REVIEW ARTICLE

Blood Serum Atherogenicity: Cellular Test for the Development of Anti-Atherosclerotic Therapy

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Abstract: Atherosclerosis is one of the main problems in modern medical practice. This multifactorial disease can remain asymptomatic for a long time before manifesting itself in cardiovascular disorders, causing ischemic heart disease, myocardial infarction and even sudden death. Many synthetic drugs have been developed to reduce the symptoms of atherosclerosis, however, their efficacy in terms of reduction of atherosclerotic lesions progression is a matter of debate. Adverse effects of the exiting therapy should also be taken into account. The development of cellular models and improved understanding of the mechanisms of atherogenesis at the vascular wall level helped establishing the "direct anti-atherosclerosis therapy" approach. In this approach, the decrease of intracellular lipid deposition and atherogenicity of human blood serum are considered primary (direct) anti-atherosclerotic effects. Screening of synthetic and natural substances for anti-atherosclerotic activity revealed a number of botanicals that could be used for direct anti-atherosclerotic therapy to treat early-stage atherosclerosis. As a result, 3 novel non-pharmaceutical products were developed (Allicor, Inflaminat and Karinat). Studies on *in vitro* and *ex vivo* models of atherogenesis confirmed their anti-atherosclerotic and anti-atherogenic activities and safety in patients. Clinical studies of Allicor, Inflminat and Karinat were carried out in subjects with diagnosed early stage atherosclerosis, demonstrating a clinically significant anti-atherosclerotic effect of the drugs. In this overview, we will present the complete process of the development of novel non-pharmaceutical products and report the results obtained in the conducted pre-clinical and clinical studies of these medications.

Keywords: Anti-atherosclerotic therapy, serum atherogenicity, cellular models, clinical trials, non-pharmaceutical products.

INTRODUCTION

Atherosclerosis is a complex and multifactorial disease. Disorders of lipid metabolism, arterial pressure and blood coagulation could be identified as possible risk factors of atherosclerosis development. Atherosclerotic process is characterized by combination of different morphological, histological and hemorheological changes in the arteries. Contrary to the common belief, atherosclerotic process can begin already at a young age and remain asymptomatic for a long period [1-4].

Current treatment of atherosclerosis consists mainly of symptomatic therapy and preventive measures. Most of the patients with diagnosed atherosclerosis have to receive a lifelong therapy, which is not free from adverse effects. The achieved improvement of the quality of life is not necessarily associated with the disease remission. Therefore, the development of direct anti-atherosclerosis therapy is necessary to induce regression of the existing atherosclerotic lesions and prevent their *de novo* formation. Despite the large amount of data from basic and clinical studies, no existing drugs were proven effective for the treatment of existing plaques, lowering the volume of lipid necrotic core and stabilizing the fibrous cap simultaneously [1-9]. Modern anti-atherosclerotic drugs can be divided into 2 big groups: statins (inhibitors of hydroxymethylglutaryl-coenzyme A reductase) and calcium antagonists. Statins are considered the most effective hypolipidemic drugs, providing a stable reduction of cholesterol level. The results of some studies demonstrated that statins may possess masked anti-atherosclerotic effects, such as inhibition of monocyte migration, suppression of cell proliferation in atherosclerotic lesions, reduction of cellular cholesterol accumulation, etc. [10-20]. The influence of aggressive statin therapy on slowing the atherosclerotic process and even regression of existing lesions has been evaluated in several clinical studies [9]. On the other hand, conducted trials detected a spectrum of serious adverse effects of statins [21]. It should also be noted that statin treatment could be expensive due to high cost of original pharmaceutical products [10, 21, 22].

The other group of drugs with anti-atherosclerotic potential is calcium antagonists [23-31]. Unfortunately, the anti-atherosclerotic effect of these medications has only been evaluated in 4 known clinical trials [9]. The significant positive result of long-term therapy with amlodipine (the most known calcium antagonist) was evaluated as the reduction in intima-medial layer thickness (IMT) of carotids arteries [32, 33]. Other clinical studies of calcium antagonists were unsuccessful or provided the lack of clinical significance [9].

Besides the abovementioned medications, estrogens, vitamins-antioxidants and cholesterol/niacin combination are considered to possess a direct anti-atherosclerotic activity, however, all known studies showed insufficient results. Treatment with estrogens resulted in a delay in atherosclerosis progression and even in a slight reduction of carotid IMT, but the patient population included only menopausal women, all of them being concomitantly treaded with statins, so the direct anti-atherosclerotic potential of estrogens could not be clearly evaluated [34, 35].

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The progress in clinical diagnostic of atherosclerosis made the timely start of the treatment possible. However, atherosclerosis can be subclinical at the early stages, and there is no need in urgent administration of aggressive synthetic drugs. Patients can stabilize the atherosclerotic process by correcting the diet and using non-pharmaceutical products with beneficial properties [36-39].

Natural medications can provide a significant therapeutic effect without serious side effects, which makes them appropriate for long-term therapy [21]. The development of natural anti-atherosclerotic medication was made possible by the discovery of human serum atherogenicity effect and the development of cellular *in vitro* and *ex vivo* models [9]. The developed models allowed performing a multiple screening of potential natural substances for anti-atherosclerotic activity [8]. The evaluation of anti-atherosclerotic activity of a large number of botanicals within the last 20 years resulted in the creation of non-pharmaceutical products with confirmed anti-atherosclerotic effect. These products provide an alternative way for treatment of the early stage atherosclerosis [9]. The study of the anti-atherosclerotic effect of the novel natural products has been performed by our group [8, 9]. In this review, we present the results of these studies and provide the evaluation of the clinical efficiency of new non-pharmaceutical drugs.

CELLULAR MECHANISMS OF ATHEROGENESIS

Atherosclerosis could be characterized by multiple changes in different body systems. It is widely accepted that the initiation of the pathological process is caused by a combination of several factors. The lipid theory of atherogenesis formulated at the beginning of the 20th century remains the most established. According to this theory, atherosclerosis is an arterial disease caused by cholesterol infiltration to the artery wall, followed by formation of connective-tissue plaques. In modern interpretation, the key role in this process is given to lipoproteins, basically to low-density lipoproteins (LDL) and high-density lipoproteins (HDL) [40].

The HDL normally takes part in the "reverse cholesterol transport", while LDL is the major cholesterol transporter in the body. The LDL could carry up to 55% of cholesterol and its esters. The LDL metabolism disorder manifested as hypercholesterolemia results in the pathological increase of the LDL concentration in the blood serum. This condition is a risk factor of atherosclerosis. LDL particles are subject to chemical modifications that alter the structure of the native lipoprotein. The modified LDL has been demonstrated to possess atherogenic activity leading to cholesterol accumulation in vascular wall cells [41-43]. Previously existing lesions and local changes in the artery wall are also significant factors promoting the development of the atherosclerotic process [41-45].

There are several known LDL modifications – desialylation, change of the particle's surface charge and density, oxidation and possible nonenzymatic glycosylation. The first three of these processes were found to be the most atherogenic. The modified LDL is able to initiate spontaneous complex formation and can serve as an antigen inducing the immune response and production of auto-antibodies to LDL's apolipoprotein B (apo B) [43, 45]. As a result, circulating immune complexes with modified LDL can be formed. Both modified LDL and LDL-containing associates are not recognized by the LDL receptor due to the altered surface charge and structure. Therefore, they do not follow the normal metabolic processing pathway, but penetrate the arterial wall cells by phagocytosis followed by degradation processes [41-45].

Accumulation of LDL complexes in the arterial intima triggers a chain of local reactions, involving the innate immune response. Resident pericyte-like cells and tissue macrophages take up modified LDL complexes and secrete signal molecules promoting the innate immunity response and attracting the circulating monocytes to the local inflammation region [46]. They penetrate the intima layer, where they differentiate and actively proliferate stimulated by granulocyte colony stimulating factors (M-GSF, GM-CSF). Mono-

cytes turn into macrophages and express scavenger receptors to accumulate and destruct the modified LDL and LDL complexes. The uncontrolled destruction of modified LDL particles leads to overload of pericyte-like cells, macrophages and smooth muscle cells with cholesterol in the process known as foam cell formation. Macrophages and pericytes that are not transformed to foam cells produce anti-inflammatory cytokines and chemokines. Another process that takes part in the developing lesion is the migration of medial smooth muscle cells (SMC) to the intima followed by their proliferation [47]. Resident intimal cells, smooth muscle cells and pericyte-like cells start to produce collagen, elastin and glycosaminoglycans forming the extracellular matrix. At this stage, particles trapped in the arterial wall become covered with the fibrous cap [48].

Atherosclerotic plaque development is a multistage process that starts from the uncontrolled lipid accumulation in the vascular intima followed by fatty lesion and then fibrous cap formation. Main function of the fibrous cap is to separate the blood flow from the inflammation site, resulting in the formation of a chronic inflammatory focus. Erosion and destabilization of the plaque damage its surface and lead to the recruitment of platelets to the inflamed region followed by thrombus formation in the plaque area. Growing thrombi are gradually over covering the atherosclerotic invagination of lumen affecting the blood circulation [49].

The last stage of atherosclerotic plaque lifecycle is induration and saturation with calcium salts, when the calcified plaques promote lesions growing on the level of arterial wall. Importantly, several independent plaques situated in the same vascular region could pass through different stages, and simultaneous presence of all forms of plaques is possible.

As no effective treatment for the late stages of atherosclerosis has been developed so far, timely preventive measures and early diagnostics of the disease have become the priorities in modern clinical practice.

USE OF CELLULAR MODELS FOR THE DEVELOPMENT OF DIRECT ANTI-ATHEROSCLEROSIS THERAPY

Development of the new pharmaceutical and non-pharmaceutical products is a long-term process needs the cascade of laboratory tests for evaluating the safety and efficiency of the discovered molecules before opening clinical studies on human population. Rapid acquisition of statistically reliable data on the activity, metabolism and direct therapeutic effect of each investigative product can be performed using bio-models. The primary principle of biomodeling is keeping the similarity between the created model and current biological system or process. Only similar module could try to understand the real mechanism of molecular effect developing in multifactorial biological environment.

Modern study of anti-atherosclerotic effects of drugs requires the development of model systems that contain all the necessary components of the early stages of the disease development at cellular level. The purpose of the studies described below was detection of natural substances with anti-atherogenic potential and prolonged effect [36-39].

In vitro Model

The *in vitro* biological model was created to evaluate the botanicals and synthetic substances for their ability to reduce the atherogenic potential of the human serum and to prevent the pathological accumulation of cholesterol in the vascular intima [7, 9, 29, 31].

The model was based on the primary culture of subendothelial cells obtained from thoracic aorta of males and females 40-65 year old within 1.5-3 hours after sudden death. Living cells isolated by collagenase treatment from the various regions of aorta were cultured at 37° C for 7-10 days in daily refreshed medium [9]. As a

result, a heterogeneous cell population with mostly pericyte-like cells, typical and modified smooth muscle cells, as well as inflammatory cells was obtained [9]. Subendothelial cells were also isolated from the healthy region of aorta cultured in parallel to receive the second cellular model with different characteristics. Another model was established by culturing the monocyte-derived macrophages isolated from the venous blood of healthy volunteers. Monocytes were cultured for 14 days at 37° C until the transformation to macrophages. The lipidosis was induced by adding the atherogenic serum to the cultured cells. Serum was prepared from venous blood of volunteers with diagnosed atherosclerosis and could be stored at -18° C [9].

Investigated product was added to the *in vitro* model, in which the cholesterol accumulation process was ongoing (obtained from atherosclerosis lesions), and the anti-atherogenic effect was estimated by measurement of intracellular cholesterol level, intracellular lipids ratio and overall percentage of serum atherogenicity lowering. Atherogenic effect of serum was defined as the ability to stimulate the statistically significant accumulation of cholesterol in cultured cells.

The prevention of cholesterol accumulation in cells cultured from the unaffected intima (anti-atherogenic effect) or drop of cholesterol level in cells cultured from fatty atherosclerotic lesions (anti-atherosclerotic effect) were regarded as positive results interpreted as the anti-atherogenic or anti-atherosclerotic activity. The positively evaluated substance could then be proposed for the next stages of medication development.

Ex vivo Model

Ex vivo model was established with participation of healthy volunteers, who received the investigational products with proved anti-atherogenic activity and safety in the form of tablets or capsules [9]. Healthy subjects should have had no clinical symptoms of atherosclerosis or any systemic inflammatory diseases. Anti-atherogenic effect could be evaluated on participants with sufficient blood serum atherogenicity, i.e. serum ability to induce the pathological accumulation of cholesterol in the vascular wall.

The first blood sample was taken prior to the investigational product administration to fix the baseline data. After the drug administration, blood sample collection was performed from each volunteer after 2, 4 and 6 hours to evaluate the short-term effects. For long-term anti-atherogenic activity evaluation, patients were tested after 4, 8, 12 and 24 hours.

The serum from each patient was added to cultured aortic cells or monocytes-macrophages as described previously. Cell culture process was the same as for the *in vitro* testing. At the end of the study, intracellular lipids, cellular protein and calculated serum atherogenic potential were evaluated. The described *ex vivo* model allowed evaluating the anti-atherogenic effect of substances taking into account the metabolic processing that occurs after its administration in the human body. Multiple blood sample collection made possible plotting the dose response curves and obtaining data on the pharmacodynamic characteristics of the studied substance.

The described *in vitro* and *ex vivo* models allowed performing screening of both synthetic and natural substances to evaluate the most effective agents with anti-atherosclerotic effects [9]. It was shown that anti-atherogenic activity of studied botanicals was statistically significant, and the administration of these substances led to rapid lowering of serum atherogenicity level. Based on the results obtained in *in vitro* tests, several non–pharmaceutical drugs were developed and promoted to clinical study level to evaluate their safety and efficiency on patients' population [8].

DEVELOPMENT OF ANTI-ATHEROSCLEROTIC DRUGS BASED ON NATURAL COMPOUNDS

ALLICOR Development

In search for the natural compounds with potential efficacy against early stage atherosclerosis, 31 substances were screened using the *in vitro* model based on cultured aortic cells [50]. Primary cell culture was isolated from atherosclerotic region of human aorta. Most of the studied substances were related to the flavonoid group. All studied agents were checked for cytotoxicity by performing a morphologic study of cultured cells, measuring the absolute protein ratio and cell viability using trypan blue.

Each of the substances was tested in the concentration range from 10^{-4} to 10^{-6} M. Eight substances had a cytotoxic effect at concentration 10^{-4} M, and 3 of them had pro-atherogenic activity, i.e. stimulated additional accumulation of cholesterol in cells. Next, 2 more pro-atherogenic substances and 4 neutral substances (with no activity) were detected. In total, 14 substances were excluded from the following studies. The main focus of the research was detection and measurement of the anti-atherosclerotic activity (reducing the cholesterol accumulation in cells) regardless of the concentration.

Natural substances of botanical origin were proposed based on the literature studies. The following botanicals were selected to be evaluated in the study: Spirulina (*Spirulina platensis*), onion (*Allium cepa*), weat germs (*Triticum vulgaris*) hill-growing saltwort (*Salsola collina*), beetroot (*Beta vulgaris*), garlic (*Allium sativum*), licorice (*Glycyrrhiza glabra*) and extract of pine straw (*Pinus sylvesrtis*). All products were tested on the *ex vivo* model with participation of healthy volunteers who had clinically diagnosed atherogenicity of the blood serum. Participating subjects were separated into small groups of 4-8 people, and passed the blood sampling after administration of a single dose of a substance.

Garlic powder in capsules (300 mg) provided the most significant reduction of serum atherogenicity after 2, 4 and 6 hours. The maximum effect was achieved already after 4 hours. After 6 hours, the baseline rate of atherogenicity was reduced by 3 folds. Previous data obtained in the studies of garlic showed its ability to reduce serum cholesterol level, lower the arterial pressure and increase the fibrinolytic activity of blood plasma. Indicated characteristics and high availability of garlic prompted the development of the new natural anti-atherosclerotic product.

Firstly, commercially produced garlic powder (Lichtwer Pharma GmbH, Germany) standardized by allicin content (1.3%) was tested using *in vitro*, *ex vivo* and *in vivo* models to evaluate the efficiency and safety of different concentrations. The obtained results clearly demonstrated that garlic reduced the accumulation of cholesterol and lowered the level of intracellular triglycerides. The investigation of garlic compounds was performed simultaneously. Several chloroform-soluble and water-soluble fractions were obtained using thin layer chromatography and tested for anti-atherosclerotic and anti-atherogenic activities in *in vitro* models based on the aortic cell culture. The majority of chloroform-soluble components showed a combined anti-atherosclerotic and anti-atherogenic activity. Water-soluble compounds mostly possessed anti-atherogenic activity, and only 2 active agents had both anti-atherogenic and anti-atherosclerotic properties.

In summary, garlic was identified as a potential active component of a novel anti-atherosclerotic medication, and the natural non-pharmaceutical commercial product named Allicor (INAT-Pharma, Russia) was developed. Allicor is produced in two different dosages of 150 and 300 mg of garlic powder in the form of tablets. The product is intended to be used for prevention and supporting therapy of early stage atherosclerosis. For a more detailed evaluation of the efficiency and safety of the new product, laboratory and clinical

studies were designed. Allicor passed through clinical trials phase I-III and was recommended as effective product for long-term therapy of early stage atherosclerosis [8].

INFLAMINAT Development

Atherosclerosis is a multifactorial process, in which lipid accumulation in the arterial wall plays a key role [51-55]. Inflammation is permanently present in vascular regions with atherosclerotic lesions [56-60]. IL-6 and IL-1 are the most important interleukins in atherosclerotic process, where IL-1 induces the local inflammation and IL-6 acts as a pro-inflammatory factor, which is a known marker of inflammatory process in coronary artery plaques. Inflammation theory of atherogenesis is widely studied on *in vivo* models to evaluate the anti-atherosclerotic effects of the anti-inflammatory therapy. During the subclinical period of atherosclerosis development, the elevated level of inflammation markers can be detected in the blood serum and used as a prognostic risk factor of atherosclerosis complications. Modern research is focused on the evaluation of the role of cytokines in chronic diseases, and the direct anti-cytokine therapy is currently under development. At present, most of the available anti-cytokine drugs are at the research stages, and others have not demonstrated any clinically significant effects and specificity. Several publications report the efficiency of different natural substances for suppressing the production of IL-1, IL-6 and Tumor necrosis factor alpha (TNFα). The aim of our research was to develop a natural non-pharmaceutical product with anti-inflammatory activity to be used in patients with diagnosed atherosclerosis as a supportive therapy [61].

Using the described *in vitro* model, 31 selected botanicals were screened for the anti-cytokine activity. Only 5 botanicals showed the ability to inhibit the IL-1 expression: violet, calendula, elder, hawthorn and St. John's wort were selected for further studies.

Different combinations of these substances were tested to evaluate the mutually potentiating anti-cytokine activity in the *ex vivo* model. The healthy volunteers received a single dose of botanicals combination and passed blood tests after 4 and 8 hours. It was demonstrated that the inclusion of St. John's wort reduced the anti-cytokine activity in all tested combinations, while the best results were achieved after administration of violet, calendula and elder. The developed combination contained 3 active agents (elder, calendula, violet) having significant effect in reducing the expression of both IL-1 and TNF-α. The resulting commercial natural non–pharmaceutical product was named Inflaminat (INAT-Pharma, Russia). It contained equal concentrations of active compounds in a capsule form [61].

Anti-cytokine and anti-atherogenic activities of Inflaminat were evaluated in a series of studies. The efficiency of the new product was evaluated in comparison with Diclofenac and Allicor. The study was conducted on healthy volunteers (males and females 52-69 year old) with clinically diagnosed atherogenicity and pro-inflammatory potential of blood serum. After the collection of the baseline blood sample, subjects received a single dose of investigational product or comparator. Blood serum was collected after 2, 4, and 8 hours and tested for residual atherogenicity and cytokine level using the *ex vivo* model. Inflaminat caused a significant reduction of the IL-1 expression for nearly 25% of the baseline value, while Diclofenac stimulated the lowering of IL-1 expression for 49%. The positive and comparable results were detected in TNF-α expression reduction on 8th hour after drugs administration; it was lowered by 9% after Inflaminat and by 39% after Diclofenac administration.

Anti-atherogenic potential of the blood serum was assessed in the same way after single dose administration of Inflamint, Allicor and Diclofenac. After 8 hours, the following results were achieved: Inlaminat showed a reduction of atherogenicity by 64% from the initial level, Allicor administration lowered serum atherogenicity by nearly 50% and Diclofenac - by 13%.

Thus, the anti-cytokine and anti-atherogenic effects of natural product Inflaminat were demonstrated. The obtained results were comparable to those for Diclofenac (nonsteroidal anti-inflammatory drug) and Allicor. This observation indicates the efficiency of the novel drug on biological models and its safety for healthy volunteers. To complete the registration of Inflaminat, full scale clinical trials should be conducted on a population of patients with already diagnosed atherosclerosis [62].

KARINAT Development

The analysis performed by the Interstate statistical committee of CIS clearly demonstrated that atherosclerosis-related disorders are the primary causes of sudden death in women (up to 73%). In total, nearly 55% of death cases in women were related to cardiovascular disorders, and this percentage was higher than in men population. Earlier, the major attention was paid to the prevention of atherosclerosis in men, but nowadays the research is inclined towards establishing new therapies for atherosclerosis prevention in women [63, 64].

To date, no effective therapy has been established for simultaneous improvement of the quality of life and prevention of atherosclerosis in menopausal women. Hormone replacement therapy turned out to be effective against osteoporosis and in alleviating the menopause symptoms, but, despite the expectations, was unable to lower the risk of atherosclerosis development, as demonstrated by large scale clinical studies [65]. In several documented cases, hormone replacement therapy had oncogenic activity and even stimulated the cardiovascular disorders. Current research aims at developing the new generation of medications for menopausal women that can reduce the menopause symptoms and prevent the beginning of atherosclerotic process. Natural phytoestrogens are used to reduce the climacteric syndromes due to their similarity to human estrogens and ability to block estrogen receptors. Non-aggressive and short-term biological activities of phytoestrogens allow their use for the long-term therapy and preventive treatment, making them an attractive possible basis for the development of new medications [66].

The search for the most effective and safe phytoestrogens was performed by screening of known substances evaluating their positive, satisfactory or negative anti-atherosclerotic effects and pharmacodynamics on *in vitro* and *ex vivo* models. No anti-atherosclerosis effect has been known for any of the studied substances. Several substances were considered promising, i.e. had no detected toxicity and provided a stable effect. Identified substances were related to three classes: isoflavonoids, proanthocyanidins and stilbens.

At the next stage of research, a list of plants containing the selected substances was formed based on phytochemical and ethnobotanics databases. The most obtainable plants with high natural occurrence and high concentration of desired substance were selected for screening such as: grape, soybean, sage, carrot, orange, garlic, licorice, onion, hop, green tea, focus, kelp, calendula, clover, hawthorn, elder and violet.

The principal part of the research was to find out the possible anti-atherosclerotic and anti-atherogenic activities of the chosen plants. Assessment was performed using the *in vitro* and *ex vivo* models based on culture of human aortic cells. The participating volunteers were healthy individuals with assessed serum atherogenicity. The anti-atherogenic potential of active agents was measured on *ex vivo* model after a single dose administration as described previously.

Most of the plants were excluded from the initial list due to the lack of activity, short-term effect, poor availability or high consumption rate of raw materials. The final list of promising botanicals was developed containing the following compounds: tannin from grape stone, garlic, hop, sage and green tea leave.

The formulation of the phytoestrogen complex was developed based on the results of the *ex vivo* studies of the dose-dependent anti-atherogenic effects. Healthy volunteers received a single dose of each substance at different concentrations to evaluate the minimal effective dose. The final proportion of active compounds was generated in accordance to minimal effective dose of each substance and proposed scheme of daily treatment. As a result of this research, Karinat (INAT-Pharma, Russia), the novel non-pharmaceutical phytoestrogenic complex, was developed. The optimal scheme of the drug administration was established based on the results of the *ex vivo* study on healthy volunteers (women 45-55 year old) who had been assessed for atherogenicity of blood serum.

The anti-atherogenic potential of Karinat, combined with the estrogen-like effect of phytoestrogens provides unique clinically relevant effects demonstrated on *in vitro* and *ex vivo* models. Next stages of the drug development consisted of clinical trials on an adequately selected patient population [67].

CLINICAL TRIALS TO EVALUATE THE ANTI-ATHEROSCLEROTIC ACTIVITY OF DEVELOPED DRUGS

To evaluate the efficiency of different botanical combinations, 3 clinical trials (CT) were conducted in Russia. In all the studies, the patients with diagnosed atherosclerosis took part voluntarily after signing the Informed consent form (ICF). All CT's procedures were performed in accordance with the Local Russian Legislation and ICH-GCP guidelines.

Statistical Analysis

The statistical analysis was performed using SPSS version 14.0 (SPSS Inc., USA) software. The results were presented as mean value ± standard deviation or median value with 95% confidence intervals, where appropriate. The difference in the average values was defined as statistically significant when $p<0.05$.

Clinical Analysis of Blood Serum

Blood serum was analyzed in order to evaluate the lipid profile and to estimate the anti-atherosclerotic effect of the administered drug. The blood samples were taken in the morning after overnight fasting. The blood was incubated at room temperature till clot formation, and serum was obtained by centrifugation and stored in freezer (minimum -20^0C). The total cholesterol, HDL cholesterol and triglycerides were measured by enzymatic method using commercially available kits (Boehringer Mannheim, Germany). The LDL cholesterol was calculated using Friedewald formula.

Calculation of Prognostic Risks

In all CTs, the 10-year prognostic risk of ischemic heart disease was calculated using Weibull model, and the 10-year prognostic risk of myocardial infarction was calculated using Cox model. All results were corrected in accordance to Moscow region correction factor.

CLINICAL TRIAL OF INFLAMINAT

The pilot double blinded randomized placebo-controlled study was aimed to evaluate the efficiency and safety of anti-inflammatory therapy with Inflaminat in patients with subclinical carotid atherosclerosis.

During the laboratory study of different botanicals for detecting their anti-atherosclerotic activity, the herbs of calendula, elder (Sambucus) and violet showed a significant inhibition of both atherosclerosis and inflammatory processes at the cellular level. The simultaneous suppressing effect of the combination of these herbs (Inflaminat) on cholesterol accumulation in cells and production of pro-inflammatory cytokines was evaluated in laboratory studies using *in vitro* and *ex vivo* models. Next, a clinical trial of the natural non-pharmaceutical drug Inflaminat was performed [61, 62].

Subject Recruitment

The CT of Inflaminat was conducted on the basis of Moscow polyclinic #202, in the out-patient department of therapy and prevention of atherosclerosis. The study included men aged 40-74 with asymptomatic atherosclerosis. The participants were recruited according to the following inclusion criteria: arterial normotension or mild arterial hypertension without permanent treatment (more than 2 months) with antihypertensive drugs (beta-blockers or calcium antagonists); diffuse thickening of the intima-medial layer of the distal centimeter of common carotid arteries (maximum intima-media thickness 1000-2000 μm); the absence of hemodynamically significant raised plaques over 10% of vascular lumen.

Exclusion criteria were: diagnosed cardiovascular or cerebrovascular disease; presence of any comorbidity that needed continuous treatment (more than 2 moths per year); indications to surgical treatment of atherosclerosis located in extracranial region of the brachiocephalic system; and the presence of contraindications to treatment with anti-inflammatory drugs.

Screening

From 265 eligible patients, 108 men were recruited for the CT. After reviewing the ICF, 78 of them agreed to participate in the trial and signed the form. All 78 patients entered the screening period. Before randomization, all patients passed the necessary procedures such as physical examination (including body mass index calculation), ECG, screening blood test, collection and discussion of family medical history to detect the possible genetic disorders and risk factors (ischemic heart disease, arterial hypertension, type 2 diabetes, myocardial infarction).

Randomization

The patients included in the study were randomized into two groups – Inflaminat or Placebo, using double blinded method and random numbers generator. Each patient received the ID (identifying code). The code table was inaccessible for participants and medical site staff during the whole period of treatment. After receiving the ID, each participant started the 6-month long therapy with the prescribed product.

Treatment Period

Participants from Inflaminat group received Inflaminat for a 6-month period, while participants from Placebo group received placebo following the same scheme. All participants passed the obligatory dietary consultation and received individual recommendations. During the active treatment period, study subjects were examined by ultrasonography (SonoScape SSI-1000, China) to detect the condition of carotids, and passed the laboratory tests. The physical examination of each patient took place at every visit, and all parameters (arterial pressure, BMI, 10-year prognostic risks) were documented by medical site staff. The participants also underwent the periodic ECG screening and blood tests (serum lipid analysis).

Results

17 of the study participants were active smokers, and no one had the diagnosed type 2 diabetes. The following results were obtained after screening of the study participants for different genetic risks factors: family history of myocardial infarction was present in 19 subjects (24.3%), family history of arterial hypertension was present in 21 subjects (26.9 %), family history of type 2 diabetes risk was present in 8 subjects (10.3 %).

Forty-six study participants (60%) were diagnosed with mild arterial hypertension during the whole study; these participants were treated only with ACE inhibitors to control the arterial pressure.

Hypercholesterolemia was the major lipid metabolism disorder diagnosed by lipid profile analysis. According to the correlation

between LDL and HDL cholesterol, nearly 50% of study participants had a normal value of serum atherogenic index (LDL:HDL cholesterol ratio). The combined hyperlipidemia was detected in 25% of patients. Based on calculated prognostic risks of the ischemic heart disease and myocardial infarction, all patients were distributed to high and extremely high risk groups. Baseline biochemical profile is presented in Table **1**.

Table 1.   Baseline biochemical profile of patients.

Characteristic	Mean		p
	Inflaminat group	Placebo group	
Number of participants	41	37	-
cholesterol, mg/dl	262±48 (247.0) (242-282)	275±65 (267.5) (233-316)	0.434
triglycerides mg/dl	66±15 (70.2) (59-72)	63±23 (54.1) (48-77)	0.446
HDL cholesterol, mg/dl	165±48 (158.3) (144-185)	180±48 (177.3) (149-211)	0.174
LDL cholesterol, mg/dl	157±90 (149.0) (119-195)	159±68 (139.5) (116-202)	0.434
Atherogenicity index	2.7±1.0 (2.6) (2.2-3.1)	3.1±0.9 (3.2) * (2.5-3.6)	0.046

* significant difference between groups – p<0.05.

The presence of subclinical atherosclerotic lesions was the general characteristic of carotid arteries. All study participants had mean carotid intima-media thickness (cIMT) above normal value (650 μm), and 70% of patients had cIMT more than 1000 μm, what was defined as a direct diagnostic criterion of subclinical atherosclerosis. Baseline cIMT characteristics are presented in Table 2. In conclusion, the randomized groups were demonstrated to be appropriate for the planned trial.

Table 2. Baseline characteristics of atherosclerosis progression.

Characteristic	Mean		p
	Inflaminat group	Placebo group	
Number of participants	41	37	-
Average c IMT rate of right common carotid artery, μ	947±99 (955) (905-988)	825±93 (835) * (766-884)	0.017
Average c IMT rate of left common carotid artery, μm	948±157 (955) (882-1015)	840±140 (830) (751-929)	0.113

* significant difference between groups – p<0.05.

During the treatment period, no significant changes in BMI were detected (p=0.600 for Inflaminat group and p=0.374 in Placebo group). The same was registered for the abdominal fat load (p=0.600 in Inflaminat group and p=1.000 in Placebo group).

The significant changes occurred in Inflaminat group: systolic blood pressure lowered by 19 mmHg on an average (p=0.060), diastolic arterial blood pressure lowered by 6 mmHg (p=0.017); total cholesterol level was decreased by 49 ml/dl (p=0.005) and LDL cholesterol by 51 mg/dl (p=0.005) (Table **3**). The 10-year prognostic risks of myocardial infarction, sudden cardiac death and ischemic heart disease were reduced by 10% on average. The most significant changes occurred in cIMT of the right common carotid artery: it was decreased by 62 μm (p=0.002) on average (Table **4**).

Table 3.   Changes in biochemical profile of patients.

Characteristic	Changes		p
	Inflaminat group	Placebo group	
Number of participants	41	37	-
Total cholesterol mg/dl	-49±42 * (-80; -19) p=0.005	-51±49 * (-97; -5) p=0.018	0.813
triglycerides mg/dl	3±23 (-14; 20) p=0.646	-19±22 (-39; 1) p=0.091	0.025
HDL cholesterol, mg/dl	-51±60 * (-94; -8) p=0.005	-25±48 (-70; 20) p=0.310	0.475
LDL cholesterol, mg/dl	-8±32 (-31; 15) p=0.445	-35±107 (-133; 64) p=0.398	0.813
Atherogenicity index	-0.7±1.4 (-1.7; 0.3) p=0.059	0.7±1.7 (-0.8; 2.2) p=0.612	0.133

* significant difference between groups – p<0.05.

Table 4.   Changes in atherosclerosis rates.

Characteristic	Changes		p
	Inflaminat group	Placebo group	
Number of participants	41	37	-
Average c IMT rate of right common carotid artery, μm	-62±48 * (-91; -32) p=0.002	42±75 (-9; 93) p=0.109	0.002
Average c IMT rate of left common carotid artery, μm	41±100 (-20; 101) p=0.135	0±100 (-67; 67) p=0.964	0.331

* significant difference between groups – p<0.05.

In the placebo-treated group, statistically significant decrease of total cholesterol was detected, apparently caused by the prescribed diet correction. However, no significant changes in LDL cholesterol, triglycerides, atherogenic index and cIMT were observed.

Safety

During the 6-months period of treatment, no serious adverse events of side effects have been registered. This result confirms the good safety profile of natural non-pharmaceutical product Inflaminat in atherosclerosis patients.

The results of the pilot double blinded randomized placebo-controlled clinical trial of Inflamat confirm its anti-atherosclerotic effect. It was demonstrated that Inflaminat had a direct anti-atherosclerotic activity and sustained the regression of atherosclerotic lesions at the early stages of the disease. This effect was characterized mostly by cIMT reduction. Treatment with Inflaminat also had positive effects on different cardiovascular risk factors and caused the lowering of the prognostic risks of ischemic heart disease and myocardial infarction. Thus, Inflaminat can be recommended as a non-pharmaceutical natural product for the long-term prevention of atherosclerosis [61, 62].

CLINICAL TRIAL OF KARINAT

During the study of different botanicals to define their anti-atherosclerotic and anti-atherogenic activities, 3 promising classes were identified: isoflavonoids, proanthocyanidins and stilbens. All the substances possessed phytoestrogenic activity and, supposedly, anti-atherosclerotic potential. Anti-atherosclerotic properties of the botanical substances were evaluated during screening. Evaluation of the anti-atherosclerotic potential was based on components' ability to reduce the atherogenic potential of human blood serum. Limited clinical laboratory trials in *in vitro* and *ex vivo* models demonstrated the anti-atherosclerotic efficiency of all investigated botanicals. As a result, the final product Karinat was developed based on the combination of studied substances. The composition of this phytoestrogen-rich complex included tannin (extracted from the grapestone), sage leaves (Salvia gen.), hop cones, garlic powder and green tea leaves powder. After successful laboratory study, the final non-pharmaceutical product was prepared for clinical trials [63, 64, 67].

The pilot double blinded, randomized, placebo-controlled study was performed to evaluate the efficiency and safety of phytoestrogen-rich complex on the basis of Moscow polyclinic #202, in outpatient department of therapy and prevention of atherosclerosis.

Subject Recruitment

131 females were examined during the pre-screening period. The following inclusion criteria were used: stable menopausal state (physiological or surgical), minimum 5 years period of menses absence, maximum cIMT not more than 0.800 mm (as defined by ultrasonographic examination of carotids); the absence of menopausal syndrome symptoms (maximum 2 points by Kupperman scale); no hormone replacement therapy during the pre- and post-menopause; no treatment with hypolipidemic drugs for 6-month period prior to the screening; and satisfactory general health state.

Exclusion criteria were: refusal of participation or signing ICF;

treatment with hypolipidemic drugs (statins, fibrates and nicotinic acid) during 6-months period prior to the screening; treatment with sugar-lowering drugs for more than 2 months per year, treatment with beta-blockers and calcium antagonists; the history of hormone replacement therapy; the history of myocardial infarction and/or diagnosed acute cerebrovascular disorders, and/or chronic cardiovascular insufficiency, and/or pulmonary thromboembolism, the history of carcinoma; uncontrolled arterial hypertension more than 145/95 mmHg; chronic renal insufficiency; hepatic cirrhosis; individual intolerance to any of Karinat compounds, and/or detected serious adverse event or adverse reaction during the treatment period.

In total, 131 women complied with the inclusion criteria; all of them signed the ICF and successfully passed the screening assessments including physical examination, BMI calculation, ECG, ultrasound examination of common carotid arteries and lipid profile tests.

Randomization

Study participants were randomized into two groups, Karinat or Placebo, using double blinding and random numbers generator. Each patient received the ID (identifying code). The code table was inaccessible for participants and medical staff during all period of the study. After receiving the ID, each study participant started the 6-month therapy with the prescribed product.

Treatment Period

Patients randomized to Karinat group received active treatment with Karinat, while Placebo group was provided with placebo capsules. The schemes of treatment by both Karinat and Placebo groups were similar. Study participants took 3 capsules daily for 6 months, and according to the clinical trial schedule each study participant had interim visits to physician. The physical examination took place at every visit, and all the parameters (anthropometric data, arterial pressure measurement, and BMI calculation) were documented. Patients also passed through periodic ultrasound examination of common carotids, laboratory lipid tests, and periodic ECG. For each study participant, the 10-year prognostic risks of ischemic heart disease, myocardial infarction and sudden cardiac death were calculated for several times on the basis of actual parameters.

Results

The mean age of 131 randomized study participants was 64.8 years. The baseline characteristics of study participants are presented in Table **5**. In general, the study participants were characterized by mild overweight, high-normal arterial blood pressure, mild risk of ischemic disease and myocardial infarction. They had also the ultrasonographic evidence of subclinical atherosclerosis.

Both randomized groups were homogeneous and had no statistically significant difference in baseline characteristics of arterial blood pressure, prognostic risks and cIMT (baseline characteristics

Table 5.   Changes in lipidological risk factors and cardiovascular diseases risks.

Characteristic	Changes dynamic in placebo group	P	Changes dynamic in Karinat group	P
Cholesterol, mg/dl	-13.3 (41.4)	0.020	-16.5 (45.8)	0.011
Triglycerides, mg/dl	-8.7 (39.8)	uncertain	-8.7 (52.8)	uncertain
HDL cholesterol, mg/dl	-3.3 (11.7)	0.038	-2.5 (11.4)	uncertain
LDL cholesterol, mg/dl	-8.2 (39.4)	uncertain	-13.0 (45.4)	0.004
Atherogenicity index	0.01 (0.83)	uncertain	0.00 (0.97)	uncertain

Table 6.   Dynamics in atherosclerosis progression.

Characteristic	Changed dynamics in placebo group	P	Changed dynamics in Karinat group	P
Average cIMT, μm	+111 (91)	uncertain	+6 (85)	uncertain
Maximum c IMT, μm	+4 (220)	uncertain	+8 (101)	uncertain
Plaque, points	+0.31 (0.55)	<0.001	+0.21 (0.59)	0.009

of cIMT are presented in Table **6**. Therefore, the randomized groups were appropriate for the conduction of the trial.

Total cholesterol levels were reduced both in Placebo and Karinat groups, but in Karinat group this decrease was due to LDL cholesterol (beneficial lipid profile changes), while in Placebo group it was caused by the changes in HDL cholesterol level (hazardous lipid profile changes) (Table **7**).

Table 7.   Baseline lipidologic characteristics of patients.

Characteristic	Placebo group	Karinat group	P
cholesterol, mg/dl	251.5 (42.2)	270.8 (54.8)	0.030
triglycerides mg/dl	125.9 (51.2)	134.4 (78.2)	uncertain
HDL cholesterol, mg/dl	73.5 (18.2)	74.4 (14.8)	uncertain
LDL cholesterol, mg/dl	152.9 (41.7)	169.6 (47.2)	0.037
atherogenicity index	2.22 (0.84)	2.38 (0.86)	uncertain

It is noteworthy that the progression of cIMT was detected in both Placebo and Karinat groups, but in Karinat-treated group, the cIMT increase accounted for 6 μm/year, p=0.009, while in placebo group the cIMT increase was more than 100 μm/year (p=0.001. Such changes in cIMT dynamics in placebo group confirm the active atherosclerosis process in postmenopausal women. Taking into account the pilot study design and short-term of therapy, none of the detected changes in cIMT were characterized as statistically significant (Table **8**).

The active and aggressive atherosclerosis progression in study participants was also confirmed by the increase of atherosclerotic plaques size (Table **8**). In Placebo group, the growth rate of existing plaques reached up to 40% per year, while in Karinat group the growth rate of already existing plaques was 27% per year. No other significant changes were detected during this pilot study.

Safety

No serious adverse events or adverse reactions were detected during the study. This result confirms the safety of the novel non-pharmaceutical natural phytoestrogen-rich complex Karinat for postmenopausal women. Thus, the first pilot double blinded randomized study confirmed that natural phytoestrogen-rich complex Karinat had an anti-atherosclerotic effect, reduced *de novo* formation of the atherosclerotic plaques and decreased the growth rate of already existing plaques by more than 1.5 folds [67].

CLINICAL TRIAL OF ALLICOR: AMAR STUDY

Garlic is one of the best known and widely used natural medications. However, the anti-atherosclerotic effects of garlic became evident only in the last century. Garlic was the most promising of

Table 8. Instrumental characteristics of atherosclerosis development rate.

Characteristic	Placebo group	Karinat group	P
Averege cIMT, μm	849 (133)	830 (138)	uncertain
Maximum c IMT, μm	981 (161)	950 (172)	uncertain
Plaque, points	0.76 (0.72)	0.77 (0.78)	uncertain

the botanicals in the described laboratory studies [9, 50]. The experiments on the *in vitro* models based on cultured cells and atherogenic blood serum clearly demonstrated the anti-atherogenic activity of the drug, namely, the reduction of cellular cholesterol accumulation. The following CT was performed to evaluate the new non-pharmaceutical natural product Allicor. The study confirmed the anti-atherosclerotic effect of Allicor at the level of the vascular wall. On the basis of the promising pre-clinical results, a double blinded randomized placebo-controlled clinical trial of Allicor was designed, which involved patients with subclinical atherosclerosis. The primary endpoint of the study was the rate of cIMT progression/regression [8].

Subjects

The CT was performed on men aged 40-74. All participants have signed the informed consent form. 257 men were screened and randomized for the study [8]. The following inclusion criteria were used: ultrasonographically detected carotid atherosclerosis; the absence of permanent treatment (more than 2 months per year) with sugar-lowering drugs, diuretics and vasoactive drugs; the maximum cIMT 1-2 mm (that needed to be confirmed during the first B-mode ultrasound examination); and arterial normotension or mild arterial hypertension (systolic blood pressure <160 mm Hg, diastolic blood pressure <90 mm Hg). The exclusion criteria were the permanent treatment (more than 2 months per year) with sugar-lowering drugs, diuretics or vasoactive drugs; indications for surgical treatment of atherosclerotic lesions localized in the extracranial brachiocephalic system; and individual intolerance to garlic and/or serious adverse events or adverse reactions detected during the treatment period.

Randomization

Before the randomization, all screened patients were assessed at the pre-randomization visit by ECG, physical examination, arterial blood pressure measurement, evaluation of family history of CHD and myocardial infarction, lipid profile test, and ultrasound examination of carotids to determine cIMT value (all baseline data are provided in Table **9**). In total, 257 patients met the inclusion criteria and were randomized into 2 treatment groups – Allicor treatment group and Placebo group. The schemes of treatment for Placebo and Allicor groups were identical, and both patients and medical staff were blinded to the individual prescribed medication. Each

Table 9.   Baseline characteristics of patients.

Variable	Evaluable Allicor recipients (n=93)	Evaluable placebo recipients (n=103)
Carotid intima-media thickness, mm	0.929±0.015	0.937±0.015
Age, years	60.4±1.1	61.5±0.8
Smoking history, n (%)	30 (32.3)	32 (31.1)
Family history, n (%)	18 (19.4)	17 (16.5)
Systolic blood pressure, mm Hg	133.3±1.3	131.9±1.4
Diastolic blood pressure, mm Hg	81.8±0.8	81.2±0.9
Total cholesterol, mMol/L	6.14±0.12	6.02±0.10
Triglycerides, mMol/L	2.17±0.14	2.23±0.12
HDL cholesterol, mMol/L	1.07±0.03	1.10±0.03
LDL cholesterol, mMol/L	4.06±0.10	3.88±0.09
Serum-induced intracellular Cholesterol accumulation (serum atherogenicity), % of control	156.2±5.1	156.4±5.2

From [8], with permission.

patient received the Identification number to blind the study process and to protect the personal data. Randomization was performed using the random number generator [8].

Treatment

After randomization, patients received the treatment product (in tablets); the standard scheme for treatment included administration of 1 tablet twice a day for 24 months. During the whole treatment period, patients had scheduled interim visits for physical assessment, re-calculation of the 10-year prognostic risks and performing the ultrasound examination of common carotids followed by cIMT measurement.

Sixty one patients were discontinued from the treatment and/or terminated their participation for various reasons. The major reason of study discontinuation was the development of adverse events and serious adverse events; 20 subjects discontinued for personal reasons and 7 patients did not show up for the follow-up evaluation [8].

Results

In total, 196 patients were eligible for the assessment of primary endpoint. The main objective of the study was to confirm the Allicor effect on the rate of cIMT progression/regression. In Allicor-treated group, there were 30 cases (32.3% of population) of further cIMT progression, while 44 patients (47.3%) from the same group have shown positive results, such as significant reduction of cIMT. In placebo group, 50 (48.5%) cases of cIMT progression were observed, and 31 patients (30.1%) have demonstrated cIMT regression in one or both carotids arteries.

The overall changes in cIMT dynamics in Allicor-treated group were significantly different (p=0.002) from the Placebo group. By the end of the study, the mean cIMT decrease in Allicor group was

0.022±0.007 mm per year (Fig. **1**). The difference of overall regression rate after 24 months of treatment between Placebo and Allicor groups remained statistically significant with stable overbalance to Allicor active treatment group (p=0.049).
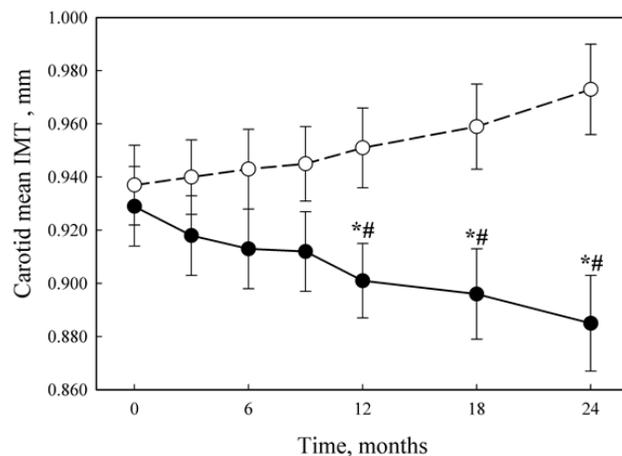

Fig. (1). The dynamics of IMT changes.

Solid circles, Allicor-treated patients; open circles, placebo patients.

*, significant IMT change as compared to baseline, *P*<0.05;

#, significant difference from placebo group, *P*<0.05.

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Statistically significant decrease of patients' serum atherogenicity (the ability of blood serum to induce intracellular cholesterol accumulation in cell culture test) was also registered. In Allicor group, serum atherogenicity decreased nearly by 30% from the baseline level already after 3 months of treatment (p=0.016), and this effect remained at the same level till the end of the study. On the contrary, in Placebo group no significant changes were observed. Positive changes in patients' serum atherogenicity correlated with the reduction of cIMT (p=0.045) (Fig. **2**).
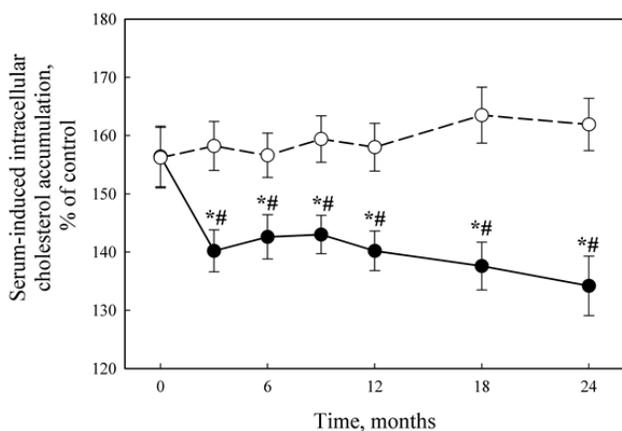

Fig. (2). The dynamics of serum atherogenicity changes.

Solid circles, Allicor-treated patients; open circles, placebo patients.

*, significant IMT change as compared to baseline, *P*<0.05;

#, significant difference from placebo group, *P*<0.05.

From [8], with permission.

The effects of Allicor on the lipid profile have also been evaluated. The major beneficial effects after 24-month treatment were detected for both total and LDL cholesterol levels in the Allicor group, while in Placebo group a statistically significant increase by 4.4% at the end of study was registered. In both groups, the level of HDL cholesterol increased significantly (p<0.05) during the study, while the changes in triglycerides did not reach the statistical significance [8].

Safety

Several adverse events (AEs) and serious adverse events (SAEs) were registered. The following fatal SAEs were observed in Allicore group: 3 myocardial infarctions and 1 pulmonary thromboembolism. In Placebo group, there were 4 myocardial infarctions, 1 fatal stroke, 1 metastatic cancer and 1 thromboembolism. Besides, 2 non-fatal gastrointestinal events, 1 non-fatal stoke and 1 prostate adenocarcinoma were registered in the Allicor group. One non-fatal myocardial infarction, two non-fatal strokes and 1 unstable angina were registered in the Placebo group [8]. Statistical analysis demonstrated that the distribution of SAEs was random, and no fatal SAEs were associated specifically with the Allicor treatment.

In conclusion, the Allicor anti-atherosclerotic activity was confirmed, and the Allicor-induced cIMT regression was demonstrated in the AMAR study. Therefore, Allicor can be used for the long-term treatment and prevention of subclinical atherosclerosis. It is noteworthy that even a single dose of this product had an anti-atherosclerotic effect for the next 12-16 h, as demonstrated in an *ex vivo* study. Moreover, Allicor can be regarded as a basis for further development of anti-atherosclerotic therapy [8].

CONCLUSION

To date, atherosclerosis is one of the most important medical and social problems, playing the key role in the pathogenesis of cardiovascular disorders, which account for more than 50% of all death cases and remain a major cause of disabling diseases in the developed countries. Direct anti-atherosclerotic therapy could not be developed for a long time because of the limited understanding of the mechanisms of atherogenesis and problems with the evaluation of the anti-atherosclerotic activity of drugs at the cellular level. Patients with different stages of the disease are forced to be treated with the same medications, while aggressive therapy at the early stages of the disease may result in the development of adverse reactions. The establishment of the concept of serum atherogenicity and the development of the appropriate models changed the situation for the better: the evaluation of the "direct anti-atherosclerotic" activity of drug substances became possible.

In the described studies, the main objective was the evaluation of the direct anti-atherosclerotic effect of natural agents and the development of the non-pharmaceutical products suitable for long supportive treatment and prevention of atherosclerosis. As a result of the studies, three natural non-pharmaceutical products have been created: Allicor, Karinat and Inflaminat. All of them are based on botanicals with laboratory proven direct anti-atherosclerotic effect. All the medications were studied on healthy subjects, tested on *in vitro* and *ex vivo* models and demonstrated a significant activity in lowering the total serum atherogenicity level. The clinical trials of the new natural products were performed in patients with diagnosed early stage atherosclerosis, and the main outcomes of the treatment were the reduction of cIMT progression rate and the clinically relevant decrease of intracellular cholesterol accumulation. Allicor was studied in pilot and basic clinical trials, while Karinat and Inflaminat only started their lifecycle with pilot studies. Further research of natural drugs efficiency will be continued.

Despite the positive results demonstrated in these studies, we are still far from the full-scale transition to direct anti-atherosclerotic therapy. The inclusion of non-pharmaceutical medications into standard treatment schemes for patients with early stage atherosclerosis will take time, because such drugs are not defined as the primary physician's choice. However, the promotion of these products as supportive therapy or supplement medications may bring a positive feedback.

LIST OF ABBREVIATION

BMI	=	Body mass index
CIS	=	Interstate statistical committee
cIMT	=	Carotid intima-media thickness
CT	=	Clinical trials
ECG	=	Electrocardiogram
GM-CSF	=	Granulocyte-macrophage colony-stimulating factor
HDL	=	High-density lipoproteins
ICF	=	Informed consent form
ID	=	Identifying code
IL	=	Interleukin
IMT	=	Intima-medial thickness
LDL	=	Low-density lipoproteins
M-GSF	=	Macrophage growth-stimulatory factors
SMC	=	Smooth muscle cells
TNFα	=	Tumor necrosis factor alpha

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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