (46) involves the production of ROS, thus pointing to a role of ROS as signaling molecules. Thus, an increase in intracellular ROS levels has two potentially important effects: damage of various cellular components or triggering of the activation of specific signaling pathways, thus ending up with the enhanced transcription of a number of genes, among them the PAI-1 gene. By contrast, hypoxia that is associated with low levels of ROS (36, 38) can be responsible for either inactivation of the ROS-stimulated pathways or activation of other signaling cascades inducing PAI-1 gene transcription.

Although it might be possible that various signaling cascades may respond to ROS, it appears that the transcriptional response of PAI-1 to ROS can be mediated through the mitogen-activated protein kinase (MAPK) and protein kinase B (PKB) pathways followed by stimulation of several transcription factors. The involvement of these two pathways in the ROS-dependent regulation of PAI-1 expression under oxidative stress is still not completely known. However, the role of MAPK and PKB signaling in the thrombin-induced ROS-dependent PAI-1 expression is supported by a few studies (46, 47, 60).

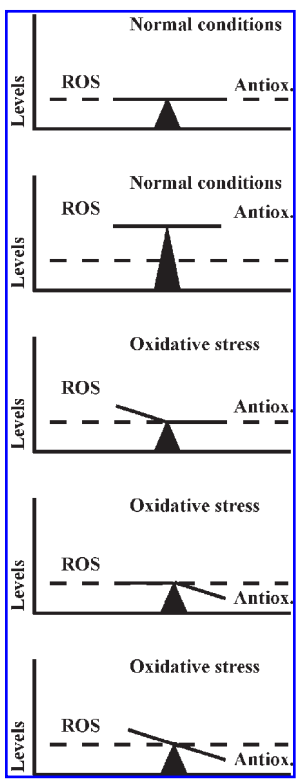

FIG. 3. Balance of ROS and antioxidants. Under normal physiological conditions, ROS formation and their removal by the antioxidant defense system are balanced at a certain level. When levels of both ROS and antioxidants rise to the same extent, no oxidative stress occurs. In general, oxidative stress is a consequence of either predominant production of ROS or depletion of antioxidants.

REDOX- AND HYPOXIA-SENSITIVE KINASES

MAPK

The MAPKs are a large family with three well characterized major pathways, including extracellular signal-regulated kinases (ERKs)-1/2, c-Jun NH_2-terminal kinase (JNK1/2/3), and p38 (p38α/β/γ/δ) MAPKs pathways. A fourth MAPK pathway in which ERK5 was involved was relatively recently identified and is the subject of intensive research. Each pathway converts various extracellular signals into intracellular responses through serial phosphorylation cascades. In general, the ERK pathway mediates cellular responses to growth and differentiation factors, including PDGF and IL-5, whereas the JNK and p38 kinases are activated by stress-related stimuli, such as heat shock, inflammation, hyperosmolarity, and UV and γ irradiation (for review, see 69).

Oxidative stress generated by exogenous application of H_2O_2, diamide, hemin, or ionizing radiation appears to be a powerful activator of ERK1/2 in different cells, including bovine tracheal monocytes (4), HeLa (123), rat oligodendrocytes (CG-49) (14), rat VSMC (90), NIH 3T3 (114), and many other cells (6). Interestingly, ERK5 has been classified as a redox-sensitive kinase based on its activation by H_2O_2 in human and rat smooth muscle cells, human umbilical vein endothelial cells (HUVEC), and fibroblasts (2, 3).

Moreover, environmental stress, such as radiation, H_2O_2, and growth factors, activates JNK1–3 (for review, see 6). The JNKs were discovered to bind and phosphorylate the DNA-binding protein c-Jun, which is a component of the activator protein (AP-1) transcription complex. AP-1 is an important

regulator of gene expression and contributes to the control of many genes. However, regulation of the JNK pathway appears to be extremely complex and is tuned by the action of >10 MAPK kinase kinases (MKKKs). Thus, due to this diversity of MKKKs, a wide range of stimuli allows the activation of the JNK pathway.

The p38 kinases were identified as modulators of TNF signaling (51, 88), but meanwhile p38 MAPKs were found to be activated by many other stimuli, including stresses such as oxidative and chemical stress, osmotic and heat shock, as well as hormones. There are at least four p38 kinases named α, β, γ and δ, of which the p38α enzyme is the best characterized. It was shown that H_2O_2 activated the p38 MAPK pathway in different cells (for review, see 6).

Interestingly, MAPKs were shown to be activated under hypoxia in different cell types and are therefore possible candidates for transmission of the hypoxic signal where ROS levels appear to be reduced (71, 92, 93, 111).

The role of ERKs to mediate regulation of PAI-1 expression was demonstrated as a response to TGF-β1 in NIH 3T3 fibroblasts (81), insulin in HepG2 cells (12), and TNF-α in HUVEC (50). In bovine aortic endothelial cells (BAEC), the dominant role of the p38 kinase for the activation of PAI-1 gene expression was shown (25). As MAPKs are involved in PAI-1 regulation and are redox-sensitive, it can be suggested that MAPKs are ROS signal transducers responsible for PAI-1 activation. However, the involvement of ROS in the induction of PAI-1 via MAPK was not directly investigated.

PKB (Akt)

PKB, also known as Akt due to its homology to the v-akt oncogene, is a serine/threonine kinase playing a key role in many cellular processes including energy metabolism and cell survival. PKB exists in three isoforms, PKBα/Akt1, PKBβ/Akt2, and PKBγ/Akt3, of which PKBα is the predominant isoform. PKB directly or indirectly controls the activity of many transcription factors. Direct phosphorylation activates cyclic AMP responsive element binding protein (33) and inactivates Foxo forkhead transcription factors (67). The indirect mechanism via inhibition of glycogen synthase kinase 3 has been shown to enhance AP-1 and nuclear factor-κB (NF-κB) activity (63).

Similar to p38 MAPK, both exogenous H_2O_2 and growth factors can activate PKB in different cell types, including NIH 3T3 fibroblasts, human embryonic kidney 293 cells, HeLa, and Jurkat cells (32). However, the finding, that H_2O_2 induced PKB (28) and p38 phosphorylation (131), together with the finding that MAPK-activated protein kinase-2, a substrate of p38, can phosphorylate PKB/Akt *in vitro* (5), suggested that oxidative stress may activate PKB either directly or via p38.

Similar to oxidative stress, hypoxia also was able to activate PKB in a phosphatidylinositol 3-kinase (PI3-K)-dependent manner (71, 76). Likewise, the PKB pathway is involved in the regulation of the nerve growth factor-induced PAI-1 expression in rat pheochromocytoma PC12 cells (118) and insulin-induced PAI-1 expression in primary rat hepatocytes (PH) (62). It was shown that wortmannin and LY294002, two PI3-K inhibitors, were able to attenuate the hypoxia-dependent up-regulation of PAI-1 gene expression, thus pointing to a role of ROS as upstream regulators of the PI3-K/PKB pathway (61, 62).

In general, the mechanisms for redox regulation of the MAPK pathways and the PKB pathway are complex and not yet resolved until the last detail, thus requiring further investigations.

REDOX- AND HYPOXIA-SENSITIVE TRANSCRIPTION FACTORS

Several transcription factors have been implicated to be involved in the redox- and hypoxia-dependent modulation of gene expression. Among these regulatory proteins, NF-κB, AP-1, the promoter-specific transcription factor Sp1, and the hypoxia-inducible factor-1 (HIF-1) appear to play central roles. Whereas the role of NF-κB and Sp1 in stress-dependent, as well as HIF-1 in hypoxia-dependent, PAI-1 regulation was shown, no data are available concerning AP-1 involvement either in ROS-dependent or in hypoxia-dependent PAI-1 regulation.

NF-κB

NF-κB denotes a family of transcription factors composed of homodimers or heterodimers, consisting of several related proteins known as p50, p52, RelA (p65), Rel B, and c-Rel. They all contain the so-called Rel homology domain, which mediates dimerization, specific DNA recognition, and the interaction with an inhibitory subunit (IκB). IκB prevents nuclear translocation of the protein and keeps it in an inactive form in the cytosol. The known mammalian forms of IκB are IκBα, IκBβ, IκBγ (p105), IκBδ (p100), IκBϵ, and BCL-3 (for review, see 16).

In general, NF-κB is activated when the IκB subunit is phosphorylated, ubiquitinated, and degraded. This occurs commonly via activation of the IκB kinase complex (IKK), consisting of the two catalytic subunits, IKKα and IKKβ, as well as the regulatory IKKγ/NEMO subunit. Once phosphorylated at two conserved serines in the N-terminal regulatory domain, IκB is ubiquitinated by a specific ubiquitin ligase, which targets IκB for degradation in the proteasome. This process releases NF-κB heterodimers, which translocate to the nucleus and activate transcription (for review, see 45, 58).

So far, two exceptions to this model of NF-κB activation are known: one is observed at NF-κB activation by UV radiation (there is no IκB phosphorylation) (72), and another one is under anoxia (phosphorylation at Tyr42) (54).

NF-κB has long been considered as a redox-sensitive transcription factor since some available data confirmed a proposed model of NF-κB activation by oxidative stress (104). This model is based on the observations that most agents activating NF-κB are known to trigger formation of ROS and that compounds with antioxidant properties can block NF-κB activation. Furthermore, in certain cell lines, including a subclone of Jurkat T-cells (105), HeLa (77), L929 fibroblasts (106), F26 fibroblasts (103), human breast MCF-7 cells, 7OZ/3 pre-B cells, and an EBV-transformed human B cell line (107), NF-κB activation was achieved by treatment with H_2O_2. In contrast, in a number of other cell types, such as

monocytic cells, astrocytoma, standard Jurkats, J-Jhan lymphoblastoid T cells, EL4.NOB-1 T cells, KB epidermal cells, and HUVEC, H_2O_2-dependent NF-κB activation was not detected (for reviews, see 16, 73). Therefore, it seems likely that H_2O_2-induced NF-κB activation is cell type-dependent. Moreover, in a recent study, it was demonstrated that antioxidants such as *N*-acetylcysteine (NAC) and pyrrolidine dithiocarbamate (PDTC) inhibited NF-κB activity independently of their antioxidant properties. NAC blocked TNF receptor-induced signaling by lowering the receptor affinity, whereas PDTC inhibited IκB-ubiquitin ligase activity (52). Thus, NF-κB activation does not seem to be a universal response to oxidative stress.

Despite the contradictions discussed above, it was anticipated that the PAI-1 gene might be regulated by NF-κB in response to oxidative stress. Although the human PAI-1 promoter does not contain a well established NF-κB binding site, it was shown that a NF-κB-like binding site 5′-T**GGGGA**GTCAGC-3′ (−678/−667) in the human PAI-1 promoter was responsible for the IL-1-dependent stimulation of an 805-bp PAI-1 promoter-driven chloramphenicol acetyltransferase construct in HepG2 cells. Further, in electrophoretic mobility shift assays, the NF-κB-binding sequence from the histocompatibility complex class II-associated invariant chain 5′-A**GGGGA**CTTTCC-3′ (86) gene competed with the PAI-1 NF-κB-like sequence 5′-T**GGGGA**GTCAGC-3′ for binding of nuclear proteins (30). However, the question whether this complex specifically contains the NF-κB subunits remains open. Thus, to what extent NF-κB contributes to PAI-1 gene expression requires further investigations.

AP-1

AP-1, which regulates genes associated with growth, differentiation, and cellular stress, is a complex formed by heterodimerization of Fos–Jun proteins or homodimerization of Jun–Jun proteins belonging to the family of basic leucine zipper domain-containing transcription factors (for review, see 110).

It has been reported that AP-1 can be activated by treatment of cells with ROS-generating agents, such as H_2O_2, UV radiation, TNF-α, and basic fibroblast growth factor (74, 83, 113). Antioxidants, such as thioredoxin, PDTC, and butylated hydroxyanisole, were also shown to activate AP-1 in HeLa and L929 cell lines (101).

ROS have been demonstrated to regulate AP-1 through several mechanisms. *In vitro* experiments have revealed that the Cys154 in c-Fos and Cys272 in c-Jun are critical redox-sensitive sites within the two proteins. Both dimerization partners can be converted to an inactive state by chemical oxidation of these cysteine residues (1). Further, it has been shown that a nuclear protein called Ref-1 (redox factor 1) facilitated AP-1 DNA binding activity due to redox modification of these cysteine residues in the DNA-binding domains of Fos and Jun proteins (127). Moreover, redox regulation of AP-1 can be achieved via the JNK cascade because it was shown that thioredoxin acts as an interacting partner of apoptosis signal-regulating kinase (ASK) 1, which activates JNK and also p38 MAPK pathways (42, 96). Within this pathway, oxidative stress such as H_2O_2 disrupts the ASK1–thioredoxin complex by oxidization of thioredoxin and thereby activates ASK1 (121) and subsequently JNK and p38. Thus, AP-1 appears to be a redox-sensitive transcription factor, which is modified either directly at redox-sensitive sites or indirectly via redox-sensitive upstream signaling components.

The human PAI-1 promoter contains an AP-1 binding site (−58/−50) that can be bound by c-Jun homodimers (10, 31). Thus, the presence of this site makes it likely that conditions leading to either enhanced or reduced ROS levels regulate PAI-1 transcription through AP-1 complexes. It was shown that the AP-1 site was responsible for the effects of IL-1 and phorbol 12-myristate 13-acetate (10, 59). However, these studies did not show that ROS were involved in the signaling chains mediating these effects.

Sp1

Sp1 was one of the first cloned and characterized mammalian transcription factors. It recognizes and specifically binds to GC-rich boxes within a number of promoters via three Cys2His2 zinc-finger motifs. Similarly, it was found that many developmental regulators, including the embryonic pattern regulator Krüppel from *Drosophila,* shared the zinc-finger motifs highly similar to those of Sp1, thereby defining a novel class of Sp1-like proteins or Krüppel-like factors. Sp1 appears to be ubiquitously expressed and plays a key role in maintaining basal transcription of a class of genes lacking the TATA or CAAT box in their promoters, among them dihydrofolate reductase, thymidylate synthase, and adenine deaminase (117).

It has been shown that, in metal-depleted nuclear extracts from human K562 cells, Sp1 DNA-binding activity is significantly decreased after incubation with H_2O_2 and glutathione (64). Another study reported that, in rat liver, H_2O_2 treatment also decreased Sp1-binding activity and this activity was restored by the treatment with dithiothreitol (7). In contrast, in cortical neurons, the basal Sp1 DNA-binding activity was low, whereas it was increased by glutathione depletion and H_2O_2 (95). The redox regulation of Sp1 occurs through reversible oxidation of cysteine residues in the interaction domain of this protein (126). Although the involvement of Ref-1 remains to be clarified, this redox-sensitive regulation via cysteine residues seems to be similar to that found in AP-1 and HIF-1α.

The human PAI-1 promoter was shown to contain two Sp1 binding sites (Sp1a, −76/−71 and Sp1b, −46/−41) that mediated responses to glucose and angiotensin II (24, 79) and were involved in the ROS-dependent activation of PAI-1 (34) (see below).

HIF(s)

HIF-1 is a dimer of HIF-1α and HIF-1β [arylhydrocarbon receptor-nuclear translocator (ARNT)], both belonging to the basic helix–loop–helix Per-ARNT-Sim transcription factor family. Two other HIF α-subunits (HIF-2α and HIF-3α), as well as two other ARNT isoforms (ARNT2 and ARNT3), have been identified. They may then give rise to the formation of several HIF isoforms composed of different α-subunits and ARNT isoforms (for review, see 124). Although other HIF isoforms appear to exist, HIF-1 is considered the

major regulator of physiologically important genes like those encoding erythropoietin, vascular endothelial growth factor, and PAI-1.

Oxygen sensitivity of the HIF-1 complex is conferred only by the HIF-1α protein via regulation of its protein stability and coactivator recruitment. Both regulation of protein stability and coactivator recruitment involve hydroxylation reactions carried out by specific prolyl and asparaginyl hydroxylases (17, 70). In addition to the O_2-dependent hydroxylation reactions that enable binding of the von Hippel–Lindau tumor suppressor protein and subsequent proteasomal degradation (75), it was found that HIF-1α can be modified by Ref-1 (53) to interact with coactivators such as CBP/p300, TIE-2, and SRC-1 in a redox-dependent manner (20, 37), thus suggesting a role for ROS in oxygen signaling.

The concept that ROS play an important role was further supported by findings demonstrating that addition of H_2O_2 to cells grown under hypoxia resulted in the destabilization of HIF-1α in Hep3B cells (53) and HIF-2α in HeLa cells (125). Furthermore, treatment with the antioxidants PDTC and NAC increased HIF-1α levels in alveolar type II epithelial cells (49). The redox processes modifying both HIF-1α and HIF-2α appeared to affect predominantly the C-terminal transactivation domain (20, 37). Within this domain, the Cys800 of HIF-1α and the Cys848 of HIF-2α seem to be critical for transactivation. Similar to AP-1, the oxidation/reduction state of these cysteines is dependent on the presence of Ref-1 (20, 37, 53, 119). Thus, it appears likely that HIF-1α may be also a redox-sensitive transcription factor. HIF-1 was shown to mediate the hypoxia-dependent induction of the rat PAI-1 and human PAI-1 gene by binding to the so-called hypoxia-responsive element-2 (HRE-2) (41, 60). Interestingly, growth factors, hormones, and coagulation factors, including PDGF, angiotensin II, insulin, and thrombin, as well as H_2O_2, were able to stimulate the HIF pathway in a ROS- and/or PI3-K/PKB-dependent manner independent from the O_2 tension (46, 62, 93). Futhermore, the MAPK/PKB pathways appeared also to influence the recruitment of the cofactor p300 (94, 99). This suggested a cross talk between these agonist-dependent pathways and the O_2 signaling cascade leading to PAI-1 expression via activation of HIF.

ROS-DEPENDENT PAI-1 EXPRESSION

The role of ROS in the regulation of PAI-1 gene expression was shown in a number of studies performed using different cell types, such as endothelial, kidney tubule epithelial, or glomerular mesangial cells, adipocytes, hepatocytes, and HepG2 hepatoma cells.

ROS-dependent PAI-1 expression in endothelial cells

Vascular endothelial cells were often used as a model system and showed induction of PAI-1 by ROS (22, 26, 34, 84, 116, 128). In these studies, oxidative stress was generated using a variety of agents that caused overproduction of ROS by different mechanisms. H_2O_2 can be generated as a by-product of oxidative hydroxylation of hypoxanthine to xanthine catalyzed by xanthine oxidase [xanthine oxidase/hypoxanthine (XO/HX) system]. Cytokines IL-1β and TNF-α induced generation of ROS in endothelial cells via not yet clarified pathways, which might involve either overproduction of ROS in the mitochondrial respiratory chain or activation of NADPH oxidase (23, 27). Hyperglycemia also can stimulate the formation of ROS by enolization of glucose (22) or by induction of superoxide production in mitochondria (34, 82). Copper- and oxygen-dependent oxidation of the amino acid homocysteine increases H_2O_2 generation (112). Nickel released from nickel subsulfide (Ni_3S_2) reacts with cytoplasmic proteins, and interaction of these Ni^{2+}–protein complexes with oxygen results in hydroxyl radical formation (85). A mechanical deformation with sinusoidal negative pressures was also used as a stimulus for ROS production, although the mechanisms behind it remained unknown (26) (Fig. 4).

In particular, in one of those studies, treatment of rat primary cardiac microvascular endothelial cells (CMEC) with XO/HX and with H_2O_2 induced PAI-1 protein synthesis and activity (84). In the same study, it was demonstrated that IL-1β induced generation of ROS such as superoxide anion radicals, H_2O_2, and hydroxyl radicals and that these ROS were responsible for the accumulation of PAI-1 in CMEC. The hydroxyl radical scavenger tetramethylthiourea (TMTU) inhibited IL-1β-mediated PAI-1 production (84).

A similar effect on PAI-1 expression was observed in a study using another proinflammatory cytokine, TNF-α (116). In the human endothelial cell line EA.hy 926, TNF-α induced

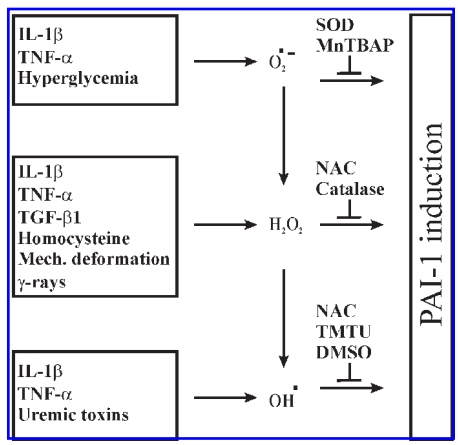

FIG. 4. Induction of PAI-1 via ROS. Different stimuli, including cytokines, chemical compounds (glucose, homocysteine, uremic toxins), and environmental stress, were reported to induce PAI-1 gene expression via overproduction of ROS such as $O_2^{-\bullet}$, H_2O_2 and $OH^{\bullet}$. These effects were shown to be blocked by application of antioxidants (NAC, TMTU, DMSO) and scavenging enzymes (SOD and its mimetic MnTBAP, catalase). Depending on the cell type and stimulus used, PAI-1 can be activated either by production of all three ROS, $O_2^{-\bullet}$, H_2O_2, and $OH^{\bullet}$, or only by overproduction of one of them. For instance, IL-1β stimulates PAI-1 induction via all these ROS in CMEC, but only via H_2O_2 in GMC. Many studies did not investigate the involvement of all three ROS. Therefore, it is likely that some of the stimuli might act via more ROS than presented.

PAI-1 mRNA, protein, and 800-bp human PAI-1 promoter-driven luciferase (Luc) gene expression through an increase in ROS production (116). The effect of TNF-α was mimicked by the treatment of the cells with H_2O_2 and abolished by the administration of NAC.

In contast to the acute generation of ROS by cytokines, chronic oxidative stress caused by the exposure of cultured HUVEC to homocysteine for 15 days accelerated the rate of cellular senescence and increased PAI-1 protein expression (128). These effects were inhibited by exogenous treatment of the cells with catalase, thus showing the possible involvement of H_2O_2.

Furthermore, overproduction of ROS, including H_2O_2, caused by the mechanical deformation of HUVEC grown on a flexible membrane base led to the fivefold induction of PAI-1 release, and this effect was inhibited by the antioxidant NAC (26).

In addition to the more direct effects of ROS on the regulation of PAI-1 expression, the indirect involvement of ROS via oxidation of LDL was also investigated, and the results were conflicting. One group found that the oxidative modification of the native, as well as glycated, LDL enhanced production of PAI-1 in HUVEC and human coronary artery endothelial cells (91, 132). Treatment of these cells with antioxidants (butylated hydroxytoluene or vitamin E) blocked LDL-induced changes in PAI-1 expression (91).

However, in another study, it was shown that HUVEC exposure to unoxidized LDL, as well as to progressively oxidized LDL, increased PAI-1 secretion, and the induction was not abolished by the preenrichment of LDL with antioxidants (probucol and α-tocopherol with vitamin C) (13). The discrepancy between these studies could be explained by the different procedures adopted for LDL modification. In one study, cells were treated with LDL glycated *in vitro* by incubation with glucose for 2 weeks. Glycated LDL was used because it was shown that glycation accelerated the oxidation of LDL *in vivo* (65, 91). In another study, LDL was exposed to oxidation by incubation with endothelial cells and then isolated from medium (13). Thus, the question whether oxidative modification of LDL, which may have an impact for several diseases, affects PAI-1 regulation remains to be clarified.

Interestingly, one study demonstrated not induction, but ROS-dependent inhibition of PAI-1 gene expression in HUVEC. ROS were produced in HUVEC after sublethal anoxia (95% N_2, 5% CO_2 for 2–24 h) and subsequent reoxygenation (95% air, 5% CO_2 for 24 or 48 h). This overproduction of ROS was responsible for the repression of PAI-1 mRNA, protein secretion, and activity during reoxygenation (108). SOD, when added during anoxia, prevented the subsequent decrease in PAI-1 activity, whereas the iron chelator deferoxamine mesylate did not prevent PAI-1 decrease. This implicated that superoxide anions, but not hydroxyl radicals produced in a Fenton reaction in the presence of iron (Fig. 2), were necessary for PAI-1 inhibition.

The lack of PAI-1 enhancement by ROS overproduction was also reported. Treatment of primary cultures of HUVEC with XO/HX for 15 min showed no effect on PAI-1 mRNA, secretion, and activity (109). In human bronchial epithelial cells BEAS-2B, nickel subsulfide (Ni_3S_2) treatment led to the higher production of ROS, such as H_2O_2 (8, 9). However, it was demonstrated that antioxidants (NAC and ascorbic acid), SOD, or NADPH oxidase inhibitors did not block induction of PAI-1 by nickel. Instead, in BEAS-2B cells, nickel induced PAI-1 expression via two separate pathways involving either AP-1 or ERK/HIF-1α in a ROS-independent way (8, 9).

Thus, the ROS involvement in PAI-1 gene regulation in endothelial cells was reported in a number of studies, although some exceptions were found. A general view cannot yet be reached, and a direct comparison of these studies is difficult because various stimuli and endothelial model systems were used (Table 1). Further, the delineation of the signaling cascades mediating the ROS effects remain incomplete, thus leaving a number of unresolved questions.

ROS-dependent PAI-1 expression in other cell types

The ROS-dependent induction of PAI-1 expression was also investigated in kidney tubule epithelial or glomerular mesangial cells (GMC) (35, 56, 80, 133). Again, oxidative stress was generated using different stimuli, such as γ-rays, cytokines (IL-1β, TGF-β1, and TNF-α), and uremic toxins. The effects of ionizing radiation can lead to production of various radicals, including superoxide, H_2O_2, hydroxyl radicals, and peroxyl and alkoxyl radicals.

Exposure of rat kidney tubule epithelial NRK52E cells to γ-rays for 24 and 48 h led to a dose-dependent increase in PAI-1 mRNA and protein levels (133). A similar induction was observed upon treatment of NRK52K cells with H_2O_2, whereas treatment with the antioxidant NAC or overexpression of catalase inhibited the radiation-induced increase in PAI-1 expression. In contrast, overexpression of mitochondrial Mn-SOD did not modulate the radiation-induced up-regulation of PAI-1, suggesting that cytoplasmic H_2O_2 rather then superoxide was involved in the regulation of PAI-1 expression in NRK52K cells (133).

TABLE 1. INDUCTION OF PAI-1 BY ROS

Cell type	*Stimulus*	*Effect on PAI-1 expression*	*Reference*
HUVEC	Hyperglycemia	Induction	22
HUVEC	Homocysteine	Induction	128
HUVEC	Mechanical deformation	Induction	26
HUVEC	Anoxia/ reoxygenation	Inhibition	108
HUVEC	XO/HX	No effect	109
BAEC	Hyperglycemia	Induction	34
BEAS-2B	Nickel	No effect	8, 9
CMEC	IL-1β	Induction	84
EA.hy 926	TNF-α	Induction	116
GMC	IL-1β	Induction	35
GMC	TGF-β1	Induction	56
HepG2/PH	Rac-1	Inhibition	47
HK-2	Uremic toxins	Induction	80
NRK52E	Ionizing radiation	Induction	133
3T3-L1	TNF-α	Induction	97
VSMC	Thrombin	Induction	46

Treatment of the rat GMC with IL-1β led to the ROS-dependent induction of PAI-1 mRNA (35). The effects of IL-1β were further increased by the actions of two different superoxide anion-generating agents, dimethylmethoxynaphthoquinone and XO/HX, as well as by the H_2O_2-generating enzyme glucose oxidase and by H_2O_2 itself, and repressed by exogenous addition of catalase. Coincubation of GMC with IL-1β, XO/HX, and the membrane-permeable SOD mimetic Mn(III) tetrakis(4-benzoic acid) porphyrin (MnTBAP) did not affect the amplification of IL-1β-dependent PAI-1 induction by ROS, thus indicating that H_2O_2 produced from superoxide anions, but not superoxide anions themselves, was mediator of this induction. These studies are in line with the studies from the NRK52 cells, thus underlining the role of cytoplasmic H_2O_2 as messenger in a signaling network involved in PAI-1 up-regulation. By contrast, the effects shown above mediated by H_2O_2 were counteracted by NO because NO donors such as *S*-nitroso-*N*-acetyl-D,L-penicillamine (SNAP) and (*Z*)-1-[*N*-(2-aminoethyl)-*N*-(2-ammonioethyl)amino]diazen-1-ium-1,2-diolate (DETA-NONOate) inhibited IL-1β-stimulated expression of PAI-1 (35). This phenomenon may be due to the reaction of NO with superoxide anions leading to the formation of peroxynitrite, thus scavenging superoxide anions (Fig. 2).

Similarly, it was demonstrated that TGF-β1-induced up-regulation of PAI-1 mRNA and protein expression in GMC was H_2O_2-dependent (56). H_2O_2 stimulated PAI-1 expression, and NAC effectively reversed TGF-β1 and H_2O_2-induced changes in PAI-1 expression (56).

Furthermore, in another kidney cell line (human renal proximal tubular cells HK-2), PAI-1 induction by uremic toxins was ROS-dependent (see below) (80).

A ROS-dependent induction of PAI-1 protein and activity was also shown in 3T3-L1 mouse preadipocytes differentiated into adipocytes and exposed to either TNF-α, insulin, or both (97). Both superoxide generated by XO/HX and H_2O_2 were potent inducers of PAI-1, and the hydroxyl radical scavengers TMTU and dimethyl sulfoxide (DMSO) completely abolished TNF-α-dependent induction of PAI-1. Interestingly, insulin potentiated the TNF-α-stimulated PAI-1 expression, although the involvement of ROS in insulin-dependent PAI-1 regulation in 3T3-L1 mouse preadipocytes was not investigated.

Although in most of these studies stimuli-dependent production of ROS mediated PAI-1 induction, it appeared that in PH and HepG2 cells the permanent production of ROS attenuated PAI-1 expression. The ROS production was achieved by overexpression of a constitutively active form of the small GTP-binding protein Rac-1. Rac-1 is a necessary component of the ROS-producing NADPH oxidase; thus, a constitutively active form initiates a continuous generation of ROS by this enzyme (47). These differences may be explained by cell type-specific variation in the participating signaling components or by the duration and level of ROS achieved during exposure. Unfortunately, due to the lack of standardized protocols used for ROS measurements, it is difficult to compare the quantity of ROS generated by the various stimuli in the different cells.

Thus, ROS, especially H_2O_2 and derived hydroxyl radicals, appeared to induce PAI-1 expression in a variety of model systems, although the observed effects appeared to be cell type-specific and dependent on the type of radical-generating stimuli (Table 1).

Mechanisms involved in the regulation of PAI-1 gene expression by ROS

Although it became clear from a number of studies that ROS play a role in the regulation of PAI-1 gene expression, the molecular mechanisms in this response are poorly understood and seem to be different depending on the cell type. In most studies, PAI-1 was investigated in the presence of either oxidative stress-generating stimuli and/or antioxidants, but only a few studies tried to elucidate the signaling pathways involved in ROS-dependent PAI-1 regulation in more detail (Fig. 5).

In human renal proximal tubular cells HK-2, PAI-1 mRNA and protein induction by uremic toxins (indoxyl sulfate and indoleacetic acid) is mediated by hydroxyl radical-dependent NF-κB activation (80). The electron paramagnetic resonance analysis showed a typical spectrum of hydroxyl radicals in the

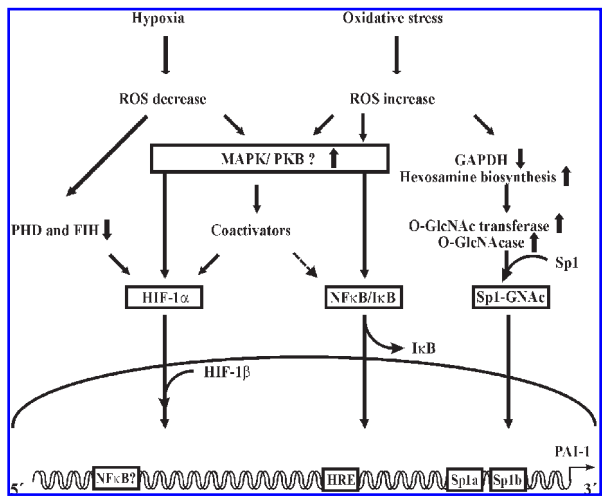

FIG. 5. PAI-1 regulation by hypoxia and ROS. Oxidative stress, as well as hypoxia, can up-regulate PAI-1 via changing ROS levels. MAPK and/or PKB signaling pathways might be responsible for the signal transduction, which involves NF-κB and HIF-1 activation, respectively, and recruitment of coactivators such as CBP/p300. Under hypoxia, HIF-1 activation is mainly caused by inhibition of prolyl and asparaginyl hydroxylases (PHD and FIH). In the nucleus, HIF dimers bind to the hypoxia-responsive element (HRE) in the PAI-1 promoter. The role of the PKB pathway for NF-κB activation was demonstrated, but it was not clarified whether NF-κB effects on PAI-1 expression were direct via the putative NF-κB-like binding site or indirect. Sp1 effects were mediated via inhibition of GAPDH and activation of hexosamine synthesis with subsequent *N*-acetylglucosamination of Sp1 via *O*-glucosamine *N*-acetyltransferase (O-GlcNAc transferase) and *N*-acetylglucosaminidase (O-GlcNAcase). Then modified Sp1 binds to Sp1a and Sp1b sites in the PAI-1 promoter. The regulation of PAI-1 expression by oxidative stress seems to be highly cell type-specific, and the role of not yet identified mechanisms is likely.

cells treated with uremic toxins. Both the antioxidants NAC and PDTC, as well as the NF-κB inhibitor sodium salicylate, dose-dependently inhibited activation of an 807-bp human PAI-1 promoter-driven Luc construct by indoxyl sulfate. These findings would be in line with the report showing the identification of the NF-κB-like site in the PAI-1 promoter (30).

Another transcription factor that might be involved in the ROS-dependent activation of PAI-1 was Sp1 (34). It was shown by using a number of inhibitors that hyperglycemia-induced mitochondrial superoxide overproduction inhibited glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity and activated the hexosamine biosynthesis pathway in BAEC, presumably by diverting the upstream metabolite fructose-6-phosphate from glycolysis to glucosamine formation. Both decreased GAPDH and increased hexosamine pathway activities were prevented by an inhibitor of mitochondrial electron transport complex II (thenoyltrifluoroacetone), an uncoupler of oxidative phosphorylation (carbonyl cyanide *m*-chlorophenylhydrazone), and by overexpression of uncoupling protein-1, showing that ROS produced in mitochondria were responsible for these effects. Treatment of cells with the SOD mimetic MnTBAP and overexpression of Mn-SOD had similar effects demonstrating the role of superoxide anions. These results seem to be in conflict with the results in which the role of H_2O_2 generated from superoxide anion, but not of superoxide anion itself, was demonstrated for PAI-1 regulation (35, 133). However, in these studies, different stimuli and different cell types were used.

It was also shown that hyperglycemia-induced activation of the hexosamine pathway increased serine/threonine O-linked *N*-acetylglycosamination of Sp1, thereby decreasing serine/threonine phosphorylation at the same sites. The involvement of *O*-glucosamine *N*-acetyltransferase and *N*-acetylglucosaminidase in the Sp1 *N*-acetylglucosamination, as well as DNA-dependent protein kinase in Sp1 phosphorylation, was previously demonstrated (48, 55).

Furthermore, hyperglycemia increased expression of a 740-bp and an 85-bp PAI-1 promoter Luc reporter gene construct. Inhibition of mitochondrial superoxide production or the glucosamine pathway prevented these changes. When the two Sp1 sites were mutated, hyperglycemia did not increase expression of an 85-bp truncated PAI-1 promoter Luc reporter. Thus, hyperglycemia-induced mitochondrial superoxide anion radical overproduction increases hexosamine synthesis and then O-linked *N*-acetylglycosamination of Sp1, which activates expression of PAI-1 (Fig. 5).

So far, the involvement of only two transcription factors, NF-κB and Sp1, in oxidative stress-dependent PAI-1 induction has been demonstrated.

ROS-dependent PA regulation

The ratio between components of the PAI/PA system is crucial for its physiological activity because PAI-1 rapidly binds to PAs in circulation. The issue of ROS-dependent regulation of PAI-1 expression appears to be even more complicated because the available data concerning PAI-1 and PAs are controversial.

The induction of PAI-1 accompanied by reduction of PA might lead to reduction of intravascular fibrinolytic activity, and such changes were observed in cells treated with LDL. Oxidative modification of the native, as well as glycated, LDL enhanced production of PAI-1 and reduced tPA in HUVEC and human coronary artery endothelial cells (91, 132). However, another group also using LDL, but oxidized by a different procedure, showed that the oxidative modification of LDL was not responsible for PAI-1 and tPA regulation in HUVEC because not only oxidized, but also native, LDL increased both PAI-1 and tPA secretion (13).

The imbalance in the PAI/PA system can also be achieved when the level of one component is increased and those of others remain unchanged. Thus, ROS induced PAI-1 expression in CMEC without affecting the levels of both tPA and uPA (84). Similarly, no induction of tPA or uPA by ROS was shown in 3T3-L1 mouse preadipocytes, whereas PAI-1 levels were induced (97). Interestingly, in another study performed in HUVEC, it was demonstrated that PAI-1 levels were unchanged by ROS, whereas tPA activity, mRNA synthesis, and antigen secretion were induced (109).

Increased expression of both tPA and its inhibitor PAI-1 by proinflammatory cytokines, not necessarily involving ROS formation, was demonstrated for a variety of cell types, including vascular endothelial cells (102). Such regulation in which not only proteases, but also a protease inhibitor are induced might be necessary for the fine-tuning of fibrinolysis. In rat GMC treated with IL-1β, the ROS-dependent induction of tPA mRNA and protein and PAI-1 mRNA was observed (35).

Both PAI-1 and tPA secretion and activity were significantly decreased by ROS in HUVEC during reoxygenation after sublethal anoxia (108). Whereas SOD prevented the decrease in both tPA and PAI-1 activity, the iron chelator deferoxamine mesylate prevented decrease of the tPA, but not of PAI-1 levels. This implicated that whereas superoxide anions were necessary for both tPA and PAI-1 inhibition, hydroxyl radicals produced in a Fenton reaction in the presence of iron were involved only in the regulation of tPA (108).

Such diversity of data points to a variety of molecular mechanisms involved in the ROS-dependent regulation of PAI-1 and PA expression.

HYPOXIA-DEPENDENT PAI-1 EXPRESSION

As a number of stimuli were able to enhance PAI-1 expression via ROS, one would expect that under conditions like hypoxia, where due to the limiting amount of oxygen ROS cannot be formed, PAI-1 expression is inhibited. However, *in vivo* studies with mice placed in a hypoxic environment (5–6% O_2) showed that plasma levels and plasma activity, as well as PAI-1 mRNA and protein in the lungs, were induced compared with those in the normoxic controls (87). Moreover, the induction of PAI-1 expression by hypoxia was confirmed in a number of cells, such as HUVEC, human saphenous vein endothelial cells (44), BAEC (122), RAW murine macrophage cell line (87), and rat PH (60, 98), and in four human liver cell lines, Chang, Hep3B, HuH7, and HepG2 (40). Thus, the mechanisms of ROS-dependent induction of PAI-1 gene expression seem to be different from the mechanisms of PAI-1 induction by low concentrations of molecular oxygen (hypoxia). It was shown that the transcription factor responsible

for the hypoxia-dependent PAI-1 activation was HIF-1 acting via HREs within the rat and human PAI-1 promoter (40, 60, 98). Interestingly, both PKB and MAPKs, including p38 MAPK and/or p42/44 MAPK, can contribute to the activation of the HIF pathway. Overexpression of PKB and the p38 upstream kinases MKK3 and MKK6 resulted in enhanced HIF-1α levels and stimulated HIF-1-dependent PAI-1 expression (61), whereas inhibition of p38 MAPK prevented thrombin-induced HIF activation and PAI-1 expression in a ROS-dependent manner (46).

Furthermore, some studies demonstrated that HIF-1 activation under hypoxia was associated with low levels of ROS. In rat PH and HepG2 cells, overexpression of constitutively activated or dominant negative GTPase Rac-1 increased or decreased, respectively, ROS production via activation of NADPH oxidase (47). This overproduction of ROS appeared to contribute to HIF-1 degradation, thus leading to the HIF-1-dependent decrease in PAI-1 mRNA, protein, and 796-bp PAI-1 promoter-driven Luc activity. Treatment with the antioxidant PDTC or coexpression of Ref-1 restored HIF-1 and PAI-1 promoter activity in Rac-expressing cells.

These investigations appear to be in contrast to studies in other cell types, including smooth muscle cells, where generation of ROS by PDGF, angiotensin II, thrombin, or H_2O_2 was able to stimulate HIF (39, 46, 93, 130). However, all together these studies demonstrate that depending on the cell type and the manner of ROS generation interference or cross talk of stress-responsive signals and hypoxia on the level of HIF-1.

CONCLUSION

At the moment, it appears that a number of oxidative stress stimuli exert an increase in PAI-1 expression via mechanisms that may vary, depending on the cell type and the stimulus used to produce ROS and to exert oxidative stress. It is tempting to speculate that ROS mainly regulate PAI-1 expression via activation of either MAPK or PI3-K/PKB pathways, whereas a different pathway was shown for the hyperglycemia-induced PAI-1 induction, namely, ROS-dependent increase in hexosamine synthesis and then O-linked *N*-acetylglycosamination of Sp1. The transcription factors involved in transferring the response to oxidative stress, in addition to Sp1, may be NF-κB and HIF-1. In contrast to the oxidative stress response, the response of the PAI-1 gene under hypoxia seems to be uniquely transferred by HIF-1. Under hypoxia, HIF-1α stability is mainly gained by inhibition of the O_2-sensitive hydroxylation of the proline and asparagine residues within HIF-1α, whereas MAPK and PKB pathways appear to play a minor, but modulatory, role. Thus, a complete model for the regulation of PAI-1 gene expression under oxidative stress and hypoxia cannot be made, and a number of issues have to be resolved in the future.

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ABBREVIATIONS

AP-1, activator protein 1; ARNT, arylhydrocarbon receptor-nuclear translocator; ASK, apoptosis signal-regulating kinase; BAEC, bovine aortic endothelial cells; CMEC, cardiac microvascular endothelial cells; DMSO, dimethyl sulfoxide; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GMC, glomerular mesangial cells; HIF, hypoxia-inducible factor; H_2O_2, hydrogen peroxide; HRE, hypoxia-responsive element; HUVEC, human umbilical vein endothelial cells; IκB, inhibitory subunit of nuclear factor-κB; IKK, IκB kinase complex; IL, interleukin; JNK, c-Jun NH_2-terminal kinase; LDL, low-density lipoprotein; Luc, luciferase; MAPK, mitogen-activated protein kinase; MKKK, mitogen-activated protein kinase kinase kinase; MnTBAP, manganese (III) tetrakis(4-benzoic acid) porphyrin; NAC, *N*-acetylcysteine; NF-κB, nuclear factor-κB; NO, nitric oxide; PA, plasminogen activator; PAI, plasminogen activator inhibitor; PDGF, platelet-derived growth factor; PDTC, pyrrolidine dithiocarbamate; PH, primary hepatocytes; PI3-K, phosphatidylinositol-3 kinase; PKB, protein kinase B; Ref-1, redox factor 1; ROS, reactive oxygen species; SOD, superoxide dismutase; TGF, transforming growth factor; TMTU, tetramethylthiourea; TNF, tumor necrosis factor; tPA, tissue PA; uPA, urokinase PA; UV, ultraviolet; VSMC, vascular smooth muscle cells; XO/HX, xanthine oxidase/hypoxanthine.

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