MINIREVIEW

A Comparison of Arteries and Veins in Oxidative Stress: Producers, Destroyers, Function, and Disease

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Reactive oxygen species (ROS) are by-products of oxygen metabolism, normally present in low levels inside cells, where they participate in signaling processes. The delicate balance in the continuous cycle of ROS generation and inactivation is maintained by enzymatic and nonenzymatic endogenous systems. Overwhelming production of ROS (by such sources as the mitochondrial electron transport chain, NADPH oxidase, xanthine oxidase, or uncoupled nitric oxide synthase), when inadequately counteracted by destruction through antioxidant systems (such as superoxide dismutase or catalase), leads to a prooxidant state also known as oxidative stress. Increased levels of ROS and markers of oxidative stress have been consistently found in such cardiovascular diseases as atherosclerosis or hypertension, although controversy still exists over the pathophysiological role of oxidative stress in these conditions. ROS can modulate vascular function either by direct oxidative damage or by activating cellular signaling pathways that lead to abnormal contractile, inflammatory, proliferative, or remodeling properties of the blood vessel. Most current research focuses on these processes in arteries, leaving veins, "the other side" of vascular biology, in obscurity. Veins are different structurally and functionally from arteries. Equipped with a smaller smooth muscle layer compared to arteries, but being able to accommodate 70% of the circulating blood volume, veins can modulate cardiovascular homeostasis and contribute significantly to hypertension pathogenesis. Although the reports on the quantitative differences in ROS production in veins compared to arteries had conflicting results, there is a clear qualitative difference in ROS metabolism and utilization between the two vessel types. This review will compare and contrast the current knowledge of ROS metabolism in arteries *versus* veins in both physiological and pathophysiological conditions. Our understanding of the mechanisms underlying vascular diseases would greatly benefit from a more thorough exploration of the role of veins and venous oxidative stress. Exp Biol Med 232:27–37, 2007

Key words: reactive oxygen species; arteries; veins; oxidative stress; vascular pathogenesis; hypertension

Overview

We will begin this review by discussing the chemistry of reactive oxygen species (ROS) and the major cellular mechanisms of ROS formation and destruction. This will be followed by a section emphasizing the structural and functional differences in arteries and veins that can influence the contribution of each of these vessel types to vascular pathogenesis. Next, a summary of existing data on the expression and activity of four of the main enzymes involved in ROS metabolism in arteries and veins will be presented. The association between vascular oxidative stress and two important cardiovascular diseases, atherosclerosis and hypertension, will be then briefly analyzed. Finally, we will highlight some of the remaining important questions in vascular ROS research.

ROS Chemistry

ROS are important vascular signaling molecules or mediators of oxidative stress. They are, by definition, highly reactive intermediates of oxygen metabolism, constantly being generated and destroyed by both environmental and

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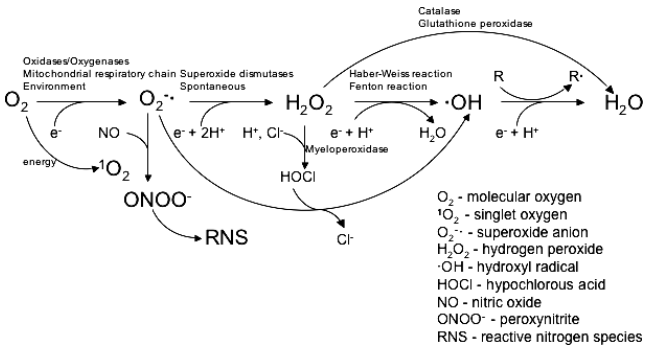

Figure 1. Reactive oxygen species metabolism: gradual addition of electrons reducing molecular oxygen.

endogenous systems. Produced by a gradual reduction of molecular oxygen, ROS include both unstable free radicals (chemical species having unpaired electrons in their outermost shell), such as the superoxide or the hydroxyl radical, and longer-lived nonfree radical oxidants, such as hydrogen peroxide (Fig. 1). The various producers and destroyers of ROS are listed in Table 1, but only the most comprehensively studied ones will be detailed in the following section. Their order does not bear any reflection of their relative importance, overall or in vascular tissues.

Cellular Mechanisms of ROS Production. *Mitochondrial Respiratory Chain.* The mitochondrial respiratory chain is the main energy source for the cell. Situated in the inner mitochondrial membrane, it catalyzes electron transfer using more than 80 peptides organized in four complexes. The transfer of electrons, shuttled by coenzyme Q and cytochrome C, usually leads to the formation of ATP by the fifth complex. However, a certain amount (1–2% *in vitro*) of electrons leak (1), principally from complex III but also from complex I, generating superoxide (2, 3). The rate of mitochondrial ROS production, the levels of mitochondrial DNA oxidative damage, and the degree of membrane fatty acid unsaturation (potentially a target of lipid peroxidation by ROS) are all inversely linked to maximum longevity in animals (4). These facts are among the evidence supporting the free radical theory of aging. Because superoxide production is directly dependent on the proton motive force, a feedback mechanism has been proposed for the uncoupling proteins (UCP 1, 2, and 3). Activated by superoxide and lipid peroxidation, these proteins seem to act by slightly reducing the proton motive force and hence energy production as a trade-off for a decreased ROS production from the mitochondrial complexes I and III (5, 6).

The Nox Family of NADPH Oxidases. The Nox family of NADPH oxidases is another major source of ROS. The classic example is the phagocytic NADPH oxidase, a multisubunit enzyme involved in host defense. Composed of two membrane-bound catalytic subunits, Nox2 (formerly known as $gp91^{phox}$) and $p22^{phox}$ (forming the central flavocytochrome b_{558}), and four cytosolic regulatory subunits, $p47^{phox}$, $p40^{phox}$, $p67^{phox}$, and Rac, the phagocytic NADPH oxidase requires for its activation a series of phosphorylation and translocation events, triggered by pathogen recognition. For more information on NADPH oxidase structure and function, please consult review references 7, 8, and 9. Deliberate generation of ROS by the professional phagocyte during the "oxidative burst" is a rapid and powerful weapon of defense against pathogens. A genetic lack of NADPH oxidase activity in patients suffering from chronic granulomatous disease, a condition characterized by recurrent, life-threatening infections, illustrates the importance of the beneficial side of ROS chemistry (7). Based on the homology with Nox2, several other members of the human Nox family have been identified, each of them seemingly having different activation requirements and expression patterns. Nox1, Nox3, and Nox4 are more similar in structure to Nox2, and they all require at least $p22^{phox}$ for activation. Duox1 and Duox2 are Ca^{2+}-activated dual oxidases with a C terminal NADPH oxidase domain and an N terminal peroxidase domain. Nox5 is closer to the Duox1/2 in structure and is also Ca^{2+} activated but lacks the peroxidase domain (9).

Xanthine Oxidoreductase. Xanthine oxidoreductase (XOR) is an enzyme that catalyzes the last steps of purine metabolism: the transformation of hypoxanthine and xanthine to uric acid, with superoxide/H_2O_2 generated as by-products. XOR possesses one molybdopterin, two iron-sulfur groups, and one FAD and functions as a 145 kDa homodimer. There are two isoforms of XOR, each of them utilizing different electron acceptors: xanthine dehydrogenase (XDH), which requires NAD^+, and xanthine oxidase (XO), which requires molecular oxygen. XDH is convertible to XO by reversible sulfhydryl oxidation or by irreversible proteolytic modifications (10). Although both isoforms have ROS-generating potential, *in-vivo* XO is by far the more important superoxide/H_2O_2 source, making XDH to XO conversion in such situations as ischemia/reperfusion or inflammation of physiopathological significance (11).

Nitric Oxide Synthase. Nitric oxide synthase (NOS), the enzyme responsible for NO generation, has three isoforms: NOS1 (the neuronal NOS), NOS2 (the inducible NOS), and NOS3 (the endothelial NOS). In physiological conditions, NOS catalyzes the transformation of L-arginine into L-citrulline and NO, using several cofactors: NADPH, FAD, FMN, and 5,6,7,8-tetrahydrobiopterin (BH_4). However, if the enzyme is depleted of BH_4 or of L-arginine, it becomes uncoupled and transfers electrons to molecular oxygen rather than the substrate L-arginine, producing superoxide. Furthermore, interaction of superoxide with NO generates peroxynitrite, the second in the family of reactive nitrogen species, capable of producing a cascade of deleterious effects through oxidation, nitration and nitrosation of molecules (12, 13).

Cellular Mechanisms of ROS Destruction. *Superoxide Dismutases.* A central role in the regulation of ROS levels is attributed to superoxide dismutases (SODs), a

Table 1. Producers and Destroyers of Reactive Oxygen Species

ROS producers	ROS destroyers
Mitochondrial respiratory chain	Superoxide dismutase family
Nox and Duox families	Catalase
Xanthine oxidase	Glutathione system
Superoxide dismutase family	Selenoproteins (glutathione peroxidase, thioredoxin reductase, etc.)
Uncoupled nitric oxide synthase	Peroxiredoxins
Monoamine oxidase	Antioxidant ROS scavengers (A, C, E vitamins, ceruloplasmin, ubiquinone, uric acid, bilirubin, etc.)
Lipoxygenase	Nitric oxide
Cyclooxygenase	Uncoupling proteins (?)
Cytochrome P450	
Haber-Weiss and Fenton reactions	
Environment (ionizing radiation, etc.)	
Nitric oxide (reactive nitrogen species)	

family of enzymes responsible for superoxide breakdown, with the consecutive production of hydrogen peroxide. This otherwise spontaneous dismutation reaction is significantly accelerated by SOD. There are three known SODs: the cytosolic CuZnSOD (SOD1), an unusually stable homodimer; the mitochondrial MnSOD (SOD2), functioning as a tetramer; and the extracellular EC-SOD (SOD3), a tetramer with a C terminal heparin-binding region. There is a great body of evidence supporting the beneficial role of SOD. Knock-out experiments showed neonatal lethality of mice lacking MnSOD and reduced lifespan and multiple function abnormalities in mice lacking CuZnSOD (14–17). Furthermore, overexpression studies of SODs strongly suggest a protective role of these enzymes in many diseases, as well as in aging (15–17). Additionally, mutations in the *SOD1* gene leading to the production of a changed, toxic variant of CuZnSOD are linked to 20%–25% of cases of familial amyotrophic lateral sclerosis (Lou Gehrig's disease), a fatal neurologic condition (18). In addition to providing protection from superoxide, SOD activity also results in production of hydrogen peroxide, a diffusible molecule far more stable than the superoxide anion. Hydrogen peroxide can act both by affecting gene expression as a signaling molecule and by continuing the ROS cascade with the formation of the hydroxyl radical. The latter, generated through a reaction with transition metals, such as Fe^{2+} *via* the Fenton/Haber-Weiss chemistry (Fig. 1), is a highly reactive radical that to our knowledge cannot be destroyed enzymatically. The only protection from its dangerous oxidative potential is therefore left to antioxidant scavengers and metal chelators.

Catalase. Catalase is a homotetrameric heme-containing enzyme that catalyzes the conversion of hydrogen peroxide into water and oxygen with one of the highest turnover rates known in enzymology ($\sim 10^7$ l/mol/sec) (19). It is usually found in peroxisomes, cellular organelles involved in multiple metabolism pathways, where it functions in hydrogen peroxide detoxification (20). Catalase, as well as other ROS enzymes, has been linked with aging.

Glutathione Redox Cycle. Another ROS-consuming system in cells is the glutathione redox cycle. Glutathione peroxidase transfers electrons from the reduced form of glutathione to hydrogen peroxide with the formation of water and oxygen; subsequently, the oxidized glutathione disulfide is reduced by glutathione reductase. There are other selenoproteins with similar activity to glutathione peroxidase, such as thioredoxin reductase and selenoprotein P, all of which work as antioxidant enzymes.

ROS Scavengers. Because of their widespread therapeutic use as antioxidants, endogenous ROS scavengers, such as vitamin C and E, should also be noted. However, when considering the antioxidant properties of such compounds, it should be appreciated that they are not enzymes, and, thus, a new molecule is needed for each superoxide anion that is scavenged. These vitamins are therefore poor ROS scavengers, and numerous other factors (such as the insufficient doses or their unknown intracellular concentration and activity) have been overlooked in some antioxidant clinical studies.

Arteries and Veins: A Comparison of Structure and Function

Arteries and veins, two separate components of the vascular system, are different structurally and functionally. Although arteries carry oxygenated blood from the heart to the peripheral tissues at a high pressure, therefore requiring a more elastic and muscular structure, veins carry blood from the tissues back to the heart at a low pressure, providing capacitance, therefore requiring more distensible, less muscular walls. The structural similarities and differences are depicted in Figure 2.

Both artery and vein are composed of similar layers: the innermost layer or the tunica intima containing endothelial cells; the tunica media, which is largely composed of smooth muscle, elastin, and collagen; and the outermost tunica adventitia containing mainly fibroblasts, collagen, and elastin. Small blood vessels called *vasa vasorum* integrate into the adventitia of larger vessels, providing nutrients to the vascular wall itself.

Several characteristics, in addition to a different

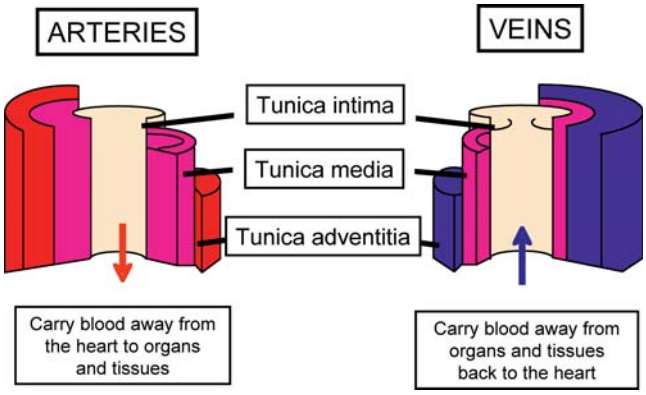

Figure 2. Schematic representation of the three layers of the blood vessel wall in an artery and a vein.

distribution and relative abundance of these layers, distinguish arteries from veins. The delineation of the three layers is more obvious in an artery compared to a vein. This is particularly illustrated when viewing the thoracic vena cava *versus* the thoracic aorta from the same rat (Fig. 3). The media of an artery, flanked by two elastic laminas, is typically thicker than that of a vein, whereas the elastic component of a vein is smaller compared to that of an artery. The greater relative contribution of the smooth muscle layer to the vascular wall thickness in arteries compared to veins can be appreciated in Figure 3, panel B. These differences are confirmed by immunohistochemical staining for α-actin, a smooth muscle marker. Larger veins possess venous valves on the luminal side of the wall, which help prevent backflow of blood. The cardiovascular system should not be envisioned as being abruptly split into the two components but rather as a gradual transition from the heart to the large elastic arteries, then smaller muscular arteries/arterioles to, finally, the capillary section, having just one endothelial layer, and then back from the peripheral tissues, through less muscular venules, to large capacitance veins possessing all the components of the vessel wall and back to the heart.

Due to these structural differences, there are also inherent differences in the contractility and synthetic properties of arteries and veins that can impact overall cardiovascular function. One can easily envision the mechanism by which arteries can affect blood pressure: by changing their tone through vasoconstriction or their structure through remodeling, they can increase total peripheral resistance, a major determinant of blood pressure. It is more difficult to picture a role for veins in the pathogenesis of hypertension. However, by accommodating 70% of circulated blood, veins can influence blood volume distribution and trigger adaptive remodeling from the arterial side that can drive a sustained increase in blood pressure (Fig. 4).

The magnitude of the contractile force developed by a vein in response to receptor-dependent and -independent agonists is less compared with that developed by an artery.

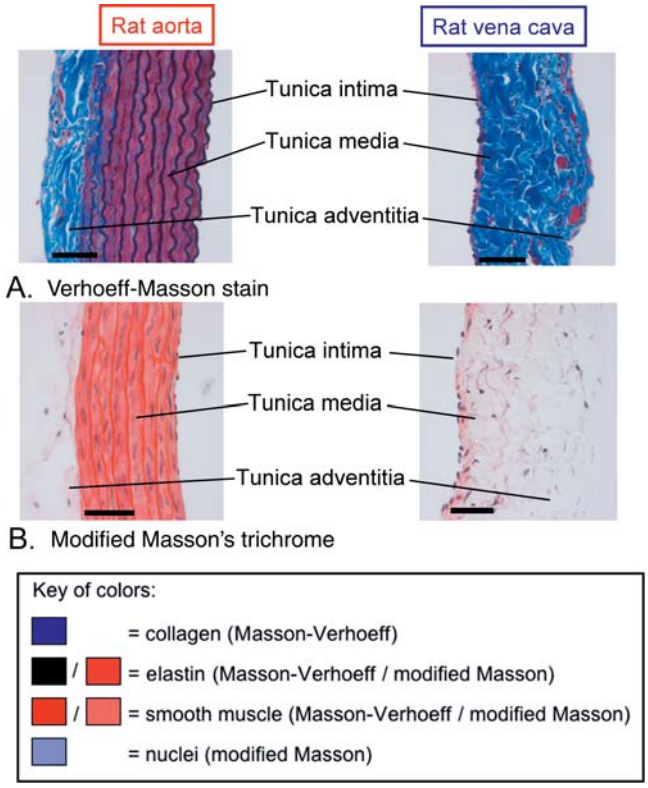

Figure 3. Magnification ×40. Bars indicate 100 μm. (A) Verhoeff-Masson histological staining of sections of rat aorta (left) and rat vena cava (right), highlighting the relative contribution of collagen (blue) and elastin (black) fibers to the composition of the blood vessel wall. (B) Modification of Masson's trichrome stain. Removing aniline blue staining of collagen reveals the smooth muscle layer (pink) on sections of rat aorta (left) and vena cava (right).

The time needed to reach half this maximal contraction, a measure of response speed, is shorter for a vein than for an artery (21). The capacity to relax in response to agonists that induce the production of endothelium-derived relaxant factors is decreased in veins compared to arteries. Similarly, specific differences exist in the contractile response of arteries and veins to a series of receptor-dependent agonists, the best studied of them being endothelin-1, a potent, though not selective, venoconstrictor.

Different properties of arterial and venous grafts used in bypass surgery, leading to different outcomes, have stimulated research on comparing these vessel types and the factors that influence their long-term patency. Venous smooth muscle cells appear to have a higher growth rate compared with their arterial counterparts, both in basal conditions (22) and in response to various mitogenic stimuli. Endothelium function is also different in veins compared to arteries. Venous endothelium produces less prostacyclin (23) and NO (24) than arterial endothelium, and its overall response to atherogenic stimuli is different.

These intrinsic properties ultimately reflect the difference in the gene expression pattern of arteries and veins. The most prominent differences in basal gene expression between arteries and veins can be seen in the signaling

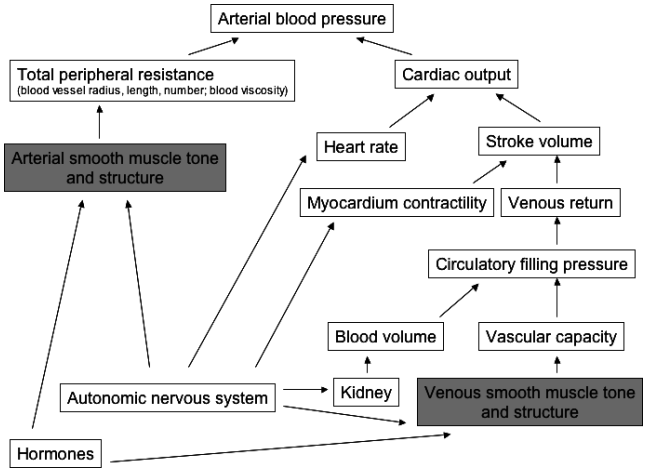

Figure 4. Diagram representing the physiology of blood pressure regulation. The shaded boxes highlight the role of arteries and veins, respectively.

molecules that regulate selective expression of Eph-B_4 in veins and ephrin-B_2 in arteries, creating a cell-cell interaction system that establishes arterial and venous identity in early angiogenesis. Other differences with significant potential for influencing specific vascular function have been identified through the use of gene arrays (25–28). Although most of these studies focus on the endothelium and the vascular smooth muscle layers of these blood vessels, fibroblasts and other components of the adventitia could also play important roles in vascular function and pathogenesis (29).

Because larger blood vessels of both kinds have different structural and functional properties compared with smaller ones, intuitively it makes sense that their potential contribution to vascular pathogenesis and dependence on ROS is also different.

Vascular ROS Metabolism

Few studies have compared basal ROS production in arteries and veins, and their conclusions were contradictory. Basal superoxide production, measured through nitroblue tetrazolium reduction to formazan, was increased in porcine venous grafts compared to arterial grafts (30). Using lucigenin-enhanced chemiluminescence, no difference in basal superoxide production was found in rings from human internal mammary artery (IMA) compared to human saphenous vein (HSV) (31). Basal hydrogen peroxide production was higher in rat vena cava compared to aorta (32). Unpublished data from our laboratory show increased superoxide production in rat veins compared to corresponding arteries as measured by lucigenin-enhanced chemiluminescence (inferior vena cava [VC] compared to thoracic aorta [Ao]: VC = 210 ± 42 % Ao; and mesenteric vein [MV] compared to mesenteric artery [MA]: MV = 267 ± 48 % MA). The increase in superoxide release, following the addition of the NOS inhibitor, L-NMMA, was greater in human arteries (IMA) compared to veins (HSV) (33). This suggests a greater basal NO production in arteries that contributes to the quenching of superoxide in comparison to veins. Accordingly, basal peroxynitrite formation was higher in IMA compared to HSV (33).

Besides this quantitative difference in ROS production between arteries and veins, there is probably also different utilization of ROS by arteries and veins, both in physiological cell signaling and in oxidative stress during vascular pathogenesis. For instance, hydrogen peroxide modulates vascular tone, acting as a contraction-inducing agent in some vascular beds and as a relaxant in others (34, 35). There is a greater contraction to H_2O_2 in veins compared to arteries, possibly reflecting a difference in K^+ channel activity and Ca^{2+} influx (34).

Below we will consider four main enzymes involved in vascular ROS metabolism: NADPH oxidase, xanthine oxidase, NOS, and superoxide dismutase. We will compare what is currently known about these enzymes in arteries and veins and highlight their potential implication in the pathology of cardiovascular diseases.

NADPH Oxidases. NADPH oxidases are perhaps the best studied enzymes involved in ROS production in the blood vessels (36). Several features of Nox enzymes expressed in blood vessels, that distinguish them from the generic phagocyte NADPH oxidase, have made researchers in the field collectively term them "the vascular oxidase." Compared with superoxide production from the phagocyte NADPH oxidase, vascular oxidase basal superoxide production is significantly lower (less than 1%; Ref. 37). Although phagocyte NADPH oxidase activity is primarily inducible, vascular oxidase has a constitutive activity that can be further increased by such agonists as angiotensin II (38). The cellular site of superoxide production by vascular oxidase also appears to be different: vascular oxidase–produced superoxide has been repeatedly detected intracellularly (36). Controversy still exists over the ability of vascular oxidase, in contrast to the phagocyte oxidase, to use NADH as an electron donor, in addition to NADPH. Finally, the physiological role of superoxide production by the blood vessel cells is distinct: instead of cytotoxic superoxide production as a defense mechanism against pathogens, ROS released by the vascular oxidase participate in cell signaling, consistent with their comparative low tissue levels.

Numerous reports on arterial NADPH oxidase subunits mRNA and protein expression vary in their results, probably because of the specific cell types (in cell culture studies), blood vessels types (in whole animal studies), or species in which they were tested. In arteries from humans and animals, Nox2, Nox4, and a very low level of Nox1 have been consistently found to be present both as mRNA and as protein (36). Besides $p40^{phox}$, the presence of which was detected only as mRNA in aorta from spontaneous hypertensive rats (SHR; Ref. 39), the other subunits, $p22^{phox}$, $p47^{phox}$, $p67^{phox}$ and Rac1, were all present in arteries as

mRNA and protein (39, 40). By comparison, venous NADPH subunit expression has been far less studied. Only one research group has compared ROS sources in human arteries and veins (Guzik *et al*.). Because human saphenous veins (HSV) and internal mammary arteries (IMA) from heterogeneous groups of patients undergoing coronary artery bypass graft surgery were used, conclusions should be drawn with caution. These studies have shown that $p22^{phox}$, $p47^{phox}$, and $p67^{phox}$ proteins, as well as $p22^{phox}$ and Nox2 mRNA, are present and increased in abundance in the HSV compared to the IMA. Nox4 mRNA expression was higher in the IMA, and Nox1 mRNA had similarly low expression in both types of vessels. In a study employing the use of specific chemical inhibitors, NADPH oxidase contribution to the total basal vascular superoxide production was found to be more important in the case of HSV compared to the IMA (31).

Cell culture studies have been performed in order to study the cell-type specific involvement of NADPH oxidase in vascular ROS production. Arterial endothelial cells express mRNA and protein of all subunits of the classical NADPH oxidase (37, 41), as well as the mRNA of Nox1 and Nox4 (36, 42, 43). Arterial endothelial Nox2 mRNA expression is 1%–3% that in leukocytes, which might explain the lower superoxide production by the vascular oxidase, in addition to lower levels of electron donors (NADH/NADPH) and specific regulation (36, 44). Arterial adventitial cells have an mRNA expression pattern similar to that of endothelial cells, confirmed at the protein level mostly by immunohistochemistry experiments (45, 46). Arterial vascular smooth muscle cells (VSMC) express Nox1, Nox4, and Nox5 mRNA; $p22^{phox}$ and $p47^{phox}$ mRNA; and protein but very low or sometimes undetectable levels of Nox2 and $p67^{phox}$. An exception is human resistance arterial VSMC, which possess a pattern of expression similar to that of the arterial endothelial cells (47). No study has yet investigated the NADPH oxidase system in venous SMC. Due to their wide availability and facile handling, human umbilical vein endothelial cell (HUVEC) culture has been frequently employed as a model for endothelial cells, but it must be appreciated that HUVECs are venous in nature and unique in function. HUVECs express the mRNA for all NADPH oxidase subunits (Nox2, $p22^{phox}$, $p47^{phox}$, and $p67^{phox}$), as well as Nox4 and very low levels of Nox1 (48).

Various endogenous and external stimuli modulate the NADPH oxidase subunits expression and/or activity. Expression of one or more of these subunits is upregulated in HUVEC culture in response to angiotensin II, ET-1, oxidized LDL, pulsatile shear stress, and PMA (36, 49, 50). They are conversely downregulated by treatment with statins, PPAR agonists, or estradiol (36, 51). Angiotensin II, TGFβ, TNFα, serum, PDGF, $PGF_{2\alpha}$, PMA, and LDL upregulated various NADPH oxidase subunits expression in the case of cultured arterial smooth muscle cell (36, 52). Long-term treatment with AT_1 receptor blockers downregulated Nox2 mRNA expression in human artery biopsies (38).

In atherosclerosis, increased arterial intracellular superoxide production is observed (36, 49, 53). This production is further increased by treatment with NADH or NADPH, suggesting that vascular oxidase might be its main source (46, 54). Additionally, expression of $p22^{phox}$, $p67^{phox}$, $p47^{phox}$, Nox2, Nox1, and Nox4 was increased in atherosclerotic human or animal arteries (36, 49). Atherosclerotic lesions, superoxide levels, and VSMC proliferation of $apoE^{-/-}$ mice were reduced when crossed with $p47^{phox\text{-}/\text{-}}$ mice, regardless of diet (55).

In hypertension, the already increased vascular superoxide generation (arterial and venous) is further increased with NADH or NADPH and lowered through treatment with the NADPH oxidase inhibitor apocynin (56, 57). Aortic mRNA expression of $p22^{phox}$ is increased in the DOCA-salt (57) and SHR (58) models of hypertension. The angiotensin II infusion model has increased expression of all NADPH oxidase subunits (59). In the same model, inhibition of NADPH oxidase activity by treatment with gp91 ds-*tat*, a chimeric peptide that blocks the association of $p47^{phox}$ with Nox2, leads to a decrease in superoxide production and an attenuation of the AngII-induced blood pressure elevation (60).

Xanthine Oxidase. Xanthine oxidase expression in blood vessels has been difficult to prove. Human small vessel arterial endothelium showed XO immunoreactivity (61), and XO mRNA was identified in cultured rat pulmonary arterial endothelial cells (62). Moreover, measurable XO activity has been detected in various disease states in arteries or cultured endothelial cells. The addition of xanthine/xanthine oxidase or uric acid in cell culture modifies cell growth and proliferation (63, 64). However, XO from the circulation can also bind to endothelial cells *via* heparin-binding sites (65), and its presence has not been detected in either arterial or venous VSMC and adventitia. The controversy is therefore still open about whether XO is functional in the blood vessel or is acquired through association with blood. When comparing the relative contribution of enzymatic sources in human arteries and veins by specific chemical inhibition, XO appeared to be a greater superoxide source in the IMA compared to the HSV (31).

Increased arterial XO activity was observed in atherosclerosis (66). Renal XO activity was increased in the SHR during the development of hypertension (67). The same model exhibited higher mesenteric artery XO activity (68). Similarly, mesenteric artery XO activity was increased in the DOCA-salt model of hypertension (69). Treatment with oxypurinol, the XO inhibitor, decreased the blood pressure of SHR, whereas it had no effect on the blood pressure of normal rats (70).

NOS. The classical view on NOS isoforms is summarized in their alternative names: the neuronal NOS (NOS1) and the endothelial NOS (NOS3), with constitutive

expression in neurons and endothelial cells, respectively, and the inducible NOS (NOS2), the only calcium-independent, transcriptionally regulated isoform found in macrophages (71). This paradigm has changed considerably in recent years: all three isoforms have been identified in arteries and veins, as well as in HUVEC culture (72–76). Furthermore, red blood cells appear to express a membrane-associated NOS3, capable of modulating vascular tone (77). No study has yet compared arteries and veins in terms of NOS isoforms expression.

Normal endothelial function, crucial in maintaining cardiovascular homeostasis, depends on normal NOS functioning, among other things. A reduction in the arterial endothelium-dependent vascular relaxation, defined as endothelial dysfunction, has been documented in atherosclerosis and hypertension. Besides decreased NO bioavailability, a malfunctioning NOS can also influence vascular function by becoming uncoupled, in the absence of L-arginine or BH_4, with consecutive production of superoxide and potentially peroxynitrite. Additional uncoupling can occur by oxidation of the existent BH_4 (78). The role of BH_4 depletion in NOS uncoupling and hypertension development is illustrated by the fact that treatment of DOCA-salt hypertensive mice with BH_4 leads, by recoupling of NOS, to lowering of blood pressure (78). Uncoupling of NOS does not occur in $p47^{phox-/-}$ mice, supporting the idea that NADPH oxidases are required for BH_4 oxidation. There is no clear evidence indicating that NOS uncoupling follows the same rules in veins as it does in arteries.

SODs. The blood vessel wall of both arteries and veins expresses all three SODs. The cytosolic CuZnSOD has ubiquitous and high expression throughout the vascular layers. Mitochondrial MnSOD is relatively less expressed compared with CuZnSOD and EC-SOD but is also ubiquitous (16). Extracellular SOD, produced largely by VSMC, is localized between arterial intima and media (79) and is thought to contribute substantially to the total SOD activity in the vasculature. In addition to its primary and important role in scavenging extracellular superoxide, EC-SOD may also be expressed intracellularly and translocated to the nucleus *via* its heparin-binding domain, which could function as a nuclear localization signal (80). Rats have lower vascular EC-SOD levels, compared with other species, due to a change in the amino acid sequence of the protein that leads to lower heparin binding, potentially influencing the results of SOD expression studies performed in this species. No difference has been found between human arteries (IMA) and veins (HSV) in terms of their CuZnSOD and MnSOD protein expression and activity (81).

Arterial expression and/or activity of CuZnSOD and MnSOD increases in several animal models of hypertension (82, 83), as well as in the initial phases of atherosclerosis, but is decreased in the later stages of this disease (84). A paradox is observed in redox regulation of MnSOD. Although upregulated in oxidative stress by redox sensitive transcription factors (15), the protein itself can be tyrosine nitrated and thus inactivated by peroxynitrite (85). EC-SOD–deficient mice had higher blood pressures in two hypertension models compared with the wild-type animals (86). Conversely, overexpression of EC-SOD improved vascular function in hypertensive animals (87).

Oxidative Stress and Disease

Increased ROS levels in the cell, resulting from their overwhelming generation or impaired destruction, have a substantial impact on normal cellular function. This imbalance between prooxidant and antioxidant factors, defined as oxidative stress, can affect cellular homeostasis either through direct oxidative damage of basic cellular components (proteins, lipids, and nucleic acids) or through the activation of various redox-sensitive signaling pathways, leading to defective cellular function, aging, disease, or apoptosis. ROS involvement in cellular signaling has been reviewed extensively elsewhere (1). In summary, a series of major signaling pathways, such as MAPK, PI3K/Akt, NF-κB, ERK, JNK, p53, and the heat shock response, can potentially be activated in response to ROS or oxidative stress.

Oxidative stress can modulate vascular function through direct oxidative damage; endothelial dysfunction; decreased NO bioavailability; impaired contractility; platelet aggregation; and ROS-mediated inflammation, proliferation, and remodeling (66, 88–92). However, the differences in the effects of oxidative stress on arterial and venous function are only beginning to be elucidated.

The presence of increased markers of oxidative stress (peroxidized lipids, oxidized proteins, increased GSSG, 8-oxo-guanine, DNA breaks, etc.) has been identified in many pathophysiological situations. However, in most cases, establishing whether oxidative stress plays a causal role or is a mere reflection of the effects of the disease process itself on cellular function has proved to be a difficult task.

Atherosclerosis. ROS appear to be involved in the pathophysiological events leading to atherosclerosis, the underlying cause of most cardiovascular diseases. Common risk factors for atherosclerosis, such as hypertension, aging, smoking, diabetes, and hypercholesterolemia, as well as local oscillatory shear, all result in increased ROS (53). ROS, in turn, contribute to atherogenesis by generating oxidized and highly oxidized LDLs, modulating adhesion molecules and chemotactic factors expression, VSMC proliferation and migration, endothelial cell apoptosis, and MMP activation with consecutive remodeling or plaque rupture. In atherosclerosis, direct and indirect evidence supports increased ROS production (36) (especially superoxide production in the neointima, the potentially important role played by extracellular superoxide being still under investigation), increased expression and/or activity of NADPH oxidase subunits and Nox isoforms (36), increased xanthine oxidase activity (66), uncoupling of NOS3,

increased lipoxygenase activity, increased oxidative damage of mitochondrial DNA, increased myeloperoxidase activity, and reduced EC-SOD and glutathione peroxidase activity. (16, 49, 53, 88, 91).

Although atherosclerosis is essentially an arterial disease, when exposed to circulatory conditions similar to those of an artery, vein grafts can also undergo atherosclerotic processes. These, together with thrombosis and intimal hyperplasia, are the main causes of the failure of venous grafts (vein graft disease) (23).

Hypertension. A great body of evidence supports the idea that ROS are involved in the pathogenesis of hypertension. Increased markers of oxidative stress are found in human hypertensive subjects, as well as in various animal models of hypertension (93–97). Treatment of these models with ROS scavengers (95, 96), inhibitors of NADPH oxidase (57, 60), inhibitors of xanthine oxidase (70), SOD mimetics, BH_4 (97) or targeted gene delivery of SOD (70), or NADPH oxidase inhibitors (98, 99) normalizes blood pressure or prevents the development of hypertension and in some cases improves vascular and renal function. Furthermore, genetic deficiency in ROS-generating enzymes protects some animals from experimental hypertension (100), whereas lack of antioxidant capacity causes increased hypertension in others (16, 93). Increased NADPH oxidase and XO expression or activity is also observed in some experimental models of hypertension (67–69).

Future Perspectives

The field of ROS and vascular pathogenesis has expanded considerably in recent years. Use of genetically engineered animals, as well as targeted gene delivery and cell culture studies, has greatly benefited our knowledge of the role of oxidative stress in vascular disease. However, a better understanding of the roles of ROS-mediated signaling in normal vascular function as well as in disease is necessary for developing better therapeutic tools for oxidative stress-related pathology. As much as we would like to be able to present a comprehensive diagram of all the differences in ROS metabolism between arteries and veins, as well as their implications on vascular function and disease, the knowledge today is simply insufficient to do so. Some of the points we envision being investigated in the future are as follows:

- Are arteries and veins exposed to the same ROS levels?
- If not, what are the molecules and mechanisms responsible for a difference, and what is their relative importance in both vessel types?
- How do these mechanisms change in pathophysiological conditions?
- What are the exact places where ROS intervene in vascular function? Is this different in veins and arteries?
- How much of the vascular ROS is necessary for signaling and vascular function, and where does oxidative stress begin?
- What is the subcellular picture of ROS production, movement, and action? Can superoxide cross membranes (Cl^- channel)? What is the role of extracellular ROS?
- What is the time course of ROS involvement in vascular disease? Are ROS a cause or an effect?
- How can we use this knowledge to develop new therapeutic tools for vascular oxidative stress?

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