ESTUDO DA PLASMASORÇÃO DE LIPOPROTEÍNA *IN VITRO* POR SORBENTE POLIMÉRICO COM GRUPOS MONOETANOLAMINA
STUDY OF THE *IN VITRO* LIPOPROTEIN PLASMASORPTION BY POLYMERIC SORBENT WITH MONOETHANOLAMINE GROUPS
ИССЛЕДОВАНИЕ ПЛАЗМОСОРБЦИИ ЛИПОПРОТЕИДОВ *IN VITRO* ПОЛИМЕРНЫМ СОРБЕНТОМ С ГРУППАМИ МОНОЭТАНОЛАМИНА

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RESUMO

Atualmente, o método de plasmasorção é utilizado para o tratamento de muitas doenças. No entanto, os plasmasorventes modernos para a correção de proteínas e lipídios têm muitas vezes uma série de desvantagens, tais como selectividade e biocompatibilidade insuficientes. Além disso, eles são muito caros. O objetivo do trabalho é estudar a adsorção *in vitro* de lipoproteínas do plasma por meio de trocadores de ânions com grupos monoetanolamina sob condições próximas às clínicas. Foram determinadas as capacidades das frações de triglicérides, lipoproteínas e o tempo ótimo de realização do procedimento de plasmasorção para os sorventes em questão e foram caracterizados seus efeitos nas propriedades aterogênicas do plasma. Os sorventes oferecidos são mais baratos que os atualmente usados porque são sintetizados com base nos polímeros sintéticos disponíveis. Espera-se que os resultados obtidos sejam utilizados para investigações adicionais que possam reduzir o custo e aumentar a disponibilidade de métodos modernos de plasmasorção.

Palavras-chave: Sorção de colesterol, remoção seletiva de componentes do perfil lipídico, purificação do plasma, aterosclerose, hipercolesterolemia.

ABSTRACT

At present, the plasmasorption method is used for the treatment of many diseases. However, the modern plasmasorbents for the correction of proteins and lipids often have a number of disadvantages, such as insufficient selectivity and biocompatibility. Besides that, they are quite expensive. The aim of the work is to study *in vitro* lipoprotein adsorption from plasma by anion exchangers with monoethanolamine groups under conditions approached to clinical ones. We determined the capacities on triglyceride and lipoprotein fractions and the optimal time of the plasmasorption procedure for the sorbents concerned and characterized their effect on the atherogenic properties of plasma. The offered sorbents are cheaper than currently used ones because they are synthesized on the basis of available synthetic polymers. The results obtained are expected to be used for further investigations that could reduce the cost and increase the availability of modern plasmasorption methods.

Keywords: cholesterol sorption, selective removal of lipid profile components, purification of plasma, atherosclerosis, hypercholesterolemia.

АННОТАЦИЯ

В современной медицине для лечения многих заболеваний используется метод плазмасорбции. Однако плазмасорбенты, применяемые для коррекции протеинов и липидов, зачастую имеют ряд недостатков, как то неудовлетворительная селективность и биосовместимость. Кроме того, они достаточно дороги. Целью настоящей работы является изучение сорбции липопротеидов из плазмы in

vitro анионитами с группами моноэтаноламина в условиях, моделирующих клинические. Для данных сорбентов определены емкости по триглицеридам и различным фракциям липопротеидов, найдено оптимальное время проведения процедуры плазмасорбции, а также охарактеризовано их влияние на атерогенные свойства плазмы. Предлагаемые сорбенты дешевле используемых в настоящее время, поскольку синтезируются на основе доступных синтетических полимерных матриц. Полученные результаты могут быть полезны для дальнейших разработок и способствовать снижению стоимости и повышению доступности плазмасорбционных методов лечения.

Ключевые слова: сорбция холестерина, селективное извлечение компонентов липидного профиля, очистка плазмы, атеросклероз, гиперхолестеринемия.

1. INTRODUCTION

The plasmadsorption method is widely used for the treatment of various pathologies caused by the increased content of certain substances in the blood. In particular, this therapeutic procedure is prescribed for the treatment of the circulatory system diseases. Atherosclerosis is the risk factor for such pathologies, whereby it can be caused by increased content of LDL and VLDL cholesterol.

Another important area of modern medicine is the extraction from the plasma of certain types of globulins. Their increased concentration is observed in patients with a hyperimmune status that can cause allergic reactions and some other pathologies. In this case, there is a need for selective extraction of these substances from the body. The method of extracorporeal immunoadsorption allows extracting the antigen-antibody reaction components from biological fluids. Along with this, it is possible to allocate by immunosorbents specific antibodies with their further elution to prepare hyperimmune sera and some costly medicines.

Plasma α - and β -globulins are associated with triglycerides, phospholipids, cholesterol, and sphingomyelin and exist in the form of lipoprotein complexes. Thus, the lipid balance as well as the content of α - and β -globulins can be adjusted by the selective sorption of lipoproteins from plasma.

As the lipoprotein particle surface is negatively charged, we chose a copolymer with monoethanolamine group as a plasmadsorbent. A quaternary nitrogen atom has a positive charge that can increase the affinity of the sorbent to lipoproteins. Besides that, secondary amine is more spatially accessible for large biological particles than tertiary or quaternary amines. The nature of the nitrogen atom substituents affects the electron density shift thus their varying allows adjusting the magnitude of the positive charge and hydrophilic-hydrophobic properties of the

sorption center.

Over the past few decades, numerous studies have been carried out in this direction. [1-5] can be noted among the latest works.

Scientific researches in the field of creating immunosorbents are also quite wide, but they are mainly devoted to the extraction of γ -globulins [6-20]. Affine membranes [9-18], various synthetic matrices [19, 20] or microspheres [19-28] are used in this case. As for the sorption of α - and β -globulins, it is studied very limitedly. In particular, Norwegian scientists examined the extraction of serum α 1-globulin associated with insulin by gel filtration and immunoadsorption methods [29].

At present there are high demands for medical sorbents, mainly these are hemocompatibility and thromboresistance. Besides that, the sorbents must be stable, chemically resistant, standardized, have sorption activity and not emit toxic substances. However, currently used plasmadsorbents often to some degree do not meet these requirements. Immunosorbents are quite expensive therefore they are often not available for the seriously ill. In this regard, the study is aimed at the development of promising biocompatible sorbents for the removal of atherogenic lipoproteins and α - and β -globulins from plasma, and at finding the optimal plasmadsorption procedure time in conditions approached to clinical ones. The developed sorbents are much cheaper than currently used in medicine ones, so in some cases, their use is more appropriate, even though they may be less selective.

2. MATERIALS AND METHODS

At the first stage, we investigated a wide range of sorbents of various classes: cation and anion exchangers, polyampholytes, neutral sorbents, and active coals, specific sorbents. The anion exchanger AMX-1 was studied in three

ionic forms: hydroxyl, bicarbonate, and chloride [30]. The process of plasmasorption was conducted under static conditions. The sorbent AMX-1 showed good adsorption of the components of the lipid profile. In this paper, we investigated the absorption of lipids by the sorbent AMX-1 under dynamic conditions simulating clinical ones.

The sorbent AMX-1 was obtained by the reaction of a chloromethylated copolymer of styrene and divinylbenzene with monoethanolamine (fig. 1). The properties of the sorbent AMX-1 are as follows: 10.1 % of chlorine, 30 % of divinylbenzene, 115 % of isooctane, the specific surface area – 110 m²/g, the total pore volume – 1.5 cm³/g, porosity – 52 %.

We carried out an in vitro experiment on plasmasorption in conditions simulating clinical ones. 200 ml of donor plasma circulated by means of a peristaltic pump through 100 ml of sorbent treated by 0.14 M sodium chloride solution. The perfusion rate was 10 ml/min. Plasma samples were taken during the sorption process and then analyzed for the content of following lipoprotein fractions: triglycerides, total cholesterol, LDL and HDL cholesterol. The analyses were carried out in the licensed Invitro laboratory on the analyzer Architect c8000 (Abbot, USA). The following methods of analyses were used:

- for triglycerides – enzymatic colorimetric method (GPO PAP)

- for total cholesterol – enzymatic method using cholesterol esterase (CHOD-PAP)

- for HDL cholesterol – direct measurement following polyanionic adsorption of other lipoproteins

- for LDL cholesterol and VLDL cholesterol – calculation method according to Fridvald's, in Equation 1 and 2:

$$C_{LDL} = C_{TC} - C_{HDL} - C_{TG} / 2.2 \quad (\text{Eq. 1})$$

$$C_{VLDL} = C_{TG} / 2.2, \quad (\text{Eq. 2})$$

Where, C_{LDL} , C_{TC} , C_{HDL} , C_{TG} , C_{VLDL} – concentrations of LDL cholesterol, total cholesterol, HDL cholesterol, triglycerides and VLDL cholesterol correspondingly, mmol/l.

3. RESULTS AND DISCUSSION:

The sorption of triglycerides and cholesterol of various lipoprotein fractions from plasma by the sorbent AMX-1 under dynamic

conditions was studied. We carried out two experiments with different samples of initial plasma (the concentration of lipids in initial plasma was larger in the first experiment than in the second one).

Figure 2 shows the change in the concentrations of the components under study during plasmasorption. The concentrations of all the components were decreasing during half an hour from the beginning of the process, and then their levels practically did not change. The content of all the studied substances in plasma did not exceed the upper reference values during the whole sorption process.

Figure 3 shows the change in concentrations, reference values, recommended levels and areas of medium and high risk for IHD for total and LDL cholesterol.

Figure 4 shows the change in levels and reference values for LDL cholesterol and HDL cholesterol, triglycerides and atherogenic coefficient. The latter was calculated by Equation 3:

$$K_a = (C_{LDL} + C_{VLDL}) / C_{HDL} \quad (\text{Eq. 3})$$

As can be seen, there is a general trend of increasing the atherogenic coefficient, this being the risk factor of atherosclerosis.

Figure 5 shows the change of the AMX-1 sorbent capacities on the lipid profile components during the sorption process. The capacities were calculated by Equation 4:

$$D = (C_0 - C_f)V_p / V_s, \text{ where} \quad (\text{Eq. 4})$$

D – dynamic sorption capacity, mmol/l;

C_0 , C_f – initial and final concentrations of a component in plasma, mmol/l;

V_p , V_s – plasma and sorbent volume, l.

It is seen that the main filling of the sorbent capacity occurs during half an hour from the beginning of the process and then it increases slightly or fluctuates around the average value.

Figure 6 shows the change in the coefficient of distribution of the components between the solid and liquid phases. It was calculated by Equation 5:

$$K_d = D / C, \text{ where} \quad (\text{Eq. 5})$$

K_d – distribution coefficient, l [plasma]/l [sorbent];

C – concentration of a component in plasma, mmol/l.

Figure 7 shows the graphs of changes in the general distribution coefficient during the experiments. It was calculated by Equation 6:

$$K_{dg} = (K_{dLDL} + K_{dVLDL}) / K_{dHDL}, \text{ where} \quad (\text{Eq. 6})$$

K_{dLDL} , K_{dVLDL} , K_{dHDL} – distribution coefficients for LDL, VLDL and HDL cholesterol correspondingly.

The numerator of this formula characterizes the extraction of the atherogenic lipoprotein fractions by the sorbent, and the denominator – the antiatherogenic one. The general distribution coefficient indicates the selectivity of the sorbent to LDL and VLDL cholesterol.

It can be stated that the distribution coefficients of the lipid profile components mainly increase during the process, thus indicating the transition of the extracted substances from plasma to the sorbent phase (Fig. 6). However, the general distribution coefficient reduces, therefore it is inexpedient to carry out the process for more than 0.5 h due to the maximum filling of the sorbent capacity upon reaching this time (Fig. 7).

It is worth noting that the sorbent AMX-1 did not cause excessive clotting of plasma that shows its good thromboresistance.

4. CONCLUSIONS:

Experiments on plasmasorption in dynamic conditions simulating clinical ones for the sorbent AMX-1 with monoethanolamine groups are carried out. The concentrations of the lipid profile components and the atherogenic coefficients of initial plasma and of plasma after sorption are determined. The influence of the sorbent AMX-1 on atherogenic properties of plasma is characterized. Its sorption characteristics such as capacities on triglycerides and lipoprotein fractions, as well as the distribution coefficients of the components are obtained. The optimal time of plasmasorption in dynamic conditions for the sorbent was found to be 0.5 h. It is shown that the sorbent AMX-1 does not cause increased plasma coagulation. The results obtained allow to consider the sorbent AMX-1 as a possible prototype for the development of promising biocompatible plasmasorbents for extraction of lipoprotein.

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Figure 1. The reaction of the syntheses of the AMX-1 sorbent

Figure 2. Dynamics of change in the triglyceride and lipoprotein concentrations in plasma during the sorption by the AMX-1 sorbent

Figure 3. Reference values and dynamics of change in the total cholesterol and LDL cholesterol concentrations in plasma during the sorption by the AMX-1 sorbent

Figure 4. Reference values and dynamics of change in the VLDL, HDL, triglycerides and atherogenic coefficient levels during the sorption by the AMX-1 sorbent

Figure 5. Dynamics of change in the AMX-1 sorbent capacities on the lipid profile components during the sorption process

Figure 6. Dynamics of change in the lipid profile component distribution coefficient during the sorption for the AMX-1 sorbent

Figure 7. Dynamics of change in the general distribution coefficient during the sorption for the AMX-1 sorbent