Pharmacology & Therapeutics 146 (2015) 104–119

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Associate editor: M. Endoh

Rabbit models for the study of human atherosclerosis: From pathophysiological mechanisms to translational medicine

Jianglin Fan [a,*], Shuji Kitajima [b], Teruo Watanabe [b], Jie Xu [c], Jifeng Zhang [c], Enqi Liu [d], Y. Eugene Chen [c,**]

[a] Department of Molecular Pathology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Shimokato 1110, Chuo-City 409-3898, Japan
[b] Division of Biological Resources and Development, Analytical Research Center for Experimental Sciences, Saga University, Saga, Japan
[c] Center for Advanced Models for Translational Sciences and Therapeutics, University of Michigan Medical Center, Ann Arbor, MI, USA
[d] Research Institute of Atherosclerotic Disease and Laboratory Animal Center, Xi'an Jiaotong University School of Medicine, Xi'an, China

ARTICLE INFO

Available online 30 September 2014

Keywords:
Atherosclerosis
Experimental animal models
Hypercholesterolemia
Transgenic rabbits
Translational medicine

ABSTRACT

Laboratory animal models play an important role in the study of human diseases. Using appropriate animals is critical not only for basic research but also for the development of therapeutics and diagnostic tools. Rabbits are widely used for the study of human atherosclerosis. Because rabbits have a unique feature of lipoprotein metabolism (like humans but unlike rodents) and are sensitive to a cholesterol diet, rabbit models have not only provided many insights into the pathogenesis and development of human atherosclerosis but also made a great contribution to translational research. In fact, rabbit was the first animal model used for studying human atherosclerosis, more than a century ago. Currently, three types of rabbit model are commonly used for the study of human atherosclerosis and lipid metabolism: (1) cholesterol-fed rabbits, (2) Watanabe heritable hyperlipidemic rabbits, analogous to human familial hypercholesterolemia due to genetic deficiency of LDL receptors, and (3) genetically modified (transgenic and knock-out) rabbits. Despite their importance, compared with the mouse, the most widely used laboratory animal model nowadays, the use of rabbit models is still limited. In this review, we focus on the features of rabbit lipoprotein metabolism and pathology of atherosclerotic lesions that make it the optimal model for human atherosclerotic disease, especially for the translational medicine. For the sake of clarity, the review is not an attempt to be completely inclusive, but instead attempts to summarize substantial information concisely and provide a guideline for experiments using rabbits.

Contents

Abbreviations: Apo, apolipoprotein; Cas9, CRISPR-associated (Cas) immune response-the protein; CETP, cholesteryl ester transfer protein; EVG, elastica van Gieson; FPLC, Fast protein liquid chromatography; HDL, high density lipoproteins; HE, hematoxylin eosin; RGENs, RNA-guided endonucleases; KO, knock-out; JW, Japanese White; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; Lp(a), lipoprotein (a); NZW, New Zealand White; PLTP, phospholipid transfer protein; RVD, repeat variable diresidue; TAL, transcription activator-like; TALEN, transcription activator-like effector nucleases; Tg, transgenic; VLDL, very low density lipoproteins; WHHL, Watanabe heritable hyperlipidemic; ZFN, zinc finger nuclease.

* Corresponding author. Tel./fax: +81 55 273 9520.
** Correspondence to: Y.E. Chen, 2800 Plymouth Rd, NCRC-26, Rm 361S, Ann Arbor, MI 48109, USA. Tel.: 734 647 5742.
E-mail addresses: jianglin@yamanashi.ac.jp (J. Fan), echenum@umich.edu (Y.E. Chen).

http://dx.doi.org/10.1016/j.pharmthera.2014.09.009
0163-7258/

1. Introduction

Atherosclerotic diseases such as myocardial infarction and stroke are still major causes of mortality globally. Atherosclerosis develops slowly throughout the stages of human life and many genetic and environmental factors are concertedly involved in the pathogenesis of this disease (Libby et al., 2011). For study of the pathophysiology and also for the development of therapeutic modalities and diagnostic tools, utilization of appropriate experimental animals is essential. In general, the ideal animal models of human atherosclerosis should possess several important characteristics (Fan & Watanabe, 2000; Vesselinovitch, 1988). They should be easy to acquire and maintain at a reasonable cost, easy to handle, and the proper size to allow for all anticipated experimental manipulations. Ideally, the animal should reproduce in a laboratory setting and have a well-defined genetic background. Finally, the animal model should share with humans the most important aspects of lipid metabolism and cardiovascular pathophysiology. Atherosclerotic lesions should develop gradually while the animal consumes a special diet, and features of the lesions (from the fatty streaks to complicated plaques with calcification, ulceration, hemorrhage and thrombosis) should mimic those of human patients with clinical sequelae (such as myocardial infarction, stroke and gangrene). Unfortunately, there is no single animal model that fulfills all of these requirements; therefore, many different animals have been used to illustrate the diverse aspects of atherosclerosis (Moghadasian, 2002). Among those used to date, rabbits are the first and one of the best models for the study of lipoprotein metabolism and atherosclerosis.

2. History of rabbit atherosclerosis models

The first experiment using rabbits to investigate atherosclerosis should date back to more than a century ago. In 1908, a Russian physician, Ignatowski, fed rabbits with a diet enriched in animal proteins (milk, meat and eggs) and observed intimal lesions with large clear cell (now called foam cell) accumulation in the aorta (Ignatowski, 1908). Later, a Russian experimental pathologist, Anitschkow, used a cholesterol diet dissolved in vegetable oil to produce aortic atherosclerosis in rabbits similar to those seen in humans and proposed a causal role of cholesterol in atherosclerosis (Steinberg, 2004). Now, a consensus has been reached in this field that, in both humans and experimental animals, it is dietary cholesterol that leads to the development of atherosclerosis (Steinberg, 2004). These pioneering studies provided the first experimental evidence and basis for the establishment of the "lipid hypothesis" of atherosclerosis (Steinberg, 2004). Since then, the rabbit model has been widely used to elucidate many facets of the pathophysiology of human atherosclerosis along with the development of therapeutics. In this regard, several significant milestones in the history of the study of atherosclerosis should be specially mentioned, which are based on tremendous breakthroughs and insights to understand the molecular and cellular mechanisms of atherosclerosis provided by rabbit models.

In the 1980s, Mahley et al. showed that the major lipoproteins elevated in cholesterol-fed rabbits are hepatically derived remnant lipoproteins, called β-VLDL, and it is these lipoproteins that are atherogenic because they are cholesterol-rich and can induce macrophages to transform into foam cells (Mahley et al., 1980). Watanabe et al. demonstrated that, in the early-stage lesions of aortic atherosclerosis of cholesterol-fed rabbits, the initial cellular event is the recruitment of intimal foam cells and these foam cells are primarily derived from blood monocytes (Watanabe et al., 1985). Later, Hansson et al. found that, in addition to monocytes, T lymphocytes are also present in the lesions of cholesterol-fed rabbits similar to those of humans (Hansson et al., 1991), suggesting that the immune response is involved in the pathogenesis of atherosclerosis. Gimbrone's, Libby's and Fogelman's groups further identified the presence of the vascular cell adhesion molecule (VCAM-1) in aortic endothelial cells of the lesions, which can be up-regulated by atherogenic lipoproteins and enhances the adhesion of monocytes to endothelial cells; they thus provided evidence that endothelial cell dysfunction induced by atherogenic lipoproteins plays an important role in monocyte adherence to endothelium and migration into the intima during atherogenesis (Cybulsky & Gimbrone, 1991; Li et al., 1993; Territo et al., 1989). Steinberg's group performed a series of studies and demonstrated that oxidization of β-VLDL and LDL actually occurs in the arterial wall of cholesterol-fed rabbits, which constitutes an essential trigger for the mediation of monocyte chemoattractant protein-1 (Yla-Herttuala et al., 1991) and macrophage colony-stimulating factor expression during lesion progression (Rosenfeld et al., 1992). It was in the rabbit that the impact of lipoproteins of different sizes on atherosclerosis was first studied. For example, it was noted more than fifty years ago that alloxan-induced type I diabetes surprisingly inhibited cholesterol-induced atherosclerosis in rabbits (Duff & Mc, 1949). This is due to the fact that large-sized triglyceride-rich lipoproteins (>75 nm diameter) that accumulated in the plasma of diabetic rabbits failed to penetrate into the arterial wall (Nordestgaard & Zilversmit, 1988). These pioneering studies using rabbits along with other animal models established the basis for the modern theory of atherosclerosis: plasma cholesterol is a critical atherogenic factor (lipid hypothesis) and inflammatory cells such as macrophages and T lymphocytes (inflammatory hypothesis) are key cellular components for the initiation and progression of atherosclerosis (Moore & Tabas, 2011). Probably, there are no more striking findings obtained from rabbit models than those of the Watanabe heritable hyperlipidemic (WHHL) rabbits, which were developed by Dr. Yoshio Watanabe at Kobe University, in Japan (Watanabe, 1980). WHHL rabbits allowed epoch-making contributions to understand LDL receptor deficiency as a cause of human familial hypercholesterolemia discovered by Goldstein and Brown (Goldstein et al., 1983).

Although the rabbit model has undoubtedly brought about many breakthroughs in the history of atherosclerosis study, there has been a reduced trend of using this model since 2000, possibly due to the development of genetically modified apoE and LDL receptor gene knock-out (KO) mice (Fig. 1). Many researchers are even reluctant to use rabbits for studying atherosclerosis because they are sometimes wrongly considered as "giant rodents". It is true that the mouse model has been a powerful tool for much research due in large part to the relative ease of genetic manipulation and the relatively short time for the development of atherosclerosis in the setting of apoE or LDL receptor deficiency (Getz & Reardon, 2012), but as an alternative model, rabbit is necessary in translational research of cardiovascular diseases. One of the best historic experiences is no less than the discovery of statin as a potent lipid-lowering drug (Shiomi et al., 2013). When Dr. Akira Ando, a Japanese scientist who discovered statin, along with Beecham Pharmaceutical Research Laboratories in England discovered compactin (the first generation of statin), they failed to prove that compactin was therapeutically effective in lowering plasma cholesterol in rats and mice, even at high doses (>500 mg/kg), regardless of marked inhibition of hepatic cholesterol synthesis (Brown & Goldstein, 2004). Later, it was realized that rodents (mice, rats and hamsters) differ from humans in many aspects of lipoprotein metabolism, as discussed in detail below (Table 1). For example, approximately two-thirds of the cholesterol pool in humans is derived from in vivo synthesis, whereas a large part of the cholesterol is derived from dietary origin in rodents (Endo, 1980). Wild-type mouse and rat are HDL mammals, while humans as well as rabbit are LDL mammals. The ability of mice and rats to excrete bile acid, which is derived from cholesterol, is much higher than in human and rabbits (Names, 2004). Dr. Endo found that compactin was more effective in rabbits than in chickens, cats, dogs and monkeys, while it was ineffective in mice, rats and hamsters (Endo, 1980). It is not a matter of the size of the model but rather whether to use proper models precisely in order to translate or extrapolate research results into a clinical setting.