Anti-atherosclerotic Drugs from Natural Products

Alexander N Orekhov1,2*

1Institute for Atherosclerosis Research, Skolkovo Innovative Center, Moscow, Russia
2Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Russia

Abstract
Atherosclerosis is the cause of more than 50% mortality in industrial countries. Atherosclerosis develops over many years, so the anti-atherosclerotic therapy should be long-term or even lifelong. Tachyphylaxis, long-term toxicity and cost amongst other issues may present problems for the use of conventional medications in the long-term. Drugs based on natural products can be a good alternative.

We have developed a series of natural compounds that are specifically designed to act at the vessel wall and modulate the atherosclerotic lesion. Clinical efficacy was determined in atherosclerosis regression studies with ultrasound examination of carotid arteries. The AMAR study (Atherosclerosis Monitoring and Atherogenicity Reduction) was designed to estimate the effect of two-year treatment with time-released garlic-based drug Allicor on the progression of carotid atherosclerosis in asymptomatic men in double-blinded placebo-controlled randomized clinical trial. The primary outcome was the rate of atherosclerosis progression, measured by high-resolution B-mode ultrasonography as the increase in carotid intima-media thickness (IMT) of the far wall of common carotid arteries. The mean rate of IMT changes in Allicor-treated group was significantly different from the placebo group in which there was moderate progression. The results of AMAR study demonstrate that long-term treatment with Allicor has a direct anti-atherosclerotic effect on carotid atherosclerosis. These results encouraged clinical trials of two other drugs based on natural products, including: Inflaminat (calendula, elder and violet), possessing anti-cytokine activity and the phytoestrogen-rich drug Kainat (garlic powder, extract of grape seeds, green tea leaves, hop cones, β-carotene, α-tocopherol and ascorbic acid), designed for postmenopausal women. As in the AMAR trial Inflaminat caused regression of carotid atherosclerosis while Kainat prevented its development.

It should be noted that the anti-atherosclerotic effects of drugs based on natural products are not inferior to the effects of such drugs as statins and calcium antagonists. Thus, natural products can be considered as promising drugs for anti-atherosclerotic therapy.

Keywords: Allicor; Anti-Atherosclerotic therapy; Atherosclerosis; Cell culture; Drugs; Imaging; Intracellular cholesterol retention; Natural products

Abbreviations: AMAR: Atherosclerosis Monitoring and Atherogenicity Reduction; CIMT: Carotid Intima-media Thickness; HDL: High-density Lipoprotein; LDL: Low Density Lipoprotein

Introduction
Development of anti-atherosclerotic drugs is based on our current knowledge and hypotheses on the mechanisms of atherogenesis. The only hypothesis that has received confirmation in the clinic is the cholesterol hypothesis. This hypothesis was proposed more than 100 years ago by Nikolai Anitschkow. The Anitschkow's hypothesis linked cholesterol hypothesis. This hypothesis was proposed more than 100 years ago by Nikolai Anitschkow. The Anitschkow's hypothesis linked atherosclerosis with high levels of total cholesterol in the blood. The modern paradigm only explains some aspects of this hypothesis, in particular as atherosclerosis is not associated with the total level of cholesterol but with atherogenic low density lipoprotein (LDL) cholesterol and anti-atherogenic high density lipoprotein (HDL) cholesterol [1-3].

Retention of intracellular lipids or lipidosis that is the accumulation of cholesterol and other lipids in the arterial cells is the most prominent manifestation of atherogenesis at the arterial cell level. Williams and Tabas [4,5] proposed so-called the Response-to-Retention model of atherogenesis. Although atherogenesis is a complex and multifactorial process, the key initiating process in atherogenesis is the sub endothelial cholesterol retention that is both necessary and sufficient to provoke lesion initiation. Retention of cholesterol transported by low density lipoprotein (LDL) in sub endothelial space of arterial wall is an absolute requirement for lesion development. According to Tabas et al. [6] the molecular basis of lipoprotein retention is associated with interaction of lipoprotein and extracellular matrix molecules. Local responses to these retained lipoproteins include inflammatory response with subsequent lesion development [7]. Specific focus is placed on the potential of these innate immune targets for therapeutic interventions to retard the progression of atherosclerosis or to induce its regression [8]. The response-to-retention model considers only the retention of cholesterol on extracellular matrix, while completely ignoring the retention of intracellular cholesterol.

Intracellular cholesterol retention is accompanied by increased proliferative activity of vascular cells and increased synthesis of extracellular matrix [9,10]. Along with the retention of intracellular cholesterol, both proliferation and fibrosis are characteristic features of atherogenesis at the arterial cell level, too. Thus, intracellular cholesterol retention may be critical event of all major manifestations of atherosclerosis on cellular level. Intracellular cholesterol retention may be regarded as a novel target for anti-atherosclerotic therapy. This allows us to consider cellular retention of cholesterol as a novel target for anti-atherosclerotic therapy. In this case the target is not the level of blood cholesterol but the level of cholesterol in vascular cells.

This review summarizes the results of basic studies shedding...
light on the mechanisms of intracellular retention of cholesterol. We describe our cellular models to search for anti-atherosclerotic agents and demonstrate the use of these models for the development of anti-atherosclerotic drugs.

Atherosclerosis develops over many years, so the anti-atherosclerotic therapy should be long-term or even lifelong. Tachyphylaxis, long-term toxicity and cost amongst other issues may present problems for the use of conventional medications in the long-term. Drugs based on natural products can be a good alternative. We reported successful translation of innovative attempts and novel drugs from natural products into clinical practice.

**Basic Studies**

Intracellular retention of cholesterol is induced by LDL. It is well known that native lipoprotein usually does not increase the cholesterol content of the cell; however the incubation of cultured cells with chemically modified LDL results in a massive accumulation of cholesterol in the cells [11]. Thus, modified, but not native, LDL is the source of cholesterol retention in arterial cells. Cells populating atherosclerotic lesions are often overloaded with lipids, and their cytoplasm is almost completely filled with lipid inclusions [12]. These cells are referred to as foam cells because of foamy appearance of their cytoplasm.

In the blood of patients with coronary and extra coronary atherosclerosis we have discovered modified (desialylated) LDL [13-16]. This naturally occurring modified LDL induces cholesterol retention in cultured arterial cells [13-16]. Circulating modified LDL is multiple modified lipoprotein characterizing by lower sialic acid, triglyceride and cholesterol contents; smaller particle size; greater density and negative charge; higher aggregative activity; and some other specific features [17]. We have discovered an enzyme, trans-sialydase, which is responsible for the desialylation of LDL particles in the blood [18].

In addition to desialylated LDL, more electronegative LDL and small dense LDL have been found in human blood [19,20]. In cooperation with researchers who found more electronegative LDL and small dense LDL we carry out comparative studies and showed that the more electronegative LDL is desialylated LDL [21] as well as desialylated LDL isolated from patient blood [13-16] is more electronegative. Desialylated LDL particle is smaller and denser than that of native LDL [22] as well as small dense LDL isolated from patients has a low content of sialic acid, i.e., it is desialylated [23].

Modified (desialylated) LDL stimulates anti-LDL auto-antibodies production [24-28]. Anti-LDL auto-antibodies and modified LDL form LDL-containing circulating immune complexes [29]. We have demonstrated LDL-containing circulating immune complexes and anti-LDL auto-antibodies in the blood of atherosclerotic patients [29-31]. We have also found a positive correlation between the levels of LDL-containing immune complexes and the severity of atherosclerosis [29-33]. LDL is able to form complexes with collagen, elastin, and proteoglycans isolated from human aortic intima [34-39]. These LDL-containing complexes induce intracellular cholesterol retention as a result of increased uptake and decreased intracellular degradation of lipoproteins in complexes [38]. Naturally occurring multiple modified LDL has tendency to associate and forms self-associates, while native LDL does not associate [37]. We found a positive correlation between intracellular cholesterol retention caused by modified LDL and the degree of LDL self-association [37,38]. LDL-associates isolated by gel filtration were shown to induce dramatic intracellular cholesterol retention. Thus, the formation of large LDL containing complexes (self-associates, immune complexes, and complexes with connective tissue matrix) is a necessary and sufficient condition for intracellular cholesterol retention.

Our knowledge of mechanisms of intracellular cholesterol retention allowed us to consider the prevention of intracellular cholesterol retention as a target for anti-atherosclerotic therapy. As a model of intracellular cholesterol retention we use primary culture of sub endothelial arterial cells and modified LDL or blood serum containing modified LDL. We use this model for the development of anti-atherosclerotic drugs.

**Cellular Models**

Cells are isolated from the subendothelial part of the human aortic intima between the endothelial lining and the media [39]. Using collagenase and elastase, viable cells are isolated from the subendothelial layer of the intima [40-42]. Isolated cells can be classified as the mixture of smooth muscle cells, pericyte-like cells, and macrophages [43-45]. The culture on which our experiments are performed is represented by this mixed population [39].

Cells isolated from atherosclerotic lesions retain all major characteristics of atherosclerotic cells when cultured. They are capable of synthesizing collagen, proteoglycans and other components of the extracellular matrix [9]. Cell cultured from fatty lesions have an enhanced proliferative activity [41], higher than that of cells cultured from unaffected intima [44,45]. Considerable part of cells cultured from atherosclerotic lesions is foam cells, which contain numerous inclusions, likely lipid droplets, which fill the entirety of the cytoplasm [41]. Excess lipids in foam cells are mainly free cholesterol and cholesteryl esters [41]. It is important that the content and composition of lipids in cultured cells within the first 10-12 days in culture remain unchanged and correspond to the respective indices of freshly isolated cells [41,45]. Thus, our investigations are carried out directly on exactly those cells that require a therapeutic action in vivo.

To induce intracellular cholesterol retention we used blood sera obtained from coronary heart disease patients [46]. These sera contain modified LDL [11-14]. As a result serum is atherogenic, i.e., it is able to cause retention of intracellular cholesterol and stimulate other atherogenic manifestations in cultured cells [10,46-48]. Atherogenic serum was added to primary culture of sub endothelial cells derived from an unaffected intima. Drug efficacy is judged by the ability to prevent the deposition of intracellular cholesterol in cultured cells. The prevention of intracellular cholesterol retention may be regarded as anti-atherosclerotic effect. In terms of arterial cells, any drug effect that does not directly prevent intracellular cholesterol retention is regarded as an indirect anti-atherosclerotic action. Only a drug that exhibits its preventive activity at the arterial level is a direct anti-atherosclerotic drug. Using this model, we have examined the effects of different drugs and chemicals.

Using our cellular model we have tested three classes of cardiovascular drugs, calcium antagonists, beta-blockers and nitrates. These drugs are widely used in clinics in the therapy of various disorders that resulted from atherosclerosis of different arteries. The effect of several calcium antagonists on intracellular cholesterol retention was tested. Verapamil and nifedipine completely inhibited the accumulation of intracellular cholesterol induced by the sera, while other calcium antagonists, such as diltiazem, nicardipine, isradipine, and darodipine, substantially reduced cholesterol accumulation [49]. The examined calcium antagonists demonstrated anti-atherogenic action in vivo by inhibiting the development of experimental atherosclerosis in animals.
Nitrates and beta-blockers have been tested to examine their effect on atherosclerotic cellular indices. Nitrates only minimally affected cholesterol levels [52]. In contrast, all the examined beta-blockers, i.e., propranolol, alpenrol, metoprolol, pindolol, and timolol, increased intracellular cholesterol retention, i.e., all of these drugs exhibited pro-atherogenic activity in culture [49,52]. If beta-blockers have a similar effect in vivo, one may assume that these drugs are atherogenic and induce their athereogenic effects at the arterial cell level. Apparently, nitrates do not follow a similar trend.

Thus, three classes of cardiovascular drugs exert a different influence on intracellular cholesterol retention. Calcium antagonists exhibit anti-atherosclerotic actions. In contrast, beta-blockers are pro-atherogenic. Nitrates do not have an effect on intracellular cholesterol retention. Our data are consistent with the results of a clinical study reported by Losaldi et al. [53] who demonstrated that long-term oral administration of propranolol aggravates coronary atherosclerosis in patients with angina of effort compared with the calcium antagonists’ nifedipine and isorobide dinitrate. Nifedipine showed the best effect on coronary atherosclerosis by suppressing the development of existing atherothrombotic lesions and preventing the appearance of new lesions. Isorobide dinitrate was less effective. These clinical observations confirming our in vitro results encourage us to develop an anti-atherosclerotic therapy using our cellular model.

Naturally, the question arises whether the anti-atherosclerotic effects revealed in in vitro cellular model can be manifested in vivo. To answer this question, an ex vivo model was developed. In the ex vivo model, instead of agents, blood sera taken from patients after oral drug administration is added to cultured cells.

Two calcium antagonists, verapamil and nifedipin, and two beta-blockers, propranolol and pindolol, were examined using the ex vivo model [52,54]. Within 2-4 hours after nifedipine or verapamil oral administration, the patients’ sera were less atherogenic; i.e., induced less intracellular cholesterol retention. In contrast, the sera of patients who received propranolol or pindolol were pro-atherogenic. Its pro-atherogenic properties manifested themselves at the arterial cell level via the rise of intracellular cholesterol accumulation. This finding allows us to assume that not only in vitro, but also in vivo, calcium antagonists and beta-blockers are anti-atherosclerotic and pro-atherogenic drugs, respectively.

The effect of nifedipine on serum properties during a course has been assessed [49]. A patient received 20 mg doses of nifedipine three times a day at an 8-hour interval for 7 days. Twenty-eight days after regular nifedipine therapy, the atherogenicity of the patient’s serum was considerably lower than at the beginning of the therapy. Directly after new dose of nifedipine, the intracellular cholesterol retention was not revealed. In contrast, after a course treatment with the beta-blocker propranolol, the patient’s serum acquired stable atherogenic properties. At the beginning of the course, the serum of this patient did not induce intracellular cholesterol retention; however, 28 days of regular propranolol therapy led to the emergence of atherogenicity, revealed even before the drug administration.

Natural Products

Ex vivo cellular model can be used to test natural products. We have investigated prevention of intracellular cholesterol retention caused by certain mushroom species and sea products. Extracts from 20 Korean mushroom species exhibit intracellular cholesterol retention revealed by cell culture test [55]. Among sea products, mollusk and krill meat were investigated. Two hours after a single dietary load with canned meat of a mollusk belonging to the genus Buccinum, the patient’s blood serum acquired marked anti-atherosclerotic properties [56]. Incubation of this serum with cultured atherosclerotic cells led to a fall in intracellular cholesterol retention. Patients of another group received a single dietary dose of Antarctic krill meat. Two hours later, the retention of cellular cholesterol induced by blood sera decreased, and four hours later, it was practically absent [56].

To develop a dietary therapy based on the krill meat, the effective dose and proper regimen have been established. The anti-atherosclerotic activity of krill meat was evaluated by the ability to reduce intracellular cholesterol retention. The dose-effect dependence was revealed by comparing the efficacy of the two doses, and we found that krill meat possesses anti-atherosclerotic effects at a dose of 10-20 g, half-maximum effect was reached at a dose of 30 g, and the maximum effect was achieved at a dose of 50 g. We believe that this approach will be useful in the development and optimization of anti-atherosclerotic dietary therapies.

We have tested numerous extracts of natural products to reveal their effects on their capacity to prevent intracellular cholesterol retention caused by atherogenic blood sera from atherosclerotic patients. Naturally, the tested agents included anti-atherosclerotic, pro-atherogenic, and neutral products. Among the anti-atherosclerotic natural products, the most effective was garlic.

We investigated the in vitro effect of garlic extract on intracellular cholesterol retention. Garlic prevented the serum-induced accumulation of free cholesterol and reduced the accumulation of cholesterol esters [57]. The effect of garlic on cholesterol esters may be explained by the action on enzymes responsible for cholesterol ester metabolism. We have shown that garlic inhibits acyl-CoA: cholesterol acyltransferase, which participates in cholesterol ester formation, and stimulates cholesterol ester hydrolase, which degrades cholesterol esters [57].

Further investigations ex vivo confirmed the in vitro effects of garlic [58]. In ex vivo experiments we applied dry garlic powder. Using ex vivo model we optimized the effective dose of oral garlic powder administration. The anti-atherosclerotic activity of garlic powder was evaluated by the ability to reduce intracellular cholesterol retention. The dose-effect dependence was revealed by comparing the efficacy of the two doses, and we found that garlic powder possesses anti-atherosclerotic effects at a dose of 50-300 mg. The minimum dose causing maximum effect was 150 mg.

Using the optimal dose of 150 mg garlic powder we have showed that long-term treatment for months and years leads to a significant reduction of intracellular cholesterol retention or its extinction [58,59]. These data stimulated us to develop a drug based on garlic powder and carried out a clinical study of the effects of this drug on atherosclerosis regression.

Translation into Clinics

We have developed the time-released garlic powder tablets referred to as Allicor that have been registered and are now being manufactured by INAT-Farma, Ltd. (Russia). The AMAR study (Atherosclerosis Monitoring and Atherogenicity Reduction) was carried out to estimate the effect of two-year treatment with Allicor on the progression of carotid atherosclerosis in asymptomatic men in a double-blinded, placebo-controlled randomized clinical trial (ClinicalTrials.gov Identifier, NCT01734707). The primary outcome was the rate of atherosclerosis progression. It was revealed that the rate of carotid atherosclerosis progression was practically absent.
progression, measured by high-resolution B-mode ultrasonography as the increase in carotid intima-media thickness (CIMT) of the far wall of common carotid arteries [59].

At the baseline, blood serum taken from patients induced 1.56-fold increase in intracellular cholesterol retention in cell culture test. In the placebo group, the mean value of serum induced intracellular cholesterol retention did not change significantly during two years. On the opposite, in Allicor-treated patients the mean level of intracellular cholesterol retention was significantly lowered already after first 3 months of treatment, and this effect was maintained during the study. We found statistically significant difference in the dynamic of changes in intracellular cholesterol retention between Allicor-treated and placebo groups. Allicor significantly reduced CIMT compared to baseline and the placebo group, while spontaneous atherosclerosis progression prevailed in the placebo group.

Our data are generally consistent with the results of a double-blinded, placebo-controlled randomized study by Koscielny et al. [60]. That study has been demonstrated that 4-year treatment with the garlic-based drug Kwai inhibited the increase in volume of atherosclerotic plaques in carotid and femoral arteries by 5-18%.

Atherosclerosis regression effect of Allicor revealed in the AMAR study is comparable with the results of most successful trials with other compounds [61-68]. Those studies employed potent lipid-lowering agents or calcium antagonists, whose beneficial effects of treatment were attributed to reduction in LDL cholesterol, the major risk factor for atherosclerosis development, or arterial wall stress.

Effects of Allicor promoted new clinical trials of two other drugs based on natural products, Inflaminat, which possesses anti-cytokine activity, and the phytoestrogen-rich drug Karinat, which is designed for postmenopausal women.

Inflammatory cytokines play significant role at every stage of atherogenesis [69-71]. So, anti-cytokine drugs may be effective for the prevention of atherosclerosis. We have developed drug Inflaminat, which is based on calendula, elder and violet. Our laboratory investigations demonstrated that Inflaminat suppresses secretion of pro-inflammatory cytokines and reduces intracellular cholesterol retention. We have carried out a pilot study (Clinical Trials.gov Identifier, NCT01743404) with Inflaminat using a protocol similar to that of the AMAR study. New study demonstrated atherosclerosis regression effects of Inflaminat and a statistically significant difference from the baseline as well as from placebo group in asymptomatic men [59].

The effective approaches to atherosclerosis prevention in postmenopausal women do not exist. Hormone replacement therapy is not acceptable due to the negative results of clinical studies, including WHI, PEPI, and HERS [72-77]. Phytoestrogens may be an alternative to hormone replacement therapy, but practically nothing is known about their effects on atherosclerosis.

We selected phytoestrogen-rich botanicals on the basis of their ability to prevent intracellular cholesterol retention in vivo test system. The following combination was chosen: garlic powder, extract of grape seeds, green tea leaves, and hop cones, all of them produced significant anti-atherogenic effects. This combination was used for development of novel isoflavonoid-rich dietary supplement Karinat. Karinat prevents intracellular cholesterol retention and is characterized by good phytoestrogen profile, providing additional amounts of biologically active polyphenols, including resveratrol, genisteine, and daidzeine. Moreover Karinat contains additional amounts of β-carotene, α-tocopherol and ascorbic acid to provide the necessary daily intake of antioxidants.

We have carried out a randomized, double-blinded, placebo-controlled pilot clinical trial to reveal possible atherosclerosis-related effects of Karinat in healthy postmenopausal women http://clinicaltrials.gov/ Identifiers, NCT01741974 and NCT01742000). The annual rate of changes in CIMT was monitored. In the Karinat group the average CIMT was not changed (statistically insignificant increase of 6 μm per year, less than 1%). The progression of existing plaques was slower by 32% per year. Thus, the use of Karinat in postmenopausal women almost completely suppresses the formation of new atherosclerotic lesions, and it slows the progression of existing lesions [59].

Conclusion

Our basic studies have demonstrated that intracellular cholesterol retention is the key initiating process in atherogenesis. On the basis of our data we have developed cellular models and an approach to prevent intracellular cholesterol retention. We have demonstrated that prevention of intracellular cholesterol retention leads to the prevention of atherosclerosis progression and/or its regression in patients. We can conclude that our basic findings were successfully translated into clinics.

Unfortunately, natural products with anti-atherosclerotic therapeutic potential are not prescribed by medical practitioners as anti-atherosclerotic agents. However, our data allows us to consider botanicals as anti-atherosclerotic prescriptions [78].

Conflict of Interest

The author confirms that this article presents no conflicts of interest.

Acknowledgement

This work was supported by the Russian Ministry of Education and Science.

References

1. Martin SS, Blumenthal RS, Miller M (2012) LDL cholesterol: the lower the better. Med Clin North Am 96: 13-26.
2. Sala F, Catapano AL, Norata GD (2012) High density lipoproteins and atherosclerosis: emerging aspects. J Geriatr Cardiol 9: 401-407.
3. Fisher EA, Feig JE, Hewing B, Hazen SL, Smith JD (2012) High-density lipoprotein function, dysfunction, and reverse cholesterol transport. Arterioscler Thromb Vasc Biol 32: 2813-2820.
4. Williams KJ, Tabas I (1995) The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol 15: 551-561.
5. Williams KJ, Tabas I (1998) The response-to-retention hypothesis of atherogenesis reinforced. Curr Opin Lipidol 9: 471-474.
6. Tabas I, Williams KJ, Borén J (2007) Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation 116: 1832-1844.
7. Insull W Jr (2009) The pathology of atherosclerosis: plaque development and plaque responses to medical treatment. Am J Med 122: S3-S154.
8. Moore KJ, Freeman MW (2008) Targeting Innate Immunity for CV Benefit. Drug Discov Today Ther Strateg 5: 15-23.
9. Orekhov AN, Tertov VV, Kudryavcov SA, Smirnov VN (1990) Triggerlike stimulation of cholesterol accumulation and DNA and extracellular matrix synthesis induced by agtherogenic serum or low density lipoprotein in cultured cells. Circ Res 66: 311-320.
10. Orekhov AN, Tertov VV, Pokrovsky SN, Adamova IYu, Martensyuk ON, et al. (1988) Blood serum atherogenicity associated with coronary atherosclerosis. Evidence for nonlipid factor providing atherogenicity of low-density lipoproteins and an approach to its elimination. Circ Res 62: 421-429.
11. Kruth HS (2011) Receptor-independent fluid-phase pinocytosis mechanisms
32. Sobenin IA, Orekhova VA, Melnichenko AA, Bobryshev YV, Orekhov AN (2013) Low density lipoprotein-containing circulating immune complexes have better prognostic value in carotid intima-media thickness progression than other lipid parameters. Int J Cardiol 166: 747-748.

33. Orekhov AN, Tertov VV, Mukhin DN, Kotleiansky VE, Glukhova MA, et al. (1989) Insolubilization of low density lipoprotein induces cholesterol accumulation in cultured subendothelial cells of human aorta. Atherosclerosis 79: 59-70.

34. Glukhova MA, Kabakov AE, Frid MG, Ormatsky OL, Belkin AM, et al. (1988) Modulation of human aorta smooth muscle cell phenotype: a study of muscle-specific variants of vinculin, caldesmon, and actin expression. Proc Natl Acad Sci U S A 85: 9542-9546.

35. Melnichenko AA, Aksenov DV, Myasoedova VA, Panasenko OM, Yaroslavov AA, et al. (2012) Pluronic block copolymers inhibit low density lipoprotein self-aggregation. Lipids 47: 995-1000.

36. Orekhov AN, Tertov VV, Mukhin DN, Kotleiansky VE, Glukhova MA, et al. (1987) Association of low-density lipoprotein with particulate connective tissue matrix components enhances cholesterol accumulation in cultured subendothelial cells of human aorta. Biochim Biophys Acta 928: 251-258.

37. Melnichenko AA, Tertov VV, Sobenin IA, Gabbasov ZA, Popov EG, et al. (1992) Three types of naturally occurring modified lipoproteins induce intracellular lipid accumulation due to lipoprotein aggregation. Circ Res 71: 218-228.

38. Rekhter MD, Andreeva ER, Mironov AA, Orekhov AN (1991) Three-dimensional cytoarchitecture of normal and atherosclerotic intima of human aorta. Am J Pathol 138: 569-580.

39. Orekhov AN, Andreeva ER, Krushinsky AV, Smolimov VN (1984) Primary cultures of enzyme-isolated cells from normal and atherosclerotic human aorta. Med Biol 62: 255-259.

40. Orekhov AN, Tertov VV, Novikov ID, Krushinsky AV, Andreeva ER, et al. (1985) Lipids in cells of atherosclerotic and uninvolved human aorta. I. Lipid composition of aortic tissue and enzyme-isolated and cultured cells. Exp Mol Pathol 42: 117-137.

41. Orekhov AN, Krushinsky AV, Andreeva ER, Repin VS, Smolimov VN (1986) Adult human aortic cells in primary culture: heterogeneity in shape. Heart Vessels 2: 193-201.

42. Yamada S, Guo X, Yoshizawa M, Li Z, Matsuayama S, et al. (2011) Primary desmoplastic cutaneous leiomyosarcoma associated with high MB1-1 labeling index: a teaching case giving rise to diagnostic difficulties on a small biopsy specimen. Pathol Res Pract 207: 726-732.

43. Orekhov AN, Kosevich YV, Repin VS, Smolimov VN (1983) Cell proliferation in normal and atherosclerotic human aorta. II. Autoradiographic observation on deoxyribonucleic acid synthesis in primary cell culture. Lab Invest 48: 71-79.

44. Orekhov AN, Tertov VV, Kikutasho SA, Khashimov KhA, Smolimov VN (1986) Primary culture of human aortic intima cells as a model for testing anti-atherosclerotic drugs. Effects of cyclic AMP, prostaglandins, calcium antagonists, antioxidants, and lipid-lowering agents. Atherosclerosis 60: 101-110.

45. Chazov EI, Tertov VV, Orekhov AN, Lyakishew AA, Perova NV, et al. (1986) Atherogenicity of blood serum from patients with coronary heart disease. Lancet 2: 595-598.

46. Tertov VV, Orekhov AN, Ryong LH, Smolimov VN (1988) Intracellular cholesterol accumulation is accompanied by enhanced proliferative activity of human aortic intimal cells. Tissue Cell 20: 849-854.

47. Tertov VV, Orekhov AN, Martsenyuk ON, Perova NV, Smolimov VN (1989) Low-density lipoproteins isolated from the blood of patients with coronary heart disease induce the accumulation of lipids in human aortic cells. Exp Mol Pathol 50: 337-347.

48. Orekhov AN (1990) In vitro models of anti-atherosclerotic effects of cardiovascular drugs. Am J Cardiol 66: 231-281.

49. Palatini P (2009) Elevated heart rate in cardiovascular diseases: a target for treatment? Prog Cardiovasc Dis 52: 46-60.

50. Schulman IH, Zachariah M, Raji L (2005) Calcium channel blockers, endothelial dysfunction, and combination therapy. Aging Clin Exp Res 17: 40-45.

51. Orekhov AN, Balzdenkov GN, Tertov VV, Ryong LH, Kozlov SG, et al. (1988) Cardiovascular drugs and atherosclerosis: effects of calcium antagonists, beta-blockers, and nitrates on atherosclerotic characteristics of human aortic cells. J Cardiovasc Pharmacol 12 Suppl 6: 566-66.
53. Loaldi A, Polese A, Montorsi P, De Cesare N, Fabbriocchi F, et al. (1989) Comparison of nifedipine, propranolol and isosorbide dinitrate on angiographic progression and regression of coronary arterial narrowings in angina pectoris. Am J Cardiol 64: 433-439.

54. Orekhov AN, Baldenkov GN, Tertov VV, Ruda MYa, Kashimov KA, et al. (1990) Antiatherosclerotic effects of calcium antagonists. Study in human aortic cell culture. Herz 15: 139-145.

55. Li HR, Tertov VV, Vasil’ev AV, Tut’el’yan VA. Orekhov AN (1989) Anti-atherogenic and anti-atherosclerotic effects of mushroom extracts revealed in human aortic intima cell culture. Drug Devel Res 17: 109-117.

56. Orekhov AN (2013) Direct anti-atherosclerotic therapy; development of natural anti-atherosclerotic drugs preventing cellular cholesterol retention. Curr Pharm Des 19: 5929-5938.

57. Orekhov AN, Tertov VV (1997) In vitro effect of garlic powder extract on lipid content in normal and atherosclerotic human aortic cells. Lipids 32: 1055-1060.

58. Orekhov AN, Grünwald J (1997) Effects of garlic on atherosclerosis. Nutrition 13: 656-663.

59. Orekhov AN, Sobenin IA, Korneev NV, Kirichenko TV, Myasoedova VA, et al. (2013) Anti-atherosclerotic therapy based on botanicals. Recent Pat Cardiovasc Drug Discov 8: 56-66.

60. Kosičieny J, Klüssendorf D, Latza R, Schmitt R, Radtke H, et al. (1999) The antiatherosclerotic effect of Allium sativum. Atherosclerosis 144: 237-249.

61. Crouse JR 3rd, Byington RP, Bond MG, Espeland MA, Craven TE, et al. (1995) Pravastatin, Lipids, and Atherosclerosis in the Carotid Arteries (PLAC-II) Am J Cardiol 75: 455-459.

62. Salonen R, Nyyssönen K, Pirkkalaa E, Rummukainen J, Belder R, et al. (1995) Kuopio Atherosclerosis Prevention Study (KAPS). A population-based primary preventive trial of the effect of LDL lowering on atherosclerosis progression in carotid and femoral arteries. Circulation 92: 1758-1764.

63. Smilde TJ, van Wissen S, Wollersheim H, Trip MD, Kastelein JJ, et al. (2001) Effect of aggressive versus conventional lipid lowering on atherosclerosis progression in familial hypercholesterolaemia (ASAP): a prospective, randomised, double-blind trial. Lancet 357: 577-581.

64. Pitt B, Byington RP, Furberg CD, Hunninghake DB, Mancini GB, et al. (2000) Effect ofamlodipine on the progression of atherosclerosis and the occurrence of clinical events. PREVENT Investigators, Circulation 102: 1503-1510.

65. Blankenhorn DH, Selber RH, Crawford DW, Barth JD, Liu CR, et al. (1993) Beneficial effects of colestipol-niacin therapy on the common carotid artery. Two- and four-year reduction of intima-media thickness measured by ultrasound. Circulation 88: 20-28.

66. Hodis HN (1995) Reversibility of atherosclerosis—evolving perspectives from two arterial imaging clinical trials: the cholesterol lowering atherosclerosis regression study and the monitored atherosclerosis regression study. J Cardiovas Pharmacol 25 Suppl 4: S25-S31.

67. Blankenhorn DH, Azen SP, Kramsch DM, Mack WJ, Cashin-Hempfl L, et al. (1993) Coronary angiographic changes with revascularization therapy: The Monitored Atherosclerosis Regression Study (MARS). Ann Intern Med 119: 969-976.

68. Zanchetti A, Rosei EA, Dal Palù C, Leonetti G, Magnani B, et al. (1998) The Verapamil in Hypertension and Atherosclerosis Study (VHAS): results of long-term randomized treatment with either verapamil or chlorthalidone on carotid intima-media thickness. J Hypertens 16: 1667-1676.

69. Libby P (2006) Inflammation and cardiovascular disease mechanisms. Am J Clin Nutr 83: 456S-460S.

70. Aidinian G, Weiswasser JM, Arora S, Abdulrahge CJ, Singh N, et al. (2006) Carotid plaque morphologic characteristics. Perspect Vasc Surg Endovasc Ther 18: 63-70.

71. Daugherty A, Webb NR, Rateri DL, King VL (2005) Thematic review series: The immune system and atherosclerosis. Cytokine regulation of macrophage functions in atherogenesis. J Lipid Res 46: 1812-1822.

72. Burger HG, MacLennan AH, Huang KE, Castelo-Branco C (2012) Evidence-based assessment of the impact of the WHI on women’s health. Climacteric 15: 281-287.

73. de Villiers TJ, Stevenson JC (2012) The WHI: the effect of hormone replacement therapy on fracture prevention. Climacteric 15: 263-266.

74. Ellis MJ, Suman VJ, Hoog J, Lin L, Snider J, et al. (2011) Randomized phase II neoadjuvant comparison between letrozole, anastrozole, and exemestane for postmenopausal women with estrogen receptor-rich stage 2 to 3 breast cancer: clinical and biomarker outcomes and predictive value of the baseline PAM50-based intrinsic subtype—ACOSOG Z1031. J Clin Oncol 29: 2342-2349.

75. Smith NL, Wiley JR, Legault C, Rice KM, Heckbert SR, et al. (2008) Effect of progestogen and progestogen type on hemostasis measures in postmenopausal women: the Postmenopausal Estrogen/Progestin Intervention (PEPI) Study. Menopause 15: 1145-1150.

76. Masood DE, Roach EC, Beauregard KG, Khalil RA (2010) Impact of sex hormone metabolism on the vascular effects of menopausal hormone therapy in cardiovascular disease. Curr Drug Metab 11: 693-714.

77. Pellegrini CN, Vittinghoff E, Lin F, Huiley SB, Marcus GM (2009) Statin use is associated with lower risk of atrial fibrillation in women with coronary disease: the HERS trial. Heart 95: 704-707.

78. Sielev M, Ahmed N, Wang Q, McDowell G, Badimon L (2012) Unique vascular protective properties of natural products: supplements or future main-line drugs with significant anti-atherosclerotic potential? Vasc Cell 4: 9.