

Regulation of Coronary Vasomotor Function by Reactive Oxygen Species

Lih Kuo^{1*}, Naris Thengchaisri¹ and Travis W. Hein¹

Abstract

Overproduction of reactive oxygen species is closely associated with cardiovascular diseases in part by impairing endothelial function and consequently compromising blood flow regulation. Superoxide and hydrogen peroxide are elevated under various disease states with reduced endothelium-derived nitric oxide bioavailability. The oxidative stress elicited by angiotensin II, C-reactive protein and tumor necrosis factor- α is mediated by the activation of different redox signaling pathways in the microvasculature. The upregulation of L-arginine consuming enzyme arginase also contributes to the reduced nitric oxide bioavailability during oxidative stress. Hydrogen peroxide exhibits vasodilator function in the coronary microcirculation and plays an important role in the physiological regulation of coronary blood flow. However, excessive production of hydrogen peroxide impairs endothelial function by reducing L-arginine availability through hydroxyl radical-mediated upregulation of arginase. This review summarizes the current knowledge on the effects superoxide and hydrogen peroxide on vasomotor function regulated by the endothelium-derived nitric oxide and prostacyclin in the coronary microcirculation.

Keywords: Oxidative stress; Microcirculation; Endothelium

A normal function of vascular endothelium, responding to physical [1], chemical [2,3], and electrical [4,5] stimuli, is essential to maintain microvascular homeostasis and regulate local blood flow by changing vasomotor tone via release of endothelium-derived vasodilators (e.g., nitric oxide, prostacyclin, C-type natriuretic peptide and hyperpolarizing factors) and vasoconstrictors (e.g., endothelin, prostaglandin H/F, thromboxane and angiotensin). Endothelial dysfunction is one of the earliest markers of vascular abnormalities observed in many cardiovascular diseases associated with oxidative stress due to excessive production of reactive oxygen species (ROS). Redox regulation of proteins by moderate levels of ROS is indispensable for signaling pathways underlying the regulation of subcellular and cellular activity as well as cardiovascular function [6-8]. Superoxide is produced by several enzyme systems in the cell and it is converted to hydrogen peroxide (H_2O_2) by superoxide dismutase. H_2O_2 itself is a potent oxidizing agent

that can be converted to hydroxyl radical in the presence of ferric compounds. H_2O_2 can be degraded by catalase to form H_2O and an oxygen molecule. Among the aforementioned ROS, superoxide and H_2O_2 are the most common and important molecules involved in the physiological and pathophysiological events [7,8]. Compared with superoxide, H_2O_2 is stable, lacks charge, has longer half-life, is cell permeable and can diffuse across longer distances. Therefore, its physical properties are suitable for second-messenger signaling [7,8]. Since a proper delivery of oxygen and nutrients to the tissue is essential for the normal function of an organ, in this brief review we will discuss the role of superoxide and H_2O_2 in the physiological/pathophysiological regulation of vasomotor activity of resistance arterioles in the microvascular beds where blood flow is primarily controlled, with special focus on the coronary microcirculation.

The elevated level of superoxide and H_2O_2 has been detected under various pathophysiological conditions, including ischemia-reperfusion, inflammation, hypertension, diabetes, and atherosclerosis. All of these conditions have been reportedly associated with endothelial dysfunction with reduced NO bioavailability. In animal models of hypertension and myocardial hypertrophy, the excessive ROS release associated with renin-angiotensin system activation has been well documented [9,10]. However, the vasomotor action of angiotensin II (AngII) in the coronary circulation is controversial in view that coronary blood flow can be reduced or increased by AngII [11,12]. It is also unclear whether AngII can modulate vascular tone by generating ROS since enhanced superoxide production by AngII has been reported [13]. Using isolated vessel approaches to eliminate the confounding influences from systemic and local effects inherited in in-vivo preparations, we found that AngII evokes a moderate vasoconstriction of coronary arterioles (50-80 μm in diameter) at low concentrations (~ 1 nmol/L) but a marked vasodilation at higher concentrations (>10 nmol/L) [14]. This concentration-dependent vasomotor reaction to AngII may explain the inconsistent coronary flow changes observed in in-vivo conditions. Interestingly, pre-treating the isolated coronary arterioles with a sub-vasomotor concentration of AngII (0.1 nmol/L) for 60 minutes caused an elevation of superoxide production in the vascular wall and inhibited NO production and endothelium-dependent, NO-mediated dilation in response to adenosine, a potent metabolic vasodilator in the heart. This inhibitory effect of AngII was prevented by AngII type 1 receptor (AT1) blocker losartan, superoxide scavenger TEMPOL, or NAD(P)H oxidase inhibitor apocynin [14]. These microvascular findings indicate that AngII, at the level without causing vasomotor activity, exerts an adverse effect on endothelium-dependent NO-mediated vasodilator function via released superoxide through AT1-mediated activation of the NAD(P)H oxidase pathway. It is speculated that the small elevation of local AngII in the heart during disease states may cause local endothelial dysfunction and subsequently elicit focal vasoconstriction and myocardial ischemia. Moreover, the increase of coronary flow, i.e., vasodilation of resistance arterioles, in response to metabolic stress might be compromised if the global level of AngII is slightly elevated in the heart. The inflammatory marker C-reactive

*Corresponding author: Lih Kuo, Professor and Kruse Family Centennial Chair, Department of Systems Biology and Translational Medicine, College of Medicine, Texas A&M Health Science Center, 702 Southwest H.K. Dodgen Loop, Temple, Texas 76504, USA; Tel: 254-742-7032; Fax: 254-742-7145; E-mail: LKUO@tamhsc.edu

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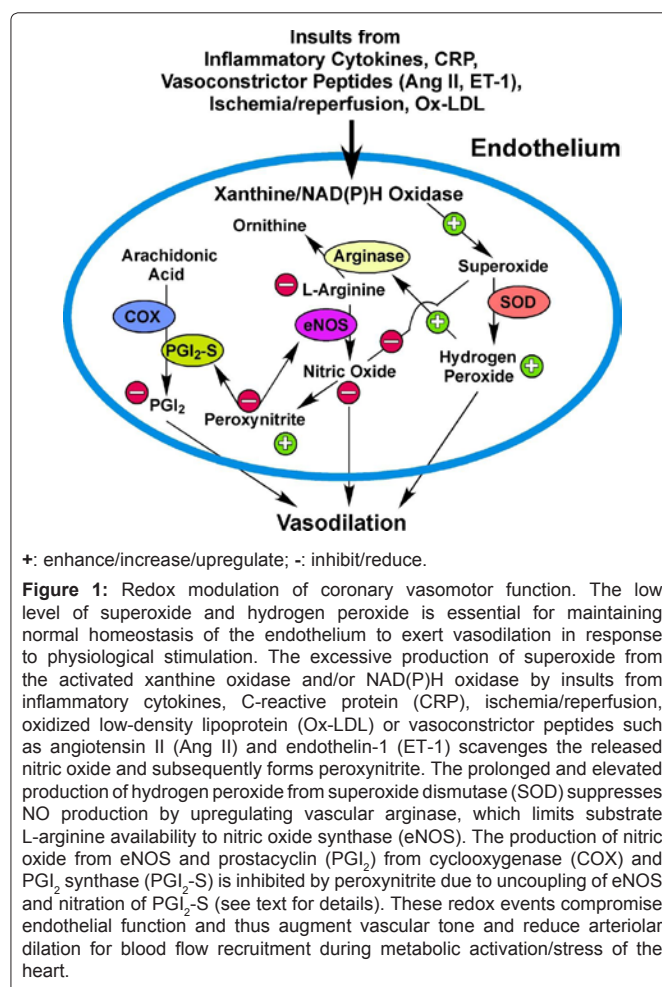
protein (CRP), which is now established as a cardiovascular risk factor, also exhibits similar adverse effects on endothelium-dependent NO-mediated vasodilatory function and NO production in both coronary [15] and retinal [16] arterioles by enhancing NAD(P)H oxidase-mediated superoxide production in the vascular wall via p38 kinase activation. The CRP also affects endothelial arachidonic acid-prostanoid pathways [17]. The formation of peroxynitrite from NO (basal release) and superoxide (CRP-stimulated release) in the endothelium appears to compromise prostacyclin (PGI₂) production, and thus PGI₂-mediated vasodilation, by inhibiting PGI₂ synthase activity through tyrosine nitration [17]. Peroxynitrite also contributes to endothelial NO synthase (eNOS) uncoupling by oxidizing the co-factor BH₄ [18] and thus reduces NO production.

Tumor necrosis factor- α (TNF- α) is a proinflammatory cytokine and an important mediator of cardiovascular complications such as acute myocardial infarction, ischemia-reperfusion injury, atherosclerosis, chronic heart failure and coronary arterial disease in association with diminished coronary blood flow. Treating the isolated coronary arterioles with a pathological concentration of TNF- α (1 ng/ml, 90 minutes) caused a significant reduction of NO release, enhanced superoxide production and JNK phosphorylation in arteriolar endothelial cells and impaired endothelium-dependent dilation to adenosine [19]. In contrast with the insults elicited by AngII and CRP, the TNF- α induced oxidative stress and endothelial dysfunction are associated with the activation of ceramide-induced activation of JNK and subsequent production of superoxide via xanthine oxidase [19,20]. In the model of ischemia-reperfusion injury of coronary arterioles, the endothelial dysfunction in relation to NO deficiency is likely related to the upregulation of arginase-I [21], an enzyme that competes with eNOS for their common substrate L-arginine. Interestingly, the upregulated arginase-I in the vascular wall also contributes, in part, to the reduced NO production and impaired endothelium-dependent dilation in coronary arterioles challenged with oxidized low-density lipoproteins [22].

H₂O₂ can be released from various types of cells, including vascular cells [23,24] and has been implicated as an endothelium-derived hyperpolarizing factor exhibiting vasodilator activity [23]. Extraluminal administration of H₂O₂ (1-100 μ mol/L) elicits concentration-dependent dilation of isolated coronary arterioles in part via an endothelium-dependent mechanism through cyclooxygenase-1-mediated release of PGE₂ [25]. H₂O₂ can also cause smooth muscle hyperpolarization and lead to vasodilation through the opening of calcium-activated potassium channels [25]. This vasodilatory response appears to play a role in coronary homeostasis by maintaining perfusion during pressure reduction (i.e., autoregulation) [26] and metabolic activation (i.e., functional hyperemia) [27], respectively, in the heart. Interestingly, in disease states, the vasodilator action of H₂O₂ appears to compensate for the impaired NO-mediated dilation linking to the uncoupling of NO synthase with its cofactor tetrahydrobiopterin [28] and to protect ischemia-reperfusion injury in the coronary microcirculation [29]. Recently, in the pig model, we found that the impaired NO-mediated vasodilation in collateral-dependent arterioles distal to chronic coronary occlusion was restored by exercise training [30]. The beneficial effect of exercise on coronary arteriolar function was abolished by catalase, suggesting the contribution of H₂O₂ in

compensating and restoring endothelium-dependent NO-mediated vasomotor function during the adaptation of coronary collateral microvessels to myocardial ischemia [30]. On the other hand, it has been reported that H₂O₂ can exert an adverse effect by reducing endothelial release of NO for vasodilation when the endothelium is exposed to a prolonged (e.g., 60 minutes) elevation of H₂O₂ (e.g., 100 μ mol/L) [31]. Interestingly, the dilation mechanisms involving cyclooxygenase, guanylyl cyclase, cytochrome-P450 monooxygenase, and potassium channel activation are not affected by H₂O₂ [31]. Supplementation of L-arginine or inhibition of arginase restores H₂O₂-impaired vasomotor function. Moreover, the adverse effect of H₂O₂ can be prevented by inhibiting hydroxyl radical production [31]. It appears that a high intravascular level of H₂O₂ selectively impairs endothelium-dependent NO-mediated dilation of coronary microvessels by reducing L-arginine availability through upregulated arginase-I. The formation of hydroxyl radical leading to arginase-I overexpression appears to be responsible for the adverse effect of H₂O₂.

Collectively, redox signaling with a low level of H₂O₂ released from cardiomyocytes and/or endothelial cells displays an indispensable role in maintaining microcirculatory homeostasis by regulating vasomotor activity in response to physiological challenges. The release of H₂O₂ from the vasculature helps to restore vasomotor function by



compensating for NO deficiency in coronary collateral microvessels adapted to chronic myocardial ischemia with exercise training. Depending upon the disease model and the pathophysiological insult, the excessive and prolonged production of superoxide, via stress kinase-activated NAD(P)H oxidase or xanthine oxidase, and the subsequent exorbitant formation of H₂O₂, appear to generate oxidative stress and inflammation, which outweighs the benefits of vasoregulation by impairing endothelial function and possibly exhausting vasodilator reserve (Figure 1). The redox signaling can be a double-edged sword in the microcirculation, which helps tissue survival in one way by improving vasomotor regulation and elicits tissue injury in the other way by causing vascular dysfunction and promoting thrombosis. The status and the balance of redox signaling in the vascular cells and their surrounding parenchymal tissues appear to modulate the homeostasis and vasomotor function of microvessels in health and disease. It is worth noting that the systemic supplementation of antioxidants showed no benefit but instead promoted possible harmful effects in cardiovascular disease prevention or therapy [32-35]. On the other hand, localized manipulation of the redox system and arginase activity in a diseased vasculature might be a useful strategy to improve flow regulation and thus enhance tissue survival.

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Author Affiliation

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¹Departments of Systems Biology & Translational Medicine, Ophthalmology and Surgery, College of Medicine, Texas A&M Health Science Center, Temple, Texas 76504, USA

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