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HISTOMORPHOLOGY OF ORGANS AND ACTIVITY OF SOME ENZYMES IN MICE AFTER IMMUNIZATION WITH POLYMER BASED ON ACRYLIC ACID OR ALUMINUM HYDROXIDE AS ADJUVANTS

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Vaccination is the best biomedical approach in avoiding diseases. Proteins and peptides purified from microorganisms or synthesized chemically are weakly antigenic and need adjuvant to provide strong immune responses. Many substances with adjuvant properties have been discovered, however, only aluminum compounds stay traditionally in clinical use. The aim of this study was to evaluate biological influence on mice after immunization with a polymer of acrylic acid with adjuvant properties.

The polymer was synthesized on the basis of glycidyl methacrylate, acrylic acid, triethylene glycol methacrylate and butyl acrylate. Histomorphology of the liver, kidney and spleen of white mice after subcutaneous administration of polymer or aluminum hydroxide was investigated. It has been established that the polymer and aluminum hydroxide did not cause changes in parenchymal organs of white mice. The structure of organs was preserved, no pathological changes were revealed.

The activities of antioxidant enzymes — superoxide dismutase (SOD), catalase and glutation reductase (GP) at the application of adjuvants were studied. Immunization of mice with aluminum hydroxide showed an increase in the activity of SOD in the kidneys by 1.5 times ($P<0.01$), catalase in liver on 8 % ($P<0.05$), and GP in kidney on 30.6 % ($P<0.001$). Polymer based on acrylic acid caused a decrease of SOD activity in the liver by 1.78 times ($P<0.01$), and an increase of catalase activity on 10.5 % ($P<0.05$). At the same time, the activity of antioxidant enzymes in the spleen and kidneys of animals after the injection of the polymer did not differ from the control group.

Keywords: ORGANS OF MICE, POLYMER, ALUMINUM HYDROXIDE, ENZYME ACTIVITY

ГІСТОМОРФОЛОГІЯ ОРГАНІВ МИШЕЙ ТА АКТИВНІСТЬ ЕНЗИМІВ ЗА ІМУНІЗАЦІЇ З ПОЛІМЕРОМ НА ОСНОВІ АКРИЛОВОЇ КИСЛОТИ ЧИ ГІДРОКСИДОМ АЛЮМІНІЮ ЯК АД'ЮВАНТІВ

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Вакцинація є найкращим біомедичним підходом для профілактики інфекційних захворювань. Білки та пептиди, очищені від мікроорганізмів або хімічно синтезовані, є слабо антигенними і потребують ад'юванта для забезпечення сильної імунної реакції. Виявлено багато речовин-ад'ювантів, однак лише сполуки алюмінію традиційно застосовуються у клінічній практиці. Мета дослідження — оцінити біологічний вплив на білих мишей полімеру акрилової кислоти, який має ад'ювантні властивості.

Полімер синтезували на основі гліцидилметакрилату, акрилової кислоти, метакрилату триетиленгліколю та бутилакрилату. У статті представлено гістоморфологічну характеристику печінки, нирок та селезінки білих мишей за підшкірного введення ад'ювантів: полімеру на основі акрилової кислоти та

гідроксиду алюмінію. Встановлено, що полімер, як і гідроксид алюмінію, який широко застосовують при виготовленні вакцин, введені спільно з БСА, не спричиняють змін у паренхіматичних органах білих мишей. Структура органів збережена, патологічних змін не виявлено.

Вивчено активності ензимів антиоксидантного захисту організму — СОД, каталази та ГП за умов застосування ад'ювантів. Після імунізації білих мишей з гідроксидом алюмінію встановлено зростання активності СОД у нирках у 1,5 разу (P<0,01), каталази у печінці — на 8 % (P<0,05) та ГП у нирках — на 30,6 % (P<0,001). За застосування полімеру на основі акрилової кислоти у печінці активність СОД знижувалась в 1,78 разу (P<0,01), а каталази — зростала на 10,5 % (P<0,05). Водночас активність ензимів антиоксидантного захисту у селезінці та нирках тварин, яким вводили полімер, не відрізнялася від контрольних груп.

Ключові слова: ОРГАНИ МИШЕЙ, ПОЛІМЕР, ГІДРОКСИД АЛЮМІНІЮ, АКТИВНІСТЬ ЕНЗИМІВ

ГИСТОМОРФОЛОГИЯ ОРГАНОВ МЫШЕЙ И АКТИВНОСТЬ ЭНЗИМОВ ПОСЛЕ ИММУНИЗАЦИИ С ПОЛИМЕРОМ НА ОСНОВЕ АКРИЛОВОЙ КИСЛОТЫ ИЛИ ГИДРОКСИДОМ АЛЮМИНИЯ В КАЧЕСТВЕ АД'ЮВАНТОВ

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Вакцинация является лучшим биомедицинским подходом для профилактики инфекционных заболеваний. Белки и пептиды, очищенные из микроорганизмов или химически синтезированные, являются слабо антигенными и требуют адъюванта для обеспечения сильной иммунной реакции. Обнаружено много веществ-адъювантнов, однако только соединения алюминия традиционно применяются в клинической практике. Цель исследования — оценить биологическое воздействие на белых мышей полимера акриловой кислоты, имеющего адъювантные свойства.

Полимер синтезировали на основе глицидилметакрилата, акриловой кислоты, метакрилата триэтиленгликоля и бутилакрилата. В статье представлена гистоморфологическая характеристика печени, почек и селезенки белых мышей при подкожном введении адъювантов: полимера на основе акриловой кислоты и гидроксида алюминия. Установлено, что полимер, как и гидроксид алюминия, широко применяемый при изготовлении вакцин, введенные совместно с БСА, не влекут изменений в паренхиматических органах белых мышей. Структура органов сохранена, патологических изменений не выявлено.

Изучены активности энзимов антиоксидантной защиты организма — СОД, каталазы и ГП при применении адъювантов. При иммунизации мышей с гидроксидом алюминия установлено повышение активности СОД в почках в 1,5 раза (P<0,01), каталазы в печени — на 8 % (P<0,05) и ГП в почках — на 30,6 % (P<0,001). При применении полимера на основе акриловой кислоты в печени активность СОД снизилась в 1,78 раза (P<0,01), а каталазы — возросла на 10,5 % (P<0,05). В то же время активность энзимов антиоксидантной защиты в селезенке и почках животных, которым вводили полимер, не отличалась от контрольных групп.

Ключевые слова: ОРГАНЫ МЫШЕЙ, ПОЛИМЕР, ГИДРОКСИД АЛЮМИНИЯ, АКТИВНОСТЬ ЭНЗИМОВ

Vaccination is the one of the main methods for the specific prevention of infectious diseases. The disadvantage of vaccination is the use of pathogens (live or attenuated viruses and bacteria) that can lead to the development of the disease. Many compounds with adjuvant properties have been experimentally investigated [2]. However, over the past 80 years, only aluminum compounds (aluminum hydroxide and aluminum phosphate) are widely used in vaccines [13]. These aluminum compounds, like adjuvants, have a number of disadvantages, including the causation of allergic reactions [8], the development of Alzheimer's disease [9], and others. In preclinical studies, polymer-

ic nanoparticles of varying composition and size [1, 5, 10, 12] were often used as antigen carriers. In previous studies, we established the better adjuvant properties of a polymer of acrylic acid, compared to that of aluminum hydroxide [5]. Subcutaneous injections of the investigated polymer did not lead to visible damage in animals throughout the observation period. There was no swelling and redness at the site of immunization, the hair of animals remained dense and with glare, the weight of the animals remained stable. There were no lethal cases among the tested animals.

The purpose of this work was to study the influence of an acrylic acid-based polymer on the morphological characteristics of the liver, kidneys and spleen of white mice.

Materials and methods

The studies were performed on white non-linear mice *Mus musculus* (analogues by age and live weight) in accordance with the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986).

Animals at age 5 months were divided into 3 groups with 5 mice in each group. Mice of the 1st experimental group were subcutaneously injected with 100 μl of a polymer solution (1.5 mg/mouse) and bovine serum albumin (BSA) at a concentration of 100 mg/ml, in a volume ratio (1:1). Mice of the 2nd experimental group were injected with a solution of 80 mg/ml of aluminum hydroxide (Sphera Sim, Ukraine) with BSA at concentration of 100 mg/ml, in a volume ratio (1:1). Control mice were injected with 100 μl of a 0.9 % solution of sodium chloride. Immunization was performed on the 1st, 14th and 28th day. The solutions were sterilized by autoclaving. The polymer was synthesized via the dispersion polymerization of a monomer mixture in heptane (*LobaChemie*, India), azoisobutyronitrile (AIBN, *Merck*, Germany) was used as initiator (5 % per monomers). Glycidyl methacrylate (GMA), butyl acrylate (BA), acrylic acid (AA), and triethylene glycol dimethacrylate (TGMDMA) were used to obtain the microsized structure. Composition of monomer mixture during synthesis: 10 % of GMA, 15 % of BA, 1 % of TGMDMA and 74 % of AA. Polymerization was carried out in flat bottom dilatometers or reactors at stirring for six hours at 70±0.2 °C pre-filled by argon. Polymer was separated and washed to remove the unreacted monomers.

One week after the last injection, animals under anesthesia (chloroform, *Sphere Sim*, Ukraine) were decapitated by cervical dislocation.

For histological studies the liver, spleen and kidneys were taken in the size 0.2–0.3 cm were fixed in 10 % formalin solution. Rinse, dehydration and formation of paraffin blocks were carried out according to the standard method. Sections with a thickness of 7 μm were made on a *Microm HM 340E* microtome and stained with hematoxylin-eosin [4].

The activity of enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GP) was determined in organ homogenates. In particular, the activity of SOD was determined by a method where principle is to restore nitrotetrazolium by superoxide radicals and expressed it in unit units per 1 mg of protein [7]. The activity of GP was determined by the rate of oxidation of reduced glutathione and expressed in μmol/ml·min [7]. The activity of catalase was determined by the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts, expressed in μmol/ml·min [7].

Statistical calculations of results (M±m) were performed using the *Microsoft Excel 2007* computer program. The probability of differences was determined by the Student's *t*-criterion.

Results and discussion

We obtained copolymer with 14 % of GMA, 12 % of BA, 4 % of TGMDMA and 70 % of AA after process of synthesis. Among particle size was 0.49 μm from TEM image. Hydrodynamic diameter of particles (from the research of the DLS) was 0.64 μm and Z-potential — 49 mV. In our previous studies, we established the adjuvant properties of polymer of acrylic acid [6]. Adjuvant properties of the polymer were compared to aluminum hydroxide, which is a component of many traditional vaccines. Experimental polymer was a stronger adjuvant because it led to an increase of specific antibodies against BSA by 2 times. A necessary stage of developing new drugs is preclinical testing on laboratory animals.

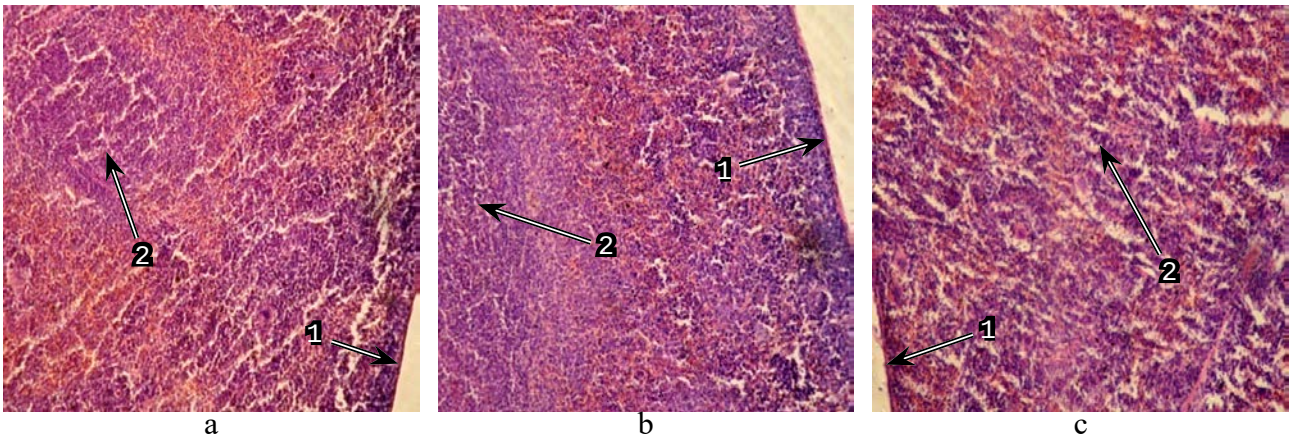

Fig. 1. Microstructure of the spleen of white mice. a — control; b — polymer; c — aluminum hydroxide; 1 — capsule; 2 — lymphatic follicle. Hematoxylin-eosin, ×100

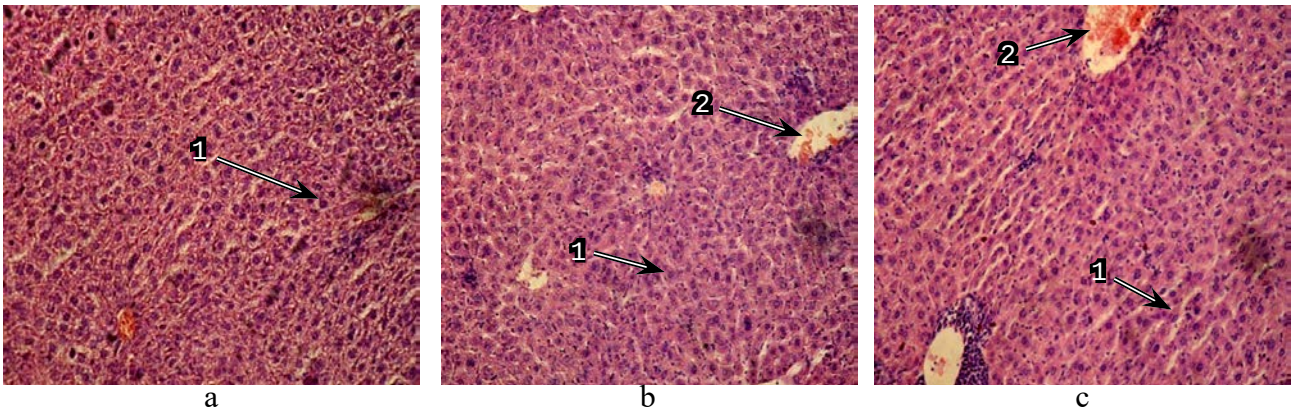

Fig. 2. Microstructure of the liver of white mice. a — control; b — a polymer; c — aluminum hydroxide; 1 — hepatocytes; 2 — a vein filled with erythrocytes. Hematoxylin-eosin, ×100

It is important to look into the morphology of organs under the action of a prototype drug to study the negative effects of drugs on the body.

We studied the structure of the spleen, liver and kidneys. It was established by macroscopic observation that the spleen of the control and experimental groups of mice hsd elastic consistency and was sickle shaped. During the microscopic examination in mice of the control group, capsules with trabeculaes, white and red pulp, trabecular veins and arteries, and lymph nodes (fig. 1) are clearly visible.

The thinning of trabeculaes (fig. 1c) was established in mice after the immunization with aluminum hydroxide. Trabecular veins and arteries were poorly differentiated. We found an increase in the number of red blood cells, platelets, monocytes and lymphocytes in the red spleen pulp under the action of aluminum hydroxide. Under the action of a polymer (fig. 1b), white pulp is poorly developed.

The macroscopic structure of the liver of the both control and experimental groups was of solid consistency, and reddish brown in color. It was observed microscopically that the structure of the organs both of the control and experimental groups were saved.

The cytoplasm of hepatocytes is in homogeneous color, the nuclei are clearly visible (fig. 2). Chromatin in the nuclei is localized predominantly on the periphery. The remains of erythrocytes are found in the veins. Structural and pathological changes in any of the experimental groups were not observed.

Macroscopic examination of the kidneys of mice in the control and experimental groups found that the organs had solid consistency and a reddish-brown color. On the slices of the organs the renal cortex and renal medulla are clearly distinguished (fig. 3).

The structure of the kidney tissue is preserved. There are no hemorrhages, the tubular

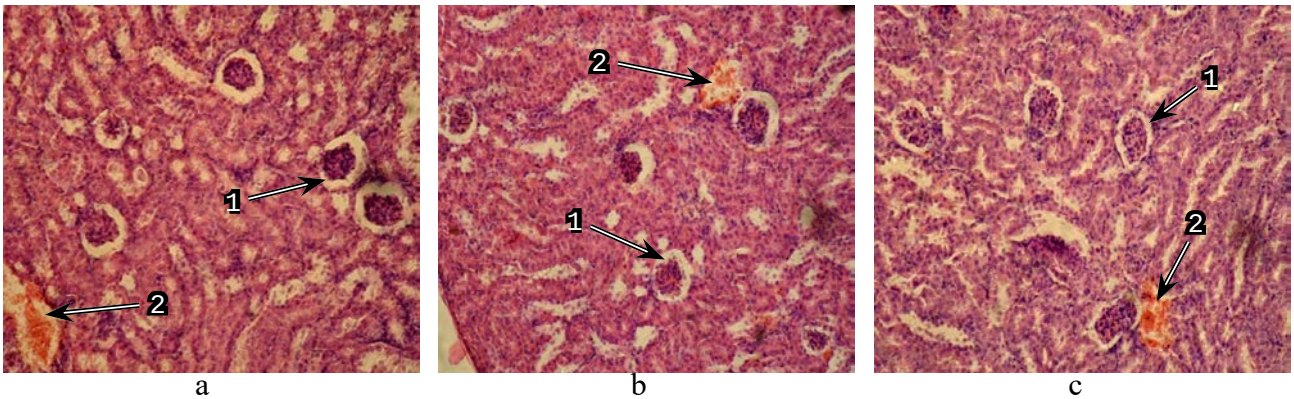

Fig. 3. Microstructure of kidney of white mice. a — control; b — polymer; c — aluminum hydroxide; 1 — vascular glomerulus; 2 — vessels with red blood cells. Hematoxylin-eosin, ×100

epithelium has not changed, lumen is well visible and the vascular glomerulus is normal size (fig. 3). Vessels are moderately blood-filled and the epithelium is without visible changes.

Since the polymer contains a large number of peroxide groups, it can act on the antioxidant protection system. In our previous studies, there was a decrease in the content of lipid hydroperoxides in the liver at the action of both a polymer of acrylic acid and aluminum hydroxide [11], indicating an effect on cellular metabolism. Interaction with reduction of activity of SOD and catalase and destruction of pulmonary tissue was established in the experimental models of acute pneumonia [3]. Therefore, activity of enzymes antioxidant protection in the liver, kidneys and spleen of mice was determined (table).

SOD activity increased in the kidneys by 1.5 times ($P<0.01$), catalase — on 8 % ($P<0.05$) in liver, and GP — on 30.6 % ($P<0.001$) in the liver after the immunization of mice with aluminum hydroxide. Under the influence of the polymer, based on acrylic acid, the activity of SOD decreased in the liver by 1.78 times ($P<0.01$), and catalase increased on 10.5 % ($P<0.05$). The detected decrease in SOD activity could be due to the increase in the concentration of H_2O_2, which is a product of the catalyzed reaction and at the same time is its inhibitor, as well as a substrate for catalase and GP [14]. Therefore, we have established the growth of catalase activity in liver. It also agrees with the absence of destructive changes in the liver after the use of the polymer as adjuvant. At the same time, the polymer did not cause changes in the activity of the investigated enzymes in the spleen and kidneys. The obtained data are consistent with the results of histomorphological analysis. The pathology of organs is detected when the activity of antioxidant enzymes is significantly reduced [3].

Consequently, based on performed histological studies, it was found that the structures of the kidneys and liver were without pathological changes in animals after immunization with both aluminum hydroxide and polymer. In the tissue of the spleen, due to the action of aluminum

Table

Activity of enzymes of antioxidant defense in tissues of mice

Enzyme	Organ	Animal groups		
		Control	1st esperimental (polymer+BSA)	2nd experimental (aluminum hydroxide +BSA)
SOD, conditional units/mg of protein	Liver	24.1±5.01	13.5±4.01**	26.03±5.12
	Kidney	16.1±1.52	15.3±2.02	24.5±3.2**
	Spleen	21.9±3.15	24.6±5.01	23.3±4.2
Catalase, μmol/ml min	Liver	4.0±0.21	4.4±0.11*	4.3±0.11*
	Kidney	2.6±0.13	2.5±0.25	2.5±0.21
	Spleen	3.5±0.21	3.7±0.47	3.4±0.36
GP, μmol/ml min	Liver	4.6±0.06	4.1±1.37	6.0±1.01***
	Kidney	1.5±0.09	1.4±0.06	1.3±0.41
	Spleen	2.2±0.1	2.4±0.55	1.7±0.33

hydroxide, the trabeculaes were insignificantly thinner, and the effect of the polymer was in the weaker development of white pulp. The results of the study of enzymatic activity of SOD, GP and catalase confirm the assumptions about the action of adjuvants on cellular metabolism, namely activation of the antioxidant defense process.

Conclusions

1. Histological studies have established that immunization both with aluminum hydroxide and the newly synthesized polymer acrylic acid-based does not cause damage of the liver, kidneys, and the spleen.

2. An activation of enzymes of antioxidant protection of SOD in kidneys, GP and catalase in liver after immunization with aluminum hydroxide, as well as growth of activity of catalase after the action of a polymer was observed.

Prospects for further research. Investigation of the influence of polymer on the organism of animals in order to create an effective and safe prophylactic agent will be studied.

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