Forum Review Article

Molecular Mechanisms Responsible for the Atheroprotective Effects of Laminar Shear Stress

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Abstract

The endothelium lining the inner surface of blood vessels of the cardiovascular system is constantly exposed to hemodynamic shear stress. The interaction between endothelial cells and hemodynamic shear stress has critical implications for atherosclerosis. Regions of arterial narrowing, curvatures, and bifurcations are especially susceptible to atherosclerotic lesion formation. In such areas, endothelial cells experience low, or oscillatory, shear stress. Corresponding changes in endothelial cell structure and function make them susceptible to the initiation and development of atherosclerosis. In contrast, blood flow with high laminar shear stress activates signal transductions as well as gene and protein expressions that play important roles in vascular homeostasis. In response to laminar shear stress, the release of vasoactive substances such as nitric oxide and prostacyclin decreases permeability to plasma lipoproteins as well as the adhesion of leukocytes, and inhibits smooth muscle cell proliferation and migration. In summary, different flow patterns directly determine endothelial cell morphology, metabolism, and inflammatory phenotype through signal transduction and gene and protein expression. Thus, high laminar shear stress plays a key role in the prevention of atherosclerosis through its regulation of vascular tone and long-term maintenance of the integrity and function of endothelial cells. *Antioxid. Redox Signal.* 11, 1669–1682.

Introduction

THE ENDOTHELIUM CONSISTS OF A MONOLAYER of endothelial cells in the lumen of blood vessels. While it functions as a mechanical barrier, more importantly it maintains anti-inflammatory, anticoagulant properties, enables physiological control of vasoregulation, and modulates vascular permeability. As a result of their unique location, endothelial cells are exposed to a highly dynamic environment in which physiology, pathology, blood dynamics, and biomechanics converge with cellular and molecular biology. Within such an environment, shear stress is a key regulator of endothelial cells. Shear stress is the force per unit area created when a tangential force of blood flow acts on the endothelium. Shear-induced mechanotransduction transforms mechanical forces to biochemical responses, activating signal transduction and endothelium-dependent gene and protein expression that determine endothelial cell phenotype. Thus, shear stress is indispensable in the long-term maintenance of blood vessel tone and structure.

In the arterial circulation, localized hemodynamic shear stress determines site-specific susceptibility to atherosclerosis and its progression (17). In arteries, certain regions such as branches, bifurcations, arterial narrowing, and curvatures are more likely to develop atherosclerotic lesions, and endothelial cells are exposed to low and oscillatory shear stress (Fig. 1) (19, 20, 66, 123).

In contrast, in the straight part of the arterial tree where blood flow is unidirectional with high velocity of laminar shear stress (streamlined blood flow where viscous forces are predominant over inertial forces), there is less lesion formation (24, 25, 76, 123).

Mechanisms that link atherosclerotic lesion formation and regional flow patterns remain complicated and difficult to dissect. Recently, the effect of shear stress on plaque vulnerability has been studied with a perivascular shear stress modifier that generates regions of lowered, increased, or lowered/oscillatory shear stress in mouse carotid arteries (19). It has been found that atherosclerosis develops invariably in regions with low or oscillatory shear stress, while it is
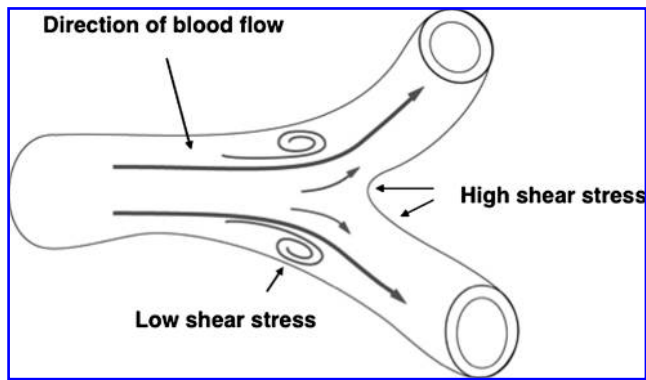

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FIG. 1. Flow pattern changes depending on the geometry of blood vessel. In "straight" regions of vasculature, endothelial cells experience ordered laminar shear stress, while at or near branch points and vascular bifurcations, endothelial cells experience low or oscillatory shear stress.

protected in regions with high shear stress. Regions of low shear stress form larger lesions containing few smooth muscle cells, less collagen, but more lipids and show more outward remodeling. These studies have demonstrated that lower shear stress and oscillatory shear stress are both essential for plaque formation. However, lower shear stress tends to induce larger lesions with vulnerable plaque, whereas oscillatory shear stress tends to induce stable lesions.

The interplay between shear stress and endothelial cells is critical for vascular homeostasis. Endothelial cells sense hemodynamic and hormonal stimuli, integrate them with signal transduction as well as gene and protein expression, and release vasoactive factors such as nitric oxide (NO), prostaglandins, hyperpolarizing factors, growth factors, and other related molecules to modulate the permeability to plasma lipoproteins, adhesion of leukocytes, and apoptosis (26, 27, 128). Abnormalities in endothelial cells' responsiveness to flow shear stress, or their failure to respond to particular types of blood flow patterns, contribute to vascular pathology, especially in the early stages of atherosclerosis (46, 70, 74, 132). This review provides a brief summary of the influence of hemodynamic shear stress on endothelial phenotype, in terms of their signal transduction, gene and protein expression, as well as structure and function.

Shear Stress-Induced Signal Transduction, Gene and Protein Expression, and Endothelial Cell Phenotype

Upon exposure to laminar shear stress, initiation of intracellular signal transduction, specifically the activation of protein kinases, is the second important molecular event in endothelial cells that modulates the activity of downstream target proteins and transcription factors, and hence the gene expression programs.

Laminar shear stress activates the signal transduction that leads to the gene and protein expression that acts against the development of atherosclerosis (15, 30, 33, 37, 55, 104, 129). In contrast, oscillatory shear stress triggers gene and protein expression in favor of the development of atherosclerosis (81, 101, 105, 107, 146).

As a result, laminar shear stress prevents endothelial cell activation through modulation of plasma lipoprotein permeability, protein expression in the leukocyte adhesion cascade, smooth muscle cell proliferation and migration, and the release of pro-thrombotic and anti-thrombotic factors, growth factors, and vasoactive substances (Fig. 2). Impairment of these shear stress-mediated processes through either physical damage or endothelial cell dysfunction under pathological conditions plays a central role in the pathogenesis of atherosclerosis.

Signal transduction, gene and protein expression, and atherosclerosis protective phenotype of endothelial cells in response to laminar shear stress

Laminar shear stress-induced NO production plays a key role in endothelial function and vascular homeostasis. In contrast, impaired endothelium-dependent vessel dilatation throughout the artery bed is the major characteristic of endothelial dysfunction that is the precursor to cardiovascular diseases including atherosclerosis.

The dominant mechanism responsible for endothelial dysfunction is the decrease in bioavailable NO, as well as the increase in reactive oxygen species (ROS) production and apoptosis. In addition to its central role in the regulation of vascular tone, NO inhibits several key steps in the initiation and development of atherosclerosis (15, 30, 37, 55, 69, 129).

The enzyme that catalyzes NO production in endothelial cells is endothelial nitric oxide synthase (eNOS). eNOS regulates diverse vascular functions, including the control of blood pressure, local vascular tone, and remodeling in response to shear stress (64, 65, 110, 117).

Although it is well known that exposure of endothelial cells to laminar shear stress stimulates the production of NO from eNOS in both cultured cells and intact vessels, the molecular mechanism by which shear stress regulates NO production is not yet clear (28, 29, 109). It appears that laminar shear stress regulates both the activity and protein expression of eNOS. eNOS activity is coordinately regulated by post-translational modifications, including phosphorylation, myristolation, palmitoylation, and subcellular localization (10). The best-characterized residues for eNOS phosphorylation are active site Ser 1177, Ser 635, and inhibitory sites Ser 116 and Thr 497 (9, 40, 94). Studies have shown that laminar shear stress stimulates serine/threonine kinase Akt (also known as protein kinase B or Rac kinase) phosphorylation on serine 473 in a vascular endothelial growth factor (VEGF) receptor-dependent manner. Activation of Akt in turn phosphorylates eNOS at Ser 1177, leading to eNOS activation and NO production (35, 44, 45, 69). However, there is also evidence that PKA contributes to shear stress-mediated eNOS phosphorylation and NO production independent of Akt (8, 11). Recent studies suggest that eNOS phosphorylation is dependent largely on its subcellular localization, and maybe the availability of the specific kinase in each location as well (10). Regardless of the upstream kinases, eNOS phosphorylation clearly plays an indispensable role on endothelial function in response to laminar shear stress.

Recently, eNOS transcription and expression has also been found to be decreased in atherosclerosis-prone regions of the mouse aorta with an *in vitro* disturbed flow model, suggesting that a sustained level of eNOS expression is necessary for the

FIG. 2. Molecular mechanisms of atherosclerosis-protective effects of laminar shear stress. Laminar shear stress maintains the anti-inflammatory and anti-apoptosis phenotype of endothelial cells through its regulation of protein expression, release of vasoactive factors, permeability of lipoproteins, adhesion and migration of leukocytes, and coagulation.
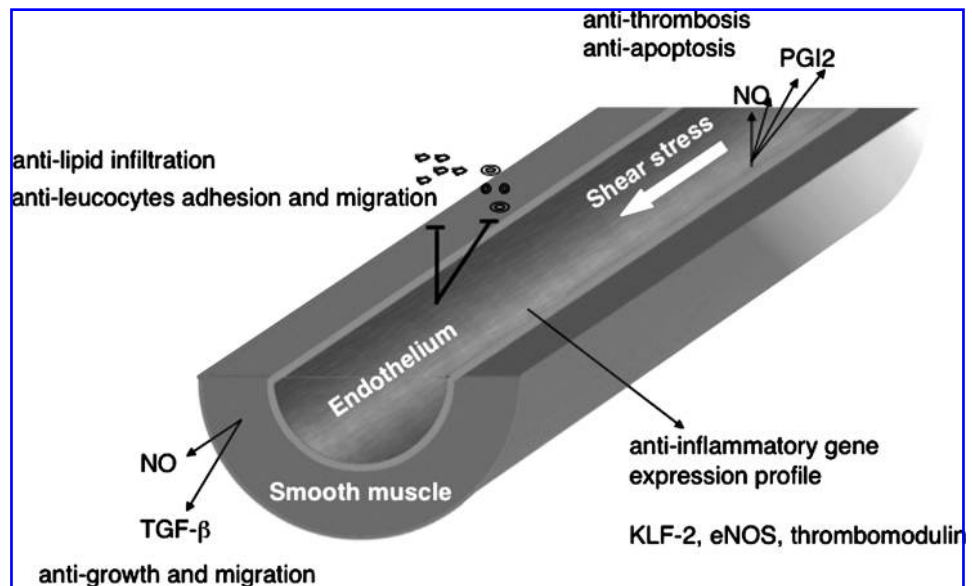
flow-mediated prevention of atherosclerosis, most likely because of its consistent supply of bioavailable NO (138).

Bioavailable NO production by laminar shear stress inhibits several key early events in the development of atherosclerosis. NO inhibits the expression of monocyte chemoattractant peptide-1 (MCP-1) and monocyte adhesion induced by cytokines and oxidized LDL, reduces vascular cell adhesion molecule-1 (VCAM-1) expression, prevents propagation of lipid oxidation, inhibits vascular smooth muscle cell proliferation, decreases platelet aggregation, and prevents cell death (15, 16, 37, 55, 129).

The role of NO in the prevention of monocytes adhesion in response to laminar shear stress is well studied. In the early stages of atherosclerosis, macrophages derived from intimal monocytes attach, spread, and migrate across the endothelium. Many studies support an inverse relationship between leukocyte adhesion and laminar shear stress. It seems probable that for conduit vessels, high shear stress inhibits leukocyte binding and chemoattractant protein expression, while low shear stress and flow reversal promote leukocyte binding and transmigration. MCP-1 signaling via its C–C motif chemokine receptor-2 has been shown to be important for monocyte adhesion to inflamed endothelium exposed to flow. There is a marked reduction in monocyte adhesion ($\sim$45%) when human pulmonary artery endothelial cell-derived MCP-1 is either neutralized with the anti-MCP-1 antibody, or inhibited by translational arrest of MCP-1 mRNA transcripts with MCP-1 antisense oligomers (90).

MCP-1 gene expression in response to shear stress is the most immediate early gene activation. Studies have suggested that this gene is probably suppressed in endothelial cells exposed to a constant high shear stress (118). On exposure to laminar shear stress, MCP-1 expression is transiently increased 2- 3-fold in human umbilical vein endothelial cells, and then decreased to basal levels after an hour. Once the gene expression is fully suppressed, it remains so even after static incubation. Additionally, the temporal gradient in shear stress increases MCP-1 gene expression (5).

Further studies have suggested that NO inhibits MCP-1 through multiple mechanisms in response to laminar shear stress (15, 98). It appears that NO inhibits MCP-1 in a protein kinase C (PKC)-epsilon and extracellular signal-regulated kinase 1/2 (ERK1/2) dependent manner. Inhibition of NO production increases MCP-1 while eNOS overexpression decreases MCP-1 (98). Other studies have also demonstrated that the anti-inflammatory effect of NO is through exocytosis of Weibel–Palade bodies, which contain von Willebrand's factor and P-selectin, as well as platelet aggregation (77, 78, 89, 93). In addition, NO also prevents MCP-1 and cytokine-induced adhesion molecule expression through nuclear factor-κB (30, 129). All these findings suggest an anti-inflammatory effect of NO in response to laminar shear stress.

Lipoprotein transport and low-density lipoprotein (LDL) metabolism appears inversely related to the availability of bioavailable NO, although the specific molecular mechanism is less well-defined (16). This hypothesis is supported by observations that LDL accumulation within the vascular wall is preferentially localized to areas with oscillatory shear stress. In these areas eNOS expression is decreased (138). In contrast, LDL incorporation and permeability have been shown decreased in straight segments, in comparison to arterial branch points that experience high shear (7, 48, 49). These findings have also been confirmed by other investigators for different areas of the vasculature in both rabbits and pigs. Additional studies are required to define the mechanisms by which LDL accumulation is affected by NO in low and oscillatory shear stress.

NO production in response to laminar shear stress is at least partly responsible for flow-induced secretion of prostacyclin (135). Shear stress is the most powerful stimulus for the release of factors that inactivate the clotting cascade. Laminar shear stress-induced secretion of prostacyclin is the first documented response of endothelial cells to shear stress (58). Prostacyclin, also known as prostaglandin I2, is the most potent natural inhibitor of platelet aggregation. Secretion of prostacyclin from endothelial cells is enhanced in a

cyclooxygenase-2 (COX-2) dependent manner. COX-2 is the enzyme that catalyzes the formation of prostacyclin. In parallel cultures, after exposure to pulsatile laminar shear stress, the prostacyclin level is significantly enhanced, concomitant with an increase in the transactivation of a COX-2 promoter (58, 127, 134). Further studies have demonstrated a positive role of NO because eNOS inhibitors N(G)-nitro-L-arginine methyl ester (100 μM, L-NAME) and N(G)-nitro-L-arginine (10 μM, LNA) cause $\sim$50% decreases in prostaglandin (135). This suggests that half of the laminar shear stress-induced production of prostacyclin is due to NO-dependent signaling.

The atherosclerosis-protective role of NO has also been demonstrated by studies on atherosclerotic lesion formation in apolipoprotein E (ApoE)/eNOS double knockout mice. eNOS deficiency increases atherosclerosis in Western-type diet-fed ApoE knockout mice (73, 80).

Laminar shear stress also maintains endothelium homeostasis through the activation of kinase signaling that promotes the viability of endothelial cells (23, 36, 71, 108). One critical molecule involved in cell survival is Akt. Activation of Akt by laminar shear stress not only contributes to the anti-apoptotic effects mediated by NO but also plays a key role in the suppression of apoptotic cell death due to either growth factor deprivation or inhibition of matrix adhesion and integrin-mediated signal transduction. Laminar shear stress activates Akt through phosphorylation of Akt in a phosphoinositide 3-kinase (PI3K)-dependent manner, which can be blocked by the PI3K inhibitors wortamannin and LY294002 (36). Upon activation, Akt phosphorylates pro-apoptotic molecule Bad on Ser 136 and Ser 112, which is required for Bad's association with 14-3-3 to sequester Bad in the cytoplasm, thereby preventing its translocation to the mitochondria to induce apoptosis. In contrast, overexpression of a dominant-negative Akt mutant significantly attenuates the apoptotic-suppressive effect of shear stress against serum depletion-induced apoptosis. These findings indicate a critical role for Akt in shear stress-mediated inhibition of endothelial cell apoptosis (36).

Recently the critical role of Akt in the prevention of atherosclerosis lesion formation has been well studied (38). On an ApoE knockout (ApoE$^{-/-}$) background, deletion of Akt1 through genetic engineering increased aortic lesion expansion compared to that of ApoE$^{-/-}$ mice. Increased endothelial and macrophage apoptosis have been found in the vessel walls of double knockout mice (ApoE$^{-/-}$ Akt1$^{-/-}$) in addition to increased inflammation and reduced eNOS phosphorylation, suggesting an indispensable role of Akt in cell survival and eNOS activation *in vivo*, particularly during the development of atherosclerosis.

Additionally, big mitogen-activated protein kinase 1 (BMK-1), which is potently stimulated by laminar shear stress, also induces phosphorylation of Bad and inhibits growth factor depletion-induced endothelial cell apoptosis. In contrast, inhibiting BMK-1 activity by overexpressing dominant-negative BMK-1 stimulates apoptosis. The role of BMK-1 in cell survival is further demonstrated by using the Bad mutant S112-136A, which abolishes the anti-apoptotic effect of constitutive active MEK-5, the upstream molecule of BMK-1 (108). These data suggest that kinase-mediated inhibition of Bad activation is likely an important mechanism involved in the anti-apoptotic effect of laminar shear stress (Fig. 3).

The gene expression profile of endothelial cells in response to different flow patterns has recently been studied intensively (81, 101, 105, 107, 146). Among transcription factors that integrate flow signaling and gene expression, the lung Kruppel-like factor (LKLF/KLF2), an endothelial transcription factor, has drawn great attention because of its critical role in mediating the anti-inflammatory effects of laminar shear stress (104). KLF2 expression is specifically induced by laminar shear stress, and it is indispensable for maintaining the atheroprotective, quiescent phenotype of endothelial cells (32).

KLF2 plays an important role at least partly because of its induction of eNOS and thrombomodulin, while reducing the expression of pro-atherogenic molecules MCP-1 and endothelin (33). Shear stress has been shown to regulate the generation of thrombomodulin, a potent activator of the protein C anticoagulant pathway and a surface receptor that binds to thrombin. Subjecting bovine aortic endothelial cells to moderate and elevated shear stress results in a mild and transient increase, followed by a significant decrease in thrombomodulin mRNA. In contrast, shear stress of low magnitude does not affect thrombomodulin mRNA levels, indicating the specific role of laminar shear stress (88). The effect of laminar shear stress in the regulation of thrombomodulin has further been confirmed in human umbilical vein endothelial cells. Laminar shear stress stimulates a sustained increase in thrombomodulin with its expression increasing $\sim$200% compared to cells maintained under static conditions (125). In addition, shear stress has also been shown to stimulate the expression of tissue plasminogen activators, as well as reduce the secretion of plasminogen activator inhibitor type-1 (34). In contrast, endothelial cells exposed to oscillatory shear stress fail to show increases in the levels of thrombomodulin and tissue plasminogen activator, further supporting the anticoagulation effect of laminar shear stress.

The protective effect of KLF2 has been further demonstrated by the fact that KLF2 overexpression dramatically induces eNOS gene expression and total enzymatic activity. In contrast, KLF2 inhibits the induction of VCAM-1 and E-selectin in response to various pro-inflammatory cytokines and subsequently, attenuates the rolling and attachment of inflammatory cells (115). The anti-inflammatory effect of KLF2 is likely due to its ability to inhibit ATF activity in response to laminar shear stress. This idea is supported by the finding that nuclear binding of ATF is dramatically reduced in a KLF2-dependent manner in human umbilical vein endothelial cells exposed to laminar shear stress (39).

Low and oscillatory shear stress regulate the endothelial secretion of several smooth muscle growth factors including platelet-derived growth factor A (PDGF-A), and endothelin-1, a growth factor acting synergistically with PDGF during smooth muscle cell proliferation. In an arteriovenous fistula model, increased mRNA levels of PDGF-A are associated with smooth muscle cell proliferation in areas that experience low blood flow compared to areas experiencing high flow (75). Similarly, a temporal gradient in shear (impulse flow and the onset of step flow) stimulates the expression of endothelial PDGF-A (5).

Endothelin-1 is a potent and long-lasting vasoconstrictor that is synthesized and released by endothelial cells. Shear stress suppresses mRNA transcript levels and the rate of endothelin-1 peptide release (86, 87, 116). More interestingly, siRNA knockdown of KLF2 specifically suppresses laminar shear stress-mediated downregulation of endothelin-1 ex-
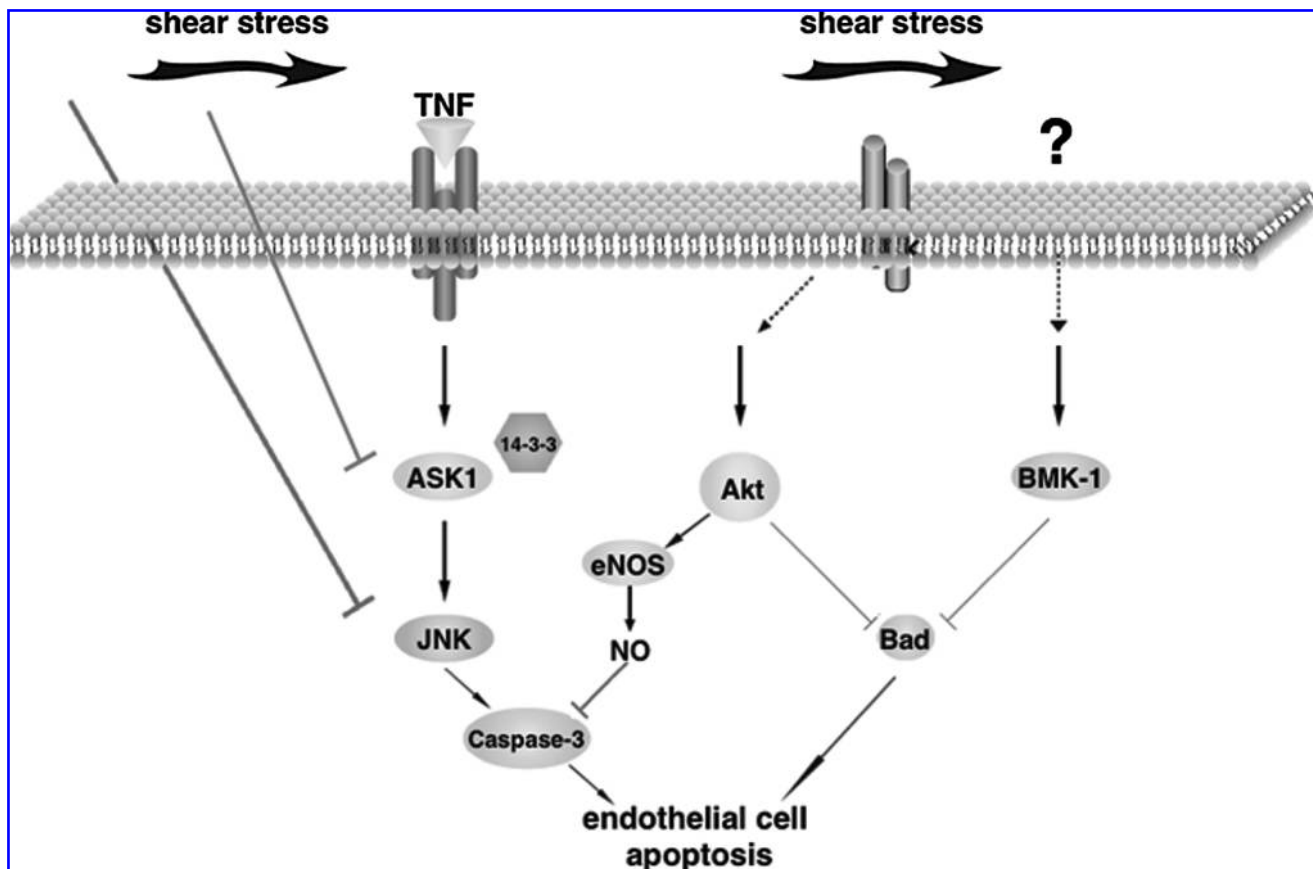
FIG. 3. **Laminar shear stress is anti-apoptotic through its activation of multiple signal transduction pathways**. It inhibits TNFα-induced ASK1 activation by increasing the binding of 14-3-3 while it activates Akt and BMK-1, which inhibiting apoptotic molecule Bad. Furthermore, nitric oxide inactivation of caspase-3 may represent additional protective mechanism.

pression, demonstrating KLF2's role in regulating the effect of shear stress on endothelin-1 (33). In addition to PDGF and endothelin-1, endothelial cells also release NO and transforming growth factor-beta (TGF-β) in response to laminar shear stress, both of which are inhibitors of vascular smooth muscle cell growth (12, 21, 28, 29).

Inhibition of atherosclerosis-prone signals by laminar shear stress

Inflammation events contribute to each step in the initiation and development of atherosclerosis. Tumor necrosis factor alpha (TNFα) stimulates signal transduction and gene expression that may promote endothelial dysfunction and atherosclerosis. For example, TNFα-induced activation of c-Jun N-terminal kinase (JNK) and p38 has been implicated in increasing endothelial cell production of matrix metalloproteinase, pro-coagulant factors, and components of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. These events can lead to oxidation, vascular remodeling, and thrombosis processes characteristic of the atherosclerotic lesion.

There has been controversy regarding the role of shear stress on JNK activation, which is likely due to the differences in flow apparatus and cell types (50, 83, 124). In human umbilical vein endothelial cells, laminar shear stress inhibits TNFα-induced JNK activation (124). Consistent with this

finding, laminar shear stress has been shown to inhibit TNFα-mediated apoptosis. Several mechanisms seem to be involved in laminar shear stress inhibition of TNFα-induced JNK activation (Fig. 4). First, activation of ERK1/2 has been linked to cell growth and survival, while JNK/p38 is associated with apoptosis. Thus, it is likely that the anti-apoptotic effect of shear stress is mediated through selective activation of ERK1/2 and inhibition of JNK induced by TNFα. This idea is further supported by observations that PD98059, a known inhibitor of ERK1/2, abolishes shear stress-induced ERK1/2 activation, as well as TNFα-mediated JNK activation. Second, laminar shear stress inactivates upstream kinase of JNK, such as apoptosis signaling kinase 1 (ASK1). Laminar shear stress has been shown to enhance the interaction between 14-3-3 and ASK1, which inhibits ASK1 activation (85). It would be interesting to find out if Akt activation is required for the inhibition of ASK1 in response to laminar shear stress. Third, laminar shear stress may alter the activity of phosphatases that contribute to JNK inactivation. In fact, laminar shear stress affects the phosphatase activity of SHP-2, which may be important for TNFα-mediated inflammation (82). Most recently, mitogen-activated protein kinase phosphatase 1 (MKP-1), a negative regulator of JNK and p38, has been found to be activated by laminar shear stress (146). It appears that MKP-1 is important in laminar shear stress-mediated inhibition of VCAM-1 expression because gene silencing of MKP-1 abolishes its inhibitory effect. These data collectively suggest
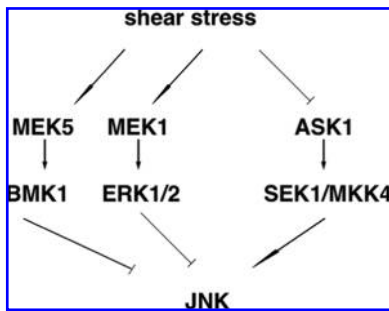
FIG. 4. **Molecular mechanisms of JNK inhibition by laminar shear stress.** Laminar shear stress inhibits ASK1, an upstream kinase of JNK, and concomitantly activates ERK1/2 and BMK-1.

that changes in phosphatase activity might be another general mechanism of the atheroprotective effect of laminar shear stress.

Laminar shear stress inhibits TNFα-mediated inflammatory events by modulating the expression level of proinflammatory proteins. Binding sites for ATF are enriched in KLF2, eNOS, and thrombomodulin, whereas TNFα-induced gene expression is mostly NF-κB-dependent (39). NF-κB and its inhibitor IκB are present in elevated levels in the cytoplasm of endothelial cells at sites exposed to disturbed blood flow (53).

Endothelial cells regulate leukocyte adhesion and migration into the blood vessel wall by modulating the secretion of chemotactic factors and the expression of cell surface molecules. Intercellular adhesion molecule-1 (ICAM-1) binds to β2-integrin on various white blood cell derivatives, while VCAM-1 mediates the adhesion of monocytes to the endothelium. VCAM-1 is also one of the earliest markers for fatty streaks and is upregulated in areas of the endothelium surrounding atherosclerotic plaques.

Leukocyte binding after exposure to shear is increased with upregulation in adhesion molecule expression. There is evidence showing that the VCAM-1 mRNA level is markedly decreased in endothelial cells exposed to laminar shear stress (2, 3, 96). In a disturbed shear system that models the *in vivo* hemodynamic characteristics of lesion-prone vascular regions, there is a distinctive, clustered-cell pattern of monocyte adhesion that strongly resembles *in vivo* leukocyte adhesion in the early and late stages of atherosclerosis. This clustered monocyte cell adhesion correlates with endothelial cell expression of ICAM-1 and E-selectin (13). In addition to studies that use cultured cells, there are also *ex vivo* studies on the effect of chronic shear stress on TNFα-induced inflammatory responses. In an *ex vivo* rabbit aorta organ culture system, TNFα-induced expression of VCAM-1 is downregulated by high shear stress (12 dynes/cm^2) while low shear stress (0.4 dynes/cm^2) has no such effect (141).

There are several proteins that bind and inhibit TNFα activation of ASK1, which then inhibits inflammatory molecule expression. Thioredoxin (Trx) is one of those proteins (111). Trx is a small thiol oxidoreductase of 12 KD whose enzymatic activity is dependent on two redox-sensitive cysteines (C32 and C35) located in its catalytic center. In its reduced form, Trx binds to the C-terminus of ASK1 and inhibits ASK1 kinase activity. TNFα activates ASK1 partly by dissociating Trx from ASK1. Trx itself is negatively regulated by its binding protein,

thioredoxin interacting protein (TXNIP, also known as vitamin D upregulating protein 1, VDUP-1). TXNIP binds to the catalytic center cysteine of Trx to inhibit its activity (106). Studies using *ex vivo* aortic segments and cultured endothelial cells have shown that laminar shear stress decreases TXNIP protein expression in endothelial cells (~50% downregulation compared to low shear) (142). The downregulation of TXNIP releases the catalytic cysteine of Trx and increases functional Trx, as evidenced by the increased Trx activity, while Trx protein levels remained unchanged. This is thought to enable increased binding of Trx to ASK1 and subsequently decrease phosphorylation of JNK and p38. It also blocks VCAM-1 expression induced by TNFα in response to laminar shear stress. Likewise, downregulation of TXNIP using RNA interference has been shown to increase the binding of Trx to ASK1, decrease JNK and p38 activation, and block TNFα -induced VCAM-1 expression. In contrast, overexpression of TXNIP increases the activation of JNK and p38. Increased expression of VCAM-1, together with other selectins, promotes leukocyte adhesion to the endothelium. These leukocytes will ultimately infiltrate below the endothelial cell surface, taking up lipid and becoming foam cells. Therefore, downregulation of TXNIP represents another atheroprotective mechanism mediated by laminar shear stress (Fig. 5). The finding that laminar shear stress inhibits JNK and TXNIP is thought to be significant, as it likely influences other aspects of endothelial function that contribute to atherosclerosis (54).

Shear Stress-Mediated Redox Regulation in Endothelial Cells

Shear stress-mediated redox regulation is becoming an increasingly interesting field to study because of ROS's role in contributing to atherosclerosis. ROS increase leukocyte adhesion molecule expression, stimulate smooth muscle cell proliferation and cell migration, promote lipid oxidation, upregulate matrix metalloproteinase, and alter vasomotor activity (68).

In healthy vessels exposed to laminar shear stress, the endothelium exerts anticoagulant effects via its secretion of NO, thromodulin, heparin sulfate, tissue factor (TF) inhibitor, and annexin V. In contrast, oscillatory shear stress induces a procoagulant phenotype of endothelial cells that may favor the formation and progression of atherothrombotic lesions. Studies have shown significantly increased release of TF mRNA
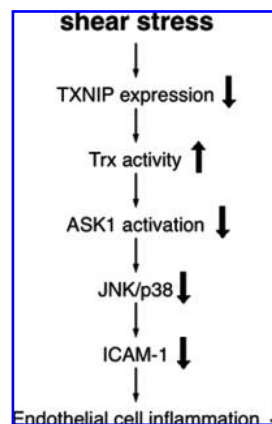

FIG. 5. **Laminar shear stress is anti-inflammatory through its downregulation of TXNIP and VCAM-1 expression**. Downregulation of TXNIP increases Trx activity, which inhibits ASK1 and downstream molecules, such as JNK and p38. Downregulation of TXNIP also decreases TNFα-induced VCAM-1 expression.

and protein expression in human endothelial cells exposed to oscillatory shear stress for 24 h, while the expression of TF inhibitor (TFPI) mRNA and protein remain unchanged relative to static conditions (91). Because TF initiates blood coagulation and contributes to vascular remodeling, it is a potential contributor to the development and progression of atherosclerosis. Conversely, cells exposed to unidirectional laminar shear show a decrease in TF activity along with a significant increase in TFPI mRNA and protein expression. It is interesting that increased expression of TF is mediated in part by ROS production in smooth muscle cells. Likewise, endothelial cells exposed to oscillatory shear stress fail to show increased expression of thrombomodulin and tissue plasminogen activator. Furthermore, when endothelial cells are exposed to oscillatory or low shear stress, the simultaneous production of superoxide O^{2-} and nitric oxide anion NO• can facilitate peroxynitrite $(ONOO^-)$ formation and tyrosine nitration (63). Tyrosine nitration has been found in human atherosclerotic lesions and may predispose vascular areas exposed to oscillatory flow to atherosclerosis.

Monocyte adhesion is another key event in the early stages of atherosclerosis. Binding of circulating monocytes to the endothelium is dependent on the expression of adhesion molecules on the cell surface, which is regulated in a ROS-dependent manner through unknown mechanisms. The antioxidant ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) inhibits TNFα-induced VCAM-1 and ICAM-1 expression in this regard (145). Chronic exposure of endothelial cells to oscillatory shear stress stimulates ROS generation through p47phox-dependent NAD(P)H oxidase, which leads to monocyte adhesion (119). Although there is also a transient increase in ROS generation upon exposure to laminar shear stress due to the activation of membrane-bound NADPH oxidase activation, sustained laminar shear stress maintains endothelial cells in a reduced environment, which inhibits leukocyte binding and inflammation by decreasing TNFα-induced expression of VCAM-1 and E-selectin. In contrast, extensive monocyte adhesion that co-localizes to areas of VCAM-1 expression is observed under low and oscillatory shear stress (3, 22, 100, 133).

ROS promote atherogenesis through several enzymes, including xanthine oxidase (OX), NADPH oxidase, and uncoupled eNOS (31, 67, 92). It has been proposed that NADPH oxidase maintains endothelial XO levels by regulating XO protein expression. Moreover, XO is responsible for the generation of ROS in response to oscillatory flow (92). Recent studies have also demonstrated that the expression of the vascular NADPH oxidase subunit NOX4 increases in response to oscillatory flow, leading to an increase in LDL oxidation relative to laminar shear stress (67).

Laminar shear stress modulates the expression of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX-1), as well as the redox status of proteins. Glutathione (GSH, γ-glutamyl-cysteinyl-glycine) is the major low-molecular-weight thiol antioxidant in endothelial cells. It serves as a substrate for GPX to eliminate lipid hydroperoxides and H_2O_2, whereby it is converted to glutathione disulfide (GSSG). Laminar shear stress induces upregulation of GSH/GSSG, which keeps endothelial cells in a reduced condition. Normally, endothelial cells are in a reduced state because GSH exists in very high concentrations (mM) while GSSG is maintained at levels <1% of total GSH.

Steady laminar shear stress inhibits H_2O_2-induced JNK activation, partly by increasing GSH/GSSG in a glutathione reductase (GR)-dependent manner. This is supported by the fact that the GR inhibitor, but not the thioredoxin reductase inhibitor, blocks the inhibitory effect of laminar shear stress on H_2O_2-mediated JNK activation (61). It appears that laminar shear stress mediates changes in the glutathione redox cycle by reducing GSSG through GR. L-buthionine-(S,R)-sulfoximine (BSO), which inhibits GSH synthesis, can partly reverse the protective effects of shear stress (57). In contrast, GSH levels decrease when cells are exposed to oscillatory shear stress (95). Although it is not clear how shear stress regulates the glutathione system, the impact is profound, because it is at least partly responsible for the inhibition of both H_2O_2 and TNFα-induced cell death.

As previously mentioned, laminar shear stress increases Trx activity by downregulating TXNIP. Trx serves both as a redox sensor and a redox-regulating molecule in keeping the thiol homeostasis of the cell; specifically, it acts as a general protein disulfide oxidoreductase and interacts with a broad range of proteins. This reaction is based on reversible oxidation of two cysteine thiol groups of Trx to a disulfide, accompanied by the transfer of two electrons and two protons (140).

In addition to Trx, GPX-1 is also regulated by laminar shear stress. GPX-1 belongs to a family of selenoproteins and plays an important role in the defense mechanisms against oxidative damage by catalyzing the reduction of a variety of hydroperoxides, with glutathione as the reducing substrate. Both GPX-1 mRNA and its activity are upregulated by laminar shear stress in a time- and force-dependent manner. Interestingly, this response is shear stress specific, because tensile stress changes neither the expression nor the activity of GPX-1 (126).

Laminar shear stress not only detoxifies hydroperoxides through increased expression and activity of GPX-1 but also provides protection to superoxide. SOD is the enzyme that catalyzes the decomposition of a superoxide into hydrogen peroxide and oxygen. Of the three forms of atheroprotective SOD found in the vascular wall (MnSOD, extracellular SOD, and Cu/Zn SOD), the expression of all of them are upregulated when endothelial cells are exposed to laminar shear stress (1, 122, 139).

Therefore, laminar shear stress keeps endothelial cells in a reduced condition by activating antioxidant enzymes (Fig. 6). GR provides reducing power by converting GSSG to GSH without changing the total glutathione level. Increased expression of SOD can detoxify superoxide by converting superoxide to H_2O_2. The H_2O_2 is further detoxified by GPX-1, which is also activated by laminar shear stress.

NF-E2-related factor 2 (Nrf-2) plays an essential role in the protection of endothelial cells through antioxidant response element (ARE)-mediated gene expression of phase II detoxification antioxidant proteins. Although both oscillatory and laminar shear stress increase Nrf-2 nuclear translocation, only laminar shear stress stabilizes Nrf-2 and induces Nrf-2-mediated gene expression (62, 136, 137). In human umbilical vein endothelial cells, Nrf-2 regulated genes such as heme oxygenase 1, NAD(P)H quinone oxidoreductase1, glutamate-cysteine ligase modifier subunit, and ferritin heavy chain are induced by laminar shear stress but inhibited by Nrf-2 siRNA (136, 137). Further studies have shown that laminar shear
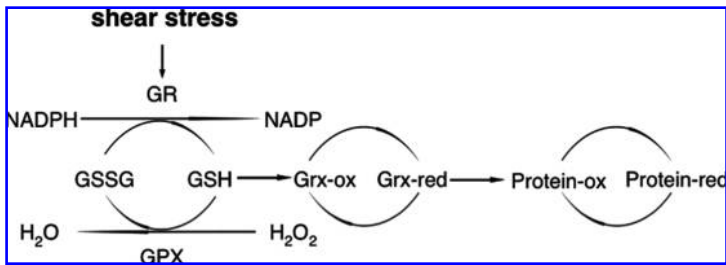
FIG. 6. Laminar shear stress maintains the reduced environment of endothelium by increasing antioxidant enzyme activity of the glutaredoxin (Grx) system. Laminar shear stress increases the ratio of GSH/GSSG by activating GR. It also increases the activity of GPX-1 and Grx that are important in maintaining the redox homeostasis.

stress stabilizes Nrf-2 protein level through lipid peroxidation (136). However, the mechanism by which Nrf-2 is activated in response to laminar shear stress requires further investigation.

S-nitrosylation is a redox-dependent post-translational modification that is highly regulated in endothelial cells. In the presence of endogenous NO, free -SH groups in proteins can be modified to -SNO (120, 121). When exposed to laminar shear stress, the level of S-nitrosylated proteins increases significantly, in part because shear stress is an important stimulus for NO production in endothelial cells (28, 29, 59). Because S-nitrosylation is a redox-sensitive modification that regulates protein function, it may partly explain certain changes in protein activity in response to laminar shear stress. To date, proteins that are known to be regulated by S-nitrosylation include the catalytic p17 subunit of caspase-3, Trx, and the GTPase p21ras (59). Increased protein S-nitrosylation results in the inhibition of caspase-3 activity, as well as the enhancement of Trx and GTPase p21 activity. Increased S-nitrosylation of various proteins may become a novel signaling mechanism regulated by laminar shear stress.

Mechanosensing of Shear Stress

Endothelial cells are very sensitive to shear stress. Upon exposure to laminar shear stress, endothelial cells deform, transmit the stress, transform mechanical forces to biochemical activities, and activate signal transduction. They adjust themselves not only biologically but also morphologically. Prolonged exposure to laminar shear stress leads to the reorientation of endothelial cells so that their longitudinal axes are parallel to the direction of blood flow (Fig. 7). During these processes, the involvement of cytoskeleton is required for stress transmission in order to link the luminal surface to junctions and focal adhesions. In addition, increased membrane fluidity may have some implications in the modulation of conformation and interaction among membrane proteins

(14). In contrast, endothelial cells do not experience reorientation in response to low or oscillatory shear stress (101, 105, 107).

The mechanisms by which the physical forces generated by blood flow are sensed and transduced into biological signals by endothelial cells are still unclear. However, several proteins are known to be important mediators in mechanotransduction. Some of these proteins are located at the surface of endothelial cells, where they can directly sense and transmit mechanical forces across the membrane to the interior of the cells. Meanwhile, nuclear membrane is also subjected to the transmission of stress mediated by the cytoskeleton.

Membrane proteins that are likely to sense the mechanical forces include integrins, platelet endothelial cell adhesion molecule-1 (PECAM-1), tyrosine kinase receptors, particularly VEGF receptor 2 (VEGFR2), G-proteins, and ion channels. Other possible flow sensors are local membrane structure such as caveolae, gap junctions, membrane lipids, and glycocalyx.

In many cases, these mechanosignaling proteins are associated with well-defined signal transduction pathways that regulate various aspects of cell function. For example, integrins integrate mechanical forces with cytoskeleton proteins, which transmit and modulate the tension between focal adhesion sites, membrane receptors, and the extracellular matrix (56, 84, 130). Therefore, when endothelial cells are aligned with laminar shear stress, there is decreased deformability and increased resistance to detachment.

In the case of PECAM-1, it undergoes rapid changes in response to flow shear stress to serve as mechanoreceptors (43, 72). PECAM-1's role in mechanosensing has been demonstrated by its tyrosine phosphorylation by directly applying mechanical force to PECAM-1 (103). In a similar way, shear stress induces a rapid tyrosine phosphorylation (<30 s) of PECAM-1 accompanied by the binding of its cytoplasmic tail to the phosphatase SHP-2. Knockdown of either PECAM-1 or SHP-2 abrogates the activation of ERK1/2 by shear stress. Additionally, VEGFR2 may mediate mechanotransduction. It
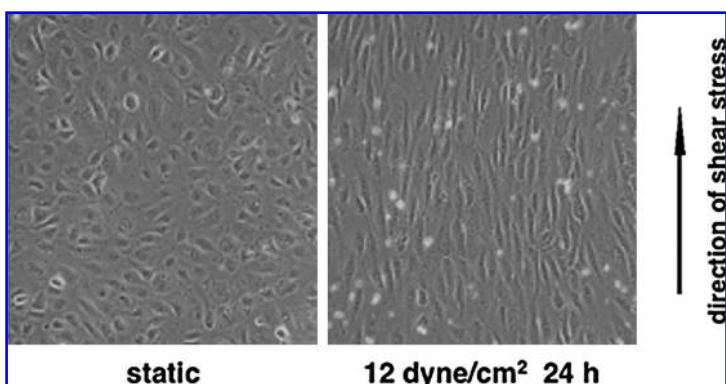

FIG. 7. Endothelial cells sense the mechanic forces and adjust themselves morphologically. Cultured human umbilical vein endothelial cells were exposed to laminar shear stress (12 dynes/cm^2) for 24 h in a parallel flow system. Cells undergo reorientation, with their longitudinal axis parallel to the direction of flow.

is activated very quickly (1–2 min) after the onset of laminar flow, becoming tyrosine phosphorylated. It then forms membrane clusters and binds to Shc and other adaptor proteins, which trigger the mechanotransduction (18, 69).

An important issue raised by these studies is the exact nature of the coordination and differential roles of these molecules in sensing the mechanical forces. The pathway upstream of integrin activation has been studied. It appears that PECAM-1, vascular endothelial cell cadherin, and VEGFR2 comprise a mechanosensory complex. More interestingly, the presence of these proteins is sufficient to confer responsiveness to flow in heterologous cells. In support of these observations *in vivo*, there has been no activation of NF-kappaB (NF-κB) or downstream inflammatory genes in regions of oscillatory shear stress in PECAM-1-knockout mice. Therefore, this mechanosensing pathway is believed to be necessary for the earliest-known events in atherogenesis (131).

The second relevant question is what determines the endothelial cells' response to the magnitudes and types of flow, and how endothelial cells distinguish laminar from oscillatory shear stress. The G proteins are one of the earliest known membrane-bound proteins that respond to shear stress (4, 52, 79, 99). When endothelial cells are exposed to shear stress, G proteins are activated within one second (52). NO production upon the onset of shear stress is both calcium-and G protein-dependent (79). More importantly, G proteins may also play a role in distinguishing flow profiles (51). Gαq and Gi respond specifically to temporal gradients of shear stress in artificial phospholipid bilayers in the absence of cytoskeleton elements (51). Studies have shown that Gαq links to PECAM-1 at cell–cell junctions while the temporal gradient has been found to be a potent mediator of their dissociation (144). These studies implicate G proteins as a primary sensor of shear stress.

Many studies have also suggested that ion channels play important roles in this process. Ion channel activation is the most rapid response of endothelial cells to shear stress, occurring almost immediately upon the onset of flow. The potassium channel in endothelial cells is the first ion channel identified using whole-cell patch-clamp recordings of single arterial endothelial cells exposed to a controlled level of laminar shear stress in capillary flow tubes (102). Recently, this channel has been cloned and expressed in *Xenopus* oocytes. Its sensitivity to flow appears to be preserved (42, 60). Blocking mechanosensitive K$^+$ channels with barium chloride or tetraethylammonium inhibits the shear-mediated increase in NO production and TGF-β release, suggesting that transmembrane ion flux and intracellular ion homeostasis are important mediators of the endothelial cell response to shear stress (42). A second type of known flow-sensitive ion channel is a chloride (Cl$^-$) channel, whose activation leads to cell membrane depolarization (6, 97). More interestingly, recent studies have shown that blocking the flow-activated Cl$^-$ current abolishes flow-induced Akt phosphorylation in bovine aorta endothelial cells, whereas blocking flow-sensitive K$^+$ currents has no effect. This suggests that flow-activated Cl$^-$ channels play an important role in regulating flow-mediated signaling in endothelial cells (47). In addition to these two channels, an ion channel that is more permeable to calcium than to sodium, can also be activated by laminar shear stress (113, 114).

Recently, some specialized structures have been identified to extend considerably further into the lumen of blood flow. For example, glycocalyx is a highly charged, glycoprotein-rich extension of the cell surface. The interaction between the glycocalyx and the membrane lipids may also play a role in mechanosensing (112). Interestingly, its thickness and distribution is particularly important because selective cleavage of glycocalyx components, glycosaminoglycan heparan sulfate, has been found to abolish both flow-mediated NO production and monolayer realignment (41, 143). Further studies are required to obtain details regarding their mechanical properties and distribution in arteries.

Concluding Remarks

Laminar shear stress is of utmost importance in maintaining vascular homeostasis. In blood vessel regions with low or oscillatory shear stress, events such as increased leukocyte adhesion, lipoprotein uptake, smooth muscle cell migration, and increased ROS generation contribute to atherosclerosis. However, these activities are inhibited by laminar shear stress. Laminar shear stress also activates signal transduction pathways and gene expression to suppress inflammation and atherosclerosis. Finally, it is responsible for maintaining the redox homeostasis of the blood vessel wall. Taken together, all of the above show that laminar shear stress is atherosclerosis-protective, while low and oscillatory shear stresses are closely associated with the pathogenesis of atherosclerosis.

Acknowledgments

Abbreviations

ApoE, apolipoprotein E; ASK1, apoptosis signaling kinase 1; BMK-1, big mitogen-activated protein kinase 1; BSO, L-buthionine-(S,R)-sulfoximine; ebselen, 2-phenyl-1,2-benzisoselenazol-3(2H)-one); ERK, extracellular signal-regulated kinase; eNOS, endothelial nitric oxide synthesis; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; Grx, glutaredoxin; ICAM-1, intercellular adhesion molecule-1; JNK, c-Jun N-terminal kinase; KLF2, lung Kruppel-like factor 2; MCP-1, monocyte chemotactic protein 1; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; OX, xanthine oxidase; Nrf-2, NF-E2-related factor 2; PDGF, platelet derived growth factor; PECAM-1, PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; platelet endothelial cell adhesion molecule-1; SOD, superoxide dismutase; TF, tissue factor; TGF-β transforming growth factor-beta; TNF-α, tumor necrosis factor; Trx, thioredoxin; TXNIP, thioredoxin interacting protein; VCAM-1, vascular cell adhesion molecule-1; VDUP-1, vitamin D upregulating protein 1.

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