PHARMACOLOGY AND TOXICOLOGY

Comparative Analysis of Efficiency and Specificity of Various Sorbents for Apheresis of Low-Density Lipoproteins

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Efficiency of sorbents for LDL apheresis was compared *in vitro*. The sorbents based on ion-exchange interaction of the ligand with LDL (Liposorber®, DALI®) exhibited minimum specificity towards the eliminated component, while immunosorbents (LNP-Lipopak®, LDR-TheraSorb®) were most efficient. By sorption capacity, the available hemosorbents are inferior to plasmasorbents, which explains low efficiency of the therapy based on single application of hemosorbents especially in patients with consederably increased content of LDL cholesterol.

Key Words: *LDL apheresis; sorbent; familial hypercholesterolemia; sorption technologies*

Elevation of concentration of cholesterol (CH) in atherogenic apoB_{100}-containing low-density lipoproteins (LDL) increases the risk of cardiovascular diseases. Familial forms of hypercholesterolemia (FHC) caused by a defect in gene encoding receptor protein for LDL is a special form of lipid metabolism disturbances. Block of the receptor-mediated binding of LDL with cells leads to LDL accumulation in the blood and development of type IIa FHC, which causes the death of patients at the age of 10-30 years from severe atherosclerosis. In some patients, even maximal hypolipidemic drug therapy is inefficient or cannot be administered by various reasons. In these cases, the target levels of total CH and LDL-CH can be attained by extracorporeal methods.

To remove LDL, a special immunosorbent was developed [12] based on dextran sulfate as the ligand (Liposorber®, Kaneka, [15]).

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To perform LDL-apheresis, a sorbent DALI® (Direct adsorption of lipoproteins, Fresenius) was developed for perfusion of the whole blood, and Liposorber DL-75 columns (Kaneka) as the concurrent product for hemoperfusion, which are currently tested in clinical trials. Moreover, hemosorbents for LDL-apheresis and matrices for perfusion of the whole blood are now extensively developed in many Russian and foreign laboratories [7].

Various systems of LDL-apheresis were compared [4,8,11,14], but it is difficult to compare the specificity and sorption capacity, because the protocols of LDL-apheresis widely vary. However, it is known that sorption capacity depends on plasma composition, incubation time of plasma and sorbent, sorbent/plasma volume ratio, and other parameters. It is necessary to obtain reliable comparative data on the parameters of sorbents tested *in vitro* with the same plasma under identical chromatography procedures.

This paper summarizes the data of comparative analysis of the sorbents used in commercial sys-

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tems of LDL-apheresis, and the sorbents currently examined during various stages of clinical testing.

MATERIALS AND METHODS

For affinity chromatography, plastic columns (Bio-Rad) were packed with the test sorbent (1 ml). The plasma from FHC patients (total CH 415 mg/dl; LDL-CH 24 mg/dl; total protein 89 g/liter) was obtained in Department of Hemodialysis, Russian Cardiology Research-and-Production Complex. After chromatography, the plasma was collected and tested for total CH, LDL-CH (Boehringer), $apoB_{100}$ by immunoturbidimetry, and lipoprotein (a) (LPa) by enzyme-linked immunosorbent assay [1]. The efficiency of removal of plasma components was assessed as the difference in plasma content of each component before and after chromatography. Elution of bound components was carried out. Sorbents 1, 2, 5, and 7 were washed with 35 ml physiological saline and eluted with 10 ml 0.6 M NaCl. Immunosorbents 3, 4, and 6 were washed with 35 ml phosphate buffered saline, eluted with 10 ml glycine buffer, and tested for total protein (spectrophotometry), total CH, $apoB_{100}$, and LPa. Protein composition of the effluent was analyzed by electrophoresis in 3-7% PAAG gradient as described elsewhere [13] with some modifications [6]. Similar assay was performed in agarose gel.

RESULTS

To compare various sorbents used in LDL apheresis, their specificity and LDL-binding capacity were examined *in vitro* in regimens approximating clinical conditions (Table 1).

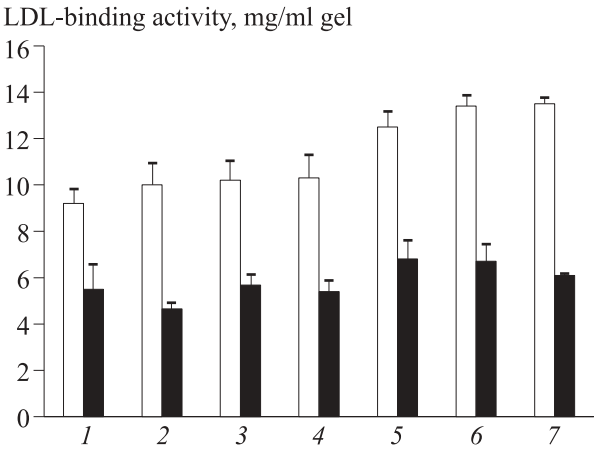

Fig. 1. Efficiency of LDL removal by various sorbents for LDL-apheresis (*m±SE*). *1*) DALI®; *2*) Liposorber L®; *3*) LNP-300; *4*) Liposorber D®; *5*) LNP-45; *6*) LNP Lipopak®; *7*) LDL-TheraSorb®. Open and closed bars show the amount of CH and $apoB_{100}$, respectively, bound by the sorbent.

LDL-binding ability of hemosorbents used in LDL apheresis of the whole blood was somewhat lower than that of sorbents used for plasma sorption (Fig. 1). In addition, in contrast to plasmasorbents, which are regenerated during the procedure and therefore have virtually unlimited LDL-binding capacity (limited only by the duration of the procedure), the available hemosorbents can be used only in one cycle. By this reason, despite simplicity and economy of hemosorption procedure, its clinical efficiency is insufficient to attain the target LDL level in many cases, especially in patients with total CH>300 mg/dl [14]. For improving the efficiency of the system, Fresenius company proposed to use several columns DALI® with volumes of 750 and 500 ml (DALI 1250). However, this configuration increases the extracorporeal volume to 690 ml, which requires additional infusion of 300 ml phy-

TABLE 1. Characteristics of Sorbents for LDL Apheresis

Sorbent	Producer or Developer	Matrix	Ligand	Regeneration ability	Perfusion fluid
Liposorber L®	Kaneka	Cellulose	Dextran sulfate	Yes	Plasma
Liposorber D®			No		Blood
LNP Lipopak®	POKARD	Agarose	Polyclonal antibodies to LDL	Yes	Plasma
LDL-TheraSorb®	Miltenyi	Agarose	Polyclonal antibodies to LDL	Yes	Plasma
DALI®	Fresenius	Polyacrylamide	Polyacrylate	No	Blood
LNP-300	SRI HPB, RC RPC	Cellulose	Polyclonal antibodies to LDL	Yes	Blood
LNP-45	Tosoh	Resins	Polyacrylate	Yes	Blood

Note. POKARD Research and Production Company (POKARD), State Research Institute of Highly Pure Biopreparations (SRI HPB), Russian Cardiology Research-and-Production Complex (RC RPC).

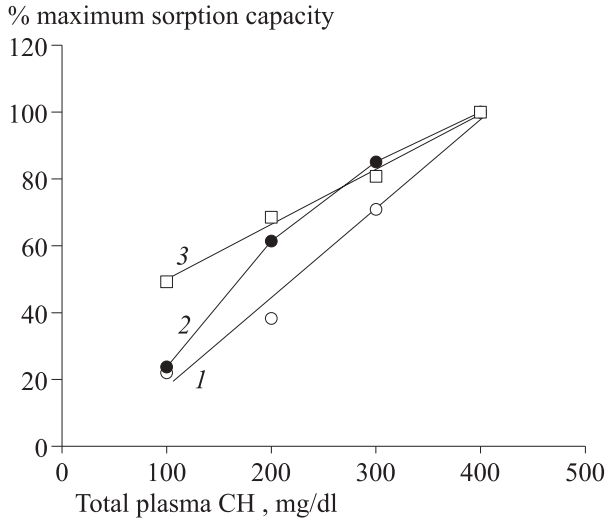

Fig. 2. Dependence of LDL-binding activity of the sorbents on total plasma CH concentration. *1*) Liposorber®; *2*) DALI®; *3*) LNP Lipopak®. The maximum sorption capacity is the amount of CH bound to the sorbent under saturation conditions, when total plasma CH concentration is 450 mg/dl. The mean of 3 measurements is presented.

siological saline before the procedure in order to avoid severe side effects [5]. In addition, the efficiency of sorption depends on concentration of the removed component, which decreases persistently. The minimal *in vitro* dependence of LDL-binding capacity on concentration of total plasma CH is characteristic of the immunosorbents, which provide maximum specific interaction, while in case of dextran sulfate cellulose, a 4-fold decrease in total CH results in a 5-fold drop of LDL-binding activity (Fig. 2).

In addition to LDL and very low-density lipoproteins (VLDL), LPa also belongs to atherogenic apoB-containing lipoproteins. This unique lipoprotein is a LDL-like particle containing apoprotein(a) characterized by a high degree of homology to plasminogen. Blood LPa concentration >30 mg/dl is associated with rapid atherosclerotic damage to coronary, carotid, peripheral arteries, and autogenous venous bypasses after myocardial revascularization [2,3,10]. At present, there are no medical drugs efficiently decreasing LPa level in human blood, but all LDL apheresis systems can remove LPa due to the presence of $apoB_{100}$ in LPa particle. High variability of LPa concentration (from 0.2 to 200 mg/dl) makes difficult reliable evaluation of the efficiency of LPa-binding potency of LDL-sorbents. Table 2 shows the results of affinity chromatography of plasma from patients with coronary heart disease with hyper-LPa (80 mg/dl) and total CH 350 mg/dl.

TABLE 2. Efficiency of LPa Removal by Various Systems of LDL Apheresis

Sorbent	LPa concentration	Decrease, %	Above the upper limit of normal, factor
LPa Lipopak®	17.4±0.4	78	Below normal
DALI®	43.8±2.8	45	1.5
LNP Lipopak®	47.4±7.3	41	1.6
LDL-TheraSorb®	48.5±2.8	39	1.6
Liposorber D®	48.7±14.5	39	1.6
LNP-300	55.8±2.5	31	1.9
LNP-45	56.7±3.8	29	1.9
Liposorber L®	62.1±8.9	22	2.1

Note. LPa concentration "before" — 80.0±9.9.

Only specific sorbent efficiently decreased LPa level below the upper limit of normal after single sorption cycle. All other systems of LDL-apheresis, including immunosorbents, removed LPa with a 20-40% efficiency.

For evaluation of sorbent specificity we used the protein/CH ratio, which is close to 0.5 in pure LDL preparation. The higher is this ratio, the greater is the percent of protein admixtures (Fig. 3, *a*). Reliability of this simple test was confirmed by vertical PAAG electrophoresis of eluates (Fig. 3, *b*). $ApoB_{100}$ is the major protein and binds to all sorbents. The least specificity towards LDL was demonstrated by the sorbents based on the ion-exchange interaction of the ligand with LDL (Liposorber® and DALI®), while the maximum specificity was observed with immunosorbents (LNP Lipopak®, LDL-TheraSorb®).

Low specificity to the removed component results in partial removal of antiatherogenic lipoproteins HDL. For example, the decrease in HDL-CH concentration on Liposorber is almost 2-fold greater than on immunosorbents (29.8±5.6% *vs.* 17.1±4.5%). DALI® also decreases HDL-CH content by 25.9±1.8%.

Comparative analysis of various plasma- and hemocompatible sorbents for LDL-apheresis showed that the sorbents based on antibodies are most specific, biologically compatible and stable during repeated cycles. The use of immunosorbents is optimal for long-term extracorporeal therapy in FHC patients.

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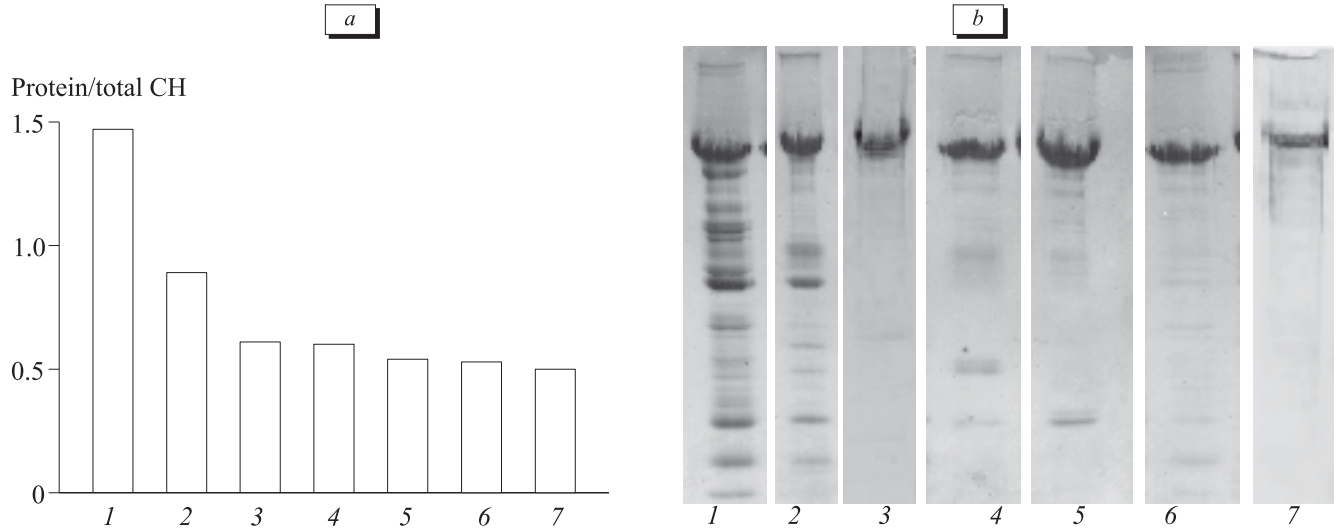

Fig. 3. Specificity of various sorbents to LDL (protein/total CH ratio) measured in the effluent of corresponding sorbent (*a*) and electrophoregrams of these effluents (*b*). *1*) Liposorber L®; *2*) DALI®; *3*) LDL-TheraSorb®; *4*) LNP-300; *5*) LNP Lipopak®; *6*) LNP-45; 7) LDL preparation.

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