Review Article

Animal Models of Hyperlipidemia: An Overview

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ABSTRACT

Several frequently used animal models of atherosclerosis are described in this mini review. Rats, Mice, rabbits, pigs, and non-human primates have been highlighted among them. Despite the fact that these animal models have helped us understand how atherosclerotic lesions form, we still don't have a suitable animal model for disease reversal. The present technique for screening method for antihyperlipidemic activity could be classified under *in vitro* and *in vivo* methods. In vivo methods include Triton, PTU-, Fructose & high fat diet induced hyperlipidemic models. In vitro methods include In vitro Assay using Caco-2 Cell Lines and Inhibition of HMG CoA Reductase. Several genetically modified and transgenic animal models that mimic human atherosclerosis have been described by researchers, although each of the existing animal models has certain drawbacks. The features of animal models utilized in atherosclerosis research are described in this review study.

Keywords: Hyperlipidemia, PTU-Induced hyperlipidemia, HFD Induced Hyperlipidemia, Triton Induced hyperlipidemia.

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INTRODUCTION

Atherosclerosis is a hardening and narrowing of the arteries. It can obstruct blood flow, putting your health at danger. The word to say atherosclerotic cardiovascular diseases. The plaques or fatty materials are building up in arteries. The composition of plaques or made up of fibrin (a clotting material in the blood), calcium, cellular waste products & fatty substances. It is cardio-pathological condition called arteriosclerotic vascular diseases. In the atherosclerosis, reducing blood flow by this narrow the channel within artery & plaques was build up, the wall of blood vessels thickens. An arterial wall thickens due to a build-up of fatty substances such as cholesterol .[1] Due to the accumulation of macrophages white blood cells & promoted by low – density lipoprotein without adequate removal of fats & cholesterol from the macrophages by functional high density lipoprotein.

Epidemiology

Every year due to coronary artery diseases 6, 10,000 people passed away in United states of America, it is the major source of killing over 3,70,000 peoples per annum, around 7,35000 people suffers from cardiac arrest per year. Due to plaque rupture 75% of population suffers acute myocardial infarction; it is mainly observing man over 45 years, whereas in woman chances of myocardial infarction are increase after the age of 45 year. Due to the protecting action of female sex hormone the chances of atherosclerosis in woman is less than man, the protecting action of hormone is lost after menopause (above 50 years) Plaque rupture is said to cause 75 percent of acute myocardial infarctions, with the largest incidence of plaque rupture occurring in males over the age of 45, while the incidence increases in women after the age of 50. [1]

In the recent study, in India about 60% population was suffering from coronary artery diseases or cardiovascular diseases. Around 10% population have admitted and some of fear about hospitalization. Around 22% patients suffering from these diseases are died Because of they were not provided therapy due to the lack of medical staff in Villages. They shouldn't give counseling and awareness about these diseases.

Their effects on low-density lipoprotein (LDL) particles and inflammation may contribute to the development of atherosclerosis. The causes of atherosclerosis are family history, smoking, hypertension, physical activity, obesity, alcohol & hypertension. [2]

The main symptoms of atherosclerosis were seen to diagnosed are Tachycardia, Bradycardia, weakness, sweating, Shortness of breath fainting, chest pain, paralysis & facial numbness. By the atherosclerosis there are many complication are also arising. Coronary artery diseases, Stroke, Renal artery diseases, Peripheral artery diseases, Aneurysms. The major risk factors are potentially controllable i.e. Hyperlipidemia, Hypertension, Smoking, Diabetic Mellitus Type 2.

The major risk factor is hyperlipidemia whereas hyperlipidemia is high level of cholesterol in arteries that

can build up by plaque formation & it may cause atherosclerosis by rupture arteries. Dyslipidemia is the level of cholesterol is higher or lower than the normal range of fats. So, by the hyperlipidemia researcher & investigator may induced and develop the diseases. There are many inducers and induction models of hyperlipidemia. It can be used in pre-clinical trials majorly & and in clinical trial we can induced naturally or by neutrionist or High fat diet. High fat diet is the best method to induced hyperlipidemia and many other animals models are also used for induction in research of hyperlipidemia or atherosclerosis.[2-3]

Pathophysiology

The formation of atheromatous plaques is known as atherogenesis. It is characterised by arterial remodelling as well as the formation of plaques, which are fatty substances. Leukocytes such as monocytes and basophils, according to one theory, assault the endothelium of the arterial lumen in cardiac muscle for unknown reasons. Atheromatous plaques occur in the tunica intima of the artery wall, which is positioned between the endothelium and the tunica media, as a result of the inflammation. The bulk of these lesions are made up of excess fat, collagen, and elastin. When plaques form, just the wall thickens without constriction, stenosis of the artery entry, also known as the lumen; stenosis is a late occurrence that may never occur and is typically the consequence of recurring plaque rupture and healing responses, rather than the atherosclerosis process alone. [3]

Endothelial dysfunction, inflammation, vascular growth, and matrix change are all involved in atherosclerosis. Vascular proliferation is linked to other cellular processes such inflammation, death, and matrix alterations, and plays a role in atherosclerosis pathobiology. In-stent restenosis, transplant vasculopathy, and venous bypass graft failure all include vascular proliferation as a key factor in their pathogenesis. As a result, targeting cell cycle regulation to decrease cellular proliferation is becoming a popular treatment method for those disorders. We will go through the present state of molecular and gene therapy methods in vascular proliferative disorders, as well as the current understanding of pathophysiological pathways. Over the last decade, our understanding of the biology of atherosclerosis and related vascular diseases has evolved, opening up new avenues for preventive and treatment interventions.

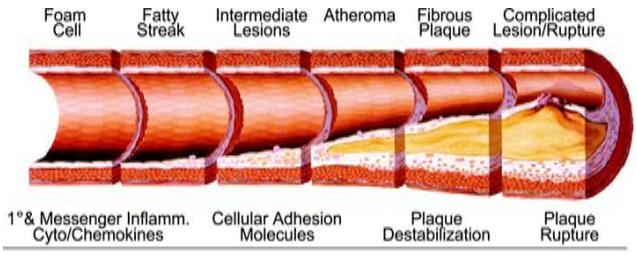

Figure 1: Stages of atherosclerosis index

Inflammation is thought to play a role in all phases of atherosclerosis, according to recent research. A major component of atherosclerosis, in addition to inflammation, is the proliferation of vascular smooth muscle cells (VSMCs) (Fig. 1). In humans, a localised buildup of VSMCs inside the intima might be a prelude to the formation of a lesion. However, the precise role of VSMCs in atherosclerosis is still a matter of dispute. VSMCs may contribute to the formatitrton of atheroma in early atherosclerosis by producing pro-inflammatory mediators such as monocyte chemoattractant protein 1 and vascular cell adhesion molecule, as well as synthesising matrix components necessary for lipoprotein retention. VSMCs, on the other hand, may play a role in preserving the plaque's integrity by forming a strong fibrous cap. Indeed, there is evidence of VSMC apoptosis, notably at the 'shoulder' area, in lipid-laden lesions with a thin and weak fibrous cap, which is linked with inflammation. Furthermore, the local inflammatory environment might increase collagenase expression while inhibiting proteolytic inhibitors

production, making the fibrous cap fragile and vulnerable to rupture. Fibroblasts and VSMCs with extracellular calcification create a fibrocalcific plaque in advanced lesions.

The origins of VSMCs in atherosclerotic plaques are mysterious. During normal growth and ageing, intimal thickening occurs. According to reports, intimal VSMCs, particularly those seen in atherosclerotic lesions, are monoclonal in origin. This would suggest that the neointima develops from the expansion of pre-existing clonal VSMCs in the area.

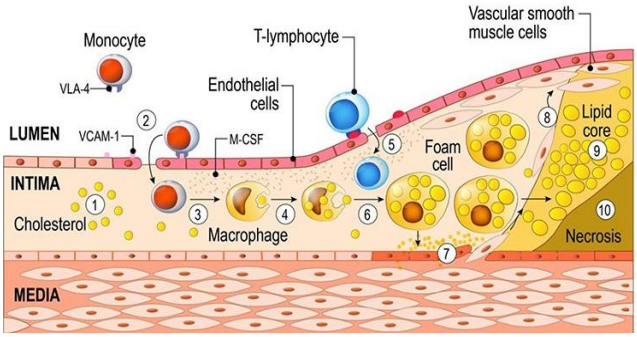

Figure 2: pathophysiology of atherosclerosis

Although substantial replication has not been observed in human atherosclerotic lesions, it may occur extremely early or at a low rate throughout the progression of atherosclerosis, or episodically at a high rate. VSMCs have been discovered in the fatty streaks of young people. Experiments show that intimal VSMCs can come from either the media or the adventitia.

Furthermore, embryonic endothelium cells have been shown to be capable of trans- differentiating into smooth muscle cell actin-expressing mesenchymal cells. According to animal research, neointimal cells can come from both bone marrow-derived and non-bone marrow-derived

circulating cells. The VSMCs that contribute to arterial remodelling in hyperlipidemia-induced atherosclerosis models, along with post-angioplasty restenosis and graft vasculopathy, may be derived from bone marrow cells. Although circulating smooth muscle progenitor cells have been discovered in human peripheral blood, their significance to human atherosclerosis has yet to be demonstrated.

In summary, the intimal smooth muscle cell appears to operate as a nidus for the formation of atherosclerotic lesions in the natural course of the disease, either via speeding lipid buildup or macrophage chemotaxis. Proliferation is most likely the first step, followed by a long-term process that creates a fibrous cap that inhibits plaque rupture. [3]

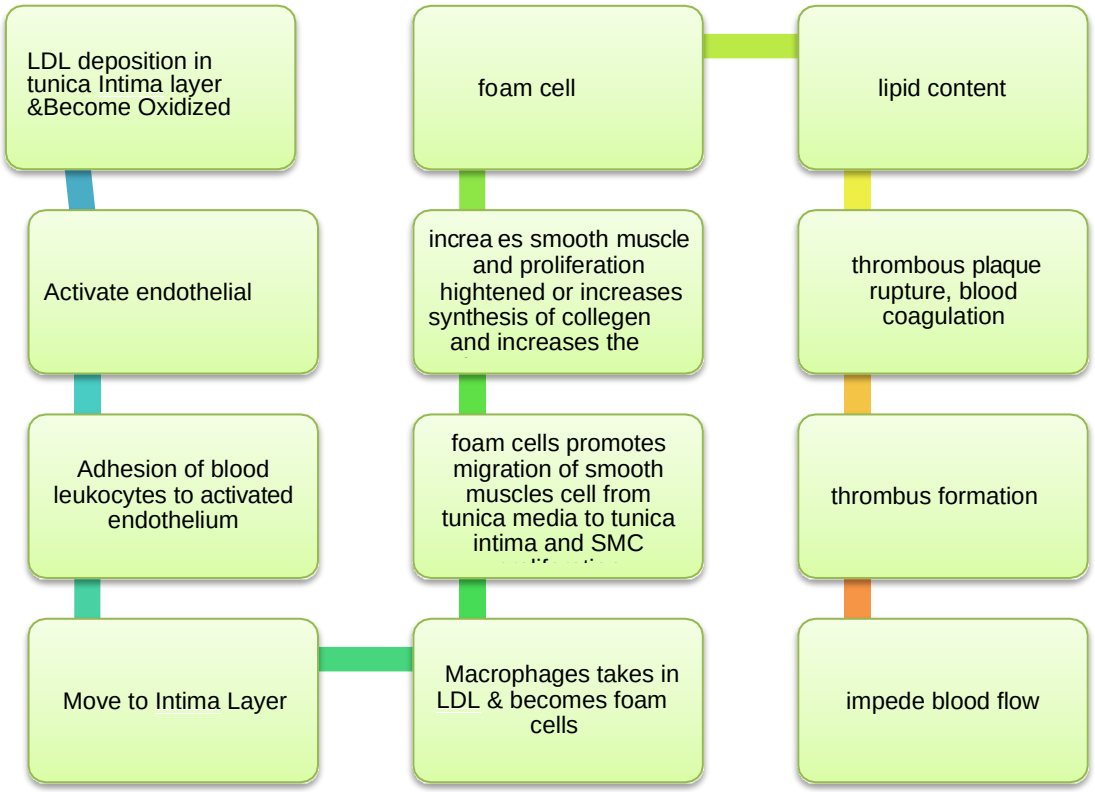

Figure 3: Summary of Pathology

Clinical Management

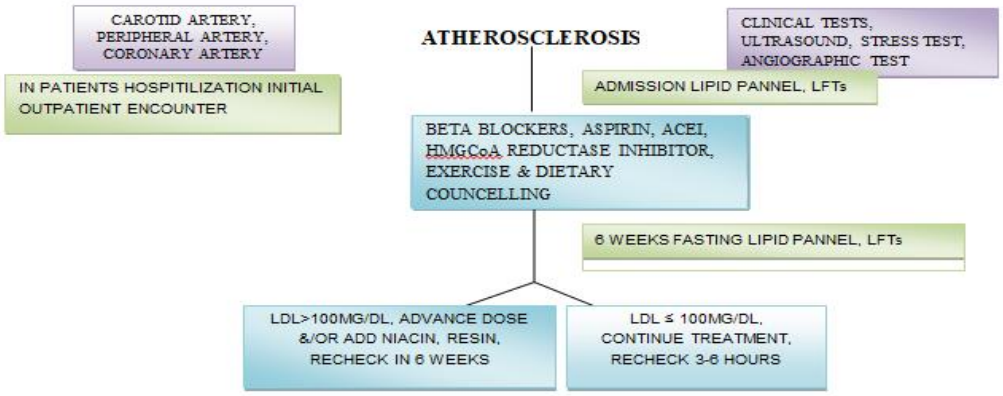

Figure 4: Management of Hyperlipidemia

Diagnosis

Detection and diagnosis pattern of atherosclerosis. After the completion of physical examination by the family history or other depending on detection pattern then further test will be suggested by the doctor or RMP or pharmacists included:-[4]

Blood Test

By this test check the blood cholesterol level & blood sugar it will be low, normal & higher the range. Cholesterol level is high it will be increase the risk of atherosclerosis. C- reactive protein test is also done. To check the inflammation in the arteries.

Electrocardiogram (ECG or EKG)

Simple and painless test to record the signals in heart.

Echocardiogram

This test is done for uses sound waves to show how blood well moves in when the heart beat & through your arteries.

Doppler Ultrasound

Your doctor may use ultrasound Doppler equipment to take your blood pressure at different spots on your hand and leg. It will display the obstructions as well as the rate of blood flow through the arteries.

Ankle- Brachial Index (ABI)

Detect the comparison between the legs and arms blood pressure. Abnormal differences may be sign of peripheral vascular diseases, usually caused by atherosclerosis.

Cardiac Catherization & Angiogram

This test is done by the insert of catheter into a blood vessels & heart. For the detection of arteries are narrowed or blockage by filling the dye to easily detect in X – ray or CT scan.

Coronary Calcium Scan

It will detect the calcium deposition in the walls of the arteries. The result of the test will be showed in score. when the calcium is present higher the score higher risk of heart diseases. It is also called heart scan and done by the computerized tomography scan (CT- scan).

Other Imaging Test–

- Magnetic resonance angiography
- Positron emission tomography.

Induction animals models of hyperlipidemia

In vivo methods

Fructose Induced Hyperlipidemia

Principle

The human liver can quickly absorb and process fructose. For thousands of years, people ingested 16–20 grams of fructose each day, mostly from fresh fruits. Dietary westernization has led in considerable increases in added fructose, resulting in normal daily fructose consumptions of 85–100 grams. When the liver is exposed to significant amounts of fructose, it undergoes fast lip genesis and TG buildup, which leads to decreased insulin sensitivity and hepatic insulin resistance/glucose intolerance. Fructose metabolism has received considerable scientific focus due to these unfavorable consequences of fructose. Small catalytic amounts of fructose, it turns out, can have a beneficial impact, lowering the glycemic response to glucose loading and improving glucose tolerance. These effects are also found in the absence of any alterations in insulin responses, non-esterified fatty acid (NEFA), or total lipid (TG) levels. [5]

Procedure

Daily for 21 days, 25% fructose in drinking water (libitium) is given to rat. The lipid profile of rat can be determined by collecting blood samples of rat's biochemical assessment. Various studies implies that by given the fructose diet will raise the fasting plasma level of leptons, which is sign that results in hyperlipidemia in rats. [6]

Triton Induced Hyperlipidemia

By parenteral administration of Triton WR 1339 (isooctyl polyoxy ethylene phenol) in adult rats.

Principle

In vivo screening technique of antihyperlipidemic activity is reported using Triton-induced hyperlipidemic rat model. Triton X-100 is a non-ionic surfactant which accelerates hepatic cholesterol synthesis and enhances intestinal lipid absorption by the emulsification process**.**

Procedure

Albino Wistar rats (160–200 g) can be utilised to test hyperlipidemic activity in vivo. The animals are kept in polypropylene cages in a well-ventilated environment at a temperature of 25°C with a light/dark cycle of 12:12 hours. Throughout the trial time, standard pellet feed and filtered tap water should be supplied. The rats are split into three groups of twelve rats each at random. Except for the control group, triton is dissolved in normal saline to achieve a 5 percent concentration and administered at a dosage of 300 mg/kg to all rats. The retro-orbital plexus is used to obtain blood samples from rats. Finally, standard diagnostic kits are used to measure blood levels of LDL, VLDL, total cholesterol, and triglycerides. [7]

Cholesterol Induced Hyperlipidemia

Procedure

Male Wistar rats (18 weeks old) were fed laboratory chow enhanced with 2% cholesterol or regular chow for 8 weeks in a room with 12 hour light–dark cycles and a constant temperature of 22±3°C.The HFD Consists of 95% commercial rat feed, 4% cholesterol & 1% Cholic acid. It is administered with vehicle for 21 days. After the completion of treatment we have used to measure blood levels of LDL, VLDL, total cholesterol, and triglycerides.

PTU-Induced Hyperlipidemia Rat Model

Principle

This procedure takes a short amount of time. Propylthiouracil induces hyperlipidemia (PTU). PTU is a hyperthyroidism medication. It causes hypothyroidism, which is characterized by elevated total cholesterol, LDL, VDL, and triglycerides.

Procedure

A large amount of cholesterol is administered into all groups 6 hours before total cholesterol, VLDL, and LDL are measured. 32 rats are required and divided into five groups. PTU is administered to all groups except the control group for 7 days at a dose of 10 mg/kg body weight of rat. PTU is administered for 7 days at a dosage of 0.01 percent. Finally, total cholesterol levels in serum, faeces, and liver extract are measured. [8]

High Fat Diet Induced Hyperlipidemia

Principle

The composition of HFD in 48 days is rat chow, Folic acid, cholesterol, dalda ghee. These are the parameters which have determined by blood collection method with different routes such as Retro orbital, tail vein collection. Lipid Profile- TC- Total Cholesterol,Triglycerides, High density lipid- Cholesterol, Low Density lipid- Cholesterol. Atherogenic Index Of Plasma - It is detected by this formula; *Log (triglyceride/ HDL- Cholesterol),* myopathy marker- Creatine kinase marker or also known as creatine phosphokinase (CPK**).** CRP- C- reactive protein**,** TNF α**,** Interleukin – 6, Adiponactin mRNA , RB-4.

Procedure

This model closely resembles hyperlipidemia in humans. This approach involves mixing a large amount of cholesterol with vegetable oil and administering it to all groups except the control group. Following the chronic treatment with high fat, the second group is given a conventional medicine, the third group is given a test sample, and the fourth group is given merely a regular diet as a control group. Collect the blood sample by an appropriate technique under minor anaesthetic at the conclusion of the 30th day. Animals are sacrificed, and organs such as the heart, liver, aorta, pancreas, spleen, and kidney are isolated, weighed, and submitted to histopathological tests. [8-10]

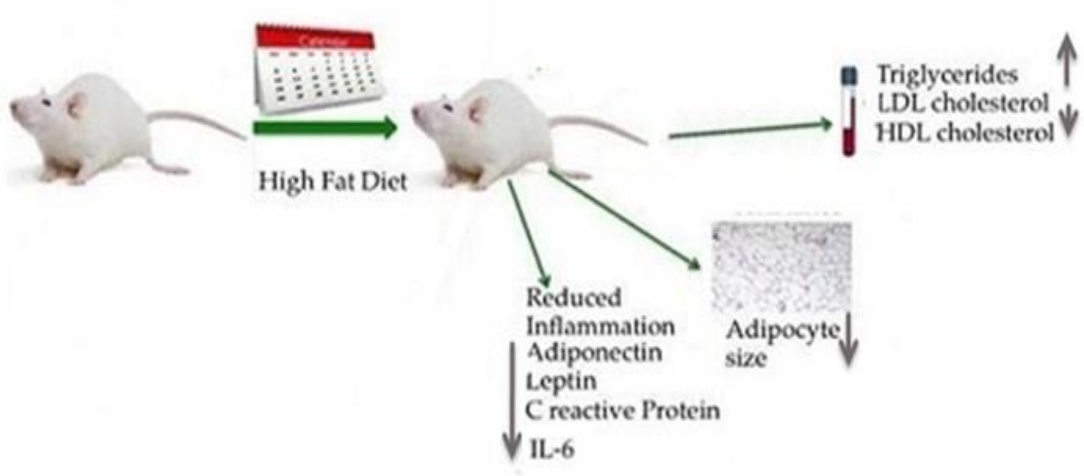

Figure 5: Mechanism of action of HFD in hyperlipidemia Model

***In Vivo* Methods**

In vitro Assay using Caco-2 Cell Lines

The lipid profile released by human intestinal epithelium-like cells from the colon cancer cell line Caco-2 is measured in this manner. Caco-2 cells are sown in well plates for two days in a mixture containing 10% foetal bovine serum, penicillin, and streptomycin. Caco-2 cells are differentiated for 4 days after being exposed to 0–5 mM sodium butyrate. Caco 2 cells will be changed into intestinal epithelium-like cells after an incubation period, and plenty of microvilli may be seen on the apical side of the cell when seen under an electron microscope. The medium containing the cultured cells is then supplemented with sodium oleate, and the differentiated Caco-2 cells secrete a lipoprotein profile into the medium via a microporous membrane. Chylomicron, VLDL, LDL, and high-density lipoproteins are among the four class fractions released. [11-13]

Inhibition of HMG CoA Reductase

HMG CoA reductase is a key regulatory enzyme in the production of cholesterol from acetyl CoA. 3-hydroxy, 3-methyl glutacyl CoA (HMG CoA) is converted to mevalonate by this enzyme. Cholesterol is not produced because this enzyme is inhibited. As a result, this in vitro model is used to assess a chemical moiety's hypolipidemic activity. This enzyme-inhibiting mechanism is how existing statin compounds show hypolipidemic action. Inhibition of HMG CoA reductase causes the liver to express LDL receptors, which decreases cholesterol levels in the blood. This is a reaction that necessitates the presence of NADPH. Commercially available HMG CoA assay kits are available. HMG CoA reductase assay buffer, HMG CoA reductase, NADPH, and an inhibitor are included in this kit (atorvastatin).The use of NADPH is assessed by a decrease in absorbance at 340 nm, which is measured by the assay kit. In addition, this sort of kit allows for the determination of pure enzyme activity. [14-16] calculate the percentage of inhibition using the following equation:

Percentage inhibition= absorbance of enzyme

absorbance of enzyme with inhibitor /absorbance of enzyme*100

CONCLUSION

The development of a hypolipidemic drug is very desirable and critical in the current situation. However, the lack of an appropriate screening technique for hypolipidemic activity is a key impediment to achieving this goal. The current methods for antihyperlipidemic activity screening methods .Methods in vitro and in vivo could be classed as in vitro and in vivo, respectively. Triton-, PTU-, and other in vivo procedures are used. Models of hyperlipidemia caused by the HFD. Alternatively, The in vitro approaches that have been documented are limited and include inhibitory activity test employing Caco-2 cell lines. HMG CoA reductase is a type of HMG CoA reductase. There is a scarcity of easy solutions. However, in vitro, it is adequately repeatable and sensitive. Anti-hyperlipidemic action can be detected using this approach.

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