ANTIOXIDANTS & REDOX SIGNALING
Volume 11, Number 7, 2009

DOI: 10.1089/ars.2008.2408

Forum Review Article

Shear Stress, Reactive Oxygen Species, and Arterial Structure and Function

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Abstract

Shear stress is well known to be a key factor in the regulation of small-artery tone and structure. Although nitric oxide is a major endothelium-derived factor involved in short- and long-term regulation of vascular caliber, it is clear that other mechanisms also can be involved. This review discusses the evidence for endothelium-derived reactive oxygen species (ROS) as mediators for shear-dependent arterial tone and remodeling. The work focuses on resistance vessels, because their caliber determines local perfusion. However, work on large vessels is included where needed. Attention is given to the shear-stress levels and profiles that exist in the arterial system and the differential effects of steady and oscillating shear on NO and ROS production. We furthermore address the relation between microvascular tone and remodeling and the effect of ROS and inflammation on the activity of remodeling enzymes such as matrix metalloproteinases and transglutaminases. We conclude that future work should address the role of H_2O_2 as an endothelium-derived factor mediating tone and influencing structure of small arteries over the long term. *Antioxid. Redox Signal.* 11, 1699–1709.

Introduction

All blood vessels are equipped with a single layer of endothelial cells. Since the pioneering work of Furchgott (25) and others, several decades ago, it has become increasingly clear that these cells play a central role in the sensing of the chemical and mechanical environment of blood vessels and the subsequent adaptation in function and structure of the blood vessels. In this review, we address the primary mechanical stimulus acting on the endothelial cells: shear stress associated with the blood flow. Based on observations on, among others, endothelial cell morphology, flow-dependent dilation, and flow-dependent remodeling, it is clear that endothelial cells have shear-stress sensors (Fig. 1). Yet an ongoing discussion continues on the nature of such sensors and the downstream signaling pathways. Reactive oxygen species (ROS) come into play here as possible mediators of shear-induced responses, in addition to, competing with, or replacing nitric oxide (NO). Shear-induced ROS and NO both influence smooth muscle cell tone and vascular structure, also a causal relation also exists between tone and vascular remodeling. Vascular tone and remodeling in turn influence the sensed shear-stress profile, allowing regulation of shear stress. Each of the elements in this process is influenced by background oxidative stress that occurs in hypertension, diabetes, and many other diseases and conditions, causing impairment of endothelial responses to shear stress.

The purpose of this review is to give an overview of the relations between shear-stress sensing, production of ROS, and functional and structural adaptation of blood vessels. We focus on the arterial system and, in particular, the resistance arteries, addressing flow-dependent dilation and remodeling as key events determining microvascular resistance and flow distribution. However, we include studies on large vessels where needed. The majority of the review addresses basic mechanisms from a physiological point of view, ignoring the influence of background oxidative stress and with a limited level of detail on the cell-signaling mechanisms [see (46, 57, 58)]. The role of ROS in vascular remodeling associated with specifically pulmonary hypertension was previously reviewed in this journal (110). Additional reviews in this issue by Morawietz and Haendeler address shear-stress sensing and ROS production in atherosclerosis and vascular aging, respectively.

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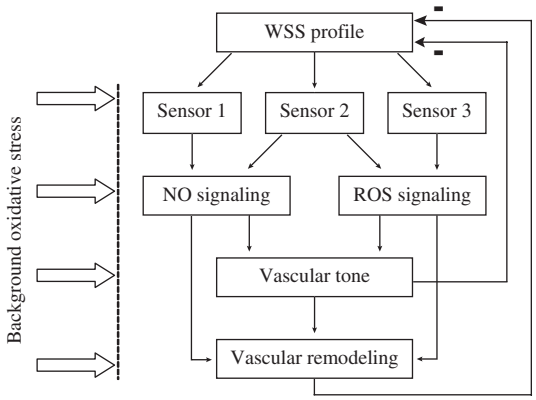

FIG. 1. Overview of interactions involved in structural adaptation to wall shear stress, showing the involvement of oxidative stress and ROS signaling.

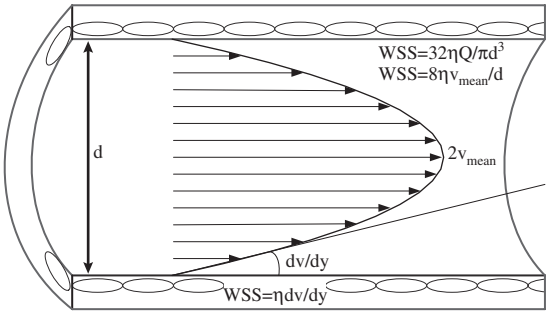

FIG. 2. Relation between flow (Q), mean and maximal velocity (v_{mean} and v_{max}, $v_{max} = 2\ v_{mean}$), and wall shear stress (WSS) under the assumption of Poiseuille flow with constant viscosity (η).

Wall Shear Stress in the Arterial Circulation

Wall shear stress (WSS) is the drag force per unit area acting on the endothelial cells. Wall shear stress is caused by the local velocity gradient (the shear rate) and is calculated as the product of shear rate and viscosity. The wall shear stress in straight vessels away from branch points is frequently estimated from bulk flow or mean velocity, by using ultrasound or MRI in large arteries and *in vivo* microscopy in small experimental animal vessels. Figure 2 shows these relations for a fully developed laminar velocity profile with constant viscosity. Estimation of WSS from these formulas should be treated with care for several reasons: measurement errors in diameter become 3 times larger when calculating WSS. Also, because of the nonnewtonian properties of blood, the velocity profile is flattened, and wall shear rate will be higher than estimated from these equations, an error that is offset by overestimation of the viscosity near the wall in small vessels. Furthermore, entrance effects cannot always be ignored, and, especially in larger vessels, both flow and diameter are unsteady. A recent detailed discussion of these issues was given by Reneman (86). Flow patterns around branching points in large vessels are extremely complex; estimation of the local WSS patterns in such areas requires computational fluid-dynamics analysis. Here, the temporal and spatial variations in WSS are considered to determine the local sensitivity for atherosclerotic plaque formation. As an example, in the carotid bifurcation, WSS is high at the flow divider and low in the bulb of the internal carotid artery, where flow reversal and recirculation occur (frequently erroneously referred to as "turbulence"). This area is known to be prone to plaque formation.

Despite its limited applicability and concerns, WSS as estimated from the Poiseuille relation is useful for understanding vascular adaptation: if the flow increases, WSS initially increases proportionally. Shear sensing and signaling then induce an increase in radius by dilation and remodeling, which will then restore WSS toward its original level. Thus, WSS is considered to be a regulated quantity, fundamental to the matching of arterial caliber to the carried flow. The third-power relation between WSS and radius would, for a constant "set point" for such regulation (*i.e.*, for equal properties of the WSS sensor and signaling pathways along the arterial bed), predict that a third-power relation holds between flow and diameter throughout the arterial bed, and that arterial branching also is characterized by such a third-power relation: $d_0^3 = d_1^3 + d_2^3$, with d_0 the radius of the mother vessel and d_1 and d_2 those of the daughter vessels. These third-power relations are commonly referred to as Murray's law (74). It should be stressed that these relations are only approximately true for the smaller arteries; for the larger arteries, branching rather fulfills a second-power relation, thus reflecting constant mean flow velocity rather than constant shear stress.

Considering that WSS is regulated, an increased WSS indicates a changed balance between the shear sensor and opposing stimuli for vasoconstriction or inward remodeling. As a trivial example, arteries generally have a much smaller diameter than their paired veins, although carrying, on average, the same flow. Arterial WSS therefore has to be larger than the venous WSS. It has been suggested that local pressure is a critical determinant of the set point for WSS (83). This is relevant for hypertension: many hypertensive disorders, once established, are characterized by little change in cardiac output. However, the flow is carried by smaller vessels (inward remodeling) or less-parallel pathways (rarefaction), indicating that the shear stress is higher in at least part of the network. This dysbalance may stem from impaired shear-stress sensing (for instance, because of oxidative stress induced by high pressure), the increased presence of vasoconstrictive peptides such as angiotensin II and endothelin, or a combination of these effects.

Because of the complex estimation of local WSS, the deviations form the Murray concept, temporal variations due to metabolic flow regulation, and the interference of pressure, oxidative stress, and local vasoactive agents with sensing of shear stress, it is not possible to derive a "true" or "healthy" single value for large or small vessels. Reneman (86) provides extensive tables for WSS reported in large vessels. In short, in common carotid arteries, mean WSS is estimated to be 1.3 Pa (or 13 dyne/cm^2) and is independent of age and gender. Peak systolic WSS is $\sim$3–4 Pa in young individuals and appears to decrease slightly with age (toward 2.5 Pa at 50–59 years). Mean WSS in femoral and brachial arteries is lower, $\sim$0.5 Pa, whereas peak values are similar, 2.5–4 Pa, with no apparent effect of age. It should be stressed that large-artery WSS values are much higher in small experimental animals as

compared with humans. Greve *et al.* (27) compared WSS derived from MRI-based velocity measurements in the infrarenal aorta. Mean WSS was 8.8 and 7.0 Pa in mice and rats *versus* 0.5 Pa in humans. Suo *et al.* (94) performed a computational fluid-dynamics analysis of mouse thoracic aorta WSS, demonstrating mean and peak values exceeding 20 and 60 Pa on the outer curvature of the aortic arch. Such studies also indicate that the velocity and shear-stress profiles in rodents are not simply "scaled-down" versions of those in humans. Thus, aortic Reynolds and Womersley numbers are much lower. Therefore, the effect of pulsatility on the flow profile is more limited, and turbulence is not likely to occur in the mouse aorta. Evidently, because of the much higher heart rate, temporal variations in WSS occur in a different frequency domain.

WSS in smaller arteries is less oscillating, and steep spatial gradients are less likely to occur, yet dynamic shear profiles do develop, and these may influence flow-induced effects through oxidative signaling. An example is the coronary circulation, in which the contracting myocardium rhythmically squeezes the vessels, in particular, the sub-endocardium, causing flow reversal in a substantial part of the vessels during systole (39). Mean WSS in the resistance vessels can be directly determined from the pressure gradient and the geometry. This way, WSS was reported to be 4.7 Pa in the cat mesentery (62). WSS, as estimated from velocity profiles and viscosity in rabbit mesenteric arterioles, ranged between 0.5 and 5 Pa (87). In the rat mesentery, Pries (83) reported WSS to decline from 10 Pa in 200-μm vessels to 1 Pa in the precapillary arterioles. Quantitative data on human microvascular shear stress seem lacking, despite the availability of clinically applicable microvascular imaging technology (80).

Overall, a need still exists for quantitative estimates of WSS in many organs and species. Species and vessel-size differences should be taken into account when attempting to apply "physiological" shear to, *e.g.*, endothelial cell cultures. The extreme case here is formed by HUVECs, which *in vivo* experience shear stresses in the order of only 0.5 Pa at term, as calculated from data provided by Link *et al.* (61).

Shear Stress–Induced ROS Signaling

In a study using electron paramagnetic resonance spectroscopy, Laurindo *et al.* (55) observed ROS generation after flow changes *in vitro* and *in vivo*. Since then, it has been well established that WSS causes the generation of not only NO but also ROS in isolated endothelial cells as well as in intact vessels and tissue. Several excellent recent reviews cover the cellular mechanisms of ROS generation (10, 34). A recent review by Ungvari *et al.* (106) in this journal discusses microvascular NADPH oxidase signaling after mechanical stimulation.

O_2^- and NO react at a diffusion-limited rate to form the peroxinitrite anion ($ONOO^-$); the role of this reaction in the bioavailability of NO and the biologic actions of $ONOO^-$ and downstream products also are discussed elsewhere (76). Both radicals and their reaction product peroxinitrite have a broad spectrum of biologic effects ultimately influencing vascular structure. The effects of various shear profiles are generally studied on cultured ECs. Although the background for such studies is frequently atherogenesis in disturbed-flow regions, effects of dynamic shear profiles are also relevant for microvascular structure: microvascular flow and shear vary because of rhythmic vasoactivity and during occlusion and reperfusion. Microvascular flow reversal is common in arcading vessels and during compression by surrounding tissue, notably during systole in coronary arterioles (101).

In cells cultured in parallel-plate or cone-and-plate shear chambers, oscillatory shear without a net forward component induces production of O_2^-. In a seminal study on isolated cells under shear, De Keulenaer *et al.* (19) showed that continuous oscillatory shear in HUVECs results in increased O_2^- production through the activity of NADPH oxidase. With bovine (41) and murine (42) aortic EC, Hwang *et al.* demonstrated an upregulation of NADPH subunits after several hours of oscillatory shear, whereas pulsatile (*i.e.*, with a net forward component) or laminar shear caused a downregulation and reduced O_2^- production. Other enzymes implicated in O_2^- generation by oscillating shear include the balance of xanthine oxidase and dehydrogenase (70, 71). O_2^- production by oscillatory shear may be counteracted by increased expression and activation of eNOS *via* H_2O_2 (13), but a net production of ROS appears to remain after prolonged oscillatory shear. Moreover, the activity of both O_2^- and NO-generating enzymes results in protein nitrosylation through $ONOO^-$ formation, as found in areas at risk in large vessels (40).

Not only oscillating flow but also onset of steady shear appears to induce ROS (8, 19). However, in the course of several hours, the effects of flow onset are compensated by antioxidant defenses, such as increased Cu/Zn SOD expression (19, 43), upregulation and activation of eNOS and subsequent downregulation, by NO, of NADPH oxidase subunits (21), increased expression of glutathione peroxidase (95), and upregulation of extracellular SOD by the smooth muscle cells (93). Thus, during continuous steady shear applied to cultured cells, ROS are produced, but their bioavailability is reduced over time and is overwhelmed by NO availability. Effects of cessation of flow on vascular responses are relevant for studies on ischemia. However, limited information is available on effects of flow cessation on flow-adapted cultured ECs. Fisher *et al.* (24) demonstrate NADPH oxidase activation and production of ROS after acute shear stop in pulmonary microvascular ECs.

Although these isolated cell studies have provided much information on shear-induced ROS signaling, questions and concerns remain. As stated, many studies use HUVECs, cells normally experiencing a very low shear. Further concerns include the influence of the extracellular matrix composition on shear responses and the possible alterations of the shear-sensor behavior in the cultured cells.

Sensors for steady and oscillating shear stress

The profoundly different responses to steady and oscillating flow might be explained by downstream signaling events in the EC. Alternatively, specific shear-stress sensors for both shear patterns may exist, with oscillating-flow sensors predominantly linked to ROS-generating pathways. This is a hypothetic view, but some arguments support it: one of the quickest responses of ECs to shear is through activation of ion channels. After onset of shear, inward rectifying or ATP-dependent K^+ channels are activated, leading to endothelial hyperpolarization. Barakat *et al.* (9) proposed that these channels provide ECs with the ability to resolve components of a complex flow signal and hence distinguish among different

types of flow. Indeed, this group (60) found differences in the response of ECs to laminar *versus* oscillatory flow: a hyperpolarization followed by depolarization compared with only minimal depolarization during oscillatory flow. Qiu (84) studied this in coronary ECs that are normally subjected to pulsatile flow because of cardiac compression. They observed the opposite response: a more sustained hyperpolarization during pulsatile flow, related to opening of K_{Ca} channels. These coronary arteries also showed more dilation to pulsatile flow than to steady flow. Irrespective of the contrasting data, such effects of flow dynamics would allow ion channels to form the mechanism by which ECs are able to distinguish between different shear-stress patterns (60). The shear-profile–dependent changes in membrane potential in turn would lead to profile-dependent transients in intracellular calcium. Alternatively, direct stimulation of calcium-permeable ion channels by shear stress could increase intracellular calcium. The transient receptor potential (TRP) channel TRPV4 has been implicated in such flow sensing (48, 52); other possibly relevant TRP channels are TRPC3 and C4, associated with oxidative stress (52). The increase in calcium after steady flow would activate eNOS (1, 59, 60, 109) (although calcium-independent flow-induced activation of eNOS has been described). During oscillatory flow in noncoronary cells, a hyperpolarization phase is absent (60), thus leading to less eNOS activation and a possible dominance of ROS-generating enzymes, whose activity is generally calcium independent.

It should be said that this is a hypothetical view that requires more studies on the relation between shear patterns, membrane potential, intracellular calcium, and NO and ROS generation. To complicate this further, a calcium-dependent NADPH oxidase, NOX5, has been described (98). NOX5 was recently shown to be functional in endothelial cells, where the calcium ionophore ionomycin enhanced ROS production by NOX5β (11). Endothelial membrane depolarization and subsequent ROS generation also was observed after cessation of flow (16, 68) and was related to the closure of K_{ATP} channels.

Alternative to shear-profile–dependent opening of ion channels, flow sensors might adapt their physical embedding in the endothelial cell after a change of shear, inducing oxidative signaling while adaptation is not yet complete (Fig. 3). Three of the many suggested flow sensors that may be relevant here are the endothelial cytoskeleton, the endothelial luminal surface layer (the glycocalyx), and the cilia.

The cytoskeleton has been proposed as a mechanotransducer that is able to sense shear *via* connections to luminal, basal, and intercellular surfaces (38). In response to laminar shear stress, endothelial cells elongate and align in the direction of the flow. Hereby, the constituents of the cytoskeleton are remodeled into the direction of the flow to reduce the load on the cell surface or to resist this load (67, 69). The morphology of an individual cell influences the shear-stress pattern acting on its surface, and the cytoskeletal reorganization may serve to minimize spatial shear-stress gradients (17). Helmke *et al*. (38) investigated the local redistribution of forces by intermediate filaments within the cell with the help of green fluorescent protein (GFP)-coupled vimentin. After onset of shear stress, the tension of the cytoskeleton is altered, resulting in the redistribution of intracellular forces through displacement of intermediate filaments (37). A larger displacement of the intermediate filaments in downstream regions compared with upstream regions was observed, together with an increase in displacement of the filaments above the nucleus instead of the basal side of the cell (38). These mechanical-force redistributions in shear-stress–adapted cells may lead to different force-transduction pathways and underlying signaling pathways, leading to a positive NO/O_2^- balance. Endothelial cells exposed to oscillatory shear-stress patterns do not display cytoskeletal remodeling, and the cells remain polygonal, as seen in cells in static cultures (36). In these cells, the lack of adaptation of the cytoskeleton in response to oscillatory shear stress, compared with laminar shear stress, may result in different mechanotransduction and signaling pathways, which might lead to an increased production of ROS (Fig. 3).

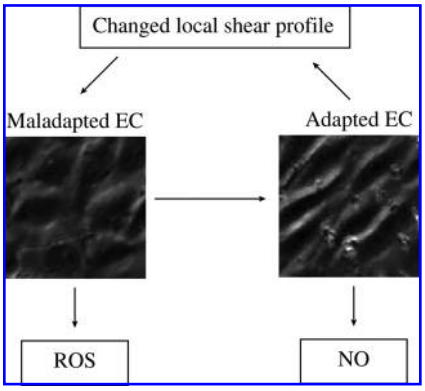

FIG. 3. Adaptation of ECs to a changed local shear profile. Maladapted ECs are suggested to generate mainly ROS. Adaptation involves changes in gene expression and cell signaling, but also changes in shape, leading to restoration of the local shear profile and the associated forces in the cytoskeleton, and generation of mainly NO.

The endothelial glycocalyx consists of glycoproteins, sulfated proteoglycans with their associated glycosaminoglycan side chains (GAGs). Located at the luminal side of the endothelial cells, the glycocalyx might well act as a mechanosensor (77). Pahakis *et al*. (77) showed that depletion of several glycoproteins, including sialic acids, heparin sulfate, and hyaluronic sulfate, blocks the endothelial production of NO under shear stress. However, these enzymes did not influence the production of prostacyclin under the same circumstances (77). The functionality of the glycocalyx as a shear-stress sensor might be influenced by the production of ROS. One of the glycocalyx components, hyaluronan, is known to be sensitive to ROS, because hyaluronan was found to react to ROS by loss of GAG residues (73).

Recently, another shear-stress sensor was proposed: cilia. Cilia, built up by microtubule bundles, are connected to the cortical actin cytoskeleton, which can act as a mechanotransducer between cilium and cell (108). Cilia also were found to be present on human umbilical vein endothelial cells (HUVECs). Iomini *et al*. (45) investigated the response of cilia to laminar shear stress (1.5 Pa) and found all cilia present to be disassembled. A possible explanation was that this disassembly was the result of a too-high shear stress applied on the HUVECs, normally not exposed to shear stresses exceeding 0.5 Pa. Data on cilia distribution on the endocardial endothelium in the developing chicken embryo support this (108). Thus, cilia may act as shear sensors in specifically low and disturbed shear-stress regimens. The downstream events after

stimulation of cilia by shear stress, including the possible generation of NO and ROS, remain to be elucidated.

Role of ROS in Shear-Dependent Vascular Tone

Effects of shear-induced ROS on small-artery structure are not easily studied in experimental settings. On the basis of the observed coupling between tone and remodeling (7), however, studying the involvement of ROS in shear-induced changes in tone could provide insight into the long-term structural effects. Two aspects are to be considered here. First, but not discussed here, background oxidative stress, as occurs in aging and diseased vessels, may add to endothelial dysfunction by interfering with endothelium-dependent dilation (*e.g.*, through quenching of NO). Second, ROS and notably H_2O_2 may form part of the endothelium-derived factors mediating vasodilation. This has been established for pharmacologically induced endothelium-dependent dilation in various isolated-vessel models (75, 90, 96). Drouin *et al*. (20) recently suggested that H_2O_2-dependent dilation in mouse cerebral arteries appears to be a physiologic eNOS-derived mechanism. Irrespective of the enzymatic source, H_2O_2 effects may involve both hyperpolarization of the SMC and alternative mechanisms for vasodilation. This involvement is opposite to the role of ROS in calcium signaling within the smooth muscle cells after the vasoconstrictor stimulation discussed earlier in this journal (100).

A few studies address H_2O_2 as a mediator in shear-induced dilation. Thus, Koller and Bagi (49) simulated reactive hyperemia in isolated coronary arterioles and found the response to this combination of pressure and flow changes to be mediated by both NO and H_2O_2. Studies on coronary arterioles obtained from patients with coronary artery disease (CAD) have pointed at H_2O_2 as the dominant factor released by the endothelium (72). Subsequent work has shown that the mitochondria constitute a major source (32, 64). Although this dominance might be specific for human vessels, more likely the shift from NO to H_2O_2 is caused by the CAD.

With human arterioles isolated from visceral fat, Phillips *et al*. (81) demonstrated that shear-induced dilation is NO dependent in the absence of known CAD. However, in the presence of CAD, flow induces production of O_2^- in these peripheral vessels, which is dismutated to H_2O_2. For obvious reasons, such a comparison of function in coronary arterioles of healthy *versus* CAD patients would be more difficult to perform.

Induction of O_2^- release by shear stress might quench simultaneously released NO. Sorop *et al*. (92) tested the effect of various flow patterns on flow-dependent dilation in coronary arterioles, vessels that are normally exposed to highly dynamic flow because of the compressing effect of the myocardium. These authors found that sinusoidal modulation of the flow did not affect the degree of dilation, even when flow reversal occurred. This indicated that only the mean flow is of relevance. Pure oscillating shear without a net forward component did not induce flow-dependent dilation (Fig. 4). However, in the presence of superoxide dismutase (SOD), such flow readily induced vasodilation, indicating that in these vessels, oscillating flow results in O_2^- generation that quenches the simultaneously released NO. Steady and oscillating shear increased fluorescence after loading by dihydroethidium at the same rate (Fig. 5), suggesting that the oscillating shear resulted in less NO generation rather than more O_2^- production. It should be said that in several cases, we observed a decrease in fluorescence after stopping flow that seems inconsistent with the mechanism of action of this indicator, irreversible binding of the oxidized form tot DNA (92). It is now clear that ethidium is not the only fluorescent product, and it has been suggested that fluorescence of 2-hydroxy-ethidium may be more specific for O_2^- generation (23). Liu *et al*. (63) observed that onset of steady flow (1–7 dyne/cm^2, 5 min) in porcine pulmonary vessels results in vasoconstriction that is converted to vasodilation in the presence of SOD, again indicating a competition between O_2^- and NO (63).

O_2^- production after a change in shear seems very rapid, as shown by the effect of SOD on the delay time of flow-induced dilation. The duration of the delay after starting flow ranges between 5 s in arterioles smaller than 100 μm (51) to around 40 s in large-conduit arteries. Sorop *et al*. (92) showed that the lag time of NO-mediated flow-dependent vasodilation in coronary arterioles was reduced from 23 to 14 s in the presence of SOD, indicating that O_2^- generation dominates over NO in the first seconds.

Taken together, available data suggest that shear induces NO and O_2^- in small arteries, the latter being dismutated to H_2O_2 and contributing to vasodilation. Early events after rapidly changing shear stress result in NO quenching and suppression of vasodilation. Longer-lasting steady flow leads to NO and H_2O_2 production in a balance that depends, among others, on pathologic conditions. Whereas the short-term effect of both NO and H_2O_2 is vasodilation, a shift from NO to H_2O_2 is likely to have consequences for regulation of vascular structure, because both mediators have partially opposite effects on growth-related signaling cascades (3).

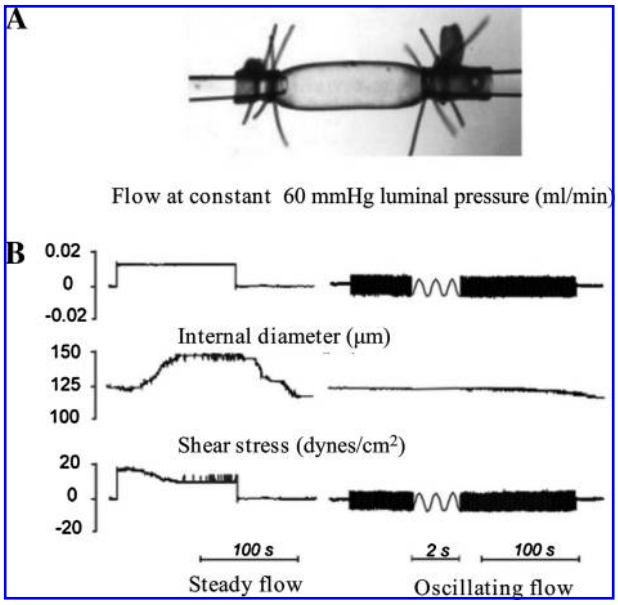

FIG. 4. (A) Example of an isolated small-artery setup, allowing independent control of pressure and flow by manipulation of left and right cannula pressures. (**B**) Isolated, cannulated small porcine coronary arteries show flow-dependent dilation to steady (*left*) but no oscillating flow (*right*). [Data from Sorop *et al*. (92)].

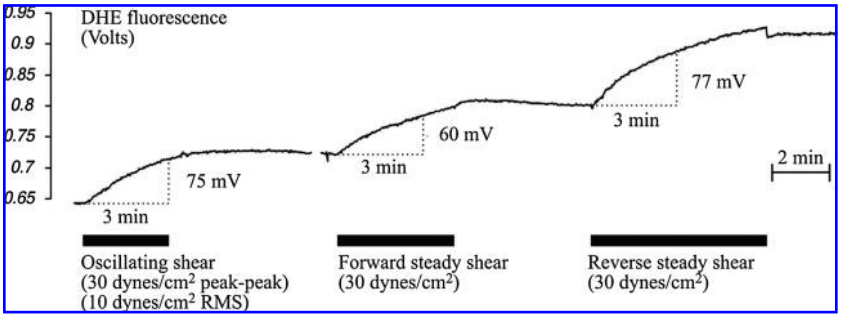

FIG. 5. Generation of O_2^-, as determined from dihydroethidium (DHE) fluorescence, in cannulated coronary arterioles (see Fig. 4A). Vessels are preincubated with DHE. After washout, the vessels are subjected to oscillating and steady shear. Although both profiles induce similar production rates of O_2^-, steady but not oscillating shear results in vasodilation (Fig. 4B). (From Sorop O, Sweeney TE, and VanBavel E, unpublished observations).

Shear Stress, ROS, and Vascular Remodeling

Blood flow–induced remodeling

Under physiologic conditions, changes in blood flow trigger an adaptive response that tends to normalize shear stress through changes in tone and, eventually, through changes in vascular structure (53, 54). Such vascular remodeling in response to altered blood flow is observed in both conduit arteries and resistance arteries. Experimental ligation of a conduit artery, such as the carotid artery, is associated with inward remodeling of the ligated artery and outward remodeling of the contralateral artery in several species. In mice, the extent and nature of the response is dependent on the strain studied (33, 50). Thus, geometric remodeling and neointima formation may be involved to various extents. In resistance arteries, normal vessel architecture is usually preserved. Hypotrophic, inward remodeling is associated with low blood flow, and hypertrophic, outward remodeling is seen after increased blood flow (82, 103, 107). Both acute and structural adaptations involve the release of nitric oxide (22, 89, 104), although conflicting data also have been obtained (15). Researchers have focused mainly on the outward remodeling in response to increased blood flow, which is indeed diminished in eNOS-knockout mice (89) and depends, among other factors, on matrix-degrading enzymes (50). A role for ROS in large-vessel flow-dependent remodeling has been shown by Castier *et al*. (14). These authors showed that molecular processes associated with outward remodeling induced by increased blood flow depend on $p47^{phox}$. Thus, mice deficient in $p47^{phox}$ or $gp91^{phox}$ were subjected to increased blood flow through an arteriovenous fistula, which increased flow without affecting blood pressure. The authors observed activation of MMPs by ROS, generated through $p47^{phox}$ (Fig. 6). Interestingly, with a similar model in rabbits, the same group found that nitric oxide may activate MMPs (102). Supportive evidence for the role of ROS in blood flow–dependent remodeling is also provided by a study of Yamamoto *et al.* (111). These authors showed that an inhibitor of xanthine oxidase, allopurinol, reduced neointima formation in a carotid artery–ligation model applied to spontaneously hypertensive rats. Considering that in several small arteries, H_2O_2 is an endothelium-dependent dilator in cases in which NO production is challenged (see earlier), it becomes relevant to test the long-term effects of endothelium-derived H_2O_2 on small-artery remodeling. There is not much information here. However, it is clear for large-artery–derived vascular SMC that extrinsic or autocrine H_2O_2 has profound effects on MAPK activity (28), DNA synthesis, and proliferation (85).

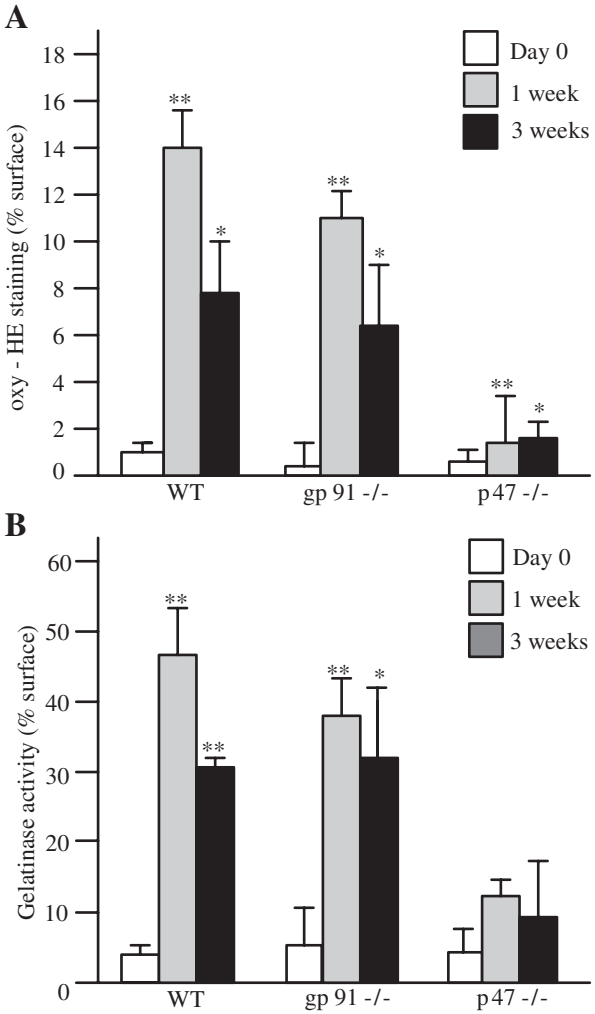

FIG. 6. Role of $p47^{phox}$ in outward remodeling after high flow. Results obtained on an arteriovenous fistula model connecting the right common carotid artery (RCCA) with the jugular vein. This model demonstrates outward remodeling in wild-type mice (not shown). (**A**) Semiquantitative analysis of oxyhydroethidine staining confirmed significant ROS production in flow-loaded arteries from wild-type and $gp91^{phox-/-}$, but not $p47^{-/-}$ mice. (**B**) Semiquantitative analysis of gelatinase fluorescence in the vessel wall demonstrates that gelatinase activity associated with remodeling is reduced in $p47^{-/-}$ mice. $^*p < 0.05$ and $^{**}p < 0.01$ *vs.* day 0. [Data from Castier *et al.* (14)].

Inflammation and ROS

Disturbed blood flow in large vessels may set the stage for pathologic types of vascular remodeling, such as aneurysm formation and atherosclerosis, through the participation of ROS and inflammation. These topics are beyond the scope of this review. Inflammatory cells may, however, also participate in flow-dependent remodeling under more-physiologic conditions. An example of such remodeling is observed during collateral artery development. Occlusion of a feeding artery causes a redirection of blood flow through small preexisting collateral arteries. In the course of days and weeks, these small arteries remodel and eventually allow sufficient blood flow to alleviate tissue ischemia. This type of remodeling, also referred to as arteriogenesis, depends on shear stress and the recruitment of monocytes (35). These inflammatory cells may contribute to the remodeling, among others, through the local release of MMPs. ROS play an essential role in this type of remodeling, as collateral artery growth induced by repetitive occlusions was shown to depend on ROS in both dogs (30) and rats (88). The role of ROS herein may relate to proinflammatory changes that attract monocytes. In addition, because inflammatory cells are also fully equipped to generate ROS, it can also be speculated that these cells act as a source of ROS during remodeling themselves.

An association between inflammation and ROS also is observed during small-artery remodeling in hypertension. The stimuli and molecular targets of ROS and the alterations observed in hypertension were reviewed by Paravicini (78). Thus, in DOCA-salt hypertensive mice, increased superoxide production, NAD(P)H oxidase activity, inflammation, and vascular remodeling are evident (47). These changes are reduced in osteopetrotic mice, which are deficient for macrophage colony-stimulating factor and consequently lack proper macrophage function. Similar findings were reported by using Ang II infusion in mice (18). Taken together, inflammatory cells participate in vascular remodeling under a variety of conditions, and ROS play a crucial role herein.

ROS activate MMPs

The activation of MMPs by ROS appears to be a key event in arterial remodeling (26). Several studies provide evidence for a link between ROS and MMPs. In cultured smooth muscle cells exposed to mechanical stress, ROS, derived from NAD(P)H oxidase, increase the expression and release of MMP-2 (29). MMP-9 also is induced by ROS in IL-1β–stimulated smooth muscle cells (31). In the context of diabetes mellitus, MMP-9 activity and expression is elevated in vascular tissues (105). This increase could be reduced by treatment with antioxidants.

In endothelial cells, lysophosphatidylcholine, a major component of oxLDL, increased gelatinolytic activity and MMP-2 release (44); inhibition of NAD(P)H oxidase attenuated the effect of LPC. Although the activation of MMPs in general may facilitate vascular remodeling by matrix degradation, ROS may also activate pathways that promote matrix stabilization. We recently found that transglutaminases, which crosslink extracellular matrix proteins (65), play an important role in the inward remodeling of small arteries (4, 6). Tissue-type transglutaminase, a prominent member of the transglutaminase family, is activated by ROS (56). The relevance of ROS-induced activation of transglutaminases, however, remains to be established in the context of remodeling.

Vascular tone and arterial remodeling

Although ROS and activation of MMPs may facilitate flow-induced vascular remodeling, these processes do not provide an explanation for the direction of remodeling (inward *versus* outward). As indicated earlier, a shift in the redox balance toward increased levels of ROS may promote vasoconstriction. Experimental work from our laboratory showed that prolonged manipulation of vascular tone directs vascular remodeling (5, 7). Thus, resistance arteries from rats and porcine hearts kept in organ culture show inward remodeling when exposed to vasoconstrictors such as endothelin-1. Conversely, vasodilator compounds inhibit or even reverse remodeling to an increase in the maximal diameter (91). Such a causal relation between vascular tone, ROS, and vascular remodeling may be highly relevant for hypertension and, perhaps, also apply to flow-induced remodeling. A vasoconstrictor substance such as angiotensin II increases ROS in murine smooth muscle cells and, subsequently, activates MMP-2 synthesis and activity (66). Catecholamines also increase ROS in cultured vascular smooth muscle cells (12). Overexpression of endothelin-1 increases ROS through an NAD(P)H-dependent mechanism, as shown in a mouse model characterized by vascular remodeling and endothelial dysfunction (2). Thus, it appears that prolonged vasoconstriction is coupled to inward remodeling, and vasoconstrictor substances can add to this both by increasing tone and the formation of ROS. This notion suggests that interference with ROS formation could alter vascular tone and remodeling. Indeed, therapeutic reduction in ROS by adding the superoxide dismutase mimetic tempol to the drinking water of SPSHR rats reduced superoxide levels, hypertension, and vascular remodeling (79).

In the treatment of human hypertension, however, trials aimed at reducing oxidative stress have been disappointing (99). In contrast, treatment of hypertension with vasodilatory compounds such as calcium channel blockers and ACE inhibitors effectively decreases blood pressure and corrects vascular structure (97). Evidence for a similar relation between vascular tone and remodeling in flow-induced remodeling is only circumstantial. Thus, Langille (53) showed that arteries exposed to reduced flow first constrict, but that the constriction becomes irreversible after several days. Here, experiments aimed at vascular remodeling through interference with vascular tone would be of great interest.

Conclusions

Shear stress is a key stimulus in the regulation of vascular structure and caliber. It is clear that the signaling pathways include NO and ROS in addition to other endothelium-derived signals. The role of ROS becomes dominant in cases in which shear stress is suddenly changed or has a pulsatile component. This may be due to either stimulation of different subsets of shear sensors by steady versus oscillating flow or activation of antioxidant pathways after prolonged constant shear. ROS signaling in response to shear also becomes more dominant whenever eNOS function is affected (*e.g.*, in various cardiovascular pathologies).

Although NO and H_2O_2 both induce vasodilation, their effects on arterial structure may well deviate. For the resistance

vessels, the link between a continuous state of partial vasodilation under shear and outward remodeling could be less effective when H_2O_2 is the dominant endothelium-derived factor. This way, resistance vessel caliber would remain smaller. Whereas feedback on shear may keep these inwardly remodeled vessels more dilated, vascular reserve and consequently the capacity for oxygen supply to tissue is limited. Future work should be done on the long-term effect of endothelium-derived ROS on small-artery tone and structure. Moreover, work should address the enzymes effectuating resistance-vessel remodeling in response to shear stress. The matrix cross-linking enzyme transglutaminase forms an interesting starting point because it is inhibited by NO but activated by ROS.

Acknowledgments

Hanke L. Matlung and Erik N.T.P. Bakker are supported by the Netherlands Heart Foundation, grant 2001T038.

Abbreviations

CAD, coronary artery disease; GAG, glycosaminoglycan side chains; GFP, green fluorescent protein; HUVEC, human umbilical vein endothelial cell; NO, nitric oxide; ROS, reactive oxygen species; TRP, transient receptor potential; WSS, wall shear stress.

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Date of first submission to ARS Central, December 16, 2008; date of acceptance, January 31, 2009.

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