

**Original article:**

**Small animal models of atherosclerosis**

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**Abstract:**

The area of cardiovascular research is day by day expanding and the small animal models play a very crucial role in this onward journey. An animal model is a non-human animal that has a disease or injury that is similar to a human condition. The choice of animal models in cardiovascular research is dictated by the size consideration alone. Atherosclerosis is a pathological process, which occurs, in large conduit arteries. They are due to focal accumulation of cells within the intima of the artery, both intra and extra cellular lipids, fibrous tissues, complex proteoglycans, mineral blood and blood products. Even though there is no one perfect animal model

that completely replicates the stages of human atherosclerosis, cholesterol feeding and mechanical endothelial injury are two common features shared by most models of atherosclerosis. Several characteristics of the rabbit make it an excellent model for the study of atherosclerosis. Several animals have been used for the study of atherosclerosis, such as the non human primates, swine, mice, guinea pigs and hamsters. With the advent of genetic engineering, transgenic mouse models have supplemented the classical dietary cholesterol induced disease models such as the cholesterol-fed hamster, rabbit, pig and monkey. This article intends to review the

various established small animal models of atherosclerosis. A thorough search of articles published in reputed journals was done by typing the keywords animal models, atherosclerosis, small animals was done to locate the information, all published articles and books were included in collecting information and data was synthesised for the present article.

**Key-words:** Atherosclerosis, small animal models, cardiovascular research

**Key Messages:** 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 aetiopathogenesis and regression of atherosclerosis.

## Introduction:

Animal Models: An Introduction

An animal model is a non-human animal that has a disease or injury that is similar to

a human condition. Animal models are the initial backbone in any research purpose even amidst the cries of anti-vivisectionists and the cardiovascular research to the credit of small animals have in the long run seen umpteen number of models accepted, discarded and still developing daily. The choice of animal models in cardiovascular research is dictated by the size consideration alone. When dealing with hemodynamics the call is for the larger vessels whereas for histopathological examination it is wise to use mice where the structure is to be serially sectioned into small pieces<sup>1</sup>. In other words -THE ANIMAL MODEL MUST BE MATCHED TO THE EXPERIMENTAL DESIGN

## Advantages and disadvantages of animal models

Due to their short life span they react and age faster. They can be sacrificed after the entire disease process is studied and as

sufficient numbers are available we can study the interactions of various factors as the physiology and anatomy of the animals may be already known<sup>2</sup>The disadvantages of animal models is that we may not be able to reproduce the same history of pathogenesis in animals as that in man.To reproduce some models maynot be feasible.Some may be difficult to perform and not possible to uniformly reproduce it. Greater variability can occur in the experimental results and there is difficulty in reducing the number of animals used (one experimental result is obtained per animal).

### **Types of animal models<sup>2</sup>**

#### **1. Experimental Model**

This type is surgically induced, should mimic the disease being studied, and be easily manipulated and readily reproducible. If this model does not reproduce the disease exactly, the correlation between animal and man

must be significant, verifiable and predictable.

#### **2. Negative Model**

This type of model does not develop the disease and is usually avoided. Can be helpful in determining why some species are resistant.

#### **3. Orphan Model**

This type of model includes diseases of animals, which do not have human counterparts, or disease similar to those in man with dissimilar aetiologies or pathogenesis.

#### **4. Spontaneous Model**

These naturally occurring diseases of animals, which mimic those occurring in man. Over 890 types have been reported.

### **Cardiovascular Research**

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The area of cardiovascular research is day by day expanding and the small animal models play a very crucial role in this onward journey. The established small

animal models are that for hypertension, atherosclerosis, and myocardial infarction .A few models have been developed in areas like arrhythmia, heart failure, pulmonary embolism, rheumatic carditis, cardiac arrest and cardiopulmonary bypass. The small animals employed in cardiovascular research are that of the rats, mice, rabbits, guinea pigs and hamsters.

### **Small Animal Models In Atherosclerosis**

Atherosclerosis is a pathological process, which occurs, in large conduit arteries. They are due to focal accumulation of cells within the intima of the artery, both intra and extra cellular lipids, fibrous tissues, complex proteoglycans, mineral blood and blood products<sup>5</sup> .Atherosclerosis has often been defined as a multifactoral disease; however, a common risk factor associated with accelerated vascular disease in man or

animals is an elevated plasma cholesterol level<sup>8</sup>.

It has been shown that disturbances in the microcirculation of the vasa vasorum affected the site of atherosclerotic predilection in high cholesterol diet fed rabbits by Nataka et al<sup>1</sup>. Several studies indicate that high HDL has an increased risk of atherogenesis.<sup>1</sup>

Even though there is no one perfect animal model that completely replicates the stages of human atherosclerosis, cholesterol feeding and mechanical endothelial injury are two common features shared by most models of atherosclerosis. The models may differ with respect to degree of dietary cholesterol supplementation, length of hypercholesterolemia, dietary regimen and type, duration and degree of mechanical endothelial injury. It is worthwhile mentioning that several animals have been used for the study of atherosclerosis, such as the non human primates<sup>9</sup>, swine<sup>10</sup>, rabbit<sup>11</sup>, mice<sup>6</sup>, guinea pigs<sup>4</sup> and hamsters<sup>12</sup>. With

the advent of genetic engineering, transgenic mouse models have supplemented the classical dietary cholesterol induced disease models such as the cholesterol-fed hamster, rabbit, pig and monkey<sup>8</sup>.

4 changes have been seen predominantly in animals fed on high cholesterol –high fat diets<sup>1</sup>.

1. Reduction in HDL concentration without apo-E
2. Increase in HDL concentration with apo-E
3. Increase in LDL in plasma
4. Appearance of VLDL in plasma

Roberts and Straus<sup>1</sup> reported that atheroma and medial sclerosis are reported in rodentia whereas fatty streaking has not been reported in them

The challenge in identifying and establishing suitable animal models has been to characterize a model which develops the same type of atherosclerosis at the same

location as seen in man<sup>1</sup>. The lesion should form over a relatively short period of time but with the same sequence as seen in the very long-term lesions characteristic of the human disease.

### Rabbit Models

Atherosclerosis was produced in this species by feeding a high cholesterol diet as early as 1900's<sup>1</sup>. The use of rabbits in the study of atherosclerosis was first mentioned in 1908<sup>13</sup>. Since then atheromatic lesions were mainly introduced by applying specific diets. Among these, a high cholesterol diet is the one which is mostly used. Due to the fact that rabbit is very sensitive to the inducement of atheromatic lesions through a high cholesterol diet, the "cholesterol fed rabbit" is deemed to be one of the most important animal models for the study of atherosclerosis.

Several characteristic of the rabbit make it an excellent model for the study of atherosclerosis. In fact despite several

dissimilarities from humans the rabbit is still likely to be the most widely used animal model for atherosclerosis. One reason for this is that rabbits are easy to feed, care for and handle, along with being inexpensive and readily available.

It has been shown that the basal release of NO (nitric oxide) is greater with the endothelium intact aortic rings from female rabbits than from male rabbits and depends on the circulating oestradiol concentration<sup>14</sup>. It has been reported that female rabbits are less prone to diet induced atherosclerotic lesions than male animals<sup>15</sup>. With cholesterol feeding they develop hypercholesterolemia (>2000mg/dl), accumulate cholesterol in intima of the large conduit arteries especially aortic arch and thoracic aorta, however the content and sites of distribution are not the same as seen in man<sup>1</sup>.

In rabbits as well as in other animal models, the earliest detectable cellular events are an increase in leucocyte margination and

endothelial penetration and the formation of macrophage derived intimal foam cells<sup>16</sup>. In rabbits, lesion morphology is altered by the percentage of cholesterol added to the diet<sup>17,18</sup>.

Microscopically, lesions resemble the thick fatty streaks with most of the lipid localised within the foam cells. When the duration of hypercholesterolemia is increased, the lipid eventually starts to accumulate extracellularly. It is rare for lesions in rabbits to develop fibrosis, haemorrhage, ulceration and thrombosis, all characteristics of lesions in humans.

Among all the strains of rabbits, the New Zealand with body weight of between 2 kg and 5 kg is the most used in the laboratory. The New Zealand strain also appears in genetically altered strains, the most important of which are the Watanabe, St Thomas and Houston RT which present genetic abnormalities in lipid metabolism<sup>11</sup>. The genetically altered strains of the rabbit are also used extensively. Rabbit

models of human lipoprotein disorders, such as the Watanabe Heritable Hyperlipidemic (WHHL) and St. Thomas' Hospital strains, models of familial hypercholesterolemia and familial combined hyperlipidemia, respectively, allow for the assessment of candidate genes for potential use in the treatment of dyslipoproteinemic patients.

The Watanabe Heritable Hyperlipidemic (WHHL)<sup>1</sup> develop massive hypercholesterolemia as a result of a single genetic defect. Fulminating atherosclerosis occurs even when the rabbits are fed a cholesterol free diet. The defect seems to reside in the genes for LDL receptors. This is the same genetic defect seen in humans with familial hypercholesterolemia. In these rabbits, the pattern of atherosclerosis development is the same as it is in humans.

### Transgenic Rabbit Models<sup>1, 5, 11</sup>

The use of transgenic rabbit models for the elucidation of the mechanisms involved in the pathogenesis of the disease is a new

area of interest. To date, transgenes for human apo A, apoA-I, apoB, apoE<sub>2</sub>, apoE<sub>3</sub> and lecithin: cholesterol acyltransferase (LCAT), as well as for rabbit apolipoprotein B mRNA-editing enzyme catalytic poly-peptide 1 (APOBEC-1), have been expressed in NZW rabbits, whereas only those for human apoA-I and LCAT have been introduced into the WHHL background<sup>5</sup>. All of these transgenes have been shown to have significant effects on plasma lipoprotein concentrations.

In both NZW and WHHL rabbits, human apoA-I expression was associated with a significant reduction in the extent of aortic atherosclerosis, which was similarly the case for LCAT in rabbits having at least one functional LDL receptor allele. Conversely, expression of apoE<sub>2</sub> in NZW rabbits caused increased susceptibility to atherosclerosis.

These studies provide new insights into the mechanisms responsible for the development of atherosclerosis,

emphasizing the strength of the rabbit model in cardiovascular disease research.

### **The Rat Model**

Rats are generally considered to be resistant to atherogenesis, although lesions have been produced by heroic measures. Naturally occurring lesions in rats are not very similar to that seen in man. Although lipid containing lesions can be produced in rats, they are generally considered to be residual lesions following an acute arteritis.

The development of genetically engineered mice with disorders of lipid metabolism, such as apolipoprotein E (apoE) and LDL receptor knockout mice, was therefore a major step forward in animal models of atherosclerosis<sup>6</sup>. These mice develop atherosclerosis spontaneously, albeit variable between mouse strains<sup>7</sup>, which can be accelerated on a high cholesterol diet. The plaques that develop are widespread and reproducible and have some architectural features reminiscent of

human lesions. These mice have formed the basis for a plethora of studies identifying specific molecules critical to atherosclerosis, in particular, those regulating monocyte adherence/chemotaxis and macrophage differentiation/foam cell development.

The major dissent has been that lesions occur at sites very different from human lesions—the aortic root and thoracic aorta for instance. Lesions in the aortic root are also foam cell-rich, rather than smooth muscle cell-rich, may not have a single definable fibrous cap, and represent xanthomata rather than clinically important advanced lesions.

Most important of all, these mice are models of atherogenesis, not advanced atherosclerosis, and they do not exhibit the single most important event in human atherosclerosis, that of plaque rupture leading to vessel occlusion.

### **Guinea Pig Models**

The most striking similarity between guinea pigs and humans is that the majority of circulating cholesterol is transported in LDL<sup>19</sup>. Guinea pigs carry the majority of cholesterol in LDL and possess cholesterol ester transfer protein and lipoprotein lipase activities, which results in reverse cholesterol transport and delipidation cascades equivalent to human situation. Guinea pigs develop atherosclerosis and gender and hormonal status affect the extent of the atherosclerotic plaques<sup>20</sup>. More recently it is shown that high cholesterol diets induce aortic cholesterol accumulation and that certain dietary components or drug treatment can reduce concentrations of cholesterol in the aorta even in the presence of very high dietary cholesterol.

Guinea pig is a model to study the inflammatory component of diet-induced atherosclerosis. Proinflammatory cytokines production and mRNA expression in the

aorta of guinea pigs fed on high cholesterol diet was seen and carbohydrate restriction showed decrease in TNF alpha expression and TNF production.

### **Hamster Model**

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. 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<sup>3</sup>.

### **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 aetiopathogenesis and regression of

atherosclerosis.

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