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Review

Contribution of the WHHL rabbit, an animal model of familial hypercholesterolemia, to elucidation of the anti-atherosclerotic effects of statins

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ABSTRACT

This year marks the 40th year since the discovery of a mutant rabbit showing spontaneous hyperlipidemia, which is the proband of the Watanabe heritable hyperlipidemic (WHHL) rabbit strain, an animal model of familial hypercholesterolemia, and the first statin, a general term for inhibitors of 3-hydroxy-3-methylglutaryl CoA reductase, a rate limiting enzyme in cholesterol biosynthesis. Nowadays, statins are the primary drug of choice for treating cardiovascular disease. Although several reviews have described clinical trials and *in vitro* studies of statins, the anti-atherosclerotic effects of statins on animal models have not been comprehensively reviewed. This review summarized the contribution of WHHL rabbits to elucidating the anti-atherosclerotic effects of statins *in vivo*. Studies using WHHL rabbits verified that statins suppress plaque destabilization by reducing unstable components (foam cells derived from macrophages, foam cell debris, and extracellular lipid accumulation), preventing smooth muscle cell reductions, and increasing the collagen content of plaques. In addition, the expression of matrix metalloproteinases and tissue factor are decreased in intimal macrophages by statin treatment. Lipid-lowering effects of statins alter plaque biology by reducing the proliferation and activation of macrophages, a prominent source of the molecules responsible for plaque instability and thrombogenicity. Although statins remain the standard treatment for cardiovascular disease, new therapeutics are eagerly awaited. WHHL rabbits will continue to contribute to the development of therapeutics.

Contents

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1. Introduction

Cardiovascular disease is a leading cause of death worldwide. In 2008, an estimated 17.3 million people died from cardiovascular disease, representing 30% of all global deaths (http://www.who.int/mediacentre/factsheets/fs317/en/index.html). Of these deaths, an estimated 7.3 million were due to coronary heart disease and 6.2 million to stroke. To overcome cardiovascular disease, various therapeutic medications, such as hypocholesterolemic, hypolipidemic, anti-inflammatory, hypotensive, and anti-oxidative drugs have been developed. Statins are the primary drug of choice for patients with hypercholesterolemia. They are prescribed to more than 30 million people worldwide and have been shown to lower the incidence of coronary events by 25–45% [1]. Statins are competitive inhibitors of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, which mediates the conversion of HMG-CoA to mevalonate and is a rate limiting enzyme in cholesterol biosynthesis. Currently, 7 statins, lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin, rosuvastatin, and pitavastatin, are available.

In development of statins, Watanabe heritable hyperlipidemic (WHHL) rabbits, an animal model of familial hypercholesterolemia (FH), have contributed to studies on the hypolipidemic and anti-atherosclerotic effects [2]. The proband of the WHHL rabbit strain was found in 1973 by Yoshio Watanabe. In this year, the first statin, compactin, was also found by Akira Endo [3], and Joseph L. Goldstein and Michael S. Brown discovered LDL receptors [4]. Therefore, 1973 was an epoch-making year in the study of lipoprotein metabolism, atherosclerosis, and cardiovascular disease.

Although many reviews have described clinical trials and *in vitro* studies of statins, the anti-atherosclerotic effects of statins in preclinical studies have not been comprehensively reviewed. In commemoration of the 40th year since discovery of the proband of WHHL rabbits and the first statin, we summarize contribution of WHHL rabbits to elucidation of the anti-atherosclerotic effects of statins *in vivo*.

2. Characteristics and history of WHHL rabbits

Fig. 1 shows the characteristics of the WHHL rabbit. The WHHL rabbit shows hypercholesterolemia [5] due to a deficiency in low-density lipoprotein (LDL) receptors [6–8] and develops atherosclerotic lesions [2,5]. Similar to humans, WHHL rabbits do not express apoB-editing enzyme in the liver [9], and cholesterol ester transfer protein (CETP) activity in plasma [10]. In addition, some WHHL rabbits show hyperinsulinemia, insulin resistance, and accumulation of visceral fats [11]. Although the original WHHL rabbits (before 1985) showed marked atherosclerotic plaques in the aorta, coronary lesions were rather limited [12]. To study cardiovascular disease in more detail, Dr. Watanabe started to develop a new line of WHHL rabbits, which had coronary lesions [2]. After two cycles of selective breeding, the incidence and degree of coronary plaques increased [2,12,13]. WHHL rabbits were then improved by the third selective breeding; leading to myocardial infarction-prone WHHL (WHHLMI) rabbits, which have inflamed thin-capped fibroatheroma in the coronary arteries (unstable coronary plaques), related to myocardial infarction [2,14].

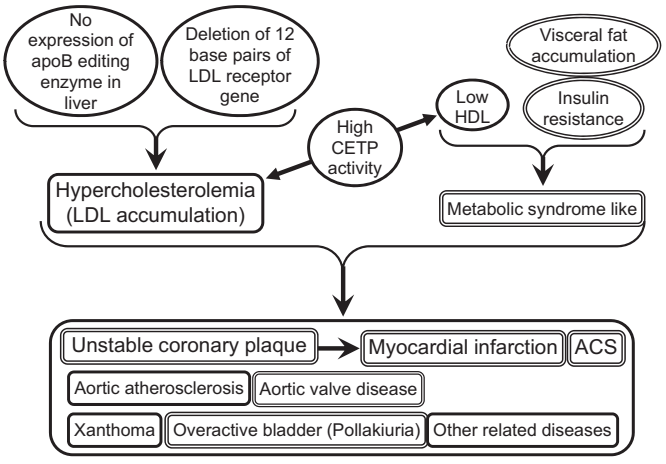

Fig. 1. Characteristics of WHHL and WHHLMI rabbits. Double lines indicate the characteristics of WHHLMI rabbits and single lines the common characteristics of WHHL and WHHLMI rabbits.

3. Species differences in the hypolipidemic effects of statins

Statins' hypocholesterolemic effects depend on the animal species; effective in hamsters, rabbits, dogs, monkeys, and hens, but ineffective in mice and rats [15–17]. In rats, the activity of hepatic HMG-CoA reductase was increased markedly by statin treatment [16]. Therefore, the main reason for the failure to cause hypocholesterolemic effects in rats was considered to be due to the abnormal induction of HMG-CoA reductase activity. Lipoprotein metabolism in mice and rats are markedly different from that in humans [18]. Mice and rats have apoB-48-containing very low-density lipoprotein (VLDL) and LDL particles in the plasma, which has been attributed to the expression of an apoB-editing enzyme in the liver [19], and no CETP activity in the plasma [20]. These features suggest that apoB-48-containing VLDL and LDL play a major role, and LDL receptor function a minor role in lipoprotein metabolism in these animals. Indeed, serum cholesterol levels were approximately 220 mg/dl (5.7 mmol/L) in homozygous LDL-receptor-KO mice fed normal chow [21], but are 500–1000 mg/dl (12.9–25.9 mmol/L) in patients with homozygous FH and WHHL rabbits (Fig. 1). Serum and LDL cholesterol levels were markedly increased by the overexpression of apoB-100 in LDL-receptor-KO mice [22] and were markedly lowered by hepatic gene transfer of apoB-editing enzyme in WHHL rabbits [9]. These studies suggested that the difference observed in serum cholesterol levels between LDL-receptor-KO mice and homozygous FH or WHHL rabbits is due to the secretion of apoB-48 containing lipoproteins from the livers of mice. In addition, apoB-48 containing lipoproteins are rapidly removed from the plasma by the heparan sulfate proteoglycans-LDL receptor-related protein pathway in the Disse space of the liver [23], and the fractional turnover rates of apoB-48 containing VLDL are approximately 7 times higher than those of apoB-100 containing VLDL [24]. These differences in lipoprotein metabolism may also be related to why the hypolipidemic effects of statins are weak in mice and rats. In contrast, WHHL or WHHLMI rabbits (homozygotes) do not express apoB-editing enzyme in the liver [9] and exhibit high CETP activity in the plasma [10] (Fig. 1). Serum cholesterol levels are 500–1200 mg/dl (12.9–31.0 mmol/L) with normal chow, and apoB-100 containing LDL particles accumulate in the plasma [2,6]. The lipoprotein metabolism in WHHL rabbits is close to that of FH [2]; however, LDL fractions prepared with ultracentrifugation (1.019–1.063 g/ml) were shown to contain small amounts of apoE and apoC, and the ratio of triglyceride/cholesterol in the LDL fraction is higher than that of humans [25]. Statins and other inhibitors of cholesterol synthesis show hypocholesterolemic effects on WHHL and WHHLMI rabbits. Therefore, the selection of animal species and animal models is important in the development of therapeutic compounds.