Oxidant stress: a key determinant of atherothrombosis

J. Loscalzo
Whitaker Cardiovascular Institute and Evans Department of Medicine, Boston University School of Medicine, 88 East Newton Street, Boston, MA 02118, U.S.A.

Abstract
ROS (reactive oxygen species) are normal products of oxidative cellular metabolism. These biochemically active free-radical derivatives of molecular oxygen serve as normal signalling molecules in the vasculature; however, an excess of vascular ROS flux occurs in the setting of risk factors for atherothrombosis or established atherothrombosis. The oxidant stress that ROS generate promotes endothelial dysfunction, causes oxidative injury to vascular cells, oxidizes lipoproteins and accelerates atherothrombogenesis. Antioxidant enzymes that are important in limiting vascular oxidant stress include the superoxide dismutases, catalase, glutathione peroxidases and glucose-6-phosphate dehydrogenase. The consequences of acquired and inherited deficiencies of these antioxidant enzymes for vascular oxidant stress, endothelial dysfunction and atherothrombosis will be reviewed.

Introduction
Normal cellular metabolism in an oxygen-rich environment leads to the production of a host of reactive oxygen intermediates, termed ROS (reactive oxygen species). These ROS serve as signalling molecules, but also have the potential to cause the adverse oxidative modification of biomolecules, including proteins, lipids and nucleic acids. In order to minimize the adverse effects of ROS, a wide range of antioxidant defences has evolved, including both low-molecular-mass antioxidant molecules and antioxidant enzymes.

In mammals, the generation of ROS is increased in the setting of inflammation, injury and repair. Under these conditions, ROS serve as simple host defences as well as signals that initiate genetic programmes important for the response to injury.

Oxidant stress in atherothrombosis
In chronic diseases such as atherothrombosis, however, ROS are responsible for the initiation and perpetuation of the pathological process, and also play an important role in acute plaque activation and the thrombotic response. Each of the known risk factors for atherothrombosis promotes vascular oxidant stress, including hypercholesterolaemia, hypertension, diabetes mellitus, tobacco use and hyperhomocysteinaemia. The principal ROS detected in the vasculature in response to risk factors for atherothrombotic disease include superoxide anion, hydrogen peroxide, lipid peroxides, hydroxyl, hydroxide, peroxynitrites and hypochlorous acid.

There are both general and specific mechanisms by which these risk factors lead to increased ROS flux in the vasculature. General mechanisms include the generation of superoxide anion by NAD(P)H oxidase in vascular smooth muscle cells and in activated or dysfunctional endothelial cells, the oxidative respiratory burst from leucocytes that enter the vessel wall in the early inflammatory response to vascular injury, and the production of oxidized arachidonate derivatives by activated platelets in the early thrombotic response to vascular injury. More specific mechanisms include the generation of oxidized low-density lipoprotein in hypercholesterolaemia, of glycoxidation products in hyperglycaemia, of redox-active compounds in tobacco smoke, and of lipid peroxides in hyperhomocysteinaemia due to the homocysteine-dependent suppression of GPx1...
(glutathione peroxidase 1) expression. Naturally occurring antioxidant defences that partly offset the adverse effects of these pathological oxidative responses include ascorbate, α-tocopherol, glutathione, thioredoxin, glutaredoxin, the superoxide dismutases, catalase, the glutathione peroxidases, glutathione reductase, paraoxonase, high-density lipoprotein and G6PD (glucose-6-phosphate dehydrogenase).

**Oxidant stress and endothelial dysfunction**
The endothelial cell serves as the front line of defence against blood-borne agents that can cause vascular injury and initiate the atherogenic process. Under normal circumstances, the endothelial cell provides a permeability barrier to blood cells and macromolecules, maintains the relaxed state of the blood vessel, has limited avidity for circulating leucocytes, suppresses vascular smooth muscle cell migration and maintains a local antithrombotic environment. Upon exposure to risk factors for atherothrombosis, however, the phenotype of the endothelial cell changes. This dysfunctional phenotype is permeable to blood cells and macromolecules, is avid for leucocytes, promotes vascular smooth muscle migration and proliferation, and supports thrombotic responses.

**Nitric oxide bioactivity in endothelial dysfunction**
The conversion of the normal into the dysfunctional endothelial phenotype is the earliest detectable vascular event in atherogenesis. Many molecular mediators are responsible for the functional properties of the normal endothelium. One of these, nitric oxide (NO), is of central importance in that it contributes to each of the principal elements of endothelial function, and can be readily assayed (bio)chemically and functionally. NO is synthesized by the NO synthase family of oxidoreductases; the endothelial isoform, eNOS, is expressed constitutively in the normal endothelial cell, where it generates endothelial NO. As is the case for all of the NO synthases, eNOS oxidizes l-arginine to l-citrulline and NO, and requires the redox cofactors NADPH, flavin adenine mononucleotide and dinucleotide, tetrahydrobiopterin and the calcium–calmodulin complex. In the absence of adequate l-arginine or tetrahydrobiopterin, this oxidoreductase undergoes ‘uncoupling’ and catalyses the reduction of molecular oxygen to superoxide anion.

Endothelial dysfunction is associated with a loss of the normal bioactivity of NO. This decrease in NO bioactivity is a consequence of decreased production by eNOS, increased oxidative inactivation of NO by ROS, or both. In atherothrombosis, there is evidence for both mechanisms at play. Asymmetric dimethylarginine is a naturally occurring l-arginine derivative that is increased in abundance in atherothrombosis, and competes for eNOS with l-arginine, impairing NO synthesis [1]. Increased superoxide flux generated by NAD(P)H oxidase, mitochondrial respiration and xanthine/xanthine oxidase leads to the oxidative inactivation of NO by the rapid formation of peroxynitrite; furthermore, eNOS itself becomes a source of superoxide anion owing to the oxidation of the requisite cofactor tetrahydrobiopterin [2] and consequent uncoupling of the enzyme.

**Examples of vascular oxidant stress**
Three examples will serve to illustrate the adverse consequences of oxidant stress for endothelial NO bioactivity in the vasculature. Hyperhomocysteinemia is a well-recognized risk factor for atherothrombosis, occurring in up to 40% of individuals with coronary, carotid or peripheral arterial disease. We have shown that homocysteine leads to increased ROS generation in cultured endothelial cells [3] and in an animal model of hyperhomocysteinemia, the cystathionine β-synthase-deficient mouse [4]. This increase in ROS is a consequence of the oxidation of the thiol functionality and, most importantly, suppression of expression of the cellular isoform of glutathione peroxidase, GPx1. This antioxidant enzyme reduces hydrogen and lipid peroxides to alcohols, utilizing glutathione in the process. A genetic deficiency of GPx1 leads to endothelial dysfunction and increased vascular oxidant stress [5], and overexpression of GPx1 restores the normal endothelial phenotype, both in cultured cells exposed to elevated homocysteine concentrations and in vivo in a cystathionine β-synthase-deficient mouse [6].

As a second example, let us consider the most common enzymopathy worldwide, G6PD deficiency. This enzyme is important as the primary source of NADPH under conditions of oxidant stress. NADPH, in turn, provides the reducing equivalents necessary for the reduction of glutathione disulphide and, thus, for the generation of reduced glutathione that is required as a co-substrate for the glutathione peroxidases. In addition, given that NADPH is a required cofactor for eNOS and for tetrahydrobiopterin synthesis from both the salvage and de novo synthesis pathways, a deficiency in this cofactor would be expected to decrease NO synthesis by eNOS. Consistent with this hypothesis, we have shown recently that inhibition of G6PD activity or expression in endothelial cells leads to a decrease in NADPH and reduced glutathione levels, a decrease in NO generation and bioactivity, and an increase in ROS generation [7]. Moreover, overexpression of G6PD in endothelial cells decreases oxidant stress in response to exogenous or endogenous ROS and increases the amount of bioavailable NO [8].

As a final example of the role of oxidant stress in atherothrombotic vascular disease, consider the case of familial, premature embolic cerebrovascular accident. We originally reported the cases of two children with childhood stroke who had a deficiency of the plasma isoform of glutathione peroxidase, GPx3 [9]. We subsequently identified seven families with a similar abnormality [10]. This enzyme is the most important antioxidant enzyme in the extracellular compartment in mammals, and reduces the lipid peroxides and hydrogen peroxide released from activated platelets to their alcohols. A deficiency of this enzyme would be expected to lead to the peroxyl-dependent oxidative inactivation of
NO. In view of the essential role of NO as a platelet inhibitor [11], a deficiency of GPx3 would be expected to promote platelet-dependent arterial thrombosis. More recent data have shown that a primary abnormality that accounts for this deficiency in GPx3 production in these families and in over 100 unrelated young stroke victims is a linked series of seven polymorphisms in the promoter of the GPx3 gene that yields a haplotype with a functional deficiency in gene expression [12].

Taken together, these data illustrate the importance of vascular oxidant stress in promoting endothelial dysfunction and atherothrombotic vascular disease. Specific, targeted delivery of antioxidant enzymes or small-molecule antioxidants can lead to improved endothelial function. The clinical applicability of these approaches, however, remains to be proven.

References

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