REVIEW ARTICLE

The Endothelium in Health and in Cardiovascular Disease

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ABSTRACT. Significant new findings in the last decade have demonstrated that the vascular endothelium is an important regulatory organ in maintaining cardiovascular homeostasis and that endothelial dysfunction is present in several cardiovascular diseases. With the production of multiple vasoactive substances the normal endothelium modulates the tone of the underlying vascular smooth muscle. These include endothelium-derived relaxing factors such as prostacyclin (PGI_2), nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) and vasoconstrictors such as endothelin-1 and angiotensin II. The antiplatelet, antithrombotic and antifibrinolytic properties of the normal endothelium contribute to the maintenance of the fluidity of the blood. Activation or injury to the endothelial cells disrupts the function of the endothelial cells leading to the development of endothelial dysfunction. Endothelial dysfunction is accompanied by vasospasm, thrombosis, and atherosclerosis. It is present in cardiovascular diseases such as hypertension, atherosclerotic heart diseases, congestive heart failure and many others. It has been shown that some therapeutic effects of drugs such as angiotensin-enzyme inhibitors is in part due to the overcoming of endothelial dysfunction.

The endothelium is a layer of cells that lines the inside of all blood vessels. Until recently, it was felt that the endothelium was a passive barrier between the blood and the vessel wall.

At present we know that the vascular endothelium participates in many important physiologic and pathophysiologic functions. These include selective permeability, lipid transport, metabolic activity, maintenance of vascular tone, thrombosis and hemostatis, immunological responses, inflammatory reaction, tumor growth and metastasis, and angiogenesis (1). The endothelium interacts with cellular and soluble factors available in the blood. It also interacts with substances present in the other layers of the vessel wall. Figure 1 shows a cross section of a vessel wall.

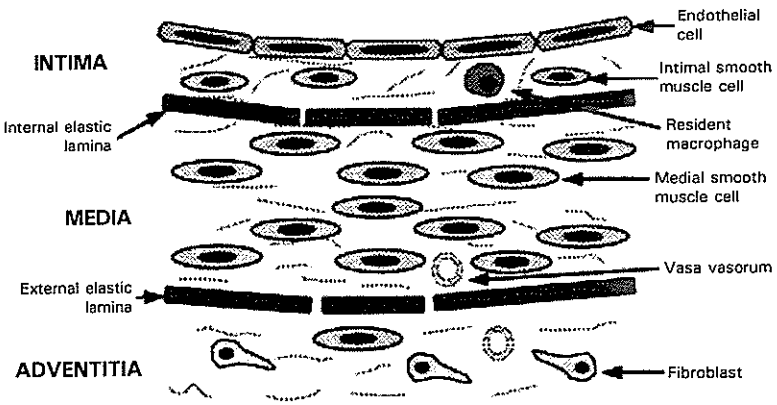

Figure 1. The vessel wall. *Reproduced from ref. 2 with permission of the author.*

As shown in Figure 1 the intima has smooth muscle cells and at times macrophages. The internal elastic lamina separates it from the media which is made of smooth muscle cells which are of great importance for the maintenance of the structural integrity and the vascular tone of the vessel. The adventitia is made of fibroblasts, connective tissue, blood vessels and nerve fibers. The endothelium carries on some of its functions by releasing autocrine and paracrine substances. Its cells act as signal transducers for the cells in the vessel wall. They regulate smooth muscle function as the muscle cells have receptors that permit this regulation to occur.

In 1976 Vane and colleagues discovered that

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prostacyclin (PGI_2) was produced by the endothelium which conveyed the identification of a metabolically active role for endothelial cells for the first time (3). The knowledge about the biology, physiology and pathology of the vascular wall has increased markedly in the past decade. This article summarizes current understanding of endothelial activities.

Today we are aware that the endothelium is involved in three main functions:

1. Serves as a macromolecular barrier
2. Offers a thrombo resistant surface
3. Participates in the modulation and regulation of vascular smooth muscle

When the endothelium is intact it serves as a macromolecular barrier. It possesses selective permeability for substances that can modulate the subendothelial functions. Under normal situations circulating low density lipoproteins (LDL) diffuse into the subendothelial space. If maintained unoxidized, these particles may cleave from the vascular wall back into the blood stream.

The provision of a thrombo resistant surface by the normal endothelium is done by maintaining a non adhesive luminal interior of the blood vessels resulting from anticoagulant, fibrinolytic and antithrombotic processes. The endothelium modulates and regulates smooth muscle tone and is involved in hemostasis, cellular proliferation, as well as in the inflammatory and immune mechanisms in the vessel wall. Because of the multiple functions of the vascular endothelium mentioned above it plays a key role in cardiovascular function in health and in disease. Injury to the endothelium or abnormal functioning of the vascular endothelium is known as endothelial dysfunction. Endothelial cell dysfunction may manifest as vasospasm, thrombus formation, atherosclerosis, or restenosis. In order to perform all its functions the vascular endothelium senses changes in its environment (Fig.2) and responds to the stimuli by producing many biologically active substances called endothelium-derived factors. These factors affects the underlying vascular smooth muscle causing relaxation, contraction or proliferation of the vascular smooth muscle cells. The biologically active substances liberated by the endothelium are small molecules, lipids, and proteins as illustrated in Fig.3.

There are multiple acronyms in use to describe many of the factors or substances related to endothelial function or liberated by the endothelium. Table 1 presents the usual meaning of letters and words frequently used to construct the acronyms related to the endothelium-derived substances.

With this table we expect to help the reader of this review article, as well as, of other articles on endothelium, to recognize the substances liberated by the endothelium

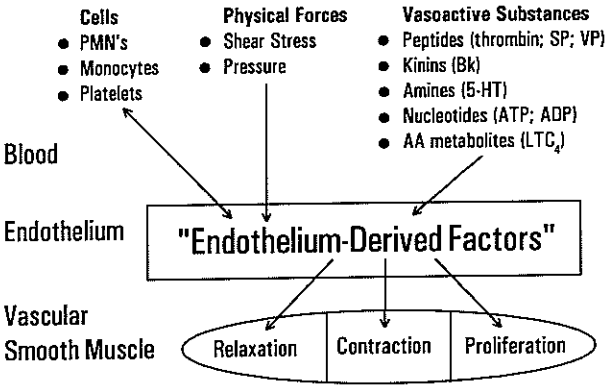

Figure 2. Modulation of the tone and structure of vascular smooth muscle by the vascular endothelium. The endothelial cell has the ability to "sense" changes in hemodynamic (physical) forces, and respond to vasoactive substances (circulating or locally produced), and mediators released from blood cells (e.g., polymorphonuclear neutrophils. PMNs) and platelets. These stimuli then trigger the synthesis/release of biologically active substances from the endothelium. ("endothelium-derived (vasoactive) factors") that modulate the tone (relaxation or contraction) and structure of underlying vascular smooth muscle. By virtue of these recently discovered properties, the vascular endothelium contributes to cardiovascular homeoastasis in a significant way. SP, substance P;VP, vasopressin:Bk, bradykinin; 5-HT, serotonin; ATP, adenosine triphosphate; ADP, adenosine diphosphate; LTC_4, leukotriene C_4. *Reproduced from reference 1 with permission.*

Endothelium - derived mediators	
Small Molecules: • Histamine • Free Radicals • EDRF (NO; R-NO)	**Proteins:** • Endothelin (ET-1) • Growth Factors (PDGF) • Adhesion Molecules (ELAM, ICAM, VCAM) • Matrix Proteins (Heparane-SO_4) • Coagulation Factors (TF, t-PA, PAI, TM, VWF) • Antigens (MHC-II) • Enzymes (ACE) • Receptors
Lipids: • Prostaglandins (PGI_2) • Leukotrienes • PAF	

Figure 3. Biologically active substances produced by the endothelial cell, which contribute to the physiologic and pathophysiologic functions of the vascular endothelium. EDRF, endothelium-derived relaxing factor; PGI_2, prostacyclin; NO, nitric oxide; R-NO nitroso compound; PDGF, platelet-derived growth factor; PAF, platelet-activating factor; ET-1, endothelin 1; PAF, platelet-activating factor, ET-1, endothelin I; ELAM, endothelial leukocyte adhesion molecule; ICAM, intracellular adhesion molecule; VCAM, vascular adhesion molecule; TF, tissue factor; t-PA, tissue plasminogen activator; PAI, plasminogen activator inhibitor; TM, thrombomodulin; VWF, von Willebrand factor; MHC-11, major histocompatibility antigen II; ACE, angiotensin-converting enzyme. *Reproduced from reference 1 with permission.*

usually identified with acronyms. As examples; EDRF means Endothelium Derived Relaxing Factor and ELAM means Endothelium Leucocyte Adhesion Molecule.

Among the normal properties of the endothelium is the maintenance of a normal fluidity of the blood as well as a nonthrombogenic surface. These processes involve many different endothelial cell regulatory mechanisms. The mediators and molecular mechanisms of

Table 1. Letters used in Acronyms

A. Adhesion	I. Intracellular
C. Contracting	L. Leucocytee
D. Derived	M. Molecule
E. Endothelium	P. Platelet
F. Factor	R. Relaxing
G. Growth	T. Tissue
H. Hyperpolarizing	V. Vascular

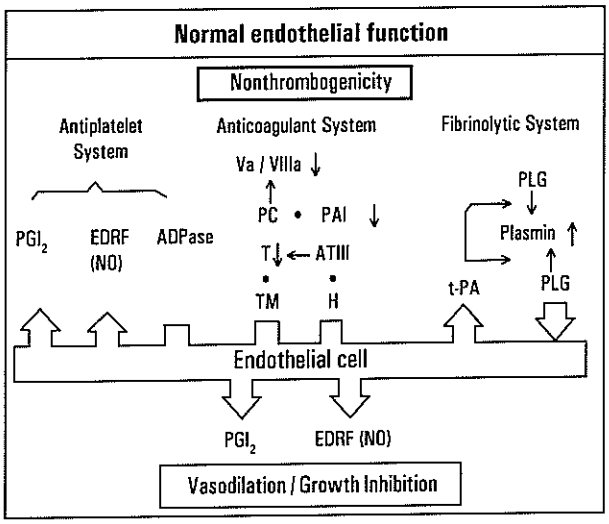

Figure 4. Mediators and molecular mechanisms of the antithrombogenic and vasodilator functions of the normal endothelium. (See text for further details). PGI_2, prostacyclin; EDRF (NO), endothelium-derived relaxing factor (nitric oxide); TM, thrombomodulin; T, thrombin; PC, protein C; H, heparin-like substance; AT III, antithrombin III; PAI, plasminogen activator inhibitor; Va/ VIIIa, coagulation factors; t-PA, tissue plasminogen activator; PLG, plasminogen. *Reproduced from reference 1 with permission.*

nonthrombogenicity of the normal endothelium do occur by provision of the endothelium of antiplatelet, anticoagulant, and fibrinolytic activities. These three systems are illustrated in Figure 4.

The antiplatelet effect is obtained by the inhibitory properties of the endothelium that depend upon the liberation of endothelial-derived prostacyclin (PGI_2) which inhibits platelet activation (4). Platelet derived endoperoxides are converted into PGI_2 by endothelial cells. Also the endothelium- derived relaxing factor, nitric oxide (NO) inhibits platelet adhesion and aggregation (5). Besides, endothelial cell produces ADPase that affects platelet activation at the vessel wall interface with the reduction of the local nucleotide concentration.

The anticoagulant effect is due to the fact that the endothelium synthesizes Heparin-like substances that bind antithrombin III and catalyze the inactivation of the coagulation proteases thrombin and factor X causing an antithrombin activity. The endothelium also produces thrombomodulin, a membrane receptor, that binds thrombin activating protein C which in the presence of protein S inactivates factors Va and VIIIa. Activated protein C also binds plasminogen cell activator (PAI) stimulating local fibrinolysis.

The fibrinolytic effect is initiated by the production and release of endothelial cell tissue plasminogen activator (t-PA) that cleaves plasminogen (PLG) and converts it into plasmin. Also regulatory control of endothelium release of plasminogen activators inhibitor - 1 (PA1-1)

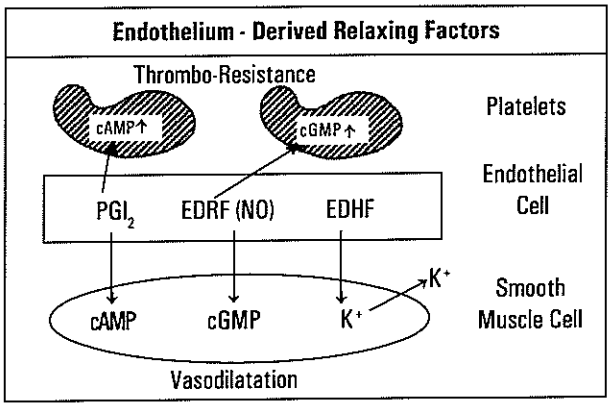

Figure 5. Endothelium-derived relaxing factors (EDRFs). Prostacyclin (PGI_2) and EDRF (identical with nitric oxide (NO) or a labile nitroso compound) relax vascular smooth muscle and inhibit platelet aggregation by elevating tissue levels of cyclic AMP (cAMP) and cyclic GMP (cGMP), respectively. Endothelium-derived hyperpolarizing factor (EDHF) relaxes vascular smooth muscle by membrane hyperpolarization presumably via activating K channels. These factors contribute to the "thrombo-resistant" and "vasodilator" function of the endothelium. *Reproduced from reference 1 with permission.*

influences the fibrinolytic process.

Concerning the third main function of the endothelium, modulation of the vascular tone by the endothelium, this is regulated by the liberation of multiple vasoactive factors substances that might have either relaxing or contracting functions. The endothelium-derived relaxing factors are illustrated in Fig.5.

The observation made by Furchgott and Zawadski in 1980 that the existence of the endothelium is needed for acetycholine induced vasorelaxation lead to the demonstration that an endothelium derived relaxing factor (EDRF) mediates endothelium induced vasorelaxation(6). The three main relaxing substances synthesized by the endothelium are endothelium derived relaxing factor (EDRF) today thought to be identical to nitric oxide (NO), prostacyclin (PGI_2) and the endothelium derived hyperpolarizing factor (EDHF). Prostacyclin is a vasorelaxant and platelet inhibitory metabolite of arachidonic acid. This was the first endothelium derived vasoactive substance discovered. Endothelium dependent

vasorelaxation is mainly mediated by nitric oxide. Its liberation is stimulated by platelet products, thrombin, hormones, neurotransmitters and shear stress among others. The vascular endothelial cells synthesize nitric oxide from L-arginine with the help of the enzyme NO-

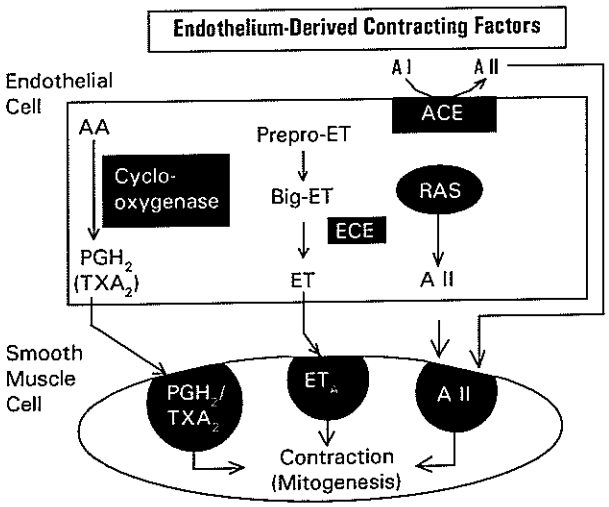

Figure 6. Endothelium-derived contracting factors (EDCFs). Endothelium-dependent contractions evoked by stretch and agonists can be prevented by inhibition of cyclooxygenase, suggesting that the mediator is probably a vasoconstrictor metabolite of arachidonic acid [TXA_2, endoperoxide (PGH_2)]. The recently discovered potent vasoconstrictor polypeptide endothelin also can be regarded as an endothelin-derived contracting factor; however, the stimuli that trigger its synthesis/release under physiological or pathological conditions are still unknown. The renin-angiotensin system (RAS) has been localized in endothelial cells, thus, angiotensin II (A II) can be regarded as an EDCF. AA, arachidonic acid; TXA_2, thromboxane A_2; ET, endothelin; ET_A; ET receptor subtype on smooth muscle; ECE, endothelin-converting enzyme; ACE, angiotensin-converting enzyme. *Reproduced from reference 1 with permission.*

synthase. This enzyme was isolated and characterized in 1991(7). In the vascular system NO is released from the endothelium stimulated by Calcium-mobilizing agonists or physical forces. NO also inhibits platelet adhesion and aggregation and smooth muscle cell growth. The integrity and liberation of NO are basic to the normal function of the endothelium. The endothelium-derived contracting factors are illustrated in Fig.6.

Vasoconstriction depending on the endothelium can be stimulated by arachidonic acid, norepinephrine, postaglandin H_2, stretch pressure and hypoxia among others. The main substances mediating endothelium dependent vasoconstriction are thromboxane A_2 and the potent 21-amino vasoconstrictor peptide, Endothelin produced by the endothelial cells (8). We also know that the renin-angiotensin system influences vascular tone. Renin produced by the kidney converts the liver secreted angiotensinogen to angiotensin I which is converted to angiotensin II by angiotensin-converting enzyme (ACE), mainly in the lumen of vascular endothelium of the lung. ACE has been localized in the vascular endothelial cells and also the synthesis of angiotensin II occurs within the vascular wall. Today angiotensin II is considered an endothelium derived contracting factor. The most potent of the vasoconstrictor factors generated by the vascular endothelium is endothelin. It was first isolated in 1988 (8). It has paracrine and systemic effects. Endothelin seems to be more important in pathophysiologic settings than in the minute to minute regulation of vascular tone (9).

Endothelial dysfunction. Dysfunction of the endothelium is the result of an imbalance between the relaxing and contracting factors, between anti and procoagulant mediators as well as growth inhibiting and growth promoting factors. This imbalance is produced by abnormal endothelial cell function generated by injury or activation of the endothelium. Many endothelium-derived substances have multiple functions. The contracting factors stimulate and the relaxing factors inhibit, the growth of vascular smooth muscle. It has been found that dysfunction of the tone-regulating mechanism may have important relevance to clinical conditions such as hypertension, atherosclerosis, diabetes and restenosis.

Abnormalities of endothelium-relaxation in essential hypertension. After the demonstration that the contractile state of vascular smooth muscle was dependent on the presence and integrity of endothelial cells, abnormal endothelial function has been recognized in several cardiovascular conditions, such as essential hypertension. Animal models show that a deficiency of endothelium-derived nitric oxide contributes to both volume-related hypertension or to hypertension resulting from resistance vessels constriction. Panza has identified an abnormality of endothelium-mediated vasodilatation in hypertensive patients that is related to decreased activity of NO (10). The precise mechanism has not been identified as yet but may involve decreased production or increased breakdown of this factor. Due to the biological significance of NO in the regulation of vascular tone, the reduction in its activity must play a role in the hypertensive process. There is also evidence that endothelin elevations may play a role in the hypertension associated with chronic renal failure. Patients with chronic renal failure and hypertension have higher plasma endothelin levels than do patients with chronic renal failure without hypertension. Other endothelium-derived factors such as prostacyclin and endothelin-I do not appear to have major significance in most types of hypertension.

Abnormalities of endothelium in atherosclerosis and hypercholesterolemia. Besides having identified the presence of endothelial dysfunction in hypertension, hypercholesterolemia and atherosclerosis it has been encountered in many cardiovascular diseases entities.

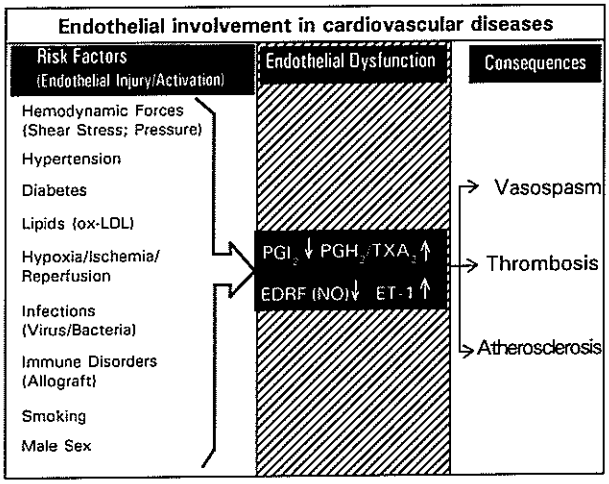

Figure 7. Common cardiovascular risk factors causing endothelial cell injury activation, which leads to endothelial dysfunction and its clinical consequences of vasospasm, thrombosis, and/or atherosclerosis. Ox-LDL, oxidized low-density lipoprotein; PGI_2, prostacyclin; TXA_2, thromboxane A_2; EDRF (NO), endothelium-derived relaxing factor (nitric oxide); ET-1, endothelin-1. *Reproduced from reference 1 with permission.*

These are congestive heart failure, thrombosis and coagulopathies, diabetic angiopathies, peripheral artery disease, inflammatory diseases, reocclusion and restenosis. It is beyond the scope of this article to review the specific

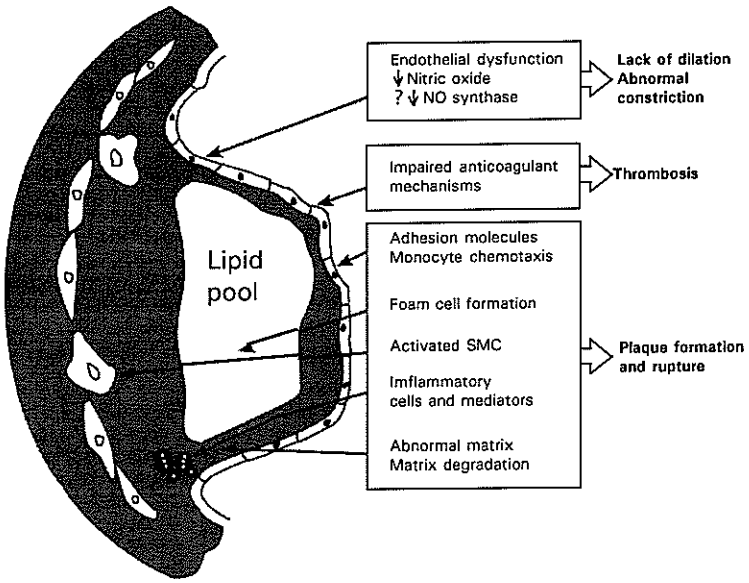

Fig .8 Important cell dysfunctions in advanced atherosclerosis lesions. *Reproduced from reference no. 11 with permission of the author.*

mechanisms of cell dysfunction of all these entities. The cardiovascular risk factors can alter the vascular endothelial functions leading to the known effects of endothelial dysfunction of vasospasm, thrombosis and atherosclerosis. Figure 7 illustrates this.

Endothelial dysfunction permits the lipids to affect the production of NO and also promote the release of endothelin, growth factors, angiotensin II, and thrombin creating an environment that is conducive to plaque formation. Hypercholesterolemia may stimulate the endothelium to increase the production of growth factors. The endothelial cells can modify LDL by oxidation. Oxidized LDL inhibits NO - dependent relaxation and causes direct contraction. This leads to vasospasm and thrombus formation. Oxidized LDL stimulates the formation of endothelin. Oxidized LDL in the arterial wall, by inhibition of endothelium-dependent relaxation and the promotion of vascular contraction and thrombus formation, is an important factor in atherogenesis. Growth factors released by endothelial cells by promoting migration and proliferation of vascular smooth muscle

cells are involved in the development of atherosclerosis lesions. Diet induced experimental atherosclerosis impairs endothelium-dependent vasorelaxation both in vitro and in vivo. Atherosclerotic human coronary arteries exhibit impaired endothelium dependent relaxation after intracoronary infusion of acetylcholine. Reduced synthesis of NO leads to the presence of more superoxide anion radicals which enhances the ability to oxidize LDL and develop atherosclerosis. The reduced release of NO and the facilitation of the production of contracting factors and growth-promoting mediators (TXA_2 PGH_2, endothelin, Angiotensin II and PDGF) can lead to vasospasm.

Figure 8 illustrates the important cell dysfunctions characteristic of advanced atherosclerosis lesions. These conditions exist throughout the pathogenesis and progression of plaque formation and precedes the functional consequences beleived to cause ischemia and plaque rupture.

Beside having identified the presence of endothelial dysfunction in hypertension, hypercholesterolemia and atherosclerosis it has been encountered in many cardiovascular disease entities. Endothelial cell injury has been considered as a basic alteration for the development of an atherogenic process. Circulating monocytes become activated and adhere to the injured endothelial surface. They migrate into the intima where they take the oxidized extracellular low density cholesterol and develop into foam cells. Concurrently growth factors liberated by platelets, endothelial cells and macrophages promote smooth muscle cell proliferation. There is synthesis of extracellular

matrix proteins all of which through time leads to the occurrence of the atherosclerotic plaque.

Endothelial dysfunction and therapy. Today, angiotensin converting enzyme (ACE) inhibitors have become a very important therapeutic tool in the treatment of hypertension and also of congestive heart failure. Clinical trials in patients with congestive heart failure suggest that ACE inhibitors have effects that go beyond their antihypertensive action by preventing or ameliorating endothelial dysfunction. The main antihypertensive effect of ACE inhibitors depend upon preventing the conversion of Angiotensin I into Angiotensin II. It is also known that angiotensin converting enzyme also facilitates the conversion of bradykinin into inactive peptides. ACE inhibitors act in part by inhibiting the bradykinin hydrolysis and so stimulate the release of prostaglandins and nitric oxide which cause vadilatation overcoming some of the spasm of the endothelial dysfunction. Many of the short term reduction in mortality from atherosclerotic heart disease obtained in recent clinical trials is believed to be due to reversal of endothelial dysfunction rather than regression of the atherosclerotic plaque.

Resumen

Hallazgos nuevos significativos han demostrado que el endotelio vascular es un órgano regulador importante para mantener la homeoastasis cardiovascular y que la disfunción endotelial ocurre en varias enfermedades cardiovasculares. Con la producción de múltiples substancias vasoactivas, el endotelio normal modula el tono del músculo liso subyacente. Estas substancias incluyen factores relajantes derivados del endotelio como la prostaciclina ($PG1_2$), el óxido nítrico (NO), el factor hiperpolarizante derivado del endotelio (EDHF) y vaso constrictores como la endotelina y la angiotensina II. Las propiedades antiplaquetarias, antitrombóticas y antifibrinolíticas del endotelio normal contribuyen al mantenimiento de la fluidez de la sangre. La activación o el daño a las células endoteliales afectan la función de éstas causando el desarrollo de la disfunción endotelial. La disfunción endotelial está acompañada de vasoespasmo, trombosis y de aterosclerosis. Esta disfunción ocurre en las enfermedades cardiovasculares como la hipertensión, enfermedad aterosclerótica del corazón, insuficiencia cardíaca congestiva y otras. Se ha demostrado que algunos de los efectos terapéuticos de los inhibidores de la enzima convertidora se debe en parte a contrarestar la disfunción endotelial.

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