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CLEARANCE OF CHEMICALLY MODIFIED HUMAN LOW DENSITY LIPOPROTEIN FROM THE RABBIT PLASMA

Clearance of native and reductively methylated low density lipoprotein (LDL) in rabbits was studied. Chemical modification (reductive methylation) of the lysyl residues of lipoprotein permitted calculation of receptor-mediated and receptor-independent degradation of LDL in rabbits.

INTRODUCTION

Low density lipoprotein, the major cholesterol carrier in bloodstream, is involved in the pathogenesis of the atherosclerosis [2-4, 16]. This process is associated with the accumulation of cholesterol in the affected arterial walls [12]. A number of cells possess a common, specific LDL degradation pathway [1, 2, 9]. This consists of an autoregulated ordered sequence of events in which LDL is first bound to a high affinity receptor on the cell surface and then internalized by endocytosis, and transported to lysosomes where free cholesterol is released [2, 3]. Receptor recognition in cells depends on functionally significant lysine residues on the LDL apoprotein [5].

We have measured the plasma clearance rate of native and chemically modified LDL in rabbits.

MATERIALS AND METHODS

New Zealand White rabbits aged 10-14 months (3-3.5 kg) on a standard diet were injected with radioactive LDL (5 to 6 x 10^7 cpm/ng of protein) in physiological saline through the marginal ear vein. At specified time intervals (up to 24 hours) 0.1 ml of blood samples in duplicate were obtained, added to 2 ml of saline and counted in a gamma counter for 10 min.

Plasma decay kinetics was determined by curve peeling using computer technique. Fractional catabolic rates (FCR) were calculated as described by M a t t h e w s [7].

Human LDL (d 1.019-1.063 g/ml) was collected from plasma by differential ultracentrifugation [6].

Reductive methylation of LDL [8] : 0.5 ml of 0.3 M sodium borate buffer pH 9.0 was mixed with 2-3 mg of LDL protein at 0^oC on an ice bath. LDL was added to 1 mg of sodium borohydride at 0 time followed by 5 µl of 7.4% aqueous formaldehyde. A total of 12 additions were made up to 1 hour. At 30 min. the reaction mixture of LDL was transferred into another vial containing 1 mg of sodium borohydride for extensive modification. After the last addition LDL was dialysed against 0.15 M NaCl, 0.01% Na_2EDTA at 7.0 at 0^oC for approximately 18 hours. To determine the extent of modification [15] 0.1 ml of both native and reductively methylated LDL were dissolved in 0.1 M sodium tetraborate, pH 9.3 (0.9 ml). To each sample 25 µl of 0.03 M 2-4-6-trinitrobenzene--sulphonic acid were added, mixed and incubated for 30 min. at room temperature. Absorbance was read at 420 nm [15].

Native and reductively methylated LDL were labelled (11) separately with ^{125}JCl and ^{131}JCl, respectively (Radiochemical Centre, Amersham). The LDL particles were subsequently freed of unbound radioiodide by gel filtration through a 1 x 20 cm column of G-10 Sephadex (Pharmacia, Uppsala, Sweden) using 0.15 M NaCl - 0.01% Na_2EDTA, pH 7.0 in the elution process [5].

RESULTS

The results from the radioassay of blood samples are expressed as the percent of initial LDL radioactivity that remained in the plasma. The date from the radioassay fixed to biexponential functions are shown on Fig. 1. The animals from experiments degrade the injected LDL rapidly. $T_{1/2}$ of native LDL particles was established to be 7.65 ±0.2 h, whereas methylated LDL was degraded slowly ($T_{1/2}$ 12.84 ±1.2 h) (Table 1). Following reductive methylation 15 lysine residues were modified in the course of our experiments. They constitute 75% of lysine residues reduction in LDL. Due to this modification methylated LDL in cleared off from circulation slower than native (Fig. 1). Fractional catabolic rate (FCR, h^{-1}) of native and chemically modified LDL is presented in Table 1.

Table 1

Plasma decay kinetics of native and methylated LDL in rabbits

Rabbit	FCR (h^{-1})			$T_{1/2}$ (h)	
	methyl. LDL	native LDL	receptor-dependent	methyl. LDL	native LDL
1	0.0694	0.0842	0.013	10.04	8.42
2	0.0404	0.0977	0.057	17.32	7.11
3	0.0430	0.100	0.057	16.11	6.93
4	0.0610	0.088	0.028	11.36	7.89
5	0.0609	0.0865	0.0256	11.36	8.01
6	0.064	0.096	0.032	10.83	7.19
7	-	0.0869	-	-	7.97
$\bar{x}$	0.0565	0.0913	0.035	12.84	7.65
SD	±0.004	±0.002	±0.007	±1.20	±0.2

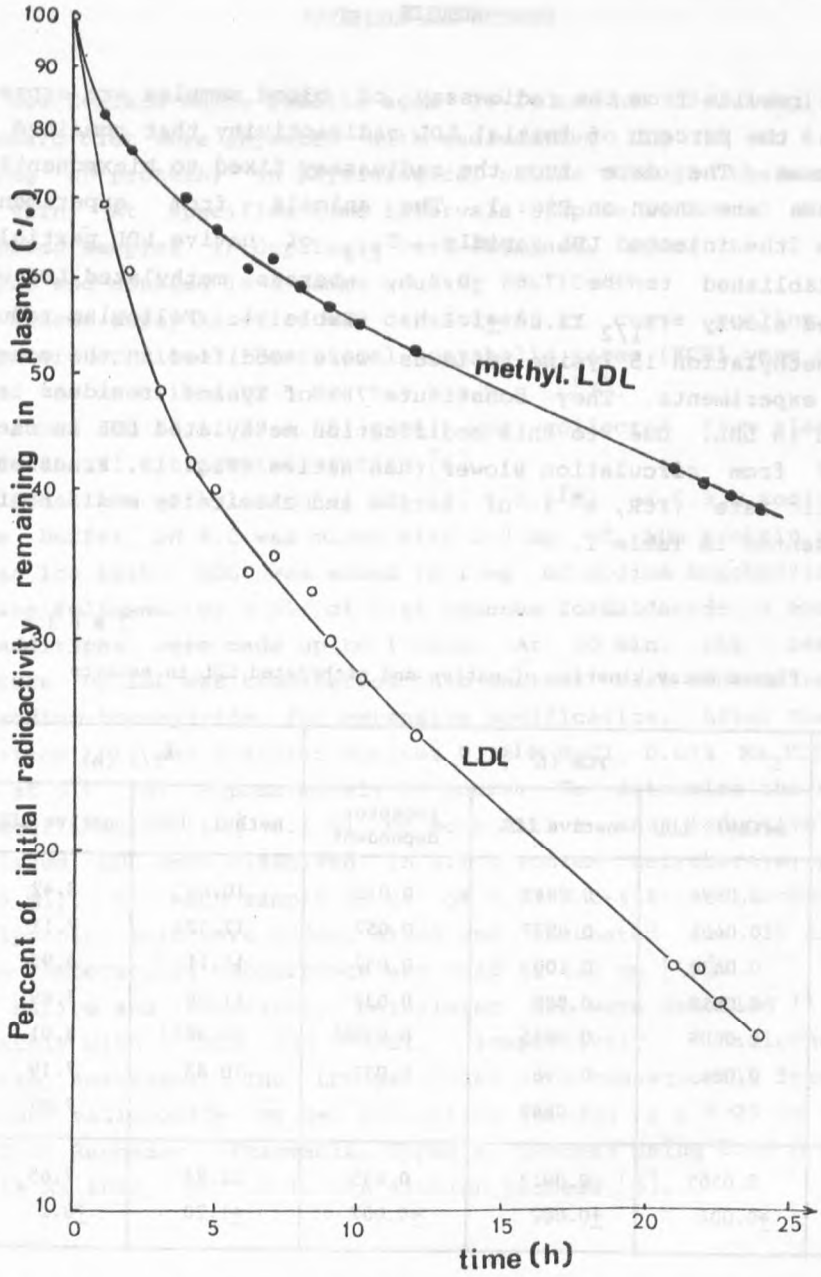

Fig. 1. Plasma decay kinetics of two LDL preparation (in rabbit 2);
o - native LDL, ● - methylated LDL

DISCUSSION

Reaction of reductive methylation of LDL [5] allows formaldehyde to form a Schiff base with the amino groups of lysine residues on apo-LDL. This compound is reduced with $NaBrH_4$ to a monomethyl derivative which undergoes futher reaction to yield dimethyllysine. This process is irreversible and provides a product chemically stable both in vivo and in vitro. Reductive methylation of at least 6 lysyl residues (30% modification of the lysine residues) abolishes LDL binding activity to specific receptors [5].

The uptake and degradation of low density lipoproteins occur in part by way of mechanisms involving the specific LDL receptor first described by Goldstein and coworkers [2]. In addition, LDL can be taken up and degraded by low affinity adsorptive endocitose that does not involve this receptor [1, 10, 12-14, 16, 17].

Our preliminary findings confirm that LDL uptake and degradation in rabbits occur not only by way of receptor - mediated process but also receptor - independent [10, 12, 16, 17].

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KATABOLIZM ZMODYFIKOWANYCH LIPOPROTEIN O NISKIEJ GĘSTOŚCI W OSOCZU KRÓLIKA

Badano metabolizm natywnych i metylowanych cząsteczek LDL w osoczu królika. Chemiczna modyfikacja (reduktywna metylacja) reszt lizynowych w apoproteinie pozwala na określenie in vivo przemiany LDL zależnej i niezależnej od specyficznych receptorów.