POSSIBLE APPROACHES TO THE USE OF PHOSPHOLIPID PREPARATIONS AS MEMBRANE CHOLESTEROL RECEPTORS

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Recent advances in the study of the theoretical basis of the pathogenesis of atherosclerosis have led to the appearance of the preconditions for targeted action against the metabolic disturbances that are characteristic of this disease. The two basic trends associated with normalization of cell, membrane, and tissue cholesterol have been identified: a search for ways of reducing the inflow of cholesterol into the cell and ways of increasing its removal from cells, or more precisely, from their membranes, by components of the plasma. The first trend has led to the creation of various adsorptive and receptor-activating methods [3], while the second is linked with the search for ways of intensifying the release of membrane cholesterol, mainly by the use of high-density lipoproteins (HDL) [6].

The essential role of phospholipids in removal of membrane cholesterol [6, 9] indicates that it may be possible, in principle, to use nonprotein phospholipid dispersion or liposomes for this purpose [2, 8-10]. This paper describes a study of the ability of emulsions of phosphatidylcholine of plant origin, together with several specific additives, for the removal of cholesterol from erythrocyte membranes of rabbits with alimentary atherosclerosis and patients with ischemic heart disease (IHD).

EXPERIMENTAL METHOD

Soy phosphatidylcholine (PCh) and the preparation "Lipostabil" (Hatterman, West Germany) based on it, and also PCh from a different plant source, similar in fatty acid composition, and containing less than 5% of impurities (lysolecithin), were used. The phospholipid emulsion, a mixture of liposomes and a micellar solution of phospholipids, was prepared as described previously [2], using hexadecyltrimethylammonium bromide (HTAB) as stabilizer in some cases. To assess the efficacy of extraction, erythrocytes were incubated with the phospholipid emulsion in the ratio of 1:1 as phospholipids at 37°C. After incubation the erythrocytes were washed 3 times with 0.9% NaCl, lipids were extracted, and concentrations

TABLE 1. Changes in Cholesterol/Phospholipids Ratio in Rabbit Erythrocytes after Incubation with Phospholipid Emulsions (M ± m)

Experimental conditions	Soy PCh		PCh used	
	lowering of Ch/PL, %	hemolysis, %	lowering of Ch/PL, %	hemolysis, %
PCh + HTAB	20,9±2,4	9,0±0,8	24,2±3,2	9,9±3,2
PCh + PD	—	—	42,0±2,8	6,5±0,9
Lipostabil	68,0±5,4	82,1±7,2		

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TABLE 2. Effect of Incubation with PCh on Properties of Erythrocyte Membranes from Patients with IHD (M ± m)

Parameters tested	Incubation with PCh	
	before	after
Ch/PL, relative units	1,08±0,1	0,76±0,05
PCh/sphingomyelin, rel. units	2,1±0,1	3,2±0,2
Fraction of linoleic acid in erythrocyte, PCh, %	24,0±1,2	3,21±0,9
S, relative units	0,662±0,002	0,625±0,001
Na,K-ATPase activity, nmoles Pi/mg protein/min	8,2±0,1	10,3±0,4

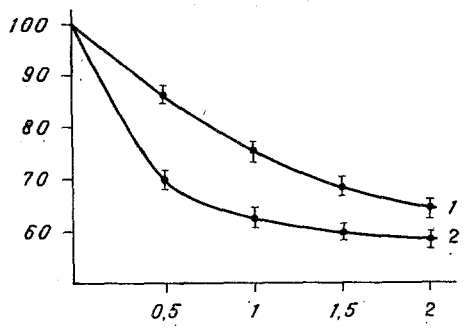

Fig. 1. Extraction of cholesterol from erythrocyte membranes by means of PCh emulsion. 1) PCh emulsion; 2) the same, with the addition of low-molecular-weight component. Abscissa, time (h); ordinate, ratio Ch/PL (%).

of cholesterol and phospholipids were determined in the extracts [4]. The degree of extraction of cholesterol was estimated by the decrease in the molar ratio of cholesterol/phospholipids (Ch/PL), or the cholesterol concentration (calculated per milliliter of cell suspension or per milligram protein). Activity of Na,K-ATPase [1] in the erythrocytes and the parameter of orderliness (S) of the spin probe 16- or 5-doxyl stearate, based on the EPR spectrum [1, 4] obtained on a Varian E-4 radiospectrometer (USA), were determined. Esterification of the cholesterol, extracted from the membranes, by the action of lecithin-cholesterol, extracted from the membranes, by the action of lecithin-cholesterol acyltransferase (LChAT) was judged by the decrease in the concentration of nonesterified cholesterol during incubation with fresh human plasma, freed from all lipoproteins (as the source of the enzyme) [5].

To determine the level of nonesterified cholesterol, enzyme kits from "Boehringer" (West Germany) were used.

Concentrations of PCh and sphinogomyelin in the erythrocytes were determined as phosphorus after fractionation by two-dimensional thin-layer chromatography. The composition of the fatty acids of PCh was analyzed by gas-liquid chromatography on a "Cromatone" chromatograph (Czechoslovakia), with flame-ionization detector, and using 15% carbowax-20 M (USA) as the stationary phase.

EXPERIMENTAL RESULTS

Comparison of the cholesterol extracting capacity of the PCh used (Table 1) showed that in 2 h they removed an equal quantity of cholesterol and reduced the molar ratio Ch/PL of rabbit erythrocytes by 21-24%. Solubilization of PCh with the aid of plant detergent (PD) increased the effectiveness of extraction up to 42%. The degree of hemolysis in this case was low, by contrast with that when Lipostabil was used, for although the latter was the most effective extracting medium for cholesterol, it caused almost total hemolysis of the erythrocytes.

Thus the plant PCh used, despite its lower content of linoleic acid, which intensifies interaction with cholesterol (54.9% compared with 70.8% in soy PCh) [10], is nevertheless capable of effectively extracting membrane cholesterol and it can be used as the basis for an extracting agent, simulating the function of HDL.

Functional similarity to HDL also was manifested as ability of the cholesterol extracted from the cells to interact with lecithin-cholesterol acyltransferase: during incubation of medium containing PCh and cholesterol extracted from cells, with a source of enzyme, the concentration of nonesterified cholesterol fell by 12.5% – from 120.5 to 106.2 μg/ml, evidence that such complexes may be involved in the reaction of lipoprotein transformation [6].

At the same time, the short time of circulation of phospholipids in the blood stream [10] makes the most rapid extraction of membrane cholesterol essential. As will be clear from the data given in Fig. 1, maximal cholesterol extraction from erythrocyte membranes with the aid of phospholipid emulsion was obtained in the course of 1.5 h, compared with 7-8 h required for the same cholesterol extraction with the aid of liposomes from soy PCh [2, 10] or HDL [4]. The addition of the low-molecular-weight component of the medium, however, shifted the extraction maximum to 0.5 h (Fig. 1).

A marked cholesterol-extracting effect of this phosphatidylcholine emulsion also exhibited toward erythrocytes of patients with IHD, in whom the molar ratio Ch/PL was rather higher in some cases than in normal individuals [1, 7]. Incubation of cells in medium with PCh led to a decrease in their relative cholesterol concentration, whereas at the same time the phosphatidylcholine/sphingomyelin ratio and the fraction of linoleic acid in PCh of the erythrocytes were increased (Table 2). This indicates some degree of transport and/or exchange with the PCh of the medium. As a result of these chemical changes in the membrane lipids, the structural and functional properties of the membranes, disturbed in IHD, were normalized [1, 7]: the reduction of microviscosity, reflected in the parameter of orderliness of the spin probe, and restoration of Na,K-ATPase activity almost up to normal values [1].

Effective extraction of cholesterol from cell membranes, simulating the function of "antiatherogenic" lipoproteins (HDL), evidently lies at the basis of the antiatherogenic action of phospholipids [9]. Overcoming difficulties connected with their solubilization, and also the too slow rate of extraction of cholesterol, compared with the rapid release of phospholipids by cells of the reticuloendothelial system [10], will facilitate the more active therapeutic use of these biologically active compounds.

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