APOLIPOPROTEIN B OF INBUILT PLASMA LIPOPROTEINS OF LIPOSOMES: IMMUNOLOGIC PROPERTIES AND DISTRIBUTION AMONG ORGANS AFTER INJECTION INTO RABBITS

Academician A. N. Klimov,* B. F. Korovkin,† A. S. Kuznetsov, and I. N. Popov

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Lipid–protein interactions in plasma lipoproteins are currently attracting the attention of many investigators. Mainly the characteristics of binding of phospholipids and cholesterol by protein components of lipoproteins (apolipoproteins, APO) of high or very low density have been studied [3, 13, 15, 18]. In addition, apo-A-1, noncovalently bound with liposomes, has been used to study the release of cholesterol from cells [22] and the reaction of lecithin-cholesterol acyltransferase [8], and apo-E, noncovalently bound with liposomes, has also been used to study the metabolism of this protein in the liver [11]. So far as apo-B, the principal protein component of low-density lipoproteins (LDL), is concerned the only information in the literature is the results of a study of binding of palmitoyllysolecithin by this protein [17].

In the investigation described below the immunologic and catabolic properties of liposomes with inbuilt apo-B were studied for the first time.

EXPERIMENTAL METHOD

LDL were isolated from blood plasma from healthy donors within the density range 1.091-1.055 g/ml by ultracentrifugation and labeled with ^{125}I by the iodine monochloride method. Apo-B was obtained after gel filtration of LDL, delipidized with sodium deoxycholate [12], on ultragel AcA-34. Protein was determined by Lowry's method.

To obtain liposomes, purified egg lecithin and cholesterol (from Sigma, USA) were used.

Apo-B was built into liposomes by the method in [9]; the lipid–protein ratio was 33 mg/mg, the cholesterol-lecithin ratio 1 M: 1 M, and the sodium deoxycholate–lipid ratio 2 mg/mg.

Liposomes were characterized by data on distribution of phospholipids, of radioactivity of apo-B, and fluorescence of rhodamine 6G after ultracentrifugation in a Ficoll density gradient (Fig. 1a). The dimensions of the liposomes were calculated from their turbidity spectrum [1].

The immunologic properties of apo-B-liposomes were assessed on the basis of the results of the ring precipitation test and binding with IgG, isolated after immunization of rabbits with human apo-B from their blood serum, immobilized on Sepharose 4B. Binding of cholesterol esters of LDL and apo-B, labeled with [^{14}C]oleate, with concanavalin-A-Sepharose also was used as a criterion of preservation of the native state of apo-B in the composition of the liposomes.

The distribution of [^{125}I]-apo-B-liposomes and of [^{125}I]-LDL among the organs was determined 3 h after intravenous injection of these preparations into rabbits with a total protein

*Academy of Medical Sciences of the USSR.
†Corresponding Member, Academy of Medical Sciences of the USSR.

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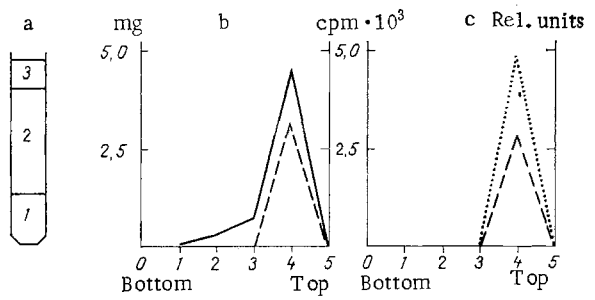

Fig. 1

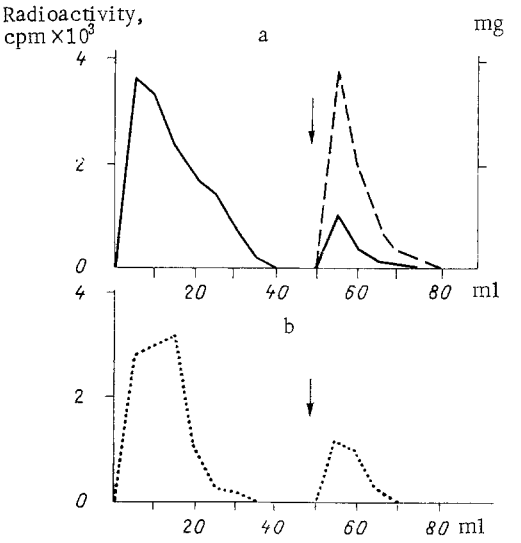

Fig. 2

Fig. 1. Ultracentrifugation of liposomes in Ficoll density gradient. a) Diagram of preparation of density gradient: 1) 1 ml of 30% Ficoll in 0.15 M NaCl + 0.4 ml liposomes, 2) 3 ml of 10% Ficoll in 0.15 M NaCl, 3) 0.15 M NaCl; b) distribution of phospholipids and radioactivity of [^{125}I]-apo-B; c) distribution of radioactivity of [^{125}I]-apo-B and intensity of fluorescence of rhodamine 6G. Continuous line — phospholipids (in mg); broken lines — radioactivity of [^{125}I]-apo-B (in cpm•10^3); dotted line — intensity of fluorescence of rhodamine 6G (in relative units). Abscissa, numbers of fractions. Conditions of centrifugation: SW 65 TI rotor; 25,000 rpm; 35 min; 10-18°C.

Fig. 2. Affinity chromatography of apo-B-liposomes on IgG-Sepharose (a) and concanavalin A-Sepharose (b). [^{125}I]-apo-B-liposomes were added in the first case, [^{14}C]-oleate—cholesterol—apo-B-liposomes in the second case. Arrow indicates change to a new eluting solution; 0.1 M glycine-HCl, pH 2.5 (a) and 5% α-methylmannopyronoside or 2% Triton X-100 (b). Continuous lines — phospholipids (in mg); broken line — radioactivity of [^{125}I]-apo-B (in cpm•10^3); dotted line — radioactivity of [^{14}C]oleate of cholesterol (in relative units). Column 2.5 × 5 cm in both cases.

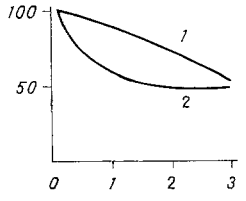

Fig. 3

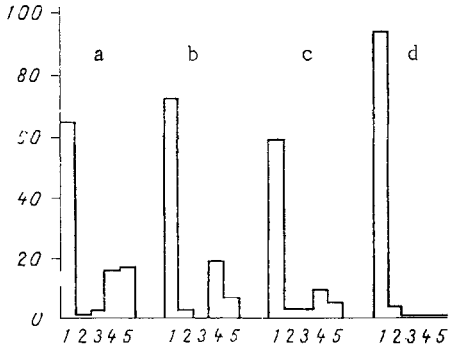

Fig. 4

Fig. 3. Elimination of [^{125}I]-apo-B-liposomes (1) and [^{125}I]-LDL (2) from blood stream. Abscissa, time (in h); ordinate, radioactivity (in %). Radioactivity determined 10 min after injection of liposomes and lipoproteins taken as 100.

Fig. 4. Distribution of liposomes and LDL among five rabbit organs. a) [^{125}I]-apo-B-liposomes, b) [^{125}I]-LDL, c) [^{125}I]albumin—apo-B-liposomes, d) [^{125}I]albumin—liposomes. 1) Liver, 2) spleen, 3) heart, 4) lungs, 5) kidneys. Ordinate, radioactivity of organs studied.

activity of $2.2 \cdot 10^9$ cpm/mg. To compare the distribution of apo-B-liposomes and liposomes without this protein among the organs, the liposomes were loaded with [^{131}I]albumin.

Phospholipids were calculated as phorphorus after ignition. Fluorescence was measured on a Turner-430 (USA) spectrofluorometer. Radioactivity was measured on an NK-350 Gamma counter. Experiments were carried out on 18 male chinchilla rabbits. The animals were killed with auxamethonium and the organs were thoroughly washed free from blood with physiological saline containing heparin.

EXPERIMENTAL RESULTS

The distribution of radioactivity of apo-B and phospholipids in the tube after ultracentrifugation of liposomes obtained in a Ficoll density gradient is shown in Fig. 1. All of the added apo-B and all phospholipids were localized in one zone at the boundary between Ficoll and physiological saline. This indicates that all the apo-B was built into the liposomes. Liposomes containing apo-B and rhodamine 6G (purified from free and adsorbed dye) had the same distribution of radioactivity of protein and of rhodamine fluorescence (Fig. 1c). The distribution of radioactivity of apo-B and of rhodamine fluorescence was similar for freshly prepared liposomes and for liposomes kept for 1 month. On the addition of Triton X-100, apo-B, phospholipids, and rhodamine 6G were localized at the bottom of the tube, evidence that the method used to estimate the stability of the liposomes was adequate. The mean diameter of apo-B liposomes was 80 ± 5.4 and 84 ± 6.7 mm respectively immediately after preparation and 1 month later. The data given above thus indicate that the liposomes were stable in the presence of apo-B with the lipid:protein ratio chosen. Incorporation of larger quantities of apo-B into liposomes raised certain difficulties and in some cases destruction of the liposomes was observed.

Apo-B liposomes gave clearly defined rings and residue in the ring precipitation test. In affinity chromatography on IgG-Sepharose with gel all the apo-B was bound but only 10% of the liposomes (as phospholipids; Fig. 2a). A similar degree of binding also was found with concanavalin A-Sepharose (Fig. 2b). The results indicate that apo-B, built into liposomes, preserves its antigenic properties; the carbohydrates of this protein face the aqueous environment and are responsible for interaction with concanavalin A, just as in the case of native LDL.

Elimination of apo-B-liposomes and LDL from the blood stream is illustrated in Fig. 3a. During the first 2 h apo-B-liposomes were eliminated more slowly than LDL. However, toward 3 h this difference was somewhat reduced. The reason for this behavior of apo-B in the composition of liposomes may be some difference in the character of localization of protein in liposomes and LDL, which is probably unavoidable in reconstructed experiments in which proteins with high molecular weight were used.

Data on the distribution of [^{125}I]-apo-B-liposomes, [^{125}I]-LDL, [^{131}I]albumin—apo-B-liposomes, and [^{131}I]albumin—liposomes among five rabbit organs are given in Fig. 4. It will be clear from Fig. 4 that [^{125}I]-apo-B-liposomes are taken up to a lesser degree by the liver and spleen, but to a greater degree by the kidneys than [^{125}I]-LDL. The distribution of [^{125}I]-apo-B-liposomes and of [^{131}]albumin—apo-B-liposomes was similar in principle, except in the lungs. However, it must be recalled that because of differences in the labels used, a completely identical character of their distribution could hardly be possible. [^{131}I]albumin—liposomes accumulated mainly in the liver. The results given above thus show that the apo-B-liposome transports its contents into the same organs as LDL, unlike liposomes from ovolecithin and cholesterol.

Many different methods of ensuring a more effective supply of medication in the composition of liposomes to the target organs and tissues are nowadays known [2, 4, 10, 14, 16]. The present investigation showed for the first time that apo-B, built into a liposome, can also be used as a ligand, facilitating the transport of drugs into cells, evidently by a receptor and nonreceptor mechanism [21]. Specific choice of target organ can be achieved by various modifications of apo-B, altering the character of distribution of LDL among the organs and tissues, as has been shown for this class of lipoproteins [6, 7, 19, 20].

The results of this investigation thus show that apo-B, built into a liposome, with a lipid:protein ratio of 33:1, preserves its antigenic properties, does not appreciably disturb the integrity of the liposomes *in vitro*, and on the whole has a similar distribution

among organs as LDL, although it is eliminated from the blood stream more slowly during the first few hours and can be used as a ligand for the oriented transport of substances to particular organs.

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