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REVIEW ARTICLE

Preclinical Evaluation Methods for Screening of Anti-Atherosclerotic Drugs: An Overview.

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ABSTRACT

Atherosclerosis is a disease of large and medium-sized muscular arteries and is characterized by endothelial dysfunction, vascular inflammation, and the buildup of lipids, cholesterol, calcium, and cellular debris within the intima of the vessel wall. This buildup results in plaque formation, vascular remodeling, acute and chronic luminal obstruction, abnormalities of blood flow and diminished oxygen supply to target organs**.** Vasomotor function, the thrombogenicity of the blood vessel wall, the state of activation of the coagulation cascade, the fibrinolytic system, smooth muscle cell migration and proliferation, and cellular inflammation are complex and interrelated biological processes that contribute to atherogenesis and the clinical manifestations of atherosclerosis. Elevated serum levels of low-density lipoprotein cholesterol overwhelm the antioxidant properties of the healthy endothelium and result in abnormal endothelial metabolism of this lipid moiety. Oxidized low-density lipoprotein is capable of a wide range of toxic effects and cell/vessel wall dysfunctions that are characteristically and consistently associated with the development of atherosclerosis. Detail study of atherosclerosis can be done by using various animal models. Animals different species mainly use for screening methods are mice, rats, rabbits, squil, hamsters, guinea pig. Various animal models are hyperlipidemic model, hypercholestermic model, hypolipidemic model, hereditary hypercholestermic model hereditary hyper lipidemic model, transgenic model. These models are used to observed effect of drug on diseased animal and find out various drugs for treatment of atherosclerosis disease.

INTRODUCTION

Atherosclerosis is a condition in which an artery wall thickens as the result of a build-up of fatty materials such as cholesterol. It is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries, in large part due to the accumulation of macrophage white blood cells and promoted by low-density lipoproteins without adequate removal of fats and cholesterol from the macrophages by functional lipoproteins. It is commonly referred to as a hardening or furring of the arteries. It is caused by the formation of multiple plaques within the arteries. [1] Hyperlipidemia is the most prevalent indicator for susceptibility to atherosclerotic heart disease. It is characterized by abnormally elevated lipid such as triglyceride, cholesterol and lipoprotein. Increase level of low density lipid and very low density lipid in the blood. This is supported by an abundance of congruent result from genetic, epidemiological, experimental animal studies and clinical trials that the presence of high plasma lipid cholesterol increases the incidence of coronary heart diseases. Atherosclerosis is the preliminary lipid disorder that affects the arteries and many factors contributing to its etiology, among them diabetes, glucocorticoid, diet, psychological factors are the major one. A crucial step in the pathogenesis of atherosclerosis is believed to be oxidative modification of low density lipid. [2][3][4] The atheromatous plaque is divided into three distinct components:

1. The center of large plaques, composed of macrophages nearest the lumen of the artery
2. The atheromas, which is the nodular accumulation of a soft, flaky, yellowish material at underlying areas of cholesterol crystals
3. Calcification at the outer base of older/more advanced lesions.

Arteriosclerosis is a general term describing any hardening of medium or large arteries. Atherosclerosis is a hardening of an artery specifically due to an atheromatous plaque. The term atherogenic is used for substances or processes that cause atherosclerosis. These complications of advanced atherosclerosis are chronic, slowly progressive and cumulative. Most commonly, soft plaque suddenly ruptures, causing the formation of a thrombus that will rapidly slow or stop blood flow, leading to death of the tissues fed by the artery in approximately 5 minutes. This catastrophic event is called an infraction. One of the most common recognized scenarios is called coronary thrombosis of a coronary artery, causing myocardial infarction. Even worse is the same process in an artery to the brain, commonly called stroke. Another common scenario in very advanced disease is claudication from insufficient blood supply to the legs, typically due to a combination of both stenosis and aneurysmal segments

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narrowed with clots. Since atherosclerosis is a body-wide process, similar events occur also in the arteries to the brain, intestines, kidneys, legs, etc. Many infarctions involve only very small amounts of tissue and are termed clinically silent, because the person having the infarction does not notice the problem, does not seek medical help; physicians do not recognize what has happened.

Signs and Symptoms:

Atherosclerosis typically begins in early adolescence, and is usually found in most major arteries, yet is asymptomatic and not detected by most diagnostic methods during life. Atheroma in arm, or more often in leg arteries, which produces decreased blood flow is called disease. According to United States data for the year 2004, for about 65% of men and 47% of women, the first symptom of atherosclerotic cardiovascular disease is heart attack or sudden cardiac death. Most artery flow disrupting events occur at locations with *less* than 50% lumen narrowing, 20% stenosis. In arterial disease, overemphasizes lumen narrowing, as opposed to compensatory external diameter enlargement. Cardiac stress testing, traditionally the most commonly performed non-invasive testing method for blood flow limitations, in general, detects only lumen narrowing of 75% or greater, although some physicians claim that nuclear stress methods can detect as little as 50%. [5]

Causes:

Atherosclerosis develops from low-density lipoprotein molecules becoming oxidized by free radicals, particularly reactive oxygen species. When oxidized low density liproteins comes in contact with an artery wall, a series of reactions occur to repair the damage to the artery wall caused by oxidized low density lipoprotein. The low density lipoprotein molecule is globular shaped with a hollow core to carry cholesterol throughout the body. Cholesterol can move in the bloodstream only by being transported by lipoproteins. The body's immune system responds to the damage to the artery wall caused by oxidized low density lipoprotein by sending specialized white blood cells to absorb the oxidized low density lipoprotein forming specialized foam cells. These white blood cells are not able to process the oxidized low density lipoprotein, and ultimately grow then rupture, depositing a greater amount of oxidized cholesterol into the artery wall. This triggers more white blood cells, continuing the cycle. Eventually, the artery becomes inflamed. The cholesterol plaque causes the muscle cells to enlarge and form a hard cover over the affected area. This hard cover is what causes a narrowing of the artery, reduces the blood flow and increases blood pressure. Some researchers believe that atherosclerosis may be caused by an infection of the vascular smooth muscle cells; chickens, for example, develop atherosclerosis when infected. Also, cytomegalovirus infection is associated with cardiovascular diseases. Hyperlipidemia is the most prevalent indicator for susceptibility to atherosclerotic heart disease. It is characterized by abnormally elevated lipid and lipoprotein levels in the blood. This is supported by an abundance of congruent result from genetic, epidemiological, experimental animal studies and clinical trials that the presence of high plasma lipid cholesterol increases the incidence of coronary heart diseases. [6-8]

Current scenario:

The frequency of clinical manifestations of atherosclerosis in Great Britain, west of Scotland in particular, is especially high. The same is true of Finland, in particular, and Scandinavia in general. Russia and many of the former states of the Soviet Union have recently experienced an exponential increase in the frequency of coronary heart disease that likely is the result of widespread economic hardship and social upheaval, a high prevalence of cigarette habituation, and a diet high in saturated fats. The frequency of coronary heart disease in the Far East is significantly lower than that documented in the West. Ill-defined genetic reasons for this phenomenon may exist, but significant interest surrounds the role of diet and other environmental factors in the absence of clinical atherosclerotic vascular disease in these populations. Atherosclerotic cardiovascular disease is also rare on the African continent, although growing evidence indicates that this too is changing as a result of rapid westernization and urbanization of the traditionally rural and agrarian African populations. The prevalence of coronary heart disease is also increasing in the Middle East, India, and Central and South America. The rate of coronary artery disease in ethnic immigrant populations in the United States approaches that of the disease in whites, supporting the role of these putative environmental factors. Various animal used for find out effect of atherosclerosis on body. Animal used are mice, rat, rabbit, Japanese sea quail, Cockerel, hamster, dog, guinea pig, cynomolgus monkey.

Mortality/Morbidity:

Atherosclerosis is the leading cause of death in the developed world, and atherosclerosis is predicted to be the leading cause of death in the developing world within the first quarter of the next century. In 2005, cardiovascular disease was responsible for 864, 5000 deaths, or 35.3% of all deaths that year. They included 151,000 deaths from myocardial infarction and 143,600 deaths from strok. An encouraging decrease in mortality due to coronary heart

disease in the developed world has occurred. Unfortunately, this decrease has not occurred in the developing world, and an exponential increase in tobacco habituation and the adoption of a Western diet high in saturated fats likely predicts the continued increase in death and disability due to coronary heart disease.

PATHOPHYSIOLOGY

Atharomatous plaques are patchy changes that develop in the tunica intima of large and medium size arteries. They consist of accumulation of cholesterol and other lipid compound's, excess smooth muscle and fat filled monocytes (foam cells). The plaque is covered with fibrous cap. As plaques grow them spread along the artery wall forming swelling that protrude in to lumen. Eventually whole thickness of the wall and long sections of vessel may be affect. Plaques may rupture, exposing subintimal material to the blood. This may cause thrombosis and vasospasm and will compromise blood flow. Arteries most commonly involved are those in the heart, brain, kidney, small intestine and lower limb. [9] The hallmark of atherosclerosis is the atherosclerotic plaque, which contains lipids, inflammatory cells, smooth muscle cells, connective tissue, thrombi, and Ca deposits. All stages of atherosclerosis from initiation and growth to complication of the plaque are considered an inflammatory response to injury. Endothelial injury is thought to have a primary role. Atherosclerosis preferentially affects certain areas of the arterial tree. Nonlaminar or turbulent blood flow leads to endothelial dysfunction and inhibits endothelial production of nitric oxide, a potent vasodilator and anti-inflammatory molecule. Such blood flow also stimulates endothelial cells to produce adhesion molecules, which recruit and bind inflammatory cells. Risk factors for atherosclerosis, oxidative stressors, angiotensin II, and systemic infection and inflammation also inhibit nitric oxide production and stimulate production of adhesion molecules, proinflammatory cytokines, chemo tactic proteins, and vasoconstrictors; exact mechanisms are unknown. The net effect is endothelial binding of monocytes and T cells, migration of these cells to the sub endothelial space, and initiation and perpetuation of a local vascular inflammatory response. Monocytes in the sub endothelium transform into macrophages. Lipids in the blood, particularly low density lipoprotein and very low density lipoprotein, also bind to endothelial cells and are oxidized in the sub endothelium. Uptake of oxidized lipids and macrophage transformation into lipid-laden foam cells result in the typical early atherosclerotic lesions called fatty streaks. Degraded erythrocyte membranes that result from rupture of vasa vasorum and intraplaque hemorrhage may be an important additional source of lipids within plaques. Macrophages elaborate proinflammatory cytokines that recruit smooth muscle cell migration from the media and that further attract and stimulate growth of macrophages. Various factors promote smooth muscle cell replication and increase production of dense extracellular matrix. The result is a sub endothelial fibrous plaque with a fibrous cap, made of intimal smooth muscle cells surrounded by connective tissue and intracellular and extracellular lipids. A process similar to bone formation causes calcification within the plaque. Atherosclerotic plaques may be stable or unstable. Stable plaques regress, remain static, or grow slowly over several decades until they may cause stenosis or occlusion. Unstable plaques are vulnerable to spontaneous erosion, fissure, or rupture, causing acute thrombosis, occlusion, and infarction long before they cause stenosis. Most clinical events result from unstable plaques, which do not appear severe on angiography; thus, plaque stabilization may be a way to reduce morbidity and mortality. The strength of the fibrous cap and its resistance to rupture depend on the relative balance of collagen deposition and degradation. Plaque rupture involves secretion of metalloproteinase's, cathepsins and collagenases by activated macrophages in the plaque. These enzymes digest the fibrous cap, particularly at the edges, causing the cap to thin and ultimately rupture. T cells in the plaque contribute by secreting cytokines. Cytokines inhibit smooth muscle cells from synthesizing and depositing collagen, which normally reinforces the plaque. Once the plaque ruptures, plaque contents are exposed to circulating blood, triggering thrombosis; macrophages also stimulate thrombosis because they contain tissue factor, which promotes thrombin generation in vivo. One of 5 outcomes may occur:

1. The resultant thrombus may organize and be incorporated into the plaque, changing the plaque shape and causing its rapid growth.
2. The thrombus may rapidly occlude the vascular lumen and precipitate an acute ischemic event.
3. The thrombus may embolize.
4. The plaque may fill with blood, balloon out, and immediately occlude the artery.
5. Plaque contents may embolize, occluding vessels downstream.

Plaque stability depends on multiple factors, including plaque composition of relative proportion of lipids, inflammatory cells, smooth muscle cells, connective tissue, thrombus and wall stress size and location of the core and configuration of the plaque in relation to blood flow. By contributing to rapid growth and lipid deposition, intraplaque hemorrhage may play an important role in

transforming stable into unstable plaques. In general, unstable coronary artery plaques have high macrophage content, a thick lipid core, and a thin fibrous cap; they narrow the vessel lumen by < 50% and tend to rupture unpredictably. Unstable carotid artery plaques have the same composition but typically cause problems through severe stenosis and occlusion or deposition of platelet thrombi, which embolize rather than rupture. Low-risk plaques have a thicker cap and contain fewer lipids; they often narrow the vessel lumen by > 50% and may produce predictable exercise-induced stable angina. Clinical consequences of plaque rupture in coronary arteries depend not only on plaque anatomy but also on relative balance of procoagulant and anticoagulant activity in the blood and on the vulnerability of the myocardium to arrhythmias. A link between infection and atherosclerosis has been observed, specifically an association between serologic evidence of certain infections such as Chlamydia pneumoniae, cytomegalovirus and coronary artery disease. Putative mechanisms include indirect effects of chronic inflammation in the bloodstream, cross-reactive antibodies, and inflammatory effects of infectious pathogens on the arterial wall. [10] Dyslipidemia, hypertension, and diabetes promote atherosclerosis by amplifying or augmenting endothelial dysfunction and inflammatory pathways in vascular endothelium. In dyslipidemia, sub endothelial uptake and oxidation of low density lipid increases; oxidized lipids stimulate production of adhesion molecules and inflammatory cytokines and may be antigenic, inciting a T cell–mediated immune response and inflammation in the arterial wall. High density lipid protects against atherosclerosis via reverse cholesterol transport, it may also protect by transporting antioxidant enzymes, which can break down and neutralize oxidized lipids. The role of hypertriglyceridemia in atherogenesis is complex, although it may have a small independent effect. [11]

Causes of atheroma:

Heredity , family history, Obesity, Gender, Diet, Increasing age, Smoking cigarettes, Diabetes mellitus, Excessive emotional stress, Hypertension, sedentary lifestyle, hyper lipidemia, excessive alcohol consumption. [9]

1. **Hereditary, family history:**
Genetic factor play a significant role in atherogenesis hereditary genetic de arrangement of lipoprotein metabolism predispose individual to high blood lipid level and familial hyper cholesteromia. [12]

2. **Obesity:** Obesity is related not only to total body weight but also to the distribution of total fat. Central or visceral obesity in which fat accumulates in trunk and in abdominal cavity is associated with much higher risk for several diseases than is excess accumulation of fat diffusely in subcutaneous tissue. [13]

3. **Gender:** Occurrence of atherosclerosis more chances all ages in male but female are less. In its pre menopausal age is probably due to high level of oestrogens and high density lipo protein both of which have antiatherogenic influence. [12]

4. **Diet:** It contains high fat and cholesterol responsible for atherosclerosis and low intake of anti oxidant. [9, 14]

5. **Increasing age:** Atherosclerosis is an age related disease. Early lesions of the atherosclerosis may be present in child hood. [12] Risk of developing atherosclerosis lesions is increases from 40 to 60 ages. [13]

6. **Smoking cigarettes:** The increase risk and severities of atherosclerosis in smokers due to reduced level of high density lipoproteins and accumulation of carbon monoxide in blood that produced carboxy heamoglobin and eventually hypoxia in arteriole wall favoring atherosclerosis. [12]

7. **Diabetes mellitus:** Atherosclerosis is more common and develops at early ages in people with both insulin dependent ant non insulin dependent diabetes mellitus. Causes of increasing severity of atherosclerosis are complex and numerous which include aggregation of platlate increase low density lipoprotein and decrease high density lipoprotein. [12]

8. Hypertension: It is other major risk factor in development of atherosclerotic ischemic heart disease. It acts probably by mechanical injury to arterial wall due to increase blood pressure. Systolic pressure of over 160mm/Hg and diastolic is over 95mm/Hg. [12]

9. Hyperlipidemia: The atherosclerotic plaque contains cholesterol and cholesterols esters. Largely derived from the lipoprotein in the blood. [12] High serum cholesterol specially when associated with low value of high density lipoprotein is strongly associated with coronary atheroma. There is increasing evidence that high serum triglycerides are independly link with coronary atheroma. [14]

10. **Life style:** It characterizes by aggressiveness, competitive drive, ambitiousness and a sense of urgency is associated with enhance risk of ischemic heart diseases compare with behaviors of relaxed and happy go lucky type. [12]

11. **Sedentary life style:** Lack of exercise is an independent risk for atherosclerosis. [14]

Dyslipidaemia:

The normal range of plasma total cholesterol concentration varies in different populations e.g. in the UK 25-30% of middle-aged people have serum cholesterol concentrations > 6.5 mmol/l, in contrast to a much lower prevalence in China. There are smooth gradations of increased cardiovascular risk with increased Low density lipoprotein-C and with reduced High density lipoprotein-C. Dyslipidaemia may be primary or secondary. The primary forms are due to a combination of diet and genetics. An especially great risk of ischemic heart disease occurs in a subset of primary type IIa hyperlipoproteinaemia caused by single-gene defects of Low density lipoprotein receptors, this is known as familial hypercholesterolemia, and the serum cholesterol concentration in affected adults is typically > 8 mmol/l in heterozygote's and 12-25 mmol/l in homozygote's. Study of familial hypercholesterolemia enabled Brown & Goldstein to define the Low density lipoprotein receptor pathway of cholesterol homeostasis. [11]

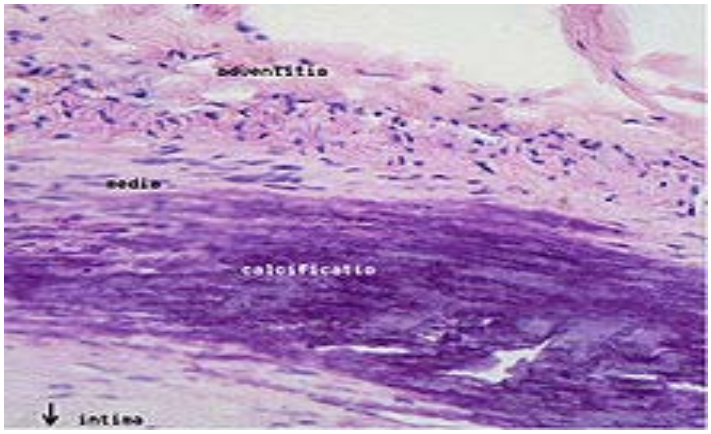

Fig.no.1: Microphotography of arterial wall with calcified (violet colour) atherosclerotic plaque (haematoxillin & eosin stain)

DIAGNOSIS

Areas of severe narrowing, stenosis, detectable by angiography, and to a lesser extent "stress testing" have long been the focus of human diagnostic techniques for cardiovascular disease, in general. However, these methods focus on detecting only severe narrowing, not the underlying atherosclerosis disease. As demonstrated by human clinical studies, most severe events occur in locations with heavy plaque, yet little or no lumen narrowing present before debilitating events suddenly occur. Plaque rupture can lead to artery lumen occlusion within seconds to minutes, and potential permanent debility and sometimes sudden death. Plaques that have ruptured are called complicated plaques. The lipid matrix breaks through the thinning collagen gap and when the lipids come in contact with the blood, clotting occurs. After rupture the platelet adhesion causes the clotting cascade to contact with the lipid pool causing a thrombus to form. This thrombus will eventually grow and travel throughout the body. The thrombus will travel through different arteries and veins and eventually become lodged in an area that narrows. Once the area is blocked, blood and oxygen will not be able to supply the vessels and will cause death of cells and lead to necrosis and poisoning. Serious complicated plaques can cause death of organ tissues, causing serious complications to that organ system. Greater than 75% lumen stenosis used to be considered by cardiologists as the hallmark of clinically significant disease because it is typically only at this severity of narrowing of the larger heart arteries that recurring episodes of angina and detectable abnormalities by stress testing methods are seen. However, clinical trials have shown that only about 14% of clinically-debilitating events occur at locations with this, or greater severity of stenosis. The majority of events occur due to atheroma plaque rupture at areas without narrowing sufficient enough to produce any angina or stress test abnormalities. Thus, since the later-1990s, greater attention is being focused on the "vulnerable plaque". [15] Though any artery in the body can be involved, usually only severe narrowing or obstruction of some arteries, those that supply more critically-important organs are recognized. Obstruction of arteries supplying the heart muscle results in a heart attack. Obstruction of arteries supplying the brain results in a stroke. These events are life-changing, and often result in irreversible loss of function because lost heart muscle and brain cells do not grow back to any significant extent, typically less than 2%. Over the last couple of decades, methods other than angiography and stress-testing have been increasingly developed as ways to better detect atherosclerotic disease before it becomes symptomatic. These have included both anatomic detection methods and physiologic measurement methods. Examples of anatomic methods include: coronary calcium scoring by CT, carotid intimal media thickness measurement by ultrasound, and IVUS. Examples of physiologic methods include: lipoprotein subclass analysis, HbA1c, hs-CRP, and homocysteine. The example of the metabolic syndrome combines both anatomic and physiologic (blood pressure, elevated blood glucose) methods. Advantages of these two approaches: The anatomic methods directly measure some aspect of the actual atherosclerotic disease process itself, thus offer potential for earlier detection, including before symptoms

start, disease staging and tracking of disease progression. The physiologic methods are often less expensive and safer and changing them for the better may slow disease progression, in some cases with marked improvement. Disadvantages of these two approaches: The anatomic methods are generally more expensive and several are invasive, such as IVUS. The physiologic methods do not quantify the current state of the disease or directly track progression. For both, clinicians and third party payers have been slow to accept the usefulness of these newer approaches.

Laboratory tests:

Fasting lipids and glucose are needed to determine if the metabolic syndrome is present. The measurement of additional biomarkers associated with insulin resistance must be individualized. Such tests might include apo b, high sensitivity CRP, fibrinogen, uric acid, urinary microalbumin and liver function tests. A sleep study should be performed if symptom of OSA is present. If PCOS is suspected based on clinical features and an ovulation. Testosterone, Luteinizing hormone, and follicle stimulating hormone should be measured. [16]

TREATMENT

1. Antihyperlipidemic drug therapy
2. Surgical treatment [17]

Classification of antihyperlipidemic: [11]

1. Statins: HMG-COA reductase inhibitors:

The rate-limiting enzyme in cholesterol synthesis is HMG-CoA reductase, which catalyses the conversion of HMG-CoA to mevalonic acid. Simvastatin , lovastatin and pravastatin are specific, reversible, competitive HMG-CoA reductase inhibitors with K_i values of approximately 1 nmol/l. Atorvastatin and rosuvastatin are long-lasting inhibitors. Decreased hepatic cholesterol synthesis up-regulates low-density lipoprotein receptor synthesis, increasing low-density lipoprotein cholesterol clearance from plasma into liver cells. The main biochemical effect of statins is therefore to reduce plasma low-density lipoprotein cholesterol. There is also some reduction in plasma triglyceride and increase in high density lipoprotein cholesterol. Several large randomized placebo-controlled trials of the effects of HMG-CoA reductase inhibitors on morbidity and mortality have been positive.

2. Fibrates:

Several fibric acid derivatives (fibrates) are available, including bezafibrate, ciprofibrate, gemfibrozil, fenofibrate and clofibrate. These cause a marked reduction in circulating very low density lipoprotein and hence triglyceride, with a modest (approximately 10%) reduction in low-density lipoprotein cholesterol and an approximately 10% increase in high density lipoproteins cholesterol. In one study, gemfibrozil reduced coronary heart disease by approximately one-third compared with placebo in middle-aged men with primary hyperlipoproteinaemia. An high density lipoproteins cholesterol intervention trial performed by the US Veterans Affairs Department in some 2500 men with coronary heart disease and low high density lipoprotein cholesterol together with low low-density lipoprotein cholesterol showed that gemfibrozil increased in high density lipoprotein cholesterol and reduced coronary disease and stroke. Event rates were linked to changes in high density lipoprotein cholesterol but not to triglycerides or to low-density lipoprotein cholesterol, suggesting that increasing in high density lipoprotein cholesterol with a fibrate reduces vascular risk. The mechanism of action of fibrates is complex. They are agonists for a subset of lipid-controlled gene regulatory elements peroxisome proliferators activated receptor Rs^4, peroxisome proliferators activated receptor ά, which are members of the super family of nuclear receptors, in humans; the main effects are to increase transcription of the genes for lipoprotein lipase, apoA1 and apoA5. They increase hepatic LDL-C uptake. In addition to effects on lipoproteins, fibrates reduce plasma C-reactive protein and fibrinogen, improve glucose tolerance, and inhibit vascular smooth muscle inflammation by inhibiting the expression of the transcription factor nuclear factor κB . As with the pleiotropic effects of statins, there is great interest in these actions, although again it is unknown if they are clinically important.

3. Drugs that inhibit cholesterol absorption:

Historically, bile acid-binding resins were the only agents available to reduce cholesterol absorption and were among the few means to lower plasma cholesterol. Decreased absorption of exogenous cholesterol and increased metabolism of endogenous cholesterol into bile acids in the liver lead to increased expression of low-density lipoprotein receptors on hepatocytes, and hence to increased clearance of low-density lipoprotein cholesterol from the blood and a reduced concentration of low-density lipoprotein cholesterol in plasma. Such resins reduce the incidence of myocardial infarction, but their effect is modest and they are bulky, unpalatable and cause diarrhea. With the introduction of statins, their role in treating dyslipidaemia was relegated largely to additional treatment in patients with severe disease (e.g. familial hypercholesterolemia).

4. Nicotinic acid or its derivatives:

Nicotinic acid is a vitamin, and as such is essential for many important metabolic processes. Quite separately from this, it has been used in gram quantities as a lipid-lowering agent. Nicotinamide inhibits hepatic triglyceride production and very low-density lipoprotein secretion, with reductions in triglyceride and low-density lipoprotein cholesterol including Lp(a), and increase in high density lipoprotien cholesterol. The mechanism is poorly understood but is believed to be initiated by an effect on lipolysis via a G-protein-coupled orphan receptor called HM74A and present in adipocyte membranes, It also influences hepatic diacylglycerol transferase. Long-term administration to survivors of myocardial infarction reduced mortality in the coronary drug project trial, but unwanted effects limit its clinical use. A modified-release preparation is better tolerated, with preserved lipid effects.

5. Fish oil derivatives:

Omega-3 marine triglycerides reduce plasma triglyceride concentrations but increase cholesterol. Plasma triglyceride concentrations are less strongly associated with coronary artery disease than is cholesterol, but there is epidemiological evidence that eating fish regularly does reduce ischaemic heart disease, and dietary supplementation with n-3 polyunsaturated fatty acids (PUFA) improves survival in patients who have recently had a myocardial infarction. The mechanism may be the potent antiarrhythmic effects of PUFA. The mechanism of action of fish oil on plasma triglyceride concentrations is unknown. Fish oil is rich in PUFA, including eicosapentaenoic and docosahexaenoic acid, and it has other potentially important effects including inhibition of platelet function, prolongation of bleeding time, anti-inflammatory effects and reduction of plasma fibrinogen. Eicosapentaenoic acid substitutes for arachidonic acid in cell membranes and gives rise to 3-series prostaglandins and thromboxanes (that is, prostanoids with three double bonds in their side-chains rather than the usual two), and 5-series leukotrienes. This probably accounts for their effects on haemostasis, because thromboxane A_3 is much less active as a platelet-aggregating agent than is thromboxane A_2, whereas PGI_3 is similar in potency to PGI_2 as an inhibitor of platelet function. The alteration in leukotriene biosynthesis probably underlies the anti-inflammatory effects of fish oil.

SCREENING METHODS

In vivo methods:

A) Triton Wistar Rat 1339 Induced hyperlipidemia [20]

Purpose and rational: The systemic administration of the surfactant triton to rats results in a biphasic elevation of plasma cholesterol and triglycerides

Requirement:

Chemical: Surfactant, Triton, momordicia diocia roxb

Animal: Wistar strain male albino rats

Procedure:

Fourty two male wistar rats weighing 190gm to 230 gm were randomly divided into 7 groups. In each group contains 6 male rats and kept in their cages for 5 days prior dosing to allow for acclimization to laboratory condition. The animals were starved for 18hr and i.p. with 10% aqueous solution of triton at 400mg /kg body weight. The test drugs employed (or) the solvent for control was administered simultaneously with triton injection. Serum analyzed made on 24hr and 48 hr after triton injection. The drug was administered in the vehicle in the same volume orally. After administration of triton, in the 24hr, blood was collected by retro orbital puncture under ether anesthesia and subject to centrifugation to obtain serum. Again, 48hr, blood was collected by retro orbital puncture under ether anesthesia and subject to centrifugation to obtain serum with 2ml syringe.

Evaluation:

Serum was analyzed for serum triglyceride, serum total cholesterol, serum high density lipoprotein cholesterol, serum low density lipoprotein cholesterol, serum very low density lipoprotein cholesterol, serum glucose .The result is evaluated by ANOVA test and Dunnet Multiple comparison test.

B) Hypolipidemic activity in rats [21]

Rational and purpose:

Hyperlipoproteinemia with increased concentrations of cholesterol and triglyceride carrying lipoproteins is considered to be the cause of arteriosclerosis with its dual sequel of thrombosis and infarction. Lipoproteins are divided into 6 major classes: chylomicrons, chylomicron remnants, very low density lipoproteins, intermediate density lipoproteins, low density lipoproteins, and high density lipoproteins. High density lipoprotein promotes the removal of cholesterol from peripheral cells and facilitates its delivery back to the liver. Therefore, increased levels of high density lipoproteins are desirable. On the contrary, high levels of very low density lipoproteins and low density lipoproteins promote arteriosclerosis. Low density lipoproteins, especially in its oxidized form, is taken up by macrophages via a scavenger

mechanism. Therefore, anti-arteriosclerotic drugs should reduce very low density lipoproteins and low density lipoproteins and/or elevate high density lipoprotein.

Requirement:

Chemical: Methanol extract of trianthema portulacasstrum

Animal: Wistar albino male rats

Procedure:

Groups of 10 male Wistar rats weighing 180–200 g are used. They are given once daily in the morning over a period of 8 days the test compounds or the standard in various doses ranging from 1 to 100 mg/kg via stomach tube in a volume of 5 ml/kg. The control group is given the solvent (e.g., PEG 400) only. Body weight of each animal is registered at the beginning and at the end of the experiment. Twenty hours prior to the experiment food but not water is withdrawn. On the morning of the first day, blood samples are taken under light ether anesthesia by retro orbital puncture. Then, the first dose is applied. During the whole period, the animals have free access to food and water. Twenty hours prior the end of the experiment, food is again withdrawn and blood samples are taken by retro orbital puncture. Immediately thereafter, the animals are sacrificed and the liver removed, blotted free from blood and weighed. Samples of liver are frozen analysis. The bloods samples are centrifuged for 2 min. Total cholesterol and total glycerin as a measure of triglycerides are determined in each blood sample. To estimate the serum lipoproteins, the serum of each rat group is pooled. The serum lipoproteins are separated by means of a preparative ultracentrifuge e.g., KONTRON TGA 65, Rotor TFT 456. The separation of fractions very low density lipoprotein, low density lipoprotein, high density lipoprotein and of the subnatant of high density lipoprotein is carried out as follows: very low density lipoprotein native density of the serum (1.006), 16 h at 40000 rpm, Low density lipoprotein density range from 1.006 to 1.04, 18 h at 40 000 rpm, High density lipoprotein density range from 1.04 to 1.21, 18 hr at 40 000 rpm, Subnatant of High density lipoprotein density > 1.21. The density is adjusted by addition of a calculated amount of NaBr solution. Cholesterol is determined using Boehringer Mannheim test combinations by the CHOD-PAP high performance method and triglycerides by means of an enzymatic assay

Evaluation:

Cholesterol is determined using Boehringer Mannheim test combinations by the CHOD-PAP high performance method and triglycerides by means of an enzymatic assay.

C) Cholesterol-diet induced atherosclerosis in rabbits [22]

Purpose and Rational:

Rabbits are known to be susceptible to hypercholesterolemia and arteriosclerosis after excessive cholesterol feeding. Therefore, this approach has been chosen by to study the effect of potential anti-arteriosclerotic drugs.

Requirement:

Chemical: Dimethyl sulfoxide

Animal: White New Zealand male rabbits

Procedure:

Usually, male rabbits from an inbred strain, e.g., white New Zealand, at an age of 8–10 weeks are used. At the beginning of the experiment, blood is withdrawn from the marginal ear vein for determination of total cholesterol, total glycerides, and blood sugar. Groups of 10 animals are used for treatment with drugs or as controls. The rabbits are switched from commercial food to a diet supplemented with 0.3–2% cholesterol and kept on this regimen for a period of 10–12 weeks. One group is kept on normal diet. During and at the end of the experiment blood is taken for analysis. Usually, cholesterol and triglyceride levels increase several-fold over the original values. The animals are sacrificed and the thoracic aorta is removed, cleaned of surrounding tissues, and longitudinally cut and opened for fixation with formaldehyde. The tissue is stained with oil red. In animals fed a normal diet, the aorta does not show any staining, whereas in cholesterol-fed rabbits the aorta shows severe atherogenic lesions.

Evaluation:

The percentage of the intimal surface covered by the oil red positive lesions is calculated with a computerized plan meter. Statistical evaluation is performed by Dunnett's or Scheffé's test.

D) Hereditary hyperlipemia in rabbits [23]

Purpose and Rational: To produced hereditary hyperlipidemia in rabbit. To study the effect of potential anti-arteriosclerotic drugs.

Requirement:

Chemical: Probucol

Animal: Female DDY mice, Homozygous Wistar Hereditary Hyperlipidemic rabbits

Procedure:

Homozygous wistar hereditary hyperlipidemic rabbits were raised in Kyoto by mating heterozygous

and/or homozygous female wistar hereditary hyperlipidemic rabbits with homozygous male Wistar hereditary hyperlipidemic rabbits. At 2 months of age, eight rabbits were divided into two groups (group A and group B). Rabbits in group A (two males, two females) were fed standard rabbit chow for 6 months. Rabbits in group B (two males, two females) were raised with rabbit chow enriched with 1% (wt/wt) probucol for 6 months. The amount of daily diet for each animal was restricted to 100 g during the study period. Water was supplied ad lib. Six months later (at the age of 8 months), the rabbits were sacrificed and their blood and aortas were taken for analysis.

Evaluation:

Plasma levels of cholesterol were measured by the enzymatic method. **S**tatistical significance was determined by the student's *t* test.

E) Hereditary hypercholesteromia in rat [24]

Purpose and Rational: In contrast to Zucker-rats, these animals are normotriglyceridemic and non-obese. The hypercholesterolemia of the RICO rat is related to a decreased rate of catabolism of chylomicrons and low density lipoprotein, but more specifically to an excessive production of these two types of lipoproteins. This strain has been proposed to study hypolipidemic drugs, particularly those designed to decrease the plasma concentrations of chylomicrons and low density lipoprotein.

Requirement:

Material: Cyclodextrin

Animal: Wistar rats, RICO rat, PHHC rat

Procedure:

In order to establish a model of hypercholesterolemia in rats, the selective inbreeding of the wistar rats that were most responsive to dietary cholesterol without any addition of cholic acid and/or thyrotoxic substances was carried out. Briefly, five pairs of rats with highest basal cholesterolemia were selected from 100 rats for parent generation. In each generation, cholesterol was then measured in the rats on a standard chow and the animals were then shifted to 2 % cholesterol diet cholesterol dissolved in 5 % beef tallow for 2 weeks and cholesterol was determined again. The rats with maximal increase in cholesterolemia were then selected for further breeding. Although there were no dramatic changes in the baseline cholesterolemia 2.0 mmol/l and even in dietary cholesterol stimulated cholesterolemia 2-3 mmol/l in males and 3-4 mmol/l in females during first 8 generations, there was a dramatic shift of dietary induced cholesterolemia to concentration ~ 10 mmol/l after the 9th generation likely due to a gene Recombination (Poledne 1986). At the same time, the other line of animals that were not responsive to dietary cholesterol was selected by breeding animals unresponsive to dietary cholesterol. However, this line was lost after thirteen generations due to the low fertility. To assess the mode of transmission of hypercholesterolemia, the polygenic hereditary hypercholesteromic animals were crossbred with the animals of control line not responsive to dietary cholesterol. The offspring (F1) displayed the dietary induced hypercholesterolemia that was at the mid level between cholesterol concentrations of both parental strains. The standard deviation of cholesterolemia of F1 generation did not differ from that of their parents. If the dietary induced cholesterolemia would be a monogenic trait, it could be predicted that distribution of cholesterolemia in offspring of F1 animals (F2 generation) should have three discrete peaks corresponding to cholesterolemia of both parental strains and F1 generation in the ratio 1:1:2, respectively. That was not a case. The cholesterolemia distribution in F2 generation displayed a single peak at concentration corresponding to that of F1 generation and very high standard deviation. That strongly suggests that hypercholesterolemia in polygenic Hereditary Hypercholesteromic rats is polygenic.

Evaluation:

To examine such a possibility, the studies of microarray gene expression in the liver of Polygenic Hereditary Hypercholesteromic rats are currently in progress.

F) Transgenic animal model [25]

Purpose and Rational: Transgenic mice lacking apolipoprotein E showed severe hypercholesterolemia and atherosclerosis.

Requirement:

Chemical: GW501516

Animal: Mice

Procedure:

The widely used model is the Apo E knockout mouse originally created by Nubuyo Maeda, University of North Carolina, and Chapell Hill, NC. These Apo E knockout mice have spontaneously elevated plasma cholesterol levels, and develop atherosclerosis even on regular chow within 3–4 months. The time dependent progression of

atherosclerosis leads to lesions similar in histopathology to those observed in humans. This animal model is used as background for atherosclerosis research and target validation.Walsh (1989) and Rubin (1991) integrated human apolipoprotein A-I gene in transgenic mice resulting in an increase of high density lipoprotein levels. Linton (1993) described the development of transgenic mice expressing high levels of human apolipoprotein B48 and human apolipoprotein B100 which are considered to be atherogenic. Transgenic mice lacking apolipoprotein E showed severe hypercholesterolemia and atherosclerosis over expression of apolipoprotein E in transgenic mice reduced plasma cholesterol and triglyceride levels, prevented hypercholesterolemia and inhibited the formation of fatty streak lesions

Evaluation:

For evaluation of the anti-atherosclerotic effect, the mice were orally treated with GW501516 for 18 weeks and atherosclerosis at the aortic valves was determined by cross sectional lesion analysis

G) Fructose induced hypertriglyceridemia in rat [26]

Purpose and Rational: Rats switched from a diet low in carbohydrates and high in protein to a high intake of fructose, develop an acute hypertriglyceridemia. Compounds are tested for inhibition of this phenomenon.

Requirement:

Chemical: *Serratia Liquefaciens*

Animal: Sprague Dawley Rat

Procedure:

Male Sprague Dawley rats weighing 200–250 g are fed over a period of one week a diet enriched in protein with reduced carbohydrate content. Groups of 10 animals are treated for 3 days daily with the test compound or the standard or the vehicle (polyethylene glycol) by oral gavage. From the second to the third day water is with held for a period of 24 h. immediately afterwards, the animals are offered 20% fructose solution and libitum for a period of 20 h. After this time which is also 20 hr after the last application of the test compound, the animals are anesthetized with ether and 1.2 ml blood is withdrawn by retro orbital puncture. The blood is centrifuged for 2 min at 16 000 *g*. Total glycerol is determined in the serum according to Eggstein and Kreutz (1966) and total cholesterol according to Richterlich and Lauber (1962).

Evaluation:

The average values of total glycerol and total cholesterol of the treated groups are compared with the control group using Student's *t*-test.

H) Hypolipidemic activity in Syrian hamsters [27]

Purpose and Rational: The Syrian hamster (*Mesocricetus auratus*) is a widely used animal to study the effects of drugs and diet on lipoprotein metabolism. Several in human approved lipid lowering drug like HMG-CoA reductase inhibitors, or cholestyramine lower plasma cholesterol in hamster. The lipoprotein and bile acid metabolism of the hamster is closer to human than the lipoprotein.

Requirement:

Material: HMG-CoA reductase inhibitors

Animal: Syrian hamsters

Procedure:

The Syrian hamster has recently emerged as a small animal model for atherosclerosis research. They are easy to handle and are more human like in their response to diet modification than most other rodents. Atherosclerosis can be induced in the Syrian hamster by feeding a diet enriched with cholesterol and saturated fat. Male Syrian hamsters weighing 95–125 g at the start of the experiment are randomly assigned to form groups of 6 animals each. After 1 month of diet they develop sub endothelial foam cells which are precursors of fatty streaks. With continued exposure to fatty diet the lesions can progress into complex plaques resembling human lesions. After 1 week on these diets, the animals are anesthetized with diethyl ether, a blood sample is taken from the superior venacava and the liver is removed and weighed. Microsomes are prepared by ultracentrifugation from the livers.

Evaluation:

The plasma is analyzed for total cholesterol using a colorimetric enzymatic assay
(Merck, CHOD-iodine, BDH). The cholesterol content of high density lipoprotein is determined using a precipitation kit

I) Effect of HMG-CoA-reductase inhibitors in vivo [28]

Purpose and Rational: A strain of rabbits with heritable hyperlipidemia, the WHHL strain, has been described by Watanabe. These animals develop digital xanthoma and aortic and coronary atherosclerosis already at an early age. This animal is considered to be a suitable model for the evaluation of preventive or even regressive effects of drugs on hyperlipidemia and atherosclerosis.

Requirement:
Chemical: Glutathione, Lovastatin
Animal: Zucker obese rat, WHHL rabbits

Procedure:

Male heterozygous WHHL rabbits weighing 1.8 to 2.5 kg at an age between 8 and 20 weeks are used. The animals are housed individually under standard conditions and are allowed to accommodate 2 weeks prior to treatment. The test compounds are suspended in 0.5% methylcellulose and are administered each day orally by gavage in the afternoon to insure an increased plasma level at night, since in man HMG-CoA reductase activity has been found to be higher at night than during daytime (Shapiro and Rodwell 1969; Shefer 1972) similar to the enzyme in rodents. The treatment is continued for 14 days. Blood samples are taken in the morning without previous feeding. Two ml of blood are drawn from the outer ear vein 5 days prior to the beginning of treatment, on days 3 and 8 of treatment and 30 days after the end of treatment for the determination of biochemical parameters. In addition, 6 ml blood are drawn at the first and the last day of treatment and 10 days after the end of treatment for determination of biochemical parameters and lipoprotein profile.

Evaluation: The separation of serum lipoproteins by gel permeation chromatography is performed according to Ha and Barter. Student's paired *t*-test is used to calculate for each group the significance of difference between mean values.

J) Lymph fistula model for cholesterol absorption [29]

Purpose and Rational: Direct evidence for an inhibitory effect on cholesterol absorption can be obtained by the lymph-fistula model in rats. This model also provides an indication as to the duration of inhibition and the relative selectivity of the compound on the absorption of cholesterol versus triglyceride and phospholipids.

Requirement:
Chemical: Telazol,
Animal: Rats

Procedure:

Rats are anesthetized by an intramuscular injection of Telazol 40 mg/kg. Silicon rubber cannulae are placed into the main mesenteric lymph duct and into the duodenum and secured with sutures. Animals are allowed to recover from surgery overnight in restraining cages while infused intraduodenally with 2% dextrose in saline containing 0.03% KCl (2.5 ml/h). Drinking water is allowed at labium during this recovery period. At 6:00 A.M. the following day, the drinking water is removed and a 2-h basal lymph sample is collected. Then, the animals are given the ACAT inhibitor at a specified dose as a single bolus into the duodenal cannula using an aqueous CMC/Tween suspension vehicle. Controls receive a bolus injection of the vehicle alone. Immediately after the drug dose, a lipid emulsion containing 0.1% cholesterol, 0.11% sodium taurocholate, 15% Intralipid (20%, Kabivitrium Inc.), 2.4% safflower oil, and 82.6% saline is infused into the duodenal cannula (3 ml/h). Then, four 2-h lymph collections are obtained. The lymph samples are extracted into hexane in the presence of a stigmasterol internal standard. Total and free cholesterol are quantitated by liquid gas chromatography.

Evaluation:

Esterified cholesterol of lymph is determined from difference between total and free cholesterol by using liquid gas chromatography.

In vitro methods:

K) Inhibition of the isolated enzyme HMG-CoA-reductase in vitro [28]

Purpose and Rational: For screening purposes, studies on the inhibition of HMG-CoA reductase obtained from rat liver microsomal fraction can be used.

Requirement:
Chemical: Dithiothreitol
Animal: Rats

Procedure:

The inhibitory activity of the test compound on HMGCoA reductase is estimated with soluble enzyme preparations obtained from the microsomal fraction of rat liver. The enzyme reaction is carried out with 50 µl partially purified HMGCoA reductase in buffer containing 25 mM Tris, 10 mM EDTA, and 10 mM dithiothreitol at pH 7.5, 20 µl of 910 µM HMG-CoA solution containing 100 nCi (3.7 KBq) of 14C-HMG-CoA and 20 µl of NADPH regenerating system (5.2 × 10–2 M glucose- 6-phosphate, 1 unit glucose-6-phosphate dehydrogenase, 5.3 × 10–3 M NADP), with the actual concentration of 50 mM NADPH. The final incubation volume is 200 ul. The main reaction is preceded by 20 min preincubation with the NADPH regenerating system at 37 °C, followed by 20 min incubation at 37 °C of the completed samples with the test compound or the standard and stopped by addition of 75 µl 2 N HClO4. After 60 min at room temperature, the samples are cooled in an

.

Sr. no.	Plant /Synthetic Drug	Animal model used	Name of Author
1	Nicotinic acid and resins, Avasimibe, fibrates, statins	Genetic mice model [30]	Brian R. Krause ,Hans M.G. Princen
2	AcylCoA:cholesterol acyltransferase	Hypocholesterolaemic animal model [31]	Hiroshi Tanaka, Teiji Kimura
3	Taurine	Hypercholesterolemia japnese squil model [32]	Murakami S, Sakurai T, Tomoike H, Sakono M, Nasu T, Fukuda N.
4	B-sitosterol	Hypercholesterolemia dogs model [33]	George W. Melchior and James F. Harwell
5	Clarithromycin	*Chlamydia pneumoniae* Induced Rabbit Model [34]	Ignatius W. Fong, Brian Chiu, Esther Viira, Dan Jang, James B. Mahony
6	Docosahexaenoic acid	Transgenic animal model [35]	Mary Sorci-Thomas, Cynthia L. Hendricks, and Mary W. Kearns
7	Sphaeranthus indicus	hyperlipidemia in rats model [36]	VV Pande, Sonal Dubey
8	Acyl CoA:cholesterol acyltransferase	Hypercholesterolemia animal model [37]	Drago R. Sliskovic, Andrew D. White
9	Sirolimus	Apolipoprotein E-Deficient Mouse Model [38]	Kun L. Ma, Xiong Z. Ruan, Stephen H. Powis, John F. Moorhead, and Zac Varghese
10	Dilemmas	Hypercholesterolemia animal model [39]	D.M. Kusters , S.J.M. Homsma
11	Ezetimibe	Apolipoprotein E-Deficient Mouse Model of Human Atherosclerosis [40]	A. L. Catapano
12	MF-tricyclic	Apolipoprotein E-Deficient Mouse Model of Human Atherosclerosis [41]	David Rott, Jianhui Zhu
13		Apolipoprotein E-Deficient Mouse Model of Human Atherosclerosis [42]	T.P. O'Neill
14		Hypercholesterolemia [43]	Amalia E. Yanni
15		Hypercholesterolemic mice model [44]	Masato Tsutsui, Yasuko Yatera Hiroaki Shimokawa
16	Colestipol	SEA quail model [45]	Audax, , Leitchfield
17	Cicaprost	hypercholesterolemic rabbit model [46]	Marina Brauna,ThomasÂ Hohlfelda, Petera Kienbauma,ArturArona Webera, Marioa Sarbiab, KarstenA Schroa

ice-bath and neutralized by addition of 75 µl 3 N potassium acetate. Supplementing the volume with water to 500 µl, the precipitate is centrifuged and 250 µl of the clear supernatant are applied to a column (0.6 × 8.0 cm) of BIORAD AG1-X8 (100–200 mesh). Mevalonolactone is eluted with water discarding the first 750 µl and collecting the next 3 500 µl. Five hundred µl of the eluate are used for measurement in duplicate, mixed in vials with 10 ml Quickscint (Zinsser) and measured in a liquid scintillation counter (Beckman). The assay is generally performed in triplicate. Lovastatin sodium is used as standard.

Evaluation:

The mean values with and without inhibitors are compared for the calculation of inhibition. IC50 values are calculated

CONCLUSION

There is no perfect animal model that completely replicates all stages of human atherosclerosis, yet these small animal models are a promising entity in exploring the etiopathogenesis and regression of atherosclerosis. But animal model are use to produce disease in animal and observe effect of drug. Animal model are use to find new chemical moiety of drug for treatment of disease and find out adverse drug reaction, side effects of drug. Animal screening is very important for developing new drug.

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