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Development of an Immunosorbent for the Removal of Atherogenic Lipoproteins from the Body in the Extracorporeal Circulation System

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Abstract

Currently, in Russia and the world, cardiovascular diseases (CVD) occupy first place among the causes of mortality in the adult population. The traditional treatment of atherosclerosis, according to modern Russian and foreign recommendations, consists in reducing the level of low-density lipoprotein cholesterol (LDL cholesterol) with statins. The article will describe the developed sorbent with high specificity and is able to effectively remove atherogenic lipoproteins from blood plasma, while it is chemically neutral and relatively inexpensive to manufacture, which allows it to be used in the future in the procedure of therapeutic LDL apheresis for the treatment of severe hypercholesterolemia. The proposed immunosorbent will increase the level of specific capacity relative to antibodies to low-density lipoproteins, and the use of relatively inexpensive components and ready-made sets of antibodies in the process of its synthesis will ensure the economic attractiveness of the final product.

Discipline: Medicine, Immunology.

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1 Introduction

Currently, in Russia and in the world, cardiovascular diseases (CVD) occupy first place among the causes of mortality in the adult population. According to the literature, up to 80% of deaths from CVD are the result of atherosclerotic vascular damage and related complications – heart attacks and strokes. At the same time, genetically determined forms of hypercholesterolemia (familial hypercholesterolemia) are known, in which atherosclerotic lesion begins at a young age and has an extremely aggressive course [1].

2 Literary Review

The traditional treatment of atherosclerosis, according to modern Russian and foreign recommendations, consists in reducing the level of low-density lipoprotein cholesterol (LDL cholesterol) with statins. Drugs of this group lead to a decrease in the concentration of LDL cholesterol by 30-50%, depending on the dose and type of statin [2-4]. However, the use of statins has significant limitations and is associated with the development of a number of serious undesirable effects (myopathy, rhabdomyolysis, impaired liver function, dyspepsia, and increased risk of hemorrhagic stroke) [5-8]. In addition, in familial hypercholesterolemia, as well as in patients with very high cardiovascular risk, statins are often insufficiently effective. 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. According to international guidelines for the treatment of familial hypercholesterolemia, LDL-apheresis is recommended for patients with a homozygous form of the disease as a vital treatment method. According to the Russian recommendations for the diagnosis and treatment of familial hypercholesterolemia, LDL-apheresis is prescribed to patients who, after 6 months of the maximum possible combined lipid-lowering therapy, have not reached the target levels of LDL cholesterol [9,10].

However, this procedure is expensive and there are only a few centers in Russia that carry out LDL apheresis for vital indications, which are located mainly in Moscow and 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 extremely difficult and involves significant costs. The development of a highly selective immunosorbent for the removal of LDL will make it possible to use the method of therapeutic apheresis on the basis of any medical organization in the region that has equipment for extracorporeal treatment [11]. The final result of the work will be a sample of a highly selective immunosorbent against low-density lipoproteins for the treatment of patients with severe hypercholesterolemia.

3 Material and Methods

Taking into account the results of research conducted in the first year of project financing, a method for the synthesis of an immunosorbent was developed, including the immobilization of athenically purified rabbit antibodies to human Apolipoprotein B on the surface of aerosil pre-activated with dextran. The synthesis of an experimental sample of an immunosorbent was carried

out as follows. 200 ml of distilled water containing 2 g of dextran was added to 15 g of aerosil. The further process of maturation of the hydrogel takes place within 24 hours at room temperature. The resulting sorbent was dried at a temperature of 110-120°C for 40 minutes with constant stirring. Then the sorbent was washed three times with distilled water of 150 ml and sieved through a sieve with a mesh size of 220-250 microns to create granules with an optimal working surface size. 0.5 ml of affine-purified rabbit antibodies to human apolipoprotein B with a concentration of 200 mcg/ml were added to 0.2 g of the carrier. The volume of the solution is due to the possibility of the complete wettability of the carrier. The mixture was left for 2 hours at a temperature of 22°C, then the carrier with immobilized antibodies was washed 5 times with distilled water with a volume of 10 ml. To exclude non-specific sorption, the resulting sorbent was treated with a 0.1% solution of human serum albumin for 2 hours.

Additional immobilization of antibodies on an aerosil and an increase in the gelation time provides an increase in specificity and an increase in sorption capacity, which, in turn, can increase the effectiveness of hypercholesterolemia therapy.

The resulting sorbent is a white powder with a granule size of 220-250 microns (Figure 1).

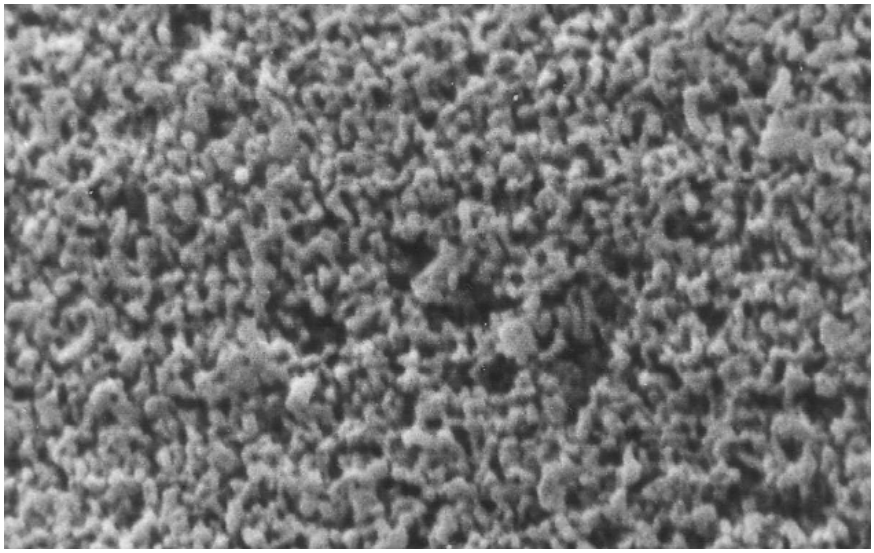

Figure 1: Sample of the developed immunosorbent during scanning electron microscopy (magnification 1:10000)

The presence of particles smaller than 150 microns in sorbents significantly increases the likelihood of their leakage through the filter during blood perfusion. That is why, when creating an immunosorbent sample, a particle size of 220-250 microns was chosen, which will increase the safety of using the sorbent during the LDL apheresis procedure.

The sorption capacity of the resulting sorbent was determined under static conditions. 10 ml of blood plasma of patients with familial hypercholesterolemia was added to 1 g of dry sorbent, the contents of the test tube were mixed for 30 minutes and the plasma was separated by centrifugation. To determine the sorption properties, a biochemical study of the plasma lipid spectrum was performed before and after its interaction with the sorbent. The concentration of total cholesterol, LDL, HDL and triglycerides were determined by standardized methods [12-14].

Table 1 shows the average values obtained from 5 independent measurements of the lipid spectrum of blood plasma before and after its contact with the sorbent (the data are presented as an arithmetic mean ± standard deviation). To determine the statistical significance of the differences between dependent samples (before and after exposure), the Wilcoxon criterion was used. The differences were considered significant at $p<0.05$ [15,16].

Table 1: Results of determining the sorption capacity of the obtained sorbent under static conditions

Indicator	TC, mmol/l	LDL cholesterol, mmol/l	HDL cholesterol, mmol/l	TG, mmol/l	atherogenicity coefficient, conl. units
Before contact with the sorbent	12.37±0.83	7.92±0.41	0.92±0.02	1.26±0.05	12.4
After contact with the sorbent	6.42±0.39*	2.78±0.27*	0.96±0.03	0.82±0.07	5.7*

Note: TC – total cholesterol, LDL cholesterol – low–density lipoprotein cholesterol, HDL cholesterol - high-density lipoprotein cholesterol, TG – triglycerides, * – the significance of differences between groups before and after contact with the sorbent at $p<0.05$.

4 Result and discussion

The statistical analysis revealed a significant decrease in LDL levels (from 7.92±0.41 to 2.78±0.27 mmol/l; p=0.018) and OHC (from 12.37±0.83 to 6.42±0.39 mmol/l; p = 0.026) in blood plasma and a decrease in the value of the atherogenicity coefficient (from 12.4 to 5.7 conl. units; p= 0.013) after contact with the sorbent, while the concentration of HDL did not change, which indicates a high sorption capacity and specificity of the resulting sorbent.

The sorption capacity of the immunosorbent was studied depending on the concentration of rabbit antibodies and human apeS. To study the sorption efficiency, a solution of Athens-purified antibodies with a concentration of 200 micrograms/ml, 150 micrograms/ml and 100 micrograms/ml was used. The presence of effective sorption with a lower concentration of antibodies will reduce the consumption of one of the most important components, and, accordingly, reduce the cost of the production process.

Sorption capacity was determined under static conditions. 10 ml of blood plasma of patients with familial hypercholesterolemia was added to 1 g of dry sorbent, the contents of the test tube were mixed for 30 minutes and the plasma was separated by centrifugation. To determine the sorption properties, a biochemical study of the plasma lipid spectrum was performed using standardized methods before and after its interaction with the sorbent (Table 2).

Table 2: Sorption capacity of immunosorbent samples depending on the concentration of antibodies to apolipoprotein B

The concentration of antibodies to apolipoprotein B	Sorption capacity (mg of substance/ml of gel)	
	Total cholesterol	LDL
200 mcg/ml	12.8±1.1	6.7±0.36
150 mcg/ml	8.6±1.0	4.8±0.4
100 mcg/ml	5.3±0.5	3.1±0.2

The presented data indicate that the largest sorption capacity is possessed by a sample of an immunosorbent, in the preparation of which affine-purified rabbit antibodies to human

apolipoprotein B with a concentration of 200 micrograms/ml were used as an immune component. Lower concentrations of the antibody solution reduce the sorption efficiency, so their use in the process of immunosorbent synthesis does not seem appropriate [7,9].

When using an antibody solution with a concentration of 150 mcg/ml, the sorption efficiency of atherogenic lipoproteins decreases by an average of 28%, and a further decrease in the concentration to 100 mcg/ml leads to a decrease in sorption capacity by 53%. Thus, the optimal concentration of a solution of antibodies to human apolipoprotein B used in the synthesis of an immunosorbent is 200 mcg/ml.

The effectiveness of binding of atherogenic lipoproteins was evaluated by affinity chromatography of blood plasma of patients with familial hypercholesterolemia on samples of the developed sorbent and an analog available on the Russian market. The immunosorbent "LNP-Lipopak®", developed by the NPF "Pokard", Russia, was used as an analogue. 10 ml of plasma was passed through a glass column with a filter containing 1 ml of the tested sorbent at a rate of 0.5 ml/min. The sorption capacity was calculated in mg/ml of the sorbent, as well as in rel.% according to

$$(C_0-C_K)/C_0\times 100\%, \qquad (1),$$

where C_0 and C_K are LDL concentrations in blood plasma before and after sorption, respectively. The data obtained are shown in Table 3.

Table 3: Comparative analysis of the sorption efficiency of the developed sorbent and its analog

Sorbent	Sorption capacity (mg of substance/ml of gel)		Sorption capacity for LDL, rel.%
	Total cholesterol (M±SD)	LDL (M±SD)	
Developed sorbent	12.8±1.1	6.7±0.36	64.9
"LNP-Lipopak ® "	13.2±1.6	6.4±0.42	63.5

The sorption capacity of the developed sorbent with respect to LDL was 6.7 ± 0.36, which is 0.3 mg/ ml more than the equivalent indicator of the analogue (6.4 ± 0.42). The calculated sorption capacity in rel.% of the developed sorbent is also higher in comparison with the analog (64.9 vs 63.5). The results obtained showed a high sorption capacity of the developed sorbent against atherogenic lipoproteins.

The sorption efficiency reflects the distribution coefficient, which is numerically equal to the ratio of the concentration of the substance bound to 1 ml of the sorbent to the concentration of the substance remaining in the plasma under equilibrium conditions. If the value of the distribution coefficient is close to 1, then sorption is not effective [17]. The distribution coefficients of the developed sorbent and analog are given in Table 4.

Table 4: Values of the distribution coefficients of the sample of the developed sorbent and analog

Sorbent	Distribution coefficient		
	Total cholesterol	LDL	HDL
Developed sorbent	4.1	4.0	1.1
"LNP-Lipopak ® "	4.2	3.7	1.3

The distribution coefficients indicate the high sorption efficiency of the claimed sorbent with respect to LDL and its selectivity since it does not reduce the concentration of anti-atherogenic HDL.

These properties characterize the great potential of the developed sorbent for its use in clinical practice after conducting preclinical and clinical trials and registration as a medical device.

The safety of the developed sorbent was determined in vitro and on laboratory animals. In vitro, the safety of the sorbent was determined by adding 5 ml of human whole blood to a test tube containing 1 g of sorbent. After 30 minutes, the blood was taken from the test tube with a syringe and examined under a microscope.

Microscopy of blood samples in contact with the sorbent did not show changes or destruction of the shaped elements, which indicates the absence of a toxic effect of the sorbent on the shaped blood elements.

The safety of the immunosorbent was also determined in laboratory animals (rats and rabbits) according to the protocol provided by Blinov et al. (2022) [18]. 1 ml of blood plasma of laboratory animals was added to 0.1 g of dry sorbent, the contents of the test tube were mixed for 30 minutes and the plasma was separated by centrifugation. Plasma was then injected into the bloodstream of the animals. Laboratory animals were observed for 7 days. During this period, there were no changes in the behavior and condition of experimental animals.

In vivo experiments on rats have shown that carrying out immunosorption procedures does not lead to adverse reactions and is not accompanied by an increase in the titer of the rat's "second" antibodies to rabbit antibodies (0.069±0.008 relative units before sorption and 0.071±0.006 relative units after 4 weekly sorption procedures on an immunosorbent, p=0.8). These indicators were no different from the antibody titer at false perfusion (0.074±0.006 relative units before sorption and 0.075±0.04 relative units after 4 weekly sorption procedures on a matrix without antibodies, p = 0.6).

Thus, the developed sorbent has a high specificity and is able to effectively remove atherogenic lipoproteins from blood plasma, while it is chemically neutral and relatively inexpensive to manufacture, which allows it to be used in the future in the procedure of therapeutic LDL apheresis for the treatment of severe hypercholesterolemia. The developed method of creating a mechanically stable immunosorbent is characterized by a high level of specific capacity relative to antibodies to Apolipoprotein B-100 without the use of expensive or toxic reagents in the synthesis process which corresponds to results studies of other drugs [19-22].

The proposed immunosorbent will increase the level of specific capacity relative to antibodies to low-density lipoproteins, and the use of relatively inexpensive components and ready-made sets of antibodies in the process of its synthesis will ensure the economic attractiveness of the final product.

5 Conclusion

An experimental sample of an immunosorbent to low-density lipoproteins has been created. The method includes adding an aqueous dextran solution to the aerosil, drying the sorbent for a day at room temperature, further washing it with distilled water three times and sieving it through a sieve with a cell size of 220-250 microns, followed by adding 0.5 ml of affine-purified rabbit antibodies to human apolipoprotein B with a concentration of 200 mcg / ml to 0.2 g of the carrier, immobilization for 2 hours at a temperature of 22 ° C and five times washing with distilled water in a volume of 10 ml, at the same time, to exclude non-specific sorption, the resulting sorbent was treated with a 0.1% solution of human serum albumin for 2 hours.

Sorption properties of the immunosorbent have been investigated. The statistical analysis revealed a significant decrease in LDL levels (from 7.92±0.41 to 2.78±0.27 mmol/l; p=0.018) and OHC (from 12.37±0.83 to 6.42±0.39 mmol/l; p = 0.026) in blood plasma and a decrease in the value of the atherogenicity coefficient (from 12.4 to 5.7 conl. units; p= 0.013) after contact with the sorbent, while the concentration of HDL did not change, which indicates a high sorption capacity and specificity of the resulting sorbent.

The effectiveness of the sorbent against atherogenic lipoproteins was studied depending on different concentrations of antibodies to apolipoprotein B. To study the effectiveness of sorption, a solution of athenically purified rabbit antibodies to apolipoprotein B in humans with concentrations of 200 mcg/ml, 150 mcg/ml and 100 mcg/ml was used. It was found that, compared with the concentration of antibodies of 200 mcg/ml when using a concentration of 150 mcg/ml, the sorption efficiency of atherogenic lipoproteins decreases by an average of 28% (from 6.7 ± 0.36 to 4.8±0.4 mmol/L), and a further decrease in the concentration to 100 mcg/ml leads to a decrease in sorption capacity by 53% (from 6.7±0.36 to 3.1±0.2 mmol/l). Thus, the optimal concentration of a solution of antibodies to apolipoprotein In humans used in the synthesis of an immunosorbent is 200 mcg/ml.

The specificity and safety of the immunosorbent were determined. The effectiveness of binding of atherogenic lipoproteins was evaluated by athene chromatography on samples of the developed sorbent and an analog ("LNP-Lipopak®", NPF "Pokard", Russia). The distribution coefficient of the sorbed component in 1 mg of sorbent was also calculated. The LDL sorption capacity of the developed sorbent was 6.7±0.36 mg/ml or 64.9 rel.%, while the sorption capacity of the analog was 6.4±0.42 mg/ml or 63.5 rel.%. The safety of the developed immunosorbent was determined in vitro and on laboratory animals. Microscopy of blood samples in contact with the sorbent showed no toxic effect on the shaped blood elements. There were also no changes in the behavior and condition of the experimental animals. The research results indicate that the developed immunosorbent has high specificity and safety.

6 Availability of Data and Material

Data can be made available by contacting the corresponding author.

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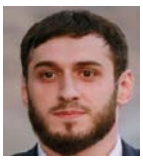

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