WHHL-RABBIT: A LOW DENSITY LIPOPROTEIN RECEPTOR-DEFICIENT ANIMAL MODEL FOR FAMILIAL HYPERCHOLESTEROLEMIA

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Received 7 July 1980

1. Introduction

Skin fibroblasts from normal human subjects possess a specific receptor for low density lipoprotein (LDL), but cells from subjects with homozygous form of familial hypercholesterolemia (FH) lack it [1–3]. Although LDL receptor-deficiency was eludicated to be a primary metabolic defect of FH, many unknown processes responsible for its pathogenesis have not yet been clarified. Thus an animal model for FH is required. A method for producing LDL receptor-deficient mice using mutagenized cultures of teratocarcinoma stem cells appeared in [4]. The lipid metabolism of rhesus monkeys with spontaneous hypercholesterolemia was analyzed [5]. In spite of these efforts, the presence of LDL receptor-deficient animal has not been reported.

WHHL-rabbit (Watanabe-heritable hyperlipidemic rabbit), characterized by its abnormally high level of serum lipids, is a strain developed by inbreeding from a mutant of spontaneous hyperlipidemia [6,7]. Here, we describe the biochemical characteristics of WHHL-rabbit including LDL receptor-deficiency in its skin fibroblasts.

2. Materials and methods

2.1. *Lipoproteins*

Lipoproteins both from normal and WHHL-rabbits were fractionated by sequential floatation at 10° C according to standard techniques [8]. For the purpose of cell culture, human LDL (d = 1.019–1.063 g/ml), high density lipoprotein (HDL, d = 1.063–1.215 g/ml) and lipoprotein-deficient serum (LPDS, $d > 1.215$ g/ml) were prepared from the blood from healthy subjects fasted for 15 h. For the study of ^{125}I-LDL in vivo, LDL fraction ranging from d = 1.030–1.055 g/ml was isolated from normal rabbit. LDL was iodinated using iodine monochloride method [9] with slight modification [10].

2.2. *Catabolism of ^{125}I-LDL in vivo*

Male normal and WHHL-rabbits (12-month-old, 3 in each group) were housed in metabolic cages and fed ORC-4 diet (Oriental Yeast Co., Tokyo) 150 and 120 g/day, respectively. A measured volume of the ^{125}I-LDL was injected into rabbits through an ear vein and 100 μl samples of blood were obtained at specified intervals. Trichloroacetic acid-insoluble ^{125}I in the diluted plasma was counted. The results were expressed as % of the radioactivity in the blood sample taken 2–3 min after the injection.

2.3. *Cells*

Cultured fibroblasts were derived from skin biopsies from both normal and WHHL-rabbits. All cells were used under similar conditions as those of humans [11].

2.4. *Assays*

Binding and degradation of ^{125}I-LDL by intact fibroblasts were determined by the method in [11]. The radioactivity which associates with the cells at 37°C represents the amount of bound plus internalized ^{125}I-LDL [12], but is expressed as binding of ^{125}I-LDL here. 3-Hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase activity and cholesterol esterification activity were assayed as in [13].

3. Results

3.1. *Blood lipids and lipoproteins*

The serum cholesterol of normal rabbits was mainly localized to HDL, but most of the elevated cholesterol content of WHHL-rabbits was localized to LDL (table 1). Elevated levels of serum triglycerides and phospholipids of WHHL-rabbits were also localized preferentially to LDL (not shown). Their levels of very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL) increased significantly, but the amount of HDL decreased. These results agree well with disc gel electrophoresis studies [7]. The ratio of free cholesterol to total cholesterol (FC/TC) in the serum of WHHL-rabbits, however, coincided with that of normal rabbits.

3.2. *Catabolism of ^{125}I-LDL in vivo*

The rate of removal of ^{125}I-LDL from the bloods of WHHL-rabbits was slower than that of normal rabbit (fig.1). About 50% of the injected ^{125}I-LDL was cleared from the plasma of normal rabbit within 4 h after injection, whereas it took 15 h in WHHL-rabbit.

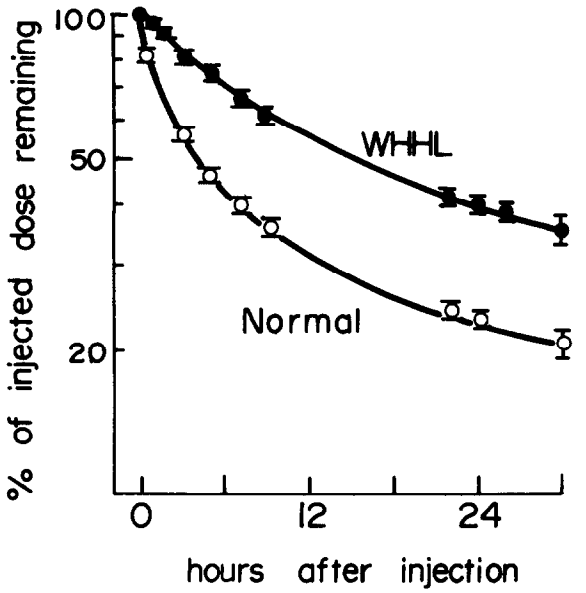

Fig.1. Percent of the total injected dose of rabbit ^{125}I-LDL that remained in trichloroacetic acid-insoluble fraction of the plasma. The figure is plotted as a function of time after intravenous injection into normal rabbit (○) or WHHL-rabbit (●). The mean ±SD (bar) represents the values obtained in 3 rabbits at each time point.

3.3. *LDL receptor activity in skin fibroblasts*

The time courses of binding and degradation of ^{125}I-LDL at 37°C by intact skin fibroblasts from a normal rabbit are illustrated in fig. 2A. The amount of ^{125}I bound to the cells reached a maximum at 2 h and remained constant thereafter, whereas degradation proceeded almost linearly after an initial 0.5 h lag period. However, neither binding nor degradation of ^{125}I-LDL took place in the cells from WHHL-rabbit (fig. 2B). The binding of ^{125}I-LDL to the normal cells was abolished almost completely by the addition of unlabeled LDL (fig. 2A), but decreased only slightly by unlabeled HDL (not shown). High-affinity binding and degradation were calculated as in [4]. The fibroblasts from normal rabbits do possess high-affinity LDL receptor activity regardless of their age, sex (table 2) or growing phase of the cells. However, LDL receptor activity was found to be deficient almost completely in all the cell strains from WHHL-rabbits (table 2).

3.4. *HMG-CoA reductase and cholesterol esterification activities*

In skin fibroblasts from WHHL-rabbits, HMG-CoA

Table 1
Serum lipoprotein concentrations in normal and WHHL-rabbits

Age (month)	Rabbit strain	Lipoprotein cholesterol (mg/dl serum)					FC/TC
		VLDL	IDL	LDL	HDL	Total	
3	Normal	1.4 ± 0.7[a]	n.d.[b]	6.2 ± 1.0	16.0 ± 0.7	23.6 ± 1.6	0.254
	WHHL	39.7 ± 17.4	43.4 ± 25.7	846 ± 287	3.0 ± 0.6	932 ± 259	0.267
9	Normal	1.1 ± 0.9	n.d.	4.7 ± 1.8	12.4 ± 4.6	18.2 ± 5.6	0.301
	WHHL	43.2 ± 26.3	64.1 ± 25.3	561 ± 32	4.4 ± 1.8	673 ± 42	0.312

[a] Mean ± SD, for 3 male animals; [b] not detected

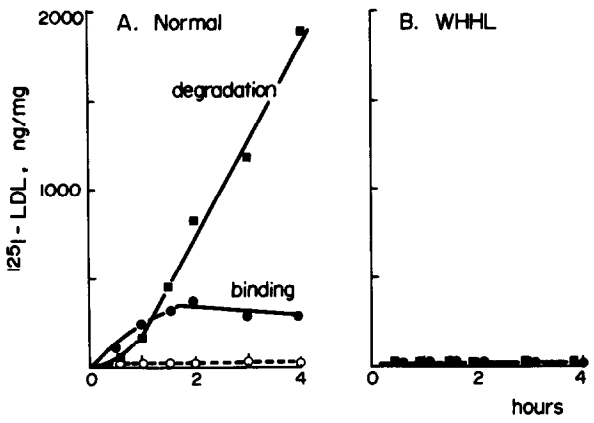

Fig.2. The effect of time of incubation at 37°C on ^{125}I-LDL binding and degradation by skin fibroblasts from normal rabbit (A) and WHHL-rabbit (B). The cells were grown in 60 mm tissue culture dishes to confluency before being washed once with phosphate buffer and incubated for 16 h with culture medium containing lipoprotein-deficient serum (LPDS medium). The ^{125}I-LDL was added to the dishes at 10 μg protein/ml final conc. The cells were then incubated at 37°C for the indicated times and analyzed for bound ^{125}I-LDL in the presence (○) or absence (●) of unlabeled LDL (150 μg protein/ml). Culture medium (1 ml) was analyzed for trichloroacetic acid-soluble ^{125}I radioactivity for the determination of proteolytic degradation of ^{125}I-LDL (■).

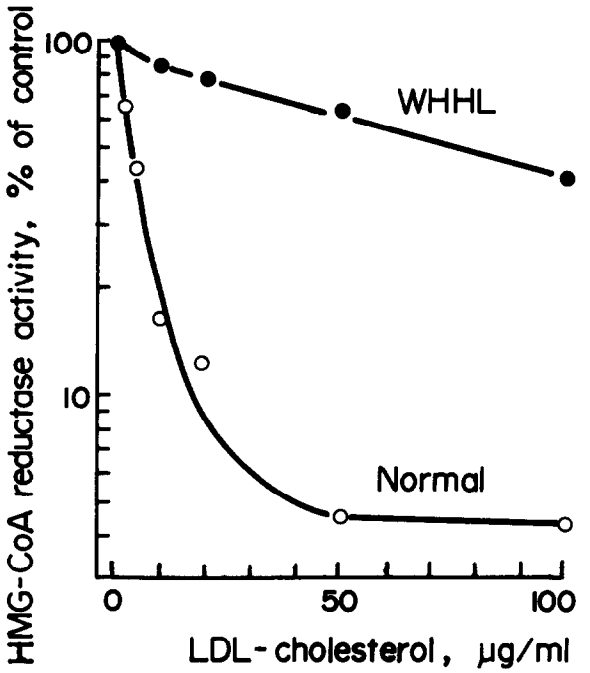

Fig.3. The effects of increasing amounts of LDL on HMG-CoA reductase activity in skin fibroblasts from normal rabbit (○) and WHHL-rabbit (●). When the cells were grown to confluency, the medium was replaced with LPDS medium and human LDL was added to the cultures at indicated concentrations. At 16 h after addition, the extracts from duplicate flasks were assayed for reductase activity.

reductase activity is higher and cholesterol esterification activity is lower than those in the cells from normal rabbits (table 2). The data in fig. 3 indicate that ~3 μg/ml of LDL-cholesterol is sufficient for 50% suppression of HMG-CoA reductase activity in the normal cells, but 74 μg/ml is required for the same effect in the mutant cells.

4. Discussion

The lipoprotein metabolism of WHHL-rabbit shows resemblance to that of FH homozygote in the following respects; (i) accumulation of serum LDL; (ii) reduced rate of clearance of ^{125}I-LDL from the blood; (iii) LDL receptor-deficiency in the cultured cells. As a consequence of LDL receptor-deficiency, suppression of HMG-CoA reductase activity and stimulation of cholesterol esterification activity by the cholesterol liberated from LDL function to a lesser extent in the mutant cells. High level of serum triglycerides and low level of HDL have been reported to be charac-

Table 2
Activities of LDL receptor, HMG-CoA reductase and cholesterol esterification in the cells from normal and WHHL-rabbits

Rabbit strain	No. cell strains	^{125}I-LDL (ng . 2 h^{-1} . mg^{-1})		HMG-CoA[a] reductase (pmol . min^{-1} . mg^{-1})	Cholesterol[a] esterification (pmol . h^{-1} . mg^{-1})
		High-affinity binding	High-affinity degradation		
Normal	3	342 ± 22	796 ± 117	4.7 ± 0.9	533 ± 38
WHHL	6	7.6 ± 5.8	10.6 ± 3.8	31.3 ± 6.5	54.5 ± 2.9

[a] The enzyme activities were assayed after3 day incubation in the medium containing 10% fetal-calf serum

teristic to FH homozygote [3]. In addition to the elevated level of serum cholesterol, these tendencies are more extreme in WHHL-rabbit.

However, similar values of FC/TC between normal and WHHL-rabbits suggest the normal functioning of lecithin–cholesterol acyltransferase in WHHL-rabbit irrespective of the HDL level. The genealogy of inbreeding of this animal suggests a single gene inheritance of the hyperlipidemia (Y.W., unpublished). In these respects, WHHL-rabbit will be a powerful tool for finding a significant role of LDL receptor-deficiency in the occurrence of clinical syndrome of hyperbetalipoproteinemia. Detailed studies on the cultured cells will be published elsewhere.

Acknowledgements

The authors are grateful to Dr K. Ohnishi, Central Research Laboratories, Sankyo Co., for his helpful advice on tissue culture. Thanks are also due to Mrs T. Nagatomo and A. Tamura for encouraging the study.

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