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Reviews

Shear-dependence of endothelial functions

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Abstract. Endothelial cells are subjected to shear forces which influence important cell functions. Shear stress induces cell elongation and formation of stress fibers, increases permeability, pinocytosis and lipoprotein internalization, is involved in the formation of atherosclerotic lesions, increases the production of tissue plasminogen activator, and enhances von Willebrand factor release and hence platelet aggregation. It decreases adherence of erythrocytes and leukocytes, and increases the release of prostacyclin, endothelium derived relaxing factor, histamine and other compounds, but decreases erythropoietin secretion. The mechanism of signal transduction to the endothelial cell is not known exactly; shear-sensitive ion channels seem to be involved. It is concluded that a better understanding of shear-dependent endothelial functions will influence pathophysiologic concepts and therapeutic interventions.
Key words. Adhesion; atherosclerosis; cytoskeleton; endothelium; hormones; shear; thrombosis.

Introduction

Endothelial cells have been regarded for a long time as an inert cellular lining of the vessel wall. The unique properties of these cells – which cover an area of about 400 m^2, have a total weight of about 1.5 kg, and may be regarded as an organ dispersed over the entire body – have been revealed in recent years. It has become evident that endothelial cells have important regulatory functions for blood flow, vascular functions and processes in the surrounding tissue. A new area of research has been opened, which gathers together investigators from different fields: rheologists, angiologists, endocrinologists, immunologists, pathologists and others. Endothelial cells are the interface between flowing blood and tissue. In this review the present state of knowledge about the influence of blood flow on endothelial functions will be discussed.

Endothelial cell shape and microstructure

The hemodynamic forces acting on the vessel wall have two principal components: 1) shear stress, a tangential, frictional force acting in the direction of blood flow on the surface of the endothelial cell, and 2) pressure-stretch, which acts perpendicularly to the vascular wall and affects endothelial cells as well as the underlying structures (smooth muscle cells, matrix). Shear stress, which varies in the vascular tree from <1 dyn/cm^2 in veins to 2–20 dyn/cm^2 (locally up to 100 dyn/cm^2) in arteries, is almost exclusively received by the endothelial cells, and affects their morphology and function.

Endothelial cells, which have a polygonal shape at rest, become gradually oriented and elongated in the direction of flow with increasing shear rate. This has been shown in vitro with cultured endothelial cells from various sources, and in vivo using an animal model with aortic stenosis[19,41,47]. Cell elongation becomes visible within 6 h after the onset of shear stress[59]. Cell elongation is preceded by changes in the architecture of the cytoskeleton, starting 2–3 h after the onset of shear stress, and at values of 2 dyn/cm^2 which are too low to induce shape changes. The actin filaments which are located at the cell periphery at rest decrease, while long, thick 'stress fibers' containing actin, myosin and α-actinin are formed in the center of the cells[26,59,84]. They share attachment sites on the cell membrane and are probably capable of applying tension to resist the shear forces acting on the endothelial cells. The endothelial cell stiffness as measured with micropipette correlates with the shear stress to which they have been previously exposed[71]. Interestingly, pulsatile shear stress gives rise to greater cell stiffness than does steady shear stress[71]. Both morphological and microstructural changes are reversible[41].

Thus a variety of endothelial cell types can be found in the human circulatory system, ranging from polygonal cells without actin stress fibers in veins to elongated, oriented cells with strong stress fibers on the arterial side. Similarly in the heart, endothelial cells in the atria or at the tip of the aortic valve, where low shear stresses are present, are round and have no actin fibers, whereas endothelial cells in the ventricle or at the base of the aortic valve are elongated, oriented and contain stress fibers[84]. When vessel walls are subjected to other flow conditions, e.g. a venous graft is used for an aortocoronary bypass, the vessel wall including the endothelial cells will adapt accordingly[65].

Influence of shear stress on endothelial cell permeability, pinocytosis and endocytosis

The permeability of endothelial cells for macromolecules such as albumin is shear-dependent. In vitro a shear stress of 1 dyn/cm^2 leads to a 4-fold increase and a shear stress of 10 dyn/cm^2 to a 10-fold increase in the transendothelial permeability of albumin, which is reversible within 1–2 h after cessation of flow[33]. Pinocytosis, i.e. fluid-phase endocytosis, measured by the uptake of horseradish peroxidase by bovine aortic endothelial cells, is increased by shear stress in the first 2 h, followed by a return to control values in subsequent hours[16]. Pinocytosis is not only stimulated by an increase in shear stress, but also by a decrease, and periodic changes of shear stress with cycles of 15 min can sustain a high pinocytosis rate. On the other hand oscillatory flow in the physiological range (about 1 Hz) has no influence on pinocytosis[16]. These observations suggest that pinocytosis, a very important mechanism of endothelial homeostasis, may be influenced by physiological states leading to changes of shear stress, e.g. by exercise.

Endocytosis of low density lipoproteins (LDL), which play a key role in atherogenesis, is shear-dependent. It has been shown that cultured bovine aortic endothelial cells internalize LDL at a greater rate when they are exposed to high shear stress (30 dyn/cm^2), and that this is due to an increased specific binding of LDL to its receptor[77]. This has clinical implications with regard to atherosclerosis.

Role of shear stress in the development of atherosclerotic lesions

Atherosclerosis, the disease responsible for the highest morbidity and mortality in industrialized countries, is certainly a multifactorial process[5], involving heredity, dietary fat content, hypertension, diabetes and smoking[4]. These factors aggravate the disease, but do not explain the specific localization of the atherosclerotic lesions in the vascular tree. With the introduction of physical methods in clinical investigation 30–40 years ago it became obvious that mechanical factors are intimately involved in the formation of atherosclerosis[22,53,79,83]. This discovery initiated intensive work in this field and was the starting point for many other investigations described in this review. The location of plaques is determined by the wall shear stress; they are found in areas where separation of the stream-lines from the vessel wall and formation of eddies occur[4]. It is now well established that areas with high shear stress which remains unidirectional during systole, i.e. the inner walls or the flow divider at a bifurcation, have a minimal thickening of the intima[36]. On the other hand, the outer walls of a bifurcation have the lowest shear stress, which oscillates between negative and positive values, and they have the most extensive intimal thickening[36]. It has been shown that increased shear stress diminishes the proliferation of smooth muscle cells[35]. Steady shear stress enhances DNA synthesis of endothelial cells during repair of mechanical denudation[3]. Turbulent flow of the same mean magnitude as laminar flow leads to a several-fold increase of cell turnover as assessed by DNA synthesis[17].

Thromboresistance of endothelial cells and thrombus formation on an injured vessel wall

Intact endothelial cells have several mechanisms to prevent adhesion of platelets or coagulation, and hence provide a thromboresistance which is extremely important. Endothelial cells produce prostacyclin and endothelium-derived relaxing factor (EDRF), substances which cause vasodilation and inhibit platelet aggregation. This antiaggregatory effect is mediated by an increased production of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), respectively. The production of prostacyclin and EDRF is regulated by shear stress, as will be discussed later. Fibrin formation is inhibited by heparin-like glycosaminoglycans and by thrombomodulin, both substances being expressed on the cell surface. Thrombomodulin avidly binds thrombin, which alters the specificity of thrombin, resulting in an activation of protein C, which in turn inactivates coagulation factors Va and VIIIa with the help of protein S present on the endothelial surface[24]. Not only fibrin production but also fibrin degradation is regulated by the endothelial cells. Tissue plasminogen activator (tPA) and plasminogen activator inhibitor type 1 (PAI-1) are produced by endothelial cells. The production of tPA is shear-stress dependent, it can be induced in cultured endothelial cells when a shear stress of ≥ 10 dyn/cm^2 is applied[20] and it has been shown that the regulation occurs on the level of gene transcription[21]. PAI-1 on the other hand is independent of shear stress and is probably not secreted by endothelial cells in vivo to a substantial degree, because of inhibition by a soluble factor produced by smooth muscle cells[15].

When the nonthrombogenic endothelial cells are removed from the vessel wall, platelets rapidly adhere to the underlying subendothelium. This process is mediated by a variety of proteins produced by endothelial cells and released into the subendothelium, for which platelets have receptors, e.g. von Willebrand factor, collagen, fibronectin and thrombospondin. Both protein synthesis and platelet adhesion are shear-dependent.

As shown in the table, shear stress decreases the synthesis of fibronectin[29], and increases the release of von Willebrand factor, a large multimeric glycoprotein, which is produced by endothelial cells[60] and induces its binding to the platelet glycoprotein GPIb, which in turn leads to an influx and intracellular accumulation of calcium, platelet activation, binding of von Willebrand

Regulation of endothelial secretion by shear stress

	Shear stress	
	increases	decreases
Substances involved in clot formation	von Willebrand factor – release – binding to platelet GP Ib Tissue plasminogen activator	Fibronectin
Vasoactive agents	Endothelin-1 Nitric oxide Prostacyclin Histamine Renin	Endothelin-1
Cytokines	Transforming growth factor-β1 Platelet derived growth factor	Erythropoietin

factor to platelet glycoprotein IIb/IIIa and formation of stable aggregates[2,14,70].

Platelet aggregation induced by high shear stress is inhibited in von Willebrand disease[81] or by the administration of a monoclonal antibody against the binding site of von Willebrand factor on GPIb[70], and is enhanced by epinephrine[27]. In contrast to platelet activation by thrombin this process is independent of cyclooxygenase. Shear-induced platelet aggregation can, therefore, not be inhibited by aspirin[56].

Shear stress determines the composition of thrombi[8,9,32,82]; low wall shear rates, as prevailing in the venous system lead to a fibrin-rich thrombus (red thrombus). High wall shear rates (>30 dyn/cm^2), as seen in arteries and stenotic areas, induce platelet aggregation and sticking to the subendothelium[56], while fibrin polymerization and hence fibrin deposition is decreased[80,88], which leads to a platelet rich thrombus (white thrombus).

Role of shear stress in cell adhesion to the vessel wall

Adhesion to endothelial cells is the crucial step in important pathophysiological events such as inflammation and metastasis. In recent years the 'rolling' of leukocytes on endothelial cells stimulated by cytokines has been attributed to selectins[43]. It is followed by adhesion to the endothelial cell via integrins[78]. Thus, the early events of inflammation have been elucidated. Selectin-mediated rolling of leukocytes on endothelial cells is shear-dependent: with increasing shear stress the rolling velocity increases and binding to the vessel wall decreases[45]. Leukocyte adhesion mediated by the CD11/CD18 adherence glycoproteins is inversely related to shear stress[44,61]. The adherence of sickle erythrocytes to endothelial cells also decreases with increasing shear stress[6]. However, shear stress does not always decrease adherence. The adhesion of tumor cells, which is a crucial phase of metastatic dissemination, is increased with increasing shear rates, a mechanism which is due to increased platelet interaction with tumor cells and the vessel wall[7].

Influence of shear stress on vasoconstriction and vasodilation

The influence of shear stress on the secretion of substances regulating the vascular tone is summarized in the table. The role of endothelin-1, an important vasoconstrictor, is controversial[85,87]. Malek and Izumo[50] provided evidence that shear stress has an inhibitory effect on endothelin-1 production. They found a dose-dependent downregulation of endothelin-1 production by measuring mRNA for endothelin in cultured endothelial cells at shear stresses between 3 and 20 dyn/cm^2. This effect was due to the level of shear stress and not to the level of fluid flow velocity, as tested with different fluid viscosities, and was similar for laminar and turbulent flow[50]. Recently Kuchan and Frangos[37] showed that shear stress can either stimulate or inhibit endothelin release, depending on the intensity and duration of the stress. Sustained low levels of shear (<2 dyn/cm^2) or brief exposure to high shear (10 dyn/cm^2) leads to prolonged endothelin release via activation of protein kinase C. Exposure to high shear for a longer period of time (>6 h) results in a marked inhibition of endothelin release via the action of nitric oxide and cGMP.

It has long been known that arteries dilate when blood flow is increased. This process is endothelium-dependent[34,64] and might be a major mechanism for the maintenance of an adequate tissue perfusion. Shear-induced endothelium-dependent vasodilation has been intensively studied in the coronary circulation of animals[38,40], and humans[54]. Blood viscosity correlates with the flow-induced dilator response[51]. Endothelium-derived relaxing factor (EDRF), which is likely to be identical with nitric oxide (NO) plays a key role in this shear-stress-induced vasodilation[63,69]. The shear-stress-induced release of EDRF has been studied in an in vitro

bioassay system using cultured bovine aortic endothelial cells[11].

Prostacyclin (PGI_2), a potent vasodilator and a very important inhibitor of platelet aggregation, is also regulated by shear stress[25,28]. In an in vitro model the sudden onset of flow leads to a rapid increase in PGI_2 production, which is decreased at a constant rate over minutes but remains elevated compared to the condition of no flow[25].

Large arteries undergo a cyclic strain or stretch with each pulse wave. The influence of such a cyclic stretch has been studied with a bioassay[62], and recently on endothelial cells grown on elastic membranes and subjected to cyclic stretch[12], and it has been found that the secretion of the vasoactive substances endothelin[12], EDRF[62] and prostacyclin[12,62] is increased by cyclic strain. Pulsatile flow results in a > 2-fold higher PGI_2 level than steady flow of the same mean shear stress of 10 dyn/cm^2 (ref. 27).

Shear stress regulates a variety of mediators, cytokines, hormones and paracrine substances

These observations are also summarized in the table. The expression of transforming growth factor-beta 1 (TGF-β1) is increased gradually with increasing shear stress in the range 0–40 dyn/cm^2 (ref. 57). Platelet derived growth factor (PDGF) is secreted by endothelial cells[68] and is known to be a vasoconstrictor, and a mitogen for vascular smooth muscle cells. It has been shown that shear stress increases the mRNA levels for PDGF[30] in human endothelial cells via activation of protein kinase C[31].

Histamine formation is increased by increasing shear stress[18]. Renin secretion is modulated by blood viscosity[13], which is a determinant of shear stress (shear stress = shear rate × viscosity).

In this context erythropoietin is interesting. Erythropoietin seems to be secreted by capillary endothelial cells of the kidney[39], and regulates erythropoiesis. By increasing the erythrocyte count it increases blood viscosity. In conditions with high blood viscosity, e.g. hypergammaglobulinemia[48] or sickle cell disease[75], erythropoietin levels were found to be low, which suggests a negative feedback control that tends to keep blood viscosity constant by lowering the hematocrit[48,75]. Indeed, we were able to show that erythropoietin production is regulated by blood viscosity and hence shear stress, at the level of mRNA[76].

Influence of shear stress on the function of other cell types

The function of a variety of other cell types has been shown to be regulated in vitro by shear stress. The production of cAMP in fibroblasts or osteoblasts is shear-dependent[67]. Osteoblasts were even found to be more sensitive to shear stress than endothelial cells[67]; this may be involved in bone remodeling by shear stress. The synthesis and secretion of very low density lipoproteins by hepatocytes is regulated by the viscosity of the culture medium[86], which may play a role in the hyperlipidemia found in nephrotic syndrome, a condition with low plasma viscosity due to hypoproteinemia. It may well be that many more functions of these, and of other – if not all – cell types, can be influenced by shear, in vitro as well as in vivo.

Possible signal transduction mechanisms

The exact mechanism of signal transduction is unknown at the present time. Information on the direction and magnitude of shear forces must be transmitted from the membrane to the cytosol, the microtubule-microfilament system[66] and the nucleus of the endothelial cell. Shear-dependent activation of various ion channels has been described; these include a nonselective, stretch-activated cation channel, allowing sodium and calcium to enter and potassium to leave the endothelial cell[42], and a shear-dependent, selective change in potassium permeability leading to hyperpolarization of the membrane potential[1,58]. It has been shown that shear-induced expression of TGF-β1 is regulated on the mRNA level via this flow-activated potassium channel[57].

Shear stress has been shown to increase the intracellular calcium by increasing the inward flux of calcium[73,74], at least in the presence of adenine nucleotides ATP and ADP[23,52], though this has not been confirmed by others[72]. In hypertensive patients a correlation was found between cytosolic free calcium in platelets and the mean shear stress to which these platelets were exposed in vivo[46]. Shear stress also stimulates endothelial membrane phospholipid metabolism: phosphatidylinositol-specific phospholipid C is activated by shear, which leads to an increase of the metabolites diacylglycerol and inositol 1-, 4-, 5-triphosphate (IP_3)[10], which are second messengers capable of activating protein kinase C and releasing calcium from intracellular stores, respectively. Stimulation of protein kinase C is involved in the production of endothelin, prostacyclin and t-PA. A further mechanism is an increase in cAMP, a modulator of several protein kinases, in endothelial cells exposed to a shear stress of 4 dyn/cm^2 (ref. 67). For the endothelin-1 gene it has been shown that the regulation by shear stress is mediated transcriptionally and is independent of protein kinase C and cAMP[49].

On the level of rapid increase in protein synthesis, evidence exists for an involvement of members of two proto-oncogene families, *fos* and *jun*, which are so-called immediate early genes characterized by rapid gene transcription and very short half-lives[55]. These proto-oncogenes *fos* and *jun* may be involved in the

regulation of various proteins, e.g. tissue plasminogen activator, plasminogen activator inhibitor, endothelin, von Willebrand factor, and TGF-β1[55].

Conclusions

Studies on endothelial cell cultures in vitro have revealed the complexity of the biology of these cells, and it seems that this represents only a beginning. Most studies have been done under 'no flow' conditions. The present review, which does not intend to be comprehensive, makes it clear that the biology of endothelial cells must be studied under shear stress, which is an important regulator of various, often contrary functions, such as vasodilation and vasoconstriction; thromboresistance and thrombogenesis; normal cell morphology and atherosclerosis. Further investigations on shear-dependence of endothelial functions will deepen our understanding of disease processes and eventually allow new therapeutic strategies.

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