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Blood biochemical parameters in mice under the action of polyphosphate esters and their complexes with antibiotics

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Complexes of polyphosphate esters with antibiotics were developed in Lviv Polytechnic National University together with scientists of Institute of Animal Biology NAAS to reduce the negative impact of antibiotics on the animal body. The conducted experiments allow assessing the effect of antibiotics, polyphosphate esters and complexes of polyphosphate esters with antibiotics on the body of laboratory animals based on biochemical markers of hepato- and nephrotoxicity. The antibiotics were administered in average daily therapeutic doses. It was found that the physiological state of mice and their blood biochemical indicators were within physiological normal values after the administration of polyphosphate ester P4 and complexes of polyphosphate ester P4+antibiotics (amoxicillin, oxytetracycline, and doxycycline). At the same time, intramuscular administration of polyphosphate ester P6 and complexes of P6+antibiotics have a certain negative effect on mice, which is manifested by changes in the activity of marker enzymes: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP). We found an increase in AST and ALT activities. P6+amoxicillin and P6+oxytetracycline complexes increased ALP activity. Complexes P4+antibiotics decreased ALP. Blood urea content decreased after the administration of polyphosphate ester P6 by 38.5%, P6+oxytetracycline by 26.9%, P6+doxycillin by 21.8%. P6+amoxicillin complex caused a significant increase by 237% in the concentration of creatinine in the blood of mice. The changes of blood creatinine concentration of other experimental groups fell within normal physiological range. Conducted studies of blood biochemical characteristics of mice under the action of new complexes of nanobiopolymer transporters with antibiotics ensured the selection of antibacterial drugs with low toxicity.

Key words: mice, antibiotics, polymers, polyphosphate ester complexes

Introduction

Drug delivery is understood as a set of methods, technologies and techniques aimed at modifying the physical and chemical, pharmacological and pharmaceutical properties of medicinal products in order to improve their effectiveness and safety [6]. Development and production of medicinal products using nanotechnology allows optimizing efficiency and minimizing side effects [4, 5, 12]. First of all, drugs with high toxicity, due to their inclusion in drug delivery systems, can be successfully used. In addition, their bioavailability improves and controlled drug release becomes possible [2, 6]. But despite this, there are not enough studies on

the toxicity of drugs created on the basis of nanoparticles or polymers.

In medical practice, among a large number of drugs with bactericidal properties, antibiotics or their synthetic analogues are most often used. They act against bacteria and tumor cells, suppressing their vital activity or destroying them. This group includes hundreds of drugs of different chemical structure. They have divergent spectrum, mechanism of action, side effects and indications for use [17]. However, the use of antibiotics is associated with toxicity for the human or animal body, which develops especially during their long-term use [1, 7]. This issue can be resolved in two ways: reducing the administered dose while maintaining its effectiveness and by "targeted delivery", when the active substance is delivered directly to the microorganism in special container molecules that are non-toxic for the body [11]. Effective targeted drug delivery systems developed in recent years include nanoscale biocompatible polymer transport systems that penetrate the membranes of bacterial cells and, in combination with existing antibiotics, are able to increase the therapeutic effect and reduce the toxic effect of the active substance on cells and, in general, on human or animal body [9, 14].

Antibiotics are often applied in veterinary practice. The slaughter of animals for meat and the consumption of milk are allowed only after a certain period of time, which depends on the drug that was used. The meat and milk obtained before the specified period are disposed of or fed to non-productive animals, depending on the conclusion of a veterinary medicine doctor [3]. Therefore, in order to reduce the negative impact of antibiotics on the animal body, the scientists of the Institute of Animal Biology NAAS together with the staff of the Lviv Polytechnic National University developed complexes of polyphosphate esters with antibiotics. It is supposed that the antibiotic in combination with polyphosphates will maintain its therapeutic properties and be less toxic than the commercial form.

The aim of this study was to assess the effect of polyphosphate ester complexes with antibiotics on the body of laboratory animals based on biochemical markers of hepato- and nephrotoxicity to select compound with reduced toxicity for veterinary medicine.

Materials and methods

The research was carried out at the Institute of Animal Biology NAAS together with the staff of the Lviv Polytechnic National University.

The polymeric transporters, polyphosphate esters, were synthesized based on N-derivatives of glutamic acid and dipolyethylene glycol(ethyl)phosphates. The molecular weight of the synthesized polymeric transporters is 1800–2400 Da. The particle size is 80–160 nm in the self-stabilized aqueous dispersed phase. Polymers were synthesized using dipolyethylene glycol(ethyl)phosphate (PEG-200÷1500), N-stearoyl glutamic acid, N,N'-dicyclohexylcarbodiimidine (DCC), N,N'-dimethylaminopyridine (DMAP), dimethylformamide (*Aldrich*). The polymer-antibiotic complexes were synthesized by adding the appropriate antibiotic [7]. The quantitative content of the antibiotic in the samples was investigated using high-performance 8liquid chromatography with a diode-matrix detector. The samples were separated on a Waters chromatograph equipped with a *Luna C 18(2)* 250×4.6 chromatographic column. Eluent flow rate was 1 ml/min. A mixture of acetonitrile and 0.2% phosphoric acid in a volume ratio of 2:8 acted as the eluent and solvent simultaneously.

White mice of the BALB/c line weighing 20–30 g were selected and randomly divided into groups: the control group receiving intramuscular administration of physiological solution, and experimental groups that included subgroups. The first experimental group consisted of 3 subgroups according to the antibiotic used, which was administered to mice intramuscularly in an average daily therapeutic dose (amoxicillin 15 mg/kg, oxytetracycline 20 mg/kg, doxycycline 4.4 mg/kg). In the second experimental group, mice were divided into three subgroups and injected intramuscularly with the complexes of polyphosphate esters and antibiotics in doses that were used in the first experimental group. The third experimental group contained two subgroups. These mice were injected with polymer P4 and P6, respectively. The mice were observed for 3 days after the use of medicines, and were decapitated. Blood biochemical analysis was performed to assess the hepato- and nephrotoxicity of the used drugs. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities, blood urea and creatinine content were measured.

Mice were kept in the vivarium of Institute of Animal Biology NAAS. All manipulations with animal were done in accordance with the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986). The protocol for animal experiments was approved by the Ethical Committee of the Institute of Animal Biology NAAS of Ukraine (Protocol no. 135 from 20.06.2023).

Statistical analysis of the results (M±m) was performed using *Microsoft Excel* software, $P<0.05$ was accepted as statistically significant.

Results and discussion

An increase in AST with the use of amoxicillin was established in all experimental groups. The maximum increase by 44% was in the group of mice that were injected intramuscularly with polymer P6 and its complex with amoxicillin by 31% (fig. 1).

Despite the increased AST activities in these experimental groups of mice, all parameters were within the normal physiological range for mice [8, 10].

At the same time, the ALT activity differed little between groups after polymer P4+antibiotic complexes administration to mice. The established changes in the activity of the enzyme were within the physiological normal range.

The ALT activity was 47.4±6.62 U/l after P4+amoxicillin administration, 67.0±8.2 U/l for P4+oxytetracycline and 69.9±13.45 U/l for P4+doxycycline. ALT activity of control group was 45.8±4.96 U/l.

Changes in ALT activity in subgroups of experimental mice that received P4 or P6 polymers were contrary. ALT activity increased by 38.2% after polymer P4 administration, but, on the contrary, it decreased by 17.7% using polymer P6 (fig. 2). These data were compared to the control.

Low ALT activity (for mice it is under 50 U/L) may be normal or indicates kidney disease [16]. At the same time, the created polymer P6+antibiotic complexes caused an increase in ALT activity in the blood of mice of all experimental groups (fig. 3).

Thus, ALT activity increased by 21% after P6+amoxicillin complex administration. P6+doxycycline complex caused increase of ALT by 106%, and P6+oxytetracycline complex — by 140%. These data were compared to ALT activity of control group (fig. 3). At the same time, changes in ALT activity after polymer P4+antibiotic complexes administrations differed little from the control group of mice. Thus, The ALT activity was 47.4±6.62 U/l after P4+amoxicillin complex administration, 67.0±8.2 U/l for P4+oxytetracycline, and 69.9±13.45 U/l for P4+doxycycline. All obtained results fell within physiological normal values.

Changes in the activity of AST and ALT enzymes in the blood of experimental mice may indicate that the functional capacity of the liver is reduced due to P6 polymer and polymer P6+antibiotic complexes administration to mice.

Another catalyst of biochemical reactions in the body is alkaline phosphatase, an enzyme that is localized mostly in the liver. The change in its activity characterizes the processes occurring in the hepatobiliary system [15].

It was found that the P6+amoxicillin and P6+oxytetracycline complexes increased the activity of the enzyme by 27.9% ($P<0.05$) and 3%, respectively (fig. 4). This is an evidence of negative influence on mice liver. Alkaline phosphatase is tightly bound to cell membranes. The established increase in the activity of the enzyme in the blood serum of mice under the action of P6+amoxicillin and P6+oxytetracycline complexes may indicate damage to their liver parenchyma.

Controversially, the activity of alkaline phosphatase decreased from 7.7% to 47% ($P<0.001$) after the administration of complexes P4+antibiotics compared to the control group. However, decreased activity of the enzyme in the blood was within the normal physiological range, and therefore, the use of polymer P4 in the combination with antibiotics did not affect the functional capacity of the liver.

An important diagnostic test of the function of both the liver and kidneys is the determination of urea. Blood urea concentration depends on the intensity of synthesis in the liver and the activity of excretion from the body by the kidneys. Blood urea concentrations are presented in table.

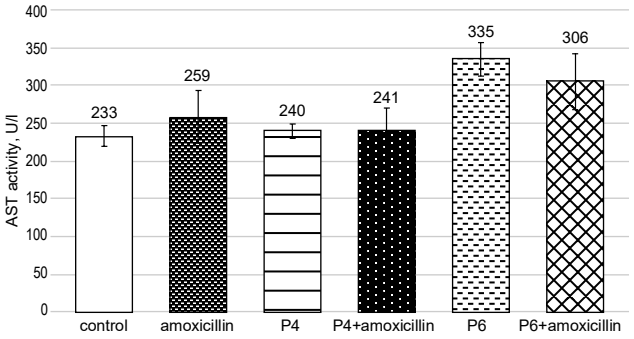

Fig.1. AST activity in blood serum of control and experimental groups of mice, U/l (n=3–4)

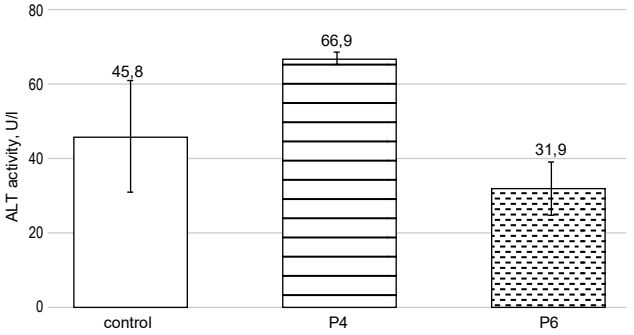

Fig. 2. ALT activity in the blood of mice under the action of polyphosphate esters, U/l (n=3)

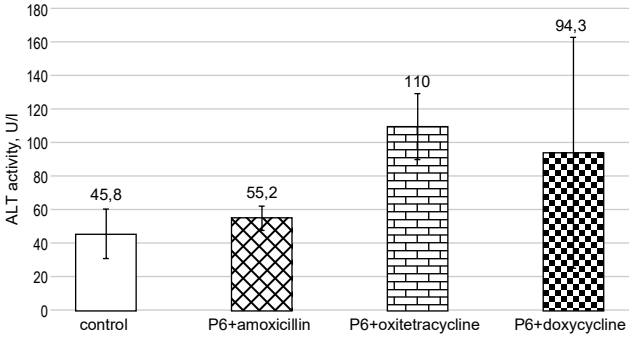

Fig. 3. Blood serum ALT activity in mice under the action of polymer P6+antibiotic complexes, U/l (n=3–4)

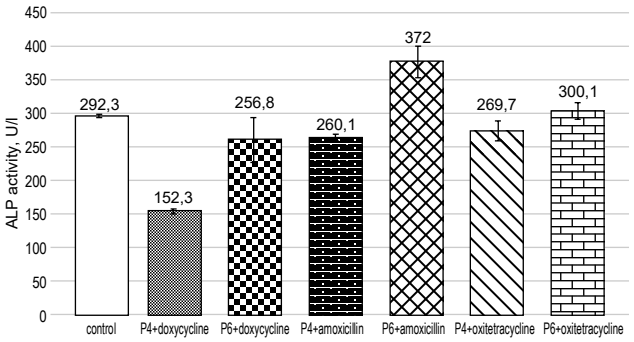

Fig. 4. Alkaline phosphatase activity in blood serum of mice under the action of polymer+antibiotic complexes, U/l (n=3–4)

Table. The concentration of urea and creatinine in the blood serum of mice, mmol/l (n=3–4)

Groups of mice	Urea concentration, mmol/l	Creatinine concentration, mmol/l
Control	8,57±0.19	51.45±0.25
Antibiotic: amoxicillin	8.23±0.69	57.80±0.15
doxycycline	8.03±1.07	57.20±4.83
oxytetracycline	7.1±1.36	56.10±0.15
Polymer P4	4.37±0,50***	58.88±3.88
P4+antibiotic complex P4+amoxicillin	5.60±0.34***	59.40±6.60
P4+doxycycline	5.73±1.00*	64.40±2.43
P4+oxytetracycline	4.71±1.27*	62.75±7.55
Polymer P6	5.27±0.54***	52.27±1.75
P6+antibiotic complex P6+amoxicillin	8.95±0.25	173.45±53.95
P6+doxycycline	6.70±1.00	55.0±1.35
P6+oxytetracycline	6.27±1.80	57.23±5.92

Note. The difference is statistically significant compared to the control group: *** — P<0.001; ** — P<0.01; * — P<0.05.

Intramuscular administration of antibiotics leads to a slight decrease of blood urea concentration by 3.9% (amoxicillin), 6.4% (doxycycline) and 17% (oxytetracycline). Polymers P4 and P6 caused the decrease in urea concentration by 49% (P<0.001) and 38.5% (P<0.001), compared to the control group. Polymer+antibiotic complexes also caused a decrease of blood urea concentration: P4+amoxicillin by 53% (P<0.001), P4+doxycycline by 49.4% (P<0.05) and P4+oxytetracycline by 44.4% (P<0.05). P6+doxycycline and P6+oxytetracycline complexes also reduce urea concentration in the blood of mice by 22% and 27%, respectively, compared to the control group. However, the changes in urea concentration in the blood of experimental groups of mice remained within the physiological normal range [13].

In contrast, intramuscular administration of the polymer P6+amoxicillin complex to mice caused an increase in urea concentration by 4.5% compared to the control group. This may indicate a negative effect on the functional capacity of the kidneys, in particular, their filtration function.

At the same time, polymer P6+amoxicillin complex caused a significant increase by 237% in the concentration of creatinine in the blood of mice (table). Creatinine is excreted by the kidneys via glomerular filtration. Its concentration in the blood reflects the degree of impairment of the filtration function of the kidney glomeruli. Increased blood creatinine concentration in mice under the action of the polymer P6+amoxicillin complex may indicate a violation of the filtration function of the renal glomeruli.

The changes of blood creatinine concentration in mice of other experimental groups were within normal physiological range.

The conducted studies of the physiological and biochemical characteristics of the animal organism under the action of new complexes of nanobiopolymer transporters with antibiotics (amoxicillin, oxytetracycline and doxycycline) ensured the selection of effective antibacterial drugs with low toxicity for their organism. It was found that the physiological state and blood parameters of the animals were within the physiological limits after polyphosphate ester complexes P4+antibiotics complexes administration in average daily therapeutic doses. These complexes are perspective candidates for medical practice. Intramuscular administration of polyphosphate ester P6 in combination with antibiotics has a negative effect on the body of experimental mice, which is manifested by changes in the activity of enzymes-markers of the state of the body, therefore it is not recommended for use. The results of this study will be used as a basis for elucidating the mechanisms and features of the action of polyphosphate esters and their complexes with antibiotics on the animal body.

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Біохімічні показники крові мишей за дії поліфосфатестерів та їх комплексів з антибіотиками

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Для зменшення негативного впливу антибіотиків на організм тварин в Інституті біології тварин НААН спільно зі співробітниками Національного університету «Львівська політехніка» створено комплекси поліфосфатестерів з антибіотиками. Проведені досліди дозволили за біохімічними маркерами гепато- і нефротоксичності оцінити вплив антибіотиків, поліфосфатерів та комплексів поліфосфатерів з антибіотиками на організм лабораторних тварин за введення їх у середньодобових лікувальних дозах. Встановлено, що фізіологічний стан і показники крові тварин за застосування окремо поліфосфатестеру Р4 та комплексів поліфосфатестер Р4+антибіотики у його складі (амоксицилін, окситетрациклін, доксициклін) були в межах фізіологічних величин. Водночас внутрішньом'язове введення поліфосфатестеру Р6 та комплексу Р6+антибіотики у його складі мають певний негативний вплив на організм дослідних мишей, що проявляється змінами активності ензимів-маркерів стану організму — аспартатамінотрансферази (АСТ), аланінамінотрансферази (АЛТ) і лужної фосфатази (ЛФ). Ми виявили підвищення активності АСТ і АЛТ. Комплекси Р6+амоксицилін і Р6+окситетрациклін підвищували активність ЛФ; комплекси Р4+антибіотики знижували активність цього ензиму. Вміст сечовини у крові знизився після введення поліфосфатестеру Р6 на 38,5%, комплексу Р6+окситетрациклін — на 26,9%, комплексу Р6+доксицилін — на 21,8%. Комплекс Р6+амоксицилін викликав вірогідне підвищення на 237% концентрації креатиніну в крові мишей. Зміни концентрації креатиніну в крові інших дослідних груп були в межах фізіологічної норми. Проведені дослідження біохімічних характеристик крові мишей за дії нових комплексів нанобіополімерних транспортерів з антибіотиками забезпечили підбір антибактеріальних препаратів з низькою токсичністю.

Ключові слова: миші, антибіотики, полімери, комплекси поліфосфатестерів

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