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REVIEW

Atherosclerosis: an update

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Abstract: Atherosclerosis is a chronic systemic inflammatory disease that affects populations of all age and income with great health related consequences. The update focuses on the topic of atheroclerosis and new data published in this field of cardiovascular disease. The article also emphasizes on the need for further research, notably in the fast developing field of immunology which may provide further therapeutic discoveries.
Keywords: atherosclerosis, cardiovascular disease, high-density lipoprotein, low-density lipoprotein, miRNAs, PCSK9, statin.

Rezumat: Ateroscleroza este o boală sistemică cronică inflamatorie care afectează populaţiile de toate vârstele cu consecinţe importante asupra sănătăţii. Această lucrare se concentrează pe tema ateroclerozei şi a datelor noi publicate în acest domeniu. Articolul subliniază, de asemenea, necesitatea unei cercetări ulterioare, în special în domeniul dezvoltării rapide a imunologiei, care ar putea oferi noi descoperiri terapeutice.
Cuvinte cheie: ateroscleroză, boli cardiovasculare, lipoproteine cu densitate mare, lipoproteine cu densitate scăzută, miRNAs, PCSK9, statine.

INTRODUCTION

Atherosclerosis is a pathological process that starts in the early decades of human life as fatty-streaks and evolves throughout the life of an individual. The contributing risk factors which also accelerate the intimal thickening are hypertension, smoking, diabetes mellitus, obesity, dyslipidemia and family history of cardiovascular disease[1]. Clinical complications of atherosclerosis are dependant of the arteries involved. Coronary atherosclerosis is the leading cause of angina pectoris and acute coronary syndromes, cerebrovascular atherosclerosis causes transient cerebral ischemia and stroke while atherosclerosis of the lower extremity vessels causes claudication and acute or chronic limb ischemia[2].

PATHOGENESIS

In the last century there has been an outstanding evolution in our understanding of the pathogenesis of atherosclerosis. This disease presents a great amount of heterogenity both in time with acute and chronic manifestations, as well as in space with certain lesion sites being more prone to disease than others[3].

It is widely accepted that the key process in the pathogenesis of atherosclerosis is represented by abnormal inflammation and subsequent lack of resolution in the arterial wall with the prime stimulus for lesion formation being lipid deposits[4].

The normal human endothelium cell of the arterial intima provides a barrier between the blood and the underlying tissues. This thin, one cell layer structure is equiped with a finely tuned system of modulators involved in the strategic balance between the anticoagulant and procoagulant factors and thus is responsible for the vascular homeosthasis[5]. It is hence our understanding that when this system is astray, the foundation of atherosclerosis begins. The endotelial cell expresses on its surface multiple antithrombotic molecules[6].

The endothelium neutralizes coagulation by providing thrombin inhibitors and preventing direct contact of blood flow with tissue factors. Heparan sulfate proteoglycan serves as a cofactor for antitrombin III which in turn inactivates the thrombin molecule. Thrombomodulin binds directly with thrombin and activates proteins S and C, which in turn regulate coagultion and inflammation. Furthermore, in case of an

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already formed thrombus, the endothelium produces tissue plasmin activator which converts plasminogen to plasmin aiding the start of fibrinolysis[7].

Underneath the endothelium, the arterial smooth muscle cell exhibits essential functions in regulating blood flow through contraction and relaxation and building extracellular matrix. With aging, smooth muscle cells proliferate and migrate into the intima between the endothelial cells contributing to a more complex structure of the inner artery layer, a process called diffuse intimal thickening[8]. This process may progress independent of lipid accumulation.

The outer most component of the artery wall, the adventitia, contains fibroblasts and mast cells, with new analysis advocating for their part in the development of atherosclerosis. A closer look at the data indicates that vasa vasorum which forms a web of micro-vessels, extends from the adventitia through the media and into the thickened intima in the atherosclerotic lession. This fragile network is susceptible to deterioration as it cannot supply the plaque growth, causing haemorrhage and subsequent progression of the atheroma[9].

Even though inflammatory cells were found in atheroma lesions dating back to the 19th century, it took decades of dedicated research to grasp the integral pathophysiological image of atheroma genesis.

Western type diets, rich in saturated fats, increase circulating apolipoprotein B particles, which in turn gather in the vascular intima and assemble with proteoglycans creating complexes that tend to be retained longer at this level and are more exposed to oxidation (via NADH/NADPH oxidases, myeloperoxidases) and glycation (in diabetic pacients)[10]. This process is particulary observed at sites of disturbed blood flow. Amidst the chemically modified lipoprotein deposits, leukocytes start to overcome the natural endothelial resistance to their adhesion, predominantly due to the adhesion molecules VCAM-1, ICAM-1 and P-selectin. An increasing number of leukocyte chemoattractant molecules (IP-10, I-TAC, MIG) commence expression on the endothelial cell, allowing passage of T cells and monocytes[11]. Of most importance is the MCP-1 protein (monocyte chemoattractant protein 1) synthesized by endothelial cells and smooth muscle cells in response to the presence of oxidised lipoproteins. MCP-1 is responsible for the migration of monocytes into the vessel wall, with studies demostrating that mice which lack this protein express attenuated atherosclerosis lesions. Leukocytes, particularly monocytes that begin to differentiate into phagocytic macrophages, start accumulating complexes of lipoproteins and proteoglycans and transform into foam cells[12].

These chemical alterations establish the early stages of atheromatous plaque formation.

Foam cells macrophages and endothelial cells generate more cytokines, proinflammatory mediators and oxidative species thus maintaining the incipient mechanism of atherosclerosis. It is generally believed this augmentation represents a normal response seen in innate immunity, independent of antigenic stimulation, but has a detrimental effect in this context. The inflammatory response may take several years and the current evidence supports that this may be a reversible stage[13].

While the first events comprise of endothelial dysfunction and migration of leukocytes, the next phase of evolution to more complex plaques is associated with smooth muscle cells.

A great number of these cells proliferate and migrate from the media to the intima, encapsulating lipid enriched macrophages and forming the extracellular matrix. This migration is facilitated by cytokines and growth factors released by foam cells and endothelial cells (IL-1, TNF-α, TGF-β)[14]. Some data propose the existence of resident vascular stem cells which act as a precursor for the intimal smooth muscle cells in disease affected arteries[15]. The smooth muscle cell population is believed to be heterogenous and more recent genetic studies have shown the possibility of phenotypic switching that results in less-differentiated forms which become macrophage-like cells and thus promote atherosclerosis.

In this stage, the fatty streaks evolve into the fibrofatty lesion. After the apoptosis of smooth muscle cells (promoted by the abundance of local cytokines and T cells), the lipid core is essentially surrounded by a fibrous capsule that is acellular in nature. This makes up for most of the volume of an atherosclerotic plaque rather than the cells. The fibrous cap structure varies greatly depending on the smooth muscle cell production with major components represented by collagen types I, III, proteoglycans and to a smaller extent elastin fibers. Collagen production is stimulated by PDGF and TGF-β derived from platelets[16].

While the first classification for atherosclerosis was purely dichotomial with "fatty streaks" and atheromatous plaque, the *American Heart Association* (AHA) introduced a new scheme which stratified the various stages based upon the temporal progression of the disease. As such, there were 6 defined types

of atherosclerotic lesions. Type I represents the minimal, barely noticeable changes with an increase in intimal macropahges and mild thickening, types II-III represent pre-atherosclerotic lesions, also refferd to as "fatty streaks" and types IV-VI are considered advanced atherosclerotic disease, with type IV – the atheroma, type V – the fibroatheroma and type VI – the complicated atherosclerotic lesion[17].

Matrix metalloproteinase (MMP) family represents calcium-dependent, zinc-containing endopeptidase enzymes that are involved in the metabolism of extracellular matrix[18]. Their role in atherosclerosis is widely debated, with several studies which generated contradictory results. It is generally accepted that MMP activation promotes smooth muscle cell migration – a key step in the atherosclerotic process while several polymorphisms (MMP3 and MMP8) are associated with higher atherosclerotic burden. High homocysteine serum levels are linked with activation of various MMPs, while nitric oxide has been shown to either activate or deactivate some of these enzymes[19].

An increasing amount of research focused on the link between monocytosis and atherosclerosis with new evidence suggesting that monocytosis itself may be an independent cardiovascular risk factor[20].

RESEARCH AND THERAPY

The main therapeutic approaches for prevention and treatment of atherosclerosis and subsequent cardiovascular disease that are currently being used in clinical practice target first and foremost modulation of circulating cholesterol levels.

On the basis of the new evidence currently being researched, it seems fair to suggest that a new objective for medication should be the immune system. Corticosteroids, cyclooxygenase inhibitors and anti-tumor necrosis factors are efficient drugs in acute inflammation or autoimmune disorders. The risk/benefit ratio in these conditions justifies their use. However, in the case of atherosclerosis, due to the chronic nature of maladaptive inflammation, there is no current therapeutic approach for anti-inflammatory medication[21]. A closer look at data indicates that new research is focused mainly on finding the best precision medicine and tailor drugs that mimic the endogenous resolution of the underlying inflammation and not just simply cut the inflammatory process (as is the mechanism for current anti-inflammatory drugs)[22].

Developments in the field of microRNAs (miRNAs) highlight these molecules as relevant components in modulation of numerous pathophysiological mechanisms like cellular adhesion, proliferation and lipid regulation. Since their discovery in 1993, the importance of miRNAs was intesively studied with the goal of understanding their roles in different systems ranging from normal human development, metabolism regulation, aging and disease progression. These are small, genetically conserved, single-stranded, noncoding RNAs, that modulate gene expression at the post-transcriptional level while being capable of detering mRNA translation and stimulate mRNA degradation. It is believed that miRNAs are involved in regulating almost two thirds of all protein-coding genes. Their role in atherosclerosis is important due to recent discoveries of miRNAs associated with control of LDL-C and HDL-C genes. These miRNAs are found in the liver and regulate the lipoprotein metabolism. Thus, miR-33 and miR-758 regulate HDL-C metabolism, miR-148a and miR-128 increase hepatic LDLR and ABCA1 expression, while inhibition of miR-148a and miR-128 in mice has been shown to decrease the serum level of LDL-C[23-24].

HDL-C represents the main system for removing cholesterol from the periphery. HDL-C is generated in the hepatocyte and enterocyte, from cholesterol and phospholipids while apolipoprotein A1 being a key component of this process. In people with ABCA1 defficiency (Tangier disease), there is a severe drop in circulating serum HDL-C, and individuals affected by this condition develop premature atherosclerosis. miR-33 is involved several phases of the reverse cholesterol transport by HDL-C modulating the expression of genes ABCA1 and CYP7A1[25].

As the protective role of HDL from observational studies is well established, further research of miRNAs that are able to upregulate this form of serum cholesterol are of particular importance, in light of current therapy failure in this regard.

The link between lowering circulating LDL cholesterol levels and the reduction of cardiovascular events and mortality in high risk population is strongly supported by our current evidence (PROSPER, JUPITER, PROVE-IT-TIMI 22, IMPROVE-IT trials). This reduction of mortality is consistent with a gradient of per unit reduction of LDL cholesterol with a continuous reduction of risk with further LDL cholesterol lowering[26].

Nonetheless, having a single primary therapeutic agent as in statin therapy has certain drawbacks. There are individuals that do not tolerate statins due to their side effects while others continue to present

high LDL levels even on the maximum recommended dosage. As such, novel medication to combat elevated LDL-C was needed, and thus, more than 40 years after the discovery of HMG-CoA reductase inhibitors, a new therapeutic target has emerged in proprotein convertase subtilisin-kexin type, or PCSK9.

While statins are perhaps the most widely-prescriped lipid lowering agent, PCSK9 monoclonal antibodies have proved themselves to be the most efficient agent.

Several observational studies have showed that most of the serum cholesterol is produced endogenously in the liver, with dietary cholesterol sharing only a small fraction. Statins competitively inhibit the HMG-CoA reductase in the liver cell, decreasing mevalonic acid production, a precursor of serum cholesterol. When cholesterol levels begin to fall, hepatocytes increase the expression of LDL-R receptors on their surface, start to form more LDL-C and LDL-R complexes which are internalized, and subsequently contribute to the serum LDL-C lowering effect[27].

In essence, the LDL endocytosis has proven to be our first efficient breakthrough in lipid-lowering therapy for the last decades and in this fashion it is easy to understand how PCSK9 therapy has emerged. While statins promote the over-expression of LDL-R in liver cells, PCSK9 therapy downregulates LDL-R degradation. The PCSK9 enzyme is a liver cell protease that contributes to internalization and lysosomal degradation of the hepatocyte LDL-R receptors, decreasing the number of LDL-R and LDL-C surface complexes and slowing serum clearance of LDL cholesterol. Observational studies showed that populations with loss of function in PCSK9 genes present with lower serum LDL and have lower mortality and overall cardiovascular events[28].

Meanwhile, statins promote to some degree the expression of the PCSK9 and subsequent LDL-R clearance, with less reduction in LDL levels. Moreover, statins have a wide array of pharmacokinetic and pharmacodynamic properties with differnt rates of absorption, bioavailability and plasma protein binding while also being subjected to hepatic metabolization, rendering broad therapeutic outcomes.

Thus, evolocumab and alirocumab (monoclonal antibodies targeting PCSK9) were developed by pharmaceutical companies and approved by the FDA and the EMA in 2015. Their indications are for treating adults with primary hypercholesterolemia or in combination with statin therapy for patients that do not reach optimum LDL levels or in patients that do not tolerate statins[29].

The new molecules were validated in 2 large randomized trials (ODYSSEY LONG TERM and FOURIER) with a highly effective reduction of LDL cholesterol, with averages between 50 and 70%, independent of concomitent statin therapy. More importantly, these antibodies were effective both in patients with heterozygous or homozygous familial hypercolesterolemia and in patients with other types of hypercholesterolemia. PCSK 9 targeted therapy has shown to be safe and well tolerated.

The ODYSSEY LONG TERM trial evaluated alirocumab versus placebo in 2341 high-risk patients treated with statins at the maximum tolerated dose and demonstrated a reduction both in LDL-C levels and cardiovascular adverse outcomes while having a great safety profile. A mean reduction of 62% of LDL serum cholesterol was obtained over a course of 24 weeks. It is worth mentioning that almost 50% of the included patients were on a "high-dose" statin regimen and further LDL-C lowering in association with alirocumab was observed in this subgroup as well. The study also noted a 25% reduction in lipoprotein(a) levels, high lipoprotein(a) levels being corelated to increased risk of atherosclerosis and cardiovascular disease, with statin therapy having mixed results as therapy[30].

Evolocumab was assessed in the OSLER and FOURIER trials. The OSLER trials enroled 4465 patients treated with conventional lipid lowering therapy who were randomized to receive evolocumab or placebo. Although the primary endpoints of the OSLER trials were safety and adverse effects, a reduction of 61% in the level of LDL cholesterol was also noted. Albeit a significant reduction of cardiovascular events was observed, the overall number of reported events was small and the authors conclued that further research was needed in this regard[31]. Consequently, the FOURIER trial, which enrolled 27.564 patients on statin therapy randomized to receive evolocumab or placebo had a primary end point based on efficacy. After 1 year of treatment, a 59% reduction in serum LDL cholesterol levels and a 2% reduction for the primary composite endpoint (cardiovascular death, myocardial infarction or stroke) was observed. This reduction of cardiovascular outcomes had an increasing trend over-time, with further benefit observed after the 1 year mark[32].

While the overall rate of adverse effects were similar in both evolocumab or alirocumab and placebo

groups, most of the noted adverse effects of PCSK9 therapy were related to local administration of the drug – injection site reactions, allergies and neurocognitive effects (memory loss).

As such, in the current updated dyslipidemia guidelines, PCSK9 inhibitors are recommended in very high-risk patients, with persistent high LDL-C levels, despite treatment with maximul available lipid lowering therapies.

Another pathway for LDL cholesterol lowering is the CETP inhibition. Cholesterylester transfer protein (CETP) is a plasma protein involved in cholesteryl ester transport among serum lipoproteins. CETP inhibition results in increasd HDL-C and decreased LDL-C levels. Although most trials with CETP inhibitors (torcetrapib, evacetrapib and dalcetrapib) have failed, REVEAL HPS TIMI 55 is a new ongoing trial which evaluates another CETP inhibitor, anacetrapib, with promising early results[33].

CONCLUSION

Despite our current advances in understanding and diagnosing atherosclerosis, this disease still remains the most important contributing factor and a great burden for cardiovascular morbidity and mortality. Furthermore, even though modern research has unveiled key mechanisms involved in the ethiopatoghenesis of atherosclerosis, there are still gaps for explaining the disease-responsible multifactorial process and the steps involved which shift the atheroma plaque from stability to instability. After several paradigm changes, atherosclerosis is now widely considered to be a progressive systemic inflammatory disease with focal and global expressions. Although the role of low-density lipoprotein cholesterol is essential and well established in pathogenesis and therapy, with newer medication focused on LDL-C lowering that showed increased benefits on cardiovascular outcomes, the inflammatory part of treatment is still lacking. The hope for new targeted agents and personalized medicine as well as identification of new pathophysiological pathways are important goals for further research.

Conflict of interest: none declared.

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