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Cellular models of atherosclerosis and their implication for testing natural substances with anti-atherosclerotic potential

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ABSTRACT

Background: Atherosclerosis remains a major problem in the modern society being a cause of life-threatening cardiovascular diseases. Subclinical atherosclerosis can be present for years before the symptoms become obvious, and first manifestations of the disease in a form of acute ischemia of organs are often fatal. The development of atherosclerosis is characterized by lipid accumulation in the aortic wall and formation of foam cells overloaded with large amounts of lipid inclusions in the cytoplasm. Current therapy of atherosclerosis is aimed mostly at the normalization of the blood lipid profile, and has no direct activity on the atherosclerotic plaque development. It is therefore necessary to continue the search for substances that possess a direct anti-atherosclerotic effect, preventing the cholesterol deposition in the arterial wall cells and reducing the existing plaques.
Purpose: Medicinal plants with potential anti-atherosclerotic activity are especially interesting in that regard, as plant-based medications are often characterized by good tolerability and are suitable for long-term therapy. The evaluation of novel active substances requires the establishment of reliable models of atherogenesis. In this review we discuss cellular models based on cultured human aortic cells. We also discuss several examples of successful application of these models for evaluation of anti-atherosclerotic activity of natural products of botanical origin based on measurable parameters, such as intracellular cholesterol accumulation.
Chapters: We describe several examples of successful screening and clinical studies evaluating natural products that can be beneficial for prevention and treatment of atherosclerosis, including the subclinical (asymptomatic) forms.
Conclusion: Several substances of botanical origin have been demonstrated to be active for treatment and prevention of atherosclerosis. The obtained results encourage future studies of naturally occurring anti-atherosclerotic agents.

Introduction

Atherosclerosis remains one of the major medical and social problems in the developed countries, since associated with it cardiovascular disorders account for a great part of overall morbidity and mortality (Simmons et al. 2012). Atherosclerosis development is a multifactorial process, which is characterized by degenerative changes in the wall of large arteries with subsequent blood vessel occlusion and ischemia of organs and tissues. Subclinical (asymptomatic) atherosclerosis is a wide spread condition in modern society. Atherosclerotic lesions exist already in relatively young people and can progress for decades until first clinical manifestations become evident (Simmons et al. 2012; Berenson et al. 1998; McGill et al. 2002; Tuzcu et al. 2001). It has been shown that middle-aged people do not present with symptoms of atherosclerosis while having atherosclerotic lesions at various stages of progression in nearly 100% of cases (Berenson et al. 1998; McGill et al. 2002; Tuzcu et al. 2001). At the same time, no specific therapy is developed so far for direct prevention and treatment of subclinical atherosclerosis, partly because current understanding of the exact mechanisms and hence relevant therapeutic targets is not sufficient.

Abbreviations: CHD, coronary heart disease; cIMT, carotid intima-media thickness; HDL, high density lipoprotein; LDL, low density lipoprotein.

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The results of epidemiological studies define a number of factors associated with the increased risk of vascular occlusion, including several clinical and biochemical syndromes (Anderson et al. 1991). Elimination of such risk factors is regarded as the most promising approach for primary prevention of atherosclerosis (Fowkes et al. 2013). These preventive measures, however, are indirect, as they are aimed to alter certain conditions, which are not immediately related to the molecular and cellular mechanisms of atherogenesis. Another therapeutic strategy implicates a "direct" therapy, which is aimed to prevent the onset and progression of atherosclerotic lesions by inhibiting the molecular and cellular mechanisms of atherogenesis in human arteries (Orekhov and Tertov 1997; Orekhov et al. 1986a; Sazonova et al. 2009). This approach to atherosclerosis prevention and treatment is currently under development.

Statins are currently regarded as most promising therapeutic agents for prevention and treatment of atherosclerosis (Stein and Raal 2014). Regular and long-term statin therapy helps preventing the development of novel and promoting the regression of existing atherosclerotic lesions. However, recent opinion is that, although statins are effective at reducing cholesterol levels, they fail to substantially improve cardiovascular outcomes. It became known that the directors of the clinical trials have succeeded in minimizing the significance of the numerous adverse effects of statin treatment (Diamond and Ravnskov 2015). However, the target level of LDL cholesterol cannot be achieved in many patients because of statin intolerance (Banach et al. 2015). Noteworthy, therapy aimed at prevention and treatment of early stages of atherosclerosis should necessarily be long-term, making the good tolerability and low cost essential. Therefore, anti-atherosclerotic drugs based on natural products can be a preferred alternative, as they have virtually no adverse effects and are suitable for long-term or even life-long treatment (Orekhov et al. 2013).

Natural products with anti-atherosclerotic potential

Natural products with potential anti-atherosclerotic properties are currently gaining attention of the researchers (Orekhov et al. 2013, Badimon et al. 2010). Many of the well-known dietary products have strong positive effects on the blood lipid profile, reducing the risk of atherosclerosis development, others can be used to reduce inflammation and modulate platelet aggregation. Nutraceuticals that can reduce cIMT can be considered as direct anti-atherosclerotic agents. The protective effect of Mediterranean diet, which includes high amounts of mono- and polyunsaturated fatty acid sources, such as olive oil, has been evaluated in several controlled studies (Mensink et al. 2003). Garlic (*Allium sativum*) has been demonstrated to contain active substances that can possess not only protective, but also curative effects on atherosclerosis (Koscielny et al. 1999). Vegetables, fruits, cereals and other dietary substances contain such biologically active molecules as polyphenols, phytosterols and phytostanols, vitamins and antioxidants and dietary fiber, all of them being considered to have more or less prominent anti-atherosclerotic activity (Badimon et al. 2010).

Several recent studies evaluated certain nutraceuticals as potential agents for development of dietary supplements for lowering the risk of cardiovascular disease development. Chitosan, a dietary fiber derived from fungal mycelium, has been demonstrated to reduce plasma and LDL cholesterol and triglycerides and increase HDL cholesterol in patients with elevated plasma triglyceride levels, not taking lipid-lowering medications (Rizzo et al. 2014, Patti et al. 2015b). It could be interesting to study the beneficial activity of chitosan on plasma lipid profile in a larger study. Another recent study has evaluated the beneficial effects of a natural supplement containing several biologically active substances: curcumin (*Curcuma longa*), silymarin (*Silybum marianum*), guggul (*Commiphora wightii*), chlorogenic acid and inulin (Patti et al. 2015a). The supplement was tested on 78 patients with metabolic syndrome as add-on therapy for 4 months. Treatment with the product resulted in significant reductions of body weight and body mass index (BMI), total cholesterol levels ($p = 0.03$) and fasting glucose levels ($p = 0.14$). Levels of LDL cholesterol were decreased as well, although statistical significance has not been achieved. Here again, a larger study is required to evaluated the protective effects of the supplement for prevention and treatment of atherosclerosis.

Despite the considerable progress made during the recent years, our understanding of possible cardioprotective effects of nutraceuticals, such as lowering of blood cholesterol and blood pressure regulation, and their impact on cardiovascular disease risk factors is still limited (Rai et al. 2013; Al-Waili et al. 2013; Ried et al. 2013; Hopkins et al. 2013; Sobenin et al. 2008a; Sobenin et al. 2010; Sobenin et al. 2009). This can partly be explained by the limited methodology for studying anti-atherosclerotic activity of drug substances, especially regarding the early stages of the pathogenesis, and the lack of adequate pathophysiological models. Therefore, efforts should be taken to develop reliable new methods for assessment of the therapeutic potential of natural products.

Mechanisms of atherogenesis in humans

Current understanding of cellular and molecular mechanisms of atherogenesis is based on the classical lipid theory of atherosclerosis, postulating that the most important event in the disease development is the accumulation of extracellular and intracellular lipids in the arterial intima (Schönfelder 1969; Konstantinov et al. 2006). Low-density lipoprotein (LDL) serves as the major source of lipid deposits in the arterial wall. During the last decades, it became obvious that atherogenesis is caused not by native LDL, but rather by its modifications, including desialylation, the change of the total surface charge (electronegativity), change of hydrated density of lipoprotein particles and oxidation (Jaakkola et al. 1993; Sobenin et al. 1998; Tertov et al. 1992a; Tertov et al. 1995a). It is likely that we actually deal with the same type of multiple atherogenic modifications, but differently evaluated by different methods of laboratory diagnostics (Tertov et al. 1995a; Tertov et al. 1995b; Tertov et al. 1996; Tertov et al. 1992b). The elevated levels of modified LDL in the bloodstream trigger additional mechanisms that enhance its atherogenic potential, most importantly, the formation of large complexes. The modified LDL species acquire the ability to spontaneous self-association because of altered surface charge. Additionally, modified LDL possesses antigenic properties, inducing the production of anti-LDL autoantibodies, which ultimately leads to the formation of LDL-containing circulating immune complexes (Sobenin et al. 2014a). Modified LDL also has high avidity for connective tissue matrix components. The resulting large LDL-containing associates are characterized by altered cellular metabolism. Rather than being taken up by the receptor-mediated internalization, these complexes are internalized by vascular cells in process of uncontrolled phagocytosis and processed differently than native LDL particles (Goldstein and Brown 1987). As a result, larger amounts of LDL-containing phagocytized particles accumulate in cellular cytoplasm mainly in the form of lipid droplets, leading to the lipid retention in the arterial wall. Such lipid-loaded cells are defined as foam cells and are a common feature of atherosclerotic lesions.

The key steps of atherosclerosis development are represented on Fig. 1. The initiation of atherosclerotic lesion depends on the atherogenic modification of circulating LDL and on local change in the endothelial permeability (Vanhoutte 2009). Modification of LDL can occur both in the bloodstream and directly in the intima, after penetration of LDL particles via the luminal endothelium. One of the earliest events of multiple modification of LDL is