Gate Keepers in the Route From Oxidative Stress to Vascular Dysfunction

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Through reading textbooks on pathophysiology, medical students already learn during their early university years that cardiovascular disease (CVD) is closely associated with oxidative stress, which apparently promotes progression of diseases like atherosclerosis, diabetes mellitus, hypertension, and ischemic heart disease. Oxidative stress is created through reactive oxygen species (ROS), eg, the superoxide anion and hydrogen peroxide, that are generated mainly within the mitochondrial respiratory chain or through activity of NADPH oxidases and reduce the bioavailability of nitric oxide (NO), which is the nodal point of endothelial vasoactive substances that alter the bioactivity of a variety of cellular molecules but are also known to regulate several classes of genes that are involved in the complex network of vascular growth and function, eg, formation of focal adhesion molecules, expression of metalloproteinases, cytokines and growth factors and, thus, when occurring in excessive amounts, may tilt the endothelial balance toward vasoconstriction and endothelial dysfunction. Furthermore, ROS can interfere with the plasma membrane phospholipid bilayer, which is easily prone to lipid peroxidation, thus resulting in the generation of a number of degradation products displaying potential detrimental bioactivity that may finally initiate vascular dysfunction. Over the last decade, a number of biomarkers of oxidative stress in vivo have been identified that caution against cardiovascular risk factors, the severity of CVD, and cardiovascular outcomes. Among these biomarkers of oxidative stress are a class of prostaglandin F₂-like compounds (F₂-isoprostanes [F₂-IsopPs]), which are generated from the nonenzymatic, free radical–catalyzed peroxidation of phospholipid-bound arachidonic acid independently of the cyclooxygenase pathway. Until recently, IsoPs were considered just as 1 class of oxidative stress markers of CVD among others because they were found to be elevated under conditions of ischemia/reperfusion and atherosclerosis but also in plasma and urine of patients at high cardiovascular risk, such as smokers and hypercholesterolemic, diabetic, and obese patients. However, a closer inspection of the biological functions of IsoPs revealed that they indeed activate specific signaling pathways that may be crucial for the pathogenesis of a large variety of diseases, not only related to heart and vascular function but also to neurodegenerative disorders like Alzheimer’s or Parkinson’s disease, which are well known to be associated with dysregulated ROS production. IsoPs were first discovered by Morrow and colleagues and were detected in all biological tissues and fluids, including plasma, urine, bronchoalveolar lavage fluid, cerebrospinal fluid, and bile. Chemically, 3 arachidonyl radicals give rise to 4 F₂-IsoP regioisomers, each of which comprises 8 racemic diastereomers. Because they are relatively stable as compared to lipoperoxides and aldehydes, they can be easily detected in body fluids and are currently considered as the most reliable markers of lipid peroxidation as recently evaluated by a multilaboratory study of the National Institute of Environmental Health Sciences. More recently, it became apparent that IsoPs exhibit significant bioactivity and (besides their feature to serve as an easy handle oxidative stress biomarker) play a role in the pathogenesis of CVD associated with oxidant injury. Namely, IsoPs were shown to contribute to the progression of atherosclerosis, to interact with platelets by either stimulatory or inhibitory pathways and to act as powerful vasoconstrictors, thus inducing hypertension, enhancing ischemia, and potentially initiating a vicious cycle of ROS generation. However, although some hints pointed toward adverse vascular effects of IsoPs, the final proof of their involvement in inhibition of angiogenesis and vascular damage so far had been missing.

This gap is filled by Benndorf et al in this issue of Circulation Research. The authors elegantly demonstrate that IsoPs exert antiangiogenic effects by interfering with vascular endothelial growth factor (VEGF) signaling pathways, the classic signaling cascade widely involved in various aspects of angiogenesis, vascular homeostasis, and vascular remodeling. Using in vitro and in vivo models of angiogenesis, ie, human coronary artery endothelial cells (HCAECs), the in vitro cardiac angiogenesis assay, as well as the in vivo chorioallantois membrane model of vasculogenesis, the authors conclusively demonstrated that different IsoPs inhibited not only VEGF-mediated endothelial cell migration but also vascular tube formation. Moreover, the authors succeeded in identifying previously unknown biologically active decomposition products of specific IsoPs, namely the cyclopentenone isoprostane derivatives X and Y, which may exert antiangiogenic effects in vivo, contribute to the progression of CVD, and may be exploited as further reliable biomarkers of CVD in the near future. The findings of Benndorf et al are challenging with regard to the well-described role of the VEGF family and its receptor system as...
a fundamental regulator in the redox cell signaling of cardiac angiogenesis. Not only VEGF expression but also VEGF signaling is positively regulated by ROS. Moreover, further proangiogenic growth factors like platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF)-2 have been shown to involve ROS within their signal transduction, thus suggesting that the conditions of ischemia/hypoxia, hypertension, and hyperglycemia that parallel CVD states and are associated with increased ROS generation should promote angiogenesis and neovascularization instead of exerting antiangiogenic effects.

Somehow, the pro- versus antiangiogenic actions of ROS in pathophysiological settings of CVD leave basic scientists and clinicians puzzled in their efforts to understand the complex nodal role of ROS playing divergent roles in cardiovascular injury and protection. The most commonly used loophole from this dilemma is the assumption of a tightly regulated balance of ROS, where high concentrations of ROS arising during severe disease states of CVD cause oxidative stress, apoptosis, and cell death, whereas low levels of ROS produced in response to growth factors, transient ischemia/hypoxia, or mild mechanical strain of the myocardium during hypertension may initiate cardiovascular repair mechanisms including endothelial cell differentiation, proliferation, and migration.

But, which signaling pathways are used for the angiogenic versus the proangiogenic route? It is well known that during angiogenesis, NADPH oxidase–derived ROS evolve in angiogenic growth factor–mediated signaling cascades and inactivate protein tyrosine phosphatases (PTP) by oxidizing an essential cysteine residue in the active site, thus, in turn, activating tyrosine kinases. The data of Benndorf et al shed light on a novel angiogenic signaling pathway that involves activation of the thromboxane A2 receptor (TBXA2R), a G–protein–coupled transmembrane eicosanoid receptor, by IsoPs (Figure). Thromboxane A2 is formed from prostaglandin H2 through the activity of thromboxane synthase and has a half-life time of 30 seconds before being catabolized to inactive thromboxane B2. The idea that at least some of the vasoactive effects of IsoPs result from interaction with the thromboxane receptor is not entirely new and has been recently suggested to underlie radial artery vasoospasm, which is a potential cause of early graft failure after coronary bypass graft surgery. Moreover, it has been previously shown that TBXA2R signaling inhibits angiogenesis, as well as VEGF-induced endothelial cell differentiation and migration. The striking news from the data of Benndorf et al, obtained by pharmacological and short hairpin RNA approaches, are that IsoPs activate the small GTPase RhoA via TBXA2R, which disturbs VEGF-induced stress fiber and focal adhesion formation. Amazingly, VEGF likewise regulates cell endothelial cell motility via RhoA downstream of the p110 catalytic subunit of phosphoinositide 3-kinase bound to 1 of 5 distinct p85 regulatory subunits, thus raising the question which molecular cues mediate the distinction of pro- versus antiangiogenic signaling pathways during CVD progression? The take-home message from the data of Benndorf et al is that RhoA activation by IsoPs appears to more robust and persistent as compared to the transient and moderate RhoA activation by VEGF. Persistent RhoA activation has been previously shown to inhibit depolymerization of F-actin and thus may increase cell adhesion and reduce the turnover of focal adhesions, which is necessary for membrane protrusion during cell migration.

The study of Benndorf et al adds an important new key element into the understanding of the balance of redox-regulated signaling pathways that, on the 1 side, promote and, on the other, deregulate angiogenesis during the gradual evolution of CVD. From this study, it can be learned that transient and low level ROS signals arising during initial stages of CVD may be exploited by the organism in growth factor-mediated signaling pathways to drive cardioprotection, neoangiogenesis, and regeneration. However, high levels of ROS, which accumulate during advanced CVD result in lipid peroxidation and the formation of bioactive IsoPs and their further reaction products, thereby aggravating CVD presumably by inhibiting neovascularization and causing vascular dysfunction. The clinical importance of the study of Benndorf et al is clearly evident not only because it offers an explanation for the decrease in capillary density frequently observed during graft surgery, but also because it offers an explanation for the decrease in capillary density frequently observed...
during oxidative stress-associated CVD, such as coronary heart disease and systemic hypertension, but also because it also opens potential new avenues of patient treatment with TXA2R antagonists, which may reverse the angiogenic activity of IsoPs and potentially reinitiate the patients own cardiovascular regeneration programs.

**Sources of Funding**

This work was supported by the Excellence Cluster Cardiopulmonary System and the SFB 604 “Multifunctional Signaling Proteins” of the German Research Foundation.

**Disclosures**

None.

**References**


**Key Words:** angiogenesis | oxidant signaling | oxidative stress | thromboxane
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Circ Res. 2008;103:907-909
doi: 10.1161/CIRCRESAHA.108.187278
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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