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PEGYLATED ANTIMICROBIALS

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INTRODUCTION

Today, there is a need for development of new technologies to create highly effective and safe antibacterial agents for the body. Medicines that were effective a few years ago are losing their positions, and their use is limited. According to the World Health Organization, the rapid increase of microorganisms' resistance to antibiotics over the past 50 years is one of the most important health threats of the 21st century[1 2 3].

Over the last decade, the development of nanotechnology and innovative approaches to the creation of new highly effective antibacterial drugs speedily progressed[4 5 6].

The most promising technology for the development of medicinal forms of antibacterial drugs is the creation of compounds with nanopolymers that perform the function of antibiotic carrier. The development of such polymers that would provide protection of antibiotics from adverse environmental conditions, stabilize their transportation to organs and tissues, and slow down destruction and removal from the body are topical[7 8 9] . At the same time, it is important that the created new antibacterial agents have good penetration into

[1] Munita J.M., Arias C.A. Mechanisms of Antibiotic Resistance. *Microbiol Spectr*. 2016. Vol.4, №2. P. 10. DOI: https://doi.org/10.1128/microbiolspec.VMBF.

[2] Padiyara P., Inoue H., Sprenger M. Global Governance Mechanisms to Address Antimicrobial Resistance. *Infectious Diseases*. 2018. Vol.11. P. 11. DOI: https://doi.org/10.1177/1178633718767887.

[3] International cooperation to improve access to and sustain effectiveness of antimicrobials / C. Ardal et al. *Lancet*. 2016. Vol.387. P. 296–307. DOI: https://doi.org/10.1016/S0140-6736(15)00470-5.

[4] Hemeg H. A. Nanomaterials for alternative antibacterial therapy. *International journal of nanomedicine*. 2017. Vol. *12*. P. 8211–8225. DOI:https://doi.org/10.2147/IJN.S132163.

[5] Nanobiotics against antimicrobial resistance: harnessing the power of nanoscale materials and technologies / N. Chakraborty et al. *Journal of nanobiotechnology*. 2022. №*20*. P. 375. DOI: https://doi.org/10.1186/s12951-022-01573-9.

[6] Gupta A., Landis R. F., Rotello V. M. Nanoparticle-Based Antimicrobials: Surface Functionality is Critical. *F1000Research*. 2016. Vol.*5*. P.364. DOI: https://doi.org/10.12688/f1000research.7595.1.

[7] Chen D. Wei-Chin Chen, Liu Shih-Jung. Nanofibers used for delivery of antimicrobial agents. *Nanomedicine (Lond).* 2015. № 10. P. 1959–1971.

[8] Polyphosphate Ester-Type Transporters Improve Antimicrobial Properties of Oxytetracycline / M. Kozak et al. *Antibiotics (Basel, Switzerland)*. 2023. №*12*. P. 616. DOI: https://doi.org/10.3390/antibiotics12030616.

[9] Martinho N., Damgé C., Reis C.P. Recent advances in drug delivery systems. *J. Biomater. Nanobiotechnol*. 2011.Vol. 2, №5. P. 510–526. DOI: https://doi.org/10.4236/jbnb.2011.225062.

the foci of the pathological process, which would contribute to the acceleration of recovery [10]. Ensuring the effectiveness of the targeted delivery of drugs leads to an increase of their concentration only in the affected area, minimizing the overall toxic effect on the body[11].

Therefore, the search for new antibiotics should be aimed at the development of drugs that have a changed molecular structure and purposefully act against the bacterial cells. The connection of antibiotic with a carrier for targeted transportation to the affected areas should provide the best possible experience for the treatment of patients.

1. PEGylation of drugs

Polymeric nanocarriers are one of the most promising, as they possess a number of specific physicochemical and biological properties that ensure their biocompatibility, biodegradability, the possibility of additional functionalization by special bioelements necessary for the delivery of pharmaceuticals in the body[12] [13].

Pharmaceutical forms, in which the active substance is conjugated with a polymer carrier, have lower toxicity, improved pharmacokinetic parameters and higher efficiency of therapeutic action[14] [15].

Polyethylene glycol (PEG) is the most promising carrier among polymers. It serves as an effective steric stabilizer for various dispersions, causing physicochemical transformation of the native molecule[16].

PEG is an ethylene oxide polymer with two terminal hydroxyl groups. The molecular weight of PEG can vary between 300 and 4000 Da, and

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[10] Radomska A., Leszczyszyn J., Radomski M. W. The Nanopharmacology and Nanotoxicology of Nanomaterials: New Opportunities and Challenges. *Advances in clinical and experimental medicine : official organ Wroclaw Medical University*. 2016.Vol. 25, № 1. P. 151–162. DOI: https://doi.org/10.17219/acem/60879.

[11] Characteristics of novel polyme rbased on pseudopolyamino acids GluLa-DPG-PEG600: binding of albumin,biocompatibility, biodistribution and potentialcrossing the blood-brain barrier in rats / B. O. Chekh et al. *The Ukrainian Biochemical Journal*. 2017. Vol. 89, № 4. P. 13–21. DOI: https://doi.org/10.15407/ubj89.04.013.

[12] Kozak M., Mitina N., Zaichenko A., Vlizlo V. Anionic Polyelectrolyte Hydrogel as an Adjuvant for Vaccine Development. *Scientia Pharmaceutica*. 2000.Vol. 88, № 4. P. 56. DOI: https://doi.org/10.3390/scipharm88040056.

[13] Synthesis and Properties of Phosphorus-Containing Pseudo-Poly(Amino Acid)s of Polyester Type Based on N-Derivatives of Glutaminic Acid /A. Stasiuk et al. *Chemistry & Chemical Technology*. 2022. Vol.16, №1. P. 51–58. DOI: https://doi.org/10.23939/chcht16.01.051.

[14] An influence of complexes of therapeutic antisense oligodeoxynucleotides with cationic polymers on cell respiration / M.R. Kozak et al. *Biopolym. Cell*. 2021. Vol 37, № 5. P. 357-368. DOI: http://dx.doi.org/10.7124/bc.000A61.

[15] Preperationandresearchofpropertiesofcombinedalginate/gelatinhydrogels /M.M.Bukartyk et al. *JournalofChemistryandTechnologies*. 2022. Vol. 30, № 1. P. 11-20.

[16] Фосфорновмісні поліестеретери похідних двоосновних природних α-амінокислот та поліетиленгліколів: патент на корисну модель 02108 Україна. 2021.

macromolecules built in a chain form both branched and linear stereochemical structures. The molecular weight of PEG and its stereochemical structure, as a rule, determine the basic properties of the future drug[17]. Polyethylene glycol as a drug carrier was first proposed for use in 1990. A characteristic feature of PEG is its good solubility in water. This is explained by the fact that the structure of hydrogen bonds in water does not change after the introduction of PEG, due to geometric similarity[18] [19]. At the same time, it facilitates overcoming of the cellular lipid membrane. PEG is biodegradable and biocompatible because it does not form toxic metabolites. PEG is also commercially available[20]. The process of connecting the native drug molecule with PEG is called PEGylation[21]. PEGylation is one of the most successful ways to improve drug delivery[22].

PEGylation of drugs promotes their protection against aggregation, opsonization and phagocytosis, extending the time of circulation in the body. An increase of the therapeutic effect of drugs is ensured due to better solubility of insoluble compounds in water and the accumulation of the drug in the target area[23]. At the same time, the toxic effect on the body is minimal[24] [25] [26].

PEGylation increases the size and molecular weight of conjugated biomolecules, increases their pharmacokinetics, pharmacodynamics, protection from enzymatic degradation, reduces the clearance in the kidneys,

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[17] Yadav D., Dewangan H. K. PEGYLATION: animportantapproachfor novel drug delivery system. *Journal of biomaterials science.Polymer edition*. 2021. Vol.*32*, №2. P. 266–280. DOI: https://doi.org/10.1080/09205063.2020.1825304.

[18] On the origin of the extremely different solubilities of polyethers in water /B.Ensing et al. *Nature communications.* 2019. № 10. P. 2893. DOI: https://doi.org/10.1038/s41467-019-10783-z.

[19] Lentz B. R. PEG as a tool to gain insight into membrane fusion. *European biophysics journal : EBJ*. 2007. Vol. 36. № 4-5. P. 315–326. DOI: https://doi.org/10.1007/s00249-006-0097-z.

[20] Mozar F.S., Chowdhury E.H. Impact of PEGylated Nanoparticles on Tumor Targeted Drug Delivery. *Current Pharmaceutical Design*. 2018. Vol. 24, № 28. P. 3283-3296. DOI: https://doi.org/ 10.2174/1381612824666180730161721 1.

[21] Zhang X., Wang H., Ma Z., Wu B. Effects of pharmaceutical PEGylation on drug metabolism and its clinical concerns. *Expert opinion on drug metabolism & toxicology*. 2014. №*10*. P.1691–1702. DOI: https://doi.org/10.1517/17425255.2014.967679.

[22] Kanikkannan N. Technologies to Improve the Solubility, Dissolution and Bioavailability of Poorly Soluble Drugs. *J Anal Pharm Res.* 2018. Vol. 7, № 1. P .44-50. DOI: https://doi.org/10.15406/japlr.2018.07.00198.

[23] PEGylation as a strategy for improving nanoparticle-based drug and gene delivery / J. S. Suk et al. *Advanced Drug Delivery Reviews*. 2016. Vol.99. Pt. A. P. 28-51. DOI: https://doi.org/10.1016/j.addr.2015.09.012.

[24] Гематологічні показники щурів за введення енрофлоксацину у складі полімеру / О. М Зеленіна та ін. *Біологія тварин*. 2020. Т. 22, № 1. С. 26-30.

[25] Зеленіна О.М., Влізло В.В. Кількість тромбоцитів крові тварин та їх індекси за введення різних форм антибіотика енрофлоксацину. *Міжнародна наукова конференція «Єдине здоров'я-2022»*. Київ, 2022. С. 65-66.

[26] Зеленіна О. М., Влізло В. В. Стан антиоксидантної системи у щурів за введення пегельованого енрофлоксацину. *Міжнародна науково-практична конференція «Сучасні епідемічні виклики в концепції «Єдине здоров'я»*, Тернопіль, 2021. С. 23.

and limits immunogenic and antigenic reactions. The kidneys filter substances based on molecular size. Therefore, PEGylated molecules, which have a higher molecular weight and a larger hydrodynamic radius than the parent molecule, are excreted much more slowly. This reduced speed increases the half-life of PEGylated molecules *in vivo*[27] [28].

PEGylated nanocarriers are attracting more and more attention due to the prolongation of circulation time in the body compared to free drugs. Accordingly, PEGylation of drugs increases their biological activity. This technology prolongs the period of «effective» half-life of the drug. PEGylation inhibits rapid drug release, reduces its allergenicity, toxicity and immunogenicity. Thereby, this new approach significantly increases the efficiency of patient treating[29].

One of the key challenges in drug delivery is the intracellular transport of drugs. This process is complicated by several factors: non-specificity of carriers or their inability to penetrate through the cell membrane; lysosomal hydrolysis of drugs (peptides, nucleic acids) that penetrated through the endocytic pathway. The solution to this problem is the use of so-called cell-penetrating peptides, first discovered in the human immunodeficiency virus. These peptides are able to transfer molecules of hydrophilic drugs attached to them through the cell membrane via peptide transduction. To avoid lysosomal destruction of cell-penetrating peptides, their covalent coupling with PEG molecules is possible, which shield them from binding to the cell membrane through the hydrazone residue. Hydrazone at pH values 5.0–6.0 is destroyed, thereby freeing the CPP (cell penetrating peptides) molecule from the PEG «screen» and enabling it to interact with the cell membrane. The controlled intracellular transport of drugs becomes possible[30] [31].

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[27] Milla P., Dosio F., Cattel L. PEGylation of proteins and liposomes: a powerful and flexible strategy to improve the drug delivery. *Current drug metabolism.* 2012. Vol. 13, № 1. P. 105–119. https://doi.org/10.2174/138920012798356934.

[28] Pegylated oleic acid: A promising amphiphilic polymer for nano-antibiotic delivery /C. A. Omolo et al. *European journal of pharmaceutics and biopharmaceutics:official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnike.* 2017.Vol. 112. P. 96–108. DOI: https://doi.org/10.1016/j.ejpb.2016.11.022.

[29] Facile Separation of PEGylated Liposomes Enabled by Anti-PEG scFv / W. Tang et al. *Nano Lett.* 2021. Vol. 21, № 23. P. 10107–10113. DOI: https://doi.org/10.1021/acs.nanolett.1c03946.

[30] Bareford L. M., Swaan P. W. Endocytic mechanisms for targeted drug delivery. *Advanced drug delivery reviews.* 2007. Vol. 59, № 8. P. 748–758. DOI:https://doi.org/10.1016/j.addr.2007.06.008.

[31] Torchilin V. P. Tat peptide-mediated intracellular delivery of pharmaceutical nanocarriers. *Advanced drug delivery reviews.* 2008. Vol. 60, № 4-5. P. 548–558.

2. Creation of PEGylated antibacterial preparations

Pharmaceutical nanotechnologies open up new opportunities for chemotherapy of infectious diseases. The ability of nanoparticles to deliver antibiotics to target cells, as well as to the foci of infection, allows increasing their efficiency and selectivity of action. Natural and synthetic polymers are used to form nanoscale carriers[32] [33].

Polymer conjugates of antibiotics provide decreased toxicity, increased solubility and prolonged activity of drugs[34]. Antibiotics can either be incorporated into nanoparticles during the polymerization process, or be bound covalently to the surface of nanoparticles after their formation. Numerous experiments have shown that the inclusion of various antibiotics in polymer nanoparticles leads to an increase in their specific activity. Consequently, polymers are effective tools for optimizing both the pharmacokinetics and pharmacodynamics of antibacterial drugs.

Antimicrobial peptides (AMPs) have therapeutic potential for localized infections. The introduction of PEGylated AMPs into the respiratory tract minimizes lung tissue toxicity while maintaining antimicrobial activity. PEGylation may be a tool of improving the pulmonary biocompatibility of AMPs intended for the treatment of pulmonary infections[35]. At the same time, oral administration of antibiotics with PEG improves their effect against pathogenic microorganisms[36].

The use of PEGylated antibacterial drugs based on nanogel showed low cytotoxicity and high bactericidal activity against gram-negative *E. coli* and *P. aeruginosa* and gram-positive *S. mutans* and *S. aureus* bacteria[37]. PEGylation has been established to improve the effectiveness of antibiotics

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[32] Kozak, M., Stasiuk, A., Vlizlo. V., Ostapiv, D., Bodnar Y., Kuzmina, N., Figurka. N., Nosova. N., Ostapiv, R., Kotsumbas. I., Varvarenko, S., &Samaryk V. Antimicrobial Action of Oxytetracycline in the Composition of Polyphosphate Ester Type Transporter that was presented at ICEID. Atlanta, Georgia, USA, 2022. P. 274.

[33] Nanomaterials-Based Combinatorial Therapy as a Strategy to Combat Antibiotic Resistance /A. León-Buitimea et al. *Antibiotics (Basel)*. 2022. Vol.11, № 6. P.794. DOI: https://doi.org/10.3390/antibiotics11060794

[34] Polymer-drug conjugates, PDEPT and PELT: basic principles for design and transfer from the laboratory to clinic / R. Duncan et al. *Journal of controlled release: official journal of the Controlled Release Society*. 2001. Vol. 74, № 1–3. P. 135–146. DOI: https://doi.org/10.1016/s0168-3659(01)00328-5.

[35] Morris C.J. Pegylation of antimicrobial peptides maintains the active peptide conformation, model membrane interactions, and antimicrobial activity while improving lung tissue biocompatibility following airway delivery. *Antimicrobial agents and chemotherapy*. 2012. Vol. 56, № 6. P. 3298–3308. DOI: https://doi.org/10.1128/AAC.06335-11.

[36] Influence of PEGylated porous silicon nanoparticles on permeation and efflux of an orally administered antibiotic / A. Raza et al. *Materials Today Advances*. 2022. Vol. 13. P .100–210. DOI: https://doi.org/10.1016/j.mtadv.2022.100210.

[37] Optimization of Cationic Nanogel PEGylation to Achieve Mammalian Cytocompatibility with Limited Loss of Gram-Negative Bactericidal Activity / G. Joann et al. *Biomacromolecules*. 2020. Vol. 21, № 4. P. 1528–1538. DOI: https://doi.org/10.1021/acs.biomac.0c00081.

used to treat infected wounds, preventing antibiotic resistance against the most common pathogenic microflora[38] [39].

Polymer conjugates with antibiotics show high activity against bacterial biofilms[40]. Biofilms are often impervious to antibiotics, which is a major cause of poor wound healing. Healing prognosis for wounds infected by biofilm-forming antibiotic resistant bacteria was worse than for infected by methicillin-resistant *Staphylococcus aureus* (*MRSA*), methicillin-resistant *S. epidermidis* (*MRSE*), and multidrug-resistant *Pseudomonas aeruginosa* (*MDR-PA*). Resistance interferes with initial treatment using standard antibiotics. Antibiotic resistance of MRSA, MRSE, and MDR-PA often results in acute infections developing into chronic wound infections. The water-soluble hydrophilic properties of low molecular weight (600 Da) branched polyethylenimine (600 Da BPEI) allow easy drug delivery and use in the environment as a topical agent for wound treatment. BPEI 600 Da was modified by polyethylene glycol to mitigate the toxicity issue. PEG-BPEI molecules reduce β-lactam resistance in MRSA, MRSE and MDR-PA bacteria and have the ability to dissolve established bacterial biofilms[41].

PEGylation can improve the effectiveness of antibiotics against resistant isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* isolated from patients[42].

The obtained nanoscale aqueous systems of conjugates of PEG-containing oligomeric carriers with the antibiotic chloramphenicol showed their high efficiency[43]. Levomycetin, immobilized on a PEG carrier, showed a high antimicrobial effect against microorganisms that were resistant to the action of free levomycetin. PEG-containing oligomer synthesized on the basis of

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[38] PEGylation of Polyethylenimine Lowers Acute Toxicity while Retaining Anti-Biofilm and β-Lactam Potentiation Properties against Antibiotic-Resistant Pathogens / A.K. Lam et al. *ACS Omega*. 2020.Vol.5, №40. P.26262–26270. DOI: https://doi.org/ 10.1021/acsomega.0c04111.

[39] Терапія післяопераційних ран армованими пов'язками на основі пектину та їх протимікробна дія / В. Влізло та ін. *Науковий вісник Львівського національного університету ветеринарної медицини та біотехнологій. Сер. Ветеринарні науки*. 2021. Т.23, №104. С. 41-46. DOI: https://doi.org/10.32718/nvlvet10407.

[40] Spellberg B. Trends in antimicrobial drug development: implications for the future. *Clinical infectious diseases.* 2004. Vol. 38, №9. P.1279–1286. DOI: https://doi.org/10.1086/420937.

[41] PEGylation of Polyethylenimine Lowers Acute Toxicity while Retaining Anti-Biofilm and β-Lactam Potentiation Properties against Antibiotic-Resistant Pathogens / A. K. Lam et al. *ACS omega*. 2020. Vol.5, №40. P. 26262–26270. DOI: https://doi.org/10.1021/acsomega.0c04111.

[42] Dual-Function Potentiation by PEG-BPEI Restores Activity of Carbapenems and Penicillins against Carbapenem-Resistant Enterobacteriaceae / H. Panlilio et al. *ACS Infect. Dis*. 2021.Vol.7, №6. P.1657–1665. DOI: https://doi.org/10.1021/acsinfecdis.0c00863

[43] Bhattacharya S., Sen D., Bhattacharjee C. *In vitro* antibacterial effect analysis of stabilized PEGylated allicin-containing extract from Allium sativum in conjugation with other antibiotics. *Process Biochemistry*. 2019. Vol. 87. P. 221–231. DOI: https://doi.org/10.1016/j.procbio.2019.09.025.

oligoperoxide with side epoxy groups is water-soluble. It is a surface-active substance capable of immobilizing the water-insoluble antibiotic chloramphenicol and forming nano-sized aqueous systems for its targeted delivery, which provide increased antimicrobial activity of the drug[44].

The study of antibacterial activity and cytotoxicity of PEGylated antibiotics of the group of aminoglycosides (gentamicin, kanamycin and neomycin) showed that their activity was decreased with an increase in the content of polyethylene glycol in these compounds. Thus, a ratio of aminoglycoside to PEG of 1 to 1 had significantly higher antimicrobial activity compared to compounds in which the ratio was 1 to 2. This decrease of antibacterial activity was found to be most prominent in the gram-positive bacteria *S. aureus*. On the other hand, PEGylation significantly reduced the cytotoxicity of antibiotics[45].

Nanoparticles made from two different polymers, namely poly(d,l-lactic-co-glycolic acid (PLGA) and methoxypoly(ethylene glycol)-b-poly(lactic-co-glycolic acid) (mPEG-PLGA). They were used to increase the efficiency of delivery of the antibiotic ofloxacin. Ofloxacin-mPEG-PLGA nanoparticles showed higher antibacterial activity. They also showed effective bacterial absorption, delayed release from the body, compared to free ofloxacin. PEGylation increased the permeability of the bacterial membrane, allowing mPEG-PLGA nanoparticles to accumulate inside the cells to a greater extent than pure PLGA nanoparticles. This nanoformulation also slowed the development of bacterial resistance compared to the free drug. It was found that the PEG-PLGA compound improved antibacterial activity compared to free ofloxacin. At the same time, pathogenic strains (*E. coli*, *P. aeruginosa*, *Proteus vulgaris*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *S. aureus*) were also more sensitive to PEGylated ofloxacin compared to its «pure» form[46].

The antibiotic ciprofloxacin was covalently attached to the chain end of poly(2-methyloxazoline) (PMOx), poly(2-ethyloxazoline) (PEtOx) and polyethylene glycol. The antimicrobial activity of these conjugates was tested against *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. It was found that the

[44] Нові поліетиленгліколькомісні олігомерні носії для доставки лікарських засобів / А.О. Рябцева. *Хімічні Каразінські читання – 2011:* зб. тез доп. ІІІ всеукраїнської наук. конф. студентів та аспірантів (Харків, 18-21 квітня 2011р.). Харків: ХНУ імені В. Н. Каразіна, 2011. С. 115.

[45] Evaluation of antimicrobial activity and cytotoxicity of pegylated aminoglycosides / Z. Ahmadiet al. *Journal of Bioactive and Compatible Polymers*. 2018. Vol. 33, №3. P. 295–309. DOI:https://doi.org/10.1177/0883911517739318.

[46] Marslin G. PEGylated ofloxacin nanoparticles render strong antibacterial activity against many clinically important human pathogens. *Colloids and surfaces B: Biointerfaces.* 2015. Vol. 132. P. 62– 70. DOI: https://doi.org/10.1016/j.colsurfb.2015.04.050.

antimicrobial activity of the conjugates increases in the order PMOx < PEtOx < PEG[47].

Improvement of the therapeutic potential of the antibiotic ciprofloxacin was achieved by encapsulation in polyethylene glycol-coated long-circulating sustained-release liposomes. The use of ciprofloxacin in liposomal form with PEG led to its delayed clearance and increased long-term concentration in blood and tissues. The therapeutic effectiveness of PEGylated liposomal ciprofloxacin against *Pseudomonas aeruginosa* has been proven[48].

A good antibacterial effect of tobramycin was shown against gram-negative bacteria, in particular *E. coli*, if the antibiotic was used in combination with PEG[49]. PEGylation of the antibiotic tobramycin led to improved antimicrobial activity against *Pseudomonas aeruginosa* compared to «pure» tobramycin[50].

3. Antimicrobial properties of PEGylated antibiotic enrofloxacin

The antibiotic enrofloxacin belongs to the third generation of fluoroquinolones, its use is effective in many types of antibiotic therapy**.** This antimicrobial preparation originates from 4-quinolone and contains a piperazine cycle and a fluorine atom, the presence of which significantly expands the spectrum of the antibacterial action. However, there is a concern about the emergence of enrofloxacin-resistant strains of bacteria, and therefore a negative effect may develop from its excessive use[51]. In addition, enrofloxacin is poorly soluble in water[52]. Therefore, it creates difficulties in obtaining optimal doses of the dissolved form and limits the bioavailability of the substance. It is hygroscopic and has a bitter taste, which additionally

[47] Conjugation of Ciprofloxacin with Poly(2-oxazoline)s and Polyethylene Glycol via End Groups / M. Schmidt et al. *Bioconjugate chemistry*. 2015. Vol. 26, № 9. P. 1950–1962. DOI: https://doi.org/10.1021/acs.bioconjchem.5b00393.

[48] Bakker-Woudenberg I.A. Improved efficacy of ciprofloxacin administered in polyethylene glycol-coated liposomes for treatment of Klebsiella pneumoniae pneumonia in rats. *Antimicrobial agents and chemotherapy*. 2001. Vol. 45, № 5. P. 1487–1492. DOI: https://doi.org/10.1128/AAC.45.5.1487-1492.2001.

[49] Jegatheeswaran S., Sundrarajan M. PEGylation of novel hydroxyapatite/PEG/Ag nanocomposite particles to improve its antibacterial efficacy. *Materials science & engineerin C Materials for biological applications*. 2015. Vol. 51. P. 174–181. DOI: https://doi.org/10.1016/j.msec.2015.02.012.

[50] PEGylation of Tobramycin Improves Mucus Penetration and Antimicrobial Activity against Pseudomonas aeruginosa Biofilms *in Vitro* / T. F. Bahamondez-Canas et al. *Molecular pharmaceutics.* 2018. Vol. 15, № 4. P. 1643–1652. DOI: https://doi.org/10.1021/acs.molpharmaceut.8b00011.

[51] Zinc(II) complexes of the second-generation quinolone antibacterial drug enrofloxacin: Structure and DNA or albumin interaction /A. Tarushi et al. *Bioorganic & medicinal chemistry*. 2010. Vol. 18, № 7. P. 2678–2685. DOI: https://doi.org/10.1016/j.bmc.2010.02.021.

[52] Trouchon T., Lefebvre S. A Review of Enrofloxacin for Veterinary Use. *Open Journal of Veterinary Medicine*. 2016. Vol. 6, № 2. P. 40-58. DOI: https://doi.org/10.4236/ojvm.2016.62006.

complicates the oral route of administration of the antibiotic enrofloxacin. In addition, it was established that enrofloxacin can cause a cytotoxic effect on the body[53]. It is indicated that some fluoroquinolone compounds have more specific antimicrobial activity than the pure substance[54]. Therefore, the search for new compounds of the antibiotic enrofloxacin with improved characteristics is urgent.

In particular, a polymer of polyethylene glycol with a molecular weight of 400 Da was synthesized. This polymer was named PEG-400[55]. The antibiotic enrofloxacin with a purity of 99.5%[56] was used for the formation of the PEGylated antibiotic enrofloxacin. During the synthesis of the PEGylated antibiotic enrofloxacin, the authors took into account the fact that the molecule of the antibiotic enrofloxacin contains reactive carboxyl groups in its structure. The molecules of the antibiotic enrofloxacin were attached[57] to the ends of the PEG-400 polyoxyethylene hydrophilic chain. High-performance liquid chromatography has shown that the purity of the PEGylated antibiotic enrofloxacin is 98–99%[58].

The synthesized model compound of enrofloxacin with PEG-400, which are covalently connected to each other, showed high antibacterial activity[59 60].

This is explained by the fact that PEG is able to affect the permeability of membranes, increasing the absorption of the antibacterial drug by cells[55].

Conducted studies of the antimicrobial activity of the PEGylated antibiotic enrofloxacin and the traditional antibiotic enrofloxacin showed the difference in their effect on reference museum strains of microorganisms *Escherichia coli* ATCC 11105. Thus, the minimum inhibitory concentration (MIC) of traditional antibiotic enrofloxacin against *Escherichia coli* ATCC 11105 was

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[53] Luan Y., Chen K., Zhao J., Cheng L. Comparative Study on Synergistic Toxicity of Enrofloxacin Combined with Three Antibiotics on Proliferation of THLE-2 Cell. *Antibiotics.* 2022.Vol. 11. P. 394. DOI: https://doi.org/10.3390/antibiotics11030394.

[54] Synthesis and antitubercular activity of palladium and platinum complexes with fluoroquinolones / L. M. Vieira et al. *European journal of medicinal chemistry*. 2009. Vol. 44, №10. P. 4107–4111. DOI: https://doi.org/10.1016/j.ejmech.2009.05.001.

[55] Синтез і дослідження антибактеріальної активності пегільованих енрофлоксацинів / Дронь І. А. та ін. *Вісник Національного університету «Львівська політехніка». Хімія, технологія речовин та їх застосування*. 2018. № 886. С. 47-51.

[56] PEGylation of antibiotic enrofloxacin and its effects on the state of the antioxidant system in rats / O. M. Zelenina et al. *Ukrainian Journal of Ecology*. 2021. Vol. 11, № 1. P. 202-208.

[57] Спосіб посилення антимікробної дії енрофлоксацину: патент на корисну модель 152046 Україна. 2022.

[58] PEGylation of enrofloxacin reduces minimum inhibitory concentrations and hepatoxic effects in rats / V. Vlizlo et al. *Medical Biodefense Conference.* Munich, 2021. P.103-104.

[59] Antimicrobial activity of the PEGylated antibiotic enrofloxacin and its functional and structural effect on the liver in rats / O. M. Zelenina et al. *Journal of Applied Pharmaceutical Science*. 2022. Vol.12, № 6. P. 68–75.

[60] Chakrabarty B., Ghoshal A.K., Purkait M.K. Effect of molecular weight of PEG on membrane morphology and transport properties. *Journal of Membrane Scienc.* 2008.Vol. 309, №1-2. P. 209–221. DOI: https://doi.org/10.1016/j.memsci.2007.10.027.

0.31 μg/ml. At the same time, the MIC of PEGylated antibiotic enrofloxacin against the museum reference strain of *Escherichia coli* ATCC 11105 was two-fold lower (0.15 μg/ml) than MIC of traditional antibiotic enrofloxacin. consequently, PEGylation of the antibiotic enrofloxacin leads to the improvement of its antimicrobial properties against *Escherichia coli*[61].

The MIC of the antibiotic enrofloxacin both in the traditional substance and in the PEGylated forms against reference museum strains of microorganisms *Staphylococcus aureus* ATCC 6538P was 0.31 μg/ml.

No differences were established between the minimum inhibitory concentration of the antibiotic enrofloxacin in the traditional substance and in the PEGylated form against field strains of gram-positive and gram-negative microorganisms.

Thus, the MIC level of the antibiotic enrofloxacin both in the traditional substance and in the PEGylated forms against *Staphylococcus epidermidis* was 1.0 μg/ml and 2.0 μg/ml against *Enterobacter*. The obtained results showed that the binding of PEG-400 to the antibiotic enrofloxacin, or the PEGylation of enrofloxacin, does not change the level of its bacteriostatic activity against field strains of gram-positive (*Staphylococcus epidermidis*) and gram-negative (*Enterobacter*) microorganisms[59].

PEGylation of the antibiotic enrofloxacin increases the size and molecular weight of conjugated biomolecules, increases their solubility in water, protects against enzymatic degradation, leads to a decrease in hepatotoxicity and nephrotoxicity, limits immunogenic and antigenic reactions, does not affect hematopoiesis and hemostasis[62] [63] [64].

CONCLUSIONS

The synthesis of the enrofloxacin antibiotic with the PEG-400 polymer (PEGylation of the antibiotic enrofloxacin) showed improved antibacterial activity, which is associated with the ability of PEG to affect the permeability of membranes, ensuring the absorption of the active substance by cells.

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[61] Зеленіна О.М., Влізло В.В. Вплив пегелювання антибіотика енрофлоксацину на його антимікробну активність. *Актуальні аспекти розвитку науки і освіти: зб. матеріалів II Міжнар. наук.-практ. конф.наук.-педагог.працівників та молодих науковців* (Одеса, 08-09 грудня 2022 р.). Одеса, 2022. С. 96-99.

[62] Активність трансаміназ і вміст білірубіну у крові щурів за введення антибіотика енрофлоксацину, ПЕГ-400 та їх комплексу / О. М. Зеленіна та ін. *Наукові доповіді НУБіП України*. 2020. № 4.

[63] Зеленіна О. М. Пегелювання антибіотика енрофлоксацину та його вплив на активність індикаторних ензимів і структуру печінки. *Аграрний вісник Причорномор'я*: зб. наук. пр. Одеса:ОДАУ, 2021. С. 31-34.

[64] Функціональний стан нирок у тварин за застосування пегельованого антибіотика енрофлоксацину / О. М. Зеленіна та ін. *Сучасні методи діагностики, лікування та профілактики у ветеринарній медицині*: тези доповідей II конференції (18-19 листопада 2021 р., Одеса). Одеса, 2021. С. 56-57.

PEGylation of the antibiotic enrofloxacin increases the size and molecular weight of conjugated biomolecules, increases their water solubility and protection against enzymatic degradation, reduces hepatotoxicity and nephrotoxicity, limits immunogenic and antigenic reactions, and does not affect hematopoiesis and hemostasis.

SUMMARY

Recently, bacterial strains resistant to the antibiotic enrofloxacin appeared which indicates the development of antibiotic resistance. Enrofloxacin is poorly soluble in water. This physical property creates difficulties in obtaining optimal doses of the dissolved form and limits the bioavailability of the substance. It is hygroscopic and has a bitter taste, which reduces the possibility of oral use. In addition, enrofloxacin can cause a cytotoxic effect on the body. Therefore, the search for new compounds and forms of the antibiotic enrofloxacin with improved characteristics is topical. PEGylation is one of the most successful ways to improve drug delivery. PEG is biodegradable and biocompatible, as it does not form toxic metabolites, and is commercially available. The development of a model compound of enrofloxacin with a PEG-400 polymer (PEGylation of the antibiotic enrofloxacin) by covalent connection showed improved antibacterial activity, which is associated with the ability of PEG to affect the permeability of membranes, ensuring the absorption of the active substance by cells. PEGylation of the antibiotic enrofloxacin increases the size and molecular weight of conjugated biomolecules, increases their water solubility and protection against enzymatic degradation, reduces hepatotoxicity and nephrotoxicity, limits immunogenic and antigenic reactions, does not affect hematopoiesis and hemostasis.

Consequently, The PEGylation of the antibiotic enrofloxacin helps to increase the therapeutic effect providing good solubility, increasing the accumulation of the active substance in the pathological area, and reducing the toxic effect on the body.

Bibliography

1. Munita J.M., Arias C.A. Mechanisms of Antibiotic Resistance. *Microbiol Spectr*. 2016. Vol. 4, № 2. P. 10. DOI: https://doi.org/10.1128/microbiolspec.VMBF.

2. Padiyara P., Inoue H., Sprenger M. Global Governance Mechanisms to Address Antimicrobial Resistance. *Infectious Diseases*. 2018. Vol. 11. P. 11. DOI: https://doi.org/10.1177/1178633718767887.

3. International cooperation to improve access to and sustain effectiveness of antimicrobials / C. Ardal et al. *Lancet.* 2016. Vol.387. P. 296–307. DOI: https://doi.org/10.1016/S0140-6736(15)00470-5.

4. Hemeg H. A. Nanomaterials for alternative antibacterial therapy. *International journal of nanomedicine*. 2017. Vol. 12. P. 8211–8225. DOI: https://doi.org/10.2147/IJN.S132163.

5. Nanobiotics against antimicrobial resistance: harnessing the power of nanoscale materials and technologies / N. Chakraborty et al. *Journal of nanobiotechnology*. 2022. № 20. P. 375. DOI: https://doi.org/10.1186/s12951-022-01573-9.

6. Gupta A., Landis R. F., Rotello V. M. Nanoparticle-Based Antimicrobials: Surface Functionality is Critical. *F1000Research*. 2016. Vol.5. P.364. DOI: https://doi.org/10.12688/f1000research.7595.1.

7. Chen D. Wei-Chin Chen, Liu Shih-Jung. Nanofibers used for delivery of antimicrobial agents. *Nanomedicine (Lond).* 2015. № 10. P. 1959–1971.

8. Polyphosphate Ester-Type Transporters Improve Antimicrobial Properties of Oxytetracycline / M. Kozak et al. *Antibiotics (Basel, Switzerland)*. 2023. № 12. P. 616. DOI: https://doi.org/10.3390/ antibiotics12030616.

9. Martinho N., Damgé C., Reis C.P. Recent advances in drug delivery systems. *J. Biomater. Nanobiotechnol*. 2011.Vol. 2, № 5. P. 510–526. DOI: https://doi.org/10.4236/jbnb.2011.225062.

10. Radomska A., Leszczyszyn J., Radomski M. W. The Nanopharmacology and Nanotoxicology of Nanomaterials: New Opportunities and Challenges. *Advances in clinical and experimental medicine : official organ Wroclaw Medical University*. 2016.Vol. 25, № 1. P. 151–162. DOI: https://doi.org/10.17219/acem/60879.

11. Characteristics of novel polyme rbased on pseudopolyamino acids GluLa-DPG-PEG600: binding of albumin,biocompatibility, biodistribution and potentialcrossing the blood-brain barrier in rats / B. O. Chekh et al. *The Ukrainian Biochemical Journal*. 2017. Vol.89, № 4. P. 13–21. DOI: https://doi.org/10.15407/ubj89.04.013.

12. Kozak M., Mitina N., Zaichenko A., Vlizlo V. Anionic Polyelectrolyte Hydrogel as an Adjuvant for Vaccine Development. *Scientia Pharmaceutica*. 2000.Vol. 88, № 4. P. 56. DOI: https://doi.org/10.3390/scipharm88040056.

13. Synthesis and Properties of Phosphorus-Containing Pseudo-Poly(Amino Acid)s of Polyester Type Based on N-Derivatives of Glutaminic Acid /A. Stasiuk et al. *Chemistry & Chemical Technology*. 2022. Vol. 16, № 1. P. 51–58. DOI: https://doi.org/10.23939/chcht16.01.051.

14. An influence of complexes of therapeutic antisense oligodeoxynucleotides with cationic polymers on cell respiration / M.R. Kozak et al. *Biopolym. Cell*. 2021. Vol 37, № 5. P. 357-368. DOI: http://dx.doi.org/10.7124/bc.000A61.

15. Preperation and research of properties of combine dalginate /gelatinhy drogels / M.M. Bukartyk et al. *Journal of Chemistry and Technologies*. 2022. Vol. 30, № 1. P. 11-20.

16. Фосфорновмісні поліестеретери похідних двоосновних природних α-амінокислот та поліетиленгліколів: патент на корисну модель 02108 Україна. 2021.

17. Yadav D., Dewangan H. K. PEGYLATION: animportantapproachfor novel drug delivery system. *Journal of biomaterials science.Polymer edition*. 2021. Vol. 32, № 2. P. 266–280. DOI: https://doi.org/10.1080/09205063.2020.1825304.

18. On the origin of the extremely different solubilities of polyethers in water /B.Ensing et al. *Nature communications*. 2019. № 10. P. 2893. DOI: https://doi.org/10.1038/s41467-019-10783-z.

19. Lentz B. R. PEG as a tool to gain insight into membrane fusion. *European biophysics journal : EBJ*. 2007. Vol.*36*. №4-5. P. 315–326. DOI: https://doi.org/10.1007/s00249-006-0097-z.

20. Mozar F.S., Chowdhury E.H. Impact of PEGylated Nanoparticles on Tumor Targeted Drug Delivery. *Current Pharmaceutical Design*. 2018. Vol. 24, № 28. P. 3283-3296. DOI: https://doi.org/10.2174/1381612824666180730161721 1.

21. Zhang X., Wang H., Ma Z., Wu B. Effects of pharmaceutical PEGylation on drug metabolism and its clinical concerns. *Expert opinion on drug metabolism & toxicology*. 2014. № 10. P. 1691–1702. DOI: https://doi.org/10.1517/17425255.2014.967679.

22. Kanikkannan N. Technologies to Improve the Solubility, Dissolution and Bioavailability of Poorly Soluble Drugs. *J Anal Pharm Res.* 2018. Vol. 7, №1. P.44-50. DOI: https://doi.org/10.15406/japlr.2018.07.00198.

23. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery /J. S. Suk et al. *Advanced Drug Delivery Reviews*. 2016. Vol. 99. Pt. A. P.28-51. DOI: https://doi.org/10.1016/j.addr.2015.09.012.

24. Гематологічні показники щурів за введення енрофлоксацину у складі полімеру / О. М Зеленіна та ін. *Біологія тварин*. 2020. Т. 22, № 1. С. 26-30.

25. Зеленіна О.М., Влізло В.В. Кількість тромбоцитів крові тварин та їх індекси за введення різних форм антибіотика енрофлоксацину. *Міжнародна наукова конференція «Єдине здоров'я-2022»*. Київ, 2022. С. 65-66.

26. Зеленіна О. М., Влізло В. В. Стан антиоксидантної системи у щурів за введення пегельованого енрофлоксацину. *Міжнародна науково-практична конференція «Сучасні епідемічні виклики в концепції «Єдине здоров'я»*, Тернопіль, 2021. С. 23.

27. Milla P., Dosio F., Cattel L. PEGylation of proteins and liposomes: a powerful and flexible strategy to improve the drug delivery. *Current drug metabolism*. 2012. Vol. 13, № 1. P. 105–119. https://doi.org/10.2174/138920012798356934.

28. Pegylated oleic acid: A promising amphiphilic polymer for nano-antibiotic delivery /C. A. Omolo et al. *European journal of pharmaceutics and*

biopharmaceutics:official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnike. 2017. Vol. 112. P. 96–108. DOI: https://doi.org/10.1016/j.ejpb.2016.11.022.

29. Facile Separation of PEGylated Liposomes Enabled by Anti-PEG scFv /W.Tang et al. *Nano Lett*. 2021. Vol. 21, № 23. P. 10107–10113. DOI: https://doi.org/10.1021/acs.nanolett.1c03946.

30. Bareford L. M., Swaan P. W. Endocytic mechanisms for targeted drug delivery. *Advanced drug delivery reviews*. 2007. Vol. 59, № 8. P. 748–758. DOI: https://doi.org/10.1016/j.addr.2007.06.008.

31. Torchilin V. P. Tat peptide-mediated intracellular delivery of pharmaceutical nanocarriers. *Advanced drug delivery reviews*. 2008. Vol. 60, № 4-5. P. 548–558.

32. Kozak, M., Stasiuk, A., Vlizlo. V., Ostapiv, D., Bodnar Y., Kuzmina, N., Figurka. N., Nosova. N., Ostapiv, R., Kotsumbas. I., Varvarenko, S., &Samaryk V. Antimicrobial Action of Oxytetracycline in the Composition of Polyphosphate Ester Type Transporter that was presented at ICEID. Atlanta, Georgia, USA, 2022. P. 274.

33. Nanomaterials-Based Combinatorial Therapy as a Strategy to Combat Antibiotic Resistance /A. León-Buitimea et al. *Antibiotics (Basel)*. 2022. Vol. 11, № 6. P. 794. DOI: https://doi.org/10.3390/antibiotics11060794

34. Polymer-drug conjugates, PDEPT and PELT: basic principles for design and transfer from the laboratory to clinic / R. Duncan et al. *Journal of controlled release: official journal of the Controlled Release Society*. 2001. Vol. 74, №1–3. P. 135–146. DOI: https://doi.org/10.1016/s0168-3659(01)00328-5.

35. Morris C.J. Pegylation of antimicrobial peptides maintains the active peptide conformation, model membrane interactions, and antimicrobial activity while improving lung tissue biocompatibility following airway delivery. *Antimicrobial agents and chemotherapy*. 2012. Vol. 56, № 6. P. 3298–3308. DOI: https://doi.org/10.1128/AAC.06335-11.

36. Influence of PEGylated porous silicon nanoparticles on permeation and efflux of an orally administered antibiotic / A. Raza et al. *Materials Today Advances*. 2022. Vol. 13. P. 100–210. DOI: https://doi.org/10.1016/ j.mtadv.2022.100210.

37. Optimization of Cationic Nanogel PEGylation to Achieve Mammalian Cytocompatibility with Limited Loss of Gram-Negative Bactericidal Activity / G. Joann et al. *Biomacromolecules*. 2020. Vol.21, №4. P.1528–1538. DOI: https://doi.org/10.1021/acs.biomac.0c00081.

38. PEGylation of Polyethylenimine Lowers Acute Toxicity while Retaining Anti-Biofilm and β-Lactam Potentiation Properties against Antibiotic-Resistant Pathogens / A.K. Lam et al. *ACS Omega*. 2020.Vol. 5, № 40. P. 26262–26270. DOI: https://doi.org/ 10.1021/acsomega.0c04111.

39. Терапія післяопераційних ран армованими пов'язками на основі пектину та їх протимікробна дія / В. Влізло та ін. *Науковий вісник*

Львівського національного університету ветеринарної медицини та біотехнологій. Сер. Ветеринарні науки. 2021. Т. 23, № 104. С. 41-46. DOI: https://doi.org/10.32718/nvlvet10407.

40. Spellberg B. Trends in antimicrobial drug development: implications for the future. *Clinical infectious diseases.* 2004. Vol. 38, № 9. P. 1279–1286. DOI: https://doi.org/10.1086/420937.

41. PEGylation of Polyethylenimine Lowers Acute Toxicity while Retaining Anti-Biofilm and β-Lactam Potentiation Properties against Antibiotic-Resistant Pathogens / A. K. Lam et al. *ACS omega*. 2020. Vol. 5, № 40. P. 26262–26270. DOI: https://doi.org/10.1021/acsomega.0c04111.

42. Dual-Function Potentiation by PEG-BPEI Restores Activity of Carbapenems and Penicillins against Carbapenem-Resistant Enterobacteriaceae / H. Panlilio et al. *ACS Infect. Dis.* 2021. Vol. 7, № 6. P. 1657–1665. DOI: https://doi.org/10.1021/acsinfecdis.0c00863.

43. Bhattacharya S., Sen D., Bhattacharjee C. *In vitro* antibacterial effect analysis of stabilized PEGylated allicin-containing extract from Allium sativum in conjugation with other antibiotics. *Process Biochemistry*. 2019. Vol. 87. P. 221–231. DOI: https://doi.org/10.1016/j.procbio.2019.09.025.

44. Нові поліетиленгліколькомісні олігомерні носії для доставки лікарських засобів /А.О Рябцева . *Хімічні Каразінські читання – 2011:* зб. тез доп. III всеукраїнської наук. конф. студентів та аспірантів (Харків, 18-21 квітня 2011 р.). Харків: ХНУ імені В. Н. Каразіна, 2011. С. 115.

45. Evaluation of antimicrobial activity and cytotoxicity of pegylated aminoglycosides / Z. Ahmadiet al. *Journal of Bioactive and Compatible Polymers*. 2018. Vol. 33, № 3. P. 295–309. DOI: https://doi.org/10.1177/0883911517739318.

46. Marslin G. PEGylated ofloxacin nanoparticles render strong antibacterial activity against many clinically important human pathogens. *Colloids and surfaces B: Biointerfaces.* 2015. Vol. 132. P. 62–70. DOI: https://doi.org/10.1016/j.colsurfb.2015.04.050.

47. Conjugation of Ciprofloxacin with Poly(2-oxazoline)s and Polyethylene Glycol via End Groups / M. Schmidt et al. *Bioconjugate chemistry*. 2015. Vol. 26, № 9. P. 1950–1962. DOI: https://doi.org/10.1021/acs.bioconjchem.5b00393.

48. Bakker-Woudenberg I.A. Improved efficacy of ciprofloxacin administered in polyethylene glycol-coated liposomes for treatment of Klebsiella pneumoniae pneumonia in rats. *Antimicrobial agents and chemotherapy*. 2001. Vol. 45, № 5. P. 1487–1492. DOI: https://doi.org/10.1128/AAC.45.5.1487-1492.2001.

49. Jegatheeswaran S., Sundrarajan M. PEGylation of novel hydroxyapatite/PEG/Ag nanocomposite particles to improve its antibacterial efficacy. *Materials science & engineerin C Materials for biological applications*. 2015. Vol. 51. P. 174–181. DOI: https://doi.org/10.1016/j.msec.2015.02.012.

50. PEGylation of Tobramycin Improves Mucus Penetration and Antimicrobial Activity against Pseudomonas aeruginosa Biofilms *in Vitro* / T. F. Bahamondez-Canas et al. *Molecular pharmaceutics*. 2018. Vol. 15, № 4. P.1643–1652. DOI: https://doi.org/10.1021/acs.molpharmaceut.8b00011.

51. Zinc(II) complexes of the second-generation quinolone antibacterial drug enrofloxacin: Structure and DNA or albumin interaction /A. Tarushi et al. *Bioorganic & medicinal chemistry*. 2010. Vol. 18, № 7. P. 2678–2685. DOI: https://doi.org/10.1016/j.bmc.2010.02.021.

52. Trouchon T., Lefebvre S. A Review of Enrofloxacin for Veterinary Use. *Open Journal of Veterinary Medicine*. 2016. Vol. 6, № 2. P. 40-58. DOI: https://doi.org/10.4236/ojvm.2016.62006.

53. Luan Y., Chen K., Zhao J., Cheng L. Comparative Study on Synergistic Toxicity of Enrofloxacin Combined with Three Antibiotics on Proliferation of THLE-2 Cell. *Antibiotics.* 2022.Vol. 11. P. 394. DOI: https://doi.org/10.3390/antibiotics11030394.

54. Synthesis and antitubercular activity of palladium and platinum complexes with fluoroquinolones / L. M. Vieira et al. *European journal of medicinal chemistry*. 2009. Vol. 44, № 10. P. 4107–4111. DOI: https://doi.org/10.1016/j.ejmech.2009.05.001.

55. Синтез і дослідження антибактеріальної активності пегільованих енрофлоксацинів / Дронь І. А. та ін. *Вісник Національного університету «Львівська політехніка». Хімія, технологія речовин та їх застосування*. 2018. № 886. С. 47-51.

56. PEGylation of antibiotic enrofloxacin and its effects on the state of the antioxidant system in rats / O. M. Zelenina et al. *Ukrainian Journal of Ecology*. 2021. Vol. 11, № 1. P. 202-208.

57. Спосіб посилення антимікробної дії енрофлоксацину: патент на корисну модель 152046 Україна. 2022.

58. PEGylation of enrofloxacin reduces minimum inhibitory concentrations and hepatoxic effects in rats / V. Vlizlo et al. *Medical Biodefense Conference.* Munich, 2021. P. 103-104.

59. Antimicrobial activity of the PEGylated antibiotic enrofloxacin and its functional and structural effect on the liver in rats / O. M. Zelenina et al. *Journal of Applied Pharmaceutical Science*. 2022. Vol. 12, № 6. P. 68–75.

60. Chakrabarty B., Ghoshal A.K., Purkait M.K. Effect of molecular weight of PEG on membrane morphology and transport properties. *Journal of Membrane Scienc.* 2008. Vol. 309, № 1-2. P. 209–221. DOI: https://doi.org/10.1016/j.memsci.2007.10.027.

61. Зеленіна О.М., Влізло В.В. Вплив пегелювання антибіотика енрофлоксацину на його антимікробну активність. *Актуальні аспекти розвитку науки і освіти: зб. матеріалів ІІ Міжнар. наук.-практ. конф.наук.-педагог.працівників та молодих науковців* (Одеса, 08-09 грудня 2022 р.). Одеса, 2022. С. 96-99.

62. Активність трансаміназ і вміст білірубіну у крові щурів за введення антибіотика енрофлоксацину, ПЕГ-400 та їх комплексу / О. М. Зеленіна та ін. *Наукові доповіді НУБіП України*. 2020. № 4.

63. Зеленіна О. М. Пегелювання антибіотика енрофлоксацину та його вплив на активність індикаторних ензимів і структуру печінки. *Аграрний вісник Причорномор'я*: зб. наук. пр. Одеса: ОДАУ, 2021. С. 31-34.

64. Функціональний стан нирок у тварин за застосування пегельованого антибіотика енрофлоксацину / О. М. Зеленіна та ін. *Сучасні методи діагностики, лікування та профілактики у ветеринарній медицині*: тези доповідей ІІ конференції (18-19 листопада 2021 р., Одеса). Одеса, 2021. С. 56-57.

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