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Review Article

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Vascular Cell Responses against Oxidative Stress and its Application

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Abstract

The history of studies in biology regarding reactive oxygen species (ROS) is approximately 40 years. During the initial 30 years, it appeared that these studies were mainly focused on the toxicity of ROS. However, recent studies have identified another action regarding oxidative signaling, other than toxicity of ROS. Basically, it is suggested that ROS are reactive, and degenerate to biomolecules such as DNA and proteins, leading to deterioration of cellular functions as an oxidative stress. On the other hand, recent studies have shown that ROS act as oxidative signaling in cells, resulting in various gene expressions. Recently ROS emerged as critical signaling molecules in cardiovascular research. Several studies over the past decade have shown that physiological effects of vasoactive factors are mediated by these reactive species and, conversely, that altered redox mechanisms are implicated in the occurrence of metabolic and cardiovascular diseases[1-3]. ROS is a collective term often used by scientist to include not only the oxygen radicals ($O2^{-\bullet}$, $^{\bullet}OH$), but also some non-radical derivatives of oxygen. These include hydrogen peroxide, hypochlorous acid (HOCl) and ozone (O3). The superoxide anion ($O2^{-\bullet}$) is formed by the univalent reduction of triplet-state molecular oxygen ($^3O_2$). Superoxide dismutase (SOD)s convert superoxide enzymically into hydrogen peroxide.[4, 5] In biological tissues superoxide can also be converted nonenzymically into the nonradical species hydrogen peroxide and singlet oxygen ($^1O_2$).[6] In the presence of reduced transition metals (e.g., ferrous or cuprous ions), hydrogen peroxide can be converted into the highly reactive hydroxyl radical ($^{\bullet}OH$).[7] Alternatively, hydrogen peroxide may be converted into water by the enzymes catalase or glutathione peroxidase. In the glutathione peroxidase reaction glutathione is oxidized to glutathione disulfide, which can be converted back to glutathione by glutathione reductase in an NADPH-consuming process.

ROS as intracellular messengers

There are various examples of growth factors, cytokines, or other ligands that trigger ROS production in nonphagocytic cells through their corresponding membrane receptors. Such ROS production can mediate a positive feedback effect on signal transduction from these receptors since intracellular signaling is often enhanced by ROS or by a pro-oxidative shift of the intracellular thiol/disulfide redox state. For example, the role of ROS has been demonstrated for nerve growth factor (NGF) signaling in neuronal cells[8], for epidermal growth factor (EGF) signaling in human epidermoid carcinoma cells[9], and for PDGF.[10, 11] Stimulation by any of these growth factors results in a transient increase in intracellular ROS through the signaling protein Rac1. Elimination of hydrogen peroxide by catalase was shown to inhibit EGF- and NGF-induced tyrosine phosphorylation of various cellular proteins, including phosphorylation of the growth factor receptor itself.

The most important insulin-responsive tissues are liver, skeletal muscle, and adipose tissue. In these tissues insulin controls several physiologically important functions, including the rate of glucose uptake, intracellular glucose metabolism, lipid metabolism, and the synthesis of proteins at the transcriptional and translational level.[12] Lower and physiologically relevant concentrations (<0.1 mM) of hydrogen peroxide are not sufficient to trigger the autophosphorylation of the insulin receptor in the absence of insulin, but do enhance the response to 100 nM insulin[13], indicating that the redox signal has a coregulatory function in insulin

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receptor activation under physiologically relevant conditions.

Target molecules of ROS

MAPK signaling cascades are regulated by phosphorylation and dephosphorylation on serine and/or threonine residues and respond to activation of receptor tyrosine kinases, protein tyrosine kinases, receptors of cytokines and growth factors, and heterotrimeric G protein-coupled receptors. Numerous studies with various experimental systems show that in particular the MAPK species JNK and p38 are strongly activated by ROS or by a mild oxidative shift of the intracellular thiol/disulfide redox state.[14-18] The extracellular signal-regulated kinase 1 (ERK-1) and ERK-2 were found to be activated in vascular smooth muscle cells by superoxide but not by hydrogen peroxide.[19] The apoptosis signaling-regulating kinase 1 (ASK1) plays a role in the activation of MKK3/6, MKK4/MKK7, and the MAPK species p38 and JNK.[20] This leads ultimately to the phosphorylation of ATF2, c-Jun, and p53.[21-23]

Screening for ASK1-associated proteins has led to the identification of thioredoxin (Trx) as the redox-sensitive target molecule.[24] Under normal conditions, Trx binds to the NH_2-terminal domain of ASK1 and inhibits its kinase activity. Deletion of the Trx-binding NH_2-terminal residues of ASK1 renders it constitutively active and no longer responsive to the inhibitory effect of Trx. ROS induce the dimerization of Trx and its dissociation from ASK1, followed by multimerization of ASK1 and activation of its kinase activity.[25, 26] Signaling factors such as redox factor-1 (Ref-1) and transcription factors such as the AP-1 complex both contain redox-sensitive cysteine motifs that regulate activity in response to oxidative stress[27].

Changes in the cytosolic Ca^{2+} level play a role in the modulation of several intracellular signal pathways, including PKC- α and calmodulin-dependent signal pathways.[28] The cytosolic Ca^{2+} level can be increased by ROS in various cell types through the mobilization of intracellular Ca^{2+} stores and/or through the influx of extracellular Ca^{2+}.[29-32] The ROS-mediated increase in the cytosolic Ca^{2+} concentration contributes to the oxidative stress-mediated activation of PKC-α[33] and to the transcriptional induction of the AP-1 proteins c-Fos and c-Jun .[34]

The adherence of leukocytes to endothelial cells is also induced by ROS.[35, 36] This effect is abolished by catalase but not by superoxide dismutase, suggesting that hydrogen peroxide and not superoxide is the effective agent.[35] Moreover, the oxidant-induced adherence of monocyte is inhibited by overexpression of redox factor-1 which may have an antioxidant property, suggesting that the induction of adherence may be mediated by oxidative stress within the cell.[37] Adhesion of neutrophils to endothelial cells involves the vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), CD11b/CD18, and L-selectin.[35, 37] In addition, ROS treatment of endothelial cells induces the phosphorylation of the focal adhesion kinase $pp125^{FAK}$, a cytosolic tyrosine kinase that has been implicated in the oxidant-mediated adhesion process.[38]

Enzymatic production of ROS

Multiple enzymatic systems produce $O_2^{\cdot-}$ and its derivatives in the vasculature, including NAD(P)H oxidases, Xanthine oxidase (XO), nitric oxide synthases (NOS), and myeloperoxidase (MPO). The relative importance of each of these proteins appears to vary with the physiological state of the vasculature.

NAD(P)H oxidases consist of multiple subunits: the electron transfer moieties (gp91phox, nox1 or nox4), p22phox, and regulatory subunits (p47phox, p67phox, and rac1). The expression pattern of these subunits varies among vascular cells (Table 1)[39]. The important role of vascular NAD(P)H oxidase will discuss in Section 4. In certain circumstances, NOS can generate $O_2^{\cdot-}$ in addition to $NO^{\cdot}$. NOS utilizes L-arginine as a substrate to synthesize $NO^{\cdot}$ in a tetrahydrobiopterin (H_4B)-dependent manner. If the concentration of L-arginine or H_4B is low, or if H_4B is oxidized, NOS becomes uncoupled and generates significant amounts of $O_2^{\cdot-}$.[40] This occurs in hypertension, where activation of NAD(P)H oxidases leads to oxidation of H_4B and production of large amounts of $O_2^{\cdot-}$ from endothelial NOS.[41] Xanthine oxidoreductase is ubiquitous and appears in two interconvertible, yet functionally distinct, forms: xanthine dehydrogenase and XO.[42] XO metabolizes hypoxanthine, xanthine, and NADH to form $O_2^{\cdot-}$ and H_2O_2. XO-generated ROS have been implicated in various clinicopathologic entities, including ischemia/reperfusion injury, hypercholesterolemia and endothelial dysfunction in chronic heart failure.[42, 43]

Recently, the role of MPO in vascular pathology has been highlighted. MPO is abundant in phagocytes and catalyzes H_2O_2 to produce HOCl and other oxidizing species.[44] It also utilizes NO˙ to generate reactive nitrogen species, thereby reducing NO˙ bioactivity and increasing oxidative stress.[45, 46]

Vascular NADPH oxidase

Vascular NAD(P)H oxidase–dependent overproduction of reactive oxygen species contributes to pathogenesis of cardiovascular diseases.[47-51] Among biologically relevant and abundant reactive oxygen species, superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) appear most important in redox signaling. Whereas $O_2^{\cdot-}$ primarily modulates vascular function by rapidly inactivating NO˙,[52] H_2O_2 impacts on vascular function via complex mechanisms. Ambient production of H_2O_2 at low levels, likely maintained by pre-assembled NAD(P)H oxidases,[50] is necessary for endothelial cell growth and proliferation.[53, 54] Under pathological conditions, however, agonists-provoked activation of vascular NAD(P)H oxidases produces H_2O_2 in large quantities, which in turn amplifies its own production, resulting in compensatory or detrimental consequences. For instance, H_2O_2 is either compensatorily responsible for endothelium-dependent vasodilatation in hypertension where NO˙ is substantially reduced,[41] or over the long term detrimentally involved in vascular smooth muscle cell proliferation and hypertrophy.[55-57]

More recently it has become clear that the vascular wall also produces superoxide, mostly via enzymes similar to the neutrophil oxidase. Furthermore, it was also discovered that the catalytic subunit gp91phox is only one member of a new family of homologous proteins termed nox (for NADPH oxidase) [58-61] and that most cells express multiple nox proteins.[62]

In expression of vascular NAD(P)H oxidases in cells and tissues, evidence that vascular cells can express gp91phox (aka, nox2), as well as nox1, nox4, and nox5, will be presented. It is thought that nox family members transfer electrons from a reduced substrate to molecular oxygen in a way similar to gp91phox. Very recent reports suggest that nox1 can interact with the phagocytic subunits p22phox, p47phox, and p67phox,[59] as well as two novel homologues of p47phox and p67phox.[59] However, it is not yet known whether these latter proteins are expressed in vascular cells.

Table 1. Expression(+) of phagocytic oxidase(phox) components in vascular cells

	VSMCs		Endothelial Cells		Adventitial Cells	
	mRNA	Protein	mRNA	Protein	mRNA	Protein
Gp91phox	-	-	+	+	ND*	+
P22phox	+	+	+	+	ND*	+
P47phox	+	+	+	+	ND*	+
P67phox	-	-	+	+	+	+

*ND indicates not determined.

Monitoring ROS formation in vivo

Traditionally, attention has focused on the development of in vivo biomarkers of oxidant stress. Essentially, the approach has been indirect and configured on the identification of chemically stable, free radical–catalyzed products of lipid peroxidation (such as isoprostanes), modified proteins (such as nitrated fibrinogen), and indices of free radical–catalyzed modification of DNA (such as 8-oxo-deoxyguanosine).[25, 63, 64] Much of the earlier literature has been confounded by limitations reflective of ex vivo methodology or intrinsic to the specific approach. These include the nonspecific route to formation of the anylate, the imprecision with which the anylate is quantified, and the possibility that ROS generation is related nonlinearly to alterations in the anylate. Finally, ROS generation can result in modification of lipids, protein, and DNA.[65-67] Approaches to quantification of ROS generation in vivo have tended to focus on a single anylate within one of these broad categories, and an integrated approach, using modern spectroscopic methods, has yet to be applied. Earlier studies have focused most commonly on products of lipid peroxidation. These have included the measurement of thiobarbituric acid–reacting substances, including malonyldialdehyde. However, these compounds can be formed nonspecifically (malonyldialdehyde is a byproduct of cyclooxygenase turnover), and ex vivo platelet activation may seriously confound measurements.[68]

An example of the more recently discovered anylates formed in vivo are the isoprostanes (iPs), chemically stable, free radical–catalyzed products of arachidonic acid.[69] These compounds are free radical–catalyzed isomers of traditional enzymatic products of arachidonic acid metabolism. They are formed initially in situ in the phospholipid domain of cell membranes subject to ROS

attack and are then cleaved by phospholipases, released extracellularly, circulated, and excreted in urine.[67, 70] A range of mass spectroscopic assays have emerged on the basis of authentic standards for individual F_2 iPs.[71-75] Current immunoassays directed against $iPF_{2\alpha}$-III (also known as 8-iso $PGF_{2\alpha}$) are more commonly used.

Oxidative stress and cardiovascular disease

Atherosclerosis is a multifactorial disease characterized by hardening and thickening of the arterial wall. The vascular areas affected by this disease contain mononuclear cells, proliferating smooth muscle cells, and extracellular matrix components. Atherosclerosis is commonly viewed as a chronic inflammatory disease and is associated with certain risk factors such as hyperlipidemia, diabetes, and hypertension. Excessive ROS production has been implicated in the pathogenesis of atherosclerosis and hypertension. [76-80] . Excessive ROS production is associated with massive macrophage apoptosis and contributes thereby to the formation of the atherosclerotic lesions.[81, 82] The process may be further enhanced by cytokines and other factors such as TNF, interleukin-1β, angiotensin II, and interferon-γ, which induce superoxide production by the membrane-bound NADPH oxidase in endothelial cells.[83-85]

Ischemia and reperfusion can lead to tissue injury and are serious complications in organ transplantation, myocardial infarction, and stroke.[86-88] Massive ROS production was identified as an important causative factor.[89-91] Xanthine dehydrogenase, which normally utilizes NAD^+ as electron acceptor, is converted under the conditions of ischemia/reperfusion into xanthine oxidase which uses oxygen as substrate. More recently, a Rac1-regulated NAD(P)H oxidase distinct from the phagocytic NAD(P)H oxidase was shown to be critically involved in ROS production in a mouse model of hepatic ischemia/reperfusion injury.[92] Also, treatment with a synthetic SOD mimetic was shown to ameliorate tissue damage in a rat model of ischemia/reperfusion injury.[93]

In hypertension, the role of the angiotensin-1 (AT1) receptor has been the subject of intense investigation in both in vitro and animal models. Ang II modulates hypertension through its effect on the renin-angiotensin system, and the stimulation of AT1 receptors in the vascular wall leads to activation of NADH/NAD(P)H oxidase in vascular cells. The resultant oxidative stress is considered a unifying mechanism for hypertension and atherosclerosis.[94, 95]

Application of cell permeable ROS inhibitors

Oxidative stress, involving elevated levels of ROS such as superoxide and peroxynitrite, has been implicated in the pathogenesis of several, if not most, forms of cardiovascular disease. Recent studies using viral-mediated gene transfer of genes that redress oxidative stress in animal models of cardiovascular disease have suggested that targeting sources of superoxide would provide a novel therapeutic strategy in cardiovascular disease. However, in vivo, gene therapy approaches relying on adenoviral vectors are associated with significant difficulties relating to a lack of target specificity and toxicity which have contributed to poor performance in several clinical trials.

Remarkably, the recent identification of a particular group of proteins with enhanced ability to cross the plasma membrane in a receptor-independent fashion has led to the discovery of a class of protein domains with cell membrane penetrating properties. The fusion of these protein transduction domain peptide sequences with heterologous proteins is sufficient to cause their rapid transduction into a variety of different cells in a rapid, concentration-dependent manner[96].

It is necessary, therefore, to establish a novel technique to introduce membrane proteins onto live cells, in particular, directly from outside of the cell. In this regard, we focused on using the protein transduction domain (PTD) of HIV-TAT protein.[97] The Tat PTD, a short basic region composed of residues 47–57 of HIV Tat protein, delivers fused peptides into live cells in vitro and in vivo (reviewed in[98]). Identification of a vascular form of the NAD(P)H oxidase as the major source of superoxide has resulted in a search for effective inhibitors. Recently, it was reported a chimeric peptide inhibitor (gp91ds-tat) that interferes with the assembly of vascular NAD(P)H oxidase components, and showed that this chimera abolished Ang II–induced aortic O_2^- generation in vitro and in vivo[99]. However, this novel strategy of "molecular transplantation" can be applied to modulate cell functions for use in Various biological fields.

Closing Remark

The most exciting aspect of protein transduction technology is the previously unheard-of ability to address specific inhibition of oxidative stress and the pathophysiology of cardiovascular diseases. These kinds effort to reduce oxidative stress, we look forward to being able to restore cardiovascular disorder or interfere with various oxidative pathways using this technology in the coming years.

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References

1. Angkeow P, Deshpande SS, Qi B, Liu YX, Park YC, Jeon BH, Ozaki M, Irani K. Redox factor-1: an extra-nuclear role in the regulation of endothelial oxidative stress and apoptosis. Cell Death Differ. 2002;9(7):717-725.
2. Hur GM, Ryu YS, Yun HY, Jeon BH, Kim YM, Seok JH, Lee JH. Hepatic ischemia/reperfusion in rats induces iNOS gene transcription by activation of NF-kappaB. Biochem Biophys Res Commun. 1999;261(3):917-922.
3. Jeon BH, Gupta G, Park YC, Qi B, Haile A, Khanday FA, Liu YX, Kim JM, Ozaki M, White AR, Berkowitz DE, Irani K. Apurinic/apyrimidinic endonuclease 1 regulates endothelial NO production and vascular tone. Circ Res. 2004;95(9):902-910.
4. Deby C, Goutier R. New perspectives on the biochemistry of superoxide anion and the efficiency of superoxide dismutases. Biochem Pharmacol. 1990;39(3):399-405.
5. Fridovich I. The biology of oxygen radicals. Science. 1978;201(4359):875-880.
6. Stadtman ER. Oxidation of free amino acids and amino acid residues in proteins by radiolysis and by metal-catalyzed reactions. Annu Rev Biochem. 1993;62:797-821.
7. Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev. 1979;59(3):527-605.
8. Suzukawa K, Miura K, Mitsushita J, Resau J, Hirose K, Crystal R, Kamata T. Nerve growth factor-induced neuronal differentiation requires generation of Rac1-regulated reactive oxygen species. J Biol Chem. 2000;275(18):13175-13178.
9. Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, Rhee SG. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. J Biol Chem. 1997;272(1):217-221.
10. Bae YS, Sung JY, Kim OS, Kim YJ, Hur KC, Kazlauskas A, Rhee SG. Platelet-derived growth factor-induced H(2)O(2) production requires the activation of phosphatidylinositol 3-kinase. J Biol Chem. 2000;275(14):10527-10531.
11. Heeneman S, Haendeler J, Saito Y, Ishida M, Berk BC. Angiotensin II induces transactivation of two different populations of the platelet-derived growth factor beta receptor. Key role for the p66 adaptor protein Shc. J Biol Chem. 2000;275(21):15926-15932.
12. O'Brien RM, Granner DK. Regulation of gene expression by insulin. Physiol Rev. 1996;76(4):1109-1161.
13. Schmid E, Hotz-Wagenblatt A, Hacj V, Droge W. Phosphorylation of the insulin receptor kinase by phosphocreatine in combination with hydrogen peroxide: the structural basis of redox priming. Faseb J. 1999;13(12):1491-1500.
14. Abe J, Kusuhara M, Ulevitch RJ, Berk BC, Lee JD. Big mitogen-activated protein kinase 1 (BMK1) is a redox-sensitive kinase. J Biol Chem. 1996;271(28):16586-16590.
15. Allen RG, Tresini M. Oxidative stress and gene regulation. Free Radic Biol Med. 2000;28(3):463-499.
16. Guyton KZ, Liu Y, Gorospe M, Xu Q, Holbrook NJ. Activation of mitogen-activated protein kinase by H2O2. Role in cell survival following oxidant injury. J Biol Chem. 1996;271(8):4138-4142.
17. Hehner SP, Breitkreutz R, Shubinsky G, Unsoeld H, Schulze-Osthoff K, Schmitz ML, Droge W. Enhancement of T cell receptor signaling by a mild oxidative shift in the intracellular thiol pool. J Immunol. 2000;165(8):4319-4328.
18. Lo YY, Wong JM, Cruz TF. Reactive oxygen species mediate cytokine activation of c-Jun NH2-

terminal kinases. J Biol Chem. 1996;271(26):15703-15707.

19. Baas AS, Berk BC. Differential activation of mitogen-activated protein kinases by H2O2 and O2- in vascular smooth muscle cells. Circ Res. 1995;77(1):29-36.
20. Pu M, Akhand AA, Kato M, Hamaguchi M, Koike T, Iwata H, Sabe H, Suzuki H, Nakashima I. Evidence of a novel redox-linked activation mechanism for the Src kinase which is independent of tyrosine 527-mediated regulation. Oncogene. 1996;13(12):2615-2622.
21. Adler V, Pincus MR, Minamoto T, Fuchs SY, Bluth MJ, Brandt-Rauf PW, Friedman FK, Robinson RC, Chen JM, Wang XW, Harris CC, Ronai Z. Conformation-dependent phosphorylation of p53. Proc Natl Acad Sci U S A. 1997;94(5):1686-1691.
22. Fuchs SY, Adler V, Pincus MR, Ronai Z. MEKK1/JNK signaling stabilizes and activates p53. Proc Natl Acad Sci U S A. 1998;95(18):10541-10546.
23. Ip YT, Davis RJ. Signal transduction by the c-Jun N-terminal kinase (JNK)--from inflammation to development. Curr Opin Cell Biol. 1998;10(2):205-219.
24. Saitoh M, Nishitoh H, Fujii M, Takeda K, Tobiume K, Sawada Y, Kawabata M, Miyazono K, Ichijo H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. Embo J. 1998;17(9):2596-2606.
25. Liu H, Nishitoh H, Ichijo H, Kyriakis JM. Activation of apoptosis signal-regulating kinase 1 (ASK1) by tumor necrosis factor receptor-associated factor 2 requires prior dissociation of the ASK1 inhibitor thioredoxin. Mol Cell Biol. 2000;20(6):2198-2208.
26. Gotoh Y, Cooper JA. Reactive oxygen species- and dimerization-induced activation of apoptosis signal-regulating kinase 1 in tumor necrosis factor-alpha signal transduction. J Biol Chem. 1998;273(28):17477-17482.
27. Hsieh MM, Hegde V, Kelley MR, Deutsch WA. Activation of APE/Ref-1 redox activity is mediated by reactive oxygen species and PKC phosphorylation. Nucleic Acids Res. 2001;29(14):3116-3122.
28. Clapham DE. Calcium signaling. Cell. 1995;80(2):259-268.
29. Hallbrucker C, Ritter M, Lang F, Gerok W, Haussinger D. Hydroperoxide metabolism in rat liver. K+ channel activation, cell volume changes and eicosanoid formation. Eur J Biochem. 1993;211(3):449-458.
30. Kumasaka S, Shoji H, Okabe E. Novel mechanisms involved in superoxide anion radical-triggered Ca2+ release from cardiac sarcoplasmic reticulum linked to cyclic ADP-ribose stimulation. Antioxid Redox Signal. 1999;1(1):55-69.
31. Doan TN, Gentry DL, Taylor AA, Elliott SJ. Hydrogen peroxide activates agonist-sensitive Ca(2+)-flux pathways in canine venous endothelial cells. Biochem J. 1994;297 (Pt 1):209-215.
32. Dreher D, Junod AF. Role of oxygen free radicals in cancer development. Eur J Cancer. 1996;32A(1):30-38.
33. Larsson R, Cerutti P. Translocation and enhancement of phosphotransferase activity of protein kinase C following exposure in mouse epidermal cells to oxidants. Cancer Res. 1989;49(20):5627-5632.
34. Maki A, Berezesky IK, Fargnoli J, Holbrook NJ, Trump BF. Role of [Ca2+]i in induction of c-fos, c-jun, and c-myc mRNA in rat PTE after oxidative stress. Faseb J. 1992;6(3):919-924.
35. Sellak H, Franzini E, Hakim J, Pasquier C. Reactive oxygen species rapidly increase endothelial ICAM-1 ability to bind neutrophils without detectable upregulation. Blood. 1994;83(9):2669-2677.
36. Roy S, Sen CK, Packer L. Determination of cell-cell adhesion in response to oxidants and antioxidants. Methods Enzymol. 1999;300:395-401.
37. Kim CS, Son SJ, Kim EK, Kim SN, Yoo DG, Kim HS, Ryoo SW, Lee SD, Irani K, Jeon BH. Apurinic/apyrimidinic endonuclease1/redox factor-1 inhibits monocyte adhesion in endothelial cells. Cardiovascular Research. 2005 (In press).
38. Schaller MD, Borgman CA, Cobb BS, Vines RR, Reynolds AB, Parsons JT. pp125FAK a structurally distinctive protein-tyrosine kinase associated with focal adhesions. Proc Natl Acad Sci U S A. 1992;89(11):5192-5196.
39. Hanna IR, Taniyama Y, Szocs K, Rocic P, Griendling KK. NAD(P)H oxidase-derived reactive

oxygen species as mediators of angiotensin II signaling. Antioxid Redox Signal. 2002;4(6):899-914.

40. Stuehr D, Pou S, Rosen GM. Oxygen reduction by nitric-oxide synthases. J Biol Chem. 2001;276(18):14533-14536.
41. Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest. 2003;111(8):1201-1209.
42. Harrison R. Structure and function of xanthine oxidoreductase: where are we now? Free Radic Biol Med. 2002;33(6):774-797.
43. Landmesser U, Spiekermann S, Dikalov S, Tatge H, Wilke R, Kohler C, Harrison DG, Hornig B, Drexler H. Vascular oxidative stress and endothelial dysfunction in patients with chronic heart failure: role of xanthine-oxidase and extracellular superoxide dismutase. Circulation. 2002;106(24):3073-3078.
44. Winterbourn CC, Vissers MC, Kettle AJ. Myeloperoxidase. Curr Opin Hematol. 2000;7(1):53-58.
45. Eiserich JP, Baldus S, Brennan ML, Ma W, Zhang C, Tousson A, Castro L, Lusis AJ, Nauseef WM, White CR, Freeman BA. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. Science. 2002;296(5577):2391-2394.
46. Gaut JP, Byun J, Tran HD, Lauber WM, Carroll JA, Hotchkiss RS, Belaaouaj A, Heinecke JW. Myeloperoxidase produces nitrating oxidants in vivo. J Clin Invest. 2002;109(10):1311-1319.
47. Brandes RP. Role of NADPH oxidases in the control of vascular gene expression. Antioxid Redox Signal. 2003;5(6):803-811.
48. Cai H, Griendling KK, Harrison DG. The vascular NAD(P)H oxidases as therapeutic targets in cardiovascular diseases. Trends Pharmacol Sci. 2003;24(9):471-478.
49. Griendling KK. Novel NAD(P)H oxidases in the cardiovascular system. Heart. 2004;90(5):491-493.
50. Li JM, Shah AM. Endothelial cell superoxide generation: regulation and relevance for cardiovascular pathophysiology. Am J Physiol Regul Integr Comp Physiol. 2004;287(5):R1014-1030.
51. Touyz RM, Schiffrin EL. Reactive oxygen species in vascular biology: implications in hypertension. Histochem Cell Biol. 2004;122(4):339-352.
52. Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res. 2000;87(10):840-844.
53. Griendling KK, Harrison DG. Dual role of reactive oxygen species in vascular growth. Circ Res. 1999;85(6):562-563.
54. Eyries M, Collins T, Khachigian LM. Modulation of growth factor gene expression in vascular cells by oxidative stress. Endothelium. 2004;11(2):133-139.
55. Ushio-Fukai M, Zafari AM, Fukui T, Ishizaka N, Griendling KK. p22phox is a critical component of the superoxide-generating NADH/NADPH oxidase system and regulates angiotensin II-induced hypertrophy in vascular smooth muscle cells. J Biol Chem. 1996;271(38):23317-23321.
56. Zafari AM, Ushio-Fukai M, Akers M, Yin Q, Shah A, Harrison DG, Taylor WR, Griendling KK. Role of NADH/NADPH oxidase-derived H2O2 in angiotensin II-induced vascular hypertrophy. Hypertension. 1998;32(3):488-495.
57. Jin ZG, Melaragno MG, Liao DF, Yan C, Haendeler J, Suh YA, Lambeth JD, Berk BC. Cyclophilin A is a secreted growth factor induced by oxidative stress. Circ Res. 2000;87(9):789-796.
58. Suh YA, Arnold RS, Lassegue B, Shi J, Xu X, Sorescu D, Chung AB, Griendling KK, Lambeth JD. Cell transformation by the superoxide-generating oxidase Mox1. Nature. 1999;401(6748):79-82.
59. Banfi B, Maturana A, Jaconi S, Arnaudeau S, Laforge T, Sinha B, Ligeti E, Demaurex N, Krause KH. A mammalian H+ channel generated through alternative splicing of the NADPH oxidase homolog NOH-1. Science. 2000;287(5450):138-142.
60. Lambeth JD, Cheng G, Arnold RS, Edens WA. Novel homologs of gp91phox. Trends Biochem Sci. 2000;25(10):459-461.
61. Lambeth JD. Nox/Duox family of nicotinamide adenine dinucleotide (phosphate) oxidases. Curr Opin Hematol. 2002;9(1):11-17.
62. Cheng G, Cao Z, Xu X, van Meir EG, Lambeth JD. Homologs of gp91phox: cloning and tissue

expression of Nox3, Nox4, and Nox5. Gene. 2001;269(1-2):131-140.

63. O'Donnell VB, Eiserich JP, Bloodsworth A, Chumley PH, Kirk M, Barnes S, Darley-Usmar VM, Freeman BA. Nitration of unsaturated fatty acids by nitric oxide-derived reactive species. Methods Enzymol. 1999;301:454-470.

64. Murphy RC, Zarini S. Glutathione adducts of oxyeicosanoids. Prostaglandins Other Lipid Mediat. 2002;68-69:471-482.

65. Lawson JA, Rokach J, FitzGerald GA. Isoprostanes: formation, analysis and use as indices of lipid peroxidation in vivo. J Biol Chem. 1999;274(35):24441-24444.

66. Lee SH, Blair IA. Oxidative DNA damage and cardiovascular disease. Trends Cardiovasc Med. 2001;11(3-4):148-155.

67. Lorch S, Lightfoot R, Ohshima H, Virag L, Chen Q, Hertkorn C, Weiss M, Souza J, Ischiropoulos H, Yermilov V, Pignatelli B, Masuda M, Szabo C. Detection of peroxynitrite-induced protein and DNA modifications. Methods Mol Biol. 2002;196:247-275.

68. Catella F, Healy D, Lawson JA, FitzGerald GA. 11-Dehydrothromboxane B2: a quantitative index of thromboxane A2 formation in the human circulation. Proc Natl Acad Sci U S A. 1986;83(16):5861-5865.

69. Roberts LJ, 2nd, Morrow JD. Products of the isoprostane pathway: unique bioactive compounds and markers of lipid peroxidation. Cell Mol Life Sci. 2002;59(5):808-820.

70. Rokach J, Khanapure SP, Hwang SW, Adiyaman M, Lawson JA, FitzGerald GA. Nomenclature of isoprostanes: a proposal. Prostaglandins. 1997;54(6):853-873.

71. Morrow JD, Awad JA, Boss HJ, Blair IA, Roberts LJ, 2nd. Non-cyclooxygenase-derived prostanoids (F2-isoprostanes) are formed in situ on phospholipids. Proc Natl Acad Sci U S A. 1992;89(22):10721-10725.

72. Lawson JA, Li H, Rokach J, Adiyaman M, Hwang SW, Khanapure SP, FitzGerald GA. Identification of two major F2 isoprostanes, 8,12-iso- and 5-epi-8, 12-iso-isoprostane F2alpha-VI, in human urine. J Biol Chem. 1998;273(45):29295-29301.

73. Li H, Lawson JA, Reilly M, Adiyaman M, Hwang SW, Rokach J, FitzGerald GA. Quantitative high performance liquid chromatography/tandem mass spectrometric analysis of the four classes of F(2)-isoprostanes in human urine. Proc Natl Acad Sci U S A. 1999;96(23):13381-13386.

74. Morrow JD, Zackert WE, Yang JP, Kurhts EH, Callewaert D, Dworski R, Kanai K, Taber D, Moore K, Oates JA, Roberts LJ. Quantification of the major urinary metabolite of 15-F2t-isoprostane (8-iso-PGF2alpha) by a stable isotope dilution mass spectrometric assay. Anal Biochem. 1999;269(2):326-331.

75. Burke A, Lawson JA, Meagher EA, Rokach J, FitzGerald GA. Specific analysis in plasma and urine of 2,3-dinor-5, 6-dihydro-isoprostane F(2alpha)-III, a metabolite of isoprostane F(2alpha)-III and an oxidation product of gamma-linolenic acid. J Biol Chem. 2000;275(4):2499-2504.

76. Cathcart MK, McNally AK, Morel DW, Chisolm GM, 3rd. Superoxide anion participation in human monocyte-mediated oxidation of low-density lipoprotein and conversion of low-density lipoprotein to a cytotoxin. J Immunol. 1989;142(6):1963-1969.

77. Auch-Schwelk W, Bossaller C, Claus M, Graf K, Grafe M, Fleck E. Local potentiation of bradykinin-induced vasodilation by converting-enzyme inhibition in isolated coronary arteries. J Cardiovasc Pharmacol. 1992;20 Suppl 9:S62-67.

78. Halliwell B. Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. Br J Exp Pathol. 1989;70(6):737-757.

79. Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res. 1994;74(6):1141-1148.

80. Alexander RW. Theodore Cooper Memorial Lecture. Hypertension and the pathogenesis of atherosclerosis. Oxidative stress and the mediation of arterial inflammatory response: a new perspective. Hypertension. 1995;25(2):155-161.

81. Kinscherf R, Wagner M, Kamencic H, Bonaterra GA, Hou D, Schiele RA, Deigner HP, Metz J. Characterization of apoptotic macrophages in atheromatous tissue of humans and heritable

hyperlipidemic rabbits. Atherosclerosis. 1999;144(1):33-39.

82. Reid VC, Mitchinson MJ, Skepper JN. Cytotoxicity of oxidized low-density lipoprotein to mouse peritoneal macrophages: an ultrastructural study. J Pathol. 1993;171(4):321-328.

83. Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest. 1993;91(6):2546-2551.

84. Mohazzab KM, Kaminski PM, Wolin MS. NADH oxidoreductase is a major source of superoxide anion in bovine coronary artery endothelium. Am J Physiol. 1994;266(6 Pt 2):H2568-2572.

85. De Keulenaer GW, Alexander RW, Ushio-Fukai M, Ishizaka N, Griendling KK. Tumour necrosis factor alpha activates a p22phox-based NADH oxidase in vascular smooth muscle. Biochem J. 1998;329 (Pt 3):653-657.

86. Gersh BJ. Current issues in reperfusion therapy. Am J Cardiol. 1998;82(8B):3P-11P.

87. Goode HF, Webster NR, Howdle PD, Leek JP, Lodge JP, Sadek SA, Walker BE. Reperfusion injury, antioxidants and hemodynamics during orthotopic liver transplantation. Hepatology. 1994;19(2):354-359.

88. Garcia JH, Lassen NA, Weiller C, Sperling B, Nakagawara J. Ischemic stroke and incomplete infarction. Stroke. 1996;27(4):761-765.

89. Downey JM. Free radicals and their involvement during long-term myocardial ischemia and reperfusion. Annu Rev Physiol. 1990;52:487-504.

90. Chien S, Li S, Shyy YJ. Effects of mechanical forces on signal transduction and gene expression in endothelial cells. Hypertension. 1998;31(1 Pt 2):162-169.

91. Chan PH. Role of oxidants in ischemic brain damage. Stroke. 1996;27(6):1124-1129.

92. Ozaki M, Deshpande SS, Angkeow P, Bellan J, Lowenstein CJ, Dinauer MC, Goldschmidt-Clermont PJ, Irani K. Inhibition of the Rac1 GTPase protects against nonlethal ischemia/reperfusion-induced necrosis and apoptosis in vivo. Faseb J. 2000;14(2):418-429.

93. Salvemini D, Wang ZQ, Zweier JL, Samouilov A, Macarthur H, Misko TP, Currie MG, Cuzzocrea S, Sikorski JA, Riley DP. A nonpeptidyl mimic of superoxide dismutase with therapeutic activity in rats. Science. 1999;286(5438):304-306.

94. Zalba G, San Jose G, Moreno MU, Fortuno MA, Fortuno A, Beaumont FJ, Diez J. Oxidative stress in arterial hypertension: role of NAD(P)H oxidase. Hypertension. 2001;38(6):1395-1399.

95. Nickenig G, Harrison DG. The AT(1)-type angiotensin receptor in oxidative stress and atherogenesis: part I: oxidative stress and atherogenesis. Circulation. 2002;105(3):393-396.

96. Schwarze SR, Ho A, Vocero-Akbani A, Dowdy SF. In vivo protein transduction: delivery of a biologically active protein into the mouse. Science. 1999;285(5433):1569-1572.

97. Watson K, Edwards RJ. HIV-1-trans-activating (Tat) protein: both a target and a tool in therapeutic approaches. Biochem Pharmacol. 1999;58(10):1521-1528.

98. Wadia JS, Stan RV, Dowdy SF. Transducible TAT-HA fusogenic peptide enhances escape of TAT-fusion proteins after lipid raft macropinocytosis. Nat Med. 2004;10(3):310-315.

99. Jacobson GM, Dourron HM, Liu J, Carretero OA, Reddy DJ, Andrzejewski T, Pagano PJ. Novel NAD(P)H oxidase inhibitor suppresses angioplasty-induced superoxide and neointimal hyperplasia of rat carotid artery. Circ Res. 2003;92(6):637-643.