PATHOGENETIC ASPECTS OF ATHEROSCLEROSIS

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Atherosclerosis is a chronic progressive disease, which develops from the moment of birth. Lipid deposit in blood vessel intima leads to gradual stenosis and impeding blood flow through tissues and eventually results in broad spectrum of clinical manifestations. Clinical manifestations are the consequence of plaque rupture which induce thrombogenesis and formation of thrombus and embolus. In this way, the process of thrombogenesis is followed by obstruction of blood vessels. Elimination of risk factors such as obesity, hyperlipidemia and hypertension improves endothelial function and reduces progression of atherosclerosis. *Acta Medica Medianae 2007;46(1):25-29.*

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Introduction

Atherosclerosis is diffuse, disseminated pathological process affecting all vascular beds and is characterized by thickness and lipid deposition in intima of the blood vessel wall. Atherosclerotic plaque represents intima lipid accumulation and wall thickness. It primarily develops in intima of abdominal aorta, in its big branches, arteries for low extremities as well as coronary and cerebral arteries. It is characterized by accumulation of extracellular and intracellular lipids, monocyte/ macrophage infiltration, formation of foamy cells, proliferation of smooth muscle cells and accumulation of fibrous-tissue proteins.

Atherosclerosis can be clinically manifested as: coronary heart disease (angina pectoris, infarction, sudden heart death), cerebrovascular disease (transient ishemic attack- TIA, stroke) and periphery vascular disease (intermittent claudication, gangrene).

Risk factors for atherogenesis

All risk factors for atherosclerosis can be divided into modifying and non-modifying. Recent investigation has shown that besides the most important traditional risk factors (dyslipidemia, hypertension, cigarette smoking and diabetes mellitus) there are also non-traditional or modern risk factors such as (increased oxidative stress, endothelial dysfunction and inflammation (1).

In spite of the fact that many pathogenic factors are included in pathogenesis of atherosclerosis, dyslipidemia still plays a central role in this process. Dyslipidemia stands for disorders in lipid concentration and composition. It results in disturbance of concentration of certain lipids (hyperlipidemia, hypercholesterolemia, hyperlipoproteinemia). Hyperlipidemia is the main cause of atherosclerosis and its related diseases, cardiovascular diseases, ischemic cerebrovascular diseases and periphery vascular diseases. The most important risk factors for atherosclerosis are: increased LDL-C, decreased HDL-C, tobacco smoking, hypertension, diabetes mellitus type 2, old age, positive family anamnesis for cardiovascular disease in first line relatives (men under 55 years of age, women under 65 years of age) (2, 3).

The firs link between cholesterol and atherosclerosis was established in 1841, when Vogel demonstrated the presence of cholesterol in atherosclerotic plaque. In 1884, Von Rokitanski propounded thrombogenetic theory, and in 1856 there emerged Virchov's response theory stating that endothelium damage was a basis for further inflammation and cell proliferation. In 1913, Anitschkov and Chalotov demonstrated that nutrition rich in cholesterol induced atherosclerotic changes. Similarly, Goldstein and Brown identified LDL particles as main atherogenic lipoproteins.

Numerous studies determined the key importance of lipid disorders in atherogenesis and that the risk of cardiovascular diseases was significantly lower if LDL-C was under 4,2 mmol/l even if other risk factors were present. Combinations of risk factors increase the risk of endothelial dysfunction and incidence of myocardial infarction (3, 4).

Cholesterol as a lipid represents the constituent part of cell membrane and it has a very important role in the synthesis of steroid hormones. Organism is provided with cholesterol in two ways: by cholesterol biosynthesis in the liver or by food intake. If organism receives more cholesterol, decreased synthesis in the liver will occur. Other cells may not synthesize cholesterol, since they obtain it from plasma. In body liquids, cholesterol is carried out by hylomicrones, low

density lipoproteins (LDL) and high density lipoproteins (HDL) particles. Hylomicrones transport triglycerides, cholesterol and other lipids from intestine to liver and fatty tissue. LDL particles transfer cholesterol to periphery tissue, and HDL particles transfer excessive cholesterol from periphery tissue to liver. Since peripheral cells cannot synthesize cholesterol, LDL is the main source of cholesterol. Mechanism of cholesterol removal from LDL has its basis in LDL receptor which is present on the cell surface. LDL binds to specific receptor, thus inducing internalization, i.e. LDL is transferred into cytosol by endocytosis, in the form of endocyte vesicle. It is taken by lysosomes and lysosome enzymes release cholesterol for cells needs. Released, unesterificated cholesterol may be used for membrane biosynthesis, or if it is not needed temporarily it may be esterificated and deposited in the cell until it is needed again.

Family hypercholesterolemia is a result of mutation only in one locus. The consequence is disturbed expression of LDL receptor. In homozygotes, complete dysfunction of LDL receptors occurs, so that they very early die of progressive form of atherosclerosis. In heterozigotes there is one half of dysfunctional LDL receptors. Since LDL receptors do not have normal LDL function, cholesterol increases concentration in blood, which is associated with deposit in the wall and development of atherosclerosis.

HDL lipoproteins have well-established protective effect. The basis of this effect is reverse transport of cholesterol. HDL transports cholesterol from periphery tissues toward liver. HDL particles also contain antioxidative enzymes (PAF-acetil-hydrolasis and paraoxonase) which break oxidated LDL particles down and neutralize their proinflammatory effect. Besides, they inhibit expression of certain adhesive molecules (VCAM-1) (3).

Hypertrigliceridemia may cause the change of structural cell membranes and activation of adenil-cyclase with the development of oxidative stress in endothelial cells, monocytes and lymphocytes. Also, aggregation and adhesion of thrombocytes as well as proliferation of smooth muscle cells is stimulated.

An independent factor for the development of atherosclerosis is increased concentration of Lp(a) in blood plasma as a consequence of increased oxidation and easy penetration of this lipoprotein particles into blood vessel intima.

Correction of hyperlipidemia presents simultaneous prevention and treatment of atherosclerosis and its consequences. Therapy of hyperlipidemia decreases mortality of cardiovascular diseases by 30-40% as well as incidence of non-fatal events (1,3). Hypertension, also, represents another important factor for the development of atherogenesis. There is activation of renin-angiotensin system (RAS) in a great number of hypertensive patients. Activation of RAS with AT II formation and subsequent activation of AT II receptors, mostly of AT type I, is included in the process of atherogenesis. Angiotensin II increases expression of inflammatory factors such as P-selectin and monocyte chemo attractant protein (MCP-1), which regulate adhesion of monocyte and other inflammatory cells. It also increases uptaking of ox-LDL and cholesterol biosynthesis in macrophages transferring them into foamy cells. It also increases gene activity for LOX-1 in culture of coronary endothelium. It leads to increase of apoptosis in human endothelial cells, activity of nicotineamid dinucleotide phosphate (NADPH) oxidasis in macrophages, and NADPH oxidase increases oxidative stress. Mechanical wall stress is the main characteristic of arterial hypertension, which leads to activation of NADPH oxidase in smooth muscle cells and consequently to oxidative stress.

Hypertension and hyperlipidemia as great risk factors for coronary disease are very often associated in many patients. Numerous studies revealed that AT II and ox-LDL do not act independently in the process of atherogenesis. These factors have the following common characteristics: they lead to formation and release of free radicals; they neutralize and decrease expression of endothelial N oxide synthetasis, which influences decrease of endothelium-dependent vasodilatation; they induce activation of redox sensitive transcription of nuclear factor kappa B(NF- kB); they have proinflammatory properties (they induce expression of adhesion molecules and cytokines, upregulation of gene for monocyte chemoattractant protein-1 and induce monocyte adhesion and apoptosis.

Experimental studies have shown that hyperlipidemia increases RAS activity. There is linear correlation between concentration of LDL cholesterol in plasma and expression of AT I receptor (4).

Hyperglycemia in diabetes increases oxidative stress, thus contributing to vascular dysfunction. It influences endothelial dysfunction by production of free radicals, by sorbitol accumulation, by non-enzyme glycolisation of macromolecules, and by direct activation of protein kinase C. Glycolisation of protein and lipids runs in each patient with diabetes and irreversibly leads to long-term vascular dysfunction In initial protein glycolisation, early glycoside products are produced. Reorganization of molecules occurs later on, partially by oxidation and final glycoside products are produced (AGE) which react with superficial receptors thus producing free radicals and decreasing the level of reduced glutathione and activating redox sensitive transcription of factors NF-kB. In diabetics, oxidative modification of LDL particles occurs in circulation in contrast to non-diabetics in whom it is usually the wall of blood vessel, because there is enough amount of antioxidants in circulation. Produced AGE LDL particles lead to expression of VCAM-1 and monocyte-endothelial interaction with formation of atherosclerotic lesions (5).

The World Health Organisation (WHO) and expert team of National Cholesterol Education Program Adult Treatment Panel (NCEP ATP III) defined metabolic syndrome as a new secondary target in therapy and reduction of cardiovascular diseases (5, 6).

Definition of metabolic syndrome implies the presence of at least three of the following parameters: waist circumference >102 cm in man and >88 cm in women; concentration of triglycerides (>1.7 mmol/l); HDL-C (<1 mmol/l in men and <1.3 in women); blood pressure (>130/85 mmHg); fasting glycemia (>6.1 mmol/l).

According to the WHO definition metabolic syndrome comprise one of the following disorders: insuline resistention (HOMA > 2.5), -impaired glucose tolerance and DM type 2, plus two of the following disorders: hypertension (>140/90 mmHg), body mass index (>30 kg/m^2), waist/hip ratio (>0.9 in men, >0.85 in women), triglyceride concentration (>1.7 mmol/l), HDL-C (<0.9 mmol/l in men, <1 mmol/l in women), albumine/creatinine ratio (>2.5 mg/mmol in men, > 3.5 mg/mmol in women).

The importance of metabolic syndrome lies in the fact that there is concordance of many risk factors for the development of cardiovascular diseases. Moreover the metabolic syndrome has great prevalence in modern society. On the basis of WHO and NCEP ATP III criteria, it has be estimated that prevalence of metabolic syndrome in USA general population is 24%, while in population of elderly Americans over 60 years of age it is 44%.(7).

Patients with metabolic syndrome are usually at very high risk for development of cardiovascular disease. Life style habits have great impact on all components of metabolic syndrome. Therefore, one of the first actions in therapy of metabolic syndrome shoud be modification of life style by losing weight and increased physical activity.

It has been established that homocysteine has toxic effect on endothelial cells. Homocysteine (Hcy) is amino acid containing sulphate. It is not present in natural proteins because of the absence of DNA code for this amino acid. Total Hcy in organism is produced from metabolism of essential amino acid methionine. Hyperhomocysteinemia is a name for higher concentrations of homocysteine in serum. Damaged endothelial cells enable better LDL penetration into subendothelial space. Hyperhomocysteinemia higher than 100 micro mol/l is associated with early thrombosis and atherosclerosis. Various studies have shown that these patients have endothelial dysfunction and oxidative stress, but their effect has not been fully elucidated yet.

Tobacco smoking significantly contributes to atherosclerosis of blood vessels. The fact that the system of prooxidants/antioxidants plays an important role in atherogenesis to some extent accounts for the significance of this risk factor in atherogenesis. Cigarettes contain a great number of prooxidants, which may directly influence the process of lipid peroxidation and decrease of antioxidant level. Also, they increase the level of peroxidation of LDL cholesterol and favors oxidation of LDL cholesterol (5,6,7,8).

Atherogenesis and theory of oxidative change of LDL

Atherogenesis represents production of atheromas (plaques made up of lipid nucleus surrounded by connective tissue), which is the main event of atherosclerosis. The basic mechanism for atheroma development and progression is endothelial damage of blood vessels. Endothelial dysfunction initiates the whole sequence of successive reactions resulting in atherosclerotic lesion. Hereby, there exists direct relationship between risk factors for atherosclerosis (smoking, hyperlipidemia, hypertension, obesity, diabetes, inflammation, infection) and endothelial dysfunction. Immune system is directly involved in initiation of atherosclerosis playing a dominant role in the development of inflammatory reaction in plaque. Atherosclerosis morphologically begins by invisible endothelial dysfunction, which may be induced by physical, mechanical, chemical, toxic, infectious and immunological factors (1,9).

There is great evidence that some pathogenetic stimuli take part in the production of reactive oxidative particles in endothelial microenvironment and that oxidative stress plays a key role in the development of endothelial dysfunction related to atherosclerosis.

Oxidative stress is identified by process of atherosclerosis, which is an early stage of endothelial dysfunction. With the development of atherosclerotic process, a great amount of free radicals is produced, which further enforces atherogenesis. Increased production of free radicals then influences four fundamental mechanisms of atherogenesis: LDL oxidation, dysfunction of endothelial cells and smooth muscle cells, growth and migration of monocytes (9).

Risk factors for atherosclerosis such as hypertension and hyperlipidemia are associated with increased production of oxidative particles. A similar situation is present in diabetics and smokers. Numerous cytokines, such as tumor necrosis factor (TNF-), interferon (INF-9), interleukines (IL-1, IL-6) and angiotensine II are of key importance in intracellular production of free radicals. High concentrations of low-density lipoproteins (LDL), particularly of oxidative form (ox-LDL), induce free radical production. The following growth factors have similar effects: thrombocyte risk factor (PDFG), epidermal growth factor (EFG) and vascular endothelial growth factor (VEGF) as well as hormones like insulin (1,8,10).

Atherogenic lesion primarily develops inside the blood vessel intima, starting from fat stripes and diffuse intima thickness, through fibrolipid plaque, up to developed lesions complicated by thrombosis, hemorrhage or calcified lesions (‘complicated lesions”).

Oxidation of LDL particles, formation of fat stripes and proliferation of smooth muscle cells represent basis for formation of atheromatous fibrolipid plaque. A few cellular forms from arterial wall in the blood are included in the eterogenetic process: endothelial cells, smooth muscle cells, macrophage, thrombocytes as well as numerous growth factors. Lipid theory hypothesis is based on increased integration and accumulation of LDL lipoprotein plasma in the blood vessel wall and their transformation into more atherogenous form, called modified LDL.

Oxidation of LDL particles represents initial process in the development of modified LDL forms, which are then ingested by macrophages. Oxidative LDL modification (ox-LDL) has been largely investigated, and therefore, the valid theory has been named "peroxide theory of atherogenesis). On predilection places for endothelial damage excess of circulated LDL is accumulated, resulting in endothelial damage manifestating with changes of permeability of endothelial barrier, procoagulant properties and release of vasoactive substances. Oxidatively modified LDL particles express chemotaxic effect on circulating monocytes as well as on T-Ly. Moreover, modified LDL lead to adhesion increase and aggregation of monocytes and T-Ly, thus forming two adhesive molecules on the endothelial surface: vascular adhesive molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1). It, also, causes release stimulation of monocyte chemotaxic protein from endothelial cells and additional monocyte mobilization from circulation. By chemotaxis, monocytes and T-Ly pass through endothelium and in subendothelial space are converted into macrophages, overtaking larger amount of modified LDL, transforming into foamy cells, which are a part of lipid nucleus of atheromas. It is considered that endothelial and smooth muscle cells are capable of LDL modification by initiating process of lipid peroxidation, after which macrophages recognize and ingest modified LDL. LDL oxidation is followed by endothelial dysfunction and subsequently, by loss of endothelium-dependent vasodilatation, initiation of inflammatory response and aggravating of anticoagulant protection for the development of thrombosis (1,4,8,9).

Macrophages may secrete oxidized LDL and superoxide anion which additionally damages endothelium. Activated macrophages excrete numerous risk factors (PDGF) stimulating proliferation of fibroblast and smooth muscle cells, TGF-R growth factor for smooth muscle cells. Transformation of macrophages into foamy cells enables smooth muscle cells to be moved from media into intima where division, synthesis and secretion of connective tissue matrix take place. In this way, development of fibromuscular proliferative lesion is enabled (4).

Advanced lesions of atherosclerosis and its complications

The first and most characteristic lesion of advanced atherosclerosis is atherosclerotic plaque consisting of lipids-rich core in central part of eccentrically thickened intima. The surface of the plaque toward lumen is covered by fibrous cap. The central part of the plaque is filled with lipid detritus which develops by increase and confluence of small collections of extracellular lipids. Lipids (mainly, LDL) directly enter blood space by insudation from plasma or by macrophage endocytosis, by means of scavenger receptor after oxidative modification and indirectly accumulated after necrosis of macrophages overfilled with lipids. Over time, the content of connective tissue (consisting of collagen and smooth muscle cells) is increased in the plaque (11).

Increasing fibrolipid plaque is substrate for the development of thrombocytic complications. Relative relationship of nucleus size and connective tissue is important to plaque prognosis. Big, eccentric lipid nucleus, great macrophage density and thin fibrous cap are risky to plaque rupture, thrombosis and acute coronary syndrome.

In mature plaques, two main components in different relation are present: soft, atheromatous mash rich in lipids and hard sclerotic tissue rich in collagen. Sclerotic component is usually far more voluminous, it stabilizes plaque preven-ting it from disruption, while atheromatous mash destabilizes plaque making it be prone to rupture. The main determinants of plaque vulnerability are the size and content of atheromatous core, thickness of fibrous cap, acute inflammation inside cap and cap stability.Predilection site for plaque rupture is edge region, where fibrous cap is the thinnest and infiltrated by macrophages. Desintegration of fibrous cap is followed by sudden exposure of highly thrombogenetic mash to blood flow.

On previously ruptured plaque or intact plaque, sudden thrombosis may emerge due to the change in thrombocyte function, coagulation and/or fibrinolysis, which is probably important response mechanism for total occlusion of blood vessel and infarction. Harmful role of macrophages after plaque rupture reflects in induction of thrombine generation and luminal thrombosis by tissue factor. Tissue factor is integral membrane protein, which binds to factor VII/VIIa and initiates coagulative cascade. It is overexpresed on the surface by lipids-overfilled with monocytes, macrophages and foamy cells in human atherosclerotic lesions. Plaque rupture is also followed by development of hematoma through fibrous cap cleft, although particular hematomas may develop inside lesion due to hemorrhage from newly formed blood vessels. Mature plaques, particularly those containing greater amount of fibrous connective tissue, may calcify, whereby mineral deposits replace dead cells and extracellular lipids. Advanced lesions are often associated with localized dilatation of the part of the blood vessel they occupy.

Such aneurisms may contain mural thrombs, and particles of thrombi, which are disseminated into systemic circulation, may cause embolia. Even if there are no complicated lesions, with the advancement of atherosclerosis the blood vessel lumen becomes narrower, blood flow is disturbed and there is great risk of further damage of endothelial cells. In this way vicious circle is closed. Stenosis of lumen is followed by tissue hypoxia, and blood pressure is raised due to reduced elasticity of blood vessels. Thereby, there is a great possibility of the rupture of changed blood vessel wall and subsequent blood overflow into surrounding tissue, particularly under condition of blood pressure increase (6,9).

Conclusion

Atherosclerosis is a dynamic process characterized by significant changes in artery biology due to chemodynamic changes caused by growth and disruption of plaque. Disturbed vascular biology associated with atherosclerosis and its risk factors includes vasomotoric dysfunction and plaque inflammation as well as prothrombous/antifibinolytic state.

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PATOGENETSKI ASPEKTI ATEROSKLEROZE

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Ateroskleroza je hronična bolest sa progresivnim tokom čiji razvoj praktično počinje od rođenja. Osnovni supstrat aterosklerotske lezije je deponovanje lipida u intimu krvnog suda, što dovodi do postupnog suženja i otežanog protoka krvi kroz tkiva. Kliničke manifestacije aterosklerotskog procesa su brojne i pre svega zavise od stepena suženja i brzine nastanka stenoze. Najčešće kliničke manifestacije su posledica rupture aterosklerotskog plaka što aktivira proces trombogeneze i dovodi do razvoja začepljenja krvnih sudova. Uklanjanje faktora rizika, kao što su gojaznost, hiperlipidemija, hipertenzija, dovodi do popravljanja endotelne disfunkcije i usporavanja patogeneze aterosklerotskog plaka. *Acta Medica Medianae 2007;46(1):25-29.*

Ključne reči: *ateroskleroza, faktori rizika, evolucija plaka*