LECTURE

ΔΙΑΛΕΞΗ

The pathogenesis and laboratory findings of atherosclerosis from hemostasis standpoint

In a brief review, the pathogenesis of atherosclerosis, as related to the mechanism of hemostasis, is discussed. The types of disorders that have been noted concerning the hypo- and dys- function of the endothelium, the decrease of fibrinolytic activity, the increase of several coagulation factors, the decrease of natural inhibitors and the proliferation and migration of smooth muscle fibers are extensively described. The findings of the author and his colleagues are presented.

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Παθογένεια
και εργαστηριακά ευρήματα
στην αρτηριοσκλήρυνση
από τη σκοπιά της αιμόστασης

Περίληψη στο τέλος του άρθρου

Key words

Atherogenesis
Atherosclerosis
Hemostasis
Platelets

1. INTRODUCTION

In this short review, I am going to discuss atherosclerosis from hemostasis and thrombosis standpoints, as well as our laboratory findings in the hemostatic system.[1,2] Vessel wall alteration was first cited by Imhotep in ancient Egypt few thousand years ago. He showed the difference of young and old people during mummification and this was written down on papyrus as well as on rocks. Since then many publications mentioned this as a degenerative process. In 1833 J.F. Lobstein and in 1904 F. Marchand named this condition "arteriosclerosis" while "atherosclerosis" is used during the last millenium.

There are various hypotheses regarding the pathogenesis of the disease. In recent years many hypotheses, theories and experimental studies on atherosclerosis were published and the pathogenesis of the disease was investigated in biochemical, molecular biology and molecular genetics perspectives. Most of the studies published in recent years were on lipid changes, from genetic defects to receptors; these studies have shown strong correlation between changes and defects in lipoprotein metabolism and development of atherosclerosis.

We are going to present our data supporting hemostatic theory that has really been introduced more than 150 years ago. This depends on abnormal deposition of blood components including fibrin in the intima. In 1856 Virchow proposed the tissue response to endothelial injury theory. Both theories got supporting evidence through experimental models. We will discuss the recent views later on in our lecture. Firstly, I would like to summarize our observations and results mainly from the hemostasis standpoint.[1–4,27,45,46,60]

I would, at this point, like to summarize what type of alterations take place in this group of cases: (a) hypo/dysfunction of endothelial cells (EC), (b) decreased and altered fibrinolytic system, (c) increase of some coagulation factors, (d) decrease of natural inhibitors activities with/without molecular abnormalities, (e) hyperactivity of platelets with some functional and biochemical alter-

ations, (f) smooth muscle cell hypertrophy, hyperplasia and also migration.[1,2,4]

a. There is increasing evidence that the EC is involved in many functions of the vascular system especially in the hemostatic mechanism. The EC has a strong antithrombotic function due to the substances that contains and synthesizes. In atherosclerosis these functions and substances are reduced (tabl. 1). The inner surface of EC has a proteoglycan layer that is one of the reasons it is non-wetable. In atherosclerosis Prostacyclin (PGI_2) production and release progressively decrease. Prostacyclin is a very potent antiaggregator and vasodilator substance and increases the formation of cAMP from ATP. Prostacyclin (PGI_2) decreases significantly in experimental and clinical atherosclerosis.[3–5] The plasma 6-keto-PGF1a levels for normal controls are 96.4 pg/mL and for atherosclerotics 37.7 pg/mL difference that was statistically significant $P<0.001$ in our patients.

EC also produces nitric oxide (NO). NO is a vasodilator, that decreases the tone of smooth muscle and also inhibits aggregation and adhesion of platelets. PGI_2 and NO act synergistically. It has been shown that the level and production of NO decreases in atherosclerosis.[5–7]

On the other hand, in 1988, Yanasigawa et al,[7,8] isolated a polypeptide from endothelial cells and named it "endothelin". Endothelin has a strong vasoconstrictor activity, increases the smooth muscle tone, stimulates platelet and mitogen formations. PGI_2 and NO counteract with endothelin. In the case of atherosclerosis endothelin level is increased.[7,12]

Tissue factor (TF) content and its availability increase in the case of atherosclerosis.[12] As we know, TF and factor VIIa have a role in the activation of extrinsic system and also TF activates factor IX in the intrinsic system of coagulation mechanism. TF expression increases in atherosclerosis with and without angina and myocardial infarction. Also, TF is released from monocytes in the cases of hypercoagulability associated with angina and myocardial infarction.

Table 1. The alteration of some endothelial cell function in the cases of atherosclerosis.

Decrease answer to venous stasis test
Decrease of PGI_2 formation and release
Decrease of NO production
Decrease and/or alteration of thrombomodulin
Decrease/increase of t-PA production and release
Increase PAI 1 production and release
Increase of endothelin production and release
Increase of TF production and availability
Increase of vWF production/release
Increase of PAF production/release

Table 2. Functional and biochemical alteration of the platelets in the cases of atherosclerosis.

Increase of platelet adhesion with and without other platelet defect
Alteration of foreign surface activation
Hyperaggregation with aggregating agents
Occasionally spontaneously platelet aggregation
Hypersecretion
Increase of platelet markers in plasma
Increase of platelet α_2-antiplasmin
Decrease of platelet AT-III
Decrease in gamma glutamyl transferase in platelets
Increase in PGF_{2a}, PGE, MDA and TXA_2
Defective glucose membrane transport to the platelets and red blood cells
Alteration of membrane phospholipids
Increase of galactose transportation to the platelets and some alteration of membrane glucoproteins
Shortening half life of the platelets
In some cases an "acquired storage pool deficiency of platelets" occurred

Table 3. Some observations in the young members of atherosclerotic families.

	Parents	Young member	Young control
Defective cuff test (%)	82.6	43.7	11.6
Decreased prostacyclin (%)	72.8	31.9	9.8
Platelets hyperadhesion, hyperaggregation and hypersecretion (%)	85.2	22.4	8.3
Glucose transport defect (%)	84.2	32.3	4.5
Increase of ß-TG, PF-4	89.9	16.7	5.2

A substance produced by the endothelial cells named TFPI (tissue factor pathway inhibitor) inhibits the action of TF, TF/VIIa and Xa. So, coagulation mechanism is inhibited by TFPI in a very early stage on the EC and its surface, a fact that was difficult to demonstrated with standard coagulation tests. This demostrates an additional observation suggesting that anticoagulant activity is different from the antithrombotic activity. In blood, Lp(a) binds TFPI and inactivates it by approximately 60–70%. Only free TFPI inhibits certain substances. In the cases of atherosclerosis Lp(a) levels increase, so that TFPI activity decreases. Some drugs, such as heparin, LMW heparin, and defibrotide stimulate the production and release of TFPI.[17] Recently, it has been shown that a molecular abnormality exist in human TFPI, in some cases of thromboembolism, especially in cases with venous thrombosis.[13,23]

Tissue plaminogen activator (t-PA), urokinase (UK) and plasminogen activator inhibitor 1 (PAI 1) are synthesized in EC. It has also been shown that fibrinolytic activity decreases in the case of atherosclerosis, due to decrease of t-PA or increase of PAI 1.

The production of vWF increases in the case of atherosclerosis so that platelet adhesion also increases. In the same condition fibrinogen and factor VIII also increase. It has been demonstrated that a stimulation of EC by thrombin, histamin, bradykinin, leukotriens, etc., increases the level of platelet activating factor (PAF). This increase is more marked in cases of atherosclerosis.[1,56] In atherosclerosis endothelial cell function is altered and so the antithrombotic role of endothelial cells decreases.

b. Fibrinolytic system activity shows different alterations in cases of atherosclerosis with and without thrombus formation. t-PA and PAI 1 is synthesized in EC and in one sense control the fibrinolytic activity. The balance between PAI 1 and t-PA is important in the cases of atherosclerosis with or without thrombosis.[25,27] Out of 250 cases with atherosclerosis PAI 1 was increased in 207 cases and t-PA was decreased in only 24 cases while it remained unchanged in 19 cases. Also, a molecular abnormality has been described in PAI 1 and PAI 2. In a group of MI (n=66) versus a healthy control group (n=20) a significant difference in PAI 2 was found. Similar differences were found in ACE patients with MI, as compared with normal control cases.[42,43]

There are several studies showing the relation between fibrinolytic system and the development of atherosclerosis. There are many animal experiments showing the inhibition of fibrinolytic system that cause atherosclerotic changes in vascular wall and promote thrombus formation. On the other hand, the activation of fibrinolytic system in animals prevents or decreases EC alteration and also smooth muscle alteration.

c. In the cases of atherosclerosis with and without thrombosis some coagulation factor levels increase and circulate in activated forms and cause hypercoagulability. We can measure their plasma levels immunologically or their procoagulant activity or their activation peptides. In atherosclerosis some factors increase such as fibrinogen, FVIII, vWF, XII, IX,X,VII,V, etc. In this group of cases prothrombin fragments 1+2 and activation peptides of IXa, Xa, XIIa also increase. Fibrinogen levels increase significantly in the case of atherosclerosis with and without thrombosis. A significantly high fibrinogen polymerisation curve occurs. Fibrinogen became more sensitive to thrombin as compared to normal control. Also, HMW fibrinogen percent increases in the patients with atherosclerosis and thus they have higher risk of thrombosis. In this group of cases VIII/vWF also increases significantly. From laboratory finding standpoint this type of cases exhibit some similarity with chronic low-grade DIC.

On the other hand, many recent publications indicate that high levels of FVIII are a risk factor for venous thrombosis as well arterial thrombosis. As we mentioned in our earlier publications, fibrinogen level increases significantly in atherosclerosis, in stroke and in POAD. The increased levels of D-dimer and FPA are the indicators of intravascular coagulation. The recognition of specialized membrane receptors and binding of fibrinogen to leucocytes as well as regulation of leucocyte-endothelium interaction by fibrinogen give an additional regulative role to the fibrinogen.[28–33,58] The half-lives of fibrinogen and platelets are decreased in the case of atherosclerosis.

d. Decreases of natural inhibitor's activities with/without molecular abnormalities were seen. In the cases of atherosclerosis, there is a resistance to heparin, while the level of heparin decreases. Heparin is produced by basophils, mast cells and EC; especially EC produces heparan sulfate. The surface of EC is usually covered by heparan sulfate which has a role in the non-wetable surface of EC with PGI_2. As we will discuss later heparin produced by EC has an inhibitory effect on platelet derived growth factor's effect on smooth muscle. Heparin's antithrombotic effect enhances through the activation of AT-III. AT-III and activated AT-III by UF heparin inactivate in a dose dependent fashion XIa, Xa, VIIa and thrombin. On the other hand, by description, LMW heparin acts through on Xa. But in practice LMW heparin also acts through AT-III but in lesser degree and this is different for the different products. We can thus say that LMW heparins are a group of similar proteoglycans but differ to their activities. Heparins obtained from different tissues and different species show also some structural differences.[34,35]

We have found that protein C and S slightly decrease in the cases of atherosclerosis, probably due to consumption. In 1997 Ozbek and Tangun mentioned the existence of FV-Leiden anomaly in the Turkish population.[37] According to Akar et al[38] in the cases of MI,CV infarct and DVT the occurence of Leiden anomaly was 25% and of PT 21020 was 9%. Gurgey et al[39] showed that 55 out of 142 adult cases with cerebral thrombosis, pulmonary thrombo-embolism, mesenterium thrombosis and DVT had molecular abnormalities such as: 35 heterozygous FV-Leiden (24.6%) and 6 homozygous cas-

es (4.2%); 9 cases of PT 20210 (6.3%) and 5 cases of combined defect of FV-Leiden and PT20210A (3.5%). Gurgey et al also reported in Azerbaijan 43 healthy cases (18 heterezygote and 1 homozygote) with methylenetetrahydrofolate reductase C677T mutation (MTHFR C667T) and also 6 cases with FV-Leiden. These cases were chosen from the general population and had no history of thrombosis.[40]

Akar et al[41] demonstrated endothelial nitric oxide synthase polymorphism in a Turkish patient with DVT and cerebrovascular accidents.[44] In another study, Sayhan et al[42,43] showed a significant difference observed in the cases of MI compared with normal healthy people. Also a deletion polymorphism in the gene encoding for ACE has been show in a certain percentage and also a significantly different PAI_2 genotype distribution between MI and control group has been shown. Furthermore, a deletion polymorphism at the converting enzyme gene in a patient with coronary artery disease has been shown. In another study made in our clinic among 35 patients with thromboembolism, it was shown that 8 patients had protein S deficiency, 7 APC-resistance, 4 antiphospholipid antibody syndrome, 3 protein C deficiency and 2 AT-III deficiency.[59] We have also presented a family with 2 cases homozygous for AT-III deficiency; the father and the mother were relatives and in the family there were 4 heterozygous patients for AT-III deficiency.[35]

e. The alterations of platelet in the cases of atherosclerosis with and without thrombosis are as follows: hyperaggregation, hypersecretion, hyperadhesion and mitogen formation and release.[45–47] Platelets do not adhere to normal endothelium but to injured EC and to subendothelium. Also, platelets adhere to functionally altered EC. The continuity of heparan sulfate layer on the surface of EC decreases and also does PGI formation so that more platelet also adhere to the EC surface. Using platelet retention test, it was shown that, more platelets adhere to the foreign surface. Using Rebuck technique we measured the differential count of platelets on formvar membrane using shadow-casting technique and electron microscope.[2,4,45,47] In normal subjects the majority of platelets were of dendritic form (66%) and only 4.5% small aggregates were observed per hundred single platelets. On the other hand in the case of atherosclerosis there were 12.6% small and 5.2% gross aggregates. This was also shown by Marion Barnhart and her group[48] in the case of transient ischaemia and cerebrovascular disorders. Certain anti-aggregating drugs like aspirin normalize this pathologic alteration. Our results of functional and biochemical alterations of platelets from atherosclerotic patients are summarised in the table 1. When platelets adhere to subendothelium they release growth factors including ß-TG, chemotactic substances, anti-heparin (PF-4) and also procoagulants (PF-3) etc., and this gives them a role in the pathogenesis of atherosclerosis. In the cases of atherosclerosis the main functions of platelets significantly increase,[45,46] fact that is observed in congenital or acquired form of atherosclerosis. Mammen[49,50] has described a congenital form named "sticky platelet syndrome". Platelet showed hyperaggregation to the aggregating agents such as adrenaline, ADP, collagen, etc. Platelet secretion due to induction increased significantly in the majority of platelets. But in some cases platelets already release their materials before the induction with aggregating agents and in those cases platelets circulate as ghost forms. Among secreted and released factors PF-3 is a procoagulant, PF-4 is an antiheparin factor, smooth muscle proliferative factor is a mitogen factor, b-TG is also a mitogen factor, etc. Alteration of platelet membrane phospholipids distribution in atherosclerosis has also been shown.[51] The total amount of membrane phospholipids was increased, but each phospholipid increased in a different rate. Also, different results were obtained with different release inducers. In the case of atherosclerosis, platelet ATIII and cAMP levels were significantly decreased. The decrease of cAMP in inactivated platelets indicated that platelets were already activated in resting condition.

On the other hand, platelet α_2-antiplasmin increases. Together with the alteration of t-PA and PAI 1 resulted in decrease of global fibrinolytic activity in the cases of atherosclerosis. We may also add the alteration of PAI 2 in the cases of MI.[1,2,4,45,46] We have demonstrated that the active transport of ^{14}C-glucose to the platelet and red cell is defective in the cases of atherosclerosis. The specific glucose binding protein is absent or decreased significantly or is molecularly altered in the cell membrane of atherosclerotics.[52,53] This defect also exists in the young members of the atherosclerotic families. Some drugs partially correct this defect such as defifrotide, diamicron and metformin. On the other hand insulin receptors are decreased in the case of atherosclerosis with diabetes mellitus or without. It has also been shown that galactose transport to the platelet is normal but the incorporation of galactose to the platelets increases and this may explain the changes of the pattern of membrane glucoprotein in the cases of atherosclerosis. The alteration of leucocytes, smooth muscle cells and others will not be discussed in this short review.

ΠΕΡΙΛΗΨΗ

Παθογένεια και εργαστηριακά ευρήματα στην αρτηριοσκλήρυνση από τη σκοπιά της αιμόστασης

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Γίνεται βραχεία ανασκόπηση της παθογένειας της αθηροσκλήρυνσης σε σχέση με το μηχανισμό της αιμόστασης. Περιγράφονται διεξοδικά ο τύπος των διαταραχών που έχουν σημειωθεί, όσον αφορά την υπολειτουργία ή δυσλειτουργία του ενδοθηλίου, τη μείωση της ινωδολυτικής δραστηριότητας, την αύξηση ορισμένων παραγόντων της πήξης, την ελάττωση των φυσικών αναστολέων, την υπερλειτουργία των αιμοπεταλίων, την υπερπλασία και διείσδυση των λείων μυϊκών κυττάρων. Παρουσιάζονται τα ευρήματα προσωπικών εργασιών του συγγραφέα και των συνεργατών του.

Λέξεις ευρετηρίου: Αθηρογένεση, Αθηροσκλήρυνση, Αιμοπετάλια, Αιμόσταση

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