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(54) Title: METHODS EMPLOYING AND COMPOSITIONS CONTAINING DEFINED OXIDIZED PHOSPHOLIPIDS FOR PREVENTION AND TREATMENT OF ATHEROSCLEROSIS

(57) Abstract: Novel synthetic forms of etherified oxidized phospholipids and methods of utilizing same for preventing and treating atherosclerosis and other related disorders are provided. In addition, methods of synthesizing esterified oxidized phospholipids and using same for preventing and treating atherosclerosis and other related disorders are also provided.

METHODS EMPLOYING AND COMPOSITIONS CONTAINING DEFINED OXIDIZED PHOSPHOLIPIDS FOR PREVENTION AND TREATMENT OF ATHEROSCLEROSIS

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to defined, oxidized LDL (oxLDL) components for prevention and treatment of atherosclerosis and related disease and, more particularly, to methods and compositions employing oxidized phospholipids effective in inducing mucosal tolerance and inhibiting inflammatory processes contributing to atheromatous vascular disease and sequalae.

Cardiovascular disease is a major health risk throughout the industrialized world. Atherosclerosis, the most prevalent of cardiovascular diseases, is the principal cause of heart attack, stroke, and gangrene of the extremities, and as such, the principle cause of death in the United States. Atherosclerosis is a complex disease involving many cell types and molecular factors (for a detailed review, see Ross, 1993, Nature 362: 801-809). The process, which occurs in response to insults to the endothelium and smooth muscle cells (SMCs) of the wall of the artery, consists of the formation of fibrofatty and fibrous lesions or plaques, preceded and accompanied by inflammation. The advanced lesions of atherosclerosis may occlude the artery concerned, and result from an excessive inflammatory-fibroproliferative response to numerous different forms of insult. For example, shear stresses are thought to be responsible for the frequent occurrence of atherosclerotic plaques in regions of the circulatory system where turbulent blood flow occurs, such as branch points and irregular structures.

The first observable event in the formation of an atherosclerotic plaque occurs when inflammatory cells such as monocyte-derived macrophages adhere to the vascular endothelial layer and transmigrate through to the sub-endothelial space. Elevated plasma LDL levels lead to lipid engorgement of the vessel walls, with adjacent endothelial cells producing oxidized low

density lipoprotein (LDL). In addition, lipoprotein entrapment by the extracellular matrix leads to progressive oxidation of LDL by lipoxygenases, reactive oxygen species, peroxynitrite and/or myeloperoxidase. These oxidized LDL's are then taken up in large amounts by vascular cells through scavenger receptors expressed on their surfaces.

Lipid-filled monocytes and smooth-muscle derived cells are called foam cells, and are the major constituent of the fatty streak. Interactions between foam cells and the endothelial and smooth muscle cells surrounding them produce a state of chronic local inflammation which can eventually lead to activation of endothelial cells, increased macrophage apoptosis, smooth muscle cell proliferation and migration, and the formation of a fibrous plaque (Hajjar, DP and Haberland, ME, J.Biol Chem 1997 Sep 12; 272(37):22975-78). Such plaques occlude the blood vessels concerned and thus restrict the flow of blood, resulting in ischemia, a condition characterized by a lack of oxygen supply in tissues of organs due to inadequate perfusion. When the involved arteries block the blood flow to the heart, a person is afflicted with a 'heart attack'; when the brain arteries occlude, the person experiences a stroke. When arteries to the limbs narrow, the result is severe pain, decreased physical mobility and possibly the need for amputation.

Oxidized LDL has been implicated in the pathogenesis of atherosclerosis and atherothrombosis, by it's action on monocytes and smooth muscle cells, and by inducing endothelial cell apoptosis, impairing anticoagulant balance in the endothelium. Oxidized LDL also inhibits anti-atherogenic HDL-associated breakdown of oxidized phospholipids (Mertens, A and Holvoet, P, FASEB J 2001 Oct; 15(12):2073-84). This association is also supported by many studies demonstrating the presence of oxidized LDL in the plaques in various animal models of atherogenesis; the retardation of atherogenesis through inhibition of oxidation by pharmacological and/or genetic manipulations; and the promising results of intervention trials with anti-oxidant vitamins (see, for example, Witztum J and

Steinberg, D, Trends Cardiovasc Med 2001 Apr-May;11(3-4):93-102 for a review of current literature). Indeed, oxidized LDL and malondialdehyde (MDA)-modified LDL have been recently proposed as accurate blood markers for 1st and 2nd stages of coronary artery disease (US Pat. Nos. 6,309,888 to Holvoet et. al. and 6,255,070 to Witztum, et al).

Reduction of LDL oxidation and activity has been the target of a number of suggested clinical applications for treatment and prevention of cardiovascular disease. Bucala, et al (US Pat. No. 5869534) discloses methods for the modulation of lipid peroxidation by reducing advanced glycosylation end product, lipid characteristic of age-, disease- and diabetes-related foam cell formation. Tang et al, at Incyte Pharmaceuticals, Inc. (US Pat. No. 5,945,308) have disclosed the identification and proposed clinical application of a Human Oxidized LDL Receptor in the treatment of cardiovascular and autoimmune diseases and cancer.

Atherosclerosis and autoimmune disease

Because of the presumed role of the excessive inflammatory-fibroproliferative response in atherosclerosis and ischemia, a growing number of researchers have attempted to define an autoimmune component of vascular injury. In autoimmune diseases the immune system recognizes and attacks normally non-antigenic body components (autoantigens), in addition to attacking invading foreign antigens. The autoimmune diseases are classified as auto- (or self-) antibody mediated or cell mediated diseases. Typical autoantibody mediated autoimmune diseases are myasthenia gravis and idiopathic thrombocytopenic purpura (ITP), while typical cell mediated diseases are Hashimoto's thyroiditis and type I (Juvenile) Diabetes.

The recognition that immune mediated processes prevail within atherosclerotic lesions stemmed from the consistent observation of lymphocytes and macrophages in the earliest stages, namely the fatty streaks. These lymphocytes which include a predominant population of CD4+ cells

(the remainder being CD8+ cells) were found to be more abundant over macrophages in early lesions, as compared with the more advanced lesions, in which this ratio tends to reverse. These findings posed questions as to whether they reflect a primary immune sensitization to a possible antigen or alternatively stand as a mere epiphenomenon of a previously induced local tissue damage. Regardless of the factors responsible for the recruitment of these inflammatory cells to the early plaque, they seem to exhibit an activated state manifested by concomitant expression of MHC class II HLA-DR and interleukin (IL) receptor as well as leukocyte common antigen (CD45R0) and the very late antigen 1 (VLA-1) integrin.

The on-going inflammatory reaction in the early stages of the atherosclerotic lesion may either be the primary initiating event leading to the production of various cytokines by the local cells (i.e endothelial cells, macrophages, smooth muscle cells and inflammatory cells), or it may be that this reaction is a form of the body's defense immune system towards the hazardous process. Some of the cytokines which have been shown to be upregulated by the resident cells include TNF-α, IL-1, IL-2, IL-6, IL-8, IFN-γ and monocyte chemoattractant peptide-1 (MCP-1). Platelet derived growth factor (PDGF) and insulin-like growth factor (ILGF) which are expressed by all cellular constituents within atherosclerotic plaques have also been shown to be overexpressed, thus possibly intensifying the preexisting inflammatory reaction by a co-stimulatory support in the form of a mitogenic and chemotactic factor. Recently, Uyemura et al. (Cross regulatory roles of IL-12 and IL-10 in atherosclerosis. J Clin Invest 1996 97; 2130-2138) have elucidated type 1 T-cell cytokine pattern in human atherosclerotic lesions exemplified by a strong expression of IFN-γ but not IL-4 mRNA in comparison with normal arteries. Furthermore, IL-12 - a T-cell growth factor produced primarily by activated monocytes and a selective inducer of Th1 cytokine pattern, was found to be overexpressed within lesions as manifested

by the abundance of its major heterodimer form p70 and p40 (its dominant inducible protein) mRNA.

Similar to the strong evidence for the dominance of the cellular immune system within the atherosclerotic plaque, there is also ample data supporting the involvement of the local humoral immune system. Thus, deposition of immunoglobulins and complement components have been shown in the plaques in addition to the enhanced expression of the C3b and C3Bi receptors in resident macrophages.

Valuable clues with regard to the contribution of immune mediated inflammation to the progression of atherosclerosis come from animal models. Immunocompromised mice (class I MHC deficient) tend to develop accelerated atherosclerosis as compared with immune competent mice. Additionally, treatment of C57BL/6 mice (Emeson EE, Shen ML. Accelerated atherosclerosis in hyperlipidemic C57BL/6 mice treated with cyclosporin A. Am J Pathol 1993; 142: 1906-1915) and New-Zealand White rabbits (Roselaar SE, Schonfeld G, Daugherty A. Enhanced development of atherosclerosis in cholesterol fed rabbits by suppression of cell mediated immunity. J Clin Invest 1995; 96: 1389-1394) with cyclosporin A, a potent suppressor of IL-2 transcription resulted in a significantly enhanced atherosclerosis under "normal" lipoprotein "burden". These latter studies may provide insight into the possible roles of the immune system in counteracting the self-perpetuating inflammatory process within the atherosclerotic plaque.

Atherosclerosis is not a classical autoimmune disease, although some of its manifestations such as the production of the plaque which obstructs the blood vessels may be related to aberrant immune responsiveness. In classical autoimmune disease, one can often define very clearly the sensitizing autoantigen attacked by the immune system and the component(s) of the immune system which recognize the autoantigen (humoral, i.e. autoantibody or cellular, i.e. lymphocytes). Above all, one can show that by passive transfer of these components of the immune system the disease can be induced in healthy

animals, or in the case of humans the disease may be transferred from a sick pregnant mother to her offspring. Many of the above are not prevailing in atherosclerosis. In addition, the disease definitely has common risk factors such as hypertension, diabetes, lack of physical activity, smoking and others, the disease affects elderly people and has a different genetic preponderance than in classical autoimmune diseases.

Treatment of autoimmune inflammatory disease may be directed towards supression or reversal of general and/or disease-specific immune reactivity. Thus Aiello, for example (US Pat. Nos. 6,034,102 and 6,114,395) discloses the use of estrogen-like compounds for treatment and prevention of atherosclerosis and atherosclerotic lesion progression by inhibition of inflammatory cell recruitment. Similarly, Medford et al (US Pat. No. 5,846,959) disclose methods for the prevention of formation of oxidized PUFA, for treatment of cardiovascular and non-cardiovascular inflammatory diseases mediated by the cellular adhesion molecule VCAM-1. Furthermore, Falb (US Pat. No. 6,156,500) designates a number of cell signaling and adhesion molecules abundant in atherosclerotic plaque and disease as potential targets of anti-inflammatory therapies.

Since oxidized LDL has been clearly implicated in the pathogenesis of atherosclerosis (see above), the contribution of these prominent plaque components to autoimmunity in atheromatous disease processes has been investigated.

Immune responsiveness to Oxidized LDL

It is known that Ox LDL is chemotactic for T-cells and monocytes. Ox LDL and its byproducts are also known to induce the expression of factors such as monocyte chemotactic factor 1, secretion of colony stimulating factor and platelet activating properties, all of which are potent growth stimulants.

The active involvement of the cellular immune response in atherosclerosis has recently been substantiated (Stemme S, et al, Proc Natl Acad Sci USA 1995; 92: 3893-97), who isolated CD4+ within plaques clones responding to Ox

LDL as stimuli. The clones corresponding to Ox LDL (4 out of 27) produced principally interferon-γ rather than IL-4. It remains to be seen whether the above T-cell clones represent mere contact with the cellular immune system with the inciting strong immunogen (Ox LDL) or that this reaction provides means of combating the apparently indolent atherosclerotic process.

The data regarding the involvement of the humoral mechanisms and their meaning are much more controversial. One recent study reported increased levels of antibodies against MDA-LDL, a metabolite of LDL oxidation, in women suffering from heart disease and/or diabetes (Dotevall, et al., Clin Sci 2001 Nov; 101(5): 523-31). Other investigators have demonstrated antibodies recognizing multiple epitopes on the oxidized LDL, representing immune reactivity to the lipid and apolipoprotein components (Steinerova A, et al., Physiol Res 2001;50(2): 131-41) in atherosclerosis and other diseases, such as diabetes, renovascular syndrome, uremia, rheumatic fever and lupus erythematosus. Several reports have associated increased levels of antibodies to Ox LDL with the progression of atherosclerosis (expressed by the degree of carotid stenosis, severity of peripheral vascular disease etc.). Most recently, Sherer et al (Cardiology 2001;95(1):20-4) demonstrated elevated levels of antibodies to cardiolipin, beta 2GPI and oxLDL, but not phosphatidyl choline or endothelial cells in coronary heart disease. Thus, there seems to be a consensus as to the presence of Ox LDL antibodies in the form of immune complexes within atherosclerotic plaque, although the true significance of this finding has not been established.

Antibodies to Ox LDL have been hypothesized as playing an active role in lipoprotein metabolism. Thus, it is known that immune complexes of Ox LDL and its corresponding antibodies are taken up more efficiently by macrophages in suspension as compared with Ox LDL. No conclusions can be drawn from this consistent finding on the pathogenesis of atherosclerosis since the question of whether the accelerated uptake of Ox LDL by the macrophages is beneficial or deleterious has not yet been resolved.

Important data as to the significance of the humoral immune system in atherogenesis comes from animal models. It has been found that hyperimmunization of LDL-receptor deficient rabbits with homologous oxidized LDL, resulted in the production of high levels of anti-Ox LDL antibodies and was associated with a significant reduction in the extent of atherosclerotic lesions as compared with a control group exposed to phopsphate-buffered saline (PBS). A decrease in plaque formation has also been accomplished by immunization of rabbits with cholesterol rich liposomes with the concomitant production of anti-cholesterol antibodies, yet this effect was accompanied by a 35% reduction in very low density lipoprotein cholesterol levels.

Thus, both the pathogenic role of oxidized LDL components and their importance as autoantigens in atherosclerosis, as well as other diseases, have been extensively demonstrated in laboratory and clinical studies.

Mucosal Tolerance in Treatment of Autoimmune Disease

Recently, new methods and pharmaceutical formulations have been found that are useful for treating autoimmune diseases (and related T-cell mediated inflammatory disorders such as allograft rejection and retroviral-associated neurological disease). These treatments induce tolerance, orally or mucosally, e.g. by inhalation, using as tolerizers autoantigens, bystander antigens, or disease-suppressive fragments or analogs of autoantigens or bystander antigens. Such treatments are described, for example, in US Pat. No. 5,935,577 to Weiner et al. Autoantigens and bystander antigens are defined below (for a general review of mucosal tolerance see Nagler-Anderson, C., Crit Rev Immunol 2000;20(2):103-20). Intravenous administration of autoantigens (and fragments thereof containing immunodominant epitopic regions of their molecules) has been found to induce immune suppression through a mechanism called clonal anergy. Clonal anergy causes deactivation of only immune attack T-cells specific to a particular antigen, the result being a significant reduction in the immune

response to this antigen. Thus, the autoimmune response-promoting T-cells specific to an autoantigen, once anergized, no longer proliferate in response to that antigen. This reduction in proliferation also reduces the immune reactions responsible for autoimmune disease symptoms (such as neural tissue damage that is observed in MS). There is also evidence that oral administration of autoantigens (or immunodominant fragments) in a single dose and in substantially larger amounts than those that trigger "active suppression" may also induce tolerance through anergy (or clonal deletion).

A method of treatment has also been disclosed that proceeds by active suppression. Active suppression functions via a different mechanism from that of clonal anergy. This method, discussed extensively in PCT Application PCT/US93/01705, involves oral or mucosal administration of antigens specific to the tissue under autoimmune attack. These are called "bystander antigens". This treatment causes regulatory (suppressor) T-cells to be induced in the gut-associated lymphoid tissue (GALT), or bronchial associated lymphoid tissue (BALT), or most generally, mucosa associated lymphoid tissue (MALT) (MALT includes GALT and BALT). These regulatory cells are released in the blood or lymphatic tissue and then migrate to the organ or tissue afflicted by the autoimmune disease and suppress autoimmune attack of the afflicted organ or tissue. The T-cells elicited by the bystander antigen (which recognize at least one antigenic determinant of the bystander antigen used to elicit them) are targeted to the locus of autoimmune attack where they mediate the local release of certain immunomodulatory factors and cytokines, such as transforming growth factor beta (TGF-β), interleukin-4 (IL-4), and/or interleukin-10 (IL-10). Of these, TGF-βis an antigen-nonspecific immunosuppressive factor in that it suppresses immune attack regardless of the antigen that triggers the attack. (However, because oral or mucosal tolerization with a bystander antigen only causes the release of TGF-βin the vicinity of autoimmune attack, no systemic immunosuppression ensues.) IL-4 and IL-10

are also antigen-nonspecific immunoregulatory cytokines. IL-4 in particular enhances (T helper Th2) Th_2 response, i.e., acts on T-cell precursors and causes them to differentiate preferentially into Th_2 cells at the expense of Th_1 responses. IL-4 also indirectly inhibits Th_1 exacerbation. IL-10 is a direct inhibitor of Th_1 responses. After orally tolerizing mammals afflicted with autoimmune disease conditions with bystander antigens, increased levels of TGF-β, IL-4 and IL-10 are observed at the locus of autoimmune attack (Chen, Y. et al., Science, 265:1237-1240, 1994). The bystander suppression mechanism has been confirmed by von Herreth et al., (J. Clin. Invest., 96:1324-1331, September 1996).

More recently, oral tolerance has been effectively applied in treatment of animal models of inflammatory bowel disease by feeding probiotic bacteria (Dunne, C, et al., Antonie Van Leeuwenhoek 1999 Jul-Nov;76(1-4):279-92), autoimmune glomerulonephritis by feeding glomerular basement membrane (Reynolds, J. et al., J Am Soc Nephrol 2001 Jan;12(1): 61-70) experimental allergic encephalomyelitis (EAE, which is the equivalent of multiple sclerosis or MS), by feeding myelin basic protein (MBP), adjuvant arthritis and collagen arthritis, by feeding a subject with collagen and HSP-65, respectively. A Boston based company called Autoimmune has carried out several human experiments for preventing diabetes, multiple sclerosis, rheumatoid arthritis and uveitis. The results of the human experiments have been less impressive than the non-human ones, however there has been some success with the prevention of arthritis.

Oral tolerance to autoantigens found in atherosclerotic plaque lesions has also been investigated. Study of the epitopes recognized by T-cells and Ig titers in clinical and experimental models of atherosclerosis indicated three candidate antigens for suppression of inflammation in atheromatous lesions: oxidized LDL, the stress-related heat shock protein HSP 65 and the cardiolipin binding protein beta 2GP1. US Patent Application 09/806,400 to Shoenfeld et al (filed Sept 30, 1999), which is incorporated herein in its entirety, discloses

the reduction by approximately 30% of atherogenesis in the arteries of genetically susceptible LDL-RD receptor deficient transgenic mice fed oxidized human LDL. This protective effect, however, was achieved by feeding a crude antigen preparation consisting of centrifuged, filtered and purified human serum LDL which had been subjected to a lengthy oxidation process with Cu^{++}. Although significant inhibition of atherogenesis was achieved, presumably via oral tolerance, no identification of specific lipid antigens or immunogenic LDL components was made. Another obstacle encountered was the inherent instability of the crude oxidized LDL in vivo, due to enzymatic activity and uptake of oxidized LDL by the liver and cellular immune mechanisms. It is plausible that a stable, more carefully defined oxidized LDL analog would have provided oral tolerance of greater efficiency. The induction of immune tolerance and subsequent prevention or inhibition of autoimmune inflammatory processes has been demonstrated using exposure to suppressive antigens via mucosal sites other than the gut. The membranous tissue around the eyes, and the mucosa of the nasal cavity, as well as the gut, are exposed to many invading as well as self- antigens and possess mechanisms for immune reactivity. Thus, Rossi, et al (Scand J Immunol 1999 Aug;50(2):177-82) found that nasal administration of gliadin was as effective as intravenous administration in downregulating the immune response to the antigen in a mouse model of celiac disease. Similarly, nasal exposure to acetylcholine receptor antigen was more effective than oral exposure in delaying and reducing muscle weakness and specific lymphocyte proliferation in a mouse model of myasthenia gravis (Shi, FD. et al, J Immunol 1999 May 15; 162 (10): 5757-63). Therefore, immunogenic compounds intended for mucosal as well as intravenous or intraperitoneal administration should optimally be adaptable to nasal and other membranous routes of administration.

Thus, there is clearly a need for novel, well defined, synthetic oxidized phospholipid derivatives exhibiting enhanced metabolic stability and efficient

tolerizing immunogenicity in intravenous, intraperitoneal and mucosal administration.

Synthesis of oxidized phospholipids

Modification of phospholipids has been employed for a variety of applications. For example, phospholipids bearing lipid-soluble active compounds may be incorporated into compositions for trans-dermal and trans-membranal application (US Pat. No. 5,985,292 to Fournerou et al) and phospholipid derivatives can be incorporated into liposomes and biovectors for drug delivery (see, for example US Pat. Nos. 6,261,597 and 6,017,513 to Kurtz and Betbeder, et al, respectively). Modified phospholipid derivatives mimicing platelet activation factor (PAF) structure are known to be pharmaceutically active in variety of disorders and diseases, effecting such functions as vascular permeability, blood pressure, heart function inhibition etc. It has been suggested that one group of these derivatives may have anti cancerous activity (U.S. Pat. No. 4,778,912 to Inoue at al.). However, the compound disclosed in U.S. Pat. No. 4,778,912 possesses a much longer bridge between the phosphate and the tertiary amine moiety than in the phosphatidyl group and therefore is not expected to be immunologically similar to ox-LDL.

Another group of modified phospholipid ether derivatives has been disclosed which was intended for chromatographic separation, but might have some physiological effect (CH Pat No. 642,665 to Berchtold).

Oxidation of phospholipids occurs in vivo through the action of free radicals and enzymatic reactions abundant in atheromatous plaque. In vitro, preparation of oxidized phospholipids usually involves simple chemical oxidation of a native LDL or LDL phospholipid component. Investigators studying the role of oxidized LDL have employed, for example, ferrous ions and ascorbic acid (Itabe, H, et al, J.Biol. Chem. 1996; 271:33208-217) and copper sulfate (George, J. et al, Atherosclerosis. 1998; 138:147-152; Ameli, S. et al, Arteriosclerosis Thromb Vasc Biol 1996; 16:1074-79) to produce

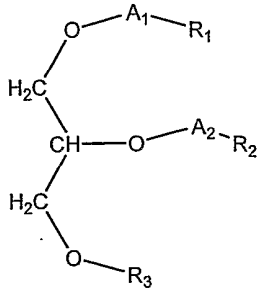
oxidized, or mildly oxidized phospholipid molecules similar to those associated with plaque components. Similarly prepared molecules have been shown to be identical to auto-antigens associated with atherogenesis (Watson A.D. et al, J. Biol. Chem. 1997; 272:13597-607) and able to induce protective anti-atherogenic immune tolerance (US Patent Application No 09/806,400 to Shoenfeld et al filed Sept 30, 1999) in mice. Likewise, Koike (US Pat. No. 5,561,052) discloses a method of producing oxidized lipids and phospholipids using copper sulfate and superoxide dismutase to produce oxidized arachadonic or linoleic acids and oxidized LDL for diagnostic use. However, the oxidation techniques employed are non-specific, yielding a variety of oxidized products, necessitating either further purification or use of impure antigenic compounds. This is of even greater concern with native LDL, even if purified.

Furthermore, in vivo applications employing oxidized phospholipids prepared as above have the disadvantage of susceptibility to recognition, binding and metabolism of the active component in the body, making dosage and stability after administration an important consideration.

Thus, there is a widely recognized need, and it would be highly advantageous to have, a novel, synthetic oxidized phospholipid and improved methods of synthesis and use thereof devoid of the above limitations.

SUMMARY OF THE INVENTION

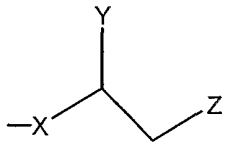
According to the present invention there is provided a compound having a formula:



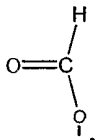
or pharmaceutically acceptable salts thereof, wherein:

(i) A_1 and A_2 are each independently selected from the group consisting of CH_2 and C=O, at least one of A_1 and A_2 being CH_2; and

(ii) R_1 and R_2 are each independently selected from the group consisting of an alkyl chain 1-27 carbons in length and

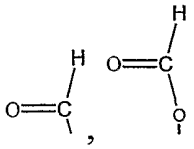


wherein X is a C_{1-24} chain, Y is selected from the group consisting of:



, -OH, –H, alkyl, alkoxy, halogen, acetoxy and aromatic functional groups; and

Z is selected from the group consisting of:



, and -OH; and

(iii) R_3 is selected from the group consisting of H, acyl, alkyl, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl cardiolipin and phosphatidyl inisitol.

According to further features in the preferred embodiments of the invention described below, R_3 is a non-phosphatidyl moeity, and as such the compound is a diglyceride.

According to yet further features in preferred embodiments of the invention described below, at least one of R_1 and R_2 is:

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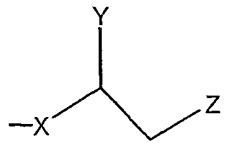
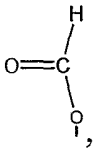
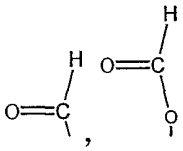


wherein X is a C_{1-24} chain, Y is selected from the group consisting of:



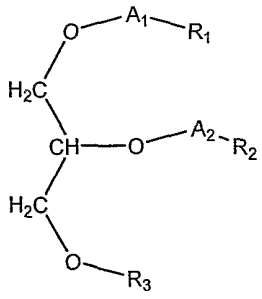
, -OH, –H, alkyl, alkoxy, halogen, acetoxy and aromatic functional groups and

Z is selected from the group consisting of:



and -OH.

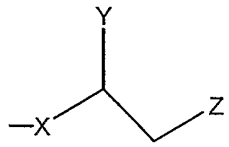
According to another aspect of the present invention there is provided a pharmaceutical composition for prevention and/or treatment of atherosclerosis, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, stenosis, restenosis and/or in-stent-stenosis in a subject in need thereof, the composition comprising, as an active ingredient, a therapeutically effective amount of a compound selected from the group having a formula:



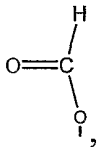
or pharmaceutically acceptable salts thereof, wherein:

(i) A_1 and A_2 are independently selected from the group of CH_2 or C=O; and

(ii) R_1 or R_2 are each independently selected from the group consisting of an alkyl chain 1-27 carbons in length and:

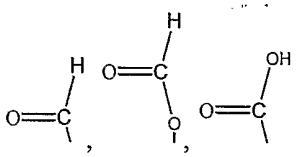


wherein X is C_{1-24}, Y is selected from the group consisting of:



, -OH and -H, alkyl, alkoxy, halogen, acetoxy and aromatic functional groups; and

Z is selected from the group consisting of:



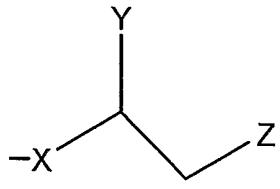
and -OH; and

(iii) R_3 is selected from the group consisting of H, acyl, alkyl, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl cardiolipin and phosphatidyl inisitol

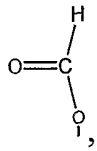
and a pharmaceutically acceptable carrier.

According to further features in the preferred embodiments of the invention described below, R_3 is a non-phosphatidyl moeity, and as such the compound is a diglyceride.

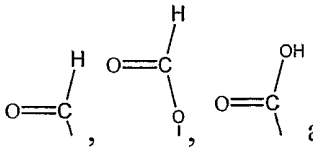
According to yet further features in preferred embodiments of the invention described below, at least one of R_1 and R_2 is:



wherein X is a C_{1-24} chain, Y is selected from the group consisting of:

 , -OH, -H, alkyl, alkoxy, halogen, acetoxy and aromatic functional groups ; and

Z is selected from the group consisting of:

 and -OH.

According to still further features in preferred embodiments of the invention described below, at least one of A_1 and A_2 is CH_2.

According to further feature in preferred embodiments of the invention described below, this molecule is known as ALLE.

According to yet further features in preferred embodiments of the invention described below, the pharmaceutical composition is designed for inducing tolerance to oxidized LDL via mucosal administration.

According to further features in preferred embodiments of the invention described below, the pharmaceutical composition is designed for nasal, oral, subcutaneous or intra- peritoneal administration, alone or in combination with additional routes of immunomodulation..

According to still further features in preferred embodiments of the invention described below, the compound reduces immune reactivity to oxidized LDL in said subject.

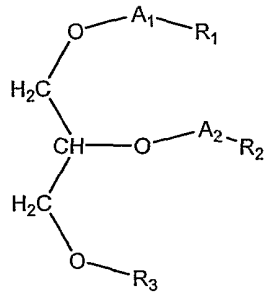
According to yet further features in preferred embodiments of the invention described below, the pharmaceutical composition is packaged and identified for use in the prevention and/or treatment of at least one disorder selected from the group consisting of atherosclerosis, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, stenosis, restenosis and/or in-stent-stenosis.

According to still further features in preferred embodiments of the invention described below, the pharmaceutical composition further comprises

a therapeutically effective amount of at least one additional compound selected from the group consisting of HMG CoA reductase inhibitors (Statins) mucosal adjuvants, corticosteroids, anti-inflammatory compounds, analgesics, growth factors, toxins, and additional tolerizing antigens.

According to still another aspect of the present invention there is provided a pharmaceutical composition for prevention and/or treatment of a disease, syndrome or condition selected from the group consisting of atherosclerosis, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, stenosis, restenosis and/or in-stent-stenosis in a subject in need thereof, comprising, as an active ingredient, a therapeutically effective amount of a synthetic LDL derivative, or pharmaceutically acceptable salts thereof, the composition further comprising a pharmaceutically acceptable carrier.

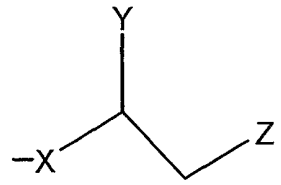
According to yet another aspect of the present invention there is provided a method of prevention and/or treatment of atherosclerosis, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, stenosis, restenosis and/or in-stent-stenosis in a subject in need thereof, the method comprising administering a therapeutically effective amount of a compound, said compound selected from the group having a formula:



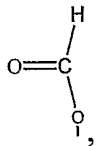
or pharmaceutically acceptable salts thereof, wherein:

(i) A_1 and A_2 are independently selected from the group consisting of CH_2 and C=O; and

(ii) R_1 or R_2 are each independently selected from the group consisting of an alkyl chain 1-27 carbons in length and:

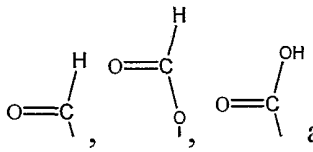


wherein X is a C_{1-24} chain, Y is selected from a group consisting of:



, -OH, -H, alkyl, alkoxy, halogen, acetoxy and aromatic functional groups; and

Z is selected from the group consisting of:

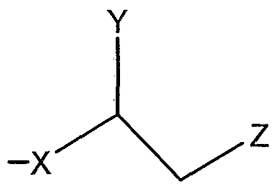


and -OH.

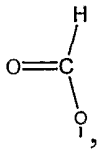
(iii) R_3 is selected from the group consisting of H, acyl, alkyl, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl cardiolipin and phosphatidyl inisitol

and a pharmaceutically acceptable carrier.

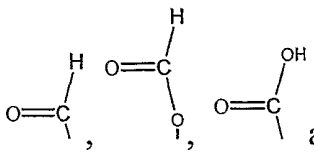
According to further features in preferred embodiments of the invention described below, at least one of R_1 and R_2 is:



wherein X is a C_{1-24} chain, Y is selected from the group consisting of:

 , -OH, -H, alkyl, alkoxy, halogen, acetoxy and aromatic functional groups; and

Z is selected from the group consisting of:

 and -OH.

According to further features in preferred embodiments of the invention described below, at least one of A_1 and $A_2$is CH_2.

According to still further features in preferred embodiments of the invention described below, the compound is 1-hexadecyl-2-(5'-oxo-pentanyl)-*sn*- glycero-3-phosphocholine ester, 3-hexadecyl-2-(5'-oxo-pentanyl)-*sn*- glycero-1-phosphocholine and racemic mixtures thereof (ALLE).

According to yet further features in preferred embodiments of the invention described below, the compound is administered via mucosal administration.

According to further features in preferred embodiments of the invention described below, the administration of the compound is nasal, oral, subcutaneous or intra- peritoneal administration, alone or in combination with additional routes of immunomodulation. According to still further features in preferred embodiments of the invention described below, the administration of the compound reduces immune reactivity to oxidized LDL in said subject.

According to further features in preferred embodiments of the invention described below, the compound is administered in addition to a therapeutically effective amount of at least one additional compound selected from the group consisting of HMG CoA reductase inhibitors (Statins), mucosal adjuvants,

corticosteroids, anti-inflammatory compounds, analgetics, growth factors, toxins, and additional tolerizing antigens.

According to yet a further aspect of the present invention there is provided a method of synthesizing an oxidized phospholipid, the method comprising: (a) providing a phospholipid backbone including two fatty acid side chains, wherein at least one of the fatty acid side chains is a mono-unsaturated fatty acid; and (b) oxidizing the double bond of the mono-unsaturated fatty acid to thereby generate the oxidized phospholipid.

According to further features in preferred embodiments of the invention described below the phospholipid backbone further includes a moiety selected from the group consisting of H, acyl, alkyl, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl cardiolipin and phosphatidyl inisitol.

According to still further features in preferred embodiments of the invention described below the mono unsaturated fatty acid is C_{2-15}.

According to yet further features in preferred embodiments of the invention described below the oxidized phospholipid is 1-palmitoyl-2-oxovaleroyl-sn-glycero-3-phosphocholine, (POVPC), and the mono-unsaturated fatty acid is 5-hexenoic acid.

The present invention successfully addresses the shortcomings of the presently known configurations by providing methods of inducing immune tolerance to oxidized LDL, which methods utilize synthetic oxidized LDL derivatives, such as for example POVPC and ALLE, which is a novel oxidized phospholipid.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred

embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIG. 1 is a flow chart depicting the synthesis of 2,5' Aldehyde lecitin ether, 1-hexadecyl-2-(5'-oxo-pentanyl)-sn-glycero-3-phosphocholine ether (for D-ALLE) or 3-hexadecyl-2-(5'-oxo-pentanyl)-sn-glycero-1-phosphocholine ether (for L-ALLE) (ALLE);

FIG. 2 is a flow chart depicting the synthesis of POVPC;

FIG. 3 is a graphic representation demonstrating inhibition of early atherogenesis in apoE-deficient mice by intra peritoneal immunization with mixed D- and L- isomers of ALLE. 5-7 week old apo-E mice were immunized with 150 μg/mouse mixed D- or L- isomers of ALLE coupled to purified tuberculin protein derivative (ALLE L+D)(n=6), 150 μg/mouse purified tuberculin protein derivative alone (PPD)(n=5) or unimmunized (CONTROL)(n=7). Atherogenesis is expressed as the area of atheromatous lesions in the aortic sinus 6 weeks following the 4^{th} immunization;

FIG. 4 is a graphic representation demonstrating inhibition of early atherogenesis in apoE-deficient mice by oral tolerance induced by feeding ALLE. 8-10 week old apo-E mice were fed mixed D- and L- isomers of ALLE: 10 μg/mouse (ALLE L+D 10 μg)(n=11) or 1mg/mouse (ALLE L+D 1 mg)(n=11); or PBS (CONTROL)(n=12) every other day for 5 days. Atherogenesis is expressed as the area of atheromatous lesions in the aortic sinus 8 weeks after the last feeding;

FIG. 5 is a graphic representation demonstrating inhibition of early atherogenesis in apoE-deficient mice by mucosal tolerance induced by

exposure to L-ALLE. 7-9 week old apo-E mice were either fed 1mg/mouse L-ALLE every other day for 5 days (OT L-ALLE)(n=11) or exposed nasally to 10 μg/mouse L-ALLE every other day for 3 days (NT L-ALLE)(n=11). Control mice were fed an identical volume (0.2 ml) of PBS (PBS ORAL)(n=12). Atherogenesis is expressed as the area of atheromatous lesions in the aortic sinus 8 weeks after the last oral or nasal exposure;

FIG. 6 is a graphic representation demonstrating suppression of immune reactivity to atheroslerotic plaque antigens induced by oral exposure to synthetic oxidized phospholipids L-ALLE and POVPC. 6 week old male apo-E mice were fed either 1mg/mouse L-ALLE (L-ALLE)(n=2) or POVPC (POVPC)(n=3) in 0.2 ml PBS; or PBS alone (CONTROL)(n=3) every other day for 5 days. One week following the last feeding the mice were sensitized with a single subcutaneous injection of 50 μg Human oxidized LDL antigen. 7 days later T-cells from inguinal lymph node were prepared as described in Materials and Methods section that follows, and exposed to the sensitizing Human ox-LDL antigen for in-vitro assessment of proliferation. Proliferation, indicating immune reactivity, is expressed as the ratio between incorporation of labeled thymidine into the T-cell's DNA in the presence and absence of human ox-LDL antigen (stimulation index, S.I.).

FIG. 7 is a graphic representation demonstrating inhibition of progression of late-stage atherogenesis in apoE-deficient mice by oral tolerance induced by synthetic oxidized phospholipids D-ALLE, L-ALLE or POVPC. 24.5 week old apo-E mice were fed 1mg/mouse L-ALLE (L-ALLE)(n=11), D-ALLE (D-ALLE)(n=9) or POVPC (POVPC)(n=10) every other day for 5 days, at 4 week intervals over a 12 week period. Control mice were fed an identical volume (0.2 ml) and regimen of PBS (CONTROL)(n=10). Atherogenesis is expressed as the area of atheromatous lesions in the aortic sinus 12 weeks after the first feeding, as compared to the lesion scores of untreated 24.5 week old mice before feeding (Time 0);

FIG. 8 is a graphic representation demonstrating reduction of triglyceride content of VLDL in 24.5 week old apoE-deficient mice induced by feeding synthetic oxidized phospholipids D-ALLE, L-ALLE or POVPC. 24.5 week old apo-E mice were fed 1mg/mouse L-ALLE (triangle) (n=11), D-ALLE (inverted triangle) (n=9) or POVPC (square) (n=10) every other day for 5 days, at 4 week intervals over a 12 week period. Control mice were fed an identical volume (0.2 ml) and regimen of PBS (circle) (n=10). Triglyceride content (Tg, mg/ml) was measured by enzymatic colorimetric method in the VLDL fractions following separation of pooled blood samples on FPLC, as described in the materials and methods section that follows;

FIG. 9 is a graphic representation demonstrating reduction of cholesterol content of VLDL in 24.5 week old apoE-deficient mice induced by feeding synthetic oxidized phospholipids D-ALLE, L-ALLE or POVPC. 24.5 week old apo-E mice were fed 1mg/mouse L-ALLE (triangle) (n=11), D-ALLE (inverted triangle) (n=9) or POVPC (square) (n=10) every other day for 5 days, at 4 week intervals over a 12 week period. Control mice were fed an identical volume (0.2 ml) and regimen of PBS (circle) (n=10). Cholesterol content (Cholesterol, mg/ml) was measured by enzymatic colorimetric method in the VLDL fractions following separation of pooled blood samples on FPLC, as described in the materials and methods section that follows.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of methods and compositions employing synthetic oxidized phospholipids effective in inducing mucosal tolerance and inhibiting inflammatory processes contributing to atheromatous vascular disease and sequalae.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the

details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Experimental and clinical evidence indicates a causative role for oxidized LDL and LDL components in the etiology of the excessive inflammatory response in atherosclerosis. Both cellular and humoral immune reactivity to plaque related oxidized LDL have been demonstrated, suggesting an important anti-oxidized LDL auto-immune component in atherogenesis. Thus, oxidized LDL and components thereof, have been the targets of numerous therapies for prevention and treatment of heart disease, cerebral-vascular disease and peripheral vascular disease.

Although the prior art teaches that oral administration of LDL can result in 30% reduction in atherogenesis, such a protective effect was observed following administration of a crude antigen preparation consisting of centrifuged, filtered and purified human serum LDL which had been subjected to a lengthy oxidation process with Cu^{++}. Although significant inhibition of atherogenesis was achieved, presumably via oral tolerance, no identification of specific lipid antigens or immunogenic LDL components was made. Another obstacle encountered was the inherent instability of the crude oxidized LDL in vivo, due to enzymatic activity and uptake of oxidized LDL by the liver and cellular immune mechanisms.

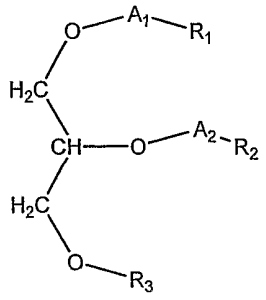
While reducing the present invention to practice, the present inventors have uncovered that administration of well-defined synthetic oxidized LDL derivative can induce immune tolerance to oxidized LDL and thus inhibit atherogenesis, while avoiding the abovementioned limitations.

Thus, according to one aspect of the present invention there is provided a method of inducing immune tolerance to oxidized LDL in a subject such as a human being. Such immune tolerance can be used in the prevention and/or

treatment of disorders associated with plaque formation, including but not limited to atherosclerosis, atherosclerotic cardiovascular disease, cerebrovascular disease, peripheral vascular disease, stenosis, restenosis and in-stent-stenosis. Some non-limiting examples of atherosclerotic cardiovascular disease are myocardial infarction, coronary arterial disease, acute coronary syndromes, congestive heart failure, angina pectoris and myocardial ischemia. Some non-limiting examples of peripheral vascular disease are gangrene, diabetic vasculopathy, ischemic bowel disease, thrombosis, diabetic retinopathy and diabetic nephropathy. Non-limiting examples of cerebrovascular disease are stroke, cerebrovascular inflammation, cerebral hemorrhage and vertebral arterial insufficiency. Stenosis is occlusive disease of the vasculature, commonly caused by atheromatous plaque and enhanced platelet activity, most critically affecting the coronary vasculature. Restenosis is the progressive re-occlusion often following reduction of occlusions in stenotic vasculature. In cases where patency of the vasculature requires the mechanical support of a stent, in-stent-stenosis may occur, re-occluding the treated vessel.

As is further detailed in the Examples section which follows, the method, according to this aspect of the present invention is effected by administering to the subject a therapeutically effective amount of a synthetic ester derivatives of oxidized phospholipid (hereinafter also referred to as an esterified oxidized phospholipid). Non-limiting examples of ester derivatives of oxidized phospholipids suitable for use by the present method include POVPC (see Examples section below for full formula), POVPC, PGPC (see Watson, AD et al J. Biol. Chem. 1997 272:13597-607) and derivatives having the general formula:

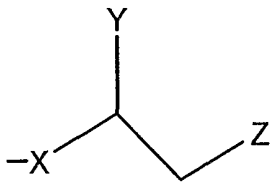
27



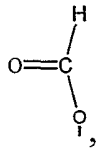
wherein:

(i) A_1 and A_2 are C=O and

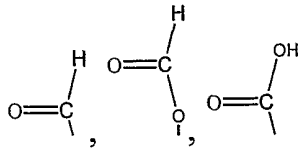
(ii) R_1 or R_2 are each independently selected from the group consisting of an alkyl chain 1-27 carbons in length;



wherein X is C_{1-24}, Y is selected from the group consisting of:

 , -OH, -H, alkyl, alkoxy, halogen, acetoxy and aromatic functional groups; and

Z is selected from the group consisting of:

 and -OH; and

(iii) R_3 is selected from the group consisting of H, acyl, alkyl, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl cardiolipin and phosphatidyl inisitol.

As used herein, the term "C_{1-n}" is defined as a carbon backbone consisting of a carbon atom covalently bonded to a chain of n-1 carbons.

In a further embodiment of the present invention, R_3 is selected from a group consisting of non-phosphatidyl moieties, and as such the resultant compound is not a phospholipid, rather a diglyceride compound. Such diglyceride compounds retain similar structure characteristics and as such in all probability would posses antigenicity and immune tolerizing activity. Thus, these compounds can also be used in prevention and/or treatment of atherosclerosis related disorders, and employed and applied similarly to the oxidized phospholipid derivatives described herein.

Recently, phospholipids and phospholipid metabolites have been clearly implicated in the pathogenesis, and therefore potential treatment of additional, non-atherosclerosis-related diseases. Such diseases and syndromes include oxidative stress of aging (Onorato JM, et al, Annal N Y Acad Sci 1998 Nov 20;854:277-90), rheumatoid arthritis (RA)(Paimela L, et al. Ann Rheum Dis 1996 Aug;55(8):558-9), juvenile rheumatoid arthritis (Savolainen A, et al, 1995;24(4):209-11), inflammatory bowel disease (IBD)(Sawai T, et al, Pediatr Surg Int 2001 May;17(4):269-74) and renal cancer (Noguchi S, et al, Biochem Biophys Res Commun 1992 Jan 31;182(2):544-50). This, the method of the invention may be used for prevention and/or treatment of non-atherosclerosis related diseases such as aging, RA, juvenile RA, IBD and cancer.

The synthetic esterified oxidized phospholipids can be synthesized using prior art approaches (Ou Z., Ogamo A., Guo L., Konda Y., Harigaya Y. and Nakagawa Y. Anal. Biochem. 227: 289-294, 1995).

Alternatively and preferably, the synthetic ester derivatives of an oxidized phospholipid utilized by this aspect of the present invention are synthesized using a novel synthesis method.

Thus, according to another aspect of the present invention there is provided a method of synthesizing an esterified oxidized phospholipid. The method is effected by first providing a phospholipid backbone including two fatty acid

side chains, at least one of the fatty acid side chains being a mono-unsaturated fatty (preferably C_{2-15}) acid followed by oxidizing the double bond of the mono-unsaturated fatty acid thereby generating the esterified oxidized phospholipid.

Examples of phospholipid backbones suitable for synthesis of an esterified oxidized phospholipid according to the teachings of the present invention include, but are not limited to lecithin, which includes two fatty acid side chains, and lysolecithin which includes a single side chain and as such must undergo an additional synthesis step of adding an additional fatty acid side chain prior to oxidation.

When utilized to synthesize POVPC, the phospholipid backbone includes 5-hexenoic acid as the mono-unsaturated fatty acid side chain.

Such a novel synthesis approach provides several advantages over prior art synthesis approaches. In this synthetic method, a defined mono unsaturated acid of desired length and structure is reacted with a molecule having lysolecithin backbone to give monounsaturated phospholipids which is then oxidize at the desired double bond.

The advantages of such a novel approach is in its specificity and simplicity. Oxidizing mono-unsaturated phospholipids having lecithin backbone results in a single, desired specific product and therefore commercial product work up and purification is made much more efficient.

Such a reaction provides specific, desired oxidized phospholipids, traversing the need to perform complicated separations and purification.

Furthermore using this method it is possible to design and synthesize oxidized phospholipids by oxidation of mono-unsaturated phospholipids with a double bond at the end of the chain, enabling the use of substantially short unsaturated acid chains in the synthetic process. Such mono unsaturated short acid chains are relatively inexpensive, and thus reducing the costs associated with synthesis. As such, the synthesis method of the present invention could therefore be conveniently adapted for large-scale manufacturing processes.

A detailed description of synthesis of an esterified oxidized phospholipid according to the teachings of the present invention is provided in the Examples section which follows.

As is clearly shown in the Examples section, immune tolerance can also be established by using an oxidized phospholipid ether (hereinafter also referred to as an etherified oxidized phospholipid).

Thus, according to another aspect of the present invention there is provided a method of inducing immune tolerance to oxidized LDL (and thus treating atherosclerosis and other related disorders) which method is effected by administering to a subject an oxidized phospholipid ether.

One example of an oxidized phospholipid ether utilizable by this aspect of the present invention is ALLE [(preferably a mixture of 1-hexadecyl-2-(5'-oxo-pentanyl)-*sn*-glycero-3-phosphocholine ether (D-ALLE) and 3-hexadecyl-2-(5'-oxo-pentanyl)-*sn*-glycero-1-phosphocholine ether (L-ALLE)], the synthesis and use of which are further detailed in the Examples section which follows.

The immune tolerance inducing compounds described herein can be administered *per se*, or in a pharmaceutical composition where it is mixed with suitable carriers or excipients.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term "active ingredient" refers to the compounds (e.g., POVPC or ALLE) accountable for the biological effect.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism

and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, inrtaperitoneal, intranasal, or intraocular injections.

Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically

compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the

active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuos infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty

acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The pharmaceutical composition of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of a disorder (e.g., atherosclerosis) or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from *in vitro* and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals. The data obtained from these *in vitro*

and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, *et al.*, 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

Dosage amount and interval may be adjusted individually to provide plasma or brain levels of the active ingredient are sufficient to induce or suppress angiogenesis (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions of the present invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling

approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as if further detailed above.

The present invention illustrates for the first time that synthetic derivatives of oxidized phospholipids can be used to prevent and treat atherosclerosis in a subject, while being devoid of limitations inherent to treatments utilizing biologically derived forms of oxidized LDL.

The present invention also provides a novel approach for synthesizing esterified oxidized phopholipids. The present invention also provides a novel form of an oxidized phospholipid ether, utilizable for treatment of atherosclerosis and related disorders.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include biochemical and immunological techniques. Such techniques are thoroughly explained in the literature. See, for example, "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th

Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; and "Methods in Enzymology" Vol. 1-317, Academic Press; Marshak et al., all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

General Materials and Methods

Animals

Apo-E deficient mice used in our experiments are from the atherosclerosis prone strain C57BL/6J-Apoe^{tm1unc}. Mice homozygous for the Apoe^{tm1unc} mutations show a marked increase in total plasma cholesterol levels which is unaffected by age or sex. Fatty streaks in the proximal aorta are found at 3 months of age. The lesions increase with age and progress to lesions with less lipid but more elongated cells, typical of a more advanced stage of pre-atherosclerotic lesion.

Strain Development: The Apoe^{tm1unc} mutant strain was developed in the laboratory of Dr. Nobuyo Maeda at University of North Carolina at Chapel Hill. The 129-derived E14Tg2a ES cell line was used. The plasmid used is designated as pNMC109 and the founder line is T-89. The C57BL/6J strain was produced by backcrossing the Apoe^{tm1unc} mutation 10 times to C57BL/6J mice (11,12).

The mice were maintained at the Sheba Hospital Animal Facility (Tel-Hashomer, Israel) on a 12-hour light/dark cycle, at 22-24°C and fed a

normal fat diet of laboratory chow (Purina Rodent Laboratory Chow No. 5001) containing 0.027% cholesterol, approximately 4.5% total fat, and water, ad libitum.

Immunization:

I. Intraperitioneal immunization with ALLE: The phospholipid ether analog (ALLE D+L) was coupled to purified protein derivative from tuberculin (PPD) at a ratio of 1.6/1. The stock solution of ALLE (D+L) was dissolved in ethanol (99 mg/ml). 5 mg ALLE (D+L), 50.5µl from stock solution was diluted to 5mg/ml with 0.25M phosphate buffer, pH 7.2, by stirring on ice. 1.5 mg of D- and L- ALLE in 300µl of phosphate buffer were added to 0.6 mg PPD dissolved in 300µl of phosphate buffer. 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimid-HCl (5 mg; Sigma, St.Louis, MO) dissolved in 50µl of water was added by stirring at 4° for 20 min. The remaining active sites were blocked with 100µl of 1M glycine. Coupled compounds were dialyzed against phosphate-buffered saline (PBS), adjusted to 3ml with PBS and stored at 4°. Immunization with 0.3ml (150µg) antigen per mouse was performed intraperitoneally 4 times every 2 weeks.

II. Subcutaneous immunization with Human oxidized LDL: Human oxidized LDL was prepared from human plasma pool (d-1.019 to 1.063g/ml by ultracentrifugation) and was Cu-oxidized overnight (by adding 15µl 1mM $CuSO_4$ to each ml of LDL previously diluted to 1mg/ml concentration). The oxidized LDL was dialyzed against saline-Tris-EDTA and filtrated. For immunization, oxidized LDL was dissolved in PBS and mixed with equal volumes of Freund's incomplete adjuvant. Immunizations were performed by single subcutaneous injection of 50µg antigen/mouse in 0.2ml volume. One to three days following the last oral administration the mice received one immunization, and were sacrificed 7-10 days post immunization.

Cholesterol Level Determination: At the completion of the experiment, 1-1.5 ml of blood was obtained by cardiac puncture, 1000U/ml

heparin was added to each sample and total plasma cholesterol levels were determined using an automated enzymatic technique (Boehringer Mannheim, Germany).

FPLC Analysis: Fast Protein Liquid Chromatography analysis of cholesterol and lipid content of lipoproteins was performed using Superose 6 HR 10/30 column (Amersham Pharmacia Biotech, Inc, Peapack, NJ) on a FPLC system (Pharmacia LKB. FRAC-200, Pharmacia, Peapack, NJ). A minimum sample volume of 300 µl (blood pooled from 3 mice was diluted 1:2 and filtered before loading) was required in the sampling vial for the automatic sampler to completely fill the 200 µl sample loop. Fractions 10-40 were collected, each fraction contained 0.5 ml. A 250 µl sample from each fraction was mixed with freshly prepared cholesterol reagent or triglyceride reagent respectively, incubated for 5 minutes at 37°C and assayed spectrophotometrically at 500nm.

Assessment of Atherosclerosis: Quantification of atherosclerotic fatty streak lesions was done by calculating the lesion size in the aortic sinus as previously described (16) and by calculating the lesion size in the aorta. Briefly, after perfusion with saline Tris EDTA, the heart and the aorta were removed from the animals and the peripheral fat cleaned carefully. The upper section of the heart was embedded in OCT medium (10.24% w/w polyvinyl alcohol; 4.26% w/w polyethylene glycol; 85.50% w/w nonreactive ingredients) and frozen. Every other section (10 µm thick) throughout the aortic sinus (400 µm) was taken for analysis. The distal portion of the aortic sinus was recognized by the three valve cusps that are the junctions of the aorta to the heart. Sections were evaluated for fatty streak lesions after staining with oil-red O. Lesion areas per section were scored on a grid (17) by an observer counting unidentified, numbered specimens. The aorta was dissected from the heart and surrounding adventitious tissue was removed. Fixation of the aorta and Sudan staining of the vessels were performed as previously described (21).

Proliferation assays: Mice were fed ALLE, POVPC or PBS as described for assessment of atherosclerosis, and then immunized one day following the last feeding with oxidized LDL prepared from purified human LDL as described above.

Proliferation was assayed eight days after immunization with the oxidized LDL as follows: Spleens or lymph nodes were prepared by meshing the tissues on 100 mesh screens. (Lymph nodes where immunization was performed, and spleens where no immunization performed). Red blood cells were lysed with cold sterile double distilled water (6ml) for 30 seconds and 2ml of NaCl 3.5% was added. Incomplete medium was added (10ml), cells were centrifuge for 7 min at 1,700 rpm, resuspended in RPMI medium and counted in a haemocytometer at 1:20 dilution (10μl cells + 190μl Trypan Blue). Proliferation was measured by the incorporation of [^{3}H] Thymidine into DNA in triplicate samples of 100μl of the packed cells (2,5'x10^6 cells/ml) in a 96 well microtiter plate. Triplicate samples of oxidized LDL (0-10μg/ml, 100μl/well) were added, cells incubated for 72 hours (37°C, 5% CO_2 and ~98% humidity) and 10μl ^{3}[H] Thymidine (0.5μCi/well) was added. After an additional day of incubation the cells were harvested and transferred to glass fiber filters using a cell harvester (Brandel) and counted using β-counter (Lumitron). For assay of cytokines the supernatant was collected without adding ^{3}[H] Thymidine and assayed by ELISA.

A separate group of mice were fed with ALLE or PBS and immunized with oxidized LDL as described above, one day following the last fed dose. Draining inguinal lymph nodes (taken 8 days after immunization) were collected from 3 mice from each of the groups, for the proliferation studies. 1x10^6 cells per ml were incubated in triplicates for 72 h in 0.2 ml of culture medium in microtiter wells in the presence 10 μg/ml oxidized LDL. Proliferation was measured by the incorporation of [^{3}H] thymidine into DNA during the final 12 h of incubation. The results are expressed as the stimulation

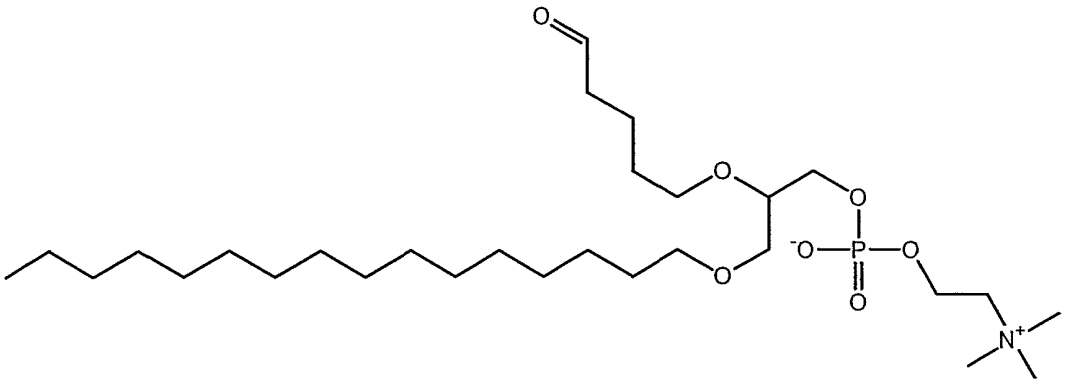
index (S.I.): the ratio of the mean radioactivity (cpm) of the antigen to the mean background (cpm) obtained in the absence of the antigen. Standard deviation was always <10% of the mean cpm.

Statistical Analysis: A one way ANOVA test was used to compare independent values. $p<0.05$ was accepted as statistically significant.

Example I

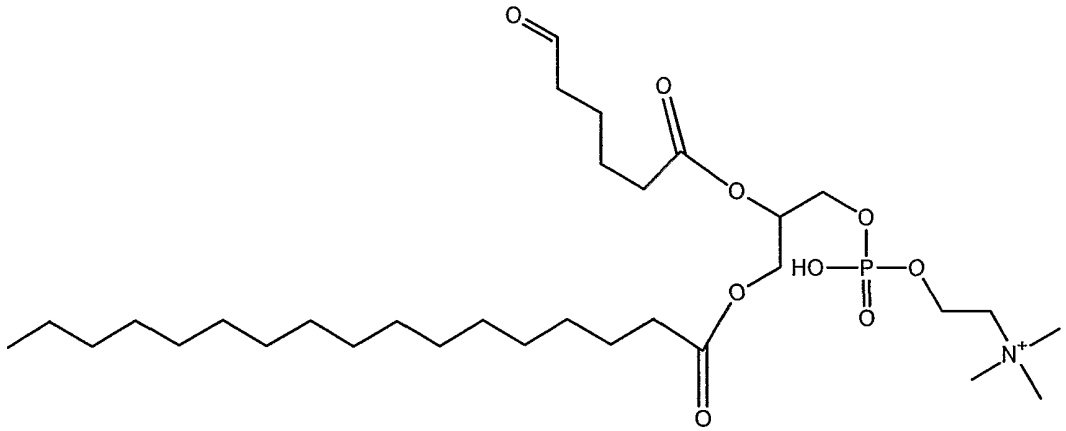
Synthesis of the tolerizing/immunizing antigens 2,5' Aldehyde Lecithin Ether (ALLE) and POVPC

ALLE



1-hexadecyl-2-(5'-oxo-pentanyl)-*sn*-glycero-3-phosphocholine ether

POVPC



1-hexadecanoyl-2-(5'-oxo-pentanoyl)-*sn*-3-phosphocholine

2,5' Aldehyde Lecithin Ether (ALLE) was synthesized according to a modification of general methods for synthesis of ether analogs of lecithins communicated by Eibl H., et al. Ann. Chem. 709:226-230, (1967), W.J. Baumann and H.K. Mangold, J. Org. Chem. 31,498 (1996), E. Baer and Buchnea JBC. 230,447 (1958), Halperin G et al Methods in Enzymology 129,838-846 (1986). The following protocol refers to compounds and processes depicted in 2-D form in Figure 1.

Hexadecyl-glycerol ether : D-Acetone-glycerol (4g) for synthesis of L-ALLE or L-Acetone glycerol for synthesis of D-ALLE, powdered potassium hydroxide (approximately 10g) and hexadecyl bromide (9.3g) in benzene (100ml) were stirred and refluxed for 5 hours, removing the water formed by azeotropic distillation (compare W.J. Baumann and H.K. Mangold, J. Org. Chem. 29: 3055, 1964 and F. Paltauf, Monatsh. 99:1277, 1968). The volume of the solvent was gradually reduced to about 20ml and the cooled mixture was dissolved in ether (100ml). The solution was washed with water (50ml) twice, and the solvent was removed in vacuo. The residue was refluxed in 100ml of methanol:water:concentrated hydrochloric acid 90:10:5 for 10 min. The product was extracted with ether (200ml) washed with water (50ml), 10% sodium hydroxide (20ml) and again with water (volumes of 20ml) until neutral. The solvent was removed in vacuo and the product (8.8g) was crystallized from hexane to give 7.4g of pure 1-hexadecyl-glyceryl ether (compound I, Figure 1) for synthesis of D-ALLE or 3-hexadecyl-glyceryl ether for synthesis of L-ALLE.

5-hexenyl-methane sulfonate: 5-Hexedecenol (1.9ml) and dry pyridine (5ml) were mixed in a culture tube fitted with teflon-protected screw cap and cooled to between –4 and –10 °C in an ice-salt bath. Methanesulfonyl chloride (10ml) was added in portions of 1ml over 60 minutes, and the mixture was kept at 4^0C for 48 hours. Ice (20g) was added, the mixture was allowed to stand for 30 minutes, and the product was extracted with ether (200ml). The organic phase was washed with water (20ml), 10% hydrochloric acid, 10% sodium

bicarbonate (20ml) and again with water (20ml). The solvent was evaporated and the crude product was mixed with silica gel 60 (100g). 14 g of 5-hexenyl-methane sulfonate was eluted with 20% ethyl acetate in chloroform.

1-Hexadecyloxy-3-trityloxy-2-propanol (for D-ALLE) or 3-Hexadecyloxy-1-trityloxy-2-propanol (for L-ALLE) (compound II): 1-Hexadecyloxy-glycerol (for D-ALLE) or 3-Hexadecyloxy-glycerol (for L-ALLE) (7.9g) and triphenylchloromethane (8.4g) and dry pyridine (40ml) were placed in a round-bottom flask fitted with reflux condenser and calcium chloride tube. The mixture was heated in an oil bath at 100°C for 12 hours. After cooling, 300ml of ether and 150ml of ice-cold water were added, and the reaction mixture was transferred to a separatory funnel. The separated ether phase was washed consecutively with 50ml of ice water, 1% potassium carbonate solution (until basic) and 50ml of water, then dried over anhydrous sodium sulfate. A second extraction of the combined aqueous phase increased the yield slightly. The solvent was evaporated, the residue was treated with 150ml of warm petroleum ether and the solution was cooled at 4^0 over night. After separation of 300-400mg of 1-hexadecyl-glyceryl ether (for D-ALLE), or 3-hexadecyl-glyceryl ether (for L-ALLE) (compound I, Figure 1), the filtrate was evaporated and the residue was recrystallized from 20ml of ethyl acetate at -30^0, yielding 8.2g of 1-Hexadecyloxy-3-trityloxy-2-propanol (for D-ALLE) or 3-hexadecyloxy-1-trityloxy-2-propanol(compound II, Figure 1)(for L-ALLE), melting point 49°C.

1-hexadecyl-2-(5'-hexenyl)-glyceryl ether (for D-ALLE) or 3-hexadecyl-2-(5'-hexenyl)-glyceryl ether (for L-ALLE) (compound IV): 1-Hexadecyloxy-3-trityloxy-2-propanol (for D-ALLE) or 3-Hexadecyloxy-1-trityloxy-2-propanol (for L-ALLE) (compound II, Figure 1) (5.5g) was etherified with 5-hexenyl-methanesulfonate with powdered potassium hydroxide in benzene solution as described above. The crude product 1-Hexadecyloxy-2-(5'hexenyloxy)-sn-3-trityloxy-propane (for D-ALLE) or 3-Hexadecyloxy-2-(5'hexenyloxy)-sn-3-trityloxy-propane (for L-ALLE)

(compound III, Figure 1)was dissolved in 100ml of methanol:water:concentrated Hydrochloric acid 90:10:5 and the mixture was refluxed for 6 hours. The product was extracted with ether, washed with water and the solvent was removed. The residue was dissolved in petroleum ether (100ml) and was kept in 4^0C overnight, causing most of the triphenyl carbinol to be deposited. The remaining product was chromatographed on silica gel 60 (40g). The pure 1-hexadecyl-2-(5'-hexenyl)-glyceryl ether (for D-ALLE) or 3-hexadecyl-2-(5'-hexenyl)-glyceryl ether (for L-ALLE) (compound IV, Figure 1) (1.8g) was eluted with 50% chloroform in petroleum ether.

1-Hexadecyl-2-(5'-hexenyl)-sn-glycero-3-phosphocholine (for D-ALLE) or 3-Hexadecyl-2-(5'-hexenyl)-sn-glycero-1-phosphocholine (for L-ALLE) (compound V): The following portion of the procedure is a modification of the method communicated by Eibl H., et al. Ann. Chem. 709:226-230, 1967.

A solution of 1-Hexadecyl-2-hexenyl-glyceryl ether (for D-ALLE) or 3-Hexadecyl-2-hexenyl-glyceryl ether (for L-ALLE) (compound IV, Figure 1) (2g) in dry chloroform (15ml) was added dropwise with stirring into a solution of dry triethylamine (3ml) and 2-bromoethyl dichlorophosphate (1.25ml) in dry chloroform (15ml) during 15minutes at –4 to -10°C in an ice-bath. The mixture was kept at room temperature for 6 hours and then for 12 hours at 40^0C. The dark brown solution was cooled to 0^0 and 0.1M potassium chloride (15ml) was added. The mixture was stirred for 60 minutes, methanol (25ml) and chloroform (50ml) were added and the organic phase was washed with 0.1M hydrochloric acid (20ml) and water (20ml). The solvent was evaporated and the crude product in a culture tube was dissolved in methanol (15ml) in an ice-salt bath. Cold trimethylamine (3 ml, -76^0C) was added and the tube was stoppered with a screw cap. The mixture was kept at 55°C for 12 hours and the solvent evaporated with a stream of nitrogen. The residue was extracted with chloroform:methanol 2:1 (25ml) and washed with 1M potassium carbonate (10ml) and twice with water (10ml). The solvent was removed in vacuo and the

products separated by chromatography on silica gel 60 (20g). Elution with 40% methanol in chloroform yielded 1.5g of 1-hexadcyl-2-(5'-hexenyl)-*sn*-glycero-3-phosphocholine (for D-ALLE) or 3-hexadcyl-2 -(5'-hexenyl)-*sn*-glycero-1-phosphocholine (for L-ALLE) (compound V, Figure 1). The structure of compound V was confirmed by NMR and mass spectrometry.

1-Hexadecyl-2-(5'-oxo-pentanyl)-sn-glycero-3-phosphocholine (for D-ALLE) or 3-Hexadecyl-2-(5'-oxo-pentanyl)-sn-glycero-1-phosphocholine (for L-ALLE) (compound VI):

Compound V (0.5g) in formic acid (15ml) and 30% hydrogen peroxide (3.5ml) was kept at room temperature over night. The product was extracted with chloroform:methanol 2:1 (50ml) and the chloroform extract was washed with 10% sodium carbonate (10ml) and water (10ml). The solvent was evaporated in vacuo and the residue (0.4g) mixed with methanol (10ml) and 10% sodium hydroxide (4 ml), then kept for 60 minutes at room temp. 80% phosphoric acid (2ml) and potassium meta periodate (0.8g) was added. The mixture was kept at room temperature overnight and chloroform:methanol 2:1 (50ml) was added. The organic phase was washed with 10% sodium bisulfite (10ml) and water (10ml). The solvent was removed in vacuo and the product (0.3g) was chromatograph on silica gel 60 (10g). The compound 1-hexadecyl-2-(5'-oxo-pentanyl)-*sn*-glycero-3-phosphocholine (for D-ALLE) or 3-hexadecyl-2-(5'-oxo-pentanyl)-*sn*-glycero-1-phosphocholine (for L-ALLE) (compound VI, Figure 1) (249mg) was eluted with chloroform methanol 1:1; exhibiting an R_f of 0.15 (TLC system, chloroform:methanol:water 60:40:8) and a positive reaction with dinitrophenylhydrazine. The chemical structure was confirmed by NMR and mass spectrometry. In an alternative process, the ethylenic group was converted to an aldehyde group by ozonization and catalytic hydrogenation with palladium calcium carbonate.

Preparation of 2-bromoethyl dichlorophosphate

2-Bromoethyl dichlorophosphate was prepared by dropwise addition of freshly distilled 2-bromoethanol (0.5M, prepared as described in Gilman Org. Synth. 12:117, 1926) to an ice-cooled solution of freshly distilled phosphorous oxychloride (0.5M) in dry chloroform over a one hour period, followed by 5 hours reflux and vacuum distillation (bp 66-68^0 at 0.4-0.5mm Hg). The reagent was stored (-20^0) under nitrogen in small sealed ampoules prior to use (Hansen W.H et al. Lipids 17(6):453-459, 1982).

1-Hexadecanoyl-2-(5'-oxo)-pentanoyl-sn-3-glycerophosphocholine (POVPC)

A mixture of 1-Hexadecanoyl-sn-3-glycerophosphocholine (compound I, Figure 2) (L-α-palmitoyl-lysophosphatidylcholine) (3g), 5-hexenoic acid (1.2ml), 1,3-dicyclohexylcarbodiamide (DCC, 4.05g) and dimethylaminopyridine (DMP,1.6g) in dichloromethane (100ml, freshly distilled from phosphorus pentoxide) was thoroughly stirred for 4 days at room temperature. The mixture was directly chromatographed on silica gel 60 (40g) and the product 1-hexadecanoyl-2-(5'-hexenoyl)-sn-3-glycerophosphocholine (compound II, Figure 2) was eluted with chloroform:methanol 25:75 (2.8g). The eluent was dissolved in 30% hydrogen peroxide:formic acid 4:15 and kept overnight at room temperature. Water (50ml) was added and the product was extracted with chloroform:methanol 2:1 (100ml) and the organic phase was washed again with water. The solvent was evaporated under vacuum, the residue was dissolved in methanol (15ml) and 10% ammonia solution (5ml) and kept for 6 hours at room temperature. The crude 1-hexadecanoyl-2-(5',6'-dihydroxy)-hexanoyl-sn-3-glycerophosphocholine (compound III, Figure 2) (structure confirmed by NMR and mass spectrometry) was not extracted. 80% phosphoric acid (3ml) and sodium metaperiodate (1g) were added to the solution and the mixture was kept overnight at room temperature.

Extraction with chloroform-methanol (2:1), chromatography on silica gel 60 (20g) and elution with chloroform-methanol 25:75 yielded 850 mg of 1-hexadecanoyl-2-(5'-oxopentanoyl)-sn-3-glycerophosphocholine (POVPC, compound IV, Figure 2) with chromatographic mobility of lecithin on TLC, and positive dinitrophenyl hydrazine reaction. The structure was assessed by NMR and mass spectrometry. Alternatively: the ethylenic group was converted to an aldehyde by ozonization and catalytic hydrogenation with palladium calcium carbonate.

Example II

Immunization against L-ALLE + D-ALLE specifically inhibits atherogenesis in genetically disposed (apoE-deficient) mice.

The present inventors have demonstrated that immunization with the stable, etherified synthetic LDL component ALLE can reduce the extent of atherosclerotic plaque formation in susceptible mice. 19 female 5-7 weeks old Apo E/C 57 mice were divided into 3 groups. In group A (n=6) the mice were immunized intreperitoneally, as described in Materials and Methods section above, with 150 μg/mouse L-ALLE + D-ALLE once every 2 weeks (0.3 ml/mouse) for 8 weeks. In group B (n=6) the mice were immunized with tuberculin toxin Purified Protein Derivative (PPD) once every 2 weeks (0.3 ml/mouse). In group C (n=7) the mice received no immunization. Mice from all three groups were bled prior to immunization (Time 0), and at one week after the second immunization for determination of anti-ox LDL antibodies, anti-ALLE antibodies and lipid profile. Mice in which the antibody response did not plateau were bled at successive 2 week intervals until plateau was reached. Atherosclerosis assessment was performed as described above, and spleens collected 6 weeks after the fourth immunization. The mice from all groups were weighed at 2 week intervals throughout the experiment. All mice were fed normal chow-diet containing 4.5% fat by weight (0.02% cholesterol) and water ad libitum.

Table I: Immunization of apoE mice with ALLE inhibits atherogenesis

Groups		*100µg/Mouse L-ALLE + D-ALLE immunization N=6*	*PPD N=5*	Control without immunization *N=7*	Statistics
Time 0	Weight	17.3±0.5	17.3±0.7	17.8±0.4	P=0.780
	Chol	435.3±47.2	436.3±49.4	412.7±44.1	P=0.919
	TG	118±9.2	111.6±10.3	120.4±14.2	P=0.865
End	Weight	20.500±0.458	21.620±0.213	20.300±0.49	P=0.123
	Chol	299.400±17.6	293.760±15.1	303.714±21.6	P=0.937
	TG	57.267±3.286	53.020±4.416	66.057±3.76	P=0.075
	Lesion size (μm^2)	101000±8276	179500±13449	210833±26714	P=0.005
	TGF-β pmol/ml	12032±2308	13963±944	12825±2340	P=0.831

As can be seen in Figure 3, the results depicted in Table I demonstrate the significant reduction in atheromatous lesions measured in the heart tissues of the ALLE immunized mice, compared to both PPD and unimmunized control mice. No significant effect is apparent on other parameters measured, such as weight gain, triglyceride or cholesterol blood levels, or immune competence, as measured by the levels of the immunosuppressive cytokine TGF-β. Thus, immunization with the synthetic oxidized LDL component ALLE (a mixture of racemic forms D- and L-) confers significant (>50%) protection from atherosclerotic lesion formation in these genetically susceptible apoE-deficient mice. A significant but less dramatic reduction in plaquing was observed in mice immunized with PPD.

Example III

Inhibition of atherogenesis in genetically predisposed (apoE-deficient) mice by induction of oral tolerance with L-ALLE and D-ALLE

Intraperitoneal immunization with the ester analogs of plaque lesion components was effective in inhibiting atherogenesis in apoE-deficient mice (Fig 1). Thus, the ability of L- and D- ALLE to suppress atherogenesis through oral tolerance was investigated. 34 male 8-10 week old Apo E/C 57 mice were

divided into three groups. In group A (n=11) oral tolerance was induced by administration by gavage of L-ALLE + D-ALLE suspended in PBS (5 mg/ml, 1 mg/mouse) for 5 days every other day. In group B (n=11) mice received 10 μg/mouse L-ALLE + D-ALLE suspended in PBS for 5 days every other day. (0.2 ml/mouse). Mice in group C (n=12) received PBS (containing the same volume of ethanol as in groups A+B). Mice were bled prior to feeding (Time 0) and at the conclusion of the experiment (End) for determination of lipid profile. Atherosclerosis was assessed in heart, aorta, and serum as described above 8 weeks after the last feeding. Mice were weighed every 2 weeks during the experiment. All mice were fed normal chow-diet containing 4.5% fat by weight (0.02% cholesterol) and water ad libitum.

Table 2: Inhibition of atherogenesis in apoE-deficient mice by oral administration of L-ALLE and D-ALLE

Groups		*PBS* N=12	*1 mg ALLE* N=11	*10 μg ALLE* N=11	Statistics
Time 0	Weight	20.7±0.6	21.5±0.8	21.1±0.8	P=0.794
	Chol	372±25	379±23	378±31	P=0.983
	TG	128	98	90	P=0.829
End	Weight	27.3±0.4	27.4±0.5	24.1±0.8	P<0.001
	Chol	302.8±16.5	248.7±24.2	320.5±15.2	P=0.031
	TG	81.4±4.3	77.6±7.5	93.3±6.2	P=0.146
	Lesion size (μm^2)	176000±13735	85278±11633	103889±14320	P<0.001
	TGF-β pmol/ml	14696±1352	13388±1489	18010±1373	P=0.07

Note: "Weight" is weight in grams; "Chol" is serum cholesterol and "TG" is serum triglycerides, expressed in mg/dL.

As can be seen from Figure 4, the results depicted in Table 2 demonstrate a striking attenuation of atherosclerotic progression measured in the tissues of mice fed low doses (10 μg – 1 mg/ mouse) of ALLE, compared to unexposed control mice. No significant effect is apparent on other parameters measured, such as weight gain, triglyceride or cholesterol blood levels, or immune competence, as measured by the levels of the immunosuppressive

cytokine TGF-β. Thus, the synthetic oxidized LDL component ALLE is a potent inducer of oral tolerance, conferring significant (>50%) protection from atherosclerosis in these genetically susceptible apoE-deficient mice, similar to the protection achieved with peritoneal immunization (see Figure 1).

Example IV

Inhibition of atherogenesis in genetically predisposed (apoE-deficient) mice by induction of oral and nasal tolerance with L-ALLE and D-ALLE

Mechanisms of mucosal tolerance are active in the nasal mucosa as well as the gut. Thus, nasal exposure and oral exposure to L- and D- ALLE were compared for their effectiveness in suppressing atherogenesis in apoE-deficient mice. 34 male 7-9 weeks old Apo E/C 57 mice were divided into 3 groups. In group A (n=11) oral tolerance was induced by administration by gavage of L-ALLE + D-ALLE suspended in PBS (5 mg/ml, 1 mg/mouse) for 5 days every other day. In group B (n=11) nasal tolerance was induced as described in Materials and Methods by administration of 10 μg/mouse/10μl L-ALLE + D-ALLE suspended in PBS every other day for 5 days. Mice in group C (n=12) received PBS administered orally and nasally (containing the same volume of ethanol as in groups A+B). Mice were bled prior to feeding (Time 0) and at the conclusion of the experiment (End) for determination of lipid profile. Atherosclerosis was assessed in heart and aorta as described above, 8 weeks after the last feeding. Mice were weighed every 2 weeks during the experiment. All mice were fed normal chow-diet containing 4.5% fat by weight (0.02% cholesterol) and water ad libitum.

Table 3: Inhibition of atherogenesis in apoE-deficient mice by nasal administration of L-ALLE and D-ALLE

Groups		*1 mg ALLE* **Oral** (N=11)	*10μg ALLE* **Nasal** (N=11)	*PBS* ***Oral*** (N=12)	Statistics
Time 0	Weight	22.1±3.0	21.1±2.5	21.1±0.8	P=0.624
	Chol	353±31	351±27	362±27	P=0.952
	TG	144	143	138	P=0.977

End	Weight	24.2±0.2	23.3±1.1	24.0±0.5	P=0.558
	Chol	418±43	328±18	343±25	P=0.084
	TG	82±7	74±6	79±5	P=0.630
	Lesion size (μm^2)	76944±17072	82708±10986	135455±12472	P=0.007

Table 3, Continued;
Note: "Weight" is weight in grams; "Chol" is serum cholesterol and "TG" is serum triglycerides, expressed in mg/dL.

As can be seen from Figure 5, the results depicted in Table 3 demonstrate effective (as effective as oral tolerance) inhibition of atherogenesis measured in the tissues of mice receiving nasal exposure to low doses (10 μg/ mouse) of ALLE, compared to unexposed control mice. Induction of nasal tolerance, like oral tolerance, had no significant effect on other parameters measured, such as weight gain, triglyceride or cholesterol blood levels, or immune competence, as measured by the levels of the immunosuppressive cytokine TGF-β. Thus, the synthetic oxidized LDL component ALLE is a potent inducer of nasal as well as oral tolerance, conferring significant (approximately 50%) protection from atherogenesis in these genetically susceptible apoE-deficient mice, similar to the protection achieved induction of oral tolerance alone.

Example V

Suppression of specific anti- ox LDL immune reactivity in genetically predisposed (apoE-deficient) mice by oral administration of L-ALLE and POVPC

Tolerance induced by mucosal exposure to oxidized analogs of LDL may be mediated by suppression of specific immune responses to the plaque-related antigens. POVPC (1-Hexadecanoyl-2-(5'-oxo-pentanoyl)-*sn*-glycerophosphocholine) is a non-ether oxidized LDL analog, which, unlike ALLE is susceptible to breakdown in the liver. Lymphocyte proliferation in response to oral exposure to both POVPC and the more stable analog ALLE was measured in apoE-deficient mice. 8 male, 6 week old Apo E/C 57 mice were divided into 3 groups. In group A (n=2) oral tolerance was induced with

1 mg/mouse L-ALLE suspended in 0.2 ml PBS, administered by gavage, as described above, every other day for 5 days. In group B (n=3) oral tolerance was induced with 1 mg/mouse POVPC suspended in 0.2 ml PBS, administered per os as described above, every other day for 5 days. The mice in group C (n=3) received oral administration of 200 µl PBS every other day for 5 days. Immune reactivity was stimulated by immunization with Human oxidized LDL as described above in the Materials and Methods section, one day after the last feeding. One week after the immunization lymph nodes were collected for assay of proliferation. All mice were fed normal chow-diet containing 4.5% fat by weight (0.02% cholesterol) and water ad libitum.

Table 4: Oral pretreatment with synthetic oxidized LDL (ALLE and POVPC) suppresses immune response to Human ox- LDL in apoE-deficient mice

Stimulation Index (SI)		
PBS	POVPC	L-ALLE
33.1±11.5	10.6±4.1	7.3±4.3
N=3	N=3	N=2
	-68%	-78%

As can be seen from Figure 6, the results depicted in Table 4 demonstrate significant suppression of immune reactivity to Human oxidized-LDL antigen, measured by inhibition of proliferation in the lymph nodes of apoE-deficient mice. Lymphocytes from mice receiving oral exposure to atherogenesis-inhibiting doses (1mg/ mouse) of ALLE or POVPC showed a reduced stimulation index following immunization with ox-LDL, as compared to control (PBS) mice. Since induction of oral, like nasal, tolerance had no significant effect on other parameters measured, such as weight gain, triglyceride or cholesterol blood levels, or immune competence (see Tables 1, 2 and 3), these results indicate a specific suppression of anti-ox-LDL immune reactivity. Thus, oral administration of the synthetic oxidized LDL component L-ALLE is an effective method of attenuating the cellular immune response to immunogenic and atherogenic plaque components in these genetically

susceptible apoE-deficient mice. Figure 4 also demonstrates a similar, if less effective inhibition of proliferation with oral administration of the less stable synthetic oxidized LDL component POVPC.

ExampleVI

Inhibition of atherogenesis in genetically predisposed (apoE-deficient) mice by induction of oral tolerance with D- and L- isomers of ALLE, and POVPC

Since feeding of ALLE and POVPC was shown to inhibit early atherogenesis and immune reactivity to plaque-related Human LDL antigen, the ability of both D- and L- isomers of the ether LDL analog, and the non-ether analog POVPC to suppress the progression of atherogenesis in older mice was compared. Their effect on the triglyceride and cholesterol fractions of VLDL was also monitored by FPLC. 40 male, 24.5 week old Apo E/C 57 mice were divided into 4 groups. In group A (n=11) oral tolerance was induced with 1 mg/mouse L-ALLE suspended in 0.2 ml PBS, administered by gavage, as described above, every other day for 5 days. In group B (n=9) oral tolerance was induced with 1 mg/mouse D-ALLE suspended in 0.2 ml PBS, administered per os, as described above, every other day for 5 days. In group C (n=10) oral tolerance was induced with 1 mg/mouse POVPC suspended in 0.2 ml PBS, administered by gavage, as described above, every other day for 5 days. Control group D (n=10) received oral administration of PBS (containing the same volume of ethanol as in groups A,B,C). Oral administration of the tested antigens took place every 4 weeks (5 oral feedings; every other day) starting at 24.5 weeks age, during 12 weeks (3 sets of feedings).

Mice were bled prior to feeding (Time 0), after the 2nd set of feeding and at the conclusion of the experiment (End) for determination of lipid profile, lipid fractionation and plasma collection. Atherosclerosis was assessed as described above in the heart and aorta and spleens collected for proliferation assay 12 weeks after the first feeding. Weight was recorded every 2 weeks

throughout the experiment. All mice were fed normal chow-diet containing 4.5% fat by weight (0.02% cholesterol) and water ad libitum.

Table 5: Inhibition of atherogenesis in apoE-deficient mice by oral administration of L-ALLE, D-ALLE and POVPC

		***PBS* (n=10)**	***1mg L-ALLE* (n=11)**	***1mg D-ALLE* (n=9)**	***1mg POVPC* (n=10)**	*statistics*
0	Weight	28.1±0.5	29±0.6	29.8±0.7	29.6±0.7	P=0.305
	Cholesterol	413±27	413±23	409±28	401±21	P=0.983
	Triglyceride	67±5	63±8	63±4	67±7	P=0.964
End	Weight	28.5±0.6	29.7±0.5	30.4±0.8	29.9±0.5	P=0.177
	Cholesterol	365±15	391±18	394±15	358±28	P=0.481
	Triglyceride	84±4	83±4	94±4	85±3	P=0.207
	Sinus Lesion μm^2	369688± 32570	233056± 12746	245938± 20474	245750± 20423	P<0.001

Note: "Weight" is weight in grams; "Cholesterol" is serum cholesterol and "Triglyceride" is serum triglycerides, expressed in mg/dL.

As can be seen from Figure 7, the results depicted in Table 5 demonstrate effective inhibition of late stage atherogenesis measured in the tissues of older mice following protracted oral exposure to moderately low doses (1mg/ mouse) of the D- and L- isomers of ALLE, and POVPC compared to PBS-fed control mice. Induction of oral tolerance had no significant effect on other parameters measured, such as weight gain, total triglyceride or cholesterol blood levels. Thus, the synthetic oxidized LDL components D-, L-ALLE and POVPC are individually potent inducers of oral tolerance, conferring nearly complete protection from atheromatous progression (as compared with lesion scores at 24.5 weeks) in these genetically susceptible apoE-deficient mice. Surprisingly, it was observed that the inhibition of atherogenesis by these oxidized LDL analogs is accompanied by a significant reduction in VLDL cholesterol and triglycerides, as measured by FPLC (Figures 8 and 9).

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and

variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

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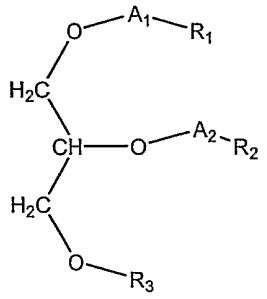
WHAT IS CLAIMED IS:

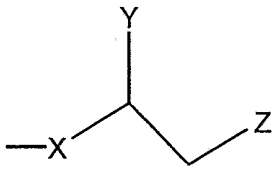
1. A compound having a formula:



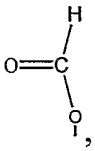
or pharmaceutically acceptable salts thereof, wherein:

(i) A_1 and A_2 are each independently selected from the group consisting of CH_2 and C=O, at least one of A_1 and A_2 being CH_2; and

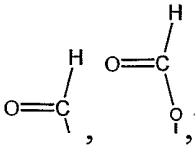
(ii) R_1 and R_2 are each independently selected from the group consisting of an alkyl chain 1-27 carbons in length and



wherein X is a C_{1-24} chain, Y is selected from the group consisting of:

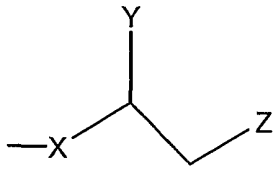
 , -OH,–H,alkyl, alkoxy halogen, acetoxy and aromatic functional groups;and

Z is selected from the group consisting of:

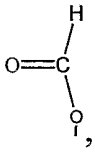
 , and -OH; and

(iii) R_3 is selected from the group consisting of H, acyl, alkyl, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl cardiolipin and phosphatidyl inisitol.

2. The compound of claim 1, wherein at least one of R_1 and R_2 is

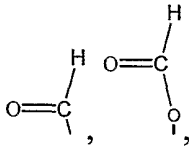


wherein X is a C_{1-24} chain, Y is selected from the group consisting of:



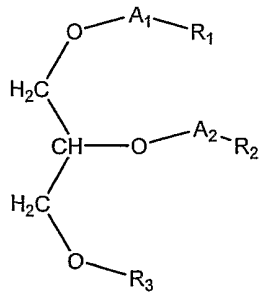
, -OH,–H,alkyl, alkoxy halogen, acetoxy and aromatic functional groups;and

Z is selected from the group consisting of:



, , and –OH.

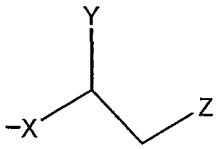
3. A pharmaceutical composition for prevention and/or treatment of atherosclerosis, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, stenosis, restenosis and/or in-stent-stenosis in a subject in need thereof, comprising, as an active ingredient, a therapeutically effective amount of a compound selected from the group having a formula:



or pharmaceutically acceptable salts thereof, wherein:

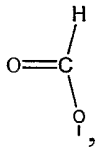
(i) A_1 and A_2 are independently selected from the group consisting of CH_2 and C=O; and

(ii) R_1 or R_2 are each independently selected from the group consisting of an alkyl chain 1-27 carbons in length and:

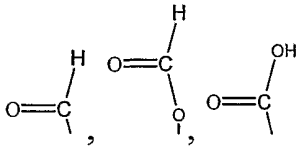


wherein X is C_{1-24}, Y is selected from the group consisting of:

:

 , -OH,–H,alkyl, alkoxy halogen, acetoxy and aromatic functional groups; and

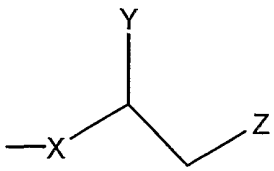
Z is selected from the group consisting of:

 and -OH; and

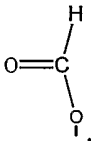
(iii) R_3 is selected from the group consisting of H, acyl, alkyl, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl cardiolipin and phosphatidyl inisitol

and a pharmaceutically acceptable carrier.

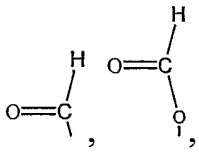
4. The composition of claim 3, wherein at least one of R_1 and R_2 is



wherein X is a C_{1-24} chain, Y is selected from the group consisting of:

 , -OH,–H,alkyl, alkoxy halogen, acetoxy and aromatic functional groups;and

Z is selected from the group consisting of:

 , and –OH.

5. The composition of claim 3, wherein at least one of A_1 and A_2 is CH_2.

6. The composition of claim 3, designed for inducing tolerance to oxidized LDL via mucosal administration.

7. The composition of claim 3, designed for nasal, oral or intra-peritoneal administration.

8. The composition of claim 3, wherein said compound reduces immune reactivity to oxidized LDL in said subject.

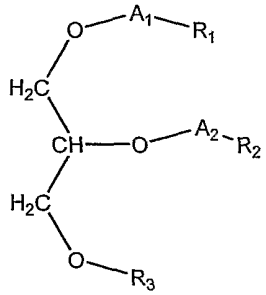
9. The composition of claim 3, packaged and identified for use in the prevention and/or treatment of at least one disorder selected from the group consisting of atherosclerosis, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, stenosis, restenosis and/or in-stent-stenosis.

10. The composition of claim 3, further comprising a therapeutically effective amount of at least one additional compound selected from the group consisting of HMGCoA reductase inhibitors (statins), mucosal adjuvants,

corticosteroids, anti-inflammatory compounds, analgesics, growth factors, toxins, and additional tolerizing antigens.

11. A pharmaceutical composition for prevention and/or treatment of a disease, syndrome or condition selected from the group consisting of atherosclerosis, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, stenosis, restenosis and/or in-stent-stenosis in a subject in need thereof, comprising, as an active ingredient, a therapeutically effective amount of a synthetic LDL derivative, or pharmaceutically acceptable salts thereof, the composition further comprising a pharmaceutically acceptable carrier.

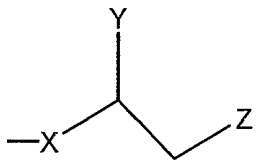
12. A method of prevention and/or treatment of atherosclerosis, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, stenosis, restenosis and/or in-stent-stenosis in a subject in need thereof, the method comprising administering a therapeutically effective amount of a compound, said compound selected from the group having a formula:



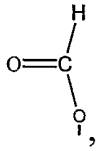
or pharmaceutically acceptable salts thereof, wherein:

(i) A_1 and A_2 are CH_2 or C=O; and

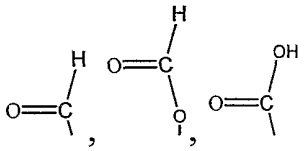
(ii) R_1 or R_2 are each independently selected from the group consisting of an alkyl chain 1-27 carbons in length and:



wherein X is C_{1-24}, Y is selected from the group consisting of:

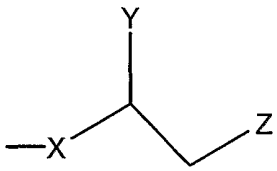
 , -OH,–H,alkyl, alkoxy halogen, acetoxy and aromatic functional groups; and

Z is selected from the group consisting of:

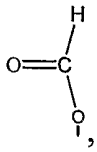
 and -OH; and

(iii) R_3 is selected from the group consisting of H, acyl, alkyl, phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl cardiolipin and phosphatidyl inisitol.

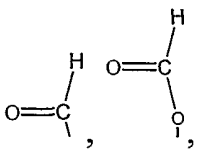
13. The method of claim 12, wherein at least one of R_1 and R_2 is



wherein X is a C_{1-24} chain, Y is selected from the group consisting of:

 , -OH,–H,alkyl, alkoxy halogen, acetoxy and aromatic functional groups;and

Z is selected from the group consisting of:

 , and –OH.

14. The method of claim 12, wherein at least one of A_1 and A_2 is CH_2.

15. The method of claim 12, wherein said compound is administered via mucosal administration.

16. The method of claim 12, wherein administration of said compound is nasal, oral or intra- peritoneal administration.

17. The method of claim 12, wherein administration of said compound reduces immune reactivity to oxidized LDL in said subject.

18. The method of claim 12, wherein said compound is administered in addition to a therapeutically effective amount of at least one additional compound selected from the group consisting of HMGCoA reductase inhibitors (statins),mucosal adjuvants, corticosteroids, anti-inflammatory compounds, analgesics, growth factors, toxins, and additional tolerizing antigens.

19. A method of synthesizing an oxidized phospholipid comprising:

(a) providing a phospholipid backbone including two fatty acid side chains, wherein at least one of said fatty acid side chains is a mono-unsaturated fatty acid C_{2-15}; and

(b) oxidizing the double bond of said mono-unsaturated fatty acid to thereby generate the oxidized phospholipid.

20. The method of claim 19, wherein said phospholipid backbone further includes a moiety selected from the group consisting of H,

phosphatidyl choline, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl cardiolipin and phosphatidyl inisitol.

21. The method of claim 19, wherein said mono unsaturated fatty acid is C_{2-15}.

22. The method of claim 19 wherein the oxidized phospholipid is POVPC, and said mono-unsaturated fatty acid is 5-hexenoic acid.

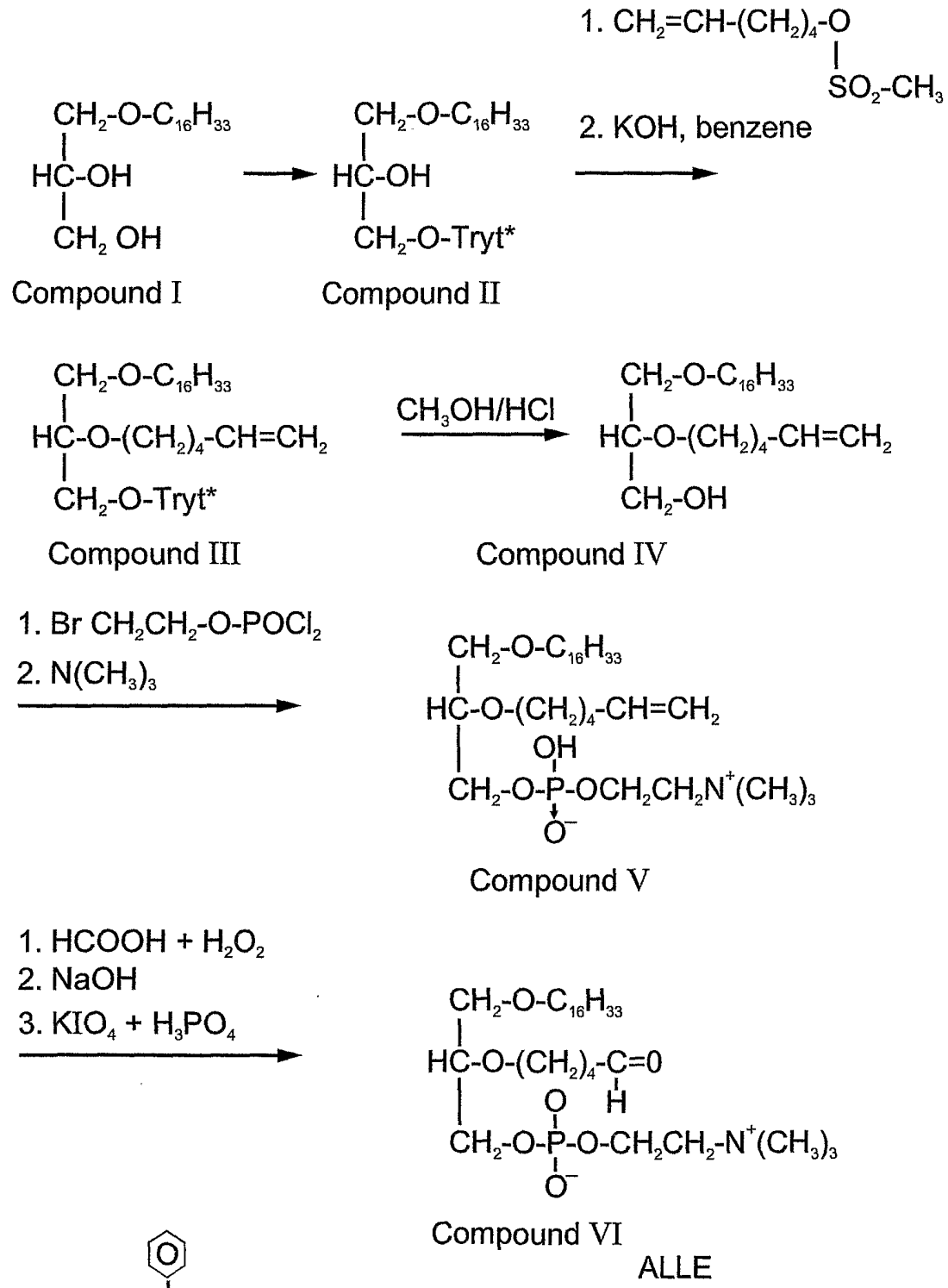
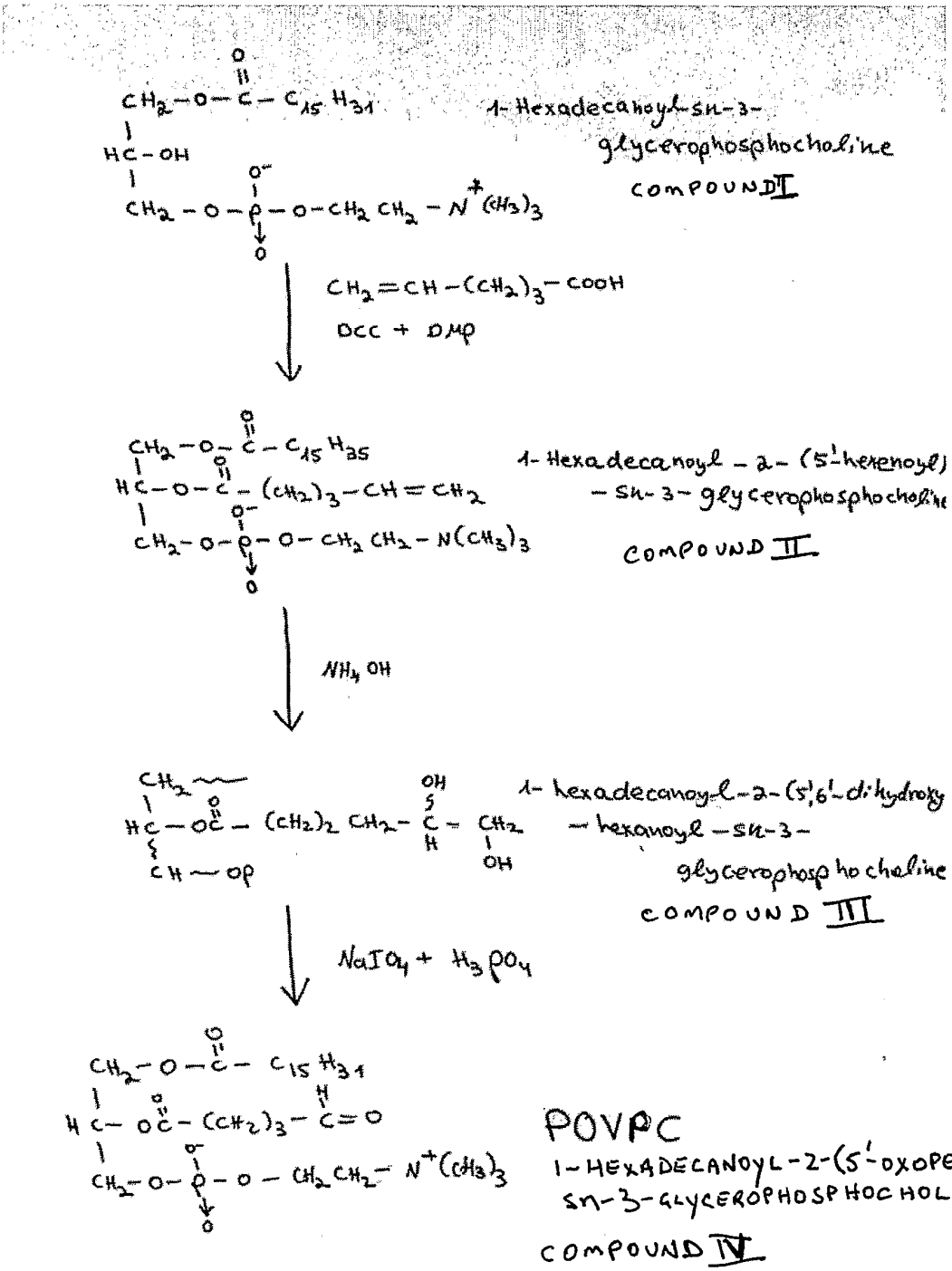

1. CH2=CH-(CH2)4-O
SO2-CH3
2. KOH, benzene
CH2-O-C16H33
HC-OH
CH2 OH
Compound I
CH2-O-Tryt*
Compound II
CH3OH/HCl
HC-O-(CH2)4-CH=CH2
CH2-OH
Compound III
Compound IV
1. Br CH2CH2-O-POCl2
2. N(CH3)3
OH
CH2-O-P-OCH2CH2N+(CH3)3
O−
Compound V
1. HCOOH + H2O2
2. NaOH
3. KIO4 + H3PO4
HC-O-(CH2)4-C=0
H
CH2-O-P-O-CH2CH2-N+(CH3)3
Compound VI
ALLE
* Tryt

Fig. 1

Figure 2: SYNTHESIS OF POVPC



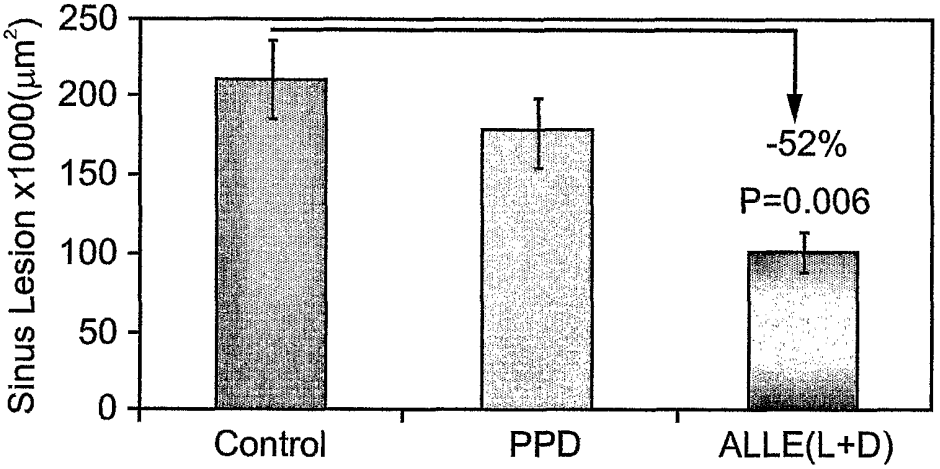

Sinus Lesion x1000(μm²)
250
200
150
100
50
0
-52%
P=0.006
Control
PPD
ALLE(L+D)

Fig. 3

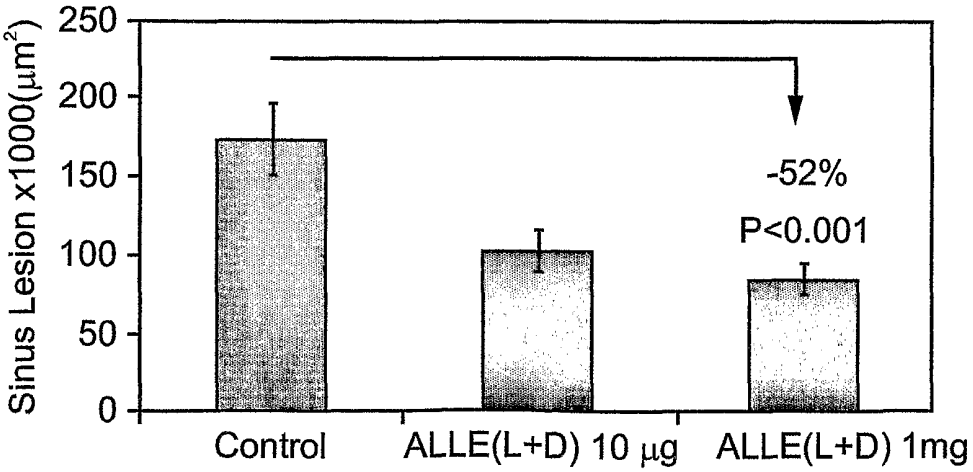

Sinus Lesion x1000(μm²)
250
200
150
100
50
0
-52%
P<0.001
Control
ALLE(L+D) 10 μg
ALLE(L+D) 1mg

Fig. 4

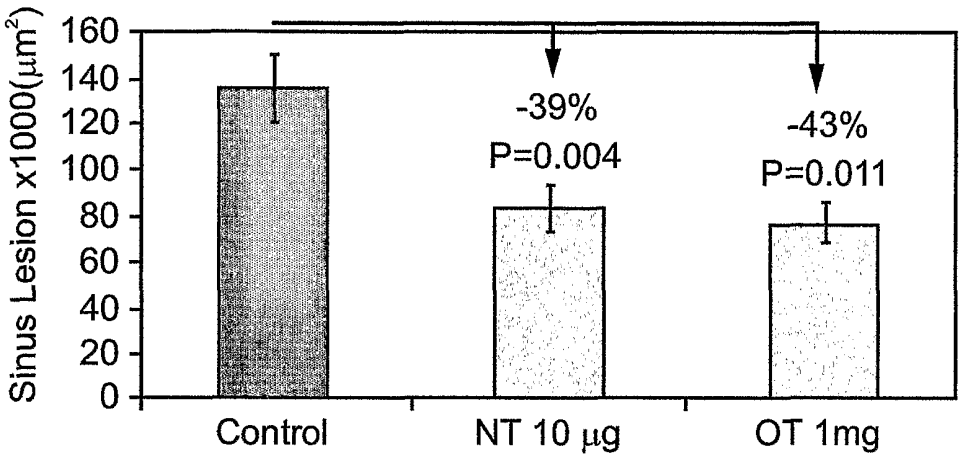

Sinus Lesion x1000(μm²)
160
140
120
100
80
60
40
20
0
-39%
P=0.004
-43%
P=0.011
Control
NT 10 μg
OT 1mg

Fig. 5

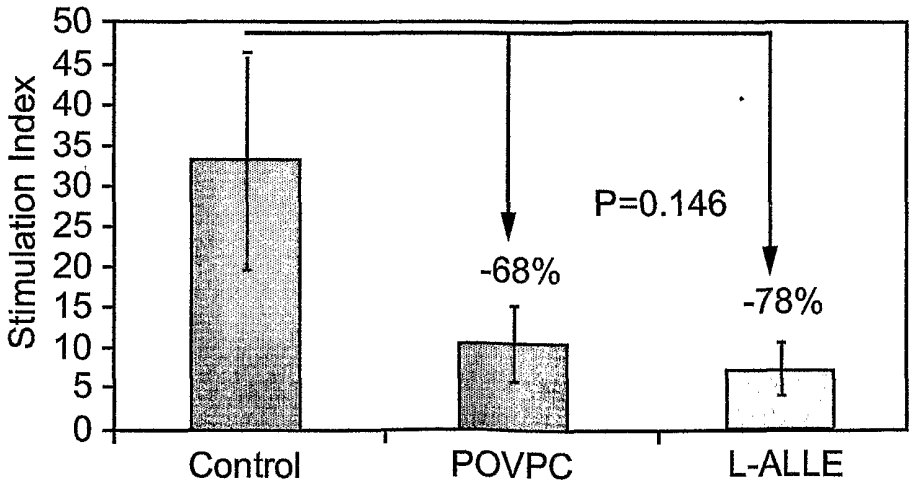

Stimulation Index
50
45
40
35
30
25
20
15
10
5
0
P=0.146
-68%
-78%
Control
POVPC
L-ALLE

Fig. 6

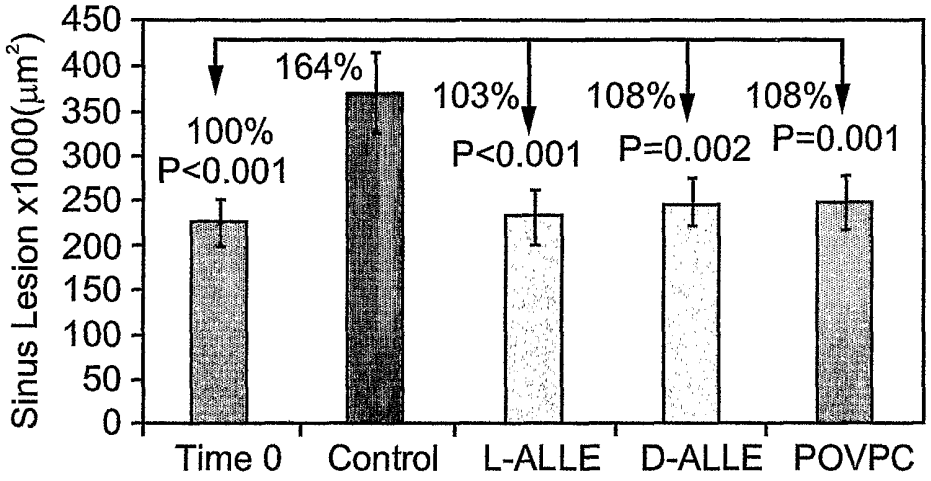

Sinus Lesion x1000(μm²)
450
400
350
300
250
200
150
100
50
0
100%
P<0.001
164%
103%
P<0.001
108%
P=0.002
108%
P=0.001
Time 0
Control
L-ALLE
D-ALLE
POVPC

Fig. 7

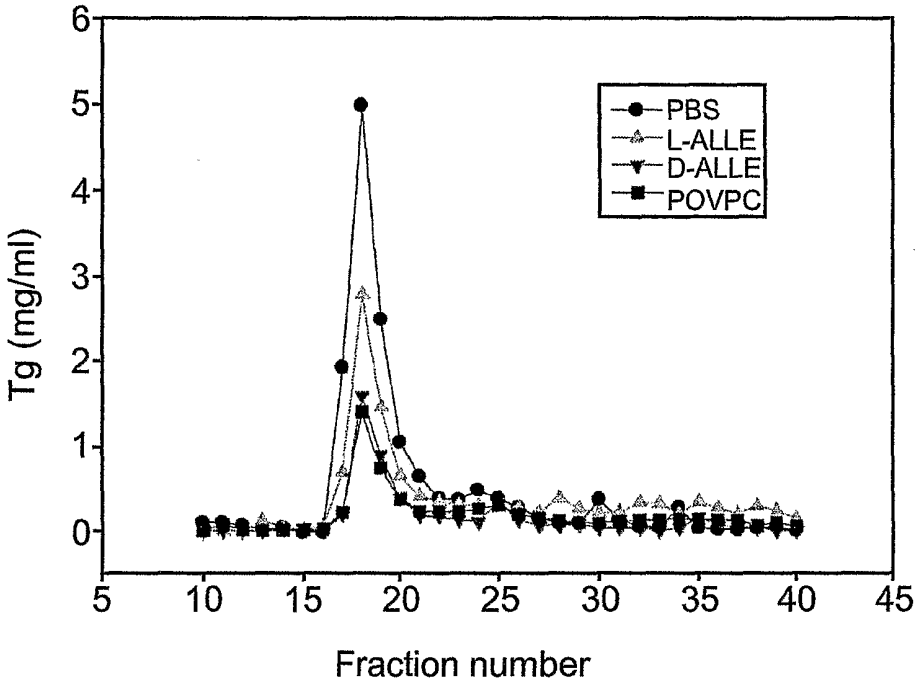

Tg (mg/ml)
Fraction number
PBS
L-ALLE
D-ALLE
POVPC

Fig. 8

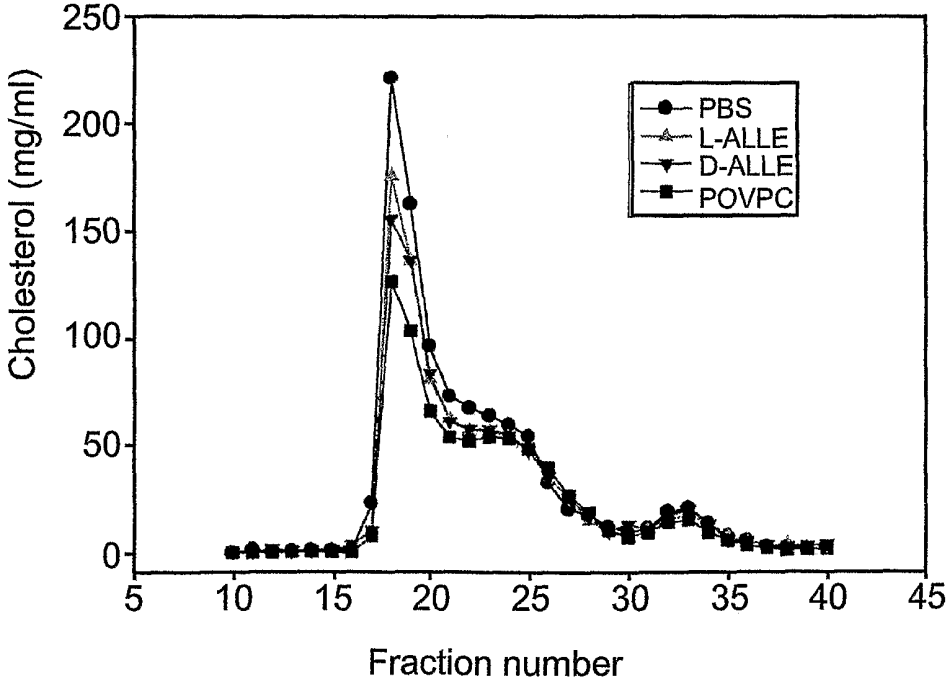

Cholesterol (mg/ml)
Fraction number
PBS
L-ALLE
D-ALLE
POVPC

Fig. 9