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Review Article

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Atheroma: From Anthropology to Molecular Biology

Ana Paula Marte Chacra [1], Anita L R Saldanha [2], Ana Paula Pantoja Margeotto [2], André Luis Valera Gasparoto [3], Tania Leme da Rocha Martinez *[2]

[1]Instituto do Coração (Incor) do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, Brazil.
[2]Nephrology Department, BP - A Beneficência Portuguesa de São Paulo, São Paulo, Brazil.
[3]Intensive Care Unit, BP - A Beneficência Portuguesa de São Paulo, São Paulo, Brazil.

*Correspondence Author: Tania Leme da Rocha Martinez; *tamar@uol.com.br*

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Abstract

Atherosclerosis was found in humans who lived thousands years ago. Paleopathologist Marc Armand Ruffer (1859-1917) identified atherosclerotic plaques in the aorta as well as in several large arteries of numerous Egyptian mummies, noting that atherosclerosis was a widespread disease in antiquity. Russian researchers tried to induce experimental atherosclerosis in an animal model, feeding laboratory animals with pure cholesterol. They demonstrated that cholesterol alone caused atherosclerotic lesions in the artery wall. Many studies have shown that blood cholesterol levels are largely determined by the amount of fat in the diet. The Study of the Seven Countries was the first epidemiological evidence that linked the increase in cholesterol to cardiovascular events. In the genetic approach, one of the first indications that coronary artery disease was related to cholesterol came from anecdotal case reports of children with xanthomas (large deposits of lipids just under the skin or attached to tendon sheaths, on the back of the hands or ankles), who had sudden death or myocardial infarction before the age of 10 years. The first cases of homozygous familial hypercholesterolemia were described. The 2019 European Guideline, for example, considers for high-risk patients, a target LDL-c < 70 mg/dl and a reduction > 50% compared to baseline; for very high risk, a target LDL-c < 55 mg/dl and a reduction > 50% compared to baseline; and for patients with acute coronary syndrome, within a two-year period, a target LDL< 40 mg/dl. In order to achieve these more aggressive goals, the combination of drugs is necessary, especially in patients with eligibility criteria for the new lipid-lowering drugs, based on molecular biology techniques, in addition to an adequate clinical judgment.

Keywords: ***Atheroma, Lipoproteins, LDL cholesterol, Ancient Medicine, Experimental atherosclerosis, Molecular Biology.***

Introduction

Atherosclerotic lesions were identified on a 5300-year-old mummy, as well as in Egyptian mummies and other ancient civilizations. For many decades of the twentieth century, atherosclerosis was considered a degenerative disease, mainly determined by passive lipid storage, while the most recent theory of atherogenesis is based on endothelial dysfunction [1,2].

A few years later, Russian researchers tried to induce experimental atherosclerosis in an animal model, feeding laboratory animals with pure cholesterol. They demonstrated that cholesterol alone caused atherosclerotic lesions in the artery wall.

In fact, in 1856 Rudolph Virchow was the first proponent of this hypothesis, but evidence of the key role of inflammation in atherogenesis occurred only in 2017 [3].

In particular, in 1913 the pathologist Nikolai N. Anitschkow (1885-1964) first showed that rabbits fed high amounts of purified cholesterol developed - in association with very high blood cholesterol – vascular lesions similar to human atherosclerosis. He accurately described the various cell types found in atherosclerotic lesions, including macrophages, lymphocytes and smooth muscle cells, partly anticipating a unifying view of the atherosclerosis's pathophysiology which links dyslipidemia with inflammation [4-6].

Atherosclerotic disease and its clinical manifestations, including acute myocardial infarction (AMI) and ischemic stroke, are the leading cause of morbidity and mortality worldwide. Among the atherogenic risk factors, the most well-documented and the one that determines a causal relationship with atherosclerotic disease is LDL-cholesterol (low-density lipoprotein) values [7]. There are multiple evidences, from experimental studies to large intervention studies, which have established not only the role of LDL-c, but also of Apolipoprotein B (apo B), including triglyceride-rich lipoproteins and their remnants and lipoprotein (a) [(Lp(a)] as active participants in the atherogenic process [7]. Despite accumulating evidence over decades, there is some skepticism about the causal nature of LDL-c and atherosclerotic disease. It is essential to identify LDL-c as a therapeutic target to reduce cardiovascular risk, especially after the emergence of new drugs that further reduce LDL-c levels, with additional risk reduction [7].

Our goal is to demonstrate that LDL-c is an important atherogenic risk factor and that any mechanism of reduction of plasma LDL-c concentrations reduces the risk of events proportional

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to the absolute reduction of LDL-c and the cumulative time of exposure to it.

Experimental evidence

The fundamental role of cholesterol in the pathogenesis of atherosclerosis was proposed more than 100 years ago by Nikolai N. Anitschkow, a young pathologist from St. Petersburg, who, while feeding rabbits a diet rich in cholesterol, observed the appearance of arterial lesions that resembled the atherosclerotic lesions of humans, publishing this experiment in 1913 [8]. The blood of these animals showed increased cholesterol and probably this "lipoid" content was deposited in the arterial wall [8].

Anitschkow went beyond the initial observations, not least because it would take time for this animal model to be accepted as an experimental model of human atherosclerosis. In the 20 years following his first paper, Anitschkow described the fundamentals related to the pathophysiology and progression of atherosclerosis, published in 1933 [9]:

Foamy cells. In the initial lesions - the fatty streaks - most of the lipids are found within the cells in the form of multiple, small lipid droplets. Because lipids are extracted during routine preparation of tissue samples, the multiple lipid droplets are seen as empty vacuoles; hence the name "foam cells".

Cholesterol buildup. In tissue sections, lipid droplets are birefringent. Anitschkow recognized birefringence as a characteristic property of liquid crystals of cholesterol esters.

White blood cell recruitment. Cholesterol-laden foam cells are white blood cells that have infiltrated the artery wall. Thus, Anitschkow anticipated that inflammation could play a role in the development of the injury.

Structurally intact endothelium. The monolayer of endothelial cells over the lesions appears to be intact, indicating that the invading blood cells must have penetrated through the endothelial cells. Although endothelial denudation occurred later, it was not a necessary antecedent for the formation of the lesion.

Anatomical and non-random distribution of lesions. There is a characteristic and reproducible pattern of lesion distribution. They occur most commonly and at points of arterial branching. Anitschkow correctly assumed that this location was determined by hemodynamic factors.

Conversion of fatty stretch marks into fibrous plaques. During long periods of feeding the animals with cholesterol (months), there is finally connective tissue deposition (conversion of the fatty strip into fibrous plaque) and development of a fibrous layer. (In human disease, it is the rupture of this fibrous coat that precipitates thrombosis and acute myocardial infarction; neither the rabbit model nor other animal models reproduce this terminal thrombotic event with any regularity.)

Reversibility. Initial lesions are partially reversible, but reversal is slow; late lesions resolve even more slowly. Most, but not all, lipids can be mobilized from advanced lesions, abandoning the fibrous layer and some cholesterol crystals.

Severity of injuries is proportional to the increase in blood cholesterol level. The extent and severity of the lesions are proportional to the degree of elevation of cholesterol in the blood and the duration of exposure to it. Anitschkow was well aware that it was the level of cholesterol in the blood that determined the size and extent of the lesions, and not necessarily the amount of cholesterol ingested.

Notion of multicausality. High cholesterol in the blood is necessary but not always enough to develop atherosclerosis.

In the 1933 review, Anitschkow points out the etiological nature of atherosclerosis [9]. For the author, although the degree of atherosclerosis was more evident depending on the degree of elevation of cholesterol in the blood, this process could be affected by other factors such as blood pressure, toxic substances and local arterial changes. In his animal model, however, such additional insults or injuries were not necessary; hypercholesterolemia was sufficient. The accuracy of this conclusion was confirmed by Watanabe's discovery in 1980, where rabbits of a certain strain had blood cholesterol levels of 600 mg/dl, uniformly developed atherosclerosis after a regular diet [10]. These rabbits had a mutation in the low-density lipoprotein cholesterol receptor (LDL-c) gene similar to that found in individuals with familial hypercholesterolemia (FH), resulting in elevated plasma LDL-c values, which is a sufficient cause of atherosclerosis [10]. However, as Anitschkow acknowledged, the rate of progression of lesions, at any LDL-c level, was significantly slowed or accelerated by other factors, such as hypertension or immune system disorders.

Making the case for the importance of hypercholesterolemia in human atherosclerosis has been an uphill battle [11]. Anitschkow was a scientist far ahead of his time. He was born in 1885 and wrote his classic article in 1913. The general acceptance of the lipid theory would have to wait more than 60 years to be recognized, when in 1984, the National Heart Institute completed the first large-scale, randomized, double-blind clinical trial showing that lowering serum cholesterol would significantly reduce the risk of myocardial infarction [12,13]. This primary prevention trial with cholestyramine, a bile salt sequestrant that lasted seven years, was a milestone in cardiovascular prevention. This and other evidence implicating cholesterol as a causal agent of atherosclerosis formed the basis for the first National Heart Institute Consensus [14] and formulation of national guidelines for the control of elevated cholesterol levels.

Epidemiological evidence

Previous studies have shown that blood cholesterol levels are largely determined by the amount of fat in the diet.

The Study of the Seven Countries was the first epidemiological evidence that linked the increase in cholesterol to cardiovascular events. This study selected seven countries ranging from Japan, where the average cholesterol values were the lowest (160 mg/dl) to countries such as Finland, with very high average cholesterol values (260 mg/dl). The saturated fat content was 2.5% (of the total caloric value) in Japan and 20% in Finland. During 10 years of the segment, for every 1,000 individuals followed, 70 deaths from myocardial infarction were recorded in Finland and only five in Japan. This study demonstrated that the risk of fatal and non-fatal coronary events was proportional to serum cholesterol values, which in turn were proportional to saturated fat intake [15,16].

The Framingham study was the one with the longest longitudinal segment time, establishing the concepts of cardiovascular epidemiology. Conceived in the city of Framingham, it followed a cohort of 6,000 individuals in order to investigate the risk factors correlated with the development of atherosclerotic disease. Over the course of 20 years, the study confirmed the importance of high LDL-c levels and reduction of high-density lipoprotein cholesterol (HDL-c), in addition to the additive effect of other risk factors such as smoking, diabetes mellitus, hypertension,

obesity, sedentary lifestyle, metabolic syndrome and excessive alcohol intake, as factors strongly correlated with atherosclerosis and its clinical manifestations, mainly coronary artery disease and cerebrovascular disease [17,18]. The Framingham data have had a major impact on the epidemiological research of ischemic heart disease, more than any other single epidemiological study, laying the foundation of cardiovascular prevention. After 60 years, the study continues to follow generations of descendants of those who participated from the beginning [19].

Several meta-analyses of prospective epidemiological studies have confirmed the consistent relationship between the magnitude of exposure to elevated LDL-c values and cardiovascular risk.

The Emerging Risk Factors Collaboration (ERFC) evaluated individual data from 302,430 participants, without prior cardiovascular disease, from 68 prospective studies, including 58 cohort studies, 4 case-control studies, and 6 randomized intervention studies. Linear elevation of plasma LDL-c values was observed to be associated with increased risk of nonfatal myocardial infarction or death from coronary heart disease. Although the authors reported an association between non-HDL cholesterol concentration and coronary heart disease risk in the primary analysis, any regression model that includes terms for non-HDL cholesterol, HDL-c, and triglycerides is a simple mathematical rearrangement of a model that includes terms for LDL-c, HDL-c, and calculated triglycerides. Therefore, in the ERFC analysis, the effect of both LDL-c and non-HDL-c was equal in predicting the risk of coronary artery disease. To confirm this fact, in a subsample of eight studies involving 44,234 individuals, the impact of LDL-c values (measured directly) on the risk of ischemic heart disease was similar to the impact of non-HDL cholesterol [20].

Genetic evidence

Probably, one of the first indications that coronary artery disease was related to cholesterol came from anecdotal case reports of children with xanthomas (large deposits of lipids just under the skin or attached to tendon sheaths, on the back of the hands or ankles), who had sudden death or myocardial infarction before the age of 10 years. The first cases of homozygous FH were described [21].

Several researchers have described FH as a genetic, monogenic alteration with a risk of early coronary artery disease secondary to elevated serum cholesterol [21,22]. The nature of the gene involved was discovered by Michael Brown and Joseph Goldstein, who identified the LDL-c receptor as the gene causing FH, demonstrating its critical role in determining blood levels of LDL-c, as reported: *"Our approach to unraveling the genetic defect in FH was to apply cell culture techniques. Our studies led to the discovery of a cell surface receptor for LDL and to elucidate the mechanism by which this receptor transports LDL particles into cells, through depressions and coated vesicles. Within the cell, LDL-c-derived cholesterol triggers several regulatory functions, including feedback inhibition of cholesterol synthesis. Soon, we discovered that FH is caused by genetic defects in the LDL receptor. These defects disrupt the normal regulation of cholesterol metabolism. In addition, LDL-c receptor studies have provided clear evidence for the selective uptake of macromolecules in cells, giving rise to the concept of receptor-mediated endocytosis"* [23].

FH is an autosomal codominant mutation-mediated disease with loss of function in the LDL-c receptor (LDLR) gene, causing increased levels of circulating LDL [24]. Other less frequent mutations have been described, such as mutation in the gene encoding apolipoprotein B (apo B) resulting in loss of function and non-recognition of the LDL receptor by apo B and mutation in genes with gain of function encoding the protein PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9), which degrades the LDL receptor, elevating plasma LDL-c levels. Regardless of the underlying genetic defect, FH is an autosomal dominant disease, characterized by markedly elevated LDL-c levels and premature atherosclerosis, particularly coronary artery disease [24,25].

The most common form is heterozygous FH, which affects between 1:200 and 1:300 individuals and which, if untreated, markedly increases the risk of early atherosclerotic disease in adults [26,27]. Homozygous FH is a rare condition with an extreme phenotype characterized by plasma LDL-c levels above 500 mg/dl from birth. If left untreated, it leads to the development, in almost all patients, of premature atherosclerosis in childhood or adolescence [25].

Although the phenotypic expression of FH is variable, the risk of cardiovascular events is proportional to the absolute magnitude and duration of exposure to elevated LDL-c levels [28,29].

Inhibitors of intestinal cholesterol absorption

With the evolution of lipid-lowering therapy, drugs with different mechanisms of action, such as ezetimibe, have been developed.

Ezetimibe is an inhibitor of dietary and bile cholesterol absorption and binds to the "Niemann-Pick C1-Like 1" protein, located in the brush border cells of the intestinal epithelium, blocking cholesterol absorption, which confers a 15% to 20% reduction in plasma cholesterol [30].

In the randomized IMPROVE-IT trial, 18,144 subjects with acute coronary syndrome were randomized to statin treatment versus statin plus ezetimibe. In the statin plus ezetimibe group, there was an additional reduction of 16 mg/dl of LDL-c, with a proportional reduction of 6.5% in major cardiovascular events at five years [31]. Benefits in further lowering LDL-c in very high-risk populations have been demonstrated, and that more aggressive targets are needed to reduce residual risk. In this context, the SHARP (Study of Heart and Renal Protection) study evaluated 9,270 individuals with chronic kidney disease and compared the association of simvastatin and ezetimibe versus placebo. The mean reduction in LDL-c levels was 33 mg/dl with the combination treatment compared to placebo, resulting in a lower incidence of major cardiovascular events, conferring a continuous risk reduction [32]. These data are in agreement with genetic data from randomized trials, demonstrating that variants in the HMGCR and NPC1L1 genes have biologically equivalent effects on cardiovascular disease risk, per unit of LDL-c reduction [33].

Treatment with statins and ezetimibe, which are low-cost oral therapies, has been widely used in patients at high risk and with more aggressive LDL-c reduction goals.

Adverse effects of statins

The efficacy of statins in reducing major cardiovascular events in secondary prevention is well established, with a highly favorable benefit/risk profile. In primary prevention, evidence of benefit is less robust, due to the low incidence of events in this population, raising a debatable relationship between benefit and risk. In this context, the 2019 publication "*Statin Safety and Associated Adverse Events: A Scientific Statement From the American Heart Association*" makes some recommendations on safety and prevalence of statin-related adverse events [34].

– Statins may cause dose-related myopathy, defined as muscle pain with no clear etiology or weakness accompanied by CK

elevations > 10 times the normal value, including rhabdomyolysis, occurring in < 0.1% of patients. The risk of myopathy and rhabdomyolysis is related to circulating concentrations of active drugs, which interfere with statin metabolism.

There is a growing appreciation and expectation of statins as a cause of muscle damage in treated patients, conveyed by negative information in the media. Such symptoms should never be ruled out by the clinician. Although muscle symptoms are very unlikely to be caused by statins, repeat treatment with the same statin at lower doses or another statin is helpful in resuming treatment (when myopathy is excluded) as it reduces the risk of cardiovascular events, particularly in high-risk patients, including those with pre-existing coronary artery disease.

- Statins may cause asymptomatic (dose-related) transaminase elevations above three times normal in approximately 1% of patients, but this alone does not indicate liver damage. Transaminase monitoring is not useful to prevent statin-related clinically hepatotoxicity, which is extremely rare, occurring in approximately 0.001%. It is not possible to predict which patients will develop severe hepatotoxicity, hence the recommendation to monitor for symptoms and warning signs in patients with pre-existing liver disease.
- Statins modestly increase the risk of developing diabetes mellitus by unidentified mechanisms, with a higher risk associated with high doses. The risk is higher in patients with multiple pre-existing risk factors for diabetes mellitus. The absolute risk of statin-induced diabetes mellitus in major trials was approximately 0.2% per year. The extent of any effect in routine clinical practice will depend on the initial risk of developing diabetes mellitus in patients. In the diabetic population, the mean increase in Glycated hemoglobin (HbA1C) with initiation of statin therapy is small with limited clinical significance.

  Statin therapy substantially reduces the risk of cardiovascular events in individuals with and without diabetes mellitus and that more cardiovascular events are prevented for each new diagnosis of diabetes mellitus.
- Statins do not increase the risk of cerebral hemorrhage in patients in the primary prevention of stroke. An increased risk is possible in secondary stroke prevention populations, but the absolute risk is very small and the benefit in overall reduction of stroke and other vascular events outweighs the risk.
- Statins such as rosuvastatin at a maximum dose of 40 mg may cause transient proteinuria and microscopic hematuria, but in the long term, including rosuvastatin, there is no long-term worsening of proteinuria or worsening of renal function. However, in the context of cardiac surgery, perioperative treatment with statins in statin-naïve patients may increase the risk of renal injury.

The summary of the above recommendations is based on numerous observational studies, registries, randomized trials, and others, in more than 30 years of clinical research, demonstrating that statins have few serious adverse effects.

With the exception of hemorrhagic stroke, the possible cause of newly diagnosed diabetes mellitus, and some rare cases of autoimmune necrotizing myositis, the adverse effects of statins can almost always be reversed by discontinuing treatment. In contrast, acute myocardial infarction or ischemic stroke permanently damage an individual's heart or brain, and can even be fatal.

In the patient population for whom statins are recommended by current guidelines, the benefit of cardiovascular risk reduction far outweighs the adverse effects.

Despite therapeutic advances in reducing LDL-c levels, the percentage of patients who achieve adequate reduction is still small, leaving this population susceptible to the progression of atherosclerosis and acute cardiovascular events. The FOURIER-OLE data provide an additional justification for intensifying efforts to reduce cardiovascular risk [35,36].

What the guidelines recommend

The benefit of intense LDL-c reductions, especially in high or very high risk populations, opens the prospect for a paradigm shift, where the concept of high doses of potent statins is modified by high-intensity personalized lipid-lowering treatment. The challenge is how to implement this strategy, considering that in order to achieve increasingly aggressive goals, it will be necessary to associate drugs other than statins, such as ezetimibe, PCSK9 inhibitors (monoclonal antibodies or inclisiram) and bempedoic acid (cholesterol synthesis inhibitor, complementary to statins) [37].

In this context, the patient's risk stratification is essential, since the risk reduction is a function of the absolute reduction of LDL-c. Based on the risk, the evaluation of how aggressive the prescription will be to achieve the recommended goals is evaluated.

The 2019 European Guideline, for example, considers for high-risk patients, a target LDL-c < 70 mg/dl and a reduction > 50% compared to baseline; for very high risk, a target LDL-c < 55 mg/dl and a reduction > 50% compared to baseline; and for patients with acute coronary syndrome, within a two-year period, a target LDL< 40 mg/dl [38]. In order to achieve these more aggressive goals, the combination of drugs is necessary, especially in patients with eligibility criteria for the new lipid-lowering drugs, based on molecular biology techniques, in addition to an adequate clinical judgment.

Conclusion

Atherosclerosis causes many other diseases in addition to coronary artery and cerebrovascular disease, including dementia, peripheral artery disease, heart failure, renal artery stenosis, carotid artery stenosis and embolization, kidney failure, aortic disease, mesenteric artery disease, erectile dysfunction, frailty, and premature aging. The "burden" of this disease is reflected by its systemic, debilitating, disabling and sometimes deadly character. Therefore, the importance of increasing efforts in prevention and treatment is justified [39].

The relationship between hypercholesterolemia and atherosclerosis took root more than 100 years ago. Advances have provided a more granular and extensive understanding of atherogenesis, and how elevated LDL-c is a causal and crucial factor in this process. There has been exponential growth in scientific tools and methods that have accelerated the understanding of the complex mechanisms that result in atherosclerosis and its consequences [39].

Evidence from randomized studies with statins alone or in combination with ezetimibe was overwhelming in pointing to the benefits of LDL-c reduction in primary and secondary prevention. Studies with PCSK9 inhibitors associated with conventional statin therapy, in patients at high or very high risk, demonstrated that additional reductions in LDL-c, even at values below 20 mg/dl, had an impact on benefits in cardiovascular outcomes, without evidence of harm. These data converge to Mendelian randomization studies,

in which patients with lower LDL-c values from birth do not present atherosclerosis [39].

Currently, the discussion seems to be on the early reduction of LDL-c values, before the beginning of the atherogenic process, since there is a therapeutic arsenal that could meet these demands, but there is still no evidence of how early these interventions should occur.

Even with guidelines, risk stratification tools, and algorithms for eligibility of patients who benefit from more aggressive LDL-c targets, few patients at high risk or in secondary prevention are among the recommended treatment targets. And in primary prevention, this scenario is less promising.

Therefore, prevention is the basis of the treatment of atherosclerosis, whether it is the prevention of the installation of the atherogenic process or the prevention of the progression of atherosclerotic lesions. Facilitating measures that can prevent these processes should be increasingly implemented.

Abbreviations

FH: Familial hypercholesterolemia
HDL-c: High-density lipoprotein cholesterol
LDL-c: Low density lipoprotein cholesterol
PCSK9: Proprotein Convertase Subtilisin/Kexin Type 9

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None.

Conflict of interest

None.

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