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Reactive oxygen species: Finding the right balance

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ROS; endothelial; inflammation; exocytosis; atherosclerosis

Introduction

Appropriate regulation of reactive oxygen species (ROS) has a significant impact on health and disease. ROS includes oxygen ions/$O_2^{\cdot}$, free radicals (superoxide/O_2^{-} and hydroxyl radicals) and peroxides (hydrogen peroxide/H_2O_2) and are the products of normal oxygen consuming metabolic process in the body. ROS are small and highly reactive molecules with important cell signaling roles when maintained at proper cellular concentrations. During times of cell stress ROS levels can greatly increase. Because of their highly reactive nature, ROS can modify other oxygen species, proteins, or lipids, a situation often termed oxidative stress. Maintaining normal cellular ROS concentrations is therefore vital to the proper physiologic function of numerous cell types throughout the body. An excess production or decreased scavenging of ROS has been implicated in the pathogenesis of diverse diseases such as neurodegeneration, diabetes, cancer, and atherosclerosis.

Kisucka et al now demonstrate that Peroxiredoxin1 (Prdx1) has an important role in the maintenance of endothelial ROS[1]. Prdx1 is an antioxidant enzyme that reduces H_2O_2, lipid peroxides, and peroxynitrite. Prior studies have shown that Prdx1-/- mice develop late onset hemolytic anemia and have increased frequency of cancer[2] due to an increase in ROS (such as H_2O_2), emphasizing the importance of Prdx1 in normal vascular homeostasis. Like many ROS, H_2O_2 can have disparate effects depending on the cell type and its local concentration. H_2O_2 can have normal regulatory functions as a second messenger molecule in signal transduction such as in the MAP kinase pathway. H_2O_2 makes a good signaling molecule because of its reactive nature and its ease of scavenging by antioxidant enzymes such as Prdx1 making for rapid signaling activation and inactivation. H_2O_2 can also be a source of oxidative stress and vascular injury. Excess H_2O_2 has been implicated in nitric oxide (NO) dysregulation and mitogenic activities that lead to intimal hyperplasia. In this study the authors demonstrate that a loss of Prdx1 accelerates the development of atherosclerosis in part by disrupting normal regulation of Weibel-Palade body release resulting in an increase in white blood cell (WBC) interactions with the vasculature[1].

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ROS in vascular biology

Endothelial cells form the vital interface between blood constituents and the vessel wall. A loss of endothelial cells leads to thrombus formation and vessel occlusion. Alterations in maintaining endothelial cells in a quiescent state leads to an increase in endothelial cell interactions with platelets and WBC, stimulating more endothelial activation and leukocyte trafficking. Dysregulation of ROS homeostasis can lead to endothelial cell dysfunction. Vascular cells themselves are sources of ROS production. The primary enzymatic sources for vascular oxygen species production are xanthine oxidase, NADH/NADPH oxidase isoforms, and endothelial Nitric Oxide synthase (eNOS)[3]. There must be a balance maintained between the production of ROS and the scavenging of ROS. Some of the important scavengers include Super Oxide Dismutase (SOD), glutathione, thioredoxin, and peroxiredoxins[3].

Oxygen species, such as nitric oxide (NO), are important players in maintaining normal physiology. NO helps to maintain vascular tone, inhibits endothelial cell stimulation, and is a regulator of platelet activation[4,5]. NO also has a key physiologic role as a second messenger in cell signaling in neurons and macrophages. A lack of NO therefore can have significant physiologic effects, and excessive NO can also contribute to cell injury by combining with superoxide to produce damaging peroxynitrite.

Kisucka et al demonstrated that Prdx1 has a functional role in endothelial cells and a lack of Prdx1 leads to an increase in WBC interactions with endothelial cells[1]. This implies that Prdx1-/- mice have an endothelial dysfunction, including a loss of normal regulation of endothelial cell degranulation as reflected by an increase in plasma von Willebrands factor (vWf) and endothelial P-selectin expression. NO has an important role in regulating Weibel-Palade body exocytosis by S-nitrosylation of the key regulatory protein N-ethylmaleimide Sensitive Factor (NSF) [4,5]. NO modification of NSF decreases NSF ATPase activity and thus blocks a critical function of NSF in promoting SNARE complex disassembly and sustaining exocytosis[4]. Prdx1-/- mice may therefore have an endothelial dysfunction by direct ROS effects or secondary to alterations in NO availability.

A lack of proper ROS scavenging can lead to a decrease in bioavailable NO and perhaps unchecked exocytosis. ROS can decrease NO bioactivity by directly interacting with and inactivating NO or by modifying other protein sites where NO may react, therefore decreasing its physiologic influence[3]. With a loss of anti-oxidant activity there may be a reduction in bioavailable NO, increased endothelial exocytosis and with it increased P-selectin expression and vWf release, such as in Prdx1-/- mice. Increased endothelial exocytosis leads to increased WBC localization and vascular inflammation. Alternatively, a loss in Prdx1 and increased H_2O_2 may drive NF-kB stimulation in endothelial cells and the elaboration of endothelial derived cytokines and adhesion molecules. It is noteworthy that Kisucka et al did not find an increase in the expression of the vascular adhesion molecules ICAM and VCAM, suggesting a loss of Prdx1 has its most significant effects on the regulation of exocytosis[1]. NO target protein regulation must also be maintained for normal cellular function. Recently Benhar et al have demonstrated that nitrosylated proteins in lymphocytes are reduced by thioredoxin[6]. The role of thioredoxin in endothelial cell biology and its effects on the state of NO target proteins remains to be determined.

Platelets also produce ROS and platelet physiology can be affected by ROS produced at the interface with endothelial cells. ROS produced by endothelial cells or during thrombus formation have the potential to influence platelet function locally and lead to a further pro-thrombotic response[7]. With agonist stimulation platelets also produce significant amounts of ROS and the enzymatic sources in platelets include NAD/NADPH oxidase and SOD[7]. In the Kisucka study, Prdx1-/- mice had no change in platelet function in vitro and in vivo[1]. This does

not exclude the possibility of Prdx having a role in platelet physiology. There are other Prdx family members expressed throughout the body. Platelets may express an alternative family member or its function made redundant by the expression of other reducing enzymes in platelets.

ROS and Atherosclerosis

Atherosclerosis is an inflammatory disease that begins at the vascular interface with an endothelial cell dysfunction. Many pathophysiologic processes contribute to the initiation of endothelial dysfunction, including elevated plasma cholesterol, hypertension, and diabetes. Each of these risk factors either result in an increase in vascular ROS or are exacerbated by an increase in ROS. Endothelial dysfunction results in increased endothelial cell activation and pro-inflammatory molecule expression culminating in inflammatory cell adhesion and migration into the developing lesion. Macrophages have a key role in atherosclerotic development and elaborate even more ROS production within the lesion. With a lack of proper ROS scavenging the cycle of inflammation and leukocyte trafficking is accelerated.

With a sustained increase in plasma LDL and ROS species production there is an increase in oxidized LDL (Ox-LDL). Ox-LDL is readily removed by macrophages, but in turn drives more macrophage stimulation. Ox-LDL also stimulates platelet activation in a platelet CD36 dependent manner [8] and platelet dependent atherothrombosis is an important part of myocardial infarction. Although Kisucka et al did not investigate whether Prdx1-/- mice on an ApoE-/- background have increased thrombotic risk, it seems reasonable to propose that unlike in Prdx1-/- mice on a normal cholesterol background, the Prdx1-/-/ApoE-/- mice may have an increase in platelet activation and thrombosis secondary to an increase in Ox-LDL.

Kisucka et al demonstrate that Prdx1-/- mice bred onto an ApoE-/- background have an exacerbation of atherosclerotic lesion development and an increase in macrophage infiltrates into the lesion[1]. The results of their studies suggest that in Prdx1-/- mice persistent endothelial cell degranulation results in monocyte recruitment, trafficking and activation. This perhaps leads to further production of ROS species that is not held in check. Interestingly, the effect of Prdx1 on atherosclerotic lesion development is more pronounced in females than males. This may suggest a difference in mechanisms of ROS scavenging in males and females or that other gender specific variables are relevant in Prdx1 function.

Summary

ROS have both beneficial and harmful effects in the vasculature. Because of their highly reactive nature ROS make rapid and transient second messenger molecules. However, this highly reactive property means that the loss of proper ROS scavenging can have deleterious effects. To minimize this potential, cells have developed multiple redundant enzymes to remove ROS, each of which may have a greater or lesser role in an individual cell type. Vascular biologists are beginning to understand the anti-oxidant systems such as perioredoxin that plays an important role in maintaining proper levels of oxidants within the vasculature.

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