AGGREGATES OF MODIFIED LOW-DENSITY LIPOPROTEINS INDUCE LIPID ACCUMULATION IN INTIMAL CELLS OF THE HUMAN AORTA IN VITRO

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Accumulation of intracellular lipids is a characteristic feature of atherosclerosis of the blood vessels in man. There is now no doubt that the source of accumulating lipids is the low-density lipoproteins (LDL) which circulate in the blood. However, the mechanism of LDL-induced lipid accumulation in the cells is not known. Some workers have demonstrated [4, 7, 13] that native LDL, isolated from the blood of healthy individuals, do not induce an increase in the lipid concentration in cells in culture. Accumulation of intracellular lipids was observed only when LDL modified chemically by treatment with malonic aldehyde or glutaraldehyde [3], by desialation [11], oxidation [12], glycosylation [14], and so on, were used. It is intriguing in this case that a wide range of different chemical modifications of LDL should give rise to the same response at the cellular level, namely, accumulation of lipids. This observation suggested to us that in all the cases described, the rise of the intracellular lipid level is based on the same process. This process may perhaps be the formation of aggregates, inducing lipid accumulation. Indirect support for this hypothesis may be given by data showing that associates of LDL with components of the connective-tissue matrix, and also with large particles of unnatural origin facilitates lipid accumulation in cells in culture [2, 10]. In the present investigation we sought experimental confirmation of this hypothesis concerning the decisive role of spontaneously formed aggregates of modified LDL in the process of intracellular lipid accumulation in intimal cells of the human aorta.

EXPERIMENTAL METHOD

LDL (1.019-1.063 g/ml) were isolated by ultracentrifugation from pooled blood from 10 clinically healthy persons and from 10 patients with coronary heart disease (CHD), in whom stenosis of the coronary arteries was demonstrated angiographically. The LDL were glycosylated in vitro for 2 weeks in the presence of 50 mM glucose [6]. The LDL were desialated by treatment with agarose-bound neuraminidase (0.01 IU/ml) for 2 h at 37°C [11]. Oxidized lipoproteins were obtained by the method in [12]. MDA-modified LDL were obtained by the method in [3]. The degree of aggregation of the lipoproteins was assessed by a method based on analysis of fluctuation (D) was used as a parameter reflecting the degree of aggregation of LDL. Gel-filtration of the lipoproteins was carried out on Sepharose CL-2B (column 22 × 0.6 cm, filtration rate 150 μl/min). Smooth-muscled cells of the unaffected intima of the aorta from men dying suddenly from myocardial infarction were isolated with the aid of collagenase and elastase and were cultured as described previously [9, 10]. On the 7th day of culture the cells were treated with medium 199 containing 5% lipoprotein-deficient serum (LDS) from a healthy person, obtained by ultracentrifugation ($\rho >$ 1.250 g/ml), and also with 100 μg/ml of native or modified LDL. All preparations were filtered (0.1 μm) immediately before addition to the culture. At the end of incubation the cells were washed and lipids extracted with a mixture of hexane and isopropranol (3:2, v/v) [5]. To determine the content of cholesterol esters (ChE) the quantity of free and esterified choleserol was measured by means of kits from the firm "Boehringer-Mannheim" (West Germany). The cell protein concentration was determined by Lowry's method [8].

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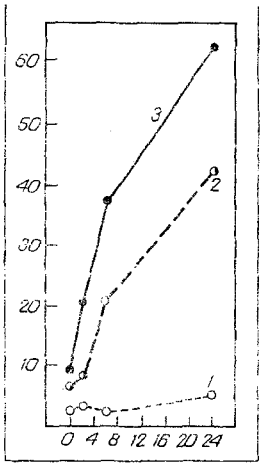

Fig. 1. Kinetics of aggregation of LDL under conditions of incubation. 1) Native LDL from healthy individual, 2) native LDL from patient with CHD, 3) MDA-modified LDL. Abscissa, incubation time (in h); ordinate, mean particle size in suspension (in relative units).

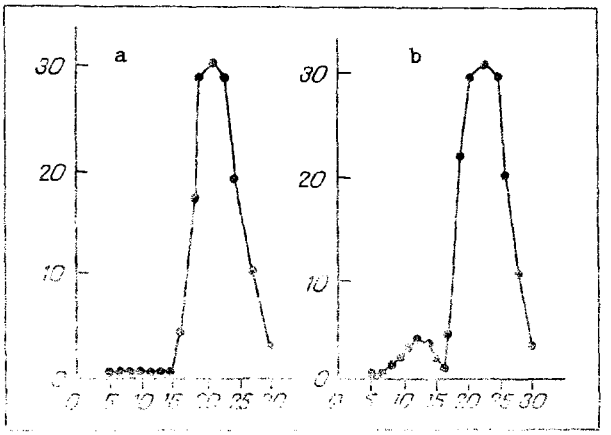

Fig. 2. LDL oxidized for 18 h at 37°C in the presence of 10^{-5} M Cu^{2+}, passed through a filter with pore diameter 0.1 μm and tested by gel-filtration before (a) and after (b) repeated incubation for 6 h at 37°C. Abscissa, serial No. of fraction; ordinate, cholesterol of LDL, μg/ml.

EXPERIMENTAL RESULTS

In the first stage we attempted to find aggregation of native LDL obtained from healthy individuals and patients with CHD, and also of LDL modified with MDA (MDA-LDL) is shown in Fig. 1. Clearly native LDL from healthy individuals, under conditions of culture, virtually did not aggregate until 6 h of incubation. Meanwhile, marked aggregation of LDL from patients with CHD and of MDA-LDL was observed. Aggregate formation also was found in preparations of lipoproteins subjected to various chemical modifications. In particular, under conditions of culture aggregation of glycosylated, desialated, and oxidized LDL was observed (Table 1).

The ability of modified LDL to aggregate spontaneously also was demonstrated by gel-filtration. The gel-filtration profile of oxidized LDL is shown in Fig. 2. The principal peak of LDL was preceded by a small peak of lipoproteins with much greater molecular weight of their particles; this peak was not detected during a study of previously filtered (0.1 μm) oxidized LDL (Fig.

TABLE 1. Aggregation of Native and Modified LDL and their Effect on CHE Content (in % of control) in Intimal Cells from Human Aorta

LDL	Accumulation of ChE	Mean particle size, relative units
Native	117±6	6,8
Patients with CDH	222±14*	22,5
Glycosylated	217±11**	30,0
Desialated	284±14**	39,5
Oxidized	286±17**	45,2
MDA-LDL	317±28*	52,3

Legend. $*p < 0.005$, $**p < 0.001$.

TABLE 2. Effect of Filtration of Preparations of Oxidized and Desialated LDL on their Ability to Increase the CHE Content in Cells of Undamaged Intima from Human Aorta

LDL	CHE content, μg/mg
Control	37±2
Native	43±2
Oxidized:	
before filtration	85±7
after filtration at 0.1 μ	39±4*
Desialated	
before filtration	108±8
after filtration at 0.1 μ	41±2**

Legend. $*p < 0.025$, $**p < 0.005$.

2a), and reappeared during investigation of lipoproteins incubated after filtration at 37°C for 6 h (Fig. 2b). Similar results were obtained for glycosylated LMP.

The ability of native and modified lipoproteins to induce CHE accumulation in cells of the unaffected intima of the human aorta was next studied. Native LDL from healthy individuals did not induce a significant increase in the intracellular ChE concentration. On the other hand, an increase was observed in the ChE concentration in cells incubated with desialated, glycosylated, and oxidized LDL and MDA-LDL (Table 1). In this case close positive correlation was found between the degree of aggregation of LDL and accumulation of ChE in cells of the unaffected intima of the human aorta ($r = +0.86$, $p < 0.001$, $n = 21$).

Next we attempted to obtain further confirmation that LDL aggregates induce ChE accumulation in the cells. For this purpose, preparations of freshly obtained and filtered (0.1 μm) modified (desialated and oxidized) LDL were incubated under conditions of culture and again filtered. The LDL were then added to a culture of intimal cells and incubated for a further 6 h, after which the intracellular CHE content was analyzed. The results given in Table 2 show that preincubated oxidized and desialated LDL induced a 2.5-3-fold accumulation of ChE. Filtration of the preparations after incubation (0.1 μm) prevented an increase in the intracellular ChE concentration during incubation for 6 h.

To sum up the information in this paper, it can be concluded that under conditions of culture in vitro aggregation of modified LDL takes place. It is these modified, and not native LDL that can induce ChE accumulation in intimal cells of the human aorta. Under these circumstances close correlation exists between the degree of aggregation and the quantity of ChE accumulated. Finally, removal of LDL aggregates from the medium prevents ChE accumulation in the cells. All these data indicate the important, if not decisive, role of aggregation of LDL in processes of accumulation of lipids by cells in vitro. It can be tentatively suggested that the formation of LDL aggregates takes place under conditions in vivo, but this hypothesis requires experimental confirmation.

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