ENDOTHELIAL CELLS ARE ACTIVE

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SUMMARY

The vascular endothelium was long regarded as simply a diffusion barrier, but now it is recognized that endothelium is a metabolically active mediator of interactions between blood and underlying smooth muscle. Metabolic functions of endothelial cells include the enzymatic destruction of serotonin, norepinephrine, the conversion of angiotensin I to potent vasoconstrictor angiotensin II and the breakdown of potent vasodilator bradykinin. Endothelial cells also synthesize and secrete vasodilator and antiaggregant prostacyclin and endothelium derived relaxing factor. These cells are normally nonthrombogenic and unstimulated platelets do not adhere to the surface but when the cells are injured platelets adhere to the exposed subendothelium. Coagulation can be initiated by damaged endothelial cells because they bind coagulation factors and may activate them. Endothelium can control coagulation by activation of anticoagulant protein C and by increasing the affinity of antithrombin III for thrombin. Endothelial cells contribute to the process of fibrinolysis by synthesizing plasminogen activators and plasminogen activator inhibitors.

Key Words: Endothelium, vascular tone, hemostasis, barrier

INTRODUCTION

Important role of endothelial cells in many important physiologic processes could be understood during the past 2 decades as a result of the ability to culture endothelial cells from an increasing number of vessels and species. Endothelial cells covering all the luminal surface of vasculature are multifunctional cells. They are modulators of hemostasis, regulators of vascular tone and gatekeeper for the movement of solute between blood and tissue. Endothelium can now be regarded as a metabolically active tissue distributed throughout the body.

Endothelial cells, which form a monolayer, look like flagstones. They are polygonal cells with dimensions varying between 10-50 μm to 25-40 μm. Cells are arranged with their long axis parallel to the blood flow. Endothelial cells rest on subendothelium which anchors the cells to the blood vessel. Subendothelium is secreted by endothelial cells and contains collagen, elastin, laminin, mucopolysaccharides, microfibrils and fibronectin. The luminal surface of endothelial cells are covered with a glycoprotein (glycocalyx) coat secreted by endothelial cells, which has negative charges that repells the similarly negatively charged circulating blood cells thus forming a nonthrombotic surface. The glycocalyx coat is not uniform but rather differentiated into domains generated by heterogenous distrubution of anionic sites which may be responsible for the physiological junctions and permeability of the layer. The highest concentration of the anionic sites are found on the fenestreal sites and they are primarily due to heparin like substances (1,2).

Endothelium as a barrier

Strategic position of the endothelial cells enables them to regulate the transport between tissues and blood. They form a selectively permeable barrier which resists the passive transfer of solutes and cells. Transendothelial passage of hydrophilic solutes is accomplished either by vesicles (endocytosis), or by passage between the cells (diffussion). Vesicles formed by the plasma membrane sometimes continue down through the cytoplasm forming channels which are important for the transport. Pits and vesicles may be involved in receptor mediated endocytosis of specific macromolecules such as LDL. Diffussion between endothelial cells is less than the diffussion between other cells like fibroblasts, smooth muscle cells because of the (tight, gap) junctions present between them (3). But there are important differences in junctions not only between vascular beds in different organs but also between different segments of a single vascular bed. In continuous capillaries, like those in the brain, testes, thymus frequent tight junctions between endothelial cells form an effective barrier between the tissues and plasma. In other tissues, the space between adjacent endothelial cells varies from 10 to 200nm. In endocrine glands, glomeruli of the kidney, intestine, endothelial cells have several openings

called "fenestrea" (approximately 700nm) in their cytoplasm. These fenestrations are covered by a proteinpolysaccharide diaphragm through which passage of substances cannot freely take place. In liver, spleen, bone marrow capillaries, endothelial cells are separated from each other, so the passage of larger molecules and even whole cells like erythrocytes is possible. Since the exchange of substances between the blood and tissues occur mainly in capillaries the heterogeneity of structure has a function (4-6). Permeability of the endothelial layer can also be influenced by substances like histamine, serotonin, bradykinin; serotonin decrease vascular permeability while histamine and bradykinin increase permeability of the layer (7).

Control of vascular tone

On the luminal surface of endothelial cells there are receptors for serotonin, histamine, kinins, angiotensin epinephrin, norepinephrine, acetylcholine and thrombin. In addition enzymes and enzyme inhibitors function on luminal surface and influence their environment by handling bioactive compounds in different ways. Endothelial cells possess multiple enzyme systems which enable them to influence blood flow, blood pressure and vascular tone (8). Pulmonary endothelial cells can metabolize a potent vasodilator, bradykinin and convert vasoinactive angiotensin I to potent vasoconstrictor angiotensin II using the same enzyme, a carboxypeptidase (angiotensin converting enzyme) (9,10). Also, serotonin, a vasoactive amine is taken by active transport and metabolized intracellularly by pulmonary endothelial cells. A potent vasodilator adonosine can also be taken and inactivated by these cells.

Endothelium contributes to local vascular tone regulation by secreting vasodilator substances such as prostacyclin (PGI2) and endothelium derived relaxing factor (EDRF) and by releasing endothelium derived contracting factors (EDCF1), (EDCF2) and endothelin (EDCF3).

Prostacyclin is the major product of the blood vessel cyclooxygenase. PGI2 is a potent vasodilator and inhibitor of platelet aggregation. Actions of prostacyclin are mediated by stimulation of adenylate cyclase in platelets and vascular smooth muscle cells. Endogenous substances leading to the generation of PGI2 are bradykinin, arachidonic acid, thrombin, trypsin, platelet derived growth factor (PDGF), epidermal growth factor, interleukin1 and adenine nucleotides. Glucocorticosteroids, testesteron, cyclooxygenase inhibitors, LDL, and vitamin K1 inhibit biosynthesis of prostacyclin in endothelial cells (11,12).

EDRF is a labile vasodilator with a half life of few seconds. EDRF was discovered by Furchott and Zawadzki who showed that the presence of endothelial layer is obligatory for the vasorelaxant action of acetylcholine (ACh) on arterial strips. When endothelium is removed from the vascular preparations ACh has no vasorelaxant effect and often evokes contraction of vascular smooth muscles. Thus the activation of muscarinic receptors on endothelium triggers the generation of EDRF. EDRF was identified as nitric oxide by Palmer et al but it can also be a labile nitrosothiol which in turn will form NO. NO is formed from the guanidino nitrogen atom of arginine, and NO synthetase in endothelium has been shown to be a Calmodulin-$Ca+^2$ dependent enzyme. EDRF potentially inhibit platelet aggregation and adhesion. Effects of EDRF both on vascular smooth muscle cells and platelets are mediated by an increase in intracellular cyclic guanosine 3'-5' monophosphate (cGMP). Substances that release EDRF from the cells include bradykinin, angiotensin II, histamine, norepinephrine, adenine nucleotides, and thrombin. Also pulsatile pressure, visible light stimulate release of EDRF (13-18).

Endothelin is a potent vasoconstrictor peptide with 21 aminoacids, the regulation of its synthesis and release is not fully understood, but in vitro studies suggest that agents such as angiotensin and thrombin or altered shear stress enhances endothelin production (19-21).

An altered ability of endothelial cells to make some of these factors has been associated with cardiovascular diseases including hypertension and atherosclerosis (19).

Antithrombotic and anticoagulant properties

The vessel wall not only forms a barrier between blood and tissues but also prevents activation of hemostatic system by creating a nonthrombotic surface. Moreover endothelium participates actively in the regulation of hemostatic processes by its ability to synthesize, secrete and express proteins (like fibrinolytic factors, inhibitors), prostaglandins and glycosaminoglycans (heparan sulfate).

Interactions of blood constituents with endothelial cells are essential for maintaining a balanced hemostasis. Since more than 80% of endothelial cells are found in capillary bed with a high ratio of cell surface area per unit blood volume, during each passage through the capillary bed, platelets are inactivated by high concentrations of prostacyclin secreted by endothelial cells. Also small amounts of activated coagulation factors are inactivated either by antithrombin III or protein C in the presence of their physiological activators which are located on the endothelial cells (22).

Healthy endothelial cells present a noncoagulant and

nonthrombotic surface to flowing blood and prevent the constituents of blood from interacting with subendothelium. The antithrombotic and anticoagulant properties of endothelial cells are due primarily to secretion of PGI2 and EDRF and to the presence of negatively charged glycosaminoglycan (mostly heparan sulfate) layer on endothelial cells. Heparan sulphates bind antithrombin and markedly increase its affinity for thrombin, thus provide the most important physiological mechanism for the inactivation of thrombin. Also endothelial cells have important role in the activation of anticoagulant protein C which is a protease circulating in plasma as a zymogen and is rapidly activated by thrombin. Activated protein C inactivates factor V and VIII and enhances activation of plasminogen. Endothelial cells markedly accelerate the activation of protein C by an endothelium associated cofactor called thrombomodulin. Another cofactor protein, protein S, which is also syntesized by endothelial cells enhances the activity of protein C (23-25).

Heparin cofactor II is a trombin directed anticoagulant and it has appreciable homology to antithrombin III. But its activity is restricted to the presence of heparin or other sulfated glycosaminoglycans which are on endothelial cell surface (22).

Procoagulant and prothrombotic properties

Under normal circumstances endothelium has nonthrombotic and anticoagulant properties but when the blood vessels are injured endothelial cells can activate coagulation mechanisms. Damaged endothelial cells may provide proteolytic enzymes and a favourable surface for the activation of factor XII; thus initiate the intrinsic pathway of coagulation. Also injured cells can synthesize tissue factor (TF, thromboplastin) and there- fore trigger the extrinsic pathway of coagulation (23-26).

TF synthesis by endothelial cells is increased by cytokines such as interleukin 1, tissue necrosis factor, and bacterial lipopolysaccharides. These agents provoke changes that alter the balance of endothelial cell behaviour in the procoagulant direction (24).

Moreover after an injury, the exposed subendothelium of the vessel activate platelets and they adhere to collagen and microfibrils. Collagen of subendothelium is capable of causing platelets in the vicinity to aggregate and adhere to collagen (23-26).

Von Willebrand factor (VWF) which is necessary for the adhesion of platelets to subendothelium, particularly in conditions of high shear stress is synthesized by endothelial cells. VWF is the carrier for clotting factor VIII. Platelets contain surface receptor for F VIII / VWF and adhere to collagen via F VIII / VWF (27-29).

Role in fibrinolysis

Fibrinolysis is an integrated part of hemostasis. Overactivity of the fibrinolytic system causes bleeding and underactivity causes thrombosis. Fibrinolytic system consists of plasminogen and those molecules that convert this inactive proenzyme into active trypsin like enzyme plasmin and their inhibitors. The formation of plasmin provides important source of localized proteolytic activity not only during fibrinolysis but also during neovascularization, tissue repair, macrophage activation, metastasis and several other physiologic processes. Since plasminogen is present in most body fluids, changes in the availability and activity of plasminogen activators (PA) and plasminogen activator inhibitors (PAIs) regulate the activity of this enzyme system. Two distinct PA are known: urokinase (uPA) and tissue plasminogen activator (tPA). Endothelial cells synthesize and secrete both tPA and uPA, thus play an important role in vascular fibrinolysis. Precise regulation of these two PAs may be controlled by synthesis and secretion or through the action of specific plasminogen activator inhibitors (30-32).

PAIs inactivate plasminogen activators by forming covalant complexes. Endothelial cells being the most important source of these inhibitors express their important role in fibrinolysis (32).

The variety of functions described above suggest that endothelial integrity is necessary for the normal functioning of blood vessels and maintaining a nonthrombotic state and that endothelial damage can initiate thrombosis.

Recent work has shown that a certain drug (Defibrotide, Prociclide [Crinos]) effects the overall growth characteristics, PGI2 secretion and protein content of cultured human umbilical vein endothelial cells. Increase in protein content is due to inceased amounts of tPA which is varified by the interaction of labelled drug with cell nuclei (33-35).

Better characterization and control of these functions will contribute to unrevealing of some vascular disorders, mainly atherosclerosis, hypertension, and will enable a more effective cure for such diseases.

REFERENCES

1- *Wehrmacher WH. Endothelium : From Whence To Whiter. Seminars in Thrombosis and Hemostasis. 1988; 14 (suppl) : 1-11.*

2- *Jaffe EA. The role of blood vessels in hemostasis. Vascular function in hemostasis. In: Williams JW,*

ed. Hematology, 4th ed. New York: Mc Graw-Hill, 1990:1322-1337.
3. Kelly DE, Woods RL, Enders AC. Bailey's Textbook of microscopic anatomy. 18th ed. Baltimore, London: Williams and Wilkins, 1984: 387.
4. Bottaro D, Shepro D, Hechtman HB. Heterogenity of intimal and Microvessel endothelial cell barriers. In vitro microvascul Research 1986; 32: 389-398.
5. Bottaro D, Shepro D, Peterson S, Hechtman HB. Serotonin, norepinephrine and histamine mediation of endothelial cell barrier function in vitro. J of Cell Phys 1986 ;128 : 189-194.
6. Simionescu M, Simionescu N, Palade GE. Morphometric data on the endothelium of blood capillaries. J Cell Biol 1974; 60 : 128-152.
7. Simionescu M, Simionescu N, Palade GE. Permeability of muscle capillaries to small hemepeptides. J Cell Biol 1975; 64: 686.
8. Kaiser L, Sparks HV. Endothelial cells not just a cellophane wrapper. Arch Intern Med 1987; 147:569-573.
9. Caldwell PRB,Seagal BC, Hsu KC, Das M, Softer RL. Angiotensin converting enzyme: Vascular endothelial localization. Scien 1976; 191: 1050-1051.
10. Johnson AR, Erdös EG. Metabolism of vasoactive peptides by human endothelial cells in culture. The J of Clin Invest 1977; 59:684-695.
11. Moncada S, Vane JR. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A2 and prostacyclin. Pharmacol Rev 1979; 30: 293-331.
12. Weksler BB, Ley WC, Jaffe EA. Stimulation of endothelial cell prostacyclin production by thrombin, tyripsin and the ionopore A 23187. J Clin Invest 1978; 62: 923-930.
13. Furchgott RF, Zwadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 299: 373-376.
14. Marletta MA. Nitric oxide: Biosynthesis and biological significance. Tibs 1989; 14 : 488-492.
15. Ignorro LJ. Biosynthesis and metabolism of endothelium derived nitric oxide. Annu Rev Pharmacol Toxicol 1990; 30:535-560.
16. Ignarro LJ. Biological actions and properties of endothelium derived nitric oxide formed and released from artery and vein. Circ Res 1989; 65:1-2.
17. Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginin. Nature 1988;333:664-666.
18. Rapoport RM, Murad F. Agonist induced endothelium dependent relaxation in rat thoracic aorta may be mediated through cGMP. Cir Res 1983; 52: 352-357.
19. Lüscher TF. Endothelium derived relaxing and contracting factors potential role in coronary artery disease. Eur Heart J 1989; 10:847-857.
20. Yanagisawa M, Kurihara H, Kimura S, et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988; 332: 411-415.
21. Inoue A, Yanagisawa M, Kimura, S. et al. The human endothelin family tree structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci 1989; 2863-2867.
22. Preissner KT. Anticoagulant potential of endothelial cell membrane components. Haemostasis 1988; 18: 271-306.
23. Thrombosis : de Gruchy's. In:Firkin F, Chesterman C, Penington D, Rush B, eds. Clinical haematology in medical practice. 5th ed. Blackwell scientific Publications, 1989: 454-474.
24. Pearson JD. Endothelial cell biology. Radiology 1991;179:9-14.
25. Williams JW. Thrombosis. In:Williams JW, ed. Hematology. 3rd ed. New York: Mc Graw Hill 1983: 1474-1481.
26. Gordon JL, Pearson JD. Biology of the vascular endothelium. In : Bloom AL, Thomas DP, eds. Haemostasis and Thrombosis .Edinburg: Churchill Livingstone, 1987: 303-308.
27. Jaffe EA, Hoyer LW, Nachman RL. Synthesis of antihemophilic factor antigen by cultured human endothelial cells. J Clin Invest 1973; 52;2757-2764.
28. Bloom AL. Physiological and clinical developments concerning factor VIII. Hemost. Thromb. In: Walter Bowie EJ, Sharp AA, eds. Butterworths, 1985; 98-147.
29. Sakariassen KS, Bolhus PA, Sixma JJ. Human blood platelet adhesion to artery subendothelium is mediated by factor VIII-vWWF bound to the subendothelium. Nature 1979;279: 636-638.
30. Hart DA, Rehemtulla A. Plasminogen activators and their inhibitors: Regulators of extracellular proteolysis and cell function. Comp Biochem Physiol 1988; 90 B (4): 691-708.
31. Schleef RR, Kutoff DJ. Fibrinolytic system of vascular endothelial cells. Haemostasis 1988;18: 328-341.
32. Kruithof EKO. Plasminogen activator inhibitors. Enzyme 1988; 40: 113-121.
33. Löbel P, Schrör K. Stimulation of vascular prostacyclin and inhibition of platelet function by oral defibrotide in cholesterol fed rabbits. Atherosclerosis 1989; 80: 69-79.
34. Bilsel S, Taga Y, Yalçın AS, Emerk K, Ulutin ON. Effect of defibrotide and endothelial cell growth supplement on human endothelial cells in culture. Hematol Rev 1989; 3:29-34.
35. Bilsel S, Yalçın AS, Taga Y, Emerk K. Interaction of 3H-defibrotide with cultured human umbilical vein endothelial cells. Thromb Res 1990;58:455-460.

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