

Animal Models of Atherosclerosis

Zaib-ul-Nisa¹, Irum Naureen², Aisha Saleem^{1*}, Muhammad Naeem³¹M.phil Researcher, School of Zoology Minhaj University Lahore, Pakistan²Assistant Professor, School of Zoology Minhaj University Lahore, Pakistan³Institute of Research and Information Mirpur AJK, PakistanDOI: [10.36348/sijap.2022.v05i02.002](https://doi.org/10.36348/sijap.2022.v05i02.002)

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*Corresponding author: Aisha Saleem

M.phil Researcher, School of Zoology Minhaj University Lahore, Pakistan

Abstract

Atherosclerotic heart disease due to the accumulation of large amount of lipids present in the blood vessels of arteries. Many animal models have been developed to study atherosclerosis, and allow for careful control of experimental conditions, food and environmental risk factors. Experimental animal models of atherosclerosis have become a valuable tool for providing information on the etiology, pathophysiology, and the mechanism of action of various drugs and compounds used in the treatment and complications of the disease. Compared to human models, the animal model is more easily manageable, as the combined effects of dietary and environmental factors can be controlled. Different models have their own advantages and disadvantages.

Keywords: Atherosclerosis, LDL, Ox-LDL, M-CSFs, SMC, CETP.

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INTRODUCTION

Atherosclerotic heart disease is a leading cause of death worldwide and presenting a severe threat to human health [1]. Atherosclerosis is due to the accumulation of large amounts of lipids in the blood vessels of the arteries that form plaques and cause stenosis of the elastic arteries of the muscles, resulting in lumen occlusion or ruptured hemorrhage. The pathological process of AS is very complex [2]. First, the excessively low-density lipoprotein slowly accumulates beneath the endothelium and becomes oxidized LDL (Ox-LDL), which causes inflammation and releases more chemokines from endothelial cells [3]. Attracted to chemokines, monocytes migrate to blood vessel intima and become macrophages upon stimulation of macrophage colony stimuli (M-CSFs) [4].

Macrophages then engulfed the Ox-LDL and convert it into foam cells, releasing a number of factors that lead to smooth muscle cell (SMC) migration and the formation of fibrous caps [5]. After that, the SMCs gradually disappear from the fibrous cap, while the infiltrating macrophages degrade the collagen-rich cap matrix. Both of these mechanisms cause thinning of the fibrous cap, which leads to plaque breakage and thrombosis [6]. The formation of atherosclerotic plaque

begins and persists with a combination of endothelial dysfunction and chronic exposure to cardiovascular risk factors that promote vascular inflammation, such as hyperlipidemia, hypertension, smoking, male gender, and diabetes.

Of these, the most important risk factor is high plasma low-density lipoprotein (LDL) levels, one of the monogenetic causes of familial hypercholesterolemia [7]. In the presence of LDL levels that allow the formation of atherosclerotic plaque, these risk factors are important in explaining the growth and development of atherosclerotic lesions [8, 9]. Excess LDL in plasma accumulates in the sub-endothelial space of the artery wall and undergoes oxidation to form oxidized LDL (Ox LDL) [10]. It then triggers an inflammatory response and stimulates the expression of chemotactic proteins such as vascular cell adhesion molecule-1 (VCAM-1), E-Selectin and P-Selectin in the endothelium [11, 12].

The expression of adhesion molecules attracts blood cells to the wall of the injured artery, the most prominent type of monocytes cell [13]. After migrating to the inner layer, monocytes differentiate into macrophages that make Ox LDL very internal and become foam cells themselves [14]. Foam cells carry

antigens to immune cells, such as circulating monocytes and T cells, the activation of which contributes to plaque growth [15]. They also secrete mediators to continue the inflammatory process in the artery wall and stimulate the transfer of smooth muscle cells from the tunica media.

Mediated by the growth factor derived from platelets, smooth muscle cells exhibit abnormally high proliferation rates and secrete extracellular matrix

proteins that aid in the formation of fibrous caps. The fibrous cap protects the core of the plaque from circulating blood cells, especially platelets, which are responsible for thrombosis associated with plaque rupture. This poor response to intractable inflammation stimulates atherosclerotic plaque growth. It is worth noting that SMCs belonging to different areas of micro vascularity have different developmental origins [16]. This may also contribute to site-specific atherosclerosis response [17].

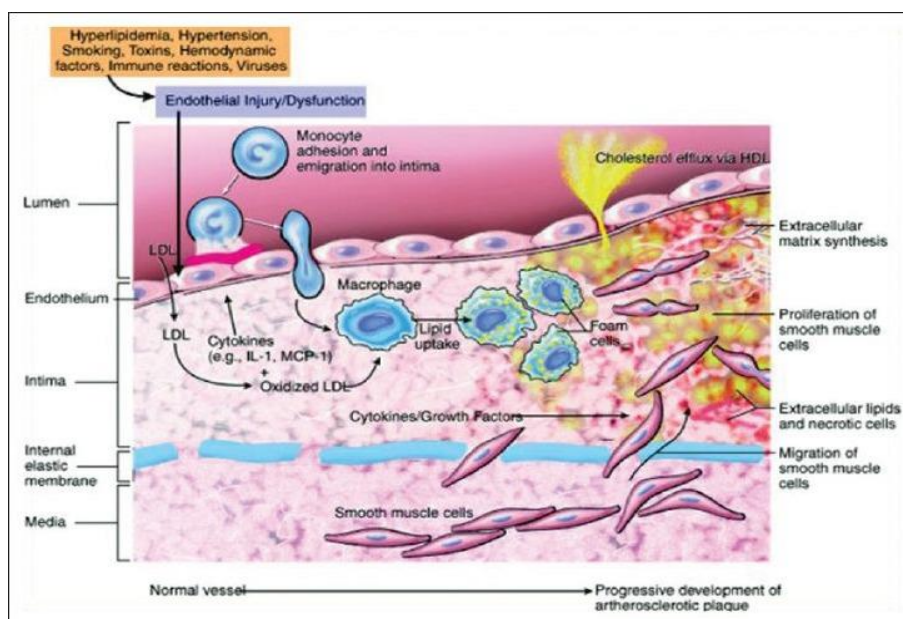


Fig 1: Normal vessel to progressive development of atherosclerosis [18]

During plaque evolution, macrophages spread and undergo apoptosis and phagocytosis [19, 20]. Depending on the performance of the aphrodisiac, apoptotic cells can be removed, reducing the size of the lesions. Or they may accumulate and become the victim of secondary necrosis, which produces a necrotic underlying feature of advanced plaques. Accumulation of apoptotic bodies will trigger inflammation and increase plaque instability [21, 22]. While foam cells have the most leukocytes in atherosclerotic lesions, studies of mouse models have affected other cell types, including neutrophils, T and B cells, and mast cells, in atherogenesis [23, 24].

These cells, although they contribute very little to the mass of the lesion, can infect atherosclerosis by secreting a variety of proteins that regulate other cells or components inside the plaque. Experimental studies using mice have shown that in T cell substrates, Th1 cells and natural killer T cells are particularly pro-atherogenic, while the role of Th2 and Th17 cells is less well understood [25, 26]. Plaque rupture is responsible for the negative consequences of acute myocardial infarction and ischemic events in brain accidents and deaths [27]. In advanced cases of atherosclerosis,

rupture of weak plaques exposes their thrombogenic compounds, thus leading to luminal thrombosis [28].

Macrophage-derived proteases, especially metal proteases, can destabilize plaques, but the exact underlying mechanism of plaque weakness remains incomplete [29].

Animal models for atherosclerosis

Experimental atherosclerosis can be encouraged in animals. The first evidence of this was seen in early 1908 by Ignatowski, who demonstrated atherosclerosis in the veins of rabbits by feeding them animal-rich foods, including meat, eggs and milk [30]. Since then, several animal models have been used to understand the mechanisms involved in both the involvement and regression of atherosclerotic lesions [31, 32]. Mice, rabbits, dogs, pigs and monkeys are well-established animal models for atherosclerosis and thrombosis. Nonhuman primates, hamsters, mice, cats and guinea pigs have also been used, but to a lesser extent [33]. Overall, an ideal animal model should represent human atherosclerosis and be workable and affordable. Although animal models have contributed to our understanding of the inclusion of atherosclerotic lesions, they have some limitations.

Table 1: Advantages and disadvantages of common animal models of atherosclerosis [34]

Animal models	Advantages	Disadvantages
Rats and mice	<ul style="list-style-type: none"> • Low price easily supply • Easy to handle and maintain. • Manageable growth. • A well establish genomic sequence allows for genomic manipulation 	<ul style="list-style-type: none"> • Generally resistant to atherogenesis. • Absence of plasma CETP activity • Most cholesterol is transmitted through HDL particles. • The small size of mice restricts repeated blood sampling and separation of small arteries.
Rabbits	<ul style="list-style-type: none"> • Easy to handle and maintain. • Relatively cheap. • High availability • Sensitive to the addition of dietary cholesterol to atherosclerosis. • Large enough to allow physical experiments. 	<ul style="list-style-type: none"> • Lesion location less compared with humans • Decreased hepatic lipase leads to hepatotoxicity after prolonged cholesterol feeding.
Pigs	<ul style="list-style-type: none"> • Similar cardiovascular system compared to humans. • Comparative patterns of plaque distribution. • High availability (for small pigs) 	<ul style="list-style-type: none"> • Large size with resultant management difficulties • High maintenance cost
Dogs	<ul style="list-style-type: none"> • Easy to work • Ideal size • High availability 	<ul style="list-style-type: none"> • Highly resistant to atherogenesis. • Differences in important aspects of their cardiovascular system compared to humans.
Hamsters	<ul style="list-style-type: none"> • low cost • High availability • Easy to handle and maintain. • It carries a significant portion of its plasma cholesterol to LDL particles and is therefore close to humans. • Sensitive to high fat foods 	<ul style="list-style-type: none"> • Wound development and absence of advance lesions. • Extremely abnormal doses and requiring treatment with cytotoxic chemical agents, such as streptozotocin
Guinea pigs	<ul style="list-style-type: none"> • Prepare diet-induced atherosclerosis. • Most cholesterol is transferred to LDL particles • Ovariectomized guinea pigs showed a similar plasma lipid profile as occurs in postmenopausal women. 	<ul style="list-style-type: none"> • Requires a permanent supplement with vitamin C, which may act as an antioxidant to interfere with atherogenesis
Nonhuman primates	<ul style="list-style-type: none"> • Originally resembling humans. • Similar omnivorous food. • Similar metabolism. • Develop metabolic syndrome with age. 	<ul style="list-style-type: none"> • Expensive. • Low availability. • Longevity (thus requiring longer experimental periods) • Potential Carriers of Dangerous Viral Zoonoses • Important moral issues.
Pigeon	<ul style="list-style-type: none"> • low cost • Easy handling. • Suffering from atherosclerosis. • Enough size. 	<ul style="list-style-type: none"> • Nonmammalian • Lipoprotein compositions and metabolism are different. • Differences in arterial histology
Chicken	<ul style="list-style-type: none"> • low cost • High availability • Naturally develop atherosclerosis in aorta and coronary arteries, feeding cholesterol accelerates pathogenesis 	<ul style="list-style-type: none"> • Non-mammal • Viral infection is associated with atherosclerosis.

Mouse model

Due to its small size and ease of genetic manipulation, the mouse is currently the most widely used model in atherosclerosis research [35]. The development of atherosclerosis is influenced by a

number of genes that interact with each other and the environment to influence the disease phenotype [36].

Rabbit model

Rabbits were the first species to be used to model atherosclerosis in research dating back to the

early 20th century, which established a direct link between high cholesterol diet and atherosclerosis [37].

Porcine model

The advantage of using porcine models is that it bears close resemblance to human counterparts in cardiac anatomy and physiology. LDL, as in humans, is the largest circulating lipoprotein in plasma. Another advantage is the highly defined gene types, which enable the development of porcine models with multiple genetic mutations [38]. Unlike the postmortem model and the previous *in vivo* model, the use of the porcine model as the *in vivo* validation of imaging tools is valuable; this failed to produce satisfactory validation. Postmortem specimens cannot mimic the components of moving cells in atherosclerotic plaques, whereas former *vivo* models are commonly observed in flexible arteries, moving coronary arteries on the beating heart, irregular heart rhythms, and moving tissues. They do not show palatial flow [39].

Pig models

Within 2–4 years on a normal diet, these pigs developed increased hypercholesterolemia, with LDL as the main circulating lipoprotein, associated with the development of coronary atherosclerosis. A mini-pig model bearing the same gene mutation but with lower cost, the hypercholesterolemia Bretoncelles Meishan (FBM) pigs, was subsequently made available [40].

Non-human primate models

The biggest advantage of using primates is that they have more in common with cardiac anatomy and physiology than humans. Abnormal cardiac physiology, plaque weakness, and spontaneous luminal thrombosis development are observed in both species, depending on the shape of the lesion [41-43]. Primates are equally susceptible to atherosclerosis; young animals are reasonably resistant to the development of atherosclerosis, but their risk of becoming susceptible increases with age [44].

Genetically modified animal models for atherosclerosis

Researchers have developed a number of genetically modified marine models using genetic technology to address many of the defects of large animals, especially to allow for the study of possible treatments for which a large number of Articles required. An interesting scientific breakthrough occurred in 1992, when Zhang *et al.*, Ape-deficient mice produced by targeting the gene were found to have five times higher plasma cholesterol levels and to have foam cell-rich deposits in their nearby aortas by the age of 3 months [45]. This model was the first line of the genetically modified marine model for atherosclerosis studies introduced to the research community. Fatty Zucker rats, cholesterol ester transfer protein (CETP) transgenic rats, LDL receptor-knockout (KO) mice, and *db/db* mice are a few of the genetically modified models developed over recent years.

Table 2: Genetically modified animal models for atherosclerosis [46]

Experimental models	Description
Apolipoprotein E knockout (ApoE ^{-/-}) mice	Apolipoprotein E (apoE), a component of lipoprotein responsible for packaging cholesterol and other fats and transporting them through the blood, is inactivated by targeting the gene. They show a plasma cholesterol concentration of 11 mm higher than in their parent C57BL / 6 mice.
LDL receptor knockout (LDLR ^{-/-}) mice	The LDL receptor (LDLR) is a cell surface receptor in liver cells that mediates apoE endocytosis to purify cholesterol-rich LDL particles from circulation. A seven- to nine-fold increase in intermediate-density lipoprotein (IDL) and LDL without significant changes in HDL causes total plasma cholesterol levels to double compared to wild species.
Scavenger receptor class B member 1 knockout (SR-BI KO) mice	The scavenger receptor class B1 (SR-BI) facilitates the increase of HDL cholesterol in the liver. It plays a key role in determining plasma cholesterol (primarily HDL) levels. Heterozygous and homozygous mutants show a 31% and 125% increase in plasma cholesterol numbers, respectively, compared to wild species.
<i>db/db</i> mice	OB-R is a high affinity receptor for leptin, an important circulatory signal for feeding, appetite and body weight regulation. The rate of fatty acid oxidation is higher in DB / DB mice with parallel onset and longer duration of hyperglycemia
<i>ob/ob</i> mice	One mutation results in a structurally defective leptin that is not bound to OB-R. Mice that are <i>ob / ob</i> have no leptin action and exhibit obesity and endothelial dysfunction.
Fatty Zucker rats	An autoimmune mutant gene (FA or fatty) that affects the action of leptin. They have high levels of lipids and cholesterol in their bloodstream and become significantly obese at 3 to 5 weeks of age, and by the age of 14 weeks, more than 40% of their body composition is lipids.
Cholesterol ester transfer protein (CETP) transgenic rats	By transferring CETP cholesterol from HDL to extremely low density lipoprotein (VLDL) and LDL, HDL mediates inhibition of reverse cholesterol transport, which promotes atherogenesis. 82% increase in non-HDL cholesterol in animals and 80% decrease in HDL cholesterol as compared to wild mice

Small and large animal models

Animal models are essential for the discovery of complex molecular mechanisms of Atherosclerosis which are conducive to studying the presence and development of Atherosclerosis and to reviewing therapeutic treatment of food and drug interventions on [47, 48]. From the early 20th century to the new millennium, researchers have used a variety of animals in the AS model for more than 100 years, including rabbits, rats, mice, pigs and nonhuman primates [49, 50].

Advantages of Small animal models

Small animals, mainly rats and rabbits, have been widely used for atherosclerosis research. Some of the reasons for the frequent use of small animal models in research include low cost, ready availability, reduction of ethical concerns over large animals (especially non-human prey), and the small size required for in vivo screening [51].

Advantages of Large animal models

Larger animal models such as pigs and non-human premature AS are suitable for research because of the shape of their vascular lesions and lipid metabolism similar to humans [52-54]. Nonhuman primates are platelet function, coagulation, and fibrinolytic system and drug pharmacokinetics resemble those in humans [55]. This has supported the use of a more accessible and less costly large animal models.

By its very nature, atherosclerotic plaque rupture is difficult to study in humans. Furthermore, because atherosclerotic plaque rupture occurs in a stochastic manner, it is difficult to identify the triggers and to investigate treatment for plaque stabilization [56]. Thus, an appropriate animal model should not only help to better understand the mechanism behind plaque breakage, but also test therapeutic measures to prevent it.

Disadvantages of animal models

Disadvantages of these models include long modeling times, high cost, complex experimental procedures, and difficulties in obtaining large amounts of data [57]. Small mammals such as rabbits and rats are cheap and can be easily genetically manipulated [58, 59]. Despite the development of porcine models of advanced human coronary atherosclerosis, there is no suitable large animal model of high-risk plaque [60, 61].

CONCLUSION

Animal models have been used to study the pathophysiology of cardiovascular disorders. There is no single animal used for experiment. Small animal models, such as mice and rabbits, generally provide valuable information on the etiology and pathophysiology of atherosclerosis. Use of small animal

models in research includes low cost, ready availability, reduction of ethical concerns. In small animal, mice are the best model for the atherosclerosis research. With advances in genetic technology, the development of small pigs is a favorable trade-off between human-like physiologies compared to non-human primate. The small animals, that is Easier to handle with more similarities to human cardiac anatomy, physiology, lipid metabolism, and atherosclerotic pathophysiology. Although the use of animal specimens has undoubtedly offered new insights into various important aspects of a disease, there are still no species that are suitable for all studies, given the multifaceted nature of heart disease.

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