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REVIEW ARTICLE

Reactive oxygen species: key regulators in vascular health and diseases

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ROS are a group of small reactive molecules that play critical roles in the regulation of various cell functions and biological processes. In the vascular system, physiological levels of ROS are essential for normal vascular functions including endothelial homeostasis and smooth muscle cell contraction. In contrast, uncontrolled overproduction of ROS resulting from an imbalance of ROS generation and elimination leads to the development of vascular diseases. Excessive ROS cause vascular cell damage, the recruitment of inflammatory cells, lipid peroxidation, activation of metalloproteinases and deposition of extracellular matrix, collectively leading to vascular remodelling. Evidence from a large number of studies has revealed that ROS and oxidative stress are involved in the initiation and progression of numerous vascular diseases including hypertension, atherosclerosis, restenosis and abdominal aortic aneurysm. Furthermore, considerable research has been implemented to explore antioxidants that can reduce ROS production and oxidative stress in order to ameliorate vascular diseases. In this review, we will discuss the nature and sources of ROS, their roles in vascular homeostasis and specific vascular diseases and various antioxidants as well as some of the pharmacological agents that are capable of reducing ROS and oxidative stress. The aim of this review is to provide information for developing promising clinical strategies targeting ROS to decrease cardiovascular risks.

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Abbreviations

ACEIs, angiotensin converting enzyme inhibitors; Ang II, angiotensin II; ARBs, angiotensin receptor blockers; ECs, endothelial cells; eNOS, endothelial NOS; GPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; HOCl, hypochlorous acid; iNOS, inducible NOS; LO, lipoxygenase; MCP-1, monocyte chemotactic protein-1; NOX, NADPH oxidase; $O_2^{\bullet -}$, superoxide ion; $OH^{\bullet -}$, hydroxyl radical; $ONOO^-$, peroxynitrite; VSMCs, vascular smooth muscle cells; XO, xanthine oxidase

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Introduction

Vessels transport blood throughout the body and provide critical nutrients to all tissues and organs. Structural and functional abnormalities of vessels result in cardiovascular diseases, such as ischaemic heart disease and stroke, which is the leading cause of death in the world (McGuire, 2016). Vascular remodelling occurs due to ageing and many pathological stimuli, including haemodynamic changes, inflammatory cytokines, cholesterol infiltration and oxidative stress.

ROS, a class of chemically reactive molecules, are now appreciated to play important roles in the regulation of a variety of biological processes. In recent years, researchers have continued to study the crucial roles of ROS in vascular homeostasis and the pathogenesis of vascular diseases, including hypertension, atherosclerosis, restenosis and abdominal aortic aneurysm. Low levels of ROS, acting as powerful signalling molecules, are essential for maintaining normal vessel functions, whereas uncontrolled overproduction of ROS exacerbates oxidative stress, resulting in vascular cell damage, the induction of proliferation and migration of vascular smooth muscle cells (VSMCs), recruitment of inflammatory cells, lipid peroxidation, activation of metalloproteinases and deposition of extracellular matrix, collectively causing vascular remodelling (Konior *et al.,* 2014; Raaz *et al.,* 2014; Vara and Pula, 2014; Kim *et al.,* 2016). An imbalance of ROS generation and elimination in pathological conditions is the reason for oxidative stress. Numerous studies have been aimed at exploring effective therapeutic strategies, such as antioxidants, for counteracting ROS and oxidative responses, ultimately protecting against the vascular diseases.

In this review, we will discuss the chemical characteristics of ROS, the balance of ROS generation and elimination and their roles in vascular homeostasis and specific vascular diseases including hypertension, atherosclerosis, restenosis and abdominal aortic aneurysm. Finally, we will discuss the recent advances on clinical strategies targeting ROS to reduce cardiovascular risks.

ROS

Chemical characteristics of ROS

ROS family comprises numerous small reactive ions and molecules that are derived from oxygen metabolism. ROS with unpaired electrons are considered as free radicals such as superoxide ion ($O_2^{\bullet -}$) and hydroxyl radical ($OH^{\bullet -}$), which are unstable and have short biological half lives. In contrast, nonradicals of ROS such as **hydrogen peroxide** (H_2O_2), singlet oxygen ($^1O_2$), peroxynitrite ($ONOO^-$) and hypochlorous acid (HOCl) are comparatively stable and have longer half lives (Droge, 2002; Vara and Pula, 2014). In particular, $O_2^{\bullet -}$, a typical extremely reactive radical with rapid spontaneous ($8 \times 10^4\ M^{-1}{\cdot}s^{-1}$) or enzymic ($2 \times 10^9\ M^{-1}{\cdot}s^{-1}$) dismutation, represents the precursor of most ROS (Fridovich, 1983). The majority of $O_2^{\bullet -}$ generated is rapidly converted to H_2O_2, which is more stable than $O_2^{\bullet -}$ and probably mediates downstream cell signallings. Compared with other ROS, H_2O_2 has a relatively long half-life, can penetrate the cell membrane easily and function as a reversible oxidant, thus representing the most ideal second messenger among ROS (Fisher, 2009; Reth, 2002). The decomposition of H_2O_2 produces the highly reactive radical $OH^{\bullet -}$, which is considered to be associated with oxidative damage due to its mostly nonselective and irreversible reactivity (Pryor, 1986; Thomas *et al.,* 2009). Various ROS molecules with a wide spectrum of chemical properties display significant heterogeneity in a number of biological processes.

Generation and elimination of ROS in the vascular system

Oxidative stress is determined an by imbalance between ROS generation and the intrinsic antioxidant defence system in favour of the first that leads to the subsequent pathogenesis of diseases (Juni *et al.,* 2013). Almost all the cells in the vascular wall, including endothelial cells (ECs), VSMCs and adventitial cells, possess the ability to generate ROS. Generally, both ROS production and elimination are dependent on enzymic and nonenzymic pathways.

Enzymic sources of ROS which are closely related to redox signalling in the vascular system have been studied extensively. **NADPH** oxidase (NOX), **xanthine** oxidase (XO) and uncoupled **NOS** are the most important sources of vascular ROS, in which **myeloperoxidase**, **lipoxygenase** (LOX), **COX** and many other amine oxidases are also included. Importantly, NOX, the only family of enzymes that produces ROS as its primary function, is the major source of ROS production in the vasculature in various conditions (Lassegue *et al.,* 2012; Montezano and Touyz, 2014). NOX contains two membrane bound subunits (gp91phox and p22phox) and several cytoplasmic subunits (p47phox, p67phox, p40phox and G protein) (Bedard and Krause, 2007). There are seven isoforms of NOX in mammals, among which NOX1, NOX2, NOX4 and NOX5 are variably expressed in the vascular system (Bedard and Krause, 2007; Muller and Morawietz, 2009; Lassegue *et al.,* 2012; Montezano and Touyz, 2014). ECs prodominantly express NOX1, NOX2, NOX4 and NOX5; VSMCs mainly express NOX1, NOX4 and NOX5; and adventitial cells express NOX2 and NOX4 (Drummond *et al.,* 2011a; Lassegue *et al.,* 2012). In particular, the various NOX differ with respect to their specific ROS generation. NOX1 and NOX2 primarily produce $O_2^{\bullet -}$ from oxygen; NOX4 has been reported to generate H_2O_2 rather than $O_2^{\bullet -}$; and NOX5 produces both $O_2^{\bullet -}$ and H_2O_2 (Dikalov *et al.,* 2008; Helmcke *et al.,* 2009). Numerous pathological stimuli, like hypertension, hypercholesterolaemia and diabetes mellitus, can activate NOX, resulting in an enhanced production of ROS (Loffredo *et al.,* 2012; Santilli *et al.,* 2015; Ellulu *et al.,* 2016). XO, mainly identified in ECs, is another source of ROS in diseased arteries (Guzik *et al.,* 2006). XO mediates hypoxanthine and xanthine oxidation, thereby producing $O_2^{\bullet -}$ and H_2O_2 as by-products (Harrison, 2004). In addition to NOX and XO, NOS also plays a critical role in both physiological and pathological conditions. **Endothelial NOS (eNOS)** produces **NO** and exerts vasoprotective effects on the endothelium under physiological conditions (Forstermann and Sessa, 2012). However, under pathological conditions, dysfunctional uncoupled eNOS no longer produces NO, but $O_2^{\bullet -}$, which

aggravates oxidative stress (Forstermann and Sessa, 2012; Li and Forstermann, 2013). Besides enzymic sources of ROS, the mitochondrial respiratory electron transport chain (ETC), which is another important source of harmful ROS, serves as a key generator in atherosclerosis and heart diseases (Li *et al.,* 2014; Muntean *et al.,* 2016). Mitochondria are responsible for utilizing oxygen for energy production and oxidative phosphorylation. During ATP formation, the oxygen consumed is converted to $O_2^{\bullet -}$, which makes ROS by-products of the mitochondrial respiratory chain (St-Pierre *et al.,* 2002; Andreyev *et al.,* 2015). Mitochondria have been conventionally considered as the main source of ROS in living cells including vascular cells (Dromparis and Michelakis, 2013). The leak of electrons to molecular oxygen from ETC, predominantly at complexes I, II and III, represents a major way of generating ROS. In addition to ETC, the mitochondrial growth factor adaptor Shc and monoamine oxidases (**MAO-A** and **MAO-B**) are also responsible for ROS production in the vascular system (Camici *et al.,* 2007; Sturza *et al.,* 2015). Importantly, ROS overproduction in mitochondria results in changed mitochondrial permeability, leading to ROS release (Zorov *et al.,* 2014). This regenerative cycle of mitochondrial ROS production and release is named 'ROS-induced ROS release', which triggers ROS burst and has a pathological impact (Zorov *et al.,* 2000; 2014).

The control of ROS steady state is critical for maintaining a healthy life. Therefore, an antioxidant system also exists in the body in order to decrease excessive ROS. The vasculature contains a number of ROS-reducing enzymes, which act as antioxidants and provide redox homeostasis. SOD, catalase and glutathione peroxidase (GPx) are the major types of antioxidant enzymes. It has been reported that there are three isoforms of SOD (SOD1, SOD2 and SOD3), all of which catalyse the dismutation of $O_2^{\bullet -}$ into H_2O_2 and oxygen (Chiarelli *et al.,* 2005). Catalase mediates the elimination of H_2O_2 by facilitating the decomposition of H_2O_2 to oxygen and water (Chelikani *et al.,* 2004). GPx, usually uses perioxide as an oxidant for another substrate, reduces H_2O_2 to water as well as organic peroxides to their corresponding alcohols (Margis *et al.,* 2008). In addition to the enzymatic degradation of ROS, various low-molecular-weight compounds can directly react with ROS (Lu *et al.,* 2010). These dietary or endogenously synthesized antioxidants, including vitamin C, vitamin E, uric acid, tripeptide GSH, phenolics, flavonoids and thiol compounds, detoxify ROS and protect against ageing and diseases (Stocker and Keaney, 2004; Vara and Pula, 2014; Bielli *et al.,* 2015).

The dynamic balance of ROS generation and elimination is vital in redox homeostasis and vascular health. Low levels of ROS, serving as second messengers within the signalling pathway, are essential for physiological cell functions. However, abnormal ROS accumulation causes increased oxidative stress and leads to vascular damage.

Biological and physiological roles of ROS in vascular health and diseases

Physiological roles of ROS in vascular cells

ECs and VSMCs are the most important cells for maintaining an intact vascular system and its homeostasis. They both represent targets of ROS and ROS signalling. Excessive ROS damage cells, which results in vascular diseases. However, low levels of ROS have been proposed to maintain normal EC and VSMC functions, including mechano-stress signal transduction, physiological angiogenesis and the permeability of ECs, as well as the differentiation and contraction of VSMCs.

ECs lining the vascular lumen are exposed to blood flow, which produces mechano-stress critical for maintaining the homeostasis of ECs. Laminar shear stress promotes the expression of signalling levels of H_2O_2 induced by NOX, which significantly activates **p38 MAPK** and **eNOS**, leading to the generation of NO and protection of ECs (Breton-Romero *et al.,* 2012). **VEGF** is a prominent regulator of angiogenesis under physiological conditions. VEGF promotes EC proliferation, migration and survival upon binding to **VEGFR1** and **VEGFR2** and activating downstream signal pathways. VEGF stimulated signalling events are at least partially dependent on endothelial ROS generation (Colavitti *et al.,* 2002; Yamaoka-Tojo *et al.,* 2004; Ikeda *et al.,* 2005). VEGF induces endothelial ROS production mainly through NOX2 and NOX4 activation (Maraldi *et al.,* 2010; Evangelista *et al.,* 2012). Meanwhile, a recent study showed that cultured human ECs stimulated by physiological amounts of VEGF exhibited increased mitochondrial ROS and enhanced cell migration (Wang *et al.,* 2011). The small GTPase Rac and IQGAP1 are critical for the localization of NOX at the leading edge of migrating ECs, resulting in the local accumulation of ROS and post-translational modification of key signalling molecules including **Akt**, **ERK** and PTP1B, which play important roles in the proliferation, migration and angiogenesis of ECs (Yamaoka-Tojo *et al.,* 2004; Kaplan *et al.,* 2011). Furthermore, low amounts of ROS are also crucial for the maintenance of the undifferentiated phenotype of endothelial progenitor cells (Ushio-Fukai and Urao, 2009). NOX2-derived ROS affect endothelial progenitor cells, thereby promoting revascularization of ischaemic tissue (Urao *et al.,* 2008). ROS are also involved in the regulation of endothelial cell permeability (Monaghan-Benson and Burridge, 2009). Adhesion protein and adherens junction have been reported to be modulated by ROS, leading to a compromised endothelial barrier (Usatyuk *et al.,* 2003; Monaghan-Benson and Burridge, 2009).

Normal levels of ROS are critical for the physiological responses of VSMCs, such as their ability to maintain a differentiated phenotype and regulate vascular tone. ROS promote VSMC differentiation from stem/progenitor cells and the phenotypic switch from a 'proliferative' state to a 'contractile' state. Xiao *et al.* reported that NOX4-induced H_2O_2 production mediates the differentiation of embryonic stem cells into VSMCs by activating SMC-specific transcription factors (Xiao *et al.,* 2009). Moreover, the interaction of Nrf3 with ***Pla2g7*** increases the generation of ROS, subsequently enhancing SMC differentiation. Enforced expression of *Pla2g7* significantly increased SMC differentiation, which could be abolished by a free radical scavenger or flavoprotein inhibitor of NOX but not by an H_2O_2 inhibitor (Pepe *et al.,* 2010; Xiao *et al.,* 2012). Consistently, Chettimada *et al.* found that addition of H_2O_2 directly induced miR-145 expression in VSMCs, which

subsequently caused VSMC differentiation (Chettimada *et al.,* 2014). Besides its effect on VSMC differentiation, vascular tone determined by VSMC-induced contraction is also regulated by ROS. Mechanistically, the effects of ROS on VSMC-induced contractions are dependent on the chemical nature of ROS and/or activation of various protein kinases. $O_2^{\bullet-}$ directly scavenges endothelial NO, exerting vasoconstrictor effects (Bae *et al.,* 2008). Meanwhile, $O_2^{\bullet-}$ contracts VSMCs through the activation of specific kinases including **Src kinases**, **Rho kinases** and ERKs (Oeckler *et al.,* 2003; Knock *et al.,* 2009).

ROS and hypertension

Extensive studies have shown that ROS play a critical role in the chronic pathogenesis of hypertension, while decreasing ROS production helps to reduce blood pressure. We are still far away from completely understanding the complicated mechanisms underlying formation of arterial hypertension. However, we have observed that an increase in ROS production is associated with hypertension in many organs, including the vascular wall, kidney and CNS.

High levels of $O_2^{\bullet-}$ and H_2O_2 have been observed to enhance **angiotensin II** (Ang II)-stimulated redox signalling in resistance arteries of hypertensive patients (Touyz *et al.,* 2005; Montezano *et al.,* 2015). Population-based observations also revealed an inverse relationship between plasma antioxidants and blood pressure (Eslami and Sahebkar, 2014; González *et al.,* 2014). Oxidative stress causes an imbalance between endothelium-derived relaxing factors and endothelium-derived contractile factors, which regulate vascular tone. Increased $O_2^{\bullet-}$ production reacts with NO, one of the most important vasodilators, by uncoupling eNOS, leading to reduced NO release, impaired endothelium-dependent relaxation and an elevated arterial pressure. Oxidative stress also increases plasma F2-isoprostanes which act on PGH/thromboxane receptors to strengthen vasoconstriction (Feldstein and Romero, 2007; Baradaran *et al.,* 2014). Another important effect of ROS in hypertension is the induction of vascular smooth muscle cell hypertrophy. H_2O_2 and NOX are believed to be the major mediators of smooth muscle cell hypertrophy, which leads to medial thickness and high systemic vascular resistance (Laude, 2005; Zhang, 2005). Moreover, ROS molecules mediate vascular fibrotic changes, including collagen and fibronectin production and accumulation in the vessel wall, which will also elevate vascular resistance (Ding, 2005; Patel *et al.,* 2006); these effects can be substantially attenuated by ROS depletion (Zaw *et al.,* 2006; Lijnen *et al.,* 2006). Although most of the studies demonstrate deleterious effects of ROS on vascular hypertensive remodelling, there are some reports showing the benefits of ROS on vessels. The endothelium regulates vascular tone not only by releasing NO but also by causing hyperpolarization, which is termed 'endothelium-derived hyperpolarizing factor'. Under physiological conditions, eNOS-derived H_2O_2 plays an important role as an endothelium-derived hyperpolarizing factor in both humans and animals, and evokes endothelium-dependent vascular relaxations (Matoba *et al.,* 2000; Feletou and Vanhoutte, 2009; Prysyazhna *et al.,* 2012). In addition, H_2O_2 produced by the mitochondria has also been shown to be an endothelium-derived hyperpolarizing factor, which is involved in flow-mediated vasodilatation in human coronary arterioles by means of **Ca^{2+}-dependent potassium channels** (Liu *et al.,* 2011). Furthermore, H_2O_2 stimulates NO production *via* the NO-**cGMP** pathway to promote vasodilatation independently of NO (Cai, 2005a,b; Yi *et al.,* 2015).

ROS production in the kidneys and renal vessels is an established contributor to the formation and maintenance of hypertension. The wide-spread expression of ROS in the renal system correlates with the abundancy of NOX in almost all parts of the kidney, including the glomeruli, podocytes, macula densa, interstitial fibroblasts, medullary thick ascending limb and distal tubule and collecting duct, among which afferent arterioles are the main sources of ROS. $O_2^{\bullet-}$ overproduction in afferent arterioles degrades NO, resulting in vasoconstriction and a reduction in glomerular filtration rate. Ang II causes endothelial dysfunction in afferent arterioles by inducing the expression of the NOX subunit p22phox, subsequently leading to hypertension (Gill and Wilcox, 2006). Moreover, Ang II-induced ROS accumulation in afferent arterioles increases the intracellular calcium concentration, which is another vasoconstrictor for hypertensive vessels (Fellner and Arendshorst, 2005). Glomerular injury can be mediated by ROS and NOX. It has been reported that glomerular injury in mice lacking P47phox is attenuated compared to that in WT mice, and the application of antioxidants alleviated the glomerular sclerosis and proteinuria (Nagase *et al.,* 2006; Taylor *et al.,* 2006; Wang *et al.,* 2015). Mitochondrial ROS generation causes the autophagy of podocytes and impairs the crosstalk between nephrin and caveolin-1, which leads to the disruption of the glomerular filtration barrier (Jia *et al.,* 2008; Ren *et al.,* 2012). Additionally, the mesangial cell proliferation and migration, extracellular matrix deposition and glomerulosclerosis involved in glomerular injury have also been attributed to ROS-mediated damaging effects (Hua *et al.,* 2012). Tubuloglomerular feedback, which plays an important role in sodium reuptake and blood pressure control, is another target of ROS. Nouri *et al.* (2007) proposed that RNA silencing of the NOX subunit p22phox can enhance single tubular glomerular filtration in Ang II-induced hypertension *via* ROS production in the macula densa. In the medulla, NOX promotes vasa recta vasoconstriction and sodium movement into the vasa recta, reducing natriuresis and consequently increasing blood pressure (Mori *et al.,* 2007; White, 2012). Furthermore, ROS have effects on sodium transport as well. $O_2^{\bullet-}$ promotes Na/K/2Cl cotransporter activity through the PKC pathway in medullary thick ascending limb preparations (Silva *et al.,* 2006). Moreover, $O_2^{\bullet-}$ scavenger and NO administration have opposite effects on the Na/K/2Cl cotransporter (Silva and Garvin, 2008). However, some discrepancies exist with regard to the marked effects of ROS on the kidney and hypertension. Several studies have shown that ROS might be beneficial (good) molecules in the development of hypertensive remodelling. Cuevas *et al.* (2012) reported that dopamine **D_2 receptor** depletion inhibits ROS production in renal proximal tubular cells and accelerates the progression of hypertension. Ohsaki *et al.* (2012) found that excessive sodium delivery to cells caused mitochondrial

H_2O_2 overproduction in medullary thick ascending limb, resulting in vasodilatation of the nearby vasa recta.

ROS mediated stimulation of the nervous system represents another specific aspect of blood pressure regulation. Hypertension caused by Ang II infusion involves an increased $O_2^{\bullet-}$ production in the CNS, while i.c.v. injection of SOD enhances Ang II-induced hypertension (Guyenet, 2006). Peterson *et al.* (2009) discovered that NOX2 and NOX4 were both linked to blood pressure regulation, while NOX2 in the subfornical organ can also modulate drinking behaviour. Lob *et al.* (2013) showed that p22phox depletion in the subfornical organ inhibited Ang II-induced hypertensive responses. The nucleus tractus solitarii is a kind of hindbrain nucleus functioning as a cardiovascular control centre, which processes signals from circumventricular organs and carotid baroreceptors. Studies have shown that NOX contributes to Ang II-induced ROS production in the nucleus tractus solitarii and reduces blood pressure in stroke-prone spontaneously hypertensive rats (Glass *et al.*, 2007; Nozoe *et al.*, 2007). H_2O_2-induced delayed hyperexcitability of nucleus tractus solitarii neurons can enhance sympathetic outflow, which contributes to hypertension (Ostrowski *et al.*, 2014). Moreover, an increased input from afferent renal nerves also activates central sympathetic nuclei in a ROS-dependent manner, which shows a complex relationship exists between the central and peripheral nerve systems (Chan *et al.*, 2006).

ROS and atherosclerosis

The classical 'oxidative stress theory' of atherosclerosis is based on the production of ROS from resident cells of the vessels and other organs and tissues, eliciting the oxidation of low-density lipoproteins (LDLs), which leads to inflammatory responses and foam cell formation within atherosclerotic plaques. An increasing number of findings have corroborated this theory, with the aim of completely understanding the role of ROS in atherogenesis so that a strategy can be developed for clinical use.

A number of studies have consistently demonstrated that ROS accumulation and oxidative stress drive atherogenesis. Among the various mechanisms, oxidation of LDLs induced by $O_2^{\bullet-}$ is well-established and widely accepted (Peluso *et al.*, 2012). Oxidized-LDLs (oxLDLs) are cytotoxic to vascular cells and promote vascular inflammation by augmenting the infiltration of monocytes/macrophages into the vessel wall, subsequently resulting in foam cell formation (Tsimikas, 2006). When polyunsaturated lipids undergo oxidation by ROS, several by-products are formed, which react with apolipoprotein (Apo) B-100 and impair its function. Modified ApoB-100 retards the removal of LDLs and prolongs the exposure of both lipids and apoB-100 to ROS attack, which further enhances the oxidation of LDLs (Rabbani *et al.*, 2010). ROS not only oxidize LDLs but also participate in oxLDLs-induced downstream cellular activities. For instance, the pro-atherogenic activation of macrophages induced by minimally oxidized LDLs is dependent on NOX2-derived ROS generation (Bae *et al.*, 2009). Interestingly, positive feedback regulation of oxLDLs-induced NOX expression has been reported, which further expands the interplay between oxLDLs and ROS (Honjo *et al.*, 2008). In addition to the effects of ROS on LDLs, studies have revealed the oxidation of high-density lipoproteins (HDLs) by HOCl, which may promote atherogenesis by counteracting the antiatherogenic effects of HDL (Besler *et al.*, 2011; Khera *et al.*, 2011).

It is well-known that atherosclerosis is a chronic inflammatory disease and inflammation mediates all stages of lesion progression (Galkina and Ley, 2009; Libby and Hansson, 2015). Macrophages, the main cellular components of atherosclerotic plaques, are extensively modified by ROS. Oxidative stress and oxLDLs enhance the release of macrophage colony-stimulating factor and **monocyte chemotactic protein-1 (MCP-1; also known as CCL2)**, which results in the attraction and adhesion of monocytes to arterial walls and promotes their differentiation into tissue macrophages tha then reside in the lesion (Hansson and Libby, 2006; Garrido-Urbani *et al.*, 2014). Moreover, ROS activate NF-κB regulatory complex and trigger the transcription of several atherosclerosis-related genes such as MCP-1, **MMP-9**, **VCAM-1** and procoagulant tissue factor that lead to vascular wall macrophage accumulation and foam cell formation (Van der Heiden *et al.*, 2010). In addition, oxidative stress also affects the function and phenotype macrophages in atherosclerosis. NOX4 has been identified as a source of ROS in macrophages and mediates oxLDLs-induced macrophage death, which is associated with necrotic cores in the advanced plaques (Lee *et al.*, 2010). Meanwhile, ROS signalling is also involved in oxLDLs-induced macrophage spreading, which may contribute to the trapping of macrophages in vessels and promotes atherosclerosis (Park *et al.*, 2009). Inflammatory cells are not only the targets of ROS but also a source of ROS. Those leukocytes that infiltrate the vascular lesion sites release a large amount of ROS as well as their granules, which contain a considerable number of molecules with killing and degradative activities such as myeloperoxidases, elastases, collagenases and lysozymes. Thus, this excessive release of ROS plus granules means vascular inflammation is deleterious in atherosclerosis.

Thrombosis is an important cardiovascular complication of atherosclerosis, which causes arterial occlusion and tissue ischaemia. It has been demonstrated that the activity of platelets plays a crucial role in thrombosis (Ellulu *et al.*, 2016). Several studies have suggested that increased ROS release can indirectly activate platelets or decrease their activation threshold, causing vessels to be prone to thrombosis (Carbonell and Rama, 2007; Lubos *et al.*, 2008; Watt *et al.*, 2012). Mechanistically, oxidative stress stimulates platelet activity by affecting calcium mobilization, inactivating NO function and interacting with arachidonic to induce the formation of isoprostanes (Violi and Pignatelli, 2014). NOX2 is involved in platelet activation and apocynin, a NOX inhibitor, has been reported to reduce platelet adhesion and atherosclerotic lesion formation (Violi and Pignatelli, 2014; Pastori *et al.*, 2015).

ROS and restenosis

Restenosis occurs after balloon angioplasty and stenting, and is characteristic of pathological VSMC accumulation leading to neointima formation. Increased ROS and NOX have been shown to be present early on after angioplasty and may be involved in the pathogenesis of restenosis (Iuliano *et al.*, 2001; Shi *et al.*, 2001; Szocs *et al.*, 2002). In balloon-injured

arteries, vascular $O_2^{\bullet-}$ production is increased and the expression of NOX1, NOX4, gp91phox and p22phox are up-regulated (Iuliano *et al.,* 2001; Shi *et al.,* 2001; Szocs *et al.,* 2002). Similar to angioplasty, bare-metal stent deployment can result in oxidative stress. In the vascular wall, a marked increased production of $O_2^{\bullet-}$ has been reported after bare-metal stent placement, which was associated with an increased expression of gp91phox and p22phox (Ohtani *et al.,* 2006). Drug-eluting stent deployment is also linked to oxidative stress. In pig trials, paclitaxel-eluting stent placement resulted in increased production of $O_2^{\bullet-}$ and decreased NO activity (Pendyala *et al.,* 2009). Paclitaxel stimulates mitochondrial ROS production by enhancing NOX activities, which contributes to oxidative stress (Laurent *et al.,* 2005; Alexandre *et al.,* 2007).

The increased proliferation and migration of VSMCs in arteries lead to neointima formation and luminal narrowing. In the vascular system, NO and Ang II are the two critical molecules that regulate the functions of VSMCs (Galougahi *et al.,* 2014). Ang II contributes to oxidative stress-induced damage to VSMCs by stimulating the overproduction of NOX-dependent ROS dependent (Wosniak *et al.,* 2009). In particular, NOX1, largely expressed in proliferating VSMCs, mediates Ang II-induced $O_2^{\bullet-}$ formation and redox-sensitive signalling pathways (Youn *et al.,* 2012). Thus, NOX1 and $O_2^{\bullet-}$ can be considered as stimulators of VSMC proliferation (Youn *et al.,* 2012). However, NO, usually acts as an inhibitor of Ang II's activity, and plays a protective role in VSMCs (Wang *et al.,* 2008). In addition to direct effects, oxidative stress induces chain reactions that result in endothelial dysfunction and macrophage activation, which, in turn, release cytokines and growth factors to stimulate VSMC proliferation and extracellular matrix remodelling (Steinberg *et al.,* 1989). Moreover, age-related changes in redox signalling and enhanced production of ROS are linked to alterations in the phenotype of VSMCs with ageing, resulting in them having an increased capacity to proliferate, migrate and synthesize extracellular matrix (Li and Fukagawa, 2010).

ROS and abdominal aortic aneurysm

Abdominal aortic aneurysm (AAA) is an important cause of sudden death, which is believed to result from an aberrant interaction between genetic factors and living environment (Emeto *et al.,* 2016). Both animal models and human clinical samples have showed that excessive oxidative stress is implicated in the vascular degeneration of AAA (Sharma *et al.,* 2011; Sawada *et al.,* 2015). A critical pathological feature of AAA is vascular wall inflammation, which is associated with a marked overproduction of ROS (Emeto *et al.,* 2014).

Studies from different animal models of AAA have revealed that ROS are involved in the pathogenesis of the lesions. In the Ang II-induced AAA mouse model, high concentrations of oxidative stress markers, 8-isoprostaneand 8-hydroxy-2′-deoxyguanosine, were detected within the diseased aortic wall (Gavrila *et al.,* 2005; Sawada *et al.,* 2015). Similarly, 8-hydroxy-2′-deoxyguanosine was also increased within aneurysmal aorta from the calcium chloride-induced mouse AAA model (Kaneko *et al.,* 2011). Additionally, in the elastase-induced rat model of AAA, Nakahashi *et al.* (2002) found that **HO-1** expression was dramatically up-regulated and was co-localized with the infiltrating macrophages. High ROS activity has also been reported in human aneurysmal aortas. Increased expression of NOX and the overproduction of $O2^{\bullet-}$ were detected in human aneurysmal segments of aortas compared with adjacent nonaneurysmal segments (Sharma *et al.,* 2011). Zhang *et al.* reported that **inducible NOS (iNOS)** mediates the excessive formation of NO-derived $ONOO^-$, which then promotes vascular oxidative injury and aneurysm progression (Zhang *et al.,* 2003). Moreover, aberrant lipid peroxidation exists in AAA patients, particularly in those with ruptured aneurysms (Dubick *et al.,* 1999). Lipid peroxidation causes the apoptosis and necrosis aortic cells, which is associated with aortic wall weakening and aneurysm formation (Kinnunen *et al.,* 2012; Ayala *et al.,* 2014).

Potential mechanisms for the contributions of ROS in AAA have been suggested but still need further investigation. NOX expression and activity are elevated in the pathogenesis of AAA. Xiong *et al.* (2009) reported that apocynin treatment inhibited NOX activity and attenuated AAA formation, which was accompanied by reduced expression of MMP-2 and MMP-9. Thomas *et al.* (2006) found that depletion of p47phox abolished NOX activity and significantly reduced the incidence and progression of Ang II-induced AAA. In addition to NOX, many other enzymes mediate ROS production in AAA lesions. iNOS and eNOS have been reported to be linked to increased production of $ONOO^-$ and $O_2^{\bullet-}$ that cause oxidative relevant damage during the pathogenesis of AAA (Zhang *et al.,* 2003; Gao *et al.,* 2012; Siu *et al.,* 2014). Inhibition of **5-LOX** by pharmacological or genetic approaches blocks leukocyte infiltration and aneurysm formation and prevents the fragmentation of the medial layers in experimental mouse models of AAA (Bhamidipati *et al.,* 2014). Similarly, a deficiency in **COX-2** markedly diminishes the incidence of AAA (Gitlin *et al.,* 2007), while the application of a COX-2 inhibitor delays the growth of AAA by inhibiting vascular inflammation (King *et al.,* 2006; Keeling *et al.,* 2007). In contrast, SODs and catalases, enzymes that reduce the oxidative burden in vessel walls, have been shown to protect against AAA formation (Sinha *et al.,* 2007; Parastatidis *et al.,* 2013).

Therapeutic strategy targeting ROS and oxidative stress

Considerable research has been performed to explore the effects of antioxidants that can reduce ROS formation or scavenge ROS in the vascular system in order to ameliorate vascular oxidative stress. Promising treatments based on antioxidants are being developed and show therapeutic effects on vascular diseases. Furthermore, many pharmacological agents used clinically in cardiovascular diseases, such as statins, ß-blockers, angiotensin converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), exhibit antioxidative effects. These medicines are not direct inhibitors or scavengers of ROS; however, they act in indirect ways by interacting with the signalling pathways or key molecules involved in ROS production and removal, to reduce vascular oxidative stress.

Antioxidants

Antioxidants are natural or synthetic compounds that are capable of neutralizing radicals and halting excessive ROS accumulation in the body. The WHO have suggested that the consumption of vegetables and fruits, rich in antioxidant vitamins, can lower the risk of chronic diseases (Nishida *et al.,* 2004). Antioxidant vitamins such as **vitamin C** (ascorbic acid) and vitamin E (α-tocopherol) are the most frequently studied antioxidants. However, other natural antioxidants from food and herbs, mainly polyphenols/flavonoids, have also been investigated for their exact roles in the vascular system.

Vitamins C and E are well known for their therapeutic and preventative effects on vascular diseases. Their mechanisms include inhibitory effects on LDL oxidation and leukocyte adhesion and an ability to improve vascular endothelial dysfunction by effectively scavenging a wide range of reactive oxygen and nitrogen species (Carr *et al.,* 2000). Vitamin C, a chain-breaking antioxidant, directly scavenges ROS and prevents the propagation of chain reactions, while vitamin E reacts directly with $O_2^{\bullet-}$, $OH^{\bullet-}$, $^1O_2$ and protects membranes from lipid peroxidation (Santilli *et al.,* 2015). Vitamin C is essential for the normal vascular functions of ECs, including stimulating endothelial repair, inhibiting apoptosis, tightening the permeability barrier and limiting NO synthesis and release (May and Harrison, 2013). More specifically, the endothelial dysfunction in chronic smokers could be improved by vitamin C through its ability to scavenge free radicals (Young *et al.,* 2006). In hypertensive patients, vitamin C has been found to dramatically lower blood pressure and reduce muscle sympathetic nerve activity (Bruno *et al.,* 2012). Similarly, vitamin E is capable of reducing ROS overload in vessels and has been proposed to prevent cardiovascular diseases (Tinkel *et al.,* 2012). Although numerous studies support the hypothesis that antioxidant vitamins can prevent oxidative stress and vascular diseases, randomized clinical trials showed unexpectedly negative results. In a randomized, double-blind, placebo-controlled factorial trial, vitamins E and C showed no effects on reducing the risk of major cardiovascular events (Sesso *et al.,* 2008). A systematic review and meta-analysis of randomized controlled trials demonstrated that supplementation with vitamins was not associated with a decreased risk of major cardiovascular events (Myung *et al.,* 2013). These data discourage the clinical use of vitamins for the treatment or prevention of cardiovascular diseases. However, the lack of benefits from these clinical trials cannot disprove the roles of antioxidants in cardiovascular diseases. Firstly, most of the studies focused on vitamins because of their easy availability, but more efficient antioxidants are still untested. Secondly, the dose of antioxidants and the therapy duration need to be optimized. Trials with larger doses and longer times may show the reversal of tenacious vascular oxidative stress. Thirdly, oral administration may not ensure a sufficient concentration of antioxidants in vascular lesions, whereas local delivery can be a better alternative for clinical use. This so-called antioxidant paradox in clinical settings requires further investigations.

Other natural antioxidants found in food and herbs have been studied for their effects in the vascular system. There are thousands of different kinds of polyphenols/flavonoids, which are widely distributed in a variety of plants and plant-derived beverages (Dauchet *et al.,* 2006). A tremendous number of studies have indicated their capacities to act as antioxidants in diseases. For example, as intake of polyphenols/flavonoids is associated with a decreased risk of cardiovascular diseases (Dauchet *et al.,* 2006; Bauer *et al.,* 2011; Ponzo *et al.,* 2015). Polyphenols/flavonoids scavenge $O_2^{\bullet-}$ and $ONOO^-$ and increase circulating NO, thus preventing vascular oxidative stress (Schroeter *et al.,* 2006; Auger, 2010; Procházková *et al.,* 2011). Green tea and concord grapes are well known for being enriched with polyphenols/flavonoids that are beneficial to vascular health. The effective components of green tea, most of which are catechins, have potent antioxidative properties. The consumption of green tea has been associated with an improvement in vascular functions and a decrease in cardiovascular death (Widlansky *et al.,* 2007; Mineharu *et al.,* 2011). The plentiful antioxidants in concord grapes directly neutralize free radicals, decrease the susceptibility of LDLs to oxidation and increase NO release, which consequently lowers the rates of cardiovascular diseases in epidemiological studies (Rissanen *et al.,* 2003; Chen *et al.,* 2010).

Other pharmacological agents

Some of widely used pharmacological agents in cardiovascular diseases, such as statins, ß-blockers, ACEIs and ARBs, are capable of regulating ROS balance and oxidative stress, resulting in cardiovascular protection. Although inhibition of ROS or scavenging ROS is not their main effects, the antioxidative properties of these medicines is of great interest.

Statins. Statins act in an indirect way by hindering the **3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase** pathway involved in cholesterol synthesis. In addition to their cholesterol-lowering characteristics, statins can also act as cholesterol-independent antioxidants. HMG-CoA reductase inhibition could normalize endothelial function and reduce oxidative stress in diabetes through the suppression of vascular NOX expression and activity and by the prevention of eNOS uncoupling (Wenzel *et al.,* 2008a). In a randomized, double-blind controlled trial, oral **atorvastatin** reduced vascular basal and NADPH-stimulated $O_2^{\bullet-}$ in saphenous vein grafts, which suggests that statin therapy could be maintained in patients undergoing CABG, independently of LDL levels (Antoniades *et al.,* 2010). Moreover, statins also produce their antioxidative actions by evoking the induction of antioxidant enzymes (SOD1, SOD3 and GPx) (Carrepeiro *et al.,* 2011).

ß-blockers. **Nebivolol** is a third-generation β-blocker that inhibits the activity of NOX and prevents NOS uncoupling in hyperlipidaemic rabbits (Mollnau *et al.,* 2003). On the other side, treatment with ebivolol could normalize endothelial function, reduce $O_2^{\bullet-}$ formation and increase NO bioavailability, which may explain its beneficial effects on Ang II-induced hypertension (Oelze *et al.,* 2006).

ACEIs and ARBs. ACEIs and ARBs can reduce NOX activity and mitochondrial $O_2^{\bullet-}$ production, inhibit XO activity and

prevent eNOS uncoupling (Imanishi *et al.,* 2008; Wenzel *et al.,* 2008b). In a prospective open-label, randomized study, ARBs were shown to significantly decrease carotid intima-media thickness, accompanied by a reduction in urine levels of 8-OHdG, a marker of oxidative stress, and increase serum levels of NOX (Ono *et al.,* 2008). Another prospective, matched case–control study showed that **irbesartan** treatment in adolescents with diabetic angiopathy could restore catalase and GPx activity and mRNA expression after exposure to high-glucose concentrations. Indicators of oxidative stress (serum malondialdehyde, fluorescent products of lipid peroxidation, MCP-1 and **$PGF_{2\alpha}$**) were dramatically decreased after treatment with irbesartan (Chiarelli *et al.,* 2005).

Other antioxidant molecules. The NOX family of enzymes are important sources of ROS production in vessels, especially those contain NOX1 or NOX2 catalytic subunits. Therefore, a number of compounds have been investigated as potential inhibitors of NOX and antioxidant molecules. Triazolopyrimidines, including VAS2870 and VAS3947, are considered to be promising inhibitors of NOX activity. VAS2870 and VAS3947 could inhibit NOX-derived ROS in several cell lines and in primary EC and VSMC cultures without effects on XO or eNOS activity (Drummond *et al.,* 2011b). Pyrazolopyridine derivatives such as GK-136901 have been identified as possible inhibitors of NOX1- and NOX4-dependent ROS formation from disrupted cell membrane preparations (Laleu *et al.,* 2010). ML171, a cell active and specific NOX1 inhibitor, potently blocks NOX1-dependent ROS production without influencing the cellular generation of ROS from other enzymes and receptors. At present, all these compounds need to be researched further.

Conclusions and perspectives

The exact mechanism of vascular diseases is complex and is not yet fully understood. ROS and oxidative stress plays an important role in the regulation of physiological angiogenesis, vascular permeability, vascular tone and vessel haemostasis, as well as the initiation, progression and development of various vascular diseases. In the past few decades, tremendous advances have been made in the vascular 'oxidative stress theory'. Antioxidative interventions have been found to be effective in experimental models of hypertension, atherosclerosis, restenosis and AAA formation. However, in contrast, randomized clinical trials of antioxidants have substantially failed. Clinical therapeutic strategies have not been established regarding the use of antioxidant regimen in vascular diseases. This drives us to carefully consider the open questions regarding ROS research. In basic research, experimental tools and standardized methods that can precisely detect the temporospatial distribution of highly unstable ROS need to be improved, in order to clarify the key activities of ROS in the cardiovascular system. In addition to antioxidants used to scavenge harmful ROS, new specific inhibitors of ROS producing enzymes may be a better choice to reduce oxidative stress. Considering the distribution and critical roles of NOX in vessels, the detection of selective NOX inhibitors deserves extensive studies. To date, clinical trials of antioxidant treatment on cardiovascular diseases have not shown positive results, which may be attributed to study design and/or therapeutic protocols. In clinical studies, antioxidant treatment should be specified and individualized with regard to antioxidant substance and dosage. Clinical trials with larger doses and longer treatment times may show beneficial effects on cardiovascular diseases. Additionally, local delivery of antioxidants, for example, using drug-eluting stents, may provide an innovative way for vascular antioxidant therapy.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.,* 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.,* 2015a,b,c,d).

Author contributions

Q.C. and Q.W. drafted the manuscript. Q.X., J.Z. and L.Z. critically reviewed the manuscript.

Conflict of interest

The authors declare no conflicts of interest.

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