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Review

Impact of apocynin on vascular disease in hypertension

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ABSTRACT

Reactive oxygen species (ROS) are generated by cell metabolism of oxygen and represent signaling molecules playing an active role in vascular biology. In pathological conditions, including hypertension, a ROS excess, together with reduced endogenous antioxidant defenses, occurs, determining a state of oxidative stress. NAD(P)H oxidase (Nox) is a major ROS source within the vasculature. A large body of literature has demonstrated that hypertension-associated vascular functional and structural changes are attributable to Nox-driven intravascular ROS generation.

Apocynin is a methoxy-catechol discovered as an inhibitor of superoxide. It has been utilized in several laboratories and in different models of hypertension as an inhibitor of Nox.

Recent evidence proposes that apocynin predominantly acts as an antioxidant. The present review will discuss the role of ROS in vascular disease in hypertension and the impact of apocynin on these vascular changes.

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1. Introduction

Reactive oxygen species (ROS) are ubiquitous reactive derivatives of O_2 metabolism detectable in all biological systems. They are involved in several intracellular signaling pathways leading to gene transcription and changes in protein synthesis. With respect to the cardiovascular system, major ROS detectable within the vasculature are superoxide anion ($\bullet O_2^-$), hydroxyl radical ($\bullet OH$), hydrogen peroxide (H_2O_2), and the reactive nitrogen species peroxynitrite ($ONOO^-$) [1]. In physiological conditions, the rate of ROS generation is counterbalanced by endogenous antioxidant molecules, which include the so-called ROS scavengers, such as ascorbic acid, or antioxidant enzymes, such as glutathione peroxidase, superoxide dismutase (SOD), and catalase. A constant balance between pro-oxidant and antioxidant compounds guarantees vascular homeostasis. In contrast, in pathological conditions, an excess of ROS generation cannot be controlled by the usual protective antioxidant mechanisms, leading to a state of oxidative stress [2].

Hypertension represents the typical clinical condition in which a state of vascular oxidative stress emerges. In hypertension, excess of $\bullet O_2^-$ production reacts with nitric oxide (NO), inducing its breakdown and producing increased concentration of the toxic $ONOO^-$, which in turn promotes a variety of negative effects on cellular function and deleterious signaling in the vasculature [3].

Within the vascular wall, ROS may be generated by different enzymatic systems including cyclooxygenase (COX), xanthine oxidase, uncoupling NO synthase (NOS), mitochondria and NAD(P)H oxidase

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(Nox). Among these, Nox is widely considered the most important source of ROS. Thus, inhibition of this enzyme might represent an interesting therapeutic target. Apocynin is a nonspecific Nox inhibitor, the vascular activity of which is supported by a large body of literature. The present review will focus on the role of ROS in vascular disease in hypertension and the impact of apocynin on these vascular changes.

2. ROS and vascular changes in hypertension

It is widely recognized that ROS members – $\bullet O_2^-$ and H_2O_2 in particular – exert a crucial role as signaling molecules to modulate vascular tone, cell proliferation and migration, extracellular matrix deposition and inflammation. When produced in excess, as occurring in hypertension, ROS represent a pathophysiological stimulus eliciting deleterious signaling within the vasculature, leading to vascular functional and structural changes (Fig. 1) [4,5]. Vascular ROS may be produced at the level of endothelial, smooth muscle and adventitial cells, and can be generated by several enzymes. As to hypertension-related vascular disease, major sources of ROS are represented by Nox and COX. The role of COX on vascular disease in hypertension has been examined in a recent review [6] and will not considered further here.

2.1. Role of Nox

Nox is a multisubunit membrane-associated enzymatic system, which utilizes NADH/NADPH as an electron donor to reduce molecular oxygen and generate $\bullet O_2^-$. It requires the assembly of cytosolic (p47phox, p67phox) and membrane bound (gp91phox/Nox1/Nox4 and p22phox) subunits to form a functional enzyme complex, as shown in Fig. 1 [3,5]. In the vasculature the Nox complex is at least partly pre-assembled, as a significant proportion of Nox subunits are co-localized intracellularly in endothelial cells [3]. Nox is functionally active in the endothelium as well as in the media and adventitia [4]. Within the vascular wall, all the Nox subunits are expressed, to varying degrees [3,4].

3. Apocynin: structure and pharmacological properties

Apocynin (4-hydroxy-3-methoxyacetophenone), was isolated from the native medicinal plant *Picrorhiza kurroa*, and has been experimentally used as an efficient inhibitor of the Nox complex [7,8]. Apocynin is able to blunt the Nox -derived $\bullet O_2^-$ release by blocking the migration of p47phox to the membrane, a critical step to favor assembly of the functional Nox complex (Fig. 1) [7,8]. Of note, the oxidation of apocynin is necessary to trigger its ability to inhibit Nox. Apocynin oxidation is guaranteed by the presence of H_2O_2 and myeloperoxidase, largely present in neutrophils, and this process leads to the formation of diapocynin, the metabolically active compound of apocynin [9,10]. The growing literature demonstrating, over the past two decades, that the Nox system is a major source of vascular ROS generation induced several laboratories to utilize apocynin as a Nox inhibitor in in vitro and in vivo studies [8]. However, other studies have also documented that apocynin is not a specific inhibitor of vascular Nox, but – rather – an important antioxidant [11]. These conclusions were based on the consideration that vascular cells do not possess myeloperoxidase, and, therefore, activated apocynin may not be formed [8,11]. According to these findings, investigators are alerted that the role of apocynin on the intravascular redox status might not be specific for Nox, and it may depend on different cell types and whether peroxidases are active or not [8].

Hypertension is associated with increased peripheral resistance, resulting predominantly from functional, structural and mechanical alterations at the level of small-resistance arteries. In this context, apocynin has shown promising application reducing the Nox activation, and leading to a dramatic modulation of vascular changes in animal models of hypertension as well as, more recently, in isolated human vessels, as detailed here below.

4. Vascular functional alterations in hypertension: role of apocynin

Functional abnormalities identify the presence of an impaired endothelial function, and are mainly assessed as a blunted agonist-evoked endothelium-dependent relaxation. A converging literature convincingly demonstrated that the impaired endothelial function associated with hypertension is linked to a decreased activity of NO, an effect documented in animal models of experimental and genetic hypertension, and in human essential hypertension [6,12]. Together with cytokines and growth factors, angiotensin (Ang) II is one of the major vasoactive compounds involved in the regulation of Nox. Ang II stimulates the activation of Nox, and increases the expression of Nox subunits, thus inducing ROS generation in vascular smooth muscle cells, endothelial cells, adventitial fibroblasts, and intact arteries as well [4,13]. Indeed, Ang II-infused rodents, a model of experimentally induced hypertension, are characterized by reduced endothelium-dependent relaxation at the level of mesenteric small arteries [14–16]. Such attenuated response was less sensitive to NO synthase inhibitors such as N^G-momomethyl-L-arginine (L-NMMA), thus indicating a reduced NO availability [15–17]. Of note, in this animal model of hypertension, chronic administration of apocynin was able not only to prevent endothelial dysfunction, but also to abrogate Nox activity as well [17,18]. In conjunction, these data suggest that activation of vascular Nox plays

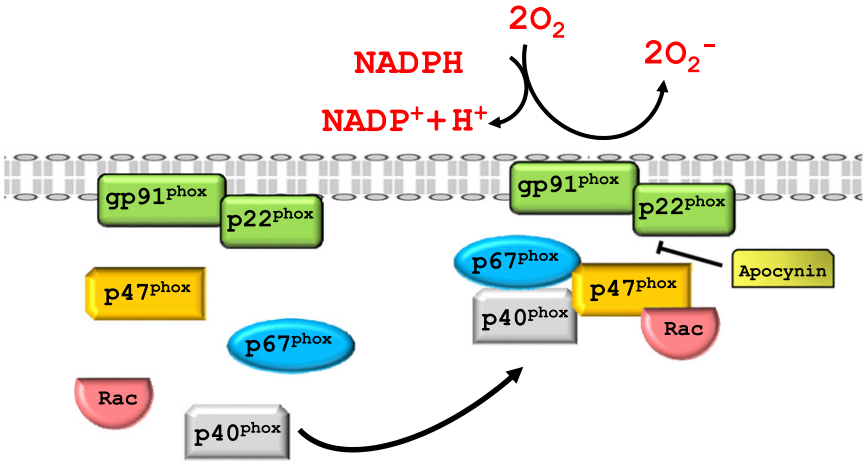

Fig. 1. NAD(P)H oxidase activation and mechanism of action of apocynin. NAD(P)H oxidase is a multisubunit membrane system, which is activated by translocation of the cytosolic subunits p47phox, p67phox, p40phox and Rac to the gp91phox/p22phox complex. It utilizes NADH/NADPH as electron donor to reduce molecular oxygen and generate $\bullet O_2^-$. Apocynin is able to blunt the NAD(P)H oxidase-derived $\bullet O_2^-$ release by blocking migration of p47phox to the membrane.