GluLa-DPG-PEG600 NANOPOLYMER BINDS PROTEINS AND SPREADS TO RATS’ ORGANS AND TISSUES

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In this article, we described the ability of GluLa-DPG-PEG600 nanopolymer based on the pseudopolyamino acids to bind proteins and its localization in rats organs and tissues after intravenous and intramuscular injections. By using electrophoresis in 5% polyacrylamide gel, it was found the ability of GluLa-DPG-PEG600 nanopolymer to bind bovine serum albumin (BSA). This indicates the GluLa-DPG-PEG600 is a potential transporter of proteins and their complexes. By means of fluorescent microscopy, it was found that GluLa-DPG-PEG600 nanopolymer labeled with fluorescein with BSA labeled with fluorescent Alexa Fluor 555 dye (GluLa-DPG-PEG600-F + BSA Alexa Fluor 555) localized in liver and brain on the 16th after intramuscular injection. On the 5th hour after intravenous injection, GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complex localized in spleen and kidney obtained. Results show a potential usefulness of GluLa-DPG-PEG600 as a transporter of drugs capable of penetrating the blood-brain barrier. Localization of GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complex in spleen suggests that GluLa-DPG-PEG600 could be used as an adjuvant for development of vaccines.

Keywords: rats, nanopolymer, pseudopolyamino acids, drug transporter, adjuvant.

INTRODUCTION

At present, the main purpose of pharmacology is to make drugs that are more effective in disease treatment and are safe for human and animals. Accordingly, scientists develop new classes of polymers that are non-toxic, stable and biodegradable platforms which can be effective drug transporters. Polymers that meet these requirements can be polymers based on the pseudopolyamino acids. The main difference between polymers based on the pseudopolyamino acids and natural polyamino acids is that polymers based on the pseudopolyamino acids do not have peptide bonds in their structure that could be changed to urethane, ester, anhydride and other chemical bonds [9]. We addressed the polymers based on the pseudopolyamino acids which have ester bonds in their structure. Particularly, lysine pseudopolyamino acids with inclusion of lactic acid providing improved cells adhesion were synthesized [2]. For tissue engineering, scientists synthesized biodegradable polymers based on the pseudopolyamino acids with...
arginine and aspartic acid in their structure [4, 6, 7]. It is were known that tyrosine-based pseudopolyamino acids polymer can transport a biologically active substances, thus, tyrosine-based pseudopolyamino acids polymers with ZnO can be used as potential anticancer drug [1, 5]. Biodegradability and prolonged time of degradation are the most important characteristics for these polymers, for example, degradation time for polymers based on glycine and lactic acid is about 10 weeks [6, 7].

We created GluLa-DPG-PEG600 nanopolymer based on the glutamic acid that has low toxic effect in rats body [3]. We also proved the ability of this nanopolymer to penetrate mammalian cells and deposit in muscle tissues by intramuscular injection (unpublished results).

The main purpose of this work is to study the ability of GluLa-DPG-PEG600 to bind bovine serum albumin (BSA) and localization of GluLa-DPG-PEG600 complex with BSA in organs and tissues of the experimental rats.

MATERIALS AND METHODS

Materials. We created GluLa-DPG-PEG600 nanopolymer that consist of glutamic and lauryl acids (GluLa), dipropylene glycol (DPF), and polyethylene glycol 600 (PEG600). To study the nanopolymer localization in rats body, fluorescein (F) was covalently attached to GluLa-DPG-PEG600 macromolecule and structure of the resulting nanopolymer (GluLa-DPG-PEG600-F) was confirmed by the NMR and IR spectroscopy analyses (Fig. 1).

Methods. Synthesis of PPAK was conducted through interaction of diol and N-protected glutamic acid, polyoxyethylene and fluorescein (at different ratios of reagents) in a solution of benzene. The solution was cooled to 280 K, while stirring adding the appropriate amount of 4-dimethylaminopyridine (DMAP) and dicyclohexylcarbodiimide (DCK). The reaction mixture was incubated at 288 K for 3 h and then 3 h at 308 K. Dicyclohexylurea (DCC) was separated by filtration, and the polymer solution was evaporated. For purification of polyesters from catalyst residues and DCK, their solutions in benzene were prepared, washed three times with 15% NaCl solution in 0,1N HCl and 15% aqueous NaCl solution to neutral pH, after that it was dried over the magnesium sulfate, then filtered and evaporated.
For studying the ability of nanopolymer to bind blood proteins, we tested 2 % water dispersion of GluLa-DPG-PEG600 and GluLa-DPG-PEG600 with 1 % water solution of BSA (molecular weight approximately 66 kDa) by using electrophoresis in 5% polyacrylamide gel [8].

To study the ability of the nanopolymer to spread in rats’ organs and tissues, we made a complex consisting of 2 % water dispersion of GluLa-DPG-PEG600-F and BSA conjugated with the Alexa Fluor 555 fluorescent dye (ThermoFisher®) in ratio of 2.5:1 and maintained it at +18 °C for 1 h. We created 2 experimental and 1 control groups of animals consisting of three mature rats Rattus norvegicus var. Alba, line Wistar, (250–300 g) in each group.

Rats from first experimental group were intramuscularly injected with 0.3 ml of water dispersion of GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes and euthanized in 16 h after the injections. Rats from second experimental group were intravenously injected with 0.3 ml of water dispersion of complexes GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 and euthanized in 5 h after the injections. Rats of control group were intact. We prepared histological samples of spleen, liver, brain and kidney by using cryostat. Localization of GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes in rats organs and tissues was determined by the fluorescent microscopy with 656 nm wavelength using Leica DM2500 microscope and Leica Application Suite software. All animals were euthanized at soft chloroform condition. All experiments were performed in accordance with the Ministry of Education and Science of Ukraine, order No 281 from 01.11.2000, the European convention for protection of vertebrate animals used for experimental and other scientific purposes (18.03.1986), EU Directive No 609 (24.11.1986), and confirmed by protocol of bioethical expertise No 58 (08.11.2016) of the Institute of Animal Biology, NAAS of Ukraine.

RESULTS AND DISCUSSION

We used electrophoresis in 5% polyacrylamide gel and found that GluLa-DPG-PEG600 nanopolymer can bind BSA (Fig. 2.). This property is important for GluLa-DPG-PEG600 as a potential transporter for proteins and drugs. Furthermore, the ability of nanopolymer to bind BSA is a significant attribute to use GluLa-DPG-PEG600 as an adjuvant, since proteins are mainly used as antigens in vaccines.

Histological study of liver, spleen, brain and kidney of rats revealed GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes in all studied organs that depended on the intramuscular or intravenous injections. On the 16th h after intramuscular injection, GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes were localized in liver and brain tissues of the experimental group of rats (Figs. 3, 4). In liver, GluLa-DPG-PEG600-F
+ BSA Alexa Fluor 555 complexes were localized in liver lobules near hepatic vessels and inside it (Fig. 3, B). Complex visualization was characterized by orange-green luminescence that matches luminescence of the Alexa Fluor 555 dye in a combination with GluLa-DPG-PEG600-F nanopolymer. This also proves that the structures with the same luminescence are lacking in liver of control group of animals (Fig. 3, A).

**Fig. 3.** Localization of GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes in liver of control (A) and experimental (B) groups of rats (luminescence microscopy, total magnification 900×)

**Comments:** 1 – luminescence of GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes; 2 – blood vessel wall of liver

Localization of GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes in liver shows the ability of GluLa-DPG-PEG600 nanopolymer, in spite of its deposit in muscles by the intramuscular injection, get into liver and engaging in xenobiotic metabolism (unpublished results) (Fig. 3, B 1).

The same results were obtained by the luminescence analysis of the histological samples of rats brain. On the 16th h after intramuscular injection, GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes with orange luminescence were localized in brain tissues of the experimental group of rats (Fig. 4, B 1). Luminescence analysis of the histological samples of brain tissues of control group of rats revealed only catecholamines luminescence (Fig. 4, A 2).

Detection of GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes in brain indicates its ability to penetrate the blood-brain barrier. Earlier, it was shown that this class of nanopolymers in complex with albumins is capable of forming rod-shaped particles with a hydrodynamic diameter of 40–70 nm that possibly allow them to penetrate the blood-brain barrier. Theoretically this is because BSA molecules were adsorbed on the surface of GluLa-DPG-PEG600-F and acted as an additional stabilizer of a dispersed phase of the nanopolymer which reduces the linear sizes of the particles by fragmentation of large particles into smaller [10].
On the 5th h after intravenous injection GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes in rats kidney were detected. These complexes were localized in the cortical layer of kidney and in the lumens of veins in a zone of proximal convoluted tu-
bules (Fig. 5, B 1). In the control group of rats, structures with the same luminescence were not detected (Fig. 5, A).
Detection of GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes in rats kidney suggests that they are excreted by the kidney.

We also detected luminescence of GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes in spleen on the 5th h after intravenous injection (Fig. 6, B). In the control group of animals, structures with the same luminescence were not detected (Fig. 6, A).

**Fig. 6.** Localization of GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes in spleen of control (A) and experimental (B) group of rats (luminescence microscopy, total magnification 900×)

**Comments:** 1 – luminescence of GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes; 2 – lymph node

Localization of GluLa-DPG-PEG600-F + BSA Alexa Fluor 555 complexes in spleen confirms that GluLa-DPG-PEG600 might be used as an adjuvant for vaccine development. It is known that in spleen take place lymphocytes antigen dependent differentiation and antibody formation processes. This can provide quick and effective immune response to vaccine antigens.

**CONCLUSIONS**

In this study, we demonstrated that GluLa-DPG-PEG600 nanopolymer can bind bovine serum albumin which suggests GluLa-DPG-PEG600 can serve as a potential transporter of proteins and their complexes. The results of histological study based on using luminescence microscopy revealed nanopolymer localization in rats liver and brain after the intramuscular injection and in spleen and kidney after the intravenous injection. This also suggests an ability of the nanopolymer to be engaged in the xenobiotic metabolism, to penetrate blood-brain barrier, and provide its effective use as a potential drug transporter and adjuvant.
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проникати крізь гематоенцефалічний бар’єр. Виявлення комплексу GluLa-DPG-PEG600-F + БСА Alexa Fluor 555 у селезінці свідчить про можливе застосування нанополімеру як ад’юванта для вакцин, оскільки відомо, що в селезінці відбуваються процеси антигензалежної диференціації лімфоцитів та утворення антитіл.

**Ключові слова:** щурі, нанополімер, псевдополіамінокислоти, транспортер лікарських засобів, ад’юvant.

**СВЯЗЬВАННЯ НАНОПОЛИМЕРА GluLa-DPG-PEG600 С БЕЛКАМИ І ЙОГО ЛОКАЛИЗАЦІЯ В ОРГАНИЗМЕ КРЫС**

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В статті показана способність нанополімера на основі псевдополіамінокислот GluLa-DPG-PEG600 сполучатися з білками крові і його розповсюдження в організмі крыс при внутримышечному і внутрішньовеному введеннях. С користуванням електрофореза в 5% поліакриламідному гелі ми обнаружили способність нанополімера GluLa-DPG-PEG600 сполучатися з бічним сироваточним альбуміном (БСА). Єто, в частоти, є важливим визначним нанополімером як потенціального транспортера білків і їх комплексів. С користуванням важкосфіновою мікроскопією ми обнаружили, що нанополімер GluLa-DPG-PEG600 меченый флуоресцентним в комплексі з БСА меченым флуоресцентним красителем Alexa Fluor 555 (GluLa-DPG-PEG600-F + БСА), на 16 годин після внутрішньовенового введення локалізується в печінці і головному мозку крыс. Через 5 годин після внутрішньовенного введення комплекса GluLa-DPG-PEG600-F + БСА Alexa Fluor 555 в організмі обнаружені в селезенці і почках крыс. Результати експериментів підтверджують можливість застосування нанополімера GluLa-DPG-PEG600 як потенціального транспортера лекарственних засобів у вакцин для антитіл. Виявлення комплекса GluLa-DPG-PEG600-F + БСА Alexa Fluor 555 в селезенці свідчить про можливість застосування у вакцин, оскільки відомо, що в селезенці відбувається процеси антигензалежної диференціації лімфоцитів та утворення антитіл.

**Ключові слова:** крыси, нанополімер, псевдополіамінокислоти, транспортер лекарственных средств, адъювант.

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