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Antioxidant enzyme deficiencies and vascular disease

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Abstract

Cellular respiration in an oxygen-rich environment leads to the generation of reactive oxygen species. These partially reduced forms of molecular oxygen can readily react with biological molecules, often modifying their normal biological function. Antioxidant enzyme mechanisms have evolved to eliminate reactive oxygen species and minimize the oxidant stress caused by their reactivity. Inherited and acquired deficiencies of key antioxidant enzymes lead to a dysregulated redox environment, which can promote pathobiology; when this redox dysfunction occurs in the blood vessel, vascular disease ensues. In this article, we consider three distinct antioxidant enzyme deficiencies – glucose-6-phosphate dehydrogenase, glutathione peroxidase-1 and glutathione peroxidase-3 – and their consequences for vascular disease.

Keywords

endothelial function; glutathione; nitric oxide; oxygen; superoxide

Normal cellular respiration in an oxygen-rich environment generates reactive oxygen species (ROS) as metabolic byproducts. The principal ROS are all partially reduced forms of molecular oxygen and include superoxide anion, hydrogen peroxide, hydroxyl radical and hydroxide anion. Cells and organisms have evolved a host of antioxidant mechanisms to inactivate ROS and minimize their toxicity, yielding their ultimate reduction to water. Excess production of ROS that outstrips antioxidant defenses defines oxidant stress, which promotes chemical modification of biological molecules and can alter their normal function. Oxidative modification of biological molecules is involved in the pathogenesis of many cardiovascular diseases and is evoked by risk factors for atherothrombotic vascular disease.

Key antioxidant enzymes that eliminate ROS include glucose-6-phosphate dehydrogenase (G6PD) and the glutathione (GSH) peroxidases (GPxs). G6PD is not only the rate-limiting enzyme for the synthesis of pentose phosphates but also the principal source of reduced NADPH. This major cellular reductant is essential for the conversion by GSH reductase of glutathione disulfide (GSSG) to GSH which, in turn, is an obligate cosubstrate for the reduction of hydrogen and lipid peroxides to water and lipid alcohols, respectively, by the GPxs.

In this overview, I will discuss our work on the effects of inherited and acquired forms of G6PD deficiency and of GPx-1 and GPx-3 deficiencies on vascular phenotype. These deficiencies –

termed oxidative enzymopathies [1] – promote oxidative stress, endothelial dysfunction and vascular disease, and serve as potentially important targets for therapeutic intervention.

G6PD deficiency

Glucose-6-phosphate dehydrogenase is the key cytosolic source of NADPH in all cells. A deficiency of G6PD has been recognized as a cause of disease since the time of Pythagoras, who warned his disciples of the danger of eating fava beans (which contain the potent oxidants, divicine and isouramil). In individuals who are G6PD-deficient, ingestion can lead to oxidant injury to erythrocytes with consequent hemolytic anemia. G6PD deficiency is the most common worldwide enzymopathy, affecting approximately 400 million individuals in the malaria belt; G6PD-deficient erythrocytes are more susceptible to parasite-induced oxidative injury and reticuloendothelial clearance than G6PD-replete erythrocytes, thereby reducing parasitemia [2].

Until recently, G6PD deficiency was believed to be important only as a mechanism for increased susceptibility of erythrocytes to oxidative injury. Beginning in 2001, we posited that G6PD deficiency can also promote endothelial dysfunction and injury. This hypothesis was based on the view that, as a source of NADPH, G6PD is a key determinant of thiol redox state via NADPH's cofactor function for GSH reductase, as well as its role as a key determinant of nitric oxide (NO) production by endothelial NO synthase (eNOS). eNOS requires GSH as well as tetrahydrobiopterin (BH₄) for optimal activity, with NADPH required for both the *de novo* and salvage pathway synthesis of BH₄. Insufficient BH₄ leads to uncoupling of eNOS, converting it from a catalyst for the oxidation of L-arginine to L-citrulline and NO, to a catalyst for the reduction of molecular oxygen to superoxide anion. In a series of studies, we demonstrated that G6PD deficiency can decrease endothelial NADPH and GSH levels and, as a result, decrease endothelial NO production and bioactivity as measured by cGMP [3–5]. This decrease in bioactive NO leads to altered NO-dependent phenotypic responses of endothelial cells, including VEGF-dependent endothelial tube formation and angiogenesis (in a murine model of G6PD deficiency) [5], as well as altered endothelial function in the forearm vasculature of otherwise healthy human subjects [6].

In more recent work, we identified an acquired form of G6PD deficiency that probably affects a sizeable portion of the hypertensive population. Plasma aldosterone concentrations have been shown to correlate with blood pressure in the Framingham population [7], even with blood pressures in the high-normal range. Aldosterone is known to promote vascular oxidant stress and, in part by that mechanism, is believed to promote vascular fibrosis and hypertension. We showed that aldosterone suppresses *G6PD* gene transcription in endothelial cells and does so by acting through the mineralocorticoid receptor to activate protein kinase A. Protein kinase A in turn increases the expression of the cAMP response element modulator to more than offset the concomitant increase in phospho-cAMP response element binding protein, which yields a net decrease in *G6PD* gene transcription [8]. As a result, aldosterone promotes endothelial dysfunction by attenuating the generation of bioactive NO by inducing a state of acquired G6PD deficiency.

In another set of experiments, we showed that aldosterone also promotes oxidant stress in vascular smooth muscle cells by inducing a state of acquired G6PD deficiency [9]. This decrease in G6PD leads to a decrease in the ratio of GSH:GSSG, and an increased susceptibility of target protein functional groups to oxidative modification. One such target is cys122 in the β -chain of guanylyl cyclase, oxidation of which renders the enzyme comparatively insensitive to activation by NO. Taken together, these findings in endothelial and vascular smooth muscle cells show that G6PD deficiency can lead to vascular dysfunction by impairing the generation of bioactive NO by the endothelium, as well as impairing the response of one of its key targets,

vascular smooth muscle guanylyl cyclase, activation of which is essential for normal vasodilation and blood pressure regulation.

Work from Stanton's group shows that hyperglycemia and diabetes mellitus also produce a state of G6PD deficiency. Hyperglycemia leads both to increased superoxide flux [10] and to activation of protein kinase A, which, in turn, phosphorylates G6PD and thereby inhibits the enzyme [11]. These findings in cultured cells were recapitulated in a diabetic rat model, supporting one mechanism by which diabetes can promote oxidant stress and cell injury [12].

Glutathione peroxidase-1 deficiency

The GPxs comprise a family of selenoproteins that reduce lipid peroxides and hydrogen peroxide to lipid alcohols and water, respectively. The members of this enzyme family work in concert with the superoxide dismutases to achieve this metabolic result. Some of the GPxs are localized to specific microenvironments to facilitate hydrogen peroxide reduction; for example, Gpx-3 is found in the extracellular compartment, where it works to reduce hydrogen peroxide generated by extracellular superoxide dismutase-3. Catalase and the peroxiredoxins also reduce hydrogen peroxide to water; however, catalase is localized to the peroxisome and has a weaker K_M than the GPxs (although it has a higher catalytic efficiency), and the peroxiredoxins study only at low concentrations of hydrogen peroxide ($<20 \mu\text{M}$), above which they are oxidatively inactivated. In an earlier work, we demonstrated that the atherosclerotic risk factor, homocysteine, promotes endothelial dysfunction by impairing the expression of GPx-1, the most prevalent of the GPxs, found in the cytosol of all cells. A deficiency of GPx-1 leads to enhanced oxidative stress and endothelial dysfunction [13]. The mechanism by which homocysteine decreases GPx-1 is unique in that it attenuates the translation of this selenoprotein rather than affecting gene transcription [14]. As a result of this acquired form of GPx-1 deficiency, NO is readily inactivated by ROS, leading to endothelial dysfunction and enhanced susceptibility to endothelial injury. In a genetic murine model of mild hyperhomocysteinemia (cystathione β -synthase deficiency), we showed that (over) expressing a GPx-1 transgene can restore normal NO bioactivity and endothelial phenotype [15]. The relevance of these observations was demonstrated in the AtheroGene population, where the risk of atherothrombotic events was independently increased by GPx-1 deficiency [16].

We also recently identified another acquired form of GPx-1 deficiency that accounts for the nephrotoxicity and ototoxicity of aminoglycoside antibiotics. This antibiotic class was developed to disrupt translational fidelity in prokaryotes. These antibiotics also perturb translation in eukaryotic cells, and we showed that this adverse effect leads to altered expression of functional GPx-1 [17]. As a selenoprotein, GPx-1 contains a selenocysteine (Sec) in its active site that is essential for optimal enzyme activity. The codon for Sec is UGA, which is normally a stop codon; however, owing to the presence of unique translational cofactors (e.g., SBP2 and eEFsec) and a stem-loop element in the 3'-untranslated region of the GPx-1 mRNA, the message is stabilized on the ribosome when UGA is encountered to facilitate incorporation of Sec via its specific tRNA into the growing polypeptide chain. Aminoglycosides perturb this process by promoting misreading of the UGA codon, leading to misincorporation of arginine for Sec at position 82, which, in turn, leads to the synthesis of an enzyme with significantly decreased specific activity, thereby promoting cellular oxidant injury [17].

Glutathione peroxidase-3 deficiency

A third antioxidant enzyme deficiency we have identified is that of GPx-3. This member of the GPx family is the only GPx found in the extracellular compartment and, as such, is a key extracellular peroxidase. In 1996, we identified a deficiency of GPx-3 as a cause of platelet-dependent thrombotic stroke in two children (brothers) [18]. In the course of this analysis, we showed that GPx-3 is essential for reducing the peroxides produced during platelet activation

to alcohol, thereby limiting the extent of platelet activation in the vascular microenvironment. By contrast, a deficiency of GPx-3 is associated with more pronounced platelet activation and thrombotic risk, largely as a result of decreasing the anti-platelet effect of bioactive NO via its oxidative inactivation. We subsequently confirmed these observations in five Israeli families with histories of young stroke [19] and, more recently, in over 114 unrelated, young, thrombotic stroke survivors [20]. Sequencing the *GPx-3* gene promoter in the stroke patients defined a unique haplotype (H₂) comprising seven tightly linked polymorphisms that is more prevalent in stroke patients than in age-matched controls. This haplotype significantly decreases promoter function (~60%), increases the risk of stroke twofold and is an independent risk factor for arterial thrombotic stroke [20], as well as cerebral venous thrombosis [21].

We recently developed a genetic murine model of GPx-3 deficiency, and found that its phenotype recapitulated that of the patients described here. Bleeding time was shortened by GPx-3 deficiency, and markers of platelet activation were increased (P-selectin levels) [22]. Furthermore, in a middle cerebral artery occlusion model of stroke, we found that GPx-3 deficiency (*GPx-3*^{+/-} and *GPx-3*^{-/-}) leads to significantly greater infarct volumes than found in *GPx-3*^{+/+} animals, with commensurately greater neurological deficits. Importantly, inhibition of platelet activation with clopidogrel in this model dramatically reduced stroke size and neurological deficit in the *GPx-3*^{+/-} and *GPx-3*^{-/-} mice.

Taken together, these findings show that GPx-3 deficiency leads to enhanced platelet activation owing to oxidative inactivation of endothelial and platelet-derived NO, which is important for attenuating the accrual of platelets to the growing platelet thrombus [23]. With vascular injury, platelet-rich thrombus propagation continues without the attenuating effect of GPx-3, and larger, more occlusive thrombi develop to produce larger, more damaging cerebral infarcts.

Expert commentary & five-year view

We have demonstrated the importance of key antioxidant enzymes in maintaining normal endothelial and vascular phenotype. Both inherited and acquired deficiencies of these protective enzymes – the oxidative enzymopathies (Figure 1) [1] – lead to important vascular pathophenotypes that underlie common vascular diseases. Understanding the mechanisms for these deficiencies is essential for identifying potential therapeutic interventions that aim to restore the function of these essential enzymes.

Key issues

- Cellular respiration in an oxygen-rich environment generates reactive oxygen species (ROS), which include superoxide anion, hydrogen peroxide, hydroxyl radical and hydroxide anion. While some of these ROS serve normal cell signaling functions (hydrogen peroxide), most require elimination to minimize toxicity.
- Antioxidant mechanisms have evolved to eliminate ROS, and include the small-molecule antioxidants as well as antioxidant enzymes, chief among which is glucose-6-phosphate dehydrogenase (G6PD), the superoxide dismutases and the glutathione peroxidases (GPxs); G6PD generates NADPH, which maintains the reduced state of glutathione necessary for the activity of the GPxs, which reduce hydrogen and lipid peroxides to water and lipid alcohols, respectively.
- G6PD deficiency is a major antioxidant enzyme deficiency that leads not only to hemolysis in the setting of oxidant stress, but also to endothelial dysfunction and vascular disease. The endothelial dysfunction of G6PD deficiency is caused by insufficient NADPH synthesis, which limits reduced glutathione and tetrahydrobiopterin stores and, as a result, bioactive nitric oxide.

- G6PD deficiency can be inherited or acquired as a result of elevated aldosterone levels, which suppress transcription of the *G6PD* gene, or of hyperglycemia, which leads to phosphorylation of the enzyme by protein kinase A, inhibiting its activity.
- A deficiency of one of the GPxs, GPx-1, also promotes oxidant stress, endothelial dysfunction and vascular disease. A major cause of GPx-1 deficiency is hyperhomocysteinemia, which decreases GPx-1 expression by impairing translation of its mRNA.
- A deficiency of another of the GPxs, GPx-3, promotes extracellular oxidant stress as this GPx family member is the key extracellular antioxidant in mammals. GPx-3 deficiency limits the bioavailability of NO in the extracellular compartment, including in plasma. As a result, platelet activation is enhanced, which can lead to arterial thrombosis. A unique haplotype in the GPx-3 promoter (H₂) is associated with an independent risk for arterial thrombotic stroke and cerebral venous thrombosis in young individuals.

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Figure 1. The central antioxidant enzyme cascade and the oxidative enzymopathies

6PG: 6-phosphogluconolactone; G6P: Glucose-6-phosphate; G6PD: Glucose-6-phosphate dehydrogenase; GPx: Glutathione peroxidase; GSH: Glutathione; GSSG: Glutathione disulfide; GSSG R: Glutathione reductase; Hcy: Homocysteine; LOH: Lipid alcohol; LOOH: Lipid peroxide(s).