Limiting reductive stress for treating in-stent stenosis: the heart of the matter?

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Restenosis after balloon angioplasty and stenting (BAS) remains an unsolved clinical dilemma for patients with coronary artery disease. A better understanding of the mechanisms that drive this phenomenon is likely to lead to more effective treatments. In this issue of the JCI, Ali et al. uncover a critical redox axis with the antioxidant enzyme glutathione peroxidase-1 (GPX1) at its hub and identify potential new therapeutic targets, such as ROS1 tyrosine kinase. This study represents a potential new approach to finding a treatment for BAS, with implications that may extend beyond BAS to other vasculopathies involving vascular remodeling.

Vascular injury and drivers of stenosis
Balloon angioplasty and stenting (BAS) remains the preferred method of treatment for flow-limiting atherosclerosis, far outnumbering coronary artery bypass grafting (1). Unfortunately, stent-mediat-ed intimal hyperplasia, a consequence of vascular smooth muscle cell (VSMC) proliferation, remains a cause of restenosis after BAS and often presents with acute coronary syndrome. Reendothelialization of the damaged vessel has been proposed to reduce intimal hyperplasia; however, current treatment options, such as drug-eluting stents, aimed at limiting VSMC proliferation may also inhibit reendothelialization. Reduced reendothelialization leads to an increased risk of thrombosis within the stented vessel, often resulting in devastating clinical consequences (2). Thus, there is a clinical need for improvements in the treatment of flow-limiting atherosclerosis. Recent advances have been made in preclinical models, including the use of mesenchymal stem cells (3) and miRNA-based strategies (4), to assist with reendothelialization. The potential for these new approaches to lead to clinical breakthroughs will likely be expedited once the mechanisms behind BAS-driven hyperplasia are better understood.

Over the past decade, there has been a groundswell of clinical and preclinical evidence that indicates the antioxidant enzyme glutathione peroxidase-1 (GPX1) is involved in the atherogenic process. Clinically, low levels of GPX1 have been associated with an increased risk of cardiovascular events in patients with coronary artery disease, independent of traditional risk factors (5), and GPX1 activity has also been shown to be reduced in excised human plaques (6). Preclinically, plaque analysis in atherosclerosis-prone mice revealed that a lack of GPX1 accelerates plaque formation under both hyperlipidemic (7) and diabetic (8) conditions. While previous studies on GPX1 in vascular injury have focused on its role in atherosclerosis, particularly the involvement of GPX1 in immune cells (9), the role of GPX1 in VSMCs, the drivers of stenosis, has not been characterized. In this issue of the JCI, Ali et al. (10) demonstrate that vascular injury–induced loss of GPX1 alters oxido-reductive stress in VSMCs and promotes in-stent stenosis. This study opens up new frontiers for in-stent stenosis research and therapy and also highlights the need to look further afield at reductive stress when dealing with imbalances in redox events. GPX1 is known to perform important functions in limiting reactive oxygen species–mediated lipid, protein, and DNA damage (11); therefore, the oxidative stress that results from a decline in GPX1 levels has been touted as the mediator of injury. The current report from Ali and colleagues necessitates reevaluation of this paradigm and identifies additional biochemical mechanisms that involve reductive stress and are modulated as a result of GPX1-dependent pathways.

GPX1: at the center of stent-induced pathologic remodeling
Using a networks approach to compare coronary vascular gene expression between de novo atherosclerosis and in-stent stenosis patients, Ali et al. (10) identified GPX1 as the network hub gene involved in pathological remodeling following stenting. Evaluation of SNPs and confirmation with GWAS revealed that mutations within GPX1 and the orphan protooncogene receptor tyrosine kinase ROS1 are tightly associated with in-stent stenosis. To elucidate the contribution of GPX1 in the development of stenosis, Ali et al. employed a model of BAS in atherosclerosis-prone (ApoE-deficient) mice lacking GPX1. Compared to animals lacking ApoE alone, mice deficient for both ApoE and GPX1 were more susceptible to BAS-mediated neointimal restenosis. Importantly, the authors determined that ROS1, which drives VSMC proliferation, migration, and survival, is low under physiological conditions, but markedly upregulated after BAS in GPX1-deficient VSMCs. Inhibition of ROS1, either pharmacologically or with siRNA, in GPX1-deficient VSMCs dramatically decreased VSMC expansion, highlighting the possibility that GPX1 mediates stenosis through ROS1 activation.

Importantly, Ali and colleagues discovered that reductive stress, defined as higher levels of reduced glutathione (GSH)
Ali and colleagues used their animal model and confirmed the presence and activation of ROS1. Furthermore, ROS1, resulting in sustained phosphorylation blocked access to the phosphatase active site of SHP-2, thereby rendering it incapable of removing phosphates from phosphorylated SHP-2. The authors identified a physical relationship between ROS1 and SHP-2. Further analysis revealed that specific cysteine sites within SHP-2 are prone to irreversible $\gamma$-glutathiolation. Computational modeling showed that $\gamma$-glutathiolation blocked access to the phosphatase active site of SHP-2, thereby rendering it incapable of removing phosphates from ROS1, resulting in sustained phosphorylation and activation of ROS1. Furthermore, Ali and colleagues used their animal model and confirmed the presence and activation of $\gamma$-glutathionylated SHP-2 increases ROS1 phosphorylation. Indeed, phosphorylated ROS1 levels were substantially elevated in mice lacking GPX1 following BAS. Importantly, ROS1 inhibition with the ROS1 inhibitor crizotinib or deglutathiolation of SHP-2 abolished GPX1-mediated increases in VSMC proliferation, but kept endothelialization intact.

### Conclusions and future directions

Together, this study by Ali et al. has resulted in several important findings that expand our understanding to include previously unidentified molecular pathways now implicated in stenosis. These findings are likely to have clinical implications. First, the finding that reductive stress over and above oxidative stress is the driver of BAS may explain the failure of antioxidants in clinical trials of vascular remodeling (12, 13). Indeed, traditional antioxidants such as vitamins E and C would have no effect on stenosis for several reasons, most notably an inability to lessen reductive stress. Future “antioxidant” studies should be directed toward the GSH-synthesizing enzyme GPX1-mediated increases in VSMC proliferation, but kept endothelialization intact.

**Figure 1. Loss of GPX1 induces oxidoreductive stress and promotes vascular remodeling.** Risk factors for vascular remodeling, such as hypertension, endothelial dysfunction, diabetes, and atherosclerotic plaques, and vascular injury during stenting are associated with reductions in the antioxidant enzyme GPX1. Reductions in GPX1 lead to redox stress, with increases in both reductive stress via GSH and oxidative stress via enhanced production of superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and peroxynitrite (ONOO$^-$). Reductive stress in turn increases $\gamma$-glutathionylation of key proteins, which in VSMCs, leads to their proliferation, migration, and survival, contributing to in-stent stenosis. Oxidative stress modifies DNA, lipids, and proteins and is known to affect pathways associated with key risk factors (pink box) to exacerbate vasculopathies. It is highly likely that oxidative stress stimulates the production of ROS1 via $\gamma$-glutathionylation of key proteins contributes to vascular remodeling associated with these risk factors. In this issue of the *JCI*, Ali et al. (10) identify GPX1 as the network hub gene capable of mediating both reductive and oxidative stress in VSMCs. Perturbations in reductive stress may turn out to be as important, if not more important, than oxidative stress, as demonstrated in in-stent stenosis, with clinical implications that may extend beyond BAS.
Commentary

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Finally, GPX1 has been shown to decrease in conditions such as diabetes (18) and atherosclerosis (6). Reductive stress, via the pathways identified by Ali and colleagues, may be important—possibly even more important than oxidative stress—in mediating the effects associated with these diseases. Thus, it is time to reassess the redox paradigm and to appreciate that one should not focus just on oxidative stress, but also consider the pathways that are altered as the result of reductive stress and induced by increased GSH as a result of GPX1 deficiency (Figure 1).

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