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Atherosclerosis in Watanabe heritable hyperlipidemic rabbit arteries

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Introduction

Coronary artery disease is still the leading cause of death in industrialized countries and the disease is caused by atherosclerosis. Atherosclerotic process begins early in the childhood, decades before first clinical cymptoms. There are four main types of atherosclerotic lesions (*Stary et al.* 1994). Lesion types I and II are very important in the early phase of atherogenesis. Clinically more important lesions are lesion types III and IV: Because the first lesion types in humans develop decades before the first clinical symptoms, it is very difficult to obtain samples from early human lesions. For that reason adequate animal models are essential for studies regarding the pathogenesis of atherosclerosis. One of the best models is Watanabe heritable hyperlipidemic (WHHL) rabbit which develops spontaneous hypercholesterolemia and atherosclerosis (*Watanabe et al.* 1980).

The WHHL-rabbit is a strain of rabbits with a consistently inherited hyperlipidemia produced by inbreeding of a mutant discovered in 1973 (*Watanabe et al.* 1980). WHHL-rabbits lack funcional low density lipoprotein (LDL) receptors (*Bilheimer et al.* 1982) which results in an increase in plasma cholesterol and triglyceride levels. Homozygous WHHL-rabbits develop atherosclerosis very early. Morphology of the lesions is very similar to that of human atherosclerotic lesions and is different from lesions in cholesterol-fed rabbits (*Rosenfeld et al.* 1990). Therefore, the WHHL-rabbit is an excelent model to study the development of atherosclerotic lesions (*Rosenfeld et al.* 1987).

Due to the lack of functional LDL-receptors the plasma cholesterol and triglyceride levels in WHHL-rabbits are abnormally high. Plasma cholesterol (P-Ch) levels can vary from 10 to 50 mmol/l and plasma triglyceride (P-Tg) from 1,5 to 20 mmol/l. In this study we analyzed atherosclerosis and standard laboratory values in our WHHL-rabbit colony.

Materials and Methods

All animals (n=20) were housed individually in steel cages under controlled environmental conditions. The rabbits received standard rabbit chow (K5, Lactamin, Sweden) and tap water ad libitum. Blood samples for all measurements were collected from central ear artery at the age of 3-6 months after 12 hour fasting. All the rabbits were clinically healthy. At the end of the study animals (mean age 2.0 years, min. - max., 0.84-4.5 years) were sacrificed and the aorta was dissected from the heart to the bifurcation. The aorta of 8 rabbits was opened longitudinally and macroscopic lesions were evaluated by a naked eye examination.

All laboratory studies were made at the Kuopio University Hospital, Kuopio, Finland. Plasma total cholesterol, HDL-cholesterol and triglyceride values were measured by Specific, Kone Instruments Corp., Finland. Liver enzyme levels were measured by Specific, Kone Instruments Corp., Finland and other blood samples by Celtac Auto MEK-8118K, Nihon Kohden, Japan. Reagents and protocols for laboratory measurements were those suggested by analyzer manufacturer.

Results

Mean, minimal and maximal values for plasma total cholesterol (P-Ch), HDL cholesterol (P-HDL) and plasma triglyceride (P-Tg) levels, and aorta lesion areas are presented in Table 1. Due to other studies aorta lesion areas were evaluated only from 8 animals. The main lesion type was a lipid-rich plque or a type IV lesion. Blood hemoglobin (B-Hb), hematoerit (B-Het), red blood cell count (B-RBC), white blood cell count (B-WBC), platelet count (B-Platelet), mean corpuscular volume (E-MCV), mean corpuscular hemoglobin (E-MCH) and mean corpuscular hemoglobin concentration (E-MCHC) are shown in Table. 2. The following plasma liver enzyme levels were also analyzed; aspartate transferase (P-AST), alanine transferase

Table 1.

	mean	min-max	n
Age	2.0 yrs	0.84-4.5	8
Lesion areas			
Aortic arch	91%	70-100	8
Thoracic aorta	48%	10-100	8
Abdominal aorta	26%	0-75	8
P-Cholesterol	20.2 mmol/l	10.5-31.9	20
P-HDL-Cholesterol	0.33 mmol/l	0.15-0.58	20
P-Triglyceride	8.0 mmol/l	2.2-19.9	20

Table 2.

	mean	min-max	n
B-Hb	137 g/l	132-141	2
B-HCT	41.3%	39.9-42.7	2
B-WBC	6.4 E9/l	5.6-7.2	2
B-RBC	6.09 E12/l	6.06-6.12	2
B-Platelet	512 E9/l	454-569	2
E-MCV	68 fi	65-72	2
E-MCH	22.5 pg	22-23	2
E-MCHC	331 g/l	330-331	2
P-AST	39.8 IU/l	12-97	20
P-ALT	53.0 IU/l	16-114	20
P-ALP	44.7 IU/l	10-114	20
P-GGT	3.1 IU/l	0-22	20
P-LDH	120 IU/l	62-220	20

Table 2. Laboratory values in normal WHHL-rabbits. Blood hemoglobin (B-HB), Hematocrit (B-Hct), white blood cell count (B-WBC), red blood cell count (B-RBC), platelet count (B-Platelet), mean corpuscular volume (E-MCV), mean corpuscular hemoglobin (E-MCH), mean corpuscular hemoglobin concentration (E-MCHC), aspartate transferase (P-AST), alanine transferase (P-ALT), alkaline phosphatase (P-ALP), γ-glutamyl transpeptidase (P-GGT) and lactate dehydrogenase (P-LDH).

(P-ALT), alkaline phosphatase (P-ALP), γ-glutamyl transpeptidase (P-GGT) and lactate dehydrogenase (P-LDH) (table 2).

Discussion

High plasma cholesterol and triglyceride levels are well known risk factors for atherosclerosis. The mean plasma total cholesterol and triglyceride levels were much higher and plasma HDL cholesterol level much lower than in normal humans or in normal New Zealand White rabbits (*Ylitalo et al.* 1994). WHHL-rabbits had severe atherosclerosis already at the early age. Although plasma lipids were very high, liver enzyme levels were close to normal human values. Livers of the WHHL-rabbits were clinically normal at the time of sacrifice and there were no clinical signs of cholesterol accumulation, bile accumulation or other types of liver disease. Other laboratory values of the WHHL-rabbits were also similar to human values and indicate that these WHHL-rabbits were healthy without any major subclinical diseases or abnormalities other than hypercholesterolemia.

Cholesterol fed rabbits usually develop severe liver damage only after a few months on cholesterol feeding even though plasma cholesterol levels are nog much higher than in normal WHHL-rabbits. This may be due to the harmful effects of exogenous cholesterol, as compared to the endogenously produced cholesterol in the WHHL-rabbits.

Our study shows that the WHHL-rabbit is an important animal model for atherosclerosis research. The WHHL-rabbit develops atherosclerotic lesions on a regular rabbit chow without any manipulations, which may cause artifacts and severe health problems. Other studies have also demonstrated that the morphology of atherosclerotic lesions in WHHL-rabbits is similar to that of human atherosclerotic lesions and differs from those lesions observed in cholesterol-fed rabbits (*Fischer Hansen et al.* 1984, *Rosenfeld et al.* 1990). All these aspects make WHHL-rabbit a valuable model in atherosclerosis research.

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