Roles of Oxidative Stress and Redox Regulation in Atherosclerosis

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Oxidative stress is believed to be a cause of aging and cardiovascular disorders. In response to inflammation or endothelial cell injury, production of reactive oxygen species (ROS) is enhanced in vascular cells. These changes contribute to the initiation of atherosclerosis. Vascular cells possess anti-oxidant systems to protect against oxidative stress, in addition to the redox system. The redox status of protein thiols is important for cellular functions. The Akt signaling pathway exerts effects on survival and apoptosis, and is regulated by the glutathione (GSH)/glutaredoxin (GRX)-dependent redox system. Sex hormones such as estrogens protect against oxidative stress by protecting the Akt signaling pathway but the physiological role of the extracellular GSH/GRX system has not been clarified, although found an increase in the levels of S-glutathionylated serum proteins in patients with atherosclerosis obliterans. The results suggested that impaired serum redox potential is a marker of the development vascular dysfunction and estrogen has a possible role in the prevention of atherosclerosis.


Key words; Oxidative stress, S-glutathionylation, Redox, Vascular function, Estradiol, Dehydroepiandrosterone

Introduction

Oxidative stress is a principle cause of the development of aging and diseases such as inflammation, infection, cancer, and cardiovascular disorders. Exogenous or endogenous sources of oxidative stress and weakened antioxidative defenses can damage macromolecules such as DNA, lipids, and proteins, and many of the enzymes expressed in vascular endothelial cells contribute to the production of reactive oxygen species (ROS), such as xanthine oxidase, NADPH-oxidase, and endothelial nitric oxide. In response to inflammation or endothelial cell injury, these ROS-producing enzymes are activated in vascular cells; in particular, decreased bioavailability of NO together with superoxide production by endothelial NO synthase (eNOS) is observed in several vascular diseases. Tetrahydrobiopterin (BH4) is an essential cofactor for eNOS and its level is regulated by guanosine triphosphate cyclohydrolase 1, a rate-limiting enzyme in BH4 synthesis, and the redox state of BH4. Under oxidative stress, BH4 is oxidized to form 7,8-dihydropterin (BH2) and biopterin, both incapable of eNOS catalysis. The redox regulation of BH4 and BH2 seems clinically important, although, the precise mechanism is not clear.

Oxidative Stress and Vascular Function

The production of ROS in vascular endothelial cells induces the oxidation of LDL and the expression of ROS-sensitive inflammatory genes. This process is involved in early atherosclerotic processes, such as the ROS-sensitive expression of vascular cell adhesion molecule-1 (VCAM-1), monocyte chemotactic protein-1 (MCP-1), and functionally, endothelial monocyte adhesion and infiltration. These changes may induce alterations in the structure and function of endothelial cells and contribute to the initiation of atherosclerosis; however, vascular cells also possess anti-oxidant systems, such as superoxide dismutase, catalase, glutathione (GSH)-synthesizing enzymes, GSH peroxidase, GSH S-transferase (GST), and so
Blood contains anti-oxidants such as vitamin C, vitamin D, vitamin E, albumin, uric acid, GSH, and GST, which are thought to protect against oxidative stress, a hypothetical etiologic factor in human aging and age-related diseases such as cardiovascular diseases.

In addition to these ROS-generating and antioxidant systems, there exists a redox system in most cells. The redox status of sulfhydryl groups is important to cellular functions such as the synthesis and folding of proteins and regulation of the structure and activity of enzymes, receptors, and transcriptional factors, and is a crucial mediator of metabolic signaling and transcriptional processes in cells. ROS are known to be important second messengers in intracellular signaling; for example, most receptor-mediated signaling pathways require ROS. When cells are exposed to oxidative stress, protein thiols are easily modified, forming inter- or intra-molecular disulfides (Pr-S-S-Pr), or S-glutathionylation with glutathione (Pr-S-SG). The S-glutathionylation of proteins occurs between protein thiols and glutathione disulfide (GSSG). The protein thiols are further oxidized to sulfenic (Pr-SOH), sulfinic (Pr-SO$_2$H) and sulfonic (Pr-SO$_3$H) (Fig. 1).

The first two steps in the modification of thiols are reversible via a catalytic reaction with glutaredoxin (GRX). S-Glutathionylation is likely the predominant physiological sulfhydryl modification due to the abundance of cellular GSH. GRX catalyzes the reduction of protein disulfides via a disulfide exchange reaction by utilizing the active site Cys-Pro-Tyr-Cys with a dithiol mechanism involving both active-site thiols and has a unique ability to reduce protein-S-S-glutathione mixed disulfide (deglutathionylation) or to participate in its formation (S-glutathionylation) through a mechanism involving monothiol, which requires only the more N-terminal active site Cys. Oxidized GRX is selectively recycled to the reduced form by GSH with the formation of glutathione disulfide (GSSG) and regeneration of GSH through coupling with NADPH and GSSG reductase, a system termed the GSH-regenerating system. These characteristic interactions with GSH distinguish GRX from TRX, which favors intermolecular disulfide substrates and is turned over by NADPH and thioredoxin reductase independent of GSH.

Mammalian GRX is known to have two isoforms, GRX1 and GRX2; GRX1 is a cytosolic form of GRX. GRX1 and S-glutathionylation are thought to be involved in a variety of cellular events such as signal transduction, stress response, and metabolic regulation, by regulating the redox status of various cellular proteins, including HIV-1 protease, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), nuclear factor, ASK1, actin, Ras, tubulin, tau and microtubule-associated protein-2, annexin A2, and protein tyrosine phosphatase 1B. We have also reported that GRX plays an important role in protecting cells from apoptosis by regulating the redox status of Akt/protein kinase B. The other form of GRX, GRX2, is distributed in the mitochondria and nucleus, and also functions as part of the cellular antioxidant defense system by regulating the redox status of mitochondrial proteins such as complex I protein. Serine/threonine kinase Akt is a critical component of an intracellular signaling pathway that exerts effects on survival and apoptosis. The unphosphorylated form of Akt is virtually inactive, and phosphorylation at
Thr-308 and Ser-473 stimulates its activity. Inactivation of Akt also occurs via dephosphorylation of the two phosphorylation sites by protein phosphatase 2A (PP2A)\(^{28}\); the activation of Akt contributes to the survival of ROS-treated cells\(^ {29}\). There exists a mechanism for the antiapoptotic effect of GRX1 via regulation of the redox state of Akt under oxidative stress. The S-glutathionylation of Akt at Cys-297 and Cys-311 by ROS is reduced by GRX1, inhibiting the association with PP2A, and the dephosphorylation of Akt by PP2A (Fig. 2)\(^ {26}\).

### Estradiol and Vascular Function

As mentioned above, vascular dysfunction is characterized by a reduction in the anti-oxidative response, an increase in inflammatory mediators and ROS. Estrogens, in contrast to aging, are known to be vasoprotective\(^ {30}\). Since the discovery of estrogen receptor (ER) subtypes, ER\(\alpha\) and ER\(\beta\), in 1996, it has been clarified that these ERs exist in the heart, brain, vessels, bones, and the mammary gland in addition to the sex organs. Estrogens, such as 17beta-estradiol (E\(2\)), play an important role in development, growth, and differentiation, and appear to have protective effects on oxidative stress mediated by ER\(\alpha\)\(^ {31}\). Inhibitory effects of E\(2\) on atherosclerosis are mediated by COX-2-derived prostacyclin\(^ {32}\) and E\(2\) induces the production of antioxidative enzymes, such as superoxide dismutase\(^ {33}\), and GST\(^ {34}\). The effects of E\(2\) are mediated mostly through ER\(\alpha\), which functions as a ligand-induced transcription factor and belongs to the nuclear receptor superfamily. Hamada et al. suggested that both ERs contributed to functional recovery after myocardial infarction in bone marrow-derived endothelial progenitor cells\(^ {35}\). ER\(\alpha\) binds to a variety of ligands and displays tissue-specific effects through estrogen-response elements (EREs). The other ER, ER\(\beta\), is expressed in cells targeted by E\(2\) including cardiomyocytes\(^ {36}\); however, the role of the ER\(\beta\)-mediated pathway in this cytoprotection and the involvement of E\(2\) in redox regulation are not well understood. We demonstrated that E\(2\) protected cardiac cells expressing ER\(\beta\) from oxidative stress concomitant with an increase in the activity of Akt. E\(2\) induced the expression of glutaredoxin (GRX) as well as \(\gamma\)-glutamylcysteine synthetase (\(\gamma\)-GCS), a rate-limiting enzyme for the synthesis of glutathione (GSH). Increase in the GRX/GSH system is involved in the cytoprotective effects of E\(2\) on the redox state of Akt, a pathway which is mediated by ER\(\beta\)\(^ {37}\).

### Dehydroepiandrosterone and Vascular Function

Vascular smooth muscle cells (VSMCs), the contractile component of blood vessels, play a critical role in the pathogenesis of atherosclerosis, as well as a variety of other major diseases, including hypertension, bronchial asthma, and vascular aneurysms. VSMCs produce adhesion molecules and chemokines following damage to the endothelium and express a set of smooth muscle-specific genes, which are characteristic of their contractile, differentiated phenotype. In contrast to skeletal and cardiac myocytes, VSMCs...
do not terminally differentiate, and they undergo phenotypic modulation in vivo and in vitro in response to environmental signals. This process involves changes in gene expression, which convert these cells from a nonproliferative contractile phenotype to a proliferating synthetic phenotype. During atherosclerosis, changes in the morphology, function and gene expression of VSMCs vary greatly at different stages. The accelerated migration, proliferation, and production of extracellular matrix components by phenotypically modulated VSMCs play a critical role in lesion development. Platelet-derived growth factors (PDGFs) are a family of homo- and heterodimers of disulfide-bonded polypeptide chains A, B, C, and D, and bind to two cell-surface receptor-tyrosine kinases, PDGF receptor (PDGFR) α and β. In response to vascular injury, VSMCs undergo rapid and quite profound changes in phenotype, including the suppression of VSMC marker genes and activation of a host of proliferative genes. PDGF-BB-induced signaling, such as the phosphatidylinositol 3-kinase/Akt pathway, is particularly important for vascular remodeling and the formation of neointimas after vascular injury, acting as a major contributor to atherosclerosis and restenosis. PDGFR-mediated signaling is regulated by the GSH/GRX1 system. The phosphorylation of PDGFR is negatively regulated by a low-molecular-weight protein tyrosine phosphatase, the activity of which is dependent on the GSH/GRX1 system. The phosphorylation of PDGFR α is important to inhibit the expression of VSMC proinflammatory mediators, including adhesion molecules like P-selectin, ICAM-1 and VCAM-1, and neutrophil chemotactic activity.

Dehydroepiandrosterone (DHEA) is a C-19 adrenal steroid. DHEA-sulfate and DHEA exist in serum and are the most abundant circulating hormones and are the most abundant circulating hormones. Similar to E2, recent studies suggest an important role for DHEA in vascular diseases. DHEA inhibits vascular inflammation through TNF-α-mediated activation of the inflammatory transcription factor, NF-κB. DHEA and the sulfated prohormone of DHEA circulate at serum concentrations higher than any other steroids, and DHEA inhibits the proliferation and migration of VSMCs independent of ERs and androgen receptors. Both the occurrence and clinical manifestation of coronary atherosclerosis have been correlated with serum levels of DHEA or DHEA sulfate. In male chronic heart failure patients, anabolic hormones involved in the depletion of DHEA sulfate are common, and deficiency of such hormones is an independent marker of poor prognosis.

Role of GRX in Serum

GSH and GSH-related enzymes, such as GSH peroxidase or GST, occur in extracellular spaces such as serum and bronchioalveolar fluid. The source of these enzymes is not entirely known; however, they are thought to play an important role in protecting the vascular system against oxidative stress. Lundberg et al. found GRX1 but not GRX2, the mitochondrial type, in human serum and suggested an extracellular function of the redox system of GSH/GRX1. The source and possible role of GRX1 in serum are not clear at present. We are interested in GRX1-dependent thioltransferase activity for the deglutathionylation of serum proteins, and whether this system is impaired with vascular dysfunction. PON1, a paraoxonase, prevents the oxidation of LDL and a decrease in its activity is associated with coronary arterial disease. Cys-283 of PON1 is thought to be glutathionylated by oxidative stress, leading to loss of the anti-atherosclerotic effect of PON1. In addition to NF-κB, Ras, Akt and PTEN inside the cell, PON1 is known to be modified by S-glutathionylation.

The number of patients suffering from arteriosclerosis obliterans (ASO) is anticipated to increase, accompanying the increase in the incidence of risk factors such as smoking, obesity, hypercholesteremia, diabetes, and hypertension. Pathologically, ASO derives from atherosclerosis, and complete occlusion by fresh or old thrombi is often observed. Most patients with ASO have no apparent clinical symptoms early on, but a diagnosis at the early stage is essential to prevent progression. Unfortunately, there are currently no specific and sensitive markers for ASO. The S-glutathionylation of proteins is initiated in the presence of GSSG and such modifications of protein thiols by oxidative stress are speculated to occur in patients with ASO. We examined levels of S-glutathionylated serum proteins and found an increase in patients with ASO (Fig. 3). The data suggest that oxidative stress and a balance of redox regulation play a role in vascular function.

Next, we estimated thioltransferase activity in serum and compared levels of S-glutathionylated serum protein in patients with peripheral arterial disease, finding a negative correlation between thioltransferase activity and the extent of S-glutathionylation. The clinical background of these cases is not defined; however, it is suggested that impairment of the redox sys-
tem in serum is a factor for the development of vascular diseases.

**Conclusion**

Oxidative stress and a weakened antioxidative defense system induce vascular cell dysfunction. The expression of ROS-sensitive inflammatory genes, such as VCAM-1 or MCP-1, enhances early atherosclerotic processes. In addition to ROS-generating and antioxidant systems, there exists a redox system in most cells, and the redox status of sulfhydryl groups is important for cellular functions. GRX1, a GSH-dependent oxidoreductase, catalyzes the reduction of protein disulfides via a disulfide exchange reaction and regulates the intracellular signaling pathway mediated by ROS or PDGF in vascular cells. Sex hormones, such as estradiol and DHEA, upregulate the GSH/GRX1 redox status, suggesting the important role of these hormones in protecting atherosclerotic processes.

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