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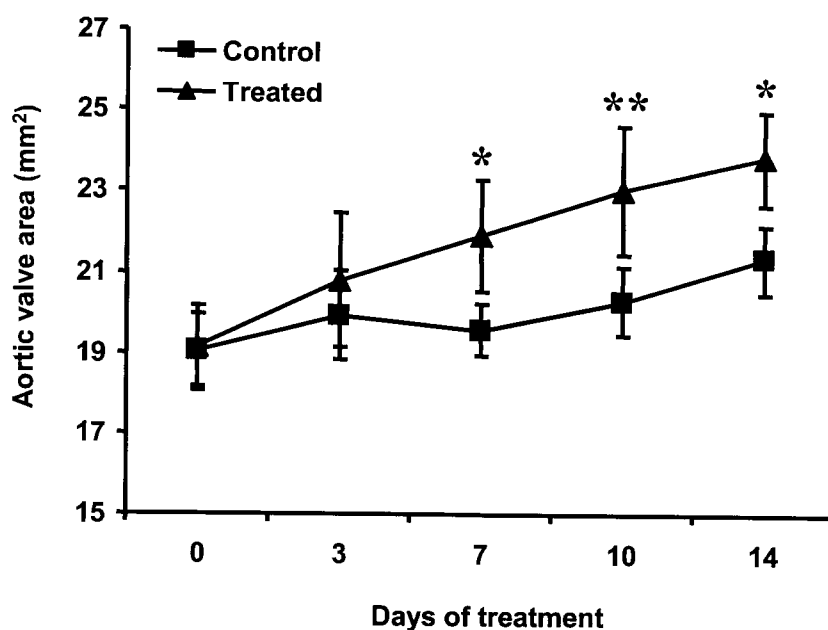
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(54) Title: METHOD AND COMPOUND FOR THE TREATMENT OF VALVULAR STENOSIS



(57) Abstract: A method for treating valvular stenosis. The method involves the administration of a therapeutically effective amount of a reverse lipid (in particular cholesterol) transport agonist to a mammal. Most preferred is an Apolipoprotein A-1 mimetic peptide/phospholipid complex, the peptide of which is defined by the amino acid sequence of SEQ ID NO 1.

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TITLE OF THE INVENTION

Method and Compound for the Treatment of Valvular Disease.

This application claims priority from US Provisional Patent Applications Serial Number 60/809,850 filed June 1, 2006, the disclosure of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0001] The present invention relates to the general field of medical methods and compounds and is particularly concerned with a method and compound for the treatment of valvular disease.

BACKGROUND OF THE INVENTION

[0002] The function of the heart is to supply the energy required for the circulation of blood in the cardiovascular system. Blood flow through all organs is passive and occurs only because arterial pressure is kept higher than venous pressure by the pumping action of the heart. The right heart pump provides the energy necessary to move blood through the pulmonary vessels and the left heart pump provides the energy that causes flow through the systemic organs.

[0003] Venous blood returns from the systemic organs to the right atrium via the superior and inferior venae cavae. It passes through the tricuspid valve into the right ventricle and from there is pumped through the pulmonic valve into the pulmonary circulation via the pulmonary arteries. Oxygenated pulmonary venous blood flows in pulmonary veins to the left atrium

and passes through the mitral valve into the left ventricle. From there, it is pumped through the aortic valve into the aorta to be distributed to the systemic organs.

[0004] Hence, in its normal operation, the left ventricle of the heart pumps oxygen-rich blood to arteries in the vasculature of the body through the aorta. As the heart pumps, the aortic valve, which is located between the left ventricle and the aorta, opens and closes to control the direction of blood flow. More specifically, during heartbeat or systole, the aortic valve is opened to allow blood to flow from the left ventricle into the aorta. Between heartbeats, or during diastole, however, the aortic valve closes to form a tight seal that prevents blood from leaking back into the ventricle.

[0005] The valves are structurally designed to allow flow in only one direction and passively open and close in response to the direction of the pressure differences across them. Typically, the aortic valve is composed of three fibrous leaflets or cusps. The leaflets are forced open against the walls of the aorta during ventricular ejection of blood but fall back during diastole, their free edges coapting to prevent blood from returning into the heart.

[0006] The aortic wall behind each aortic valve cusp bulges outward, forming three structures known as sinuses of Valsalva. The two most anterior valvular aortic cusps are known as the left and right coronary cusps because of the origin of the left and right coronary arteries from the respective sinuses of Valsalva and the remaining valvular posterior cusp is known as the non-coronary cusp.

[0007] For any of several reasons, it can happen that the aortic valve is somehow damaged and may become stenosed. When this happens, the aortic valve does not open to its normal extent and the flow of blood from the

heart into the aorta is hindered. This leads to a heart condition that is commonly known as aortic valve stenosis.

[0008] Common etiologies for aortic valve stenosis include congenital abnormality, rheumatic fever or degeneration with calcification in the aging patient. A bicuspid valve is the most common congenital abnormality, and often a raphe in one of the cusps indicates failure of the commissure to develop. Rarely, a unicuspid or quadricuspid valve can be present at birth. Although the bicuspid valve may not be initially stenotic, fibrosis and thickening lead eventually to reduced orifice size with calcification. Indeed, mechanical shear stress typically leads to calcific injury.

[0009] Rheumatic fever scars the leaflet margins, and eventually the commissures fuse and calcify. More than 50% of adults with aortic stenosis are found to have a bicuspid valve, but fibrosis and calcification may make it difficult to determine whether the valve is bicuspid or tricuspid.

[0010] In the aging patient with degenerative aortic valve stenosis, calcium deposits usually develop at the sinuses and annulus, whereas the margins of the leaflets often remain free of calcifications.

[0011] Currently, there are many proposed theories for the cellular pathophysiology of aortic valve stenosis. Such theories include cardiovascular risk factors initiating a response to injury, mechanical shear stress, auto-immune phenomena causing degeneration, chronically raised stroke volume and altered calcium metabolism (such as found in renal failure, Paget's disease, etc.).

[0012] Regardless of its etiology, aortic stenosis produces an

increase in systolic left ventricular pressure. Systolic hypertension in the ventricular chamber is compensated by concentric hypertrophy of the myocardial wall, allowing the wall stress to remain normal. The less compliant, thickened left ventricle becomes more dependent on the atrial contribution to diastolic filling, such that left ventricular performance can deteriorate when atrial contraction is lost, for example during atrial fibrillation or atrial-ventricular dissociation. The abnormal relaxation and increased stiffness of the thickened left ventricle during diastole also result in diastolic dysfunction and elevations of left ventricular and left atrial diastolic pressures.

[0013] Myocardial failure can eventually develop from chronic severe valvular obstruction and depression of the contractile state. Left ventricular dilatation can also occur in some patients. Myocardial oxygen consumption remains high owing to elevation of systolic pressure in the left ventricle and increase in left ventricular mass. In addition, the increased left ventricular diastolic pressure reduces the pressure gradient necessary for myocardial perfusion. Thus, significant aortic stenosis creates conditions in which high myocardial oxygen demands are inadequately supported by reduced oxygen supply, which leads to subendocardial ischemia.

[0014] Eventually, with a decline in the inotropic state of the myocardium, the ejection fraction is decreased to below the normal range (with or without associated dilatation of the left ventricle). Further elevation of the left ventricular end diastolic pressure (secondary to diastolic dysfunction with or without systolic dysfunction) results in pulmonary venous hypertension. The increased myocardial oxygen demands in aortic stenosis with the underperfused subendocardial myocardium can produce angina pectoris, arrhythmias, and even sudden death.

[0015] The development of any of the cardinal symptoms in the

setting of severe aortic stenosis indicates substantial mortality risk and is an indication for surgical therapy. The average life expectancy after symptom onset is 2-3 years, less if the symptoms are due to heart failure. Because symptoms, and perhaps sudden death, often accompany physical exertion, vigorous activities and competitive sports should be avoided by patients with aortic stenosis, even if it is only mild to moderate in severity. Hence, aortic stenosis is associated not only with high mortality but also with substantial morbidity.

[0016] Calcific aortic stenosis accounts for a large percentage of aortic stenosis cases. The condition is characterized by the build-up of calcified nodules on the upper or superior surface of the aortic valve leaflets. These nodules decrease the flexibility of the leaflets, thereby limiting their mobility and capacity to fully open.

[0017] Three techniques have been employed to correct aortic stenosis, namely valve replacement, intra-operative decalcification or debridement of the heart valve and balloon valvuloplasty.

[0018] Valve replacement, during open-heart surgery, is currently the standard therapy for symptomatic aortic stenosis. Ten-year survival rates for isolated aortic valve replacement are relatively good, even in elderly patients. However, this technique requires the patient to be healthy enough to undergo sternotomy (chest opening) and open heart surgery. The operative mortality for this procedure, particularly in the elderly, is relatively large.

[0019] There are two types of prosthetic heart valves, namely mechanical valves that are composed of only materials that are not derived from living organisms and bio-prosthetic valves that are composed in whole or in part of biological material. Mechanical valves currently in use have a ball-

cage construction, a tilted disc construction (1 or 2 discs) or a hinged leaflet construction.

[0020] Bio-prosthetic valves generally comprise a supporting stent and a plurality of leaflets. The leaflets are generally composed of biological material, while the stent, when present, generally comprises non-biological material, at least in part. The biological material of the leaflets can be autologous tissues such as pericardium, fascia lata or cardiac valves. Alternatively, this material can be derived from homologous tissue such as non-autologous human tissue for human implantation or can be xenogeneic.

[0021] Each type of prosthetic heart valve has advantages and disadvantages. Mechanical heart valves are durable and, hence, more likely to result in long-lasting function but require careful chronic anticoagulation because of thrombo-embolic risk. Chronic anticoagulation therapy, however, carries with it a risk of haemorrhage similar in incidence to that of the residual risk for thrombotic events.

[0022] Bio-prosthetic valves initially approximate the haemodynamic properties of the natural valve. They carry a smaller risk of complications secondary to thrombus than do mechanical valves. Such valves, however, carry a significantly higher risk of calcification than mechanical valves. Since treatment of a functionally compromised bio-prosthetic heart valve frequently requires replacement with a new valve (and hence a second open-heart surgery), limitations on the useful life expectancy of a bio-prosthetic heart valve are both a serious medical problem for the patient and a financial drain on the medical system.

[0023] Furthermore, all prosthetic heart valves are somewhat stenotic. Prosthetic dysfunction secondary to thrombosis or calcification can

lead to increased obstruction or the development of regurgitation. Regurgitation can also result from a perivalvular leak, that is a leak in the area of the sewing ring of the valve. Turbulence associated with valve dysfunction can cause haemolysis and anemia. Even normally functioning prosthetic valves can cause haemolysis in some patients.

[0024] Endocarditis is another potential and major complication in patients with prosthetic heart valves. Antibiotic prophylaxis has to be administered prior to dental, gastro-intestinal and genito-urinary surgery and other procedures associated with bacteraemia.

[0025] Furthermore, some patients have aortic dimensions that are not large enough to easily accommodate conventional replacement valves. Hence, there is a significant number of patients for whom valve replacement is impossible, impractical, or undesirable.

[0026] Intra-operative mechanical debridement or decalcification of the aortic valve was used for many years prior to the advent of mechanical replacement valves. In this technique, the aorta is entered surgically but, rather than replace the valve manually, the surgeon removes the calcified deposits, using suitable surgical tools. Recently, ultrasonic debridement has also been demonstrated to be effective to remove calcific deposits. Nevertheless, this technique still requires the patient to be healthy enough to survive and recuperate from thoracic surgery, and involves all the costs and risks attendant with such surgery.

[0027] A third technique for correcting aortic stenosis involves percutaneous balloon aortic valvuloplasty. In this procedure, an inflatable balloon catheter is advanced to the aortic valve and inflated to compress and fracture the calcified nodules in an attempt to increase leaflet mobility.

Although this procedure eliminates many of the risks and disadvantages attendant with the preceding two techniques, re-stenosis is very common within one year, limiting the usefulness of the technique to temporarily mitigating symptoms for those patients who are poor surgical candidates or refuse surgery.

[0028] Hence, there exists a need for a non-surgical treatment of aortic valve stenosis and other valvular diseases.

[0029] The present invention differs significantly from the prior art and current trends by providing a method for not only preventing the progression of aortic stenosis but also for reducing the degree of stenosis using a reverse lipid transport agonist.

[0030] An object of the present invention is therefore to provide a novel non-surgical treatment of valvular disease.

SUMMARY OF THE INVENTION

[0031] In a broad aspect, the invention provides a method for preventing or treating a valvular stenosis in a subject, the method comprising administering to a subject in need thereof a therapeutically effective amount of a reverse lipid transport agonist. For example, the lipid transport agonist is a reverse cholesterol transport agonist.

[0032] In some embodiments of the invention, the valvular stenosis is an aortic valve stenosis, a calcific valve stenosis, or any other valvular stenosis.

[0033] In some embodiments of the invention, the reverse lipid

transport agonist is selected from the group consisting of: an HDL, a peptide with HDL-like physiological effects, a peptide with HDL-like physiological effects complexed to a lipid, an HDL-mimetic agents, a CETP modulator, an SRB1 modulators, an LXR/RXR agonist, an ABCA1 agonists, a PPAR agonist and an Apolipoprotein A-I (ApoA-1) mimetic peptide/phospholipid complex.

[0034] In this latter case, administering the ApoA-1 mimetic peptide/phospholipid complex may include injecting the ApoA-1 mimetic peptide/phospholipid complex in the subject. Examples of dosages in this case are of from about 1 μ g to about 10 g per kg body weight of the subject, about 1 mg to about .5 g per kg body weight of the subject, and about 25 mg per kg body weight of the subject.

[0035] For example, the ApoA-1 mimetic peptide has the sequence of SEQ ID NO: 1 found herein below, and the ApoA-1 mimetic peptide may be complexed with egg sphingomyelin and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC).

[0036] In some embodiments, the subject is a mammal, for example a human.

[0037] In another broad aspect, the invention provides a method for preventing or treating a valvular calcification in a subject, the method comprising administering to a subject in need thereof a therapeutically effective amount of a reverse lipid transport agonist.

[0038] In yet another broad aspect, the invention provides a method for controlling a valvular stenosis in a subject, the method comprising increasing reverse cholesterol transport in a subject in need thereof.

[0039] In yet another broad aspect, the invention provides a method for preventing or reversing valvular stenosis, the method comprising administering to a patient in need thereof a reverse lipid transport agonist.

[0040] In yet another broad aspect, the invention provides a method for controlling a valvular stenosis in a subject, the method comprising administering to a subject in need thereof a therapeutically effective amount of a reverse lipid transport agonist.

[0041] For example, controlling the valvular stenosis may include reducing a rate of progression of the valvular stenosis, or reversing, at least in part, the valvular stenosis.

[0042] In yet another broad aspect, the invention provides the use of a reverse lipid transport agonist for controlling valvular stenosis in a subject.

[0043] In yet another broad aspect, the invention provides the use of a reverse lipid transport agonist for the manufacture of a pharmaceutical composition of matter for controlling valvular stenosis in a subject

[0044] In a variant, the method comprises the administration of a therapeutically effective amount of a compound, referred to hereinafter as compound A, that mimics biologic properties of Apolipoprotein A-1 (ApoA-1). Compound A and other suitable compounds are described in US Patent Nos. 6,287,590, issued Sep. 11, 2001, and 6,506,799, issued Jan. 14, 2003, which are hereby incorporated by reference in their entirety. Indeed, it is believed that in view of current knowledge regarding the action of the compounds and molecules described in these Patents, results similar to those presented herein are obtainable with these compounds and molecules.

[0045] Advantageously, the proposed method replaces relatively invasive or relatively ineffective existing treatments for valvular diseases. Also, in addition to slowing the progression of valvular disease, the proposed method also reverses valvular stenosis. Accordingly, the proposed treatment not only has a potential to slow down or stop the progression of aortic valve stenosis and other valvular diseases, but also to cause the regression of aortic valve stenosis and other valvular diseases.

[0046] Aortic valve stenosis (AVS) is the most common valvular heart disease, and standard curative therapy remains open-heart surgical valve replacement. To determine if reverse lipid transport agonists have a potential to prevent or treat AVS, ApoA-1 mimetic peptide infusions were tested to determine if they could induce regression of AVS. To that effect, fifteen New-Zealand White male rabbits received a cholesterol-enriched diet and vitamin D₂ until significant AVS was detected by echocardiography. Animals were then randomized to receive saline (control group, n=8) or an ApoA-1 mimetic peptide (treated group, n=7), 3 times per week for 2 weeks. Serial echocardiograms and post mortem valve histology were performed. Aortic valve area improved significantly in the treated group compared to controls after 7 ($21.9 \pm 3.6 \text{ mm}^2$ vs. $19.6 \pm 1.8 \text{ mm}^2$, $P=0.019$) (increases of 14.2% and 3.9%), 10 ($23.0 \pm 4.1 \text{ mm}^2$ vs. $20.3 \pm 2.4 \text{ mm}^2$, $P=0.006$) (19.8% vs. 7.6%) and 14 days of treatment ($23.8 \pm 3.1 \text{ mm}^2$ vs. $21.3 \pm 2.4 \text{ mm}^2$, $P=0.012$) (24.6% vs. 12.9%). Aortic valve thickness decreased by 21% within 14 days of treatment in the treated group ($0.094 \pm 0.034 \text{ cm}$ vs. $0.075 \pm 0.033 \text{ cm}$) whereas it was unchanged in controls ($P=0.0006$). Lesion extent at the base of valve leaflets and sinuses of Valsalva was smaller in the treated compared to control group ($52.8 \pm 12.5\%$ vs. $66.7 \pm 9.9\%$, $P=0.032$). A strong trend towards decreased calcification areas was also observed ($1.6 \pm 2.0\%$ vs. $6.9 \pm 6.7\%$, $P=0.063$). Therefore, infusions of ApoA-1 mimetic peptide lead to regression of experimental AVS.

[0047] Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of preferred embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] In the appended drawings:

[0049] Figure 1 illustrates aortic valve area values obtained through echocardiographic measurements obtained during the ApoA-1 mimetic peptide treatment period. Day "0" corresponds to the end of cholesterol plus vitamin D₂ diet and the beginning of ApoA-1 mimetic peptide treatment period, * $P \leq 0.05$; ** $P \leq 0.01$. (b), Aortic valve thickness values, *** $P \leq 0.001$;

[0050] Figure 2 illustrates aortic valve thickness values obtained through echocardiographic measurements during the ApoA-1 mimetic peptide treatment period. Day "0" corresponds to the end of cholesterol plus vitamin D₂ diet and the beginning of ApoA-1 mimetic peptide treatment period, * $P \leq 0.05$; ** $P \leq 0.01$., *** $P \leq 0.001$;

[0051]

[0052] Figure 3 illustrates a comparison of the percentage of lesion area (LA) over the region of analysis (ROA) in aortic valves from control and treated groups, * $P = 0.032$.

[0053] Figure 4 illustrates a correlation between aortic valve area (AVA) and the percentage of lesion area (LA) over the region of analysis (ROA)

in aortic valves from both groups; and

[0054]

[0055] Figure 5 illustrates the correlation between aortic valve thickness on echocardiography and the percentage of calcification area in the sinus of Valsalva area in animals from both groups. Note that a value (square) from one control animal is hidden behind two triangles at the bottom left of the figure.

DETAILED DESCRIPTION

[0056] The present description refers to many public documents, the contents of which are hereby incorporated by reference in their entirety. Histopathological, experimental and clinical data suggest that calcific aortic valve stenosis (AVS) is an active disease process with lipoprotein deposition, inflammation and active leaflet calcification (1). Although there are some similarities between AVS and atherosclerosis, their pathophysiology and treatments differ significantly. In terms of pathophysiology, a bicuspid aortic valve is present and contributes to the disease in approximately half the patients with AVS, whereas atherosclerosis is not due to a structural congenital abnormality. Rheumatic heart disease can also lead to AVS, while it has not been linked to atherosclerosis. Calcific AVS of the elderly, the most frequent type in the western countries, often leads to isolated aortic valve replacement without the need for associated coronary bypass surgery. In terms of medical treatments, statins have been shown to be protective in patients with coronary disease and to halt progression or induce regression of atherosclerosis (3); in

contrast, the progression of AVS was not prevented by intensive statin therapy in a recent randomized clinical trial (4). Similarly, angiotensin-converting enzyme inhibitors have been shown to be cardioprotective in several large-scale clinical trials but have failed to slow progression of AVS. In summary, atherosclerosis and AVS are distinct diseases, affecting different patient populations.

[0057] There is an inverse relationship between plasma levels of HDL-cholesterol and coronary artery disease. Studies in animals with experimental atherosclerosis have demonstrated that ApoA-1 Milano/phospholipid complexes rapidly mobilize cholesterol and thereby reduce atherosclerotic plaque burden (5, 6). In addition, two clinical studies have suggested that infusions of reconstituted HDL could induce rapid improvement of coronary atherosclerosis (7, 8). We have hypothesized that an HDL-based therapy may also induce regression of AVS. If this could be achieved safely, medical treatment of AVS and its regression may transform our clinical approach of this frequent disease. Apolipoprotein A-I (ApoA-1) is a structural component of HDL that mediates many of its beneficial effects including enhanced reverse cholesterol transport (9). The peptide used in our study is capable of forming an amphipathic alpha-helix in the presence of lipids, as is the case for ApoA-1 (10, 11). We have therefore tested the ability of this ApoA-1 mimetic peptide complexed with phospholipids, mimicking nascent HDL, to induce regression of calcific AVS in a previously described rabbit model (12).

[0058] As is well known, the main function of lipoproteins in plasma is to transport lipids, such as cholesterol and triglycerides. For transport in plasma, cholesterol, normally as cholesterol esters, and the triglycerides are included into lipoprotein particles in which they form a hydrophobic core. The core is surrounded by a surface coat containing phospholipids, unesterified cholesterol and proteins called apolipoproteins. The latter are responsible for

the lipid transport and, in addition, some may interact with many of the enzymes involved in lipid metabolism. To date, at least 10 apolipoproteins have been identified, namely: A-1, A-2, A-4, B-48, B-100, C-I, C-II, C-III, D and E.

[0059] We have hypothesized that a strategy directed to increasing the efficiency of the reverse lipid transport mechanism through the use of a suitable compound is a possible approach in the treatment of valvular disease. There is also a possibility that calcific deposits might be removed, thereby effectively curing valvular stenosis.

[0060] The compound used to test this hypothesis, hereinafter referred to as Compound A, is a lipoprotein that mimics biologic properties of apolipoprotein A-I (ApoA-1). This type of compound, an Apo A-I mimetic or agonist, is described in further detail in U.S. Patent No. 6,376,464 titled "Lipid complexes of APO A-1 agonist compounds," issued to Dasseux *et al.* on April 23, 2002. This document is hereby incorporated by reference in its entirety.

[0061] Briefly, these compounds include peptides, or analogues thereof, which are capable of forming amphipathic alpha-helices in the presence of lipids and which mimic the activity of ApoA-1. They are therefore referred-to as ApoA-1 agonists. The agonists have as their main feature a "core" peptide composed of 15 to 29 amino acid residues, preferably 22 amino acid residues, or an analogue thereof wherein at least one amide linkage in the peptide is replaced with a substituted amide, an isostere of an amide or an amide mimetic.

[0062] These ApoA-1 agonists are based, in part, on the discovery that altering certain amino acid residues in the primary sequence of the 22-mer consensus sequence disclosed in Venkatachalapathi *et al.*, 1991, Mol.

Conformation and Biol. Interactions, Indian Acad. Sci. B: 585-596 (PVLDEFREKLNEELEALKQKLK; hereinafter "Segrest's consensus 22-mer" or "consensus 22-mer") that were thought to be critical for activity, yields synthetic peptides which exhibit activities that approach, or in some embodiments even exceed, the activity of native ApoA-1. It was discovered that replacing three charged amino acid residues in Segrest's consensus 22-mer peptide (Glu-5, Lys-9 and Glu-13) with a hydrophobic Leu residue provides peptides that mimic the structural and functional properties of ApoA-1 to a degree that was unprecedented in the art.

[0063] Compound A comprises a peptide having the sequence:

[0064] H-Pro-Val-Leu-Asp-Leu-Phe-Arg-Glu-Leu-Leu-Asn-Glu-Leu-Leu-Glu-Ala-Leu-Lys-Gln-Lys-Leu-Lys-OH (SEQ ID NO: 1)

[0065] and was synthesized by Polypeptide Laboratories (Torrance, CA, USA). Purity assessed by high performance liquid chromatography and mass spectral analysis was greater than 98%. The peptide was complexed with egg sphingomyelin and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (Avanti Polar Lipids) (10) in a 1:1:1 weight ratio by mixing the components in saline and performing multiple heating and cooling cycles until the solution appeared perfectly clear. The solution containing the complexes was lyophilized in aliquots for long-term storage. Fresh solution was reconstituted every week under sterile conditions and kept at 4°C. Biological activity of the complex was tested in rats for its ability to mobilize cholesterol and to raise HDL-cholesterol in blood following an intravenous injection of the peptide (data not shown).

[0066] Based on their known biological activity and structures, it is believed that other compounds, such as the compounds presented in the

above-mentioned U.S. Patent No. 6,376,464, will show effects similar to Compound A.

[0067] The term "treating" or "treatment" of a state, disease, disorder or condition includes:

[0068] (1) preventing or delaying the appearance of clinical symptoms of the state, disease, disorder or condition developing in a subject that may be afflicted with or predisposed to the state, disease, disorder or condition but does not yet experience or display clinical or subclinical symptoms of the state, disease, disorder or condition;

[0069] (2) inhibiting the state, disease, disorder or condition, i.e., arresting or reducing the development of the state, disease, disorder or condition or at least one clinical or subclinical symptom thereof; or

[0070] (3) relieving the state, disease, disorder or condition, i.e., causing regression of the state, disease, disorder or condition or at least one of its clinical or subclinical symptoms.

[0071] The benefit to a subject receiving treatment is either statistically significant or at least perceptible to the subject or to the physician.

[0072] The term "subject" includes mammals (especially humans) and other animals, such as domestic animals (e.g., household pets including cats and dogs) and non-domestic animals (such as wildlife).

[0073] A "therapeutically effective amount" means the amount of a compound that, when administered to a subject for treating a state, disease,

disorder or condition, is sufficient to effect such treatment. The "therapeutically effective amount" will vary depending on the compound, the state, disease, disorder or condition and its severity and the age, weight, physical condition and responsiveness of the subject receiving treatment.

[0074] Pharmaceutical Compositions

[0075] The pharmaceutical composition of the present invention comprises at least one compound of the present invention and a pharmaceutically acceptable excipient (such as a pharmaceutically acceptable carrier or diluent). Preferably, the pharmaceutical composition comprises a therapeutically effective amount of the compound(s) of the present invention. The compound of the present invention may be associated with a pharmaceutically acceptable excipient (such as a carrier or a diluent) or be diluted by a carrier, or enclosed within a carrier which can be in the form of a capsule, sachet, paper or other container.

[0076] Examples of suitable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, polyhydroxyethoxylated castor oil, peanut oil, olive oil, gelatin, lactose, terra alba, sucrose, dextrin, magnesium carbonate, sugar, cyclodextrin, amylose, magnesium stearate, talc, gelatin, agar, pectin, acacia, stearic acid or lower alkyl ethers of cellulose, silicic acid, fatty acids, fatty acid amines, fatty acid monoglycerides and diglycerides, pentaerythritol fatty acid esters, polyoxyethylene, hydroxymethylcellulose and polyvinylpyrrolidone.

[0077] The carrier or diluent may include a sustained release material, such as glyceryl monostearate or glyceryl distearate, alone or mixed with a wax.

[0078] The pharmaceutical composition may also include one or more pharmaceutically acceptable auxiliary agents, wetting agents, emulsifying agents, suspending agents, preserving agents, salts for influencing osmotic pressure, buffers, sweetening agents, flavoring agents, colorants, or any combination of the foregoing. The pharmaceutical composition of the invention may be formulated so as to provide quick, sustained, or delayed release of the active ingredient after administration to the subject by employing procedures known in the art.

[0079] The pharmaceutical compositions of the present invention may be prepared by conventional techniques, e.g., as described in Remington: The Science and Practice of Pharmacy, 20th Ed., 2003 (Lippincott Williams & Wilkins). For example, the active compound can be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier, which may be in the form of an ampoule, capsule, sachet, paper, or other container. When the carrier serves as a diluent, it may be a solid, semi-solid, or liquid material that acts as a vehicle, excipient, or medium for the active compound. The active compound can be adsorbed on a granular solid container, for example, in a sachet.

[0080] The pharmaceutical compositions may be in conventional forms, for example, capsules, tablets, aerosols, solutions, suspensions or products for topical application.

[0081] The route of administration may be any route which effectively transports the active compound of the invention to the appropriate or desired site of action. Suitable routes of administration include, but are not limited to, oral, nasal, pulmonary, buccal, subdermal, intradermal, transdermal, parenteral, rectal, depot, subcutaneous, intravenous, intraurethral, intramuscular, intranasal, ophthalmic (such as with an ophthalmic solution) or topical (such as with a topical ointment).

[0082] Example

[0083] A complex animal model of aortic valve stenosis has been developed in rabbits. The model resulted in aortic valve stenosis characterized by a calcification similar to what is observed in a clinical setting in humans.

[0084] Methods**[0085] Animals and Experiments**

[0086] An animal model adapted from that described by Drolet *et al.* (12) was used. Fifteen male New-Zealand White rabbits (2.7-3.0 kg, aged 12-13 weeks) were fed with a 0.5% cholesterol-enriched diet (Harlan, Indianapolis, Indiana) plus vitamin D₂ (50000 UI per day; Sigma, Markham, Canada) in the drinking water until significant AVS, as defined by a decrease $\geq 10\%$ of aortic valve area (AVA) or of the transvalvular velocities ratio (V_1/V_2), could be detected by echocardiography (12.9 $\pm$ 2.4 weeks).

[0087] The animals then returned to a standard diet (without vitamin D₂) to mimic cholesterol lowering and were randomly assigned to receive either saline (control group, n=8) or the ApoA-1 mimetic peptide (treated group, n=7). Rabbits were given injections through the marginal ear vein of saline or of the ApoA-1 mimetic peptide (25 mg/kg) complexed with phospholipids (Compound A), 3 times per week for 2 weeks. Echocardiograms were performed serially (see Echocardiography Methods) every 3 to 4 days throughout the randomized treatment period. Two days after their last infusion, the animals underwent a final echocardiogram and were sacrificed, and the aortic valves were harvested for histological analyses. Blood samples were obtained through the ear artery at baseline, prior to treatment and before

sacrifice. Total cholesterol, HDL cholesterol, triglycerides and calcium levels were measured with an automated filter photometer system (Dimension RxL Max, Dade Behring, IL).

[0088] Animal care and procedures complied with the Canadian Council on Animal Care guidelines and were approved by the institutional ethics committee for animal research.

[0089] Echocardiography

[0090] Transthoracic echocardiographic studies were performed at baseline, on a weekly basis starting at 8 weeks of hypercholesterolemic diet until significant AVS developed, and then after 4, 7, 10 and 14 days of ApoA-1 mimetic peptide or saline control treatments. Studies were carried out with an S12 probe using a standard echocardiographic system (Sonos 5500, Philips Medical Imaging, MA). Intra-muscular injections of ketamine (45 mg/kg) and midazolam (0.75 mg/kg) were used for sedation.

[0091] Parasternal long and short-axis views of the aortic valve were recorded to assess leaflet morphology. Left ventricular outflow tract (LVOT) diameter was measured in a zoomed parasternal long-axis view, and LVOT cross-sectional area (CSA_{LVOT}) was calculated according to: $CSA_{LVOT} = \pi (D_{LVOT}/2)^2$. LVOT velocity (V1) and velocity time integral (VTI_{LVOT}) were obtained with pulsed-wave Doppler sampled proximal to the aortic valve in the apical 5-chamber view. Continuous wave (CW) Doppler interrogation across the aortic valve was used to obtain transvalvular maximal velocity (V2) and VTI (VTI_{AO}) in the same view. V1/V2 ratio was calculated in the pre-treatment period to determine AVS development. Aortic valve area (AVA) was obtained at each time point by the continuity equation: $AVA = CSA_{LVOT} \times (VTI_{LVOT}/VTI_{AO})$. Aortic valve thickness in its middle portion was measured at

end-diastole in a zoomed parasternal long-axis view at baseline, before randomized therapy, and at the final echocardiographic exam.

[0092] The average of three consecutive cardiac cycles was used for each measurement. Special care was taken to obtain similar imaging planes on serial examinations by reviewing previous recordings before follow-up study. All echocardiographic imaging and measurements were performed throughout the protocol by the same experienced investigator blinded to randomized treatment assignment.

[0093] Histomorphometry

[0094] The ascending aorta and aortic valve were opened longitudinally and the 3 valvular cusps were separated. Two cusps were immediately frozen in an embedding medium (OCT Tissue-Tek; Sakura, USA) and stored at -80°C. The third cusp was immersion-fixed in 10% buffered formalin at 4°C for 24 hours and embedded in paraffin. Stained or immunohistochemically labeled tissue sections obtained from the central third of each cusp were analyzed with a computer-based digitizing image system (Image Pro Plus, version 5.1) using a light microscope (BX41, Olympus) connected to a digital video camera (Qcolor3, Olympus). The region of analysis (ROA) was composed of 1000 μm of the Valsalva sinus from the leaflet base and 500 μm of the leaflet from the leaflet base. Lesion area (LA) and leaflet lesion length (LLL) were measured.

[0095] Histochemistry

[0096] Hematoxylin-phloxin-safran, von Kossa and Sirius red (F3B, BDH, UK) stained sections were prepared for routine examination, tissue

calcification and collagen studies respectively. Collagen fibers types I and III were quantified as previously described (13), on Sirius red picric acid stained sections under polarized light. For immunohistochemistry evaluation, all sections were preincubated with either mouse IgG2a monoclonal antibody against rabbit macrophage (RAM11, Dako, Canada) (1:100 dilution) or rabbit smooth muscle cell α -actin (Clone 1A4, Dako, Canada) (1:200 dilution). Species-appropriate biotinylated secondary antibodies were applied, followed by streptavidin horseradish peroxidase complex, visualized with azoethylcarbazon (AEC) and counterstained by Mayer's hematoxylin. Smooth muscle cells, macrophages and calcification areas were quantified in the ROA on digital images acquired at 40X magnification. Images from each section were digitally captured with the same illumination settings, and automatic computer-based analysis was performed with the same color threshold for all specimens. Data were expressed as percent labelled area in ROA.

[0097] For assessment of tissue free cholesterol, 5 μ m cryosections fixed in 4% paraformaldehyde in PBS (pH 7.4) were stained with filipin (13). Sections were incubated for 1 hour at room temperature in filipin complex (Sigma, Canada) dissolved in DMSO and diluted in PBS, mounted in Vectashield (Vector Laboratories, USA) and viewed by fluorescence microscopy using a Zeiss Axiovert 200M microscope with the DAPI filter set. Images were acquired with an AxioCam MRm digital camera mounted with a 0.63X C-mount adapter. Filipin data were expressed as arbitrary units of fluorescence intensity.

[0098] Statistical Analysis

[0099] Data are presented as mean $\pm$ standard deviation. For the "pre-treatment" period, repeated measures analysis of variance (ANOVA) models were used to study the echocardiographic and serum measurements

across time and between groups (treated vs. control groups). Models with time, group and group x time interaction as independent variables were used and comparisons between groups at a given time point were undertaken only in the presence of a significant group x time interaction. Otherwise, global conclusions were drawn based on the main group effects of the model. For the randomized treatment period, repeated measures analysis of covariance (ANCOVA) models were used to study the echocardiographic and serum measurements across time and between groups (treated vs. control groups), adjusted for the baseline value of the response variable. The group x time interaction was also included in the ANCOVA models and comparisons between groups at a given time point were undertaken only in the presence of a significant group x time interaction. Otherwise, global conclusions were drawn based on the main group effects of the model. Histological variables were compared between treated and control groups using Student t-test. Relationships between histomorphometry and echocardiographic variables were evaluated using Pearson correlation coefficient. All analyses were performed with SAS release 8.2 (SAS Institute Inc., Cary, NC) and conducted at the 0.05 significance level.

[0100] Results

[0101] Serum Lipids and Calcemia

[0102] There was no significant difference between groups during the pre-treatment period (the hypercholesterolemic diet period) for serum levels of total cholesterol ($P=0.942$), HDL-cholesterol ($P=0.787$), triglycerides ($P=0.906$), and calcemia ($P=0.727$). During the 2-week treatment period, total cholesterol levels were also statistically similar in both groups ($P=0.470$). Values were 20.46 ± 3.52 mmol/L and 20.13 ± 5.18 mmol/L at the time of randomization and 13.78 ± 6.24 mmol/L and 17.57 ± 10.32 mmol/L at end of

treatment in the control and treated groups respectively. There was no statistically significant difference between groups for HDL-cholesterol levels during the treatment period ($P=0.374$). HDL-cholesterol was 0.50 ± 0.20 mmol/L and 0.50 ± 0.15 mmol/L at the time of randomization and 0.39 ± 0.17 mmol/L and 0.45 ± 0.17 mmol/L at end of treatment. During this period, triglyceride levels were also similar ($P=0.544$). There was no significant difference between groups for calcemia during the treatment period ($P=0.832$), with values of 3.31 ± 0.29 mmol/L and 3.15 ± 0.37 mmol/L before randomization and 3.22 ± 0.11 mmol/L and 3.22 ± 0.12 mmol/L at the end of treatment in both groups.

[0103] Development of AVS During the Period of Hypercholesterolemic Diet and Vitamin D₂ Supplementation

[0104] AVS induction time by the cholesterol plus vitamin D₂ diet was similar for control and treated groups (12.8 ± 2.2 vs. 13.0 ± 2.9 weeks; $P=0.852$). There was a significant difference between AVA at baseline and at the end of the hypercholesterolemic diet period ($P<0.0001$). AVA decreased in both groups, and values were almost identical between control and treated rabbits (from 24.2 ± 4.1 mm² at baseline to 19.0 ± 2.7 mm² in controls and from 24.7 ± 3.9 mm² to 19.1 ± 2.6 mm² in the treated group). There were therefore no significant differences in AVA between groups during the period of AVS development ($P=0.852$). Thus, AVA decreased by $20.5\pm4.2\%$ and $21.6\pm3.7\%$ before randomized treatment in the control and treated groups.

[0105] The V_1/V_2 ratio was also significantly different between measurements at baseline and at the end of hypercholesterolemic diet ($P<0.0001$). V_1/V_2 ratios decreased between these time points with no significant differences among groups ($P=0.914$).

[0106] Evolution of AVS With Treatment– Echocardiography

[0107] As illustrated in Figure 1, during the treatment period (from AVS detection to after 2 weeks of treatment), significant group x time interaction was observed for AVA ($P=0.013$). Using repeated measures ANCOVA models, echocardiographic measurements revealed significant increases of AVA in the treated group as compared to controls after 7 days ($21.9 \pm 3.6 \text{ mm}^2$ vs. $19.6 \pm 1.8 \text{ mm}^2$, $P=0.019$) (relative increases of $14.2 \pm 3.5\%$ vs. $3.9 \pm 3.4\%$), 10 days ($23.0 \pm 4.1 \text{ mm}^2$ vs. $20.3 \pm 2.4 \text{ mm}^2$, $P=0.006$) (relative increases of $19.8 \pm 3.5\%$ vs. $7.6 \pm 4.2\%$) and 14 days of treatment ($23.8 \pm 3.1 \text{ mm}^2$ vs. $21.3 \pm 2.4 \text{ mm}^2$, $P=0.012$) (relative increases of $24.6 \pm 2.0\%$ vs. $12.9 \pm 3.5\%$).

[0108] Aortic valve thickness was assessed by echocardiography and measured before randomized therapy and after 14 days of treatment. Significant group x time interaction was also observed for aortic valve thickness ($P=0.005$). No significant difference was found between groups at the randomization time point but a significant decrease of aortic valve thickness was observed in the treated group as compared to the control group after 14 days of treatment. Aortic valve thickness was $0.094 \pm 0.034 \text{ cm}$ before randomization and $0.075 \pm 0.033 \text{ cm}$ at end of treatment in treated rabbits whereas it was $0.080 \pm 0.024 \text{ cm}$ and $0.080 \pm 0.026 \text{ cm}$ at both time points in controls.

[0109] Histology

[0110] All the animals presented aortic valve lesions. Lesions consisted in a cap of neotissue composed of multiple layers of foam cells giving place gradually to fibrotic material sparse with foam cells from about half-way deep in the lesion. Lesions were generally progressing from the sinotubular area, covering the whole sinus of Valsalva and extending to the cusp base and

up to one half to two thirds of the proximal leaflet arterialis. In contrast, lesions on the leaflet ventricularis were not common and were less severe.

[0111] Histomorphometry

[0112] As shown in Figure 3 the percentage of LA per ROA decreased significantly in the ApoA-1 mimetic peptide treated group as compared to the control group ($52.8 \pm 12.5\%/\text{ROA}$ vs. $66.7 \pm 9.9\%/\text{ROA}$, $P=0.032$). Interestingly, when the percentage of LA/ROA on histomorphometry and echocardiography-determined AVA from both groups were pooled, a negative correlation was found between the percentage of LA/ROA and AVA ($r=-0.54$, $P=0.039$, Figure 4. Similarly, analysis of pooled data from both groups revealed a negative correlation between AVA and %LLL (*i.e.* the percentage of total leaflet length occupied by lesion or $\text{LLL}/\text{total leaflet length} \times 100$) ($r=-0.70$, $P=0.004$). However, the difference between the treated and control groups for %LLL did not reach statistical significance ($55.7 \pm 24.3\%$ vs. $72.3 \pm 11.7\%$, $P=0.109$).

[0113] Histochemistry

[0114] Foci of calcifications were observed in the majority of animals. The percentages of rabbits with calcium deposits in the lesion core in the sinuses of Valsalva were 57% (4/7) in the treated group and 88% (7/8) in controls. Quantification of the percentage of the sinus of Valsalva area occupied by calcifications on von Kossa staining revealed a strong trend towards a 77% decrease in the treated group compared to controls ($1.6 \pm 2.0\%$ vs. $6.9 \pm 6.7\%$; $P=0.063$). This percentage of calcification foci area within the sinus area was significantly correlated with echocardiography-determined aortic valve thickness ($r=0.63$, $P=0.012$) using pooled data from both study groups.

(Figure 5).

[0115] Free cholesterol, as detected by filipin staining, was present throughout the whole aortic valve lesion areas in almost all control animals, whereas the fluorescent signal tended to be lower at the luminal edge of lesions in most animals treated with the ApoA-1 mimetic peptide. Assessment of the fluorescent signal within the first 10 μm at the luminal edge of lesions showed a 41% decrease in free cholesterol in treated animals compared to controls but this difference did not reach statistical significance (221 ± 54 vs. 377 ± 229 arbitrary units of fluorescence intensity, $P=0.231$). The macrophage areas (34.5%) were about twice as large as α -actin positive areas (17.1%), with no significant differences between groups. The percentage of collagen type III fibers (light green) was higher than collagen type I fibers (red-yellow) in the ROA ($19.7 \pm 5.5\%$ /ROA for type III vs. $6.6 \pm 4.1\%$ /ROA for type I in controls, $P=0.00009$; $19.1 \pm 11.3\%$ /ROA for type III vs. $5.5 \pm 5.6\%$ /ROA for type I in treated rabbits, $P=0.015$). Percentages of collagen fibers (types I and III) in the ROA were however similar between both groups ($P=0.671$ and $P=0.883$ respectively).

[0116] Discussion

[0117] These experiments demonstrate that infusions of an ApoA-1 mimetic peptide lead to significant regression of experimental AVS. Compared to the control group, ApoA-1 mimetic peptide infusions induced greater improvement of AVA and a significant reduction in aortic valve thickness. These favorable changes in AVS severity on echocardiography were accompanied on histological sections by a significant decrease in lesion extent in the leaflet base region and a strong trend towards decreased calcifications.

[0118] A rabbit model of AVS developed by Drolet et al. (12) in which aortic valve calcification occurs significantly and reproducibly was used, allowing to mimic the clinical condition. After a cholesterol-enriched diet and vitamin D₂ supplementation for approximately 13 weeks, echocardiographic measurements revealed a 21% decrease in AVA. Two-dimensional imaging showed increased valve thickness and echogenicity compatible with leaflet sclerosis and areas of calcification. Histological examination confirmed leaflet thickening and calcium deposition both in the sinuses of Valsalva and at the leaflet base. This study provides echocardiographic and histological evidence of the beneficial effects of an ApoA-1 mimetic peptide on experimental calcific AVS. The increase in AVA was observed as early as 7 days after the initiation of active treatment, to reach an improvement of 24% at 14 days. AVA indeed almost returned, with the ApoA-1 mimetic peptide infusions, to the normal value present prior to starting the hypercholesterolemic diet. In contrast, discontinuation of the cholesterol-enriched diet with vitamin D₂ supplementation in the control group (to mimic lipid-lowering) only led to a mild increase in AVA, which confirms the beneficial effects of the peptide. Aortic valve thickness was also significantly reduced after only 14 days of treatment with the peptide, as shown by echocardiography. Interestingly, AVA determined by echocardiography correlated inversely with indices of lesion extent on histology. ApoA-1 mimetic peptide infusions also led to a significant reduction in the percentage of lesion area in the region of histological analysis around the valvular leaflet base. Furthermore, the large reduction in the extent of valvular calcifications that almost reached statistical significance at the arbitrarily chosen 0.05 level is likely to be of clinical importance, given that calcific AVS of the elderly is the most frequent form of stenosis encountered in developed countries. This suggests that the presence of aortic valve calcifications not only does not preclude obtaining favorable results with the peptide but that this approach may even regress the valvular calcifications themselves. This finding may also be applicable to mitral valve and/or anular calcifications.

[0119] An ApoA-1 mimetic peptide complexed with phospholipids stimulates reverse cholesterol transport in a manner similar to native ApoA-1 (14). The higher circulating levels of total cholesterol in rabbits treated with the peptide compared to controls (observed after 2 weeks of treatment) may indicate enhanced mobilization of tissue cholesterol.

[0120] This example suggests that similar results are obtainable in humans using any suitable HDL-based therapy, such as for example one or more infusion(s) or bolus(es) of HDL or peptide (with or without lipids) with HDL-like effects, orally administered HDL-mimetic agents, and/or the administration of cholesteryl ester transfer protein (CETP) modulators, or scavenger receptor class B, member 1 (SRB1) modulators or liver X receptor (LXR)/retinoid X receptor (RXR) agonists, or ATP-binding cassette transporter-1 (ABCA1) agonists, or peroxisome proliferator-activated receptor (PPAR) agonists, among others.

[0121] While the experiments described herein concerned the regulation aortic valve stenosis, one of ordinary skilled in the art will readily appreciate that these experiments may be predictive of biological effects in humans or other mammals and/or may serve as models for use of the present invention in humans or other mammals for any other similar valve diseases.

[0122] Although the present invention has been described hereinabove by way of preferred embodiments thereof, it can be modified, without departing from the spirit and nature of the subject invention as defined in the appended claim.

References

1. Freeman RV, Otto CM. Spectrum of calcific aortic valve disease: pathogenesis, disease progression, and treatment strategies. *Circulation* 2005;111:3316-26.
2. Carabello BA. Aortic stenosis. *N Engl J Med* 2002;346:677-82.
3. Nissen SE, Nicholls SJ, Sipahi I. Effect of very high intensity statin therapy on regression of coronary atherosclerosis. The ASTEROID trial. *JAMA*. 2006;295:1556-65.
4. Cowell SJ, Newby DE, Prescott RJ, et al. Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression (SALTIRE) Investigators. A randomized trial of intensive lipid-lowering therapy in calcific aortic stenosis. *N Engl J Med* 2005;352:2389-97.
5. Ameli S, Hultgardh-Nilsson A, Cercek B, et al. Recombinant apolipoprotein A-I Milano reduces intimal thickening after balloon injury in hypercholesterolemic rabbits. *Circulation* 1994;90:1935-41.
6. Shah PK, Yano J, Reyes O, et al. High-dose recombinant apolipoprotein A-I(milano) mobilizes tissue cholesterol and rapidly reduces plaque lipid and macrophage content in apolipoprotein e-deficient mice. Potential implications for acute plaque stabilization. *Circulation* 2001;103:3047-50.

7. Nissen SE, Tsunoda T, Tuzcu EM, et al. Effect of recombinant ApoA-1 Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *JAMA* 2003;290:2292-300.
8. Tardif JC, Grégoire J, L'Allier PL, et al. Effects of reconstituted high-density lipoprotein infusions on coronary atherosclerosis: a randomized controlled trial. *JAMA* 2007;297:1675-82.
9. Meyers CD, Kashyap ML. Pharmacologic augmentation of high-density lipoproteins: mechanisms of currently available and emerging therapies. *Curr Opin Cardiol* 2005;20:307-12.
10. Khan M, Lalwani ND, Drake SL, Crockatt JG, Dasseux JLH. Single-dose intravenous infusion of ETC-642, a 22-Mer ApoA-1 analogue and phospholipids complex, elevates HDL-C in atherosclerosis patients. *Circulation* 2003;108(Suppl):IV-563-4.
11. Bodary PF, Shen Y, Westrick RJ, et al. Gene transfer of an APO-A-I mimetic peptide reduces atherosclerosis in mice. *J Am Coll Cardiol*. 2004;43(Suppl A):465A-6.
12. Drolet MC, Arsenault M, Couet J. Experimental aortic valve stenosis in rabbits. *J Am Coll Cardiol* 2003;41:1211-7.
13. Busseuil D, Zeller M, Cottin Y, et al. Late neointimal tissue growth behind the stent after intravascular gamma-radiation. *Int J Radiat Oncol*

Biol Phys 2004;58:259-66.

14. Navab M, Anantharamaiah GM, Reddy ST, et al. Apolipoprotein A-I mimetic peptides. *Arterioscler Thromb Vasc Biol* 2005;25:1325-31.
15. Navab M, Anantharamaiah GM, Reddy ST, et al. Human apolipoprotein A-I and A-I mimetic peptides: potential for atherosclerosis reversal. *Curr Opin Lipidol* 2004;15:645-9.

WHAT IS CLAIMED IS:

1. A method for preventing or treating a valvular stenosis in a subject, said method comprising administering to a subject in need thereof a therapeutically effective amount of a reverse lipid transport agonist.
- 5 2. A method as defined in claim 1, wherein said lipid transport agonist is a reverse cholesterol transport agonist.
3. The method as defined in claim 1, wherein said valvular stenosis is an aortic valve stenosis.
- 10 4. The method as defined in claim 1, wherein said valvular stenosis is a calcific valve stenosis.
- 15 5. The method as defined in claim 1, wherein said reverse lipid transport agonist is selected from the group consisting of a high density lipoprotein (HDL), a peptide with HDL-like physiological effects, a peptide with HDL-like physiological effects complexed to a lipid, an HDL-mimetic agent, a cholesteryl ester transfer protein (CETP) modulator, a scavenger receptor class B, member 1 (SRB1) modulator, a liver X receptor / retinoid X receptor (LXR/RXR) agonist, an ATP-binding cassette transporter-1 (ABCA1) agonist and a peroxisome proliferator-activated receptor (PPAR) agonist.
- 20 6. The method as defined in claim 1, wherein said reverse lipid transport agonist is an Apolipoprotein A-1 (ApoA-1) mimetic peptide/phospholipid complex.
7. The method as defined in claim 6, wherein administering said ApoA-1 mimetic peptide/phospholipid complex includes injecting said ApoA-1

mimetic peptide/phospholipid complex in said subject.

8. The method as defined in claim 7, wherein said ApoA-1 mimetic peptide/phospholipid complex is injected at a dosage of from about 1 μ g to about 10 g per kg body weight of said subject.
- 5 9. The method as defined in claim 8, wherein said ApoA-1 mimetic peptide/phospholipid complex is injected at a dosage of from about 1 mg to about .5 g per kg body weight of said subject.
- 10 10. The method as defined in claim 9, wherein said ApoA-1 mimetic peptide/phospholipid complex is injected a dosage of about 25 mg per kg body weight of said subject.
11. The method as defined in claim 6, wherein said ApoA-1 mimetic peptide has a sequence set forth in SEQ ID NO: 1.
12. The method as defined in claim 6, wherein said ApoA-1 mimetic peptide is complexed with egg sphingomyelin and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC).
15
13. The method as defined in claim 6, wherein said agonist comprises a polypeptide having a sequence set forth in SEQ ID NO: 1.
14. The method as defined in claim 1, wherein said subject is a mammal.
15. The method as defined in claim 14, wherein said subject is a human.
- 20 16. A method for preventing or treating a valvular calcification in a subject, said method comprising administering to a subject in need thereof a therapeutically effective amount of a reverse lipid transport agonist.

17. A method for controlling a valvular stenosis in a subject, said method comprising increasing reverse cholesterol transport in a subject in need thereof.
- 5 18. A method for preventing or reversing valvular stenosis, said method comprising administering to a patient in need thereof a reverse lipid transport agonist.
19. A method for controlling a valvular stenosis in a subject, said method comprising administering to a subject in need thereof a therapeutically effective amount of a reverse lipid transport agonist.
- 10 20. The method as defined in claim 19, wherein controlling said valvular stenosis includes reducing a rate of progression of said valvular stenosis.
21. The method as defined in claim 20, wherein controlling said valvular stenosis includes reversing, at least in part, said valvular stenosis.
- 15 22. Use of a reverse lipid transport agonist for controlling valvular stenosis in a subject.
23. Use of a reverse lipid transport agonist for the manufacture of a pharmaceutical composition of matter for controlling valvular stenosis in a subject.

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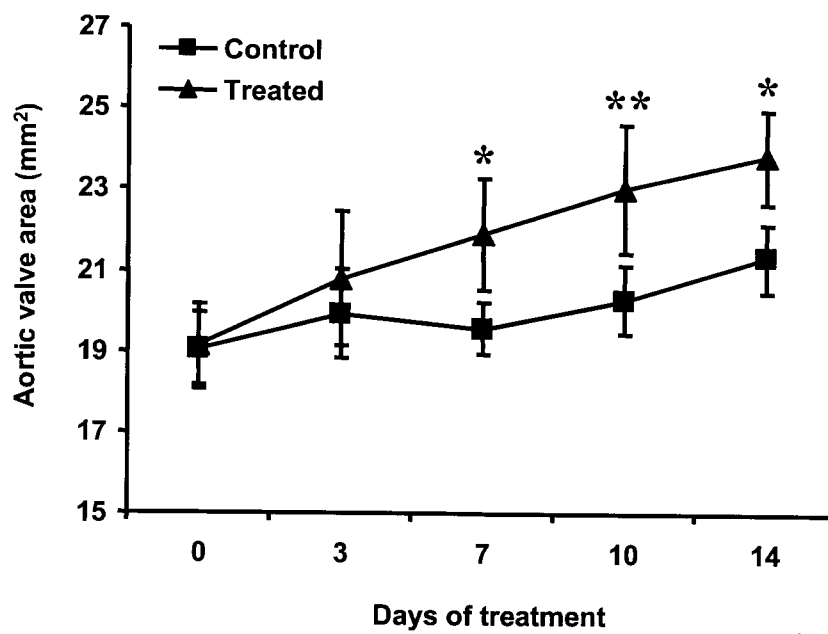


Fig 1

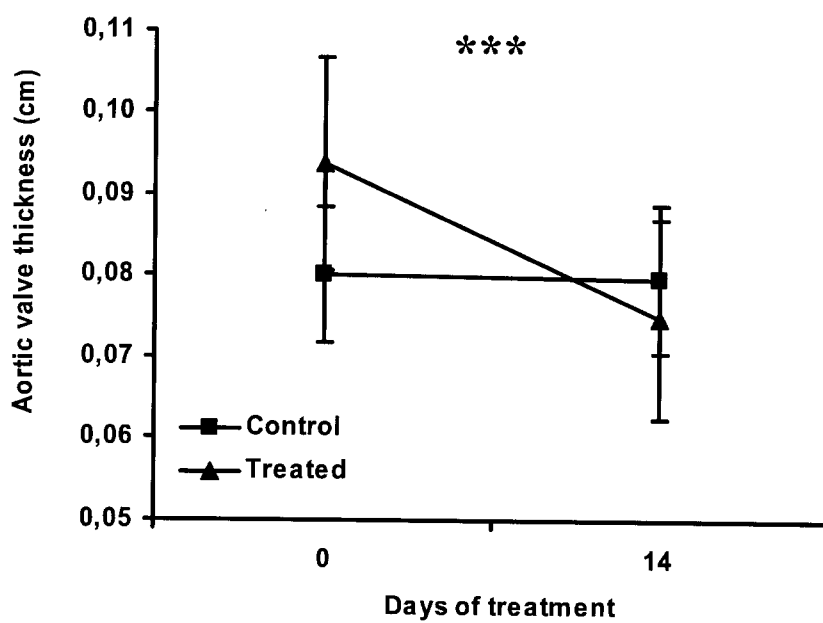


Fig 2

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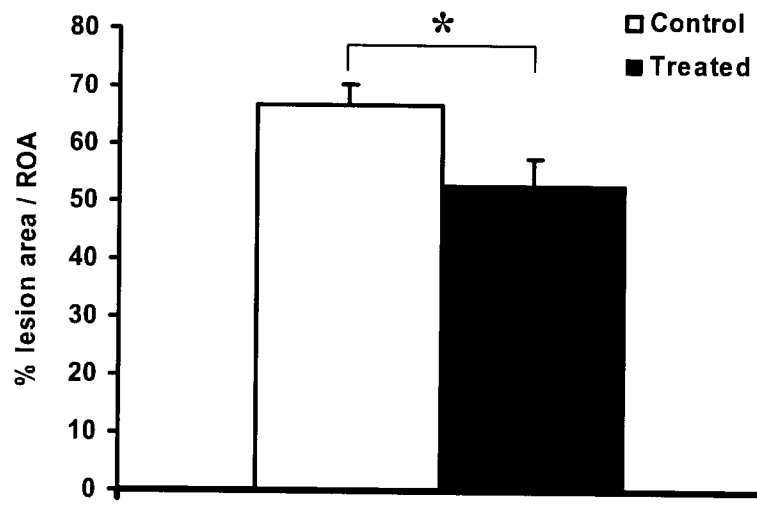


Fig 3

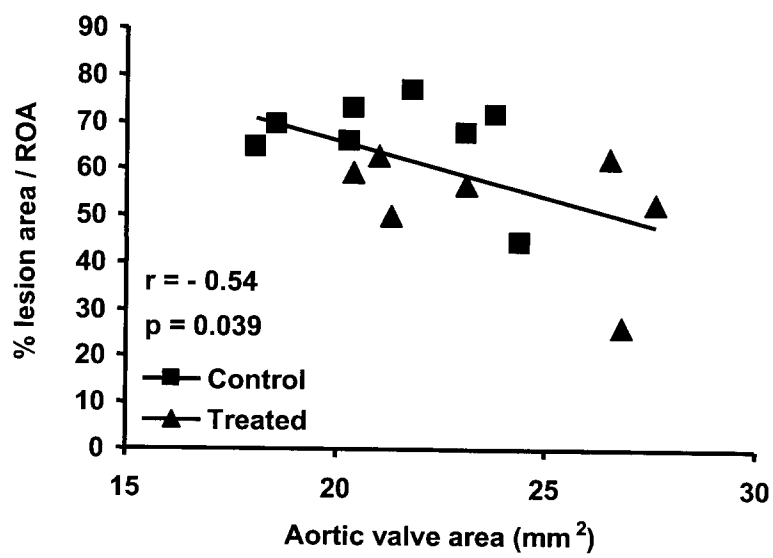


Fig 4

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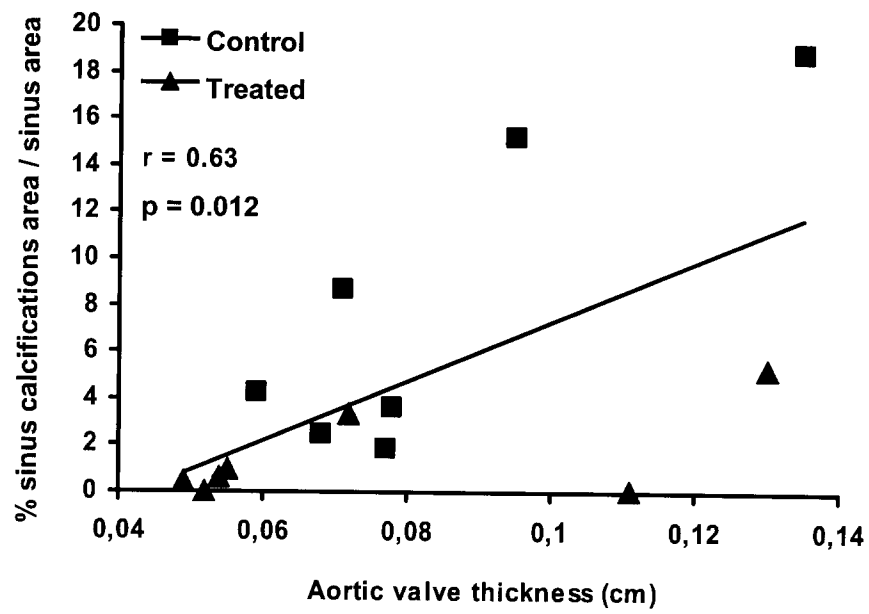


Fig 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2007/000895

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 38/17** (2006.01) , **A61K 31/661** (2006.01) , **A61K 31/688** (2006.01) , **A61P 9/10** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: **A61K 38/17** (2006.01) , **A61K 31/661** (2006.01) , **A61K 31/688** (2006.01) , **A61P 9/10** (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: CAPLUS, BIOSIS, DELPHION, GENOMEQUEST, CANADAIAN PATENT DATABASE

Keywords: SEQ ID NO:1, ApolipoproteinA-1 (ApoA-1), dyslipidemia, hypercholester*, valv*, stenosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO9916459 A1 (DASSEUX J-L) [FR/US] et al. 8 April 1999 see pages 85, 86 and 89-91 Examples 9.5, 9.6, 10 and 11 Table X, Figure 10 claims 1, 19, 29, 33, 34, 41, 42, 44-48 and 56	1-4, 6-15 and 17-23
Y	ROSSEBØ, AB AND PEDERSEN, TR Hyperlipidemia and aortic valve disease. Current Opinion in Lipidology 2004, Vol. 15, No. 4. pp. 447-451 ISSN 0957-9672 see whole document	1-4, 6-15 and 17-23

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 September 2007 (10-09-2007)

Date of mailing of the international search report

12 October 2007 (12-10-2007)

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INTERNATIONAL SEARCH REPORTInternational application No.
PCT/CA2007/000895**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. ☒ Claim Nos. : 1-21

because they relate to subject matter not required to be searched by this Authority, namely :

Claims 1-21 is directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search under Rule 39.1(iv) of the PCT. Regardless, this Authority has carried out a search based on the alleged effects of the product defined in claims 1-21.

2. ☒ Claim Nos. : 5 (fully); 1-4 and 14-23 (partially)

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

see extra sheet

3. ☐ Claim Nos. :

because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

see extra sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

claims 6-13 (fully); claims 1-4, 14, 15 and 17-23 (partially) as they relate to ApoA-1 mimetic peptide/phospholipid complex

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Box No. II

The terms “reverse lipid transport agonist” or “reverse cholesterol transport agonist” encompasses products with insufficient technical features. Furthermore, the application provides support within the meaning of Article 6 and disclosure within the meaning of Article 5 for only a limited number of such products. In the present case, the claims so lack clarity and support and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been established for the parts of the application which appear to be clear and supported, namely the reverse lipid transport agonist defined as an Apolipoprotein A-1 mimetic peptide/phospholipid complex.

Box No. III

This International Searching Authority found multiple inventions (groups) in this international application as follows:

Group 1. claims 6-13 (fully); claims 1-4, 14, 15 and 17-23 (partially)

A method for controlling, preventing or treating a valvular stenosis in a subject comprising administering to a subject an Apolipoprotein A-1 (ApoA-1) mimetic peptide/phospholipid complex.

Group 2. claims 1-5, 14, 15 and 17-23 (partially)

A method for controlling, preventing or treating a valvular stenosis in a subject comprising administering to a subject a high density lipoprotein (HDL).

Group 3. claims 1-5, 14, 15 and 17-23 (partially)

A method for controlling, preventing or treating a valvular stenosis in a subject comprising administering to a subject a peptide with HDL-like physiological effects alone or complexed with a lipid.

Group 4. claims 1-5, 14, 15 and 17-23 (partially)

A method for controlling, preventing or treating a valvular stenosis in a subject comprising administering to a subject HDL mimetic agent.

Group 5. claims 1-5, 14, 15 and 17-23 (partially)

A method for controlling, preventing or treating a valvular stenosis in a subject comprising administering to a subject a cholesteryl ester transferase protein (CETP) modulator.

Group 6. claims 1-5, 14, 15 and 17-23 (partially)

A method for controlling, preventing or treating a valvular stenosis in a subject comprising administering to a subject a scavenger receptor class B member 1 (SRB1) modulator.

Group 7. claims 1-5, 14, 15 and 17-23 (partially)

A method for controlling, preventing or treating a valvular stenosis in a subject comprising administering to a subject a liver X receptor/retinoid X receptor (LXR/RXR) agonist.

Group 8. claims 1-5, 14, 15 and 17-23 (partially)

A method for controlling, preventing or treating a valvular stenosis in a subject comprising administering to a subject an ATP-binding cassette transporter-1 (ABCA1) agonist.

Group 9. claims 1-5, 14, 15 and 17-23 (partially)

A method for controlling, preventing or treating a valvular stenosis in a subject comprising administering to a subject a peroxisome proliferator-activated receptor (PPAR) agonist.

Group 10. claim 16

A method of preventing or treating a valvular calcification in a subject comprising administering to a subject a reverse lipid transport agonist.

Box No. III (continued from previous page)

The requirements of unity of invention are not fulfilled in that there is no technical relationship among the inventions as they do not involve one or more of the same or corresponding technical features. The expression "special technical features" means those features which define a contribution which each of the claimed inventions considered as a whole makes over the prior art.

Based on *a priori* considerations, the subject matter of groups 1-9 relate to a method involving stenosis utilizing molecules that are from diverse classes of structurally unrelated classes. Compounds that have utility in treating stenosis were already known. For example, patients with aortic stenosis treated with statins controlled stenosis progression (see e.g. Novaro et al., Circulation 2001, 104:2205-2209).

The subject matter of group 10 differs from that of groups 1-9 in that the former is directed to a different method i.e. a method for preventing or treating valvular calcification.

Based on *a posteriori* considerations, it was known at the claim date of the present application that PPAR agonists and RXR agonists can be used to treat or prevent stenosis (see e.g. US2004073297, April 15, 2004, Biotronik Mess & Therapie). As such, no single general inventive concept unites the subject matter of the multiple inventions outlined above.

International application No.
PCT/CA2007/000895

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO9916459 A1	08-04-1999	AU746090B B2	18-04-2002
		AU9779098 A	23-04-1999
		AU2005201985 A1	26-05-2005
		CA2304805 A1	08-04-1999
		CN1247254C C	29-03-2006
		CN1280501 A	01-17-2001
		EP1019073 A1	19-07-2000
		EP1019073 A4	03-11-2004
		HU0002381 A2	28-10-2000
		IL135318D D0	20-05-2001
		JP2001518282T T	16-10-2001
		NO20001599 A	02-05-2000
		NO20001599 D D0	08-03-2000
		NZ503717 A	28-08-2002
		PL339629 A1	02-01-2001
		RU2215747 C2	10-11-2003
		UA71553 C2	16-10-2000
		US6004925 A	21-12-1999
		US6329341 B1	11-12-2001
		US6376464 B1	23-04-2002
		US6573239 B1	03-06-2003
		US6602854 B1	05-08-2003
		US6630450 B1	07-10-2003
		US6716816 B1	06-04-2004
		US6753313 B1	22-06-2004
		US7189689 B2	13-03-2007
		US2003203842 A1	30-10-2003
		US2004181034 A1	16-09-2004
		US2005215467 A1	29-09-2005