

Minireview

Experimental atherosclerosis A historical overview

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Abstract

Almost one-hundred years ago the first evidence of experimental atherosclerosis was reported. Over the past century, significant advances have been made in the development of animal models of human coronary artery disease. In this minireview, induction of atherosclerotic lesions in several animal models including rodents (mice, rabbits, rats, hamsters, guinea pigs), avian (pigeons, chickens, quail), swine, carnivora (dogs, cats), and non-human primates is discussed. The limitations and advantages of the animal models of atherosclerosis have been summarized. The transgenic/knockout animal models have greatly enhanced our understanding of atherosclerosis. Compared to wild-type counterparts, the knockout/transgenic animals develop atherogenesis faster without a need for a highly atherogenic diet. Although almost all investigations support a causal role for increased plasma cholesterol levels in the development of atherosclerotic vascular disease, an increasing body of evidence indicates serious involvement of other factors including oxidative stress, inflammation, infection and other emerging risk factors. © 2002 Elsevier Science Inc. All rights reserved.

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Introduction

The first evidence of experimental atherosclerosis came into view in 1908 when Ignatowski (1) reported thickening of the intima with formation of large clear cells in the aorta of rabbits fed with a diet rich in animal proteins (meat, milk, eggs). At the same time, other studies described atheromatous changes in the aortas of the experimental animals by injection of cultures of staphylococci (2) or by mechanical factors (hypertension due to hanging the animals up by their hind limbs) (3). However, these experimental procedures failed to reproduce atherosclerotic changes in subsequent studies (4,5). On the other hand, in agreement

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with the findings of Ignatowski (1), Steinbiss was able to develop atherosclerosis in the aorta of rabbits by feeding them internal organs including liver and adrenals (6). Similarities between these experimental findings with those seen in humans were the core evidence for “metabolic” hypothesis of atherosclerosis. A combination of mechanical factors (hypertension) (3) and animal proteins (egg yolk) resulted in a significant hypertrophy of the aortic intima in rabbits (4). These as well as original findings of Ignatowski (1) indicated a causal role for animal proteins. However, other investigators believed that the causal factor for atherogenesis is lipids, but not proteins of the animal tissues (7). Therefore, the “cholesterol theory” of atherosclerosis was introduced.

To test the latter theory of atherogenesis, rabbits were fed with pure cholesterol dissolved in vegetable oil; the animals developed typical atherosclerotic lesions similar to those seen in humans (8). In this regard, it should be noted that Lemoine’s findings from human cases previously suggested a role for lipids in atherosclerosis (9). “Cholesterol theory” still remains at the center of atherosclerotic vascular disease development. The atherogenic role of cholesterol has been tested in an ever-increasing number of laboratory animals including wild-types, naturally defective or genetically modified animal models of atherosclerosis. The results of almost all of these animal studies demonstrate that increased plasma cholesterol level is a reliable method for induction of atherogenesis. However, a few studies showed either a paradoxically negative correlation or no correlation between plasma cholesterol levels and severity of atherogenesis.

The aim of this review is to summarize the evidence for the etiologic role of cholesterol in experimental atherosclerosis. Similarly, evidence which does not fully support the “cholesterol theory” is discussed.

Cholesterol and atherogenesis

The original work of Ignatowsky (1) was used as a basis for further investigation in experimental atherosclerosis (10,11). Reviewing the experimental evidence, Katz, Stamler and Pick came to the conclusion that dietary cholesterol is needed for induction of atherosclerosis in experimental animals (10,11). Since then, a strong association between certain types of dyslipidemia including hypercholesterolemia, hypertriglyceridemia, and combined hyperlipidemia and development of atherosclerotic lesions has been documented by a number of clinical trials and epidemiological and experimental studies. Thus, hypercholesterolemia is a major risk factor for coronary heart disease. To better understand the relation between disorders of cholesterol metabolism and atherogenesis, a number of animal models have been used. In this regard, until recently, dietary lipid manipulation and use of naturally defective animals such as Watanabe Heritable Hyperlipidemic (WHHL) rabbits have been the focus of most experimental settings. Nowadays, gene deletion technology has allowed researchers to produce a variety of transgenic/knockout animal models closely resembling particular human lipoprotein disorders. Despite this revolutionary achievement, many of these genetically modified animals have to be fed with cholesterol to accelerate atherogenesis; this indicates an inevitable role for “cholesterol feeding” in atherosclerosis research. The application of various animal species in “atherosclerosis research” is discussed below:

Rodents

Mice

Ten inbred strains of the mouse were fed with a diet containing 1.25% (w/w) cholesterol, 0.5% (w/w) cholic acid and 15% (w/w) fat and the order of susceptibility to atherogenesis was, from the least to the most susceptible strains as follows: BALB/cJ, C3H/J, A/J, SWR/J, NZB/J, <129/J, AKR/J, DBA/2J, <C57L/J, and C57BL/6J (12). Atherogenic diet caused an increase (from 1.1 to 4.3 times on average) in plasma total cholesterol in all ten strains, while plasma triglyceride increased only in 3 and decreased in the other 7 strains. More interestingly, atherogenesis was observed only in the last 4 strains mentioned above; no significant correlation ($r=0.3$) was found between plasma total cholesterol levels and atherogenesis (12). Because of difficulties in induction of atherogenesis—most likely due to high HDL level and low cholesterol absorption rate—the wild-type mice are not commonly used in this field of research. Nevertheless, early stage of atherosclerotic lesion development (fatty streaks) can be induced by means of atherogenic diets in C57BL/6J mice (13).

Rabbits

The rabbit was the first animal model used in atherosclerosis research (1). This animal species shares several aspects of lipoprotein metabolism—except for deficiency in hepatic lipase—with humans. The rabbit also develops advanced atherosclerotic plaques; however, extremely high plasma cholesterol levels are needed for this to occur. Since it absorbs cholesterol efficiently, this high level of plasma cholesterol can be induced by high fat/high cholesterol diet (14). Atherogenic diets are usually associated with development of atherosclerotic lesions in the aortic arch and thoracic aorta rather than abdominal aorta which is almost always affected in humans. Watanabe Heritable Hyperlipidemic (WHHL) rabbits with natural LDL-receptor deficiency have been used as a model of human familial hypercholesterolemia (15); St. Thomas' Hospital (STH) strain resembles human hypertriglyceridemia and combined hyperlipidemia (16,17). Both strains develop advanced atherosclerotic lesions.

Rats

The rat is an atherosclerosis-resistant species. Unlike humans and similar to mice, rats do not have plasma cholesteryl ester transfer protein (CETP), and high density lipoprotein (HDL) is the major carrier of plasma cholesterol. Rats are generally hypo-responsive to dietary cholesterol; thus, hyperlipidemia and atherogenesis may only be induced in rats by high cholesterol/high fat diets containing cholic acid and thiouracil (18). The mechanism of action of cholic acid is two fold: an increase in cholesterol absorption and a concomitant suppression of cholesterol 7α -hydroxylase activity that results in decreased cholesterol excretion. Thiouracil induces clinical hypothyroidism with consequent decreased low density lipoprotein (LDL)-receptor activity and hypercholesterolemia. Several strains of rats with heritable hyperlipidemia, some of these associated with atherogenesis, have been described (19–21).

Hamsters

Hamsters may develop hypercholesterolemia and early atherosclerosis on atherogenic diets. For instance, a diet containing 0.2% (w/w) cholesterol and 10% (w/w) coconut oil caused a 4-fold increase in plasma total cholesterol levels and formation of fatty streaks in the ascending aorta of male Golden Syrian hamsters over a period of 2 months (22). No lesions were

found in descending and abdominal aorta. The atherogenic diet resulted in more than 200% increase in very low density lipoprotein/intermediate density lipoprotein (VLDL/IDL) cholesterol, a 20% increase in LDL cholesterol and a 45% decrease in HDL cholesterol concentrations compared to controls (22). These observations suggest significant differences in lipoprotein cholesterol distribution and localization of atheromatous lesions between hamster and man. Moreover, a similar high fat diet caused a marked increase in body weight without changes in plasma lipid levels and atherogenesis in female Syrian hamsters. On other hand, male hamsters developed hyperlipidemia and atherogenesis without significant changes in body weight (23).

Guinea pigs

Like humans, guinea pigs have lipoprotein (a) in their plasma; a significant accumulation of this atherogenic lipoprotein was found in their atherosclerotic lesions (24). Thus, this species may be suitable for investigating the relationship between lipoprotein (a) and atherosclerosis.

Avian

Pigeons

Two strains of pigeons have attracted the attention of researchers in atherosclerosis field. The White Carneau (WC) strain develops spontaneous atherosclerosis (25,26) and the Show Racer (SR) pigeons are resistant to atherogenesis even when fed a high cholesterol diet (26).

The WC strain absorbs cholesterol efficiently and develop atheromas in thoracic aorta, abdominal aorta, brachiocephalic, iliac, carotid, renal, and coronary arteries.

Quail

There are both atherogenesis resistant and susceptible strains of Japanese quail; the susceptible strain is responsive to cholesterol feeding (27). Diet-induced hypercholesterolemia is associated with a significant shift in lipoprotein profile. While HDL is the major plasma lipoprotein on cholesterol-free diet, chylomicrons and VLDLs predominate in cholesterol-fed birds (28). In addition, cholesterol feeding also increased the percentage of cholesteryl ester in both LDL and VLDL but had no effect on HDL composition (29). Increased levels of plasma cholesterol correlate with increased frequency and severity of atherosclerotic lesions. Generally, the lesions are composed of foam cells, cholesterol clefts, increased extracellular matrix and cellular component. The lesions may advance and result in further complications such as narrowing, stenosis and infarction; the nature of lesions is similar to those in humans (27). Altogether the development of advanced atherosclerotic lesions, myocardial infarction, response to dietary cholesterol, defined plasma lipoprotein profile along with known genetic background make the atherosclerosis-susceptible strain of Japanese quail attractive in dyslipidemia and atherosclerosis research (27–31).

Chickens

Criticism of the rabbit model of experimental atherosclerosis, namely of the relatively long-induction period of the disease (4–7 weeks), led to search for other animal models. Katz and Stamler were able to induce atherosclerotic lesions in both abdominal and thoracic aorta in chicks within 2 weeks by cholesterol feeding (2). Later on, development of spontaneous atherosclerosis was observed in the abdominal aorta of chickens in both sexes over 3 years

even on cholesterol free diets (32,33). Cholesterol feeding accelerates the formation of lesions with increasing content of lipids within the lesions. Discontinuation of cholesterol feeding may result in regression of early lesions. Generally, lesions in chicken are not advanced and have a minimum of complications. This can be a major limitation of this model in studying human atherosclerosis. A strain of chicken has been reported to be HDL deficient; these chickens (Wisconsin Hypo-Alpha Mutant, WHAM) have a 70–90% reduction in plasma HDL cholesterol and apo AI levels. An atherogenic diet was associated with increased VLDL and LDL cholesterol in controls and WHAM chickens, respectively. Both groups of chickens developed atherosclerosis to a similar extent (33).

Swine

An early study reported spontaneous atherosclerosis in swine (34). An extremely high cholesterol (4% w/w) is needed to induce advanced atherosclerotic lesions in coronary arteries of miniature pigs (35). On the other hand, naturally defective pigs develop hypercholesterolemia and atherosclerosis in the coronary, iliac and femoral arteries on low fat cholesterol-free diet (36,37). Further studies demonstrated that the extent and complexity of atherosclerotic lesions correlated with both the degree and duration of hypercholesterolemia in naturally defective pigs with *Lpb5* and *Lpu1* mutations (37).

Carnivora

Dogs

Spontaneous atherosclerosis is rare in dogs; even high cholesterol diet does not result in the development of advanced atherosclerotic lesions in dogs (38). However, induction of experimental atherosclerosis in dogs may be possible by the use of high fat/high cholesterol (up to 5% w/w) diet deficient in essential fatty acids (38,40).

Hypercholesterolemia and atherosclerosis were also induced in foxhounds by dietary means (41).

Cats

Cats have not been frequently used in atherosclerosis research, simply because they are resistant to atherosclerosis. Recently, both spontaneous and diet-induced atherosclerosis have been reported in domestic cats (42,43).

Non-human primates

Being the closest animal species to man makes non-human primates an attractive model; it is assumed that data derived from these animals are more directly applicable to man. During the 1950's several laboratories successfully induced experimental atherosclerotic lesions in monkeys. For example, Taylor et al. (44) documented development of atherosclerotic lesions in moderately hypercholesterolemic monkeys. These investigators (45) also reported development of atherosclerotic lesions and fatal myocardial infarction in monkeys with diet-induced hypercholesterolemia. Similarly, Mann and Andrus (46) showed that a high fat/high cholesterol diet could cause an elevation in the serum cholesterol and S_{f0-35} lipoproteins in an adult rhesus monkey. This abnormal lipid profile was associated with development of extensive atherosclerotic lesions in the aorta and all its major branches including the coronary and cerebral vessels. These lesions were similar to those seen in humans. Altogether, these

important observations led several investigators including Mann and colleagues (47), Taylor (48), Lindsay and Chaikoff (49), Armstrong and Warner (50) and Armstrong (51) to review and characterize dyslipidemia and atherosclerosis in non-human primates. The location of atheromas varies among different strains of monkeys. Recently familial LDL receptor deficiency with atherosclerosis has been reported in rhesus monkey (52,53).

Genetically-modified animal models

DNA technology has allowed us to create a number of knockout and transgenic mouse and rabbit models for studying atherosclerosis. The major advantage of these animals over their wild-type counterparts is easy and rapid induction of atherosclerosis. For example, apolipoprotein E-knockout (apo E-KO) mice develop advanced atherosclerotic lesions even when fed with regular mouse chow (54). However, addition of cholesterol to the chow reduces the induction period (55). Similarly, LDL receptor knockout or human apo B₁₀₀ transgenic mice develop advanced atherosclerotic lesion when fed a high cholesterol diet (56, 57). Development of complicated atherosclerotic lesions has been also reported in cholesteryl ester transfer protein (58) and apo E* Leiden (59) transgenic mice.

Both New Zealand and Watanabe rabbits have been used to generate several types of transgenic animal models. Among them, rabbits expressing human apo (a) develop severe atherosclerosis (60); the lesions in these transgenic rabbits with Watanabe background are more complex than those in New Zealand background indicating interactions between apo (a) and high plasma LDL cholesterol levels (61). Characteristics of genetically-modified mice and rabbits with dyslipidemia and atherosclerosis have been summarized elsewhere (62).

Beyond cholesterol

A number of recently published experimental studies provide evidence for involvement of factors other than increased plasma cholesterol levels in atherogenesis. One of the first observations was paradoxical effects of probucol on atherogenesis in both apo E-KO (54) and LDL receptor deficient (63) mice. Probucol with strong antioxidant and cholesterol-lowering effects increased atherogenesis in apo E-KO mice by 3 folds (54). Similarly, simvastatin treatment at a dose of 100 mg/kg body weight for 6 weeks did not change plasma cholesterol levels but significantly reduced cholesterol contents of the aorta in apo E-KO mice (64). In agreement with this finding, Wilson et al (65) reported an increase in coronary endothelial nitric oxide (NO) synthase along with a decrease in oxidative stress by simvastatin treatment in hypercholesterolemic pigs. Plasma cholesterol levels were not affected by simvastatin treatment. These observations suggest that the antiatherosclerotic effects of simvastatin are mediated through other mechanisms than its cholesterol-lowering activity.

Several other compounds reduced the extent and severity of atherosclerotic lesions without affecting plasma cholesterol levels in apo E-KO mice. For example, administration of antioxidant N,N'-diphenyl 1,4-phenylenediamine (DPPD) (0.5% w/w) to apo E-KO mice resulted in a significant decrease in atherosclerosis without reducing plasma cholesterol levels (66). A marked reduction in atherosclerosis by dietary vitamin E was accompanied by no change in plasma cholesterol levels in apo E-KO mice (67). Likewise, antiatherogenic effects of the angiotensin-converting enzyme inhibitors captopril (68), ramipril (69), fosinopril (70)

or the angiotensin-II receptor antagonist losartan (71) in apo E-KO mice were independent of plasma cholesterol lowering effects. Similarly, iron-deficient diet (72) or red wine consumption (73) did not significantly reduce plasma cholesterol, but significantly reduced the extent and severity of atherosclerotic lesions in apo E-KO mice. Reduced LDL oxidation are suggested as a key factor recognized in antiatherosclerotic effects of all the above-mentioned compounds in apo E-KO mice.

Comments

Atherosclerosis research is turning one hundred year old. Over the past century, a variety of experimental procedures have been used to investigate the pathophysiology of atherosclerosis. During this period several theories have been formed to explain the etiology of atherosclerosis. “Cholesterol theory” was one of the first hypotheses and still remains at the center of almost all research activities in this field. Early studies conducted in rabbits and then in other wild-type animals used dietary cholesterol to induce atherosclerosis. Discovery of Watanabe rabbits in late 1970’s (74) followed by introduction of St. Thomas’ Hospital rabbits significantly improved experimental atherosclerosis research. These strains of rabbits imitate human familial hypercholesterolemia and familial combined hyperlipidemia, respectively. These achievements were followed by further discovery of naturally defective animal models including Wisconsin hypo-alpha mutant chickens, hypercholesterolemic pigs, hyperlipidemic rats, lipoprotein lipase deficient cats, LDL-receptor deficient monkeys, as well as atherosclerosis-susceptible Japanese quail and White Carneau pigeons for studying the relation between lipid disorders and atherosclerosis.

The atherosclerosis-susceptible White Carneau pigeons excrete less natural sterols as compared to atherosclerosis-resistant Show Racer pigeons (75). Similarly, the metabolism of dietary cholesterol varies between Watanabe (LDL receptor-deficient) and New Zealand White rabbits. A marked increase in the bile acid pool size and hepatic cholesterol concentration was observed in cholesterol-fed New Zealand White rabbits, but not in homozygote Watanabe counterparts fed with the same diet (76). Because LDL particles are not taken up by hepatocytes for conversion to bile acids and excretion, they accumulate in plasma and are taken up by macrophages through scavenger receptors leading to foam cell formation and development of atherosclerotic plaques in Watanabe rabbits. Significantly decreased levels of HDL and apo AI in plasma of naturally defective pigs with *Lpb5* mutation can explain development of advanced atherosclerotic lesions in these animals (77).

The year 1992 can be considered as a world-shattering stage in experimental atherosclerosis. In this year, the first line of knockout animal models, namely apo E-KO mice was developed. This innovation is followed by production of an ever-increasing number of knockout/transgenic animal models. All these accomplishments substantially enhanced our understanding of this disease.

We now have a good understanding of the etiology of the disease which has help us to discover new therapeutic approaches in both primary and secondary prevention of coronary heart disease. Among them, cholesterol-lowering agents particularly the statins (78) have been extremely promising. However, as discussed earlier, recent studies indicate that reduction of plasma cholesterol levels should not be the only tactic in the treatment or preven-

tion of atherosclerotic vascular disease. Oxidative stress (79), inflammation (80), hyperhomocyst(e)inemia (81), infection (*Chlamydia pneumoniae*) (82) and other emerging risk factors should be carefully considered in the management of this potentially fatal disease.

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