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IN-VITRO STUDY OF THE PROPERTIES OF COMPONENTS FOR THE SYNTHESIS OF SORBENT FOR LOW-DENSITY LIPOPROTEIN APHERESIS

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ABSTRACT

The research was carried out based on the Stavropol State Medical University. This work aimed to study the fundamental possibility of creating an immunosorbent for LDL apheresis based on non-porous highly dispersed silicon dioxide and monospecific polyclonal antibodies. In the course of the study, a matrix was selected for the synthesis of an immunosorbent based on aerosil modified with dextran. Affinity-purified rabbit polyclonal antibodies to human apolipoprotein B (ApoB) were immobilized onto the matrix. The obtained sample of the immunosorbent under in-vitro conditions showed good sorption properties to LDL, which makes it promising for further work on the creation of a sorbent for LDL-apheresis. It is concluded that the fundamental possibility of creating an immunosorbent based on non-porous highly dispersed silicon dioxide and monospecific polyclonal antibodies has been shown, which makes it possible to effectively bind and remove LDL from the blood plasma.

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Introduction

Cardiovascular diseases are a big problem for world health and the cause of their development in about 80% of cases is atherosclerotic vascular lesions. It is possible to influence the occurrence and course of diseases of the circulatory system only by influencing their main pathological sign - dyslipidemia. Traditional treatment of atherosclerosis, according to modern Russian and foreign recommendations, involves lowering the level of low-density lipoprotein cholesterol (LDL-C) using various classes of drugs [1-5]. However, in some cases, even after using the maximum possible and tolerable combination lipid-lowering therapy, the target LDL-C levels cannot be achieved. An alternative to drug therapy for such patients is extracorporeal methods of treatment, in particular, therapeutic apheresis, in which 80 to 100% of low-density lipoproteins (LDL-apheresis) are removed from the patient's blood [2, 6]. However, this procedure is expensive and in Russia, there are a limited number of centers performing LDL apheresis for health reasons (Moscow, St. Petersburg). Taking into account the fact that apheresis is performed weekly or once every 2 weeks, obtaining this method of treatment for patients from other regions is difficult and associated with significant costs.

Sorption technologies are of particular interest, which make to selectively remove substances lying based on the

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pathogenesis of the disease when plasma or whole blood is passed through the columns. At the same time, the sorbent does not interact with other non-complementary molecules, without exerting a negative effect on the blood composition. Currently, there are a large number of sorbents [7-9], the action of which is based on various mechanisms of interaction of LDL.

Despite the variety of sorbents for LDL-apheresis, their effect on the level of LDL-C is comparable, except for minor differences in the binding of HDL-C [10-15].

In the work of E.V. Altynova *et al.* [16] carried out a comparative in vitro analysis of six hemosorbents for LDL apheresis. It has been shown that the most efficient sorption of cholesterol and apoB100-containing lipoproteins is possessed by the hemosorbents "LDL-45" ® ("Tosoh", Japan), "SF LPM" ® (Russia), and plasmasorbent "LDL Lipopak" ® (POCARD, Russia). Sorbents based on the ion-exchange interaction of the ligand with LDL, such as Liposorber D® (Kaneka, Japan) and DALI® (Fresenius, Germany), had the lowest specificity for LDL, the most specific were the immunosorbent " LDL-300" ® (Russia) and " LDL-45" ® ("Tosoh", Japan).

However, despite the high efficiency of existing sorbents for LDL apheresis, this procedure is not available to everyone who needs it [17, 18]. This is primarily due to the high cost of sorbents.

This work aims to study the fundamental possibility of creating a universal immunosorbent selective for LDL based on non-porous highly dispersed silicon dioxide and monospecific polyclonal antibodies.

Materials and Methods

The research was carried out based on the Stavropol State Medical University. Aerosil A-380 with the basic substance SiO2 content of 99% was used in the studies. This sorbent is non-porous silica with a particle shape close to spherical. An aqueous dextran solution was used as a modifier. About 10 g of Aerosil has added 150 ml of distilled water containing 1 g of dextran. The resulting suspension was left to mature at room temperature for 24 hours. The maturation time is due to the course of condensation processes with the participation of aerosil silanol groups - if the maturation time is less than a day, the hydrogel does not fully ripen, and if the time exceeds 24 hours, aging processes are observed. The resulting sorbent was sieved through a sieve with a mesh size of 150-200 μm and washed with distilled water. Affinity-purified rabbit antibodies to human apolipoprotein B (apo B) were added to the wet sorbent. Immobilization was carried out for 2 hours at a temperature of 22°C. Upon completion of the immobilization process, the resulting sorbent was washed with distilled water and treated with a solution of human serum albumin to activate unbound functional groups to exclude nonspecific sorption.

The sorption properties of the sample obtained during the study were evaluated using affinity chromatography [19, 20]. 10 ml of human blood plasma with a concentration of total cholesterol 355.7 ± 12.3 mg / dL (9.2 ± 0.32 mmol / L) and LDL - 216.5 ± 7.3 mg / dL (5.6 ± 0.2 mmol / L) was passed through a glass column with a filter containing 1 ml of the tested sorbent at a rate of 0.3 ml / min. After affinity chromatography, blood plasma was collected to study the dynamics of cholesterol and LDL parameters. To assess the efficiency of binding of LDL, the sorption capacity of the sample was calculated.

Results and Discussion

The most widespread in clinical practice are methods of plasma adsorption of LDL using columns with heparin-sepharose and dextran sulfate. However, the drug heparin-sepharose most often used for plasma sorption has several disadvantages - along with atherogenic lipoproteins, it eliminates high-density lipoproteins (HDL) from plasma, which have anti-atherogenic properties, and also removes hepatic triglyceride lipase and proteins of the blood coagulation system [21]. To avoid such disadvantages, in this study, aerosil was considered as a carrier - anhydrous amorphous silicon dioxide, which belongs to the group of synthetic active highly dispersed mineral fillers. It is obtained as a result of high-temperature vapor-phase hydrolysis of silicon tetrachloride in a stream of oxygen, followed by condensation in water vapor. Aerosil has good sorption properties (absorbs from 15 to 60% of various liquids without changing the appearance and flowability of the powder), while aerosil-containing pharmaceutical systems are indifferent and do not exhibit irritating and toxic effects. In the USA, silicon dioxide (aerosil) is also allowed as a food additive in the amount of 2%. At present, silica particles, due to their large surface area, sufficient pore size, good biocompatibility, and biodegradability are considered promising carriers in biomedicine [22-26].

The main feature of the development of an immunosorbent is the correct choice of antibodies, which subsequently bind to the antigen and eliminate it. In this work, we selected affinity-purified rabbit polyclonal antibodies to human apolipoprotein B (ApoB). ApoB is the main apolipoprotein of chylomicrons, very low-density lipoproteins, and their residues. The concentration of apoB in the blood is now considered a more reliable indicator of the risk of developing atherosclerosis than total cholesterol or low-density lipoprotein cholesterol [27]. A ready-made set of rabbit polyclonal antibodies was chosen for several reasons: firstly, they only bind to human apoB and do not react with other plasma proteins, and secondly, their production process is not too laborious, which affects the final cost. To obtain rabbit antisera to human apoB, rabbits are immunized with native human LDL. Antigen (1 mg of protein) is diluted in an equivalent volume of Freund's complete adjuvant and injected intraperitoneally. Immunization is carried out three times, with an interval of 7-10 days. In the course

of immunization of rabbits with autologous LDL, sera with a high titer of specific antibodies with a high affinity for human LDL are obtained [28].

The study of the sorption properties of the obtained sample showed the following results. During the test experiment, the ability of the sorbent to absorb LDL cholesterol was studied. In a test tube containing 1 g of sorbent, 20 ml of plasma was added with a known concentration of LDL cholesterol. The suspension was incubated for 30 min. at room temperature, followed by precipitation of the sorbent by centrifugation. In the supernatant, the concentration of LDL cholesterol was determined. It was revealed that the concentration of LDL cholesterol after interaction with the sorbent decreases by an average of 56.5%, which is comparable with the indicators of other sorbents used in the LDL apheresis **(Figure 1)**.

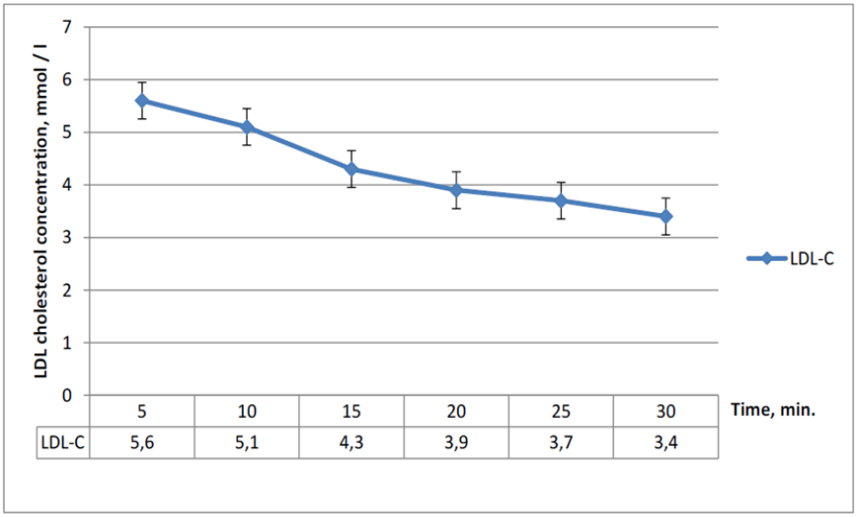

Figure 1. Change in the Concentration of LDL Cholesterol when the Blood Plasma is Passed through the Immunosorbent

The sorption capacity of the sample was determined based on the amount of bound cholesterol and LDL **(Table 1)**.

Table 1. Sorption Capacity of Various Sorbents for LDL Apheresis

Sorbent	Sorption capacity (mg substance / ml gel)	
	Cholesterol	apoB100
Test sample	10.1±0.92	5.5±0.71
Liposorber D ® (Kaneka, Japan)	10.4±1.01	5.4±0.47
DALI ® (Fresenius, Germany)	9.9±0.62	5.5±1.05
"LDL Lipopak" ® (POCARD, Russia)	13.4±0.48	6.7±0.76

As can be seen from the data presented, the obtained laboratory sample of the immunosorbent has a sufficient sorption capacity to proatherogenic liporotheids and its indicators do not differ statistically significantly from the known sorbents.

The sorption efficiency reflects the distribution coefficient, which is equal to the ratio of the concentration of the substance bound to 1 ml sorbent, to the concentration of the substance remaining in the plasma under conditions of reaching equilibrium. Let us assume that when 10 ml of blood plasma is perfused through 1 ml of sorbent, it is completely saturated. If the distribution coefficient is close to 1, then the concentration of the substance inside the sorbent is equal to the concentration of the substance in the external solution, and the sorption is not effective [16]. As follows from the results of the study, the laboratory sample of the immunosorbent has a sufficiently high distribution coefficient, and, therefore, a good binding capacity to LDL-C **(Table 2)**.

Table 2. Distribution Coefficients for Various Sorbents used for LDL- apheresis

Sorbent	Distribution coefficient	
	Cholesterol	apoB100
Test sample	3.2	3.0
Liposorber D ® (Kaneka, Japan)	3.3	2.9
DALI ® (Fresenius, Germany)	3.1	3.0
"LDL Lipopak" ® (POCARD, Russia)	4.7	3.8

Thus, as a result of the studies carried out, the fundamental possibility of creating an immunosorbent based on non-porous highly dispersed silicon dioxide and monospecific polyclonal antibodies has been shown, which makes it possible to

effectively bind and remove LDL from the blood plasma.

Conclusion

In the study, a matrix was selected for the synthesis of an immunosorbent based on aerosil modified with dextran, which meets the basic requirements: hemocompatibility, inertness, chemical stability, high porosity, which allows immobilizing a significant amount of the protein-ligand. The use of relatively inexpensive components and ready-made sets of antibodies in the process of creating a sorbent for LDL-apheresis will ensure the economic attractiveness of the final product. Further work is needed in this direction, the purpose of which will be to create a relatively cheap sorbent based on a hemocompatible carrier and a synthetic ligand with sufficient specificity, which would have the ability to regenerate.

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