Plasma Glutathione Peroxidase Activity is Potentially a Key Regulator of Vascular Disease-Associated Thrombosis

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In the current issue of *Circulation*, Jin et al provide insight into how a deficiency in the activity of the primary plasma peroxide-metabolizing enzyme glutathione peroxidase-3 (GPx-3) creates a prothrombotic state with vascular dysfunction. Mice deficient in GPx-3 are shown to have elevated soluble P-selectin (a marker of platelet and prothrombotic activity) and enhanced responses to experimental conditions promoting pulmonary thromboembolism and platelet-dependent cerebral infarctions in a no-flow ischemia–reperfusion stroke model. Initial studies on the properties of platelet aggregation in 2 brothers with a cerebral thrombotic disorder identified a deficiency in plasma glutathione peroxidase-3 activity as a key factor explaining the hyperreactivity of their platelets. Subsequent studies examining the consequences of polymorphisms in the human GPx-3 gene associated with decreases in plasma GPx-3 expression provided evidence that it was a risk factor for ischemic stroke in young adults and children. A deficiency in GPx-3 has been associated with increases in extracellular peroxide-related oxidants and decreased bioavailable nitric oxide that are thought to contribute to promoting platelet activation. Thus, plasma glutathione peroxidase activity may be a key factor in determining when disease processes activating platelets result in arterial thrombosis.

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It is well established that increased peroxide levels in plasma promote platelet hyperreactivity, and that nitric oxide is a key mechanism for inhibiting platelet aggregation and vasoconstriction associated with thrombosis. As discussed in the article by Jin et al, platelet activation generates superoxide and peroxides that appear to promote aggregation by mechanisms including peroxide-mediated stimulation of intracellular calcium release, which further triggers aggregation. Platelet-derived superoxide also ablates the inhibitory effects of nitric oxide that is even generated within the platelets themselves (Figure). Because a scavenger of peroxide (and other reactive species, including superoxide and peroxynitrite) was observed to selectively prevent the marked increase in stroke infarct volume seen in GPx-3-deficient mice, the acute effects of elevated levels of peroxide and other reactive species appear to be a key factor in enhancing thrombosis in this animal model. Although the role of GPx-3 as the primary plasma enzyme-metabolizing peroxide is the most obvious explanation for how this system functions to prevent platelet hyperreactivity, additional important factors potentially contribute to this system’s role in regulating thrombosis.

The availability of nitric oxide appears to be another factor that is influenced by GPx-3. Data in the study by Jin et al show that arteriolar vasodilation in the skeletal muscle microcirculation to acetylcholine receptor stimulation of endothelial-derived nitric oxide is markedly attenuated in GPx-3-deficient mice. However, this attenuation is absent in the presence of drugs releasing nitric oxide. Thus, GPx-3 may also normally function to maintain nitric oxide release from the endothelium, and this, together with scavenging oxidants and improving the bioactivity of nitric oxide generated by platelets themselves, would suppress thrombosis by stimulating cGMP production in platelets. Plasma cGMP levels were measured as indicators of the actions of endogenous nitric oxide, and the GPx-3 mice had markedly lower levels of cGMP. Although cellular sources other than platelets may be contributing to the cGMP being measured, decreased plasma cGMP is consistent with a deficiency in GPx-3 also being associated with decreased bioavailability of nitric oxide. Although many factors could contribute to a decrease in nitric oxide availability, it is possible that processes directly influenced by the enzymatic activity of GPx-3 could be contributing factors. For example, the glutathione peroxidase activity of GPx-3 could function normally to remove peroxides that potentially influence endothelial nitric oxide synthase activity by processes such as stimulating superoxide generation by oxidase enzymes that promote a scavenging of nitric oxide. Glutathione peroxidase enzymes may also have roles in metabolizing nitric oxide oxidation products, such as peroxynitrite, in ways that prevent their depletion of tetrahydrobiopterin, a cofactor needed for preventing nitric oxide synthase uncoupling or efficient nitric oxide generation. In addition, GPx-3 could participate in the regeneration of nitric oxide from thiols nitrosated by the metabolism and/or oxidation of nitric oxide. Thus, GPx-3 appears to have a role in preserving nitric oxide and its ability to prevent thrombosis.

The availability of thiols, such as glutathione and cysteine in plasma and thioredoxin in the extracellular environment, potentially used for the metabolism of peroxides by GPx-3, is likely to be a major factor in controlling the function of this system, even in the presence of adequate levels of GPx-3 expression. Although low micromolar levels of glutathione and cysteine are present in plasma, there is little evidence...
for an efficient regeneration of oxidized thiols once they have been produced. For example, the levels of glutathione in human plasma are in the range of 1 μmol/L, and this appears to be too low to adequately support GPx-3 activity. Although the levels of cysteine in plasma are generally in the range of 10 μmol/L, the concentrations of oxidized cysteine are much greater. This has resulted in the consideration of thioredoxin activity in plasma and/or on the surface of cells as a reducing system for GPx-3. Previous studies have documented that cardiovascular risk factors and age appear to have major effects on the plasma redox status of glutathione and cysteine in the direction of oxidation. Diet also appears to be a major factor in the diurnal variation in human plasma glutathione and cysteine redox. Thus, although the actual cofactors used by GPx-3 for the metabolism of peroxide in plasma are not well defined, the availability of thiols is likely to also be a critical factor in determining how this system influences thrombosis and other aspects of vascular dysfunction in aging and cardiovascular disease processes.

Genetic deficiencies in GPx-3 appear to enhance thrombosis and stroke in humans, and the study by Jin et al provides valuable insight into how the regulation of platelet and vascular function participate in this process. Although the thiols in the extracellular environment available for use as cofactors for the metabolism of peroxides by GPx-3 are not known, it is possible that limitations in cofactor availability or their oxidation could be a key factor in promoting thrombosis. For example, a shift toward oxidized thiols is seen at early stages of atherosclerosis development in humans, and correlations exist between multiple risk factors for cardiovascular disease (eg, age, obesity, diabetes mellitus, and cigarette smoking) and increased oxidation of plasma glutathione and cysteine redox. In addition, increased cysteine oxidation appears to activate a proinflammatory state in endothelium associated with increases in expression of adhesion proteins, including P-selectin. It is possible that even in the absence of a GPx-3 genetic deficiency, the availability of reduced thiols used by this enzyme in aging-associated cardiovascular disease processes could also be a major factor in the expression of thrombosis-related outcomes. Thus, many questions related to how GPx-3 controls platelet aggregation in vivo, and the thiols used by GPx-3 and their influence on thrombosis-related processes, remain to be investigated.

**Figure.** Roles for plasma glutathione peroxidase-3 (GPx-3) activity in controlling the function of platelet and vascular regulatory systems that potentially determine thrombosis in vivo. ROOH represents extracellular hydrogen peroxide, lipid, and other peroxides that potentially participate in activating platelets and promoting a loss of nitric oxide (NO) bioactivity for inhibiting aggregation. RSH and RSSR represent the reduced and oxidized forms of thiols such as glutathione, cysteine, and thioredoxin-like proteins that are potentially used by GPx-3 for the metabolism of ROOH. RSNQ are nitrosated thiols which can be metabolized by GPx-3 to generate NO. Some of the ways GPx-3 could influence superoxide anion (O₂⁻)–mediated attenuation of the bioactivity of NO are also shown.

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**References**


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Correction

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