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Review

Hydrogen peroxide regulation of endothelial function: Origins, mechanisms, and consequences

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Abstract

Increased production of reactive oxygen species (ROS) has been implicated in the pathogenesis of cardiovascular diseases. Enzymatic systems such as the mitochondrial respiratory chain, vascular NAD(P)H oxidases, xanthine oxidase, and uncoupled endothelial nitric oxide synthase (eNOS) produce superoxide anion ($O_2^{\bullet -}$) in vascular cells. While some $O_2^{\bullet -}$ rapidly degrades by reacting with nitric oxide ($NO^{\bullet}$), the $O_2^{\bullet -}$ signal preserved by dismutation into hydrogen peroxide (H_2O_2) exerts prolonged signaling effects. This review focuses on patterns and mechanisms whereby H_2O_2 modulates different aspects of endothelial cell function including endothelial cell growth and proliferation, endothelial apoptosis, endothelium-dependent vasorelaxation, endothelial cytoskeletal reorganization and barrier dysfunction, endothelial inflammatory responses, and endothelium-regulated vascular remodeling. These modulations of endothelial cell function may at least partially underlie H_2O_2 contribution to the development of vascular disease.
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Keywords: Hydrogen peroxide; Endothelial function; Superoxide; Nitric oxide; Growth; Actin cytoskeleton; Barrier function; Inflammation; Vascular remodeling; Vascular NAD(P)H oxidases; Uncoupled endothelial nitric oxide synthase; Xanthine oxidase; Mitochondrion; Nox

Increased production of reactive oxygen species (ROS) has been implicated in the pathogenesis of cardiovascular diseases such as atherosclerosis, restenosis, hypertension, diabetic vascular complications and heart failure [1–7]. One electron reduction of molecular oxygen forms superoxide anion ($O_2^{\bullet -}$), which serves as a progenitor for hydrogen peroxide (H_2O_2). By rapidly inactivating nitric oxide ($NO^{\bullet}$), $O_2^{\bullet -}$ contributes to endothelial dysfunction [7]. H_2O_2 however modulates endothelial cell function via intricate mechanisms. Ambient production of $O_2^{\bullet -}$ and subsequently H_2O_2 at low levels, maintained by basal activity of pre-assembled endothelial NAD(P)H oxidases [3], or potential leakage from mitochondrial respiration [8], is necessary for endothelial cell growth and proliferation. Under pathological conditions, agonists-provoked activation of vascular NAD(P)H oxidases and subsequently activated xanthine oxidase or uncoupled eNOS produce H_2O_2 in large quantities [9–11], leading to detrimental consequences [12,13]. These dual-faced functions of H_2O_2 are similar to what is characteristic of $NO^{\bullet}$: vasodilating and protective at physiological concentrations but microbicidal and apoptotic when produced in large quantities. The current review discusses enzymes responsible for endothelial production of ROS, patterns and mechanisms whereby H_2O_2 influences different aspects of endothelial cell function and H_2O_2 contribution to vascular disease development.

1. Production and metabolism of hydrogen peroxide

In mammalian cells, potential enzymatic sources of ROS include the mitochondrial electron transport chain, the arachidonic acid metabolizing enzymes lipoxygenase and

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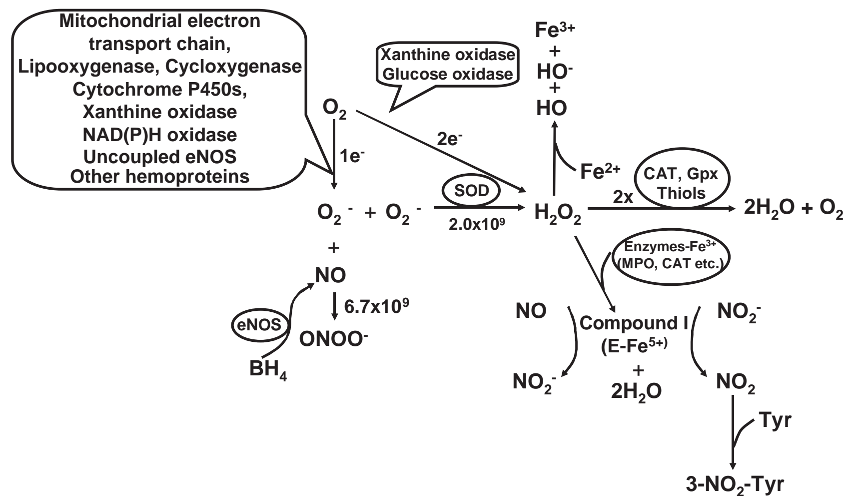

Fig. 1. Biochemical pathways of hydrogen peroxide generation and metabolism. Molecular oxygen undergoes one or two-electron reduction (1 or 2e-) to form superoxide ($O_2^{\bullet-}$) or hydrogen peroxide (H_2O_2) respectively. The majority of the bioactive H_2O_2 however, is derived from spontaneous or SOD (superoxide dismutase)-catalyzed (at the reaction speed of 2.0×10^9 mol/L^{-1}.s^{-1}) dismutation of $O_2^{\bullet-}$. Degradation of H_2O_2 involves intracellular catalase (CAT), extracellular glutathione peroxidase (Gpx) or small molecules like thiols. Besides directly serving as a signaling intermediate, H_2O_2 also indirectly exerts its biological effects via metabolites such as hydroxyl radical ($HO^\bullet$) or compound I (product of H_2O_2 oxidation of Fe^{3+}-containing enzymes such as myeloperoxidase, MPO). Of note, $O_2^{\bullet-}$ rapidly scavenges $NO^\bullet$ at the reaction speed of 6.7×10^9 mol/L^{-1}.s^{-1}, representing one of the mechanisms whereby bioactive $NO^\bullet$ diminishes independent of regulation of $NO^\bullet$ synthase.

cycloxygenase, the cytochrome *P450*s, xanthine oxidase, NAD(P)H oxidases, uncoupled nitric oxide synthase (NOS), peroxidases, and other hemoproteins. These systems primarily catalyze one electron reduction of molecular oxygen to form $O_2^{\bullet-}$ which rapidly inactivates $NO^\bullet$ to form peroxynitrite. Under ambient conditions, some $O_2^{\bullet-}$ is dismutated to H_2O_2 spontaneously or catalyzed by superoxide dismutase (SOD). Of interest, loss of $NO^\bullet$ could lead to enhanced formation of H_2O_2. Some enzymes, such as xanthine oxidase and glucose oxidase, can directly produce H_2O_2 by donating two electrons to oxygen. In the presence of heavy metals, H_2O_2 undergoes Fenton reaction to form highly reactive hydroxyl radical ($HO^\bullet$). When bound to peroxidases such as catalase, H_2O_2 forms compound I which oxidizes $NO^\bullet$ to nitrogen dioxide anion (NO_2^-) and react with NO_2^- to form nitrogen dioxide radical ($NO_2^\bullet$). $NO_2^\bullet$ in turn participates in nitrating events such as formation of nitrotyrosines [14,15]. Fig. 1 illustrates biochemical pathways of H_2O_2 generation and metabolism. When produced transiently at high concentrations, H_2O_2 and $HO^\bullet$ are capable of directly oxidizing proteins and lipids, and causing DNA strand breaks. At relatively lower pathophysiological concentrations (nano- to micromolar range) however, H_2O_2 plays important signaling roles in endothelial cells, and these are the focus of the current review.

2. Vascular NAD(P)H oxidases

Accumulating evidence has characterized a predominant role of vascular NAD(P)H oxidases in generating ROS in different vascular cells including endothelial cells, vascular smooth muscle and fibroblasts [1–6]. In endothelial cells, though there is much to be learned, it is clear that p47^{phox} modulates enzymatic activity by interacting with membrane and cytosolic components to form the active complex. The gp91^{phox} [also called Nox2, Nox for *N*AD(P)H *ox*idases, representing a family of novel NAD(P)H oxidases] is the catalytic core of this complex [16–19]. Studies using deficient mice or inhibitory peptide targeting Nox2 have established an essential role of Nox2 in producing ROS in endothelial cells [17,18,20,21]. Functions of the newly cloned gp91^{phox} homologues; Nox1, Nox4 and Nox5 however, remain obscure. Nox4 seems to express more abundantly in endothelial cells compared to other Nox proteins, representing the major catalytic unit of the endothelial NAD(P)H oxidase responsive to growth halting [22,23]. Nox1 on the other hand, was upregulated by oscillatory shear stress, mediating ROS-dependent leukocyte adhesion to endothelium [24]. Furthermore, VEGF receptor-dependent activation of Nox1 induced angiogenic tube formation of endothelial cells [25]. The observations that Nox1 mediates growth signaling while Nox4 is growth suppressive in endothelial cells seems similar to what have been observed in vascular smooth muscle [26,27]. It seems odd that the same ROS-producing Nox proteins mediate different cellular responses. It is possible that the function of each Nox protein is dependent on its distinctive subcellular localization, and is subject to specific regulations by selective agonists [4]. For example, in vascular smooth muscle cells, Nox1 localizes to caveolae while Nox4 is found in focal adhesions [28]. In endothelial cells however, Nox4 was found at endoplasmic reticulum [23] whereas Nox2 is localized to peri-nuclear cytoskeletal structure [17].

Novel homologues of Nox-regulating proteins p47^{phox} and gp67^{phox} have been identified in epithelial cells (p41^{phox} and p51^{phox} respectively), serving as potent positive

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regulators for Nox1 [29–32]. In the same cells longer Nox proteins with peroxidase tails, namely Duox1 and Duox2, have been cloned [33,34] and shown to be functional [35,36]. These proteins are yet studied for their presence and function in endothelium and vascular smooth muscle. Of note, p22*phox* is the only other membrane component of the vascular NAD(P)H oxidases. Overexpression of p22*phox* led to stabilization of Nox1 and Nox4 proteins [37]. Recent studies have elegantly characterized physical and functional interactions between p22*phox* and Noxs (Nox1 and Nox4) in vascular smooth muscle [38,39]. Similar interactions might also occur in endothelial cells. In addition, a recent study reported that p22*phox* expression correlates well with expression of Nox4 in human arteries and that of Nox2 in veins [40].

In addition, vascular NAD(P)H oxidase-derived H_2O_2 is able to amplify ROS production in vascular cells. To date at least five different mechanisms are involved in this propagation, including enhanced intracellular iron uptake, and activation of sources of mitochondria, NAD(P)H oxidases, xanthine oxidase and uncoupled eNOS [1]. These feed-forward mechanisms form a vicious circle to amplify and sustain H_2O_2 production in large quantities, contributing to prolonged pathological signaling.

3. Hydrogen peroxide regulation of endothelial cell growth and proliferation

H_2O_2 generated by xanthine oxidase or glucose oxidase stimulated endothelial cell growth [41]. Consistently, scavenging H_2O_2 by adenoviral overexpression of catalase or cytosolic glutathione peroxidase inhibited endothelial cell proliferation [42,43]. These data indicate a role of H_2O_2 in growth signaling. Indeed, H_2O_2 is downstream of Flk1/KDR, proceeding activation of ERK1/2 [44]. The growth regulating p90RSK, an important downstream effector of ERK1/2, is activated by H_2O_2 in endothelial cells [45]. Furthermore, H_2O_2 feed-forwardly increases endothelial VEGF expression and secretion via activation of PKC and NFκB [46], and regulates expression and function of other growth factors [47]. Growth signaling induced by agonists other than growth factors may also require H_2O_2. For instance, cyclic strain induces early growth factor 1 (Egr-1) expression and this requires H_2O_2-dependent activation of ERK1/2 [48].

Furthermore, H_2O_2 increases activity of eIF4E, an eukaryotic initiation factor of protein synthesis [49], and is effective in promoting endothelial cell tube formation [50,51]. Hypoxia induced activation of ERK1/2 and p38 MAPK in lung endothelial cells is mediated by H_2O_2 [52]. This may partially underlie hypoxia-induced angiogenesis. Indeed, endogenously produced H_2O_2 upregulates VEGF expression and angiogenesis in vivo [53]. The signaling events involved in H_2O_2 modulation of endothelial cell growth, and later discussed endothelial apoptosis are illustrated in Fig. 2. Whereas a modest increase in endothelial cell growth and angiogenesis is beneficial in repairing ischemic damage [54], excessive growth contributes to atheroma formation post injury [53]. In addition, when H_2O_2 diffuses to adjacent vascular smooth muscle, it could induce hypertrophy [13]. Indeed, endogenously

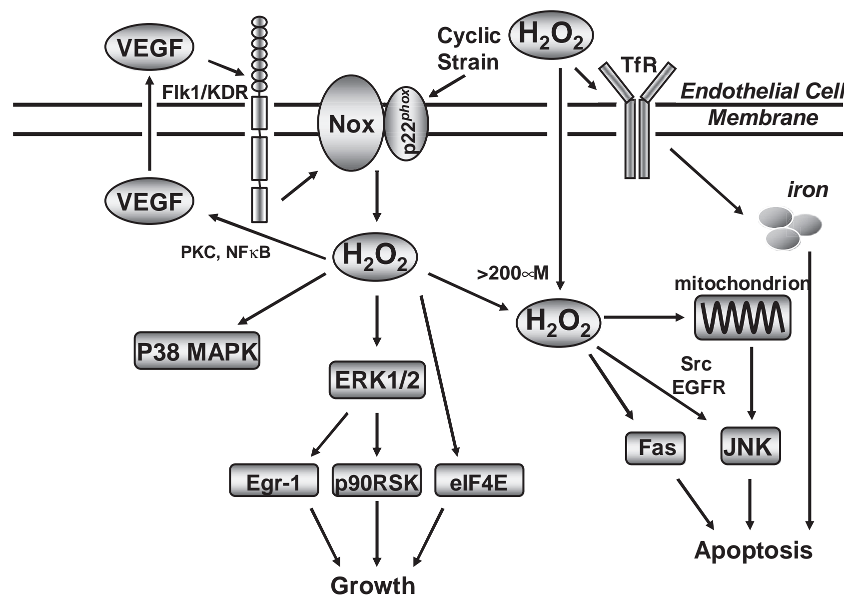

Fig. 2. Signaling events mediating hydrogen peroxide modulation of endothelial cell growth and apoptosis. Mitogenic stimuli such as vascular endothelial growth factor (VEGF) and cyclic strain activate vascular NAD(P)H oxidases to form superoxide ($O_2^{\bullet-}$) and subsequently hydrogen peroxide (H_2O_2). H_2O_2 in turn mediates activations of p38 MAPK, ERK1/2 and transcriptional factors involved in grow signaling. Of interest, H_2O_2 also feed-forwardly upregulates endothelial cell expression of VEGF. On the other hand, excessively produced H_2O_2 exceeding 200 μmol/L induces endothelial apoptosis. This response seems to involve transferrin receptor (TfR)-dependent intracellular iron uptake, and activations of mitochondrion, FAS and JNK/c-Jun pathway.

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overproduced H_2O_2 augmented angiotensin II induced vascular hypertrophy [55].

4. Hydrogen peroxide regulation of endothelial apoptosis

Cultured *umbilical* vein endothelial cells are particularly prone to oxidative damage and often used to study H_2O_2 induced apoptosis. Of note, *aortic* endothelial cells undergo no significant cell death with exogenous addition of H_2O_2 less than 200 μmol/L [56–58]. However, 50–100 μmol/L H_2O_2 was apoptotic for *umbilical* vein endothelial cells. Endogenous H_2O_2, stimulated by oxidized LDL, induced endothelial cell apoptosis via JNK activation [59]. Mitochondrion-dependent ROS propagation is likely involved because mitochondria-targeted antioxidant abrogated H_2O_2 activation of JNK and apoptosis [60]. Studies by other groups also support a role for JNK/c-Jun in H_2O_2 induced apoptosis of endothelial cells [59,61,62]. Of note, Chen et al. previously characterized upstream events of JNK activation by H_2O_2, which involve Src-dependent activation of EGFR [63]. Additionally, H_2O_2 upregulates Fas expression [64], enhances intracellular ion uptake [65], and induces mitochondrial DNA damage [66], all of which may directly trigger apoptosis.

Importantly, endothelial cell apoptosis has been implicated in atherosclerosis [67,68] although molecular mechanisms underlying H_2O_2 induced endothelial cell apoptosis remain to be fully elucidated. It is however interesting to note that H_2O_2 induced apoptosis is inhibitable by unidirectional laminar shear stress, which exerts this effect by attenuating JNK activation and enhancing glutathione reductase-dependent glutathione redox-cycling [69,70]. Furthermore, *S*-nitrosylation of thioredoxin 1 [71] and ERK5 activation [72] was found protective of endothelial cells from apoptosis.

5. Hydrogen peroxide regulation of endothelial barrier function and actin cytoskeleton

Relatively high concentrations of H_2O_2 (>200 μmol/L) increase endothelial permeability [73], and this seems mediated by activations of PKC [74], phosphodiesterase [75], small G protein Rho [76], Src tyrosine kinase [77], p38 MAPK [78], and an increase in intracellular calcium [79]. Tight junction protein occludin was found downstream of ERK1/2 in mediating H_2O_2-induced barrier dysfunction [80]. In addition, H_2O_2 downregulation of intracellular cAMP content appears important in inducing barrier dysfunction [75,81]. Decreased PKA activity can itself account for many of the activating effects that result in barrier dysfunction or at least permit those changes [82]. Crosstalks or spatial regulations among above-described

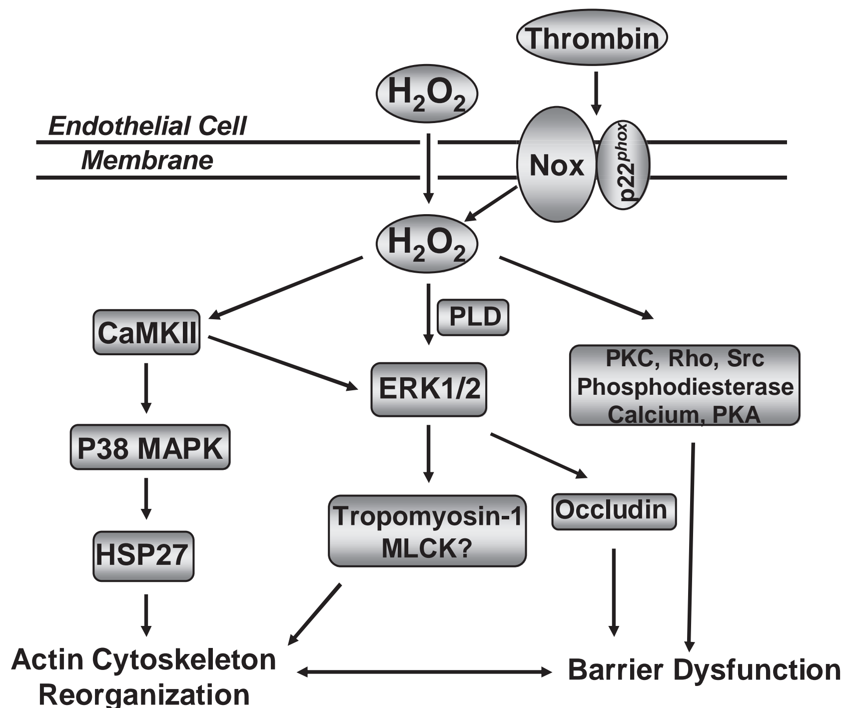

Fig. 3. Signaling events mediating hydrogen peroxide modulation of endothelial actin cytoskeleton and barrier function. Intracellular hydrogen peroxide (H_2O_2) produced in response to extracellular stimuli such as thrombin is capable of inducing phospholipase D (PLD) or calcium/calmodulin-dependent protein kinase II (CaMKII) dependent activation of ERK1/2, and CaMKII-dependent activation of p38 MAPK. By activating downstream effectors tropomyosin-1/MLCK or heat shock protein 27 (HSP27) respectively, both pathways are involved in H_2O_2 induced reorganization of actin cytoskeleton, which is associated with modulation of barrier function. Various kinases or signaling intermediates such as PKC, PKA and calcium have been implicated in H_2O_2 induction of endothelial barrier dysfunction. The inter-relationships among these players however remain to be elucidated.

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signaling events however remain to be revealed. Caveolin-1 is expressed abundantly in lipid rafts of endothelial cell membrane, where it plays an important role in modulating barrier function [83]. Whether caveolin-1 is required for H_2O_2 modulation of endothelial barrier function however is unclear although our unpublished data suggest that H_2O_2 induces rapid Src-dependent phosphorylation of caveolin-1. Of note, at various concentrations, $NO^{\bullet}$ is able to enhance or offset H_2O_2-induced loss of endothelial barrier function [84,85].

It has been shown that cytoskeletal reorganization modulates vascular permeability [86]. Likewise, H_2O_2 regulation of barrier function is linked to its effects on endothelial actin cytoskeleton. p38 MAPK phosphorylation of actin binding protein HSP27 mediates reorganization of actin cytoskeleton in H_2O_2-stimulated endothelial cells [87]. In keeping with this, H_2O_2 induced formation of actin stress fibers was found to be mediated by CaMKII-dependent activation of p38 MAPK/HSP27 and ERK1/2 [88]. Whereas HSP27 phosphorylation was completely prevented by blockade of p38 MAPK, inhibition of ERK1/2 only transiently attenuated HSP27 phosphorylation [88]. These data suggest that ERK1/2 has different downstream effectors in modulating actin cytoskeleton.

It was previously shown that endothelial MLCK is involved in H_2O_2 induced actin reorganization [89]. MLCK also plays an important role in thrombin induced endothelial barrier dysfunction [86]. Thrombin has been shown to stimulate endothelial NAD(P)H oxidase activation and subsequent H_2O_2 production [90]. Recent studies further demonstrated that thrombin upregulates $p22^{phox}$ expression via p38 MAPK and PI-3K/AKT [91]. Given these observations, it is interesting to speculate that MLCK lies downstream of ERK1/2 in mediating H_2O_2 induced actin reorganization. In addition, H_2O_2 phosphorylation of tropomyosin-1 is ERK1/2-dependent, preceding its co-localization with developing actin filaments [92]. Of note, H_2O_2 activation of phospholipase D is also involved in actin reorganization in endothelial cells [93], likely serving as upstream activator of ERK1/2 [94]. The signaling events described above are summarized as part of the Fig. 3. Importantly, H_2O_2 regulation of endothelial cytoskeleton and barrier function may not only mediate ischemia–reperfusion induced *acute* vascular injury [73], but also potentiate vascular inflammation to contribute to *chronic* vascular disease [95].

6. Hydrogen peroxide regulation of endothelium-dependent vasorelaxation and eNOS expression

Earlier studies demonstrated that H_2O_2 produces endothelium-dependent and independent vasorelaxation [96,97]. The endothelium-dependent vasorelaxation caused by H_2O_2 in large vessels depends on eNOS, as L-NAME (NOS inhibitor) abolishes it [98,99]. Indeed, H_2O_2 can acutely stimulate eNOS production of $NO^{\bullet}$ via PI3-K/AKT and ERK1/2, [58,100]. When eNOS was uncoupled to produce $O_2^{\bullet -}$ rather than $NO^{\bullet}$ in hypertensive or atherosclerotic large

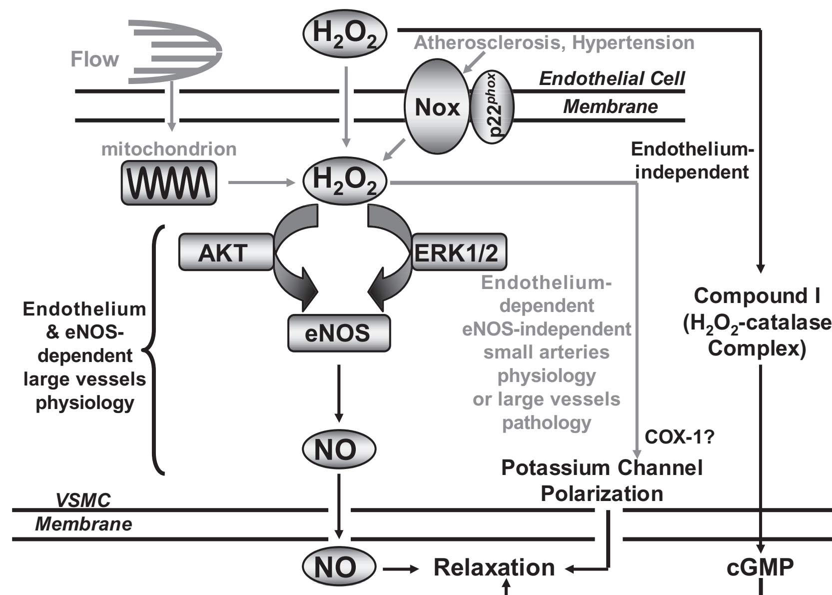

Fig. 4. Mechanisms underlying endothelium-dependent or independent vasorelaxation induced by hydrogen peroxide. Depending on size of the blood vessels and availability of bioactive nitric oxide ($NO^{\bullet}$), and physiological versus pathological environment, hydrogen peroxide (H_2O_2) mediates endothelium-dependent or independent vasorelaxation via $NO^{\bullet}$-dependent or independent mechanisms. H_2O_2 seems to activate eNOS under physiological conditions in large vessels, resulting in endothelium and $NO^{\bullet}$-dependent relaxation. In small vessels such as coronary arterioles, mitochondrial respiratory chain-derived H_2O_2 is found to be responsible for flow-mediated vasodilatation that is independent of $NO^{\bullet}$. Under pathological conditions such as atherosclerosis and hypertension, H_2O_2 produced by large vessels mediates compensatory, endothelium-dependent but $NO^{\bullet}$-independent relaxation. Additionally, under unclear conditions, H_2O_2 may also cause endothelium-independent relaxation via compound I-mediated direct activation of smooth muscle cyclic GMP.

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vessels, endothelium-derived H_2O_2 mediated compensatory relaxation via unknown mechanisms [10,101]. One possibility is direct polarization of vascular smooth muscle.

Indeed, H_2O_2 was found to be an endothelium-derived hyperpolarizing factor in small arteries [102,103] and an activator of potassium channel in large cerebral arteries [104]. In human coronary arterioles, flow-induced vasodilatation is mediated by endothelium-derived H_2O_2 [105]. In these arterioles, the enzymatic source of H_2O_2 appears to be the mitochondrial respiratory chain whereas in large vessels, vascular NAD(P)H oxidases are responsible for H_2O_2 production [6,8]. This seems consistent with previous observations that $NO^{\bullet}$ plays a lesser role in vasodilatation of small arteries. Of note, in coronary arterioles, H_2O_2 induced endothelium-dependent vasorelaxation is $NO^{\bullet}$-independent involving activation of cyclooxygenase 1 and smooth muscle potassium channel [106]. Therefore, it is clear that H_2O_2 is capable of mediating endothelium-dependent vasorelaxation, $NO^{\bullet}$-dependently or independently. The underlying mechanisms however are diverse depending on the size of the blood vessels and the availability of functional eNOS to produce $NO^{\bullet}$ [107,108].

H_2O_2 can also stimulate endothelium-independent vasorelaxation with unknown mechanisms [109–111]. Early studies demonstrated that H_2O_2 can directly activate cyclic GMP via a compound I/H_2O_2 complex [109,110]. Additional recent studies suggest that this might be true [112]. Known and hypothetical mechanisms underlying H_2O_2 induced vasorelaxation are summarized schematically in Fig. 4.

Of note, H_2O_2 potently upregulates eNOS expression in vitro and in vivo [37,56,113]. Oscillatory shear stress upregulation of eNOS requires H_2O_2 activation of CaMKII [114]. These observations are consistent with previous findings that eNOS is upregulated in animal models of atherosclerosis, diabetes or aging, where vascular H_2O_2 production is increased [115,116]. Rapidly accumulating evidence suggests that eNOS can become uncoupled to produce $O_2^{\bullet -}$ under conditions such as hypertension and atherosclerosis, likely consequent to tetrahydrobiopterin deficiency and oxidation. Thus upregulation of eNOS could be less beneficial or even detrimental while functioning as an oxidase. Indeed, our recent study indicates that endothelial NAD(P)H oxidase-derived H_2O_2 downregulates tetrahydrobiopterin salvage enzyme dihydrofolate reductase (DHFR) and DHFR/eNOS ratio in response to angiotensin II, leading to tetrahydrobiopterin deficiency and subsequently uncoupling of eNOS [9].

7. Hydrogen peroxide regulation of endothelial inflammatory responses

Exposure of endothelial cells to H_2O_2 (50–100 μmol/L) increased surface expression of intercellular adhesion molecule-1 (ICAM-1) [117]. Furthermore, endogenously produced H_2O_2 mediates ischemia–reperfusion induced upregulation of ICAM-1 [118] and MCP-1 (monocyte chemoattractant protein-1) [119]. H_2O_2 is also required for TNFα induction of ICAM-1 and VCAM-1 (vascular cell adhesion molecule-1) [120], and upregulation of MCP-1 by TNFα [121] or hyperglycemia [122]. Additionally, activation of NFκB by H_2O_2 was found responsible for TNFα induction of ICAM-1 [123] and angiotensin II upregulation of VCAM-1 [124].

Endothelial expression of platelet activating factor (PAF) [125] and P-selectin [126] is inducible by H_2O_2. All these molecules including ICAM-1, VCAM-1, MCP-1, PAF and P-selectin have been shown to mediate neutrophil adhesion to endothelium (Fig. 5). PAF and P-selectin also mediates

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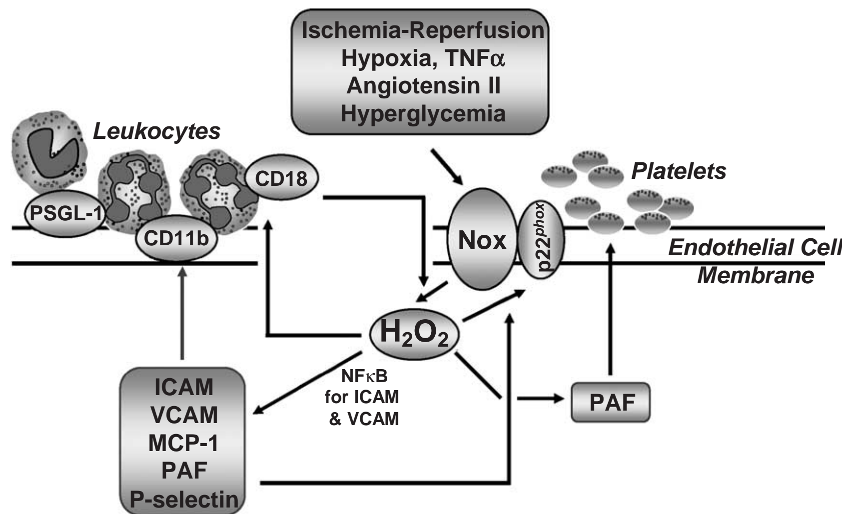

Fig. 5. Mechanisms whereby hydrogen peroxide promotes endothelial inflammatory responses. By activating redox-sensitive transcription factors such as NFκB, hydrogen peroxide (H_2O_2) mediates endothelial induction of inflammatory proteins such as VCAM, ICAM and MCP-1 in response to extracellular stimuli such as TNFα, hypoxia and angiotensin II. H_2O_2 also activates platelet and neutrophil interactions with endothelium to facilitate inflammation of the endothelium.

platelet activation and endothelium–platelet interaction. Upregulation of Mac 1 (CD11b and CD18) in neutrophils [127] by H_2O_2 stimulates more H_2O_2 release from activated neutrophils [128], forming a vicious circle. Taken together, these data strongly suggest that H_2O_2 mediates endothelial expression of inflammatory proteins and enhanced platelet–endothelium interaction, which in turn contributes to development of atherosclerotic vascular disease. The events described above are summarized in Fig. 5.

8. Hydrogen peroxide regulation of endothelium-mediated vascular remodeling

Emerging evidence seems to suggest that H_2O_2 plays an important role in mediating vascular remodeling. First of all, H_2O_2 increases endothelial MMP-2 expression [12]. TGF-β1 and macrophages are important regulators of tissue fibrosis and remodeling. TGF-β1 induction of macrophage colony-stimulating factor, and IL-1 induction of plasminogen activator inhibitor-1 (PAI-1) in endothelial cells were found dependent on H_2O_2 activation of NFκB [129,130]. PAI-1 plays an important role in vascular remodeling induced by thrombosis [131] or chronic eNOS inhibition [132]. Recent studies demonstrated that PAI-1 is also critically important for angiotensin II or salt-induced vascular remodeling in vivo [133]. Thus by mediating TGF-β1 and PAI-1 signaling and MMP release, H_2O_2 contributes to endothelium-regulated vascular remodeling, which has been shown to regulate stability of atherosclerotic plaques [134].

9. Contribution of hydrogen peroxide to vascular disease

To date, the specific, individual ROS that is most relevant to vascular signaling pathophysiologically is yet identified. Nevertheless, selectively overproducing or removing H_2O_2 significantly altered atherogenesis in animal models. Mice overexpressing p22^{phox} had markedly increased atheroma formation in a carotid ligation model [53]. This response was associated with enhanced H_2O_2 production in the vessel wall, and was abolished by scavenging H_2O_2 with ebselen, implicating a critical role of H_2O_2 [53]. Parallel studies from another group confirmed the same notion [135]. Yang et al. cross bred transgenic mice overexpressing Cu/Zn-SOD or catalase, with mice deficient in apolipoprotein E (apoE$^{-/-}$), to examine a specific role of H_2O_2 versus $O_2^{\bullet -}$ in atherogenesis [135]. They found that whereas overexpressing Cu/Zn-SOD had no effect on atherosclerotic lesion formation, overexpression of catalase, or co-overexpression of catalase and Cu/Zn-SOD markedly retarded atherosclerosis in all aspects [135]. These observations were consistent with the findings by Tribble et al. that overexpression of Cu/Zn-SOD failed to prevent atherosclerosis in high fat diet-fed apoE$^{-/-}$ mice [136]. Taken together, these data suggest that H_2O_2 is more atherogenic than $O_2^{\bullet -}$. It is true that Cu/Zn-SOD is intracellular and overexpression of Cu/Zn-SOD can not protect $NO^{\bullet}$ during its trafficking to vascular smooth muscle. Thus extracellular SOD might be more important in determining $NO^{\bullet}$ bioavailability and be more anti-atherosclerosis. Indeed, evidence gained from ecSOD-null mice and adenovirus-mediated over-expression of ecSOD supports that ecSOD is the main determinant of $NO^{\bullet}$ bioavailability in the vessel wall, and is thus involved in blood pressure regulation [137,138]. However, impact of ecSOD overexpression on athero-sclerosis is yet to be reported although mice deficient in ecSOD developed similar atherosclerotic lesions compared to wild-type mice [139]. Therefore, whereas $O_2^{\bullet -}$ is important in directly modulating $NO^{\bullet}$ bioavailability and serving as the precursor for H_2O_2 [7], relatively lasting H_2O_2 seems more important in mediating atherogenic signaling.

In summary, $O_2^{\bullet -}$ and H_2O_2 are produced in vascular cells by multiple enzymatic systems including vascular NAD(P)H oxidases, mitochondrion, xanthine oxidase and uncoupled eNOS. While some $O_2^{\bullet -}$ spontaneously degrades by reacting with $NO^{\bullet}$, $O_2^{\bullet -}$ signal preserved by dismutation into H_2O_2 exerts prolonged signaling effects. This may explain why direct scavenging H_2O_2 but not $O_2^{\bullet -}$ is more effective in athero-protection. Although there is much to be learned, the impact of H_2O_2 on different aspects of endothelial cell function as discussed throughout the review may at least partially underlie H_2O_2-mediated development of atherosclerotic vascular disease.

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References

[1] Cai H.. NAD(P)H oxidase-dependent self-propagation of hydrogen peroxide and vascular disease. Circ Res 2005;96:818–22.
[2] Brandes RP, Kreuzer J. Vascular NADPH oxidases: molecular mechanisms of activation. Cardiovasc Res 2005;65:16–27.
[3] Li JM, Shah AM. Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology. Am J Physiol Regul Integr Comp Physiol 2004;287:R1014–30.
[4] Griendling KK. Novel NAD(P)H oxidases in the cardiovascular system. Heart 2004;90:491–3.
[5] Touyz RM, Schiffrin EL. Reactive oxygen species in vascular biology: implications in hypertension. Histochem Cell Biol 2004; 122:339–52.
[6] Cai H, Griendling KK, Harrison DG. The vascular NAD(P)H oxidases as therapeutic targets in cardiovascular diseases. Trends Pharmacol Sci 2003;24:471–8.

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[7] Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 2000;87:840–4.

[8] Liu Y, Zhao H, Li H, Kalyanaraman B, Nicolosi AC, Gutterman DD. Mitochondrial sources of H_2O_2 generation play a key role in flow-mediated dilation in human coronary resistance arteries. Circ Res 2003;93:573–80.

[9] Chalupsky K., Cai H.. Endothelial dihydrofolate reductase: critical for nitric oxide bioavailability and role in angiotensin II uncoupling of eNOS. Proc Natl Acad Sci U S A 2005;102:9056–61.

[10] Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, et al. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest 2003;111:1201–9.

[11] McNally JS, Davis ME, Giddens DP, Saha A, Hwang J, Dikalov S, et al. Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. Am J Physiol Heart Circ Physiol 2003;285:H2290–7.

[12] Belkhiri A, Richards C, Whaley M, McQueen SA, Orr FW. Increased expression of activated matrix metalloproteinase-2 by human endothelial cells after sublethal H_2O_2 exposure. Lab Invest 1997;77:533–9.

[13] Zafari AM, Ushio-Fukai M, Akers M, Yin Q, Shah A, Harrison DG, et al. Role of NADH/NADPH oxidase-derived H_2O_2 in angiotensin II-induced vascular hypertrophy. Hypertension 1998;32:488–95.

[14] Brennan ML, Wu W, Fu X, Shen Z, Song W, Frost H, et al. A tale of two controversies: defining both the role of peroxidases in nitrotyrosine formation in vivo using eosinophil peroxidase and myeloperoxidase-deficient mice, and the nature of peroxidase-generated reactive nitrogen species. J Biol Chem 2002;277: 17415–27.

[15] Baldus S, Eiserich JP, Brennan ML, Jackson RM, Alexander CB, Freeman BA. Spatial mapping of pulmonary and vascular nitrotyrosine reveals the pivotal role of myeloperoxidase as a catalyst for tyrosine nitration in inflammatory diseases. Free Radic Biol Med 2002;33:1010.

[16] Bayraktutan U, Blayney L, Shah AM. Molecular characterization and localization of the NAD(P)H oxidase components gp91-phox and p22-phox in endothelial cells. Arterioscler Thromb Vasc Biol 2000;20:1903–11.

[17] Li JM, Shah AM. Intracellular localization and preassembly of the NADPH oxidase complex in cultured endothelial cells. J Biol Chem 2002;277:19952–60.

[18] Frey RS, Rahman A, Kefer JC, Minshall RD, Malik AB. PKCzeta regulates TNF-alpha-induced activation of NADPH oxidase in endothelial cells. Circ Res 2002;90:1012–9.

[19] Li JM, Shah AM. Mechanism of endothelial cell NADPH oxidase activation by angiotensin II: role of the p47phox subunit. J Biol Chem 2003;278:12094–100.

[20] Gorlach A, Brandes RP, Nguyen K, Amidi M, Dehghani F, Busse R. A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. Circ Res 2000;87:26–32.

[21] Furst R, Brueckl C, Kuebler WM, Zahler S, Krotz F, Gorlach A, et al. Atrial natriuretic peptide induces mitogen-activated protein kinase phosphatase-1 in human endothelial cells via Rac1 and NAD(P)H oxidase/Nox2-activation. Circ Res 2005;96:43–53.

[22] Ago T, Kitazono T, Ooboshi H, Iyama T, Han YH, Takada J, et al. Nox4 as the major catalytic component of an endothelial NAD(P)H oxidase. Circulation 2004;109:227–33.

[23] Buul JD, Fernandez-Borja M, Anthony EC, Hordijk PL. Expression and localization of NOX2 and NOX4 in primary human endothelial cells. Antioxid Redox Signal 2005;7:308–17.

[24] Sorescu GP, Song H, Tressel SL, Hwang J, Dikalov S, Smith DA, et al. Bone morphogenic protein 4 produced in endothelial cells by oscillatory shear stress induces monocyte adhesion by stimulating reactive oxygen species production from a nox1-based NADPH oxidase. Circ Res 2004;95:773–9.

[25] Kobayashi S, Nojima Y, Shibuya M, Maru Y. Nox1 regulates apoptosis and potentially stimulates branching morphogenesis in sinusoidal endothelial cells. Exp Cell Res 2004;300:455–62.

[26] Lassegue B, Sorescu D, Szocs K, Yin Q, Akers M, Zhang Y, et al. Novel gp91(phox) homologues in vascular smooth muscle cells: nox1 mediates angiotensin II-induced superoxide formation and redox-sensitive signaling pathways. Circ Res 2001;88:888–94.

[27] Geiszt M, Kopp JB, Varnai P, Leto TL. Identification of renox, an NAD(P)H oxidase in kidney. Proc Natl Acad Sci U S A 2000;97: 8010–4.

[28] Hilenski LL, Clempus RE, Quinn MT, Lambeth JD, Griendling KK. Distinct subcellular localizations of Nox1 and Nox4 in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2004;24:677–83.

[29] Geiszt M, Lekstrom K, Witta J, Leto TL. Proteins homologous to p47phox and p67phox support superoxide production by NAD(P)H oxidase 1 in colon epithelial cells. J Biol Chem 2003;278:20006–12.

[30] Banfi B, Clark RA, Steger K, Krause KH. Two novel proteins activate superoxide generation by the NADPH oxidase NOX1. J Biol Chem 2003;278:3510–3.

[31] Takeya R, Ueno N, Kami K, Taura M, Kohjima M, Izaki T, et al. Novel human homologues of p47phox and p67phox participate in activation of superoxide-producing NADPH oxidases. J Biol Chem 2003;278:25234–46.

[32] Kawahara T, Kuwano Y, Teshima-Kondo S, Takeya R, Sumimoto H, Kishi K, et al. Role of nicotinamide adenine dinucleotide phosphate oxidase 1 in oxidative burst response to toll-like receptor 5 signaling in large intestinal epithelial cells. J Immunol 2004;172: 3051–8.

[33] Edens WA, Sharling L, Cheng G, Shapira R, Kinkade JM, Lee T, et al. Tyrosine cross-linking of extracellular matrix is catalyzed by Duox, a multidomain oxidase/peroxidase with homology to the phagocyte oxidase subunit gp91phox. J Cell Biol 2001;154:879–91.

[34] Lambeth JD. Nox/Duox family of nicotinamide adenine dinucleotide (phosphate) oxidases. Curr Opin Hematol 2002;9:11–7.

[35] Geiszt M, Witta J, Baffi J, Lekstrom K, Leto TL. Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense. FASEB J 2003;17:1502–4.

[36] Forteza R, Salathe M, Miot F, Conner GE. Regulated H_2O_2 production by Duox in human airway epithelial cells. Am J Respir Cell Mol Biol 2005;32:462–9.

[37] Laude K, Cai H, Fink B, Hoch N, Weber DS, McCann L, et al. Hemodynamic and biochemical adaptations to vascular smooth muscle overexpression of p22phox in mice. Am J Physiol Heart Circ Physiol 2005;288:H7–12.

[38] Hanna IR, Hilenski LL, Dikalova A, Taniyama Y, Dikalov S, Lyle A, et al. Functional association of nox1 with p22phox in vascular smooth muscle cells. Free Radic Biol Med 2004;37:1542–9.

[39] Ambasta RK, Kumar P, Griendling KK, Schmidt HH, Busse R, Brandes RP. Direct interaction of the novel Nox proteins with p22phox is required for the formation of a functionally active NADPH oxidase. J Biol Chem 2004;279:45935–41.

[40] Guzik TJ, Sadowski J, Kapelak B, Jopek A, Rudzinski P, Pillai R, et al. Systemic regulation of vascular NAD(P)H oxidase activity and nox isoform expression in human arteries and veins. Arterioscler Thromb Vasc Biol 2004;24:1614–20.

[41] Ruiz-Gines JA, Lopez-Ongil S, Gonzalez-Rubio M, Gonzalez-Santiago L, Rodriguez-Puyol M, Rodriguez-Puyol D. Reactive oxygen species induce proliferation of bovine aortic endothelial cells. J Cardiovasc Pharmacol 2000;35:109–13.

[42] Zanetti M, Katusic ZS, O'Brien T. Adenoviral-mediated overexpression of catalase inhibits endothelial cell proliferation. Am J Physiol Heart Circ Physiol 2002;283:H2620–6.

[43] Faucher K, Rabinovitch-Chable H, Barriere G, Cook-Moreau J, Rigaud M. Overexpression of cytosolic glutathione peroxidase (GPX1) delays endothelial cell growth and increases resistance to toxic challenges. Biochimie 2003;85:611–7.

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[44] Colavitti R, Pani G, Bedogni B, Anzevino R, Borrello S, Waltenberger J, et al. Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR. J Biol Chem 1998;277:3101–8.
[45] Abe J, Okuda M, Huang Q, Yoshizumi M, Berk BC. Reactive oxygen species activate p90 ribosomal S6 kinase via Fyn and Ras. J Biol Chem 2004;275:1739–48.
[46] Chua CC, Hamdy RC, Chua BH. Upregulation of vascular endothelial growth factor by H_2O_2 in rat heart endothelial cells. Free Radic Biol Med 1999;25:891–7.
[47] Eyries M, Collins T, Khachigian LM. Modulation of growth factor gene expression in vascular cells by oxidative stress. Endothelium 2004;11:133–9.
[48] Wung BS, Cheng JJ, Chao YJ, Hsieh HJ, Wang DL. Modulation of Ras/Raf/extracellular signal-regulated kinase pathway by reactive oxygen species is involved in cyclic strain-induced early growth response-1 gene expression in endothelial cells. Circ Res 1996;84:804–12.
[49] Duncan RF, Peterson H, Hagedorn CH, Sevanian A. Oxidative stress increases eukaryotic initiation factor 4E phosphorylation in vascular cells. Biochem J 1999;369:213–25.
[50] Shono T, Ono M, Izumi H, Jimi SI, Matsushima K, Okamoto T, et al. Involvement of the transcription factor NF-kappaB in tubular morphogenesis of human microvascular endothelial cells by oxidative stress. Mol Cell Biol 2003;16:4231–9.
[51] Yasuda M, Ohzeki Y, Shimizu S, Naito S, Ohtsuru A, Yamamoto T, et al. Stimulation of in vitro angiogenesis by hydrogen peroxide and the relation with ETS-1 in endothelial cells. Life Sci 2004;64:249–58.
[52] Parinandi NL, Kleinberg MA, Usatyuk PV, Cummings RJ, Pennathur A, Cardounel AJ, et al. Hyperoxia-induced NAD(P)H oxidase activation and regulation by MAP kinases in human lung endothelial cells. Am J Physiol Lung Cell Mol Physiol 1999;284:L26–38.
[53] Khatri JJ, Johnson C, Magid R, Lessner SM, Laude KM, Dikalov SI, et al. Vascular oxidant stress enhances progression and angiogenesis of experimental atheroma. Circulation 2005;109:520–5.
[54] Sellke FW, Simons M. Angiogenesis in cardiovascular disease: current status and therapeutic potential. Drugs 2001;58:391–6.
[55] Weber DS, Rocic P, Mellis AM, Laude K, Lyle AN, Harrison DG, et al. Angiotensin II-induced hypertrophy is potentiated in mice overexpressing p22phox in vascular smooth muscle. Am J Physiol Heart Circ Physiol 2002;288:H37–42.
[56] Cai H, Davis ME, Drummond GR, Harrison DG. Induction of endothelial NO synthase by hydrogen peroxide via a Ca(2+)/calmodulin-dependent protein kinase II/janus kinase 2-dependent pathway. Arterioscler Thromb Vasc Biol 2002;21:1571–6.
[57] Cai H, Li Z, Dikalov S, Holland SM, Hwang J, Jo H, et al. NAD(P)H oxidase-derived hydrogen peroxide mediates endothelial nitric oxide production in response to angiotensin II. J Biol Chem 2004;277:48311–7.
[58] Thomas SR, Chen K, Keaney Jr JF. Hydrogen peroxide activates endothelial nitric-oxide synthase through coordinated phosphorylation and dephosphorylation via a phosphoinositide 3-kinase-dependent signaling pathway. J Biol Chem 2004;277:6017–24.
[59] Lin SJ, Shyue SK, Liu PL, Chen YH, Ku HH, Chen JW, et al. Adenovirus-mediated overexpression of catalase attenuates oxLDL-induced apoptosis in human aortic endothelial cells via AP-1 and C-Jun N-terminal kinase/extracellular signal-regulated kinase mitogen-activated protein kinase pathways. J Mol Cell Cardiol 1999;36:129–39.
[60] Chen K, Thomas SR, Albano A, Murphy MP, Keaney Jr JF. Mitochondrial function is required for hydrogen peroxide-induced growth factor receptor transactivation and downstream signaling. J Biol Chem 2002;279:35079–86.
[61] Wang N, Verna L, Hardy S, Zhu Y, Ma KS, Birrer MJ, et al. c-Jun triggers apoptosis in human vascular endothelial cells. Circ Res 1999;85:387–93.
[62] Ramachandran A, Moellering D, Go YM, Shiva S, Levonen AL, Jo H, et al. Activation of c-Jun N-terminal kinase and apoptosis in endothelial cells mediated by endogenous generation of hydrogen peroxide. Biol Chem 1998;383:693–701.
[63] Chen K, Vita JA, Berk BC, Keaney Jr JF. c-Jun N-terminal kinase activation by hydrogen peroxide in endothelial cells involves SRC-dependent epidermal growth factor receptor transactivation. J Biol Chem 2003;276:16045–50.
[64] Suhara T, Fukuo K, Sugimoto T, Morimoto S, Nakahashi T, Hata S, et al. Hydrogen peroxide induces up-regulation of Fas in human endothelial cells. J Immunol 2000;160:4042–7.
[65] Kotamraju S, Tampo Y, Keszler A, Chitambar CR, Joseph J, Haas AL, et al. Nitric oxide inhibits H_2O_2-induced transferrin receptor-dependent apoptosis in endothelial cells: role of ubiquitin-proteasome pathway. Proc Natl Acad Sci U S A 2004;100:10653–8.
[66] Ballinger SW, Patterson C, Yan CN, Doan R, Burow DL, Young CG, et al. Hydrogen peroxide- and peroxynitrite-induced mitochondrial DNA damage and dysfunction in vascular endothelial and smooth muscle cells. Circ Res 2004;86:960–6.
[67] Dimmeler S, Zeiher AM. Vascular repair by circulating endothelial progenitor cells: the missing link in atherosclerosis? J Mol Med 2004;82:671–7.
[68] Stoneman VE, Bennett MR. Role of apoptosis in atherosclerosis and its therapeutic implications. Clin Sci (Lond) 2002;107:343–54.
[69] Hermann C, Zeiher AM, Dimmeler S. Shear stress inhibits H_2O_2-induced apoptosis of human endothelial cells by modulation of the glutathione redox cycle and nitric oxide synthase. Arterioscler Thromb Vasc Biol 2002;17:3588–92.
[70] Hojo Y, Saito Y, Tanimoto T, Hoefen RJ, Baines CP, Yamamoto K, et al. Fluid shear stress attenuates hydrogen peroxide-induced c-Jun NH2-terminal kinase activation via a glutathione reductase-mediated mechanism. Circ Res 2004;91:712–8.
[71] Haendeler J, Hoffmann J, Tischler V, Berk BC, Zeiher AM, Dimmeler S. Redox regulatory and anti-apoptotic functions of thioredoxin depend on *S*-nitrosylation at cysteine 69. Nat Cell Biol 2002;4:743–9.
[72] Pi X, Yan C, Berk BC. Big mitogen-activated protein kinase (BMK1)/ERK5 protects endothelial cells from apoptosis. Circ Res 2004;94:362–9.
[73] Lum H, Barr DA, Shaffer JR, Gordon RJ, Ezrin AM, Malik AB. Reoxygenation of endothelial cells increases permeability by oxidant-dependent mechanisms. Circ Res 1993;70:991–8.
[74] Siflinger-Birnboim A, Goligorsky MS, Del Vecchio PJ, Malik AB. Activation of protein kinase C pathway contributes to hydrogen peroxide-induced increase in endothelial permeability. Lab Invest 1992;67:24–30.
[75] Suttorp N, Weber U, Welsch T, Schudt C. Role of phosphodiesterases in the regulation of endothelial permeability in vitro. J Clin Invest 1993;91:1421–8.
[76] Hippenstiel S, Tannert-Otto S, Vollrath N, Krull M, Just I, Aktories K, et al. Glucosylation of small GTP-binding Rho proteins disrupts endothelial barrier function. Am J Physiol 2003;272:L38–43.
[77] Kevil CG, Okayama N, Alexander JS. H(2)O(2)-mediated permeability II: importance of tyrosine phosphatase and kinase activity. Am J Physiol Cell Physiol 1996;281:C1940–7.
[78] Usatyuk PV, Vepa S, Watkins T, He D, Parinandi NL, Natarajan V. Redox regulation of reactive oxygen species-induced p38 MAP kinase activation and barrier dysfunction in lung microvascular endothelial cells. Antioxid Redox Signal 2000;5:723–30.
[79] Siflinger-Birnboim A, Lum H, Del Vecchio PJ, Malik AB. Involvement of Ca2+ in the H_2O_2-induced increase in endothelial permeability. Am J Physiol 1997;270:L973–8.
[80] Kevil CG, Oshima T, Alexander B, Coe LL, Alexander JS. H(2)O(2)-mediated permeability: role of MAPK and occludin. Am J Physiol Cell Physiol 2001;279:C21–30.
[81] Hastie LE, Patton WF, Hechtman HB, Shepro D. H_2O_2-induced filamin redistribution in endothelial cells is modulated by the cyclic

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AMP-dependent protein kinase pathway. J Cell Physiol 1997;172: 373–81.
[82] Patterson CE, Lum H. Update on pulmonary edema: the role and regulation of endothelial barrier function. Endothelium 1996;8: 75–105.
[83] Minshall RD, Tiruppathi C, Vogel SM, Malik AB. Vesicle formation and trafficking in endothelial cells and regulation of endothelial barrier function. Histochem Cell Biol 1997;117:105–12.
[84] Chang J, Rao NV, Markewitz BA, Hoidal JR, Michael JR. Nitric oxide donor prevents hydrogen peroxide-mediated endothelial cell injury. Am J Physiol 2001;270:L931–40.
[85] Okayama N, Kevil CG, Correia L, Jourd'heuil D, Itoh M, Grisham MB, et al. Nitric oxide enhances hydrogen peroxide-mediated endothelial permeability in vitro. Am J Physiol 1997;273:C1581–7.
[86] Dudek SM, Garcia JG. Cytoskeletal regulation of pulmonary vascular permeability. J Appl Physiol 2004;91:1487–500.
[87] Huot J, Houle F, Marceau F, Landry J. Oxidative stress-induced actin reorganization mediated by the p38 mitogen-activated protein kinase/heat shock protein 27 pathway in vascular endothelial cells. Circ Res 1998;80:383–92.
[88] Nguyen A, Chen P, Cai H. Role of CaMKII in hydrogen peroxide activation of ERK1/2, p38 MAPK, HSP27 and actin reorganization in endothelial cells. FEBS Lett 1998;572:307–13.
[89] Zhao Y, Davis HW. Hydrogen peroxide-induced cytoskeletal rearrangement in cultured pulmonary endothelial cells. J Cell Physiol 1998;174:370–9.
[90] Holland JA, Meyer JW, Chang MM, O'Donnell RW, Johnson DK, Ziegler LM. Thrombin stimulated reactive oxygen species production in cultured human endothelial cells. Endothelium 2003; 6:113–21.
[91] Djordjevic T, Pogrebniak A, BelAiba RS, Bonello S, Wotzlaw C, Acker H, et al. The expression of the NADPH oxidase subunit p22phox is regulated by a redox-sensitive pathway in endothelial cells. Free Radic Biol Med 1998;38:616–30.
[92] Houle F, Rousseau S, Morrice N, Luc M, Mongrain S, Turner CE, et al. Extracellular signal-regulated kinase mediates phosphorylation of tropomyosin-1 to promote cytoskeleton remodeling in response to oxidative stress: impact on membrane blebbing. Mol Biol Cell 2003;14:1418–32.
[93] Hastie LE, Patton WF, Hechtman HB, Shepro D. Metabolites of the phospholipase D pathway regulate H_2O_2-induced filamin redistribution in endothelial cells. J Cell Biochem 1996;68:511–24.
[94] Andresen BT, Rizzo MA, Shome K, Romero G. The role of phosphatidic acid in the regulation of the Ras/MEK/Erk signaling cascade. FEBS Lett 1986;531:65–8.
[95] Siflinger-Birnboim A, Malik AB. Regulation of endothelial permeability by second messengers. New Horiz 1986;4:87–98.
[96] Thomas G, Ramwell P. Induction of vascular relaxation by hydroperoxides. Biochem Biophys Res Commun 1993;139:102–8.
[97] Rubanyi GM, Vanhoutte PM. Oxygen-derived free radicals, endothelium, and responsiveness of vascular smooth muscle. Am J Physiol 1999;250:H815–21.
[98] Zembowicz A, Hatchett RJ, Jakubowski AM, Gryglewski RJ. Involvement of nitric oxide in the endothelium-dependent relaxation induced by hydrogen peroxide in the rabbit aorta. Br J Pharmacol 1993;110:151–8.
[99] Yang Z, Zhang A, Altura BT, Altura BM. Hydrogen peroxide-induced endothelium-dependent relaxation of rat aorta involvement of Ca2+ and other cellular metabolites. Gen Pharmacol 2001;33: 325–36.
[100] Cai H, Li Z, Davis ME, Kanner W, Harrison DG, Dudley Jr SC. Akt-dependent phosphorylation of serine 1179 and mitogen-activated protein kinase kinase/extracellular signal-regulated kinase 1/2 cooperatively mediate activation of the endothelial nitric-oxide synthase by hydrogen peroxide. Mol Pharmacol 2000;63:325–31.
[101] Laursen JB, Somers M, Kurz S, McCann L, Warnholtz A, Freeman BA, et al. Endothelial regulation of vasomotion in apoE-deficient mice: implications for interactions between peroxynitrite and tetrahydrobiopterin. Circulation 2003;103:1282–8.
[102] Matoba T, Shimokawa H, Nakashima M, Hirakawa Y, Mukai Y, Hirano K, et al. Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in mice. J Clin Invest 2000;106:1521–30.
[103] Yada T, Shimokawa H, Hiramatsu O, Kajita T, Shigeto F, Goto M, et al. Hydrogen peroxide, an endogenous endothelium-derived hyperpolarizing factor, plays an important role in coronary autoregulation in vivo. Circulation 2003;107:1040–5.
[104] Iida Y, Katusic ZS. Mechanisms of cerebral arterial relaxations to hydrogen peroxide. Stroke 2003;31:2224–30.
[105] Miura H, Bosnjak JJ, Ning G, Saito T, Miura M, Gutterman DD. Role for hydrogen peroxide in flow-induced dilation of human coronary arterioles. Circ Res 2001;92:e31–40.
[106] Thengchaisri N, Kuo L. Hydrogen peroxide induces endothelium-dependent and -independent coronary arteriolar dilation: role of cyclooxygenase and potassium channels. Am J Physiol Heart Circ Physiol 2001;285:H2255–63.
[107] Cosentino F, Barker JE, Brand MP, Heales SJ, Werner ER, Tippins JR, et al. Reactive oxygen species mediate endothelium-dependent relaxations in tetrahydrobiopterin-deficient mice. Arterioscler Thromb Vasc Biol 1987;21:496–502.
[108] van Hinsbergh VW. NO or H(2)O(2) for endothelium-dependent vasorelaxation: tetrahydrobiopterin makes the difference. Arterioscler Thromb Vasc Biol 1999;21:719–21.
[109] Wolin MS, Burke TM. Hydrogen peroxide elicits activation of bovine pulmonary arterial soluble guanylate cyclase by a mechanism associated with its metabolism by catalase. Biochem Biophys Res Commun 2003;143:20–5.
[110] Iesaki T, Gupte SA, Kaminski PM, Wolin MS. Inhibition of guanylate cyclase stimulation by NO and bovine arterial relaxation to peroxynitrite and H_2O_2. Am J Physiol 2003;277:H978–85.
[111] Ellis A, Pannirselvam M, Anderson TJ, Triggle CR. Catalase has negligible inhibitory effects on endothelium-dependent relaxations in mouse isolated aorta and small mesenteric artery. Br J Pharmacol 2003;140:1193–200.
[112] Fujimoto S, Mori M, Tsushima H. Mechanisms underlying the hydrogen peroxide-induced, endothelium-independent relaxation of the norepinephrine-contraction in guinea-pig aorta. Eur J Pharmacol 2003;459:65–73.
[113] Drummond GR, Cai H, Davis ME, Ramasamy S, Harrison DG. Transcriptional and posttranscriptional regulation of endothelial nitric oxide synthase expression by hydrogen peroxide. Circ Res 1996;86: 347–54.
[114] Cai H, McNally JS, Weber M, Harrison DG. Oscillatory shear stress upregulation of endothelial nitric oxide synthase requires intracellular hydrogen peroxide and CaMKII. J Mol Cell Cardiol 2004;37:121–5.
[115] Kanazawa K, Kawashima S, Mikami S, Miwa Y, Hirata K, Suematsu M, et al. Endothelial constitutive nitric oxide synthase protein and mRNA increased in rabbit atherosclerotic aorta despite impaired endothelium-dependent vascular relaxation. Am J Pathol 1993;148: 1949–56.
[116] Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, et al. Mechanisms underlying endothelial dysfunction in diabetes mellitus. Circ Res 2004;88:E14–22.
[117] Bradley JR, Johnson DR, Pober JS. Endothelial activation by hydrogen peroxide. Selective increases of intercellular adhesion molecule-1 and major histocompatibility complex class I. Am J Pathol 2001;142:1598–609.
[118] Ziegelstein RC, He C, Hu Q. Hypoxia/reoxygenation stimulates Ca2+-dependent ICAM-1 mRNA expression in human aortic endothelial cells. Biochem Biophys Res Commun 2003;322:68–73.
[119] Lakshminarayanan V, Lewallen M, Frangogiannis NG, Evans AJ, Wedin KE, Michael LH, et al. Reactive oxygen intermediates induce monocyte chemotactic protein-1 in vascular endothelium after brief ischemia. Am J Pathol 2004;159:1301–11.

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[120] Chen XL, Zhang Q, Zhao R, Ding X, Tummala PE, Medford RM. Rac1 and superoxide are required for the expression of cell adhesion molecules induced by tumor necrosis factor-alpha in endothelial cells. J Pharmacol Exp Ther 2003;305:573–80.
[121] Chen XL, Zhang Q, Zhao R, Medford RM. Superoxide, H_2O_2, and iron are required for TNF-alpha-induced MCP-1 gene expression in endothelial cells: role of Rac1 and NADPH oxidase. Am J Physiol Heart Circ Physiol 2004;286:H1001–7.
[122] Takaishi H, Taniguchi T, Takahashi A, Ishikawa Y, Yokoyama M. High glucose accelerates MCP-1 production via p38 MAPK in vascular endothelial cells. Biochem Biophys Res Commun 2003; 305:122–8.
[123] True AL, Rahman A, Malik AB. Activation of NF-kappaB induced by H(2)O(2) and TNF-alpha and its effects on ICAM-1 expression in endothelial cells. Am J Physiol Lung Cell Mol Physiol 2000;279: L302–11.
[124] Pueyo ME, Gonzalez W, Nicoletti A, Savoie F, Arnal JF, Michel JB. Angiotensin II stimulates endothelial vascular cell adhesion molecule-1 via nuclear factor-kappaB activation induced by intracellular oxidative stress. Arterioscler Thromb Vasc Biol 2000;20:645–51.
[125] Lewis MS, Whatley RE, Cain P, McIntyre TM, Prescott SM, Zimmerman GA. Hydrogen peroxide stimulates the synthesis of platelet-activating factor by endothelium and induces endothelial cell-dependent neutrophil adhesion. J Clin Invest 1988;82:2045–55.
[126] Okayama N, Coe L, Oshima T, Itoh M, Alexander JS. Intracellular mechanisms of hydrogen peroxide-mediated neutrophil adherence to cultured human endothelial cells. Microvasc Res 1999;57:63–74.
[127] Fraticelli A, Serrano Jr CV, Bochner BS, Capogrossi MC, Zweier JL. Hydrogen peroxide and superoxide modulate leukocyte adhesion molecule expression and leukocyte endothelial adhesion. Biochim Biophys Acta 1996;1310:251–9.
[128] Shappell SB, Toman C, Anderson DC, Taylor AA, Entman ML, Smith CW. Mac-1 (CD11b/CD18) mediates adherence-dependent hydrogen peroxide production by human and canine neutrophils. J Immunol 1990;144:2702–11.
[129] Hong YH, Peng HB, La Fata V, Liao JK. Hydrogen peroxide-mediated transcriptional induction of macrophage colony-stimulating factor by TGF-beta1. J Immunol 1997;159:2418–23.
[130] Okada H, Woodcock-Mitchell J, Mitchell J, Sakamoto T, Marutsuka K, Sobel BE, et al. Induction of plasminogen activator inhibitor type 1 and type 1 collagen expression in rat cardiac microvascular endothelial cells by interleukin-1 and its dependence on oxygen-centered free radicals. Circulation 1998;97:2175–82.
[131] Lang IM, Moser KM, Schleef RR. Elevated expression of urokinase-like plasminogen activator and plasminogen activator inhibitor type 1 during the vascular remodeling associated with pulmonary thromboembolism. Arterioscler Thromb Vasc Biol 1998;18:808–15.
[132] Kaikita K, Schoenhard JA, Painter CA, Ripley RT, Brown NJ, Fogo AB, et al. Potential roles of plasminogen activator system in coronary vascular remodeling induced by long-term nitric oxide synthase inhibition. J Mol Cell Cardiol 2002;34:617–27.
[133] Weisberg AD, Albornoz F, Griffin JP, Crandall DL, Elokdah H, Fogo AB, et al. Pharmacological inhibition and genetic deficiency of plasminogen activator inhibitor-1 attenuates angiotensin II/salt-induced aortic remodeling. Arterioscler Thromb Vasc Biol 2004;25: 365–71.
[134] Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002;90:251–62.
[135] Yang H, Roberts LJ, Shi MJ, Zhou LC, Ballard BR, Richardson A, et al. Retardation of atherosclerosis by overexpression of catalase or both Cu/Zn-superoxide dismutase and catalase in mice lacking apolipoprotein E. Circ Res 2004;95:1075–81.
[136] Tribble DL, Gong EL, Leeuwenburgh C, Heinecke JW, Carlson EL, Verstuyft JG, et al. Fatty streak formation in fat-fed mice expressing human copper-zinc superoxide dismutase. Arterioscler Thromb Vasc Biol 1997;17:1734–40.
[137] Jung O, Marklund SL, Geiger H, Pedrazzini T, Busse R, Brandes RP. Extracellular superoxide dismutase is a major determinant of nitric oxide bioavailability: in vivo and ex vivo evidence from ecSOD-deficient mice. Circ Res 2003;93:622–9.
[138] Fennell JP, Brosnan MJ, Frater AJ, Hamilton CA, Alexander MY, Nicklin SA, et al. Adenovirus-mediated overexpression of extracellular superoxide dismutase improves endothelial dysfunction in a rat model of hypertension. Gene Ther 2002;9:110–7.
[139] Sentman ML, Brannstrom T, Westerlund S, Laukkanen MO, Yla-Herttuala S, Basu S, et al. Extracellular superoxide dismutase deficiency and atherosclerosis in mice. Arterioscler Thromb Vasc Biol 2001;21:1477–82.

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