VII Annual scientific and practical conference of Farmak's School of young scientists with international involvement «Science and modern pharmaceutical manufacturing»

(NOVEMBER 21, 2019, «FARMAK» JSC, KYIV, UKRAINE)

INTRODUCTION

BRIDGE BETWEEN INDUSTRIAL AND BASIC SCIENCE IN THE LIGHT OF VII ANNUAL SCIENTIFIC AND PRACTICAL CONFERENCE OF FARMAK'S SCHOOL OF YOUNG SCIENTISTS WITH INTERNATIONAL INVOLVEMENT «SCIENCE AND MODERN PHARMACEUTICAL MANUFACTURING»

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Pharmaceutical science is dealing with research and development of medicinal products for human and animal use.

School of young scientists (SYS) of «Farmak» JSC was established in 2006 under patronage of the founder of «Farmak» JSC Dr. Filya Zhebrovska and became an important informal platform for the knowledge and experience exchange between specialists of the R&D department. How to implement the guidelines of European Medicines Agency (EMA) and appropriate good practices in the development of new medicines represented the key questions for discussion at SYS. Young employees of Farmak had a chance to share their observations, findings, conclusions, questions and even problems searching for their solution.

SYS was becoming more and more popular and the number of its participants consecutively increased in the last decade. Moreover, Ukrainian scientists from universities and academies started to attend these meetings.

Later, seminars were grown up to the 1st Scientific and practical conference of Farmak's SYS «Science and modern pharmaceutical manufacturing», which took place in 2013 in Kyiv. After that, Farmak held such conferences annually. Delegates discuss how to develop complex generics and biosimilar drugs in accordance with the EMA and Food and Drug Administration (FDA) guidelines, conduct bioequivalence studies, validate processes, establish pharmaceutical manufacturing, ensure proper quality control, etc.

From year to year, this platform has become increasingly popular, attracting not only Farmak employees, but also young scientists and students from recognized pharmaceutical and biotechnological universities of Ukraine. Some young scientists of Farmak defended their scientific dissertations. To date, Farmak has 5 Doctors of Sciences and 44 Candidates of Sciences.

Dr. Andrew Goy, Technical Director of «Farmak» JSC, proposed organizing an international SYS conference in Farmak (Kyiv, Ukraine).

Below you could find abstracts submitted to VIIth Annual scientific and practical conference of Farmak's SYS with international involvement «Science and modern pharmaceutical manufacturing» which was held on November 21, 2019, at «Farmak» JSC (Kyiv, Ukraine) from Austria, Hungary, Ireland, Italy, Germany, UK, USA as well as young scientists and students from Ukraine.

Hopefully, forthcoming exchange of knowledge and experience in the field of basic and industrial (pharmaceutical) science will promote the further efficient development of expertise and ability to solve complicated tasks in modern drug development. It could also assist to develop productive partnership between international basic/applied science and pharmaceutical industry.

PLENARY SESSION ABSTRACTS

LIGHT-TRIGGERED REDOX ACTIVITY OF $GdYVO_4:Eu^{3+}$ NANOPARTICLES

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Despite the better understanding of tumor biology and improved diagnostic devices observed in last years, cancer remains a major public health problem worldwide being the second leading cause of death. According to Word Health Organization report, cancer is responsible for an estimated 9.6 million deaths in 2018. Unfortunately, conventional anti-cancer treatments have many disadvantages including high toxicity, effects on healthy cells, tissues and organs and variety of side effects, etc.

It is now generally accepted that the oxidative stress caused by imbalance on Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) production and scavenging is closely related to a broad spectrum of diseases including cancer. ROS mainly including superoxide anions ($O_2^{\cdot}$), hydroxyl radicals (• OH), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) as well as RNS, like nitric oxide (•NO), nitrogen dioxide radical (•NOO), and peroxynitrite anion ($ONOO^-$) are known to have double-edged sword properties in the determination on cell fate. ROS and RNS are continuously generated in small amounts in normal cells. Endogenously produced ROS and RNS are involved in various biological functions such as blood pressure modulation, immune system control, production of energy, cellular signaling and growth, synthesis of various biological compounds, *etc*. Intracellular ROS and RNS levels is regulated via a cell antioxidant defense system (superoxide dismutase, catalase, glutathione peroxidases, peroxiredoxins and other enzymes and antioxidants) converting excess of ROS and RNS into less reactive species (O_2 or H_2O, for instance). When the action of cell antioxidants become depleted, the level of ROS and RNS increases causing oxidative stress through the oxidation of lipids (lipid peroxidation), protein and DNA damage, *etc*. Interestingly, for survival, proliferation, and metastasis, cancer cells maintain ROS and RNS levels close to the cell-death threshold, whereas normal cells maintain redox homeostasis with low level of basal ROS and RNS. Therefore, cancer cells are more vulnerable to further oxidative stress induced by ROS generative agent, as well as to ROS and RNS level reduction by the antioxidants. That is why both strategies have been currently proposed for cancer treatment.

One of the prospective approaches in cancer and other diseases treatment is used redox-active nanomaterials, which can affect the ROS production/scavenging balance in living cells. In this report, we show the prospectivity of $GdYVO_4:Eu^{3+}$ nanoparticles (VNPs) as unique redox material with a dual (pro- and antioxidant) action. Redox properties of $GdYVO_4:Eu^{3+}$ NPs have been studied in water solutions and lipid suspensions, which mimic biological environment, and in a cell culture at UV- irradiation and during dark storage. It was shown that under UV irradiation VNPs increase sufficiently the ROS amount in the water solutions and lipid suspensions due to NPs photocatalytic properties. Moreover, pro-oxidant activity of pre-irradiated VNPs was revealed in water solutions, lipid suspension and cell culture without any external stimuli (in the darkness). This pro-oxidant activity is shown to be associated with superoxide ($O_2^{\cdot}$) and hydroxyl radical (•OH)

generation and depended on the size of NPs and, consequently, concentration of surface defects, such as oxygen vacancies V_O. At the same time, VNPs, which were kept in darkness for about 4 days, demonstrate strong anti-oxidant activity associated with ROS scavenging. Possible mechanisms of dark ROS generation and scavenging in $GdYVO_4:Eu^{3+}$ NPs are considered.

INFLUENZA UNIVERSAL VACCINES

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Current influenza virus vaccines necessitate annual updates due to antigenic shift of circulating seasonal virus strains, and do not provide protection against potential pandemic influenza virus strains. Here I will discuss a universal influenza virus vaccine approach, which has the potential to provide protection against any possible influenza virus strain, alleviating annual influenza virus vaccination. This approach is based on sequential vaccination with antigens containing chimeric influenza virus hemagglutinins (HA) that share the conserved HA stalk domain but have an antigenically highly diverse HA head domain. By virtue of their antigenic characteristics, such vaccines continuously boost antibodies against the conserved subdominant HA stalk, overcoming the HA head immune dominance. High titer induction of HA stalk antibodies using chimeric HA vaccines provides protection against challenge with multiple influenza virus strains and subtypes in mouse and ferret animal models. Such vaccination approaches have also been proven immunogenic in human volunteers.

MITIGATION STRATEGIES AGAINST EMERGING AND ZOONOTIC VIRAL DISEASES

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Introduction

This lecture will discuss mitigation strategies against emerging and/or zoonotic diseases like Rift Valley Fever (RVF), Highly Pathogenic Avian Influenza (HPAI), and African Swine Fever (ASF). An emerging infectious disease is a disease that has newly appeared or the incidence has increased in the past 20 years and is expected to increase further in the near future. Many emerging diseases are also zoonotic, i.e. they spread between animals and people and have an animal reservoir, and are a constant threat to global human and animal health. Viruses are notorious for encroaching on previously untouched habitats and hosts mainly due to changes to local ecosystems, they are transported inconspicuously by travel, and can be accidentally imported via infected carrier hosts before the onset of clinical symptoms. In this presentation, our efforts to develop vaccines and point-of-need (PON) diagnostic tests against various viral diseases will be discussed.

Results

RVF is a mosquito-borne zoonotic disease that presents a substantial threat to human and animal health. It is caused by the RVF virus (RVFV). The wide distribution of competent vectors in non-endemic areas coupled with global climate change poses a significant threat to the transboundary spread of RVFV. In the last decade, an improved understanding of the molecular biology of RVFV has facilitated significant progress in the development of novel vaccines, including DIVA (differentiating infected from vaccinated animals) vaccines. We have developed a DIVA-compatible, subunit RVF vaccine which is safe and efficacious in target animals.

In 2014–2015, highly pathogenic avian influenza H5 viruses including H5N1, H5N2, and H5N8 subtypes (so-called H5N*x* viruses) were detected in U.S. wild birds, with the H5N2 and H5N8 viruses also causing major outbreaks in U.S. domestic poultry. Vaccination is one of the most effective ways to control influenza outbreaks and protect animal and public health. Therefore, we developed a Newcastle Disease Virus-based H5 (NDV-H5) vaccine that expressed the hemagglutinin from the H5N2 virus and evaluated its efficacy in chickens. Results showed that both, live and inactivated NDV-H5 vaccines completely protected chickens from lethal challenge with the highly pathogenic H5N2 virus. Furthermore, one dose of the live NDV-H5 vaccine also provided excellent protection to chickens immunized by coarse spraying. Our results suggest that the NDV-based H5 vaccine is able to protect chickens against intercontinental highly pathogenic H5N*x* viruses and can be used by mass application to protect the poultry industry and decrease the potential of human exposure.

ASF virus (ASFV) causes high morbidity and mortality in swine for which there is currently no commercially available vaccine. The disease is highly contagious and poses a serious threat to the swine industry worldwide. Since its introduction to the Caucasus region in 2007, a highly virulent, genotype II strain of ASFV has continued to circulate and spread into Eastern Europe and Russia, and most recently into Western Europe, China and various countries of Southeast Asia. These outbreaks highlight the urgent need to develop effective vaccines against ASFV. Our approaches to develop subunit and modified live virus (MLV) vaccines will be discussed. The subunit approach is based on prime-boost vaccination regiment, combining ASFV antigens encoded by DNA plasmids and recombinant ASFV proteins with the aim to activate both, humoral and cellular immunity. The approach to develop MLV vaccines is based on gene-deleted ASF viruses using CRISP-Cas9 knock-out technology.

In addition, novel point-of-need (PON) diagnostic methods for the detection of ASFV nucleic acids and ASF-specific antigens will be presented. Molecular PON diagnostics for ASFV is performed using the portable GeneReach Pockit™ and Biomeme Franklin™ systems. Side-by-side comparisons revealed equivalent or nearly equivalent sensitivity and specificity of the portable PCR machines to regulatory, laboratory-based thermocyclers. A Lateral Flow Immunoassay test (LFT) for the sensitive and specific detection of ASFV-specific antigens in the blood of pigs was also developed. The LFT strip demands a 10 ml sample, 15-min test time, water and no buffers, and the read-out is visual. PON diagnostics allow fast and accurate detection of pathogens under field diagnostic settings, and produce rapid actionable information for decision makers. In addition, a major driver for the PON platforms

is the ability to diagnose infectious diseases where there is limited infrastructure.

Conclusion

In summary, continuous investment in research to develop better diagnostics, antivirals and vaccines that will mitigate the impact of emerging and zoonotic viral diseases is needed. Innovative ways to predict and rapidly diagnose and control novel emerging/zoonotic diseases will be crucial to limit the danger of these pathogens to humans and animals.

NMR AND CHEMOMETRICS IN THE CHARACTERIZATION OF HETEROGENEOUS POLYSACCHARIDE DRUGS

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Polysaccharides are ubiquitous in animals and plants, where they play important roles in diverse physiological situations. They often comprise a heterogeneous mixture of polymers, varying in size and substitution pattern, whose composition can change between batches. One of the best-known examples of a polysaccharide drug is heparin. Heparin, which is included in the World Health Organisation's «Essential Drugs List» originates from animal sources and is used to prevent the formation of blood clots and is one of the world's most widely sold polysaccharide drugs (Szajek A.Y. et al., 2016).

Heparin chains can vary in length and comprise various combinations of differently sulfated disaccharide building blocks, making heparin an extremely heterogeneous mixture. Stringent quality control of these drugs during the production process is critical. However, determining their quality is more complex than with traditional small molecule pharmaceuticals. Nuclear magnetic resonance (NMR) spectroscopy is the leading technique in the characterization of complex polysaccharide compounds, does not require prior separation of the constituent components and provides a broad range of information, ranging from a chemical fingerprint to structural constraints.

We discuss the current state-of-the art in the use of qualitative and quantitative mono- and bi-dimensional NMR spectroscopy coupled with chemometric techniques to validate that a product meets the required specifications, as well as to establish the structural equivalence of the generic product with the originator (Rudd T.R., Yates E.A., 2017).

Rudd T.R., Yates E.A. (2017) Guerrini in NMR in Glycosciences and Glycotechnology. K. Kato, T. Peters (Eds.). Royal Society of Chemistry, Cambridge, p. 305–334.

Szajek A.Y., Chess E., Johansen K. et al. (2016) The US regulatory and pharmacopeia response to the global heparin contamination crisis. Nat. Biotechnol., 34(6): 625–630. doi: 10.1038/nbt.3606

IN-DEPTH INVESTIGATION OF TETRASACCHARIDE FRACTION OF SODIUM ENOXAPARIN

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Heparins and low molecular weight heparins (LMWHs) are the most common anticoagulant and antithrombotic drugs used in cardiovascular medicine. The widespread LMWH is sodium enoxaparin, obtained by alkaline depolymerization of heparin benzyl ester. Enoxaparin belongs, like heparin, to the family of glycosaminoglycan species, whose polysaccharide chains are made up of alternating 1→4 linked residues of uronic acids (L-iduronic, IdoA, and D-glucuronic) and D-glucosamine with a different sulfation pattern. The depolymerization process involves characteristic structural modification due mostly to modifications of the monosaccharide units at the site of cleavage. The depolymerization step in alkaline aqueous phase produces, in addition to β-elimination of uronic acid residues, a partial transformation of glucosamines into mannosamine, and a 6-O desulfation of glucosamine leading to the formation of derivatives marked 1,6 anhydro.

Aim of the project was to demonstrate the similarity of generic enoxaparin to Originator product by several structural and biological characteristics, applying sensitive analytical methods and justifying adequately eventual differences in accordance with the guidelines of both EMA and FDA. The comparability work outlines peculiar characteristics of enoxaparin due to both biological source (heparin) and mode of depolymerization and was based on subsequent levels of investigation from the whole enoxaparin to oligosaccharide families. In particular, tetrasaccharide fraction was expected to be more representative of the depolymerization conditions than longer chains, so in-depth investigation of this oligomeric family provides detailed structural data on sequences and in wider terms of the whole enoxaparin.

The objective of the present research was the isolation and structural elucidation of tetramers of enoxaparin by LC-MS and NMR. Preparative size exclusion chromatography was performed to isolate the whole tetrasaccharide fraction of enoxaparin. Then a multi-step fractionation on IPRP-HPLC was executed allowing to separate firstly three families of tetramers based on their overall negative charge and the presence of 1,6 anhydro. A second level of fractionation was performed for each family to isolate narrow tetramer fractions.

Each species was characterized by NMR and IPRP-HPLC/ESI-QTOF-MS allowing to elucidate the sequence of at least thirty tetramers. Besides several isomeric structures obtained by depolymerization process, characterized by the presence of 4,5-unsaturated uronic acid residue at the non-reducing end, several other tetramers coming from the natural non-reducing end of the parent heparin chains have been identified. The characterization of these tetramers provided further structural information about the heparin biosynthesis.

THE CURIOUS CASE OF CIPROFLOXACIN: HOW TO DEAL WITH «DIFFICULT» DRUGS MOLECULES TO IMPROVE THEIR BIOPHARMACEUTICAL PROPERTIES

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Amorphisation — a transformation of a crystalline solid into its disordered form, is one of the approaches that has been more and more widely utilised to improve bioavailability of active pharmaceutical ingredients (APIs). Ciprofloxacin (CIP) is a second-generation fluoroquinolone with a wide spectrum of antibiotic activity. In solution, the carboxylic acid and piperazine groups of CIP ionise depending on pH of the surrounding medium. However, in the neutral pH range CIP is zwitterionic and has an overall neutral charge, making it practically insoluble in aqueous media. The poor solubility of CIP is furthermore coupled with poor perme-

ability, thus amorphisation was investigated as a means of improving the inadequate biopharmaceutical properties of CIP.

This presentation shows the solid-state transformations of CIP, with an emphasis on the ionisation state (zwitterionic or unionised) of this molecule. Ball milling, cryomilling, and spray drying were used to prepare partially and fully amorphous CIP. CIP proved to be very difficult to amorphise, with only spray drying from pure water resulting in a fully amorphous product. It was shown that, while most processing methods resulted in the more stable zwitterionic form of the drug, spray drying from an ethanol/water mixture produced the unionised form. The zwitterion was found to convert to the unionised form upon heating to its melting point, and the reverse transformation occurred when unionised CIP was exposed to high humidity. CIP was found to amorphise more readily when co-processed with acidic pharmaceutical polymers forming amorphous polymeric drug salts (ASDs) via mechanochemistry. No decrease in antibiotic efficacy was observed, and significant improvements in the minimum inhibitory concentration and minimum bactericidal concentration of CIP were obtained with ASDs containing HPMCAS-LG and HPMCAS-MG. Therefore, ASDs may be a viable alternative for formulating CIP with improved solubility, bioavailability and antimicrobial activity.

ADVANCED TECHNOLOGIES IN 3D-CELL CULTURES

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A long-standing trend in pharmaceutical development, toxicology and biomedicine has been the establishment of complex *in vitro* 3D cell cultures whose structures and function resemble human tissues. Many scientific studies have confirmed that the organotypic 3D cell structures within a tissue significantly influence the behavior of living cells. This enhancement of biological model systems is not only intended to circumvent expensive animal experiments, but also to replace the currently existing and inadequate *in vitro* models of 2D cell cultures. Advantages of employing 3D cell cultures include the improved *in vivo*-like situation where cells are surrounded by its natural extra cellular matrix (ECM) and in direct cell contact with each other. In addition, the existence of extensive cell-cell and cell-ECM interactions, analogous to the *in vivo* situation, promote the recovery of natural structures and functions of the original tissue biology. The microfluidic cultivation of cells in a non-planar orientation allows the determination of physiologically relevant information, which are necessary for the disease research and will determine the demand in the next few years. Since such cultures are more suitable for mimicking or imaging typical organ microarchitectures and morphology of a living organism, tremendous effort is spent in the development of high-throughput microfluidic 3D cell models. Therefore, microfluidics represents a multifaceted technology, which can handle several processes at one time such as culture, replenishment of medium, sampling, mixing, capture, and subsequent detection. An ideal spheroid culture should promote growth of cells by supplying needed nutrition, moisture, oxygen as well as remove degradation products at the same time. Microfluidic technology offers all these privileges to cell culture. The enhanced predictive power of 3D multi-cellular spheroids in comparison to conventional monolayer cultures makes them a promising drug screening tool. However, clinical translation for pharmacology and toxicology is lagging its technological progression. Even though spheroids show a biological complexity resembling native tissue, standardization and validation of drug screening protocols are influenced by continuously changing physiological parameters during spheroid formation. To meet the growing need to improve the predictive power of toxicological, pharmaceutical and preclinical studies, the microfluidic multi-cellular spheroid array chip builds on the advancement of organ-on-a-chip technology that establishes, cultivates and supports a wide variety of multi-cellular spheroids. The spheroid array chip thus closes an important technological gap, enabling rapid and easy production of spheroids of defined size and cell types. These aspects are particularly important because, in addition to the cell type, spheroid age and size are a critical parameters of any spheroid and organoid culture. With the help of our biochip, different cell types can be established, treated and analysed as a high-content manner. Due the different sizes and geometries of the wells, different amounts of cells are captured within the cavities and thus, they aggregate to a 3D cell culture. Spheroid growth and size is restricted by the height and the depth of the well. It is well known that applied spheroid size and their size variation has a profound impact on the experimental outcome since spheroid size influences bioactivity, drug penetration barrier as well as expression profiles. Applications of the chip are tailor — made toxicity-, differentiation- and cell staining protocols for specific cell lines of various types and origins.

Research objective

Drug toxicity often goes undetected until clinical trials, the most costly and dangerous phase of drug development. Both the cultures of human cells and animal studies have limitations that cannot be overcome by incremental improvements in the drug testing protocols. A new generation of preclinical models — in form of integrated human tissue platforms, would be transformative to drug screening and predictive modeling of disease. We established three three-dimensional microtissues providing (i) hierarchical tissue-specific architectures, (ii) functional representation of human biology of health, (iii) real-time biological readouts, and (iv) compatibility with high-throughput/high-content microfluidic platforms for studies of drug toxicity and over long periods of time.

Materials and methods

For determining the effect of spheroidal age on drug screening results, spheroid quality was monitored by analyzing ultrastructural morphology and organo-specific metabolic functionalities for early-stage, mid-stage and late-stage spheroids. Following the identification of spheroidal ages, drug diffusivity, toxicity and resistance are determined to describe the interplay between spheroidal age and efficacy of drugs when employing *in vitro* 3D cell culture models. Further, an established spheroid microarray biochip offers the opportunity to generate multiple spheroids of diverse reproducible sizes in a single channel under microfluidic conditions. The channels are connected to a pump which enables a continuous supply of nutrients as well as removal of degradation products at the same time. A biocompatible silicone material is adopted for the microfluidic chip. By varying the size of microtumors on chip, all these parameters are affected and offers therapy-related studies with different emphasis.

Results and conclusions

Aside from already well-established physiological parameters, spheroidal size and age are additional critical parameters that impacts drug diffusivity and toxicity in 3D cell culture models. Liver HepG2 spheroids were generated and maintained on a self-assembled ultra-low attachment nanobiointerface and characterized regarding time-dependent changes in morphology, functionality as well as anti-cancer drug resistance. We demonstrated that spheroidal aging directly influences drug response due the evolution of spheroid micro-structure and organo-typic functions, that alter inward diffusion, thus drug uptake.

INFLUENZA VIRUS REPLICATION AND THE INDUCTION OF INNATE IMMUNE RESPONSES

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Introduction

The influenza A virus is an infectious agent that usually causes a mild respiratory disease and induces innate immune responses through the activation of host cell pathogen sensor retinoic acid-inducible gene-I (RIG-I). However, infections with highly pathogenic influenza viruses, such as the H5N1 subtypes or the 1918 H1N1 pandemic virus, can lead to an innate immune dysregulation and severe disease. It is believed that influenza virus RNA and the viral RNA-dependent RNA polymerase play a key role in this innate immune dysregulation process. The RNA genome of the influenza virus is replicated and transcribed by the viral RNA-dependent RNA polymerase in the context of viral RNA-nucleoprotein (vRNP) complexes. The RNA polymerase is a complex enzyme that consists of a central core composed of the viral proteins PB1, PB2 and PA. Various mutations in the RNA polymerase have been linked to host adaptation and viral virulence, but it is presently unclear what molecular mechanism underlies the link between RNA polymerase differences, viral pathology and human disease.

Research objective

Understand the molecular mechanism that links influenza virus RNA synthesis, host adaptation, and the induction of innate immune responses during influenza virus infection.

Methods

We used RNA polymerase activity assays, RIG-I pull-downs, RT-PCRs and deep-sequencing. RNA was isolated from transfected cells expressing viral ribonucleoproteins, infected cells and tissues of ferrets infected with the 1918 H1N1 pandemic virus, H5N1 avian influenza, or the 2009 H1N1 pandemic virus. As control viruses we used seasonal human H1N1 and H3N2 influenza A viruses. *In vitro* activity assays were used to demonstrate how the viral RNA polymerase makes viral RNA.

Results

Analysis of RNA isolated from transfected cells, cells infected with pandemic and avian influenza viruses, and lung tissues of ferrets and mice infected with the 1918 pandemic virus or H5N1 strains shows that short subgenomic viral RNAs of <125 nt potently activate RIG-I and induce innate immune signalling. We call these viral RNAs mini viral RNAs (mvRNAs) to differentiate them from other viral RNAs. The polymerases of the 1918 H1N1 pandemic virus and H5N1 subtypes are particularly efficient at generating mvRNAs *in vitro* and *in vivo*. Introduction of avian adaptive mutations into the template exit channel of RNA polymerases from low pathogenic viruses leads to an increase in mvRNA synthesis. Analysis of mvRNA sequences suggests that they are formed through an intramolecular copy-choice mechanism, while *in vitro* experiments show that mvRNA synthesis is regulated by an interaction between the polymerase template exit channel and RNA structures in the template.

Conclusion

We find that the influenza virus RNA polymerase generates mvRNAs that are preferentially bound by RIG-I and able to trigger strong innate immune responses. Our findings suggest that different influenza viruses produce different mvRNA levels and that this process underlies virus-induced innate immune responses and severe disease.

ANTI-INFLUENZA DRUGS FOR THE CONTROL OF INFLUENZA

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Introduction

Influenza virus infection is responsible for millions of hospitalizations and thousands of deaths each year. The emergence of potentially pandemic influenza strains and seasonal influenza epidemics reminds us that we are limited in the strategies available to control influenza infection. Annual vaccination is still the most efficient way to prevent an infection with influenza viruses; however, antigenic mismatch allows viruses to escape neutralizing antibodies, thus rendering the annual seasonal vaccine ineffective. Because we cannot rely solely on vaccines or predict the strain of influenza virus that will cause the next epidemic or pandemic, new anti-influenza drugs with broad reactivity are needed to alleviate an ongoing health and economic burden caused by influenza. Neuraminidase inhibitors have demonstrated effectiveness for the prevention and control of influenza infection and remain the drug of choice for influenza treatment; however, as drug-resistant variants continue to emerge naturally and through selective pressure applied by use of antiviral drugs, the efficacy of these drugs declines. To overcome the limitations of neuraminidase inhibitors, new therapeutic agents against influenza viruses which include baloxavir, favipiravir and monoclonal antibodies have been developed. Enisamium iodide (Amizon®, Farmak) is currently approved for clinical use for the treatment of influenza in 11 countries. We sought to evaluate enisamium efficacy against influenza A and B virus infections.

Methods

In vitro experiments using primary differentiated normal human bronchial epithelial (NHBE) cells with assessment of viral titers and M-gene expression, as well as *in vivo* study in ferrets were done. Permeability of enisamium into differentiated normal human bronchial epithelial cells and its cytotoxicity were also assessed, and comparisons with other cell lines were made.

Results

Enisamium markedly inhibited multiple subtypes of influenza A viruses, including seasonal H1N1, 2009 pandemic H1N1, seasonal H3N2, the zoonotic H5N1 and H7N9, neuraminidase inhibitor-resistant variant carrying the H275Y NA substitution (N1 numbering), and influenza B virus. In time-of-addition experiments in differentiated normal human bronchial epithelial cells, enisamium treatment within 4 h after A(H1N1) virus inoculation resulted in 100-fold or greater reductions in virus titers, suggesting that it affects an early stage of the virus life cycle. Treatment of ferrets with FAV00A (200 mg/

kg once daily for 7 days) initiated 24 h after inoculation with 10^5 $TCID_{50}$ of influenza A/Wisconsin/67/2005 (H3N2) virus didn't cause changes in disease signs of infection, but caused significant decrease of virus titers in the upper respiratory tract.

Conclusion

Enisamium exhibits a broad antiviral effect against multiple influenza A and B viruses in NHBE cells and provides some benefits in a ferret model. Thus, supporting the reported clinical efficacy against influenza virus infections.

PHARMACOKINETICS PRINCIPLES IN PRECLINICAL TOXICITY STUDIES

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Understanding the pharmacokinetics (PK) of a drug candidate is a well-recognized fundamental requirement to ensure successful development. PK investigations generally are done early in the discovery phase during the screening of potential candidates when selecting a lead compound. These early PK studies are normally performed at dose levels comparable to those expected to produce beneficial pharmacological action. However, distinct challenges often arise during the execution of toxicity studies when dose levels are increased substantially to induce toxicity and establish preliminary therapeutic windows. The challenges include the availability of validated bioanalytical methods to reliably assess systemic exposure, along with formulation strategies to ensure adequate bioavailability for the intended administration route and doses. In this talk we will present approaches commonly used in the execution of preclinical toxicity studies and examples of challenges encountered in our practice. The challenges posed by agent toxicology are substantially different for the development of novel cancer chemopreventive agents than they are for cancer therapeutics. Because chemopreventive agents are designed for administration to otherwise healthy individuals, they must be designed and developed with the goal of identifying pharmacologically active doses that induce zero or minimal toxicity. By contrast, although a key goal in the development of cancer chemotherapeutics is to maximize therapeutic ratios, the induction of toxicity by a pharmacologically active dose of a chemotherapeutic drug is seen as a less significant limitation to its clinical use than is the induction of toxicity by a chemopreventive agent.

THE IMPORTANCE OF MEMBRANE TRANSPORTERS IN DRUG DEVELOPMENT

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The impact on the role of membrane transporters in drug absorption, distribution, metabolism and excretion (ADME) has been extensively studied since discovery of the first membrane transporters about two decades years ago. During this period, the scientific field has rapidly evolved and better learned to understand the pivotal role drug transporters have on small molecular xenobiotics. Approximately two dozens of transporters out of the over 400 transporters expressed in the human body are found to be particularly important for the transport of xenobiotics. As a result of this importance, regulatory agencies such as FDA and EMA; and governments worldwide have implemented criteria and guidelines for studying these transporters during drug development to assure drugs are safer for patients. In addition, these insights and knowledge developed in past years in the field of transporter sciences also tremendously helped the pharmaceutical industry understand *in vivo* observations related to ADME, and efficacy of drugs. The effect of all this knowledge is that safer and more efficacious drugs make it to the market. The current presentation will give an overview of the most important transporters for the pharmaceutical industry and their role in small molecular drug development. The clinical implications of these transporters, and *in vitro* methods of how these interactions are studies will be explained.

CANCER VACCINE AS A NOVEL COMPONENT OF CANCER THERAPY

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One key hallmark of cancer is the tumor's ability to evade and escape immune surveillance. Cancer cells in concert with supporting stromal cells creates a microenvironment masking tumors from immune system recognition and penetration. Cancer therapeutic vaccine is a type of immunotherapy to train or educate body's immune system to recognize and destroy cancer cells. Most cancer therapeutic vaccines are personalized treatment utilize individual's own tumor or blood cells with *ex vivo* preparations before introduced back into patients. *Ex vivo* prepared tumor tissue exposes tumor's neoantigens to educate immune system for recognition and elimination. Patient's white blood cells are modified *ex vivo* to recognize cancer cells. Many cancer therapeutic vaccine strategies have shown promising results; particularly, the most recent CAR-T for blood cancers. However, tumor environments are difficult for immune cell to penetrate. More recent cancer vaccine strategies include combinations with immune checkpoint blockade, angiogenic blockade and other immunotherapy agents to further boost the efficacy of the cancer vaccine approach. Additionally, personalize neoantigen discovery and presentation strategy are also under development. At this presentation, we will provide a fundamental view of the basics of cancer therapeutic vaccine, regulatory issues, operational challenges, and continue on to take a panoramic view of current advancement.

DECODING X-LINKED PIGMENTARY RETICULATE DISORDER

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Viruses and their unique replication cycles can generate a variety of aberrant nucleic acids that activate innate immune sensors, resulting in type I interferon production. Mutations affecting genes involved in these sensing mechanisms can lead to immunodeficiency or autoinflammatory disorders in humans. Throughout the last six years, our group studied an X-linked reticulate pigmentary disorder (XLPDR, MIM:301220), a rare syndrome characterized by recurrent lung infections and sterile multiorgan autoinflammation. Originally, we have reported that the syndrome is caused by a hypomorphic mutation in the POLA1 gene, encoding the catalytic subunit of DNA polymerase-α, which initiates the DNA replication process.

Due to mutation, XLPDR patients display reduced expression of POLA1 protein. Unexpectedly, we uncovered that POLA1 deficiency leads to overexpression of multiple interferon-stimulated genes. Further genetic analysis favors cGAS and MAVS as the dominant pathways behind the autoinflammatory manifestations of the disease. However, until recent, the origin of immunodeficiency in XLPDR remains elusive. Here we report that XLPDR patients have reduced NK cell numbers and functionality, which is behind the XLPDR-associated immunodeficiency and recurrent lung infections.

Altogether, linking to molecular data and disease manifestations, we established that previously uncharacterized XLPDR syndrome is the NK cell deficiency, combined with type I interferonopathy. This offer several directions for development a specialized XLPDR therapy that can be extended on the over immunodeficient and autoimmune conditions.

From left to right:
1-st row: Victor Margitich, Cristina Gardini, David Boltz, Svetlana Yefimova, Petro Starokadomsky, Lidia Tajber , Roman Pogranichniy;
2-nd row: Roelof de Wilde, Aartjan te Velthuis, Miguel Muzzio, Marco Guerrini, Christoph Eilenberger, Jürgen A. Richt, Rudolf Zinell, Yuri Malyukin, Donald Rao

NEW DIAGNOSTIC METHODS FOR DISEASE DETECTION AND PROGRESS – FIELD APPLICATIONS

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It has been estimated that the world production of food animals is reduced by more than 20% due to disease at the same time as the world population is growing and raising the demand for more meat and other animal products. Such a reduction of food animals has a negative impact on human health and the population. We also know that the unprecedented flow of commodities and people give pathogens of all kinds of opportunities to spread and multiply around the world, and in addition, climate change can enable them to extend their range, colonizing new areas and spreading to new species.

There are many new diseases that have emerged in the past (influenza virus, Ebola, Q-fever, acute respiratory syndrome, Nipah virus, hantavirus, and others) in domestic animals and these pathogens can also infect humans. The reservoir for these diseases quite often comes from wild or domestic animals. It has been estimated that pathogens shared with wild or domestic animals cause more than 60% of infectious disease outbreaks in humans. Also, endemic and enzootic zoonoses have caused millions of deaths every year in humans and hundreds of billions of dollars of economic damage in the past 20 years. Detection of different pathogens from infected animals is essential. We are detecting new pathogens in veterinary and human medicine daily. The technology is available to detect different microorganisms at low cost in laboratory or field settings. The role of today's scientist is not only to be able to use these tools in the laboratory setting but also advance knowledge on pathogens and causality of disease. If detecting pathogens is becoming more simple and information is readily available, the new generation of scientists should be able to interpret results properly and accurately diagnose animal diseases.

New and old technologies in combination with interpretation of test results will be discussed more in detail. New ideas for young scientists will be discussed during the presentation.

WORKSHOP AND POSTER SESSION ABSTRACTS

PHYTO-CHEMICAL STUDIES OF PHALLUS IMPUDICUS MUSHROOM

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Introduction

Despite modern scientific advances in the treatment of cancer, folk medicine has not lost its value, and thousands of seriously ill patients are turning to it as the last hope for healing. Humankind has long learned to use the gifts of nature in the form of infusions, using ethyl alcohol 40% (vodka) as extractant. To the population of Ukraine, mainly in the countryside, well-known is common stinkhorn (Phallus impudicus), which is used solely for medicinal purposes.

In folk medicine, water-alcohol tinctures from fresh or dried mushrooms, as well as raw ones, are used. It is used both locally and internally for all kinds of diseases: gastritis, gastric and intestinal ulcers, cardiovascular diseases, thrombophlebitis, fibroids, mastopathies, ovarian cysts, prostate adenoma, any malignant tumours, sexual weakness, psoriasis and eczema, gout, consequences of chemo- and radiotherapy, to prevent metastases and recurrence of cancer.

The antitumor properties of stinkhorn, as a so-called «higher fungi», may be related to polysaccharides, β-glucans, which activate specific cellular immunity, activating inhibited cytotoxic T-lymphocytes, or natural killers that begin to produce perforin proteins, which ruin cancer cells. Also found fungal «phytoncides», which have viral inhibitory effect, ranging from rhino- to human immunodeficiency virus. Champions in the amount of these volatile substances are Japanese shiitake mushrooms (Lentinula edodes) and common stinkhorn (Phallus impudicus).

Aim of the study

Carrying out phytochemical studies of stinkhorn fungus tinctures. Freshly (harvested on its own in July 2018) and dried raw materials are used in the work. Drying of the fruiting body of the mushroom was carried out in cool conditions at a temperature

of (2–8)° C, as at higher temperature the fungus enters the stage of maturity (intensive growth) in which the mushroom according to the literature is conditionally edible. Since mushroom tinctures are prepared in traditional medicine using vodka, it is interesting to note how ethanol strength affects the extraction of biological active substances (BAS).

Tinctures were prepared in the ratio of raw material: the extractant as 1:40 in terms of dry matter, or 1: 5 without taking into account the moisture content of the mushroom's fruiting body. Different concentrations of ethanol were used as extractants; 10, 20, 30, 40, 50, 60, 70, 80, 90%. Tinctures were obtained by maceration (infusion). In tinctures, the content of polysaccharides, extractive substances was determined, and the main groups of BAS were identified.

Results

Using color reactions, were identified the main groups of ALS in tinctures: polysaccharides (Felling reagent, red precipitate), phenolic compounds (iron (III) chloride, brown colour), steroid substances (sulfuric acid, concentrated, pink colour), aminoacids (ninhydrin, blue-violet colour), nitrogen-containing compounds (alkaloids) (Dragendorf reagent, orange-red colour) and iridoids (Stall reagent, cyan colour).

When determining the amount of extractives, it has been found that the maximum extraction ensures the use of ethanol at concentrations of 10, 20 and 30%. In the case of dry stinkhorn fungus, the amount of extractives, provided by ethanol concentrations of 10, 20 and 30%, is 2–3 times more than when using freshly picked mushroom.

When determining the quantitative content of polysaccharides by gravimetric method in raw materials, their content was found to be 12.28%. Comparing this value with polysaccharides of shiitake, maitake, reishi, cordyceps and coriolus fungi, stinkhorn fungus is only inferior to shiitake. When determining the number of polysaccharides in tinctures, it has been found that the maximum content of these substances is 4.0–4.5% at extraction with aqueous-alcoholic solution of 20%, 30% and 40%.

Conclusion

The study of the chemical composition of the common stinkhorn fungus (Phallus impudicus), the development of substance production technology and the establishment of pharmacological activity is a promising area in the search for new drugs.

HYPOGLYCEMIC PROPERTIES OF AMINO ACID CHROME COMPOUNDS (III)

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Introduction

For the treatment of various forms of diabetes, insulin preparations and other blood glucose lowering drugs — derivatives of sulfonylureas (butamide, cyclamide, chlorocyclamide) and booguanides (glibutide, glibenclamide) are used. The disadvantage of these drugs is the need to use the parenteral route of administration, the possibility of allergic disorders and toxic effects on the bone marrow and parenchymal organs. All these disadvantages make it necessary to find alternative hypoglycemic drugs. One of these drugs is chromium (III) salts, which affect the functioning of the liver, pancreas and carbohydrate metabolism. This group of compounds causes a distortion of the glycemic curve and galactosuria after galactose loading, which allows them to be considered as potential sugar-lowering drugs.

Usually, chromium salts (III) take part in the regulation of blood glucose levels: they are part of the chemical activator of the «glucose tolerance factor». Interacting with insulin these compounds «capture» glucose in the blood and transport it to cells, providing its cleavage and excretion, and potentiates the action of insulin in peripheral cells. With deficiency, there is a decrease in glucose uptake by the lens of the eye, glucose utilization for lipogenesis, and a decrease in glycogen synthesis from glucose. All these disorders are treated by the administration of chromium and insulin compounds.

Recently, there has been an increasing interest in the use of complex chromium compounds (III) as low-toxic biologically active compounds for which hypoglycemic bioactivity has been demonstrated. Chromium compounds (III) with amino acids are non-toxic and used as food additives.

The purpose of the study is to examine the hypoglycemic properties and toxic effects of the complex compound of chromium (III) with cysteine (b-metkapto-a-aminopropionic acid — H_2Cys).

Tasks

1. To establish that with the introduction of chromium (III), especially its organic complex compounds: chromium glycinate (III), chromium nicotinate (III), — improves the absorption of glucose by tissues.

2. Using polarographic studies of the interaction of chromium (III) and insulin with mitochondria, analyze the effect of trivalent chromium at the cellular level with the formation of complex compounds with insulin.

Materials and methods

The following methods of analysis were used during the study:

- Elemental analysis of the substance for Nitrogen and Hydrogen was carried out on a «Carlo Erbe»analyzer, chromium was analyzed by photometric method with diphenylcarbazide;
- X-ray diffraction analysis was performed on a DRON-2.0 X-ray diffractometer at T=300 C.

Results and discussion

The synthesized complex of chromium (III) cysteine [Cr(HCys)3] is intracomplex salt with bidentally coordinated cysteine, the pH of the salt solution medium, which is 4.8–5. The mechanism of the biological action of chromium cysteine (III) has been found to be associated with adsorption on the surface of the lipid membranes, which results in alteration of the intracellular potential difference and the stiffness of lipid membranes.

In one study, the effect of the drug on the blood sugar level of white rats under a glucose load was determined. The study was performed on adult rats — males of the Vistar line. The weight of rats was 250–290 g. Experimental hyperglycemia was modeled by intragastric administration of a 40% d-glucose solution at a dose of 1600 mg/kg. Blood samples for glucose concentration measurement was taken from the tail vessels in the original state and 60 min after the introduction of glucose into the stomach. After that, animals of the experimental group were intraperitoneally administered a solution of chromium cysteine at a dose of 1 mg/kg, animals were taken 120, 180 and 240 min after glucose administration.

After the introduction of glucose solution into the stomach, its concentration in the blood increased by 23–25% compared to the initial state, and in the group of control animals remained at an elevated level for more than 4 hours. 60 min after intraperitoneal injection of 1 mg/kg chromium hydrocysteine solution (III) at a dose of 1 mg/kg, the blood glucose concentration in the experimental rats decreased and was less than 55% compared to the control. In the future, the blood glucose concentration of the animals of the experimental group remained low during the hour of the experiment compared to the control. Hyperglycemia was observed in the control rat group within 4 hours after the carbohydrate load.

The next step of the study was an acute toxicity. Studies were performed on an adult white mice. Chromium hydrocysteine tris (III) preparation was injected into the peritoneum while increasing the dose. After indicative determination of the dosage of chromium trishydrocysteine (III), the test substance was administered as a 1% solution to 48 mice (8 in each group) weighing 15–22 grams. Six increasing doses of the drug were tested: 1500; 1900; 2300 hours;

2700; 2900; 3100 mg/kg. The mice were monitored for 10 days after injection. The time of death of the animals in each group was recorded. No death of animals at a dose of 1500 mg/kg occurred, and at the administered dose of 3100 mg/kg — all mice in the group died. The LD_{50} was calculated using the least-squares method and showed that LD_{50} for chromium tris-hydrocysteine (III) was 2568 mg/kg. Compound of chromium complex (III) with cysteine is low toxic and belongs to class IV toxicity (according to I.V. Sanotsky's classification).

The hypoglycemic activity of the cysteine complex of chromium (III) is comparable to the activity of a glycine derivative with a similar chelate structure, and significantly higher than the activity of complexes with nicotinic acid.

The toxicity index for the amino acid complexes of chromium (III) varies in a number of compounds $[Cr(HCys)_3]\cdot H_2O > [Cr_2(Nik)_2(OH)_4]\cdot 4H_2O \approx [Cr(Gly)_3]\cdot H_2O > [CrAsp(MMSCl)_2]$ and is substantially lower than that of inorganic chromium salts (III).

Conclusion

1. Tris-hydrocysteine chromium (III) when administered intraperitoneally at a dose of 1 mg/kg causes a significant decrease in the concentration of glucose in the blood of white rats.

2. The distinguishing features of chromium tris-hydrocysteine (III) are high rate of decrease in blood sugar, as well as availability and simplicity of synthesis, high yield of the product, pH of the environment 4.8–5.

3. Given the low toxicity and significant hypoglycemic activity of chromium (III) tris-hydrocysteine, this compound may be recommended for further pharmacological studies as a potential hypoglycemic agent and also for use as a dietary supplement.

DEVELOPMENT OF EMULSION BASES OF THE 1st AND 2nd KIND FOR EXTEMPORAL SEMI-SOLID DOSAGE FORMS

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Introduction

World experience shows that in most countries of the world the priority is the preservation and development of extemporal formulations, as this provides an individual approach to the needs of the patient. In Ukraine today, the concept of the development of «personal medicine» is promising.

In such circumstances, extemporaneous drug production takes on new meaning. Today it is positioned as the creation of a drug for the needs of each individual patient. The advantage of extemporaneous drugs is the individualization of medical care for each patient, the choice of the most optimal ratio of ingredients, a wide selection of doses, taking into account the genetic, age, gender characteristics of the human body. However, unfortunately, the most of the extemporal prescriptions are outdated, given the pace of development of modern pharmacy and the production of new substances.

An analysis of the range of dosage forms that pharmacies are preparing in reserve showed that solid dosage forms take 1.5%, semi-solid dosage forms take 26%, liquid dosage forms take 72.5%. Semi-sold DFs (SSDF) are available on fat (25.5%), emulsion (51.1%) and hydrophilic (23.4%) bases.

After analyzing the composition of semi-sold DFs, it was found that the emulsion bases do not change over time and require a qualitative improvement. Taking into account a number of advantages of emulsion bases, our aim is the development of new carriers of this type for SSDF, as they today are the most promising, due to the high specificity of exposure and high levels of bioavailability of APIs in the composition of these bases.

Emulsion systems are thermodynamically unstable, therefore, for improving of the stability emulsifiers are used. They must meet the following requirements: ensure the formation of a stable emulsion; be chemically indifferent; show no toxic effect, do not cause skin irritation; have no disagreeable odor; must be approved for use.

Research objective

Development and research of model samples of emulsions the first and the second kind based on a mixture of emulsifiers OLIVEM® 1000 (cetearyl olivat/sorbitan olivat), Span 80 (sorbitan oleate) and Span 60 (sorbitan monostearate). Justification of the composition of the emulsion base with a view to the further creation of extemporal semi-solid drugs and cosmetics.

Materials and methods

For the preparation of emulsion bases, the following emulsifiers were used: OLIVEM® 1000 (cetearyl olivate/sorbitan olivat) (Hallstar Italy), Span 80 (sorbitan oleate; PhEur); Span 60 (sorbitan mono; PhEur; Croda, United Kingdom), corn oil (PhEur), purified water SPhUC 2.0).

Colloidal stability was determined by centrifugation on a laboratory centrifuge LabAnalyt DM 0412 for 5 min. at a speed of 6000 s-1. Thermostability was determined in a thermostat (TS-80 M-2) at a temperature of 42.5±2.5 °C for 7 days. Determination of the pH of the model samples was carried out by potentiometric method in 10% aqueous extraction from the base on a pH meter pH 150 MI (RF). The type of emulsion obtained was determined by dilution. Rheological studies were carried out on a BROOKFIELD HB DV-II PRO viscometer (USA) in the range of shear rates from 18.6 s-1 to 93 s-1 (SC4-21 spindle for a chamber with a volume of 8.3 ml) at a temperature of 20 °C. The mechanical value was calculated stability (MS) and dynamic rarefaction coefficient.

In the preparation of emulsion bases, a high-high temperature regime was used. The aqueous and oil phases were heated to a temperature of 70±5, mixed (in the manufacture of samples based on emulsions of the first kind, the phase inversion method was used) was emulsified using a laboratory homogenizer (Homogenizer HG-15A) for 30 min at 2000 rpm until a uniform consistency. Physicochemical studies of the samples were carried out after 24 hours after complete cooling and structuring of the system.

Results

The resulting emulsion bases are a mass of creamy consistency of a white-cream color, without visible inclusions, with a slight smell of corn oil.

The first series of experimental samples of emulsion bases was investigated to establish the range of concentrations of the oil phase and emulsifier. To create the basics with a minimum amount of oil phase, samples were prepared with 5, 10 and 15% corn oil, in which Olivem 1000 in concentrations of 2, 4, 6 and 8% (taking into account the manufacturer's recommendations — 1.5–6%).

Test samples that contained 2% Olivem 1000 exfoliated during storage; when using an emulsifier in the concentration range of 4–8%, the samples were stable, had satisfactory organoleptic characteristics, but differed in terms of viscosity. It is naturally noted that an increase in the content of the oil phase and the complex emulsifier contributes to an increase in reoparameters. Based on the findings of physicochemical and organoleptic characteristics bases containing Olivem 1000 (4–6%) and 15% corn oil were selected for the further study.

The second series of experimental designs was aimed at creating and studying emulsion bases of the 2-nd kind. In order to establish the optimal ratio of emulsifiers, their concentration and ratio were varied within the concentration range recommended by the manufacturer Span 80 from 0.5 to 10% and Span 60 from 0.5 to 6%. The content of corn oil remained constant, since increasing its concentration is economically feasible. It was noted that samples containing up to 6% sorbitan oleate exfoliated during emulsification Samples containing only one Span 80 emulsifier at a concentration of 6% and 8% were stratified during the colloidal stability test, which indicates an insufficient emulsifier content to stabilize a significant amount of the aqueous dispersed phase.

To study the extrusion properties of the bases, a dynamic rarefaction coefficient was calculated, which has average values of 69. High Kd values indicate the possibility of a better application of drugs during mechanical grinding, which characterize better rarefaction in the mixing mode, better distribution of APIs made in the base, and easy filling of tubes, which confirms the graph of viscosity versus shear rate. Based on the research results of physicochemical and organoleptic characteristics bases containing Span 80 from 6 to 10% and Span 60 from 4 to 6% and 50% corn oil were selected for the further study.

Conclusion

The emulsion bases of the first and second kind with different concentrations of emulsifiers mixture and correspondingly consistent properties can be used as the basis for the preparation of dermatological drugs and protective cosmetics, for the prevention of age-related skin changes and other.

BIOPHARMACEUTICAL STUDIES OF SUPPOSITORIES CONTAINING DRY EXTRACTS OF BUTCHER'S BROOM ROOT (RUSCUS AESCULUS) AND HORSE CHESTNUT SEEDS (AESCULUS HIPPOCASTANUM)

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Introduction

According to WHO official data, about 10% of people worldwide suffer from haemorrhoids. The main reason for this is a sedentary lifestyle that leads to impaired circulation in the pelvic organs and thrombosis.

Today, 26 brands of medicines for topical use for the treatment of haemorrhoids (C05A) and anal fissure are registered in the pharmaceutical market of Ukraine. Of these, 12 are domestic products, the rest — imported. Analysing the composition of active pharmaceutical ingredients (API) of domestic preparations, it can be noted that the formulations are repeated by different manufacturers and do not fully correspond to the modern directions of drug treatment of haemorrhoids. Based on the vascular concept of the development of the disease, as active ingredients dry extract of ruscus rhizomes, dry horse chestnut seed extract and shark liver oil were selected.

In recent years, scientists have paid great attention to the study of butcher's-broom, which contains sapogenins (ruscogenin). There are publications that present the results of pharmacological studies of venotonic action that exceeds the effect of aescin. At the Department of Pharmaceutical Technologies, research was conducted on the development of suppositories containing dry extracts of ruscus rhizome, chestnut seeds and shark liver oil.

Research objective

Investigation of biopharmaceutical properties of suppositories, namely dehydrating properties, dynamics of biologically active substances (BAS) release on the model of diffusion through semipermeable membrane and diffusion into agar gel to determine the type of suppository base.

Materials and methods

The generally accepted methods used in the pharmaceutical development of suppositories technology were used: determination of the dehydrating activity of suppositories, the study of the dynamics of biologically active substances release from suppositories through semipermeable membrane, the study of the dynamics of biologically active substances diffusion in agar.

Results

In determining the rational type of suppository base, in addition to pharmaco-technological indicators, it is also important to investigate their effect on the bioavailability of API. Frequent in use at the stage of pharmaceutical development of composition is the study of the dynamics of release through a semipermeable membrane or diffusion into an agar gel. A modified technique of agar plates was used based on physicochemical determination of substances diffusion into agar gel, to which a reagent was added (solution of KOH forms yellow coloration with compounds of flavonoid structure) in order to predict bioavailability. Measured coloured area at intervals of 0.5 — 1.0 — 2.0 — 3.0 — 6.0 — 24.0 h. Dynamics of zones coloration for suppositories made on the fat basis: 0 — (8.5±0.2) — (10.2±0.2) — (10.8±0.2) — (12.2±0.2) — (15.3±0.2) mm, for suppositories made on a hydrophilic basis: 0 — (8.3±0.2) — (9.5±0.2) — (10.0±0.2) — (11.2±0.2) — (12.5±0.2) mm. The results of the BAS release indicate the advantage of the composition where the fat base was used, but there is no significant difference between the samples.

One of the biopharmaceutical indicators of rectal suppositories quality is the study of dehydrating properties, that is, the ability to «draw» physiological fluids from the cell of the inflammatory process. Anestezol and Hemoproct preparations were used for comparison. The results of the studies showed that suppositories made on hydrophilic macrogol base absorb 580% of the liquid for 6 hours of the experiment. Reference drug Anestezol absorbed 500% of liquid, Hemoproct 475%. High dehydrating activity can lead to cell destruction and exacerbate the inflammatory process of hemorrhoids.

Conclusion

It is rational to choose for further research a composition where solid fat is used as the suppository base. This choice can be argued by the fact that the vast majority of rectal suppositories are made on a fat basis.

SYNERGISTIC ACTION ON MICROORGANISMS OF THE NATURAL ORIGIN BIOCIDES

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Introduction

According to recent studies of World Health Organization (WHO), almost half of clinical isolates of methicillin-resistant strains of *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Escherichia coli* are resistant to 3-rd generation cephalosporins, fluoroquinolones and carbapenems. Likewise, the resistance of representatives of the genus *Candida* is increasingly reported against fluconazole (93%), amphotericin B (35%) and echinocandins (7%). Reducing the number of resistant microorganisms can be achieved by using alternative compounds of natural origin, such as bacteriocins, microbial peptides, surfactants (SA) and essential oils (EO). The latter contain aldehydes, alcohols and phenolic compounds and thus are effective antimicrobial agents. That is why EO can be used instead of antibiotics and synthetic compounds in the cosmetic, food and pharmaceutical industries. However, the minimum inhibitory concentrations (MIC) of EO are rather high (400–1600 μg/ml), leading to high EO content in the various products. Simultaneously, EO in such concentrations are known to cause severe damage to the central nervous system, and aspiration pneumonia. The concentration of EO can be reduced without affecting their properties if they are used in combination with other biocides. The aim of this study to investigate the antimicrobial activity and synergic activity on biofilms of *Nocardia vaccinii* IMV B-7405 surfactants, essential oils and their mixtures.

Materials and methods

N. vaccinii IMV B-7405 was grow in a liquid nutrient medium. As a carbon source was used purified glycerol at concentration of 1% (v/v). The amount of synthesized extracellular surfactants (g/l) was determined by weighting method after extraction from a supernatant of culture fluid with a modified Folch mixture. Antimicrobial properties of the surfactants were determined by index of the minimum inhibitory concentration (MIC). To determine the synergism of the antimicrobial action, preparations of surfactant and a solution of essential oil with a concentration 2 times less than the MIC value of each of the preparations were used. The ratio of preparations in the mixture was 50:50. The degree of biofilm destruction was determined by spectrophotometric method.

Results

In the following studies, we established a synergism of the antimicrobial activity of tea tree EO and surfactants of *N. vaccinii* IMV B-7405 against *Pseudomonas* sp. MI-2, *S. aureus* BMS-1, *E. coli* IEM-1 and *Bacillus subtilis* BT-2. MIC of essential oil in the test cultures were 625–156 μg/ml, and in the presence of surfactants they decreased by 2 to 260 times. MIC of the mixtures of EO and surfactant were three orders of magnitude lower against *S. aureus* BMS-1 and *B. subtilis* BT-2 than MIC established for essential oil only.

Further experiments showed that surfactants of *N. vaccinii* IMV B-7405 exhibited a synergistic effect when mixed with cinnamon and lemongrass EO. Thus, MIC of EO against *Candida albicans* D-6, *C. tropicalis* PE-2 and *C. utilis* BMS-65 were in the range of 312–156 μg/ml, and if EO were added to the surfactant solution, their MIC decreased to 9.7–39 μg/ml.

In addition to antimicrobial activity, essential oils have the ability to degrade biofilms. The mechanism of biofilm degradation under the activity of EO is associated with the presence of phenolic terpenoids (thymol, carvacrol) in their composition. Our studies have shown that in addition to synergistic antimicrobial action, a mixture of *N. vaccinii* IMV B-7405 surfactants with of cinnamon and lemongrass EO was effective for the degradation of yeast biofilms. The highest degree (43–60%) of degradation of *C. albicans* D-6, *C. tropicalis* PE-2 and *C. utilis* BMS-65 biofilms was observed under the influence of microbial surfactants and essential oils at a concentration of 300 μg/ml. The use of a mixture of surfactants and EO in a ratio of 1:1 was accompanied by an increase in the degree of biofilms degradation to 70%.

Conclusion

Therefore, our own research is one of the first to demonstrate the synergistic antimicrobial activity of essential oils with microbial surfactants.

CO-CULTIVATION OF MICROORGANISMS AS A FACTOR OF INCREASING ANTIMICROBIAL AND ANTIADHESIVE PROPERTIES OF SYNTHESIZED METABOLITES

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Introduction

Antibiotics are one of the most common products on the pharmaceutical market; their productive capacity in the world is more than 100 thousand tons costs more than 20 billion $. They are used in many branches of industries, but because of their distribution in the modern world, there is a problem of the emergence of new resistance mechanisms in microorganisms, which leads to the emergence of multiresistant strains that are capable of forming biofilms and are not sensitive to several antibiotic substances.

Today, scientists consider antimicrobial peptides, bacteriocins and surfactants of microbial origin as an alternative to antibiotics and chemically synthesized biocides. Microbial surfactants are secondary metabolites that are synthesized in the form of complex of similar compounds. Their composition and ratio are depending on cultivation conditions and can change in different cultivation conditions. Obtaining a product with a certain activity is an important condition for the use of such compounds. In recent years, the reports about the possibility of regulating the antimicrobial activity of surfactants in the presence of the culture medium competitive microorganisms have begun to appear. At the Department of Biotechnology and Microbiology of the National University of Food Technologies it was found that the addition of competitive microorganisms (*Escherichia coli* IEM-1 and *Bacillus subtilis* BT-2) in *Nocardia vaccinii* IMV B-7405 cultivation medium was accompanied by increasing of surfactants antimicrobial activity.

The purpose of this work is to study the ability to destroy biofilms for the action of surfactants synthesized by *N. vaccinii* IMV B-7405 and *Rhodococcus erythropolis* IMV Ac-5017 in the presence of yeast and bacterial cells, respectively

Materials and methods

The cultivation of *N. vacinnii* IMV B-7405 and *R. erythropolis* IMV Ac-5017 was carried out in a liquid medium with glycerol and ethanol, respectively. Live and inactivated yeast cells (*Candida tropicalis* PE-2 and *Candida utilis* BVS-65) and bacterial cells (*B. subtilis* BT-2 (vegetative and spore culture), *E. coli* IEM-1) were added into the medium at the beginning of the process and in the middle of the exponential growth phase of surfactants producers. Surfactants were extracted from supernatant by the Folch mixture. The degree of destruction of the biofilm was determined by spectrophotometric method.

Results

It was established that the addition of inducer cells into the culture medium of *N. vaccinii* IMV B-7405 and *R. erythropolis* IMV Ac-5017 was accompanied by the synthesis of surfactants, which effectively destroyed bacterial biofilms. Thus, the degree of *E. coli* IEM-1 and *B. subtilis* BT-2 (vegetative and spore cells) biofilms destruction for the action of surfactants (6.25–25 μg/ml) synthesized in the presence of living and inactivated cells *C. utilis* BVS-65 and *C. tropicalis* PE-2 in the medium was averaged 73–95%, while surfactants obtained on a medium without inductors could destroy test cultures biofilm by 39–60%.

Similar patterns were observed while studying the degree of destruction of bacterial biofilms for the actions of surfactants, synthesized by *R. erythropolis* IMV Ac-5017 in the presence of bacterial inducers. The highest degree (58–73%) of *E. coli* IEM-1 and *B.subtilis* BT-2 (spore cells) biofilm destruction was observed using surfactants (12.5–50 μg/ml) obtained by culturing the strain IMV Ac-5017 in the presence of live and inactivated bacterial cells compared with the results (35–42%) obtained for the actions of similar concentration surfactants, synthesized without inducer cells.

Conclusion

The data obtained indicate the possibility of regulation of ability to destroy biofilms by microbial surfactants.

PRECONDITIONS TO THE DEVELOPMENT OF A HERBAL MEDICINAL PRODUCT WITH A DESINSIBILIZING ACTIVITY

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Introduction

Currently, there is an annual increase of allergopathologies worldwide mainly caused by adverse environmental conditions, changes in the body's immune reactivity, and poor nutrition. According to statistics, 20% of the population today suffers from various allergic diseases. The results of studies on epidemiology of allergic diseases indicate to their wide spread and steady growth.

First-generation antihistamines, classic low selectivity histamine (H1) receptors blockers with a pronounced sedative effect (such as diphenhydramine, chloropyramine, clemastine), and second generation — relatively selective antagonists of peripheral histamine H_1 receptors (such as loratadine, astemizole, cetirizine) are widely used for treatment of allergic diseases. However, it should be noted that H_1-blockers have a number of undesirable effects, for example, the first generation drugs have a sedative effect, and those of the second generation are not recommended for use in case of hepatic and/or renal diseases, which, of course, limits their use.

Therefore, it is relevant to create anti-allergic drug products based on herbal drugs (HDs). A group of combined newgalenic herbal medicines is particularly promising, they can be considered as an alternative and/or addition to the main treatment. It is supported by the practice of using HDs and medicines based on it, an increase in their use due to availability, relatively low toxicity, and the possibility of a long-term use almost without any side effects.

Research objective

To study preconditions to the development of a combined herbal medicinal product with a multifunctional action for treatment of al-

lergic diseases accompanied by immune dysfunction, to identify possible stages of the pharmaceutical development of a herbal medicinal product (extract).

Materials and methods

Literature review on HDs are used to treat allergic and related diseases. Collection of information for the development of a combined herbal medicinal product (HMP), including the study of the possibility to use recommendations on pharmaceutical development.

Results

Herbal products, due to the presence of various biologically active substances, can have a gentle effect on the body. They have an effect on various aspects of the complex etiopathogenesis of allergies, and restore disturbed immune response functions. In this regard, it is promising to develop a combined HMP with a multifunctional action for treatment of allergic diseases accompanied by immune dysfunction, which may include plants with a bactericidal, anti-allergic, and anti-inflammatory activity (for example: blackberry, wild pansy, mullein, elder, burdock, etc.), with antispasmodic and anti-sclerotic effect (for example: hazel, clover, birch, dog rose, ginkgo, asparagus, etc.), with reparative and immunocorrective effect (for example, licorice, ginseng, elecampane, aloe, echinacea, etc.). According to literature data, these types of HDs do not cause side effects such as drowsiness, adverse effects on the liver and/or kidneys, etc. However, it should be taken into account that many plants themselves are often allergens (for example, pollen of a flowering plant). Therefore, the main task of the search is a composition of HDs with biologically active substances, which increase the nonspecific resistance of the body, normalizing the immune status.

According to Guidelines «Medicines. Good manufacturing practice. ST-N of the Ministry of Health of Ukraine 42-3.0: 2011», pharmaceutical development is «comprehensive experimental studies aimed at the scientific justification of the drug composition in a dosage form, manufacturing process and its control, the choice of packaging materials, as well as the study of physical-chemical, biological and microbiological properties. It is necessary to carry out these studies during the product lifecycle in order to create a high-quality drug, its registration, and quality assurance in mass production». The purpose of the pharmaceutical development is to create a drug of the appropriate quality and constantly release products with specified functional characteristics. It is also important to understand that quality cannot be fully verified in the product; quality should be included into its development (built-in quality).

If, in case of development of drugs from a chemical substance, everything described in the Guidelines is quite clear, in case of herbal medicines, certain difficulties may arise. So, it is never possible to procure or purchase HDs (the initial substance for the production of HMP) with the same content of markers or active substances. However, it is possible to control the process of their extraction and processing. Therefore, the stages of the pharmaceutical development in case of the proposed combined HMP, we are presented as follows:

1) Study of the quality of the initial HDs (incoming control).

The quality of the initial HDs is confirmed by pharmacopoeial methods, pharmacognostic, chemical, technological, biological research methods, which play a critical role in the pharmaceutical development.

2) Development of the composition of the proposed HMP.

The proposed composition will be represented by a mixture of HDs (see above), which selected on the basis of literature data on effectiveness of the use of antiallergic, anti-inflammatory, and immunocorrective properties in the pharmacotherapy of allergic diseases, confirmed by screening pharmacological studies.

3) Development of the production technology (laboratory schedule with possible scaling), determination of critical parameters of the production technology.

It is planned to develop laboratory schedules for obtaining of a HMP with a desensitizing effect in the form of a powdered HDs species for packaging in a box and filter bag, as well as a mixture for extemporaneous preparation in a pharmacy. In addition, a scheme for production of a dry extract with specified standardization criteria for manufacture of various dosage forms (tablets, capsules, etc.) will be developed.

4) Development of quality control methods for intermediate products and HMP which will be unified for any control point of the technological process (analytical support of the production process).

5) Study of the pharmacological activity of the obtained HMPs.

6) Stability study of all HMPs.

Conclusion

A detailed literature analysis of herbal medicinal products was carried out to develop a combined herbal medicinal product with a multifunctional action for treatment of allergic diseases accompanied by immune dysfunction. The relevance of its development is indicated.

It is shown that pharmaceutical development is an integral component of the quality and effectiveness of the HMP. The stages of the pharmaceutical development of the intended HPMs are demonstrated.

ANALYSIS OF PHARMACOPEAN AND TRADE NAMES OF CARBOMERS

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Introduction

Carbomers today are the main auxiliaries used to develop gel medicines. There are over 40 brands under which carbomers are manufactured for use in various industries. However, not all carbomers are allowed to be used in pharmaceutical drug development due to their chemical purity. Carbomers were first described in the US Pharmacopoeia/National Formulary (USP/NF), subsequently in the Pharmacopoeias of Europe (Ph. Eur), Britain (BP), Japan (JPE). Each pharmacopoeia gives different names to carbomers, classifications, and requirements that pharmaceutical carbomers must meet, which leads to confusion in terminology. The trade names of carbomers, which differ from the pharmacopoeial names, and which are not mentioned in all pharmacopoeias, also exacerbate the situation of misunderstanding. Moreover, the State Pharmacopoeia of Ukraine does not contain a monograph on carbomers.

Research objective

Analysis of pharmacopoeial monographs on carbomers of different countries.

Methods of the study

Pharmacopoeial data were used as materials: US (USP/NF), Europe (Ph. Eur) and Japan (JPE). In the preparation of the material used methods of information retrieval, systematization of theoretical and practical material, comparative and descriptive generalization.

Results

Carbomers are a group of synthetic high molecular weight polymers of acrylic acid, crosslinked with allylic ether of sucrose or pentaerythritol. Carbomers are divided into 5 groups, the main difference between the polymers is related to the type of substituent and the density of crosslinking, as well as the presence of hydrophobic comonomers: Carbopol™ homopolymers, Carbopol™ copolymers, Carbopol™ interpolymers, Pemulen™ polymers, Noveon™ polycarbophil. The manufacturer of carbomers, Lubrizol Corporation, identifies the group of «pharmaceutical carbomers» as toxicologically safe to use.

USP (32)/NF (27) contains the monographs Carbomer Copolymer, Carbomer Interpolymer, Carbomer Homopolymer, Carbomer 934, Carbomer 934P, Carbomer 940, Carbomer 941, Carbomer 1342.

The Pharmacopoeia of Europe (Ph. Eur. 8) has the Carbomers monograph of a general nature and places requirements for carbomers on such parameters as the presence of free acrylic acid (not more than 0.25%, liquid chromatography), benzene (not more than 2 ppm, gas chromatography), heavy metals (not more than 20 ppm), sulfate ash (not more than 4% in 1 g of sample), and weight loss on drying, which should not exceed 3% (1 g of sample). In the list of Japanese Pharmaceutical Excipients (JPE), Carbopol™ homopolymers are designated as carboxyvinyl polymer. The European Pharmacopoeia monograph, unlike USP/NF and JPE, covers only polymers that have been manufactured without the use of benzene.

The USP/NF Carbomer Copolymer monograph is represented by the Pemulen™ TR-1 NF and Pemulen™ TR-2 NF products, the Carbomer Interpolymer monograph by the Carbopol™ Ultrez 10 NF and Carbopol™ ETD 2020 NF polymers; Polycarbophil monograph — Noveon™ AA-1 USP polymer. The most numerous is the monograph «Carbomer Homopolymer», which is represented by polymers Carbopol 71G NF Polymer, Carbopol 971P NF Polymer, Carbopol 981 NF Polymer, Carbopol 974 NF

Polymer, Carbopol 5984 EP Polymer, Carbopol 980 NF Polymer. USP/NF also includes carbomers polymerized in benzene: Carbopol™ 934 NF Polymers, Carbopol™ 934P NF Polymers, Carbopol™ 940 NF Polymers, Carbopol™ 941 NF Polymers, Carbopol™ 1342 NF NF Polymers. European Pharmacopoeia Ph. Eur. 8 includes carbomers obtained without the use of benzene and corresponding to polymers «Carbomer Homopolymer» by USP/NF, i.e. trademarks Carbopol 71G NF Polymer, Carbopol 971P NF Polymer, Carbopol 981 NF Polymer, Carbopol 974 NF Polymer, Carbopol 5984 EP Polymer, Carbopol 980 NF Polymer.

Conclusion

The normative regulation of carbomeric polymers by Pharmacopoeias of the USA, Europe, Japan and Ukraine was analysed. The difference in the structure of pharmacopoeial monographs on carbomers, in pharmacopoeial and trade names of carbomers has been determined.

APPLICATION OF MICROBIAL PROTEASES IN COSMETICS AND PHARMACEUTICALS

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Proteinases have been used in cosmetics and pharmaceuticals for a fair period of time, but until recently they were mainly enzymes of plant or animal origin — papain, elastase, hyaluronidase, trypsin. They are capable of destroying microbial cells and skin tissues, while having antimicrobial, cleansing and rejuvenating effect. Recently, microbial proteinases have also been used in similar applications: cosmetic giants, such as «Janssen Cosmetics» and «Shiseido» have released a series of cosmetics, which contains subtilisin, generated from *B. licheniformis*. The interest in microbial enzymes is due to their high specific activity and lower cost, comparing to enzymes, obtained from another sources.

The purpose of this work was to establish the possibility of using the enzyme preparation Cytal-R, which is a complex of proteolytic enzymes and other hydrolases with antimicrobial action, in the composition of pharmaceuticals and cosmetics.

Selected objects of research — Cytal-R and cosmetics based on hydrolytes of medicinal plants — were developed at the department of Industrial Biotechnology of Igor Sikorsky Kyiv Polytechnic Institute. The subject of the study was the lytic (antimicrobial) and proteolytic action of these cosmetics and compositions with the enzyme preparation Cytal-R.

The lytic action of the samples was determined by the turbidimetric method based on the percentage degradation of the cell suspension of *B. cereus* and *S. aureus*, and proteolytic activity — by the method of water-alcohol titration. Cytal-R was added to the pharmaceutical and cosmetic compositions at a concentration of 5–60 mg/ml. The pharmaceutical compositions were made using typical excipients of soft and liquid drugs — polyethylene glycol, glycerol, silicon dioxide and others.

The influence of fillers, stabilizers and preservatives in the composition of the pharmaceutical composition on the proteolytic and lytic activity of the enzyme preparation Cytal-R is determined and the possibility of developing a soft finished form with high (90–100%) residual activity of the enzyme complex and storage stability is shown. The established concentration ranges of excipients and their influence on the political activity of Cytal-R allowed us to offer several variants of a soft formulation of antiseptic drug, which is characterized by multifunctionality and complex effect on the wound process. The composition of antiseptic ointment based on polyoxyethylene-400, polyethylene glycols-600 and 1000, proxanol 268 and glycerol was developed.

The use of the enzyme preparation in the composition of functional cosmetics based on plant hydrolytes showed their significant (2–4 times) activating effect in the liquid form and, conversely, inhibition of the antimicrobial and proteolytic activity of the drug in gel form. Thus, the proteolytic activity of the individual tonic, gel and enzyme samples separately was, respectively, 1.5; 1.1 and 5.6 units/ml, and the compositions of tonic enzyme and gel-enzyme — 9.4 and 4.3 units/ml. The high proteolytic activity of the tonic enzyme composition causes the effect of removing dead skin cells and improving their overall condition. The created and researched composition of a tonic enzyme based on plant hydrolysates can be recommended as a cosmetic agent with antiseptic and regenerative action.

Therefore, the paper demonstrates the feasibility of using the enzyme preparation Cytal-R in the composition of pharmaceutical antiseptic and functional cosmetics, as well as proposed compositions of soft dosage forms based on this enzyme.

DEVELOPMENT OF MICROCAPSULES BASED ON CMC AND CHITOSAN BY THE MEMBRANE EMULSIFICATION METHOD

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Introduction

Membrane emulsification makes it possible to produce special high-tech products with a high degree of uniformity of particles. It's a simple method that has received increasing attention over the last 10 years, with potential applications in many fields (Schröder V. et al., 2002). Experimental studies which have focused mainly on investigations of process parameters, such as membrane type, average pore size and porosity, crossflow velocity, transmembrane pressure and emulsifier, are reviewed. With careful choice of these parameters, emulsions with narrow emulsion droplet size distributions have been produced with average droplet sizes ranging between 2 and 10 times the nominal membrane pore diameter. The effects of individual parameters are reasonably well understood, particularly at a qualitative level. Results can be explained by a direct influence of the membrane pore size, diameter and distribution.

Objective

Unlike conventional capsules, microcapsules made on the basis of chitosan and its complexes have the properties of dissolving at a certain pH environment, which prevents the drug from directly contacting the external environment with complete dissolution of the microcapsules. Unfortunately, the number of polymers that can be used to create microcaps is limited. This is due to toxicity, lack of swelling and biodegradation. The purpose of the work is to obtain microcapsules, based on chitosan and its complexes by the method of membrane emulsification, to find out the optimal ratio of polymer systems when microcapsules are created, to investigate the optimal parameters for carrying out membrane emulsification, to study the kinetics of drug release at different temperatures and pH.

Materials and methods

The dispersed phase is chitosan with a molecular weight of 400,000 Da (Fluka, Japan) and CMC with a molecular weight of 450,000 Da (Aldrich). The n-hexane C6H14 (Mr=86; 18 g/mol) was used as the dispersion medium. Track membranes based on polyethylene terephthalate were also used during the experiment; d=0.1 μm (Institute for Nuclear Research, IAID, Russian Federation). Tween80 polyoxyethylene sorbinate monooleate (C64H124O26), Mr=1309.65 (Tokyo Chemical Industry Co. Ltd., Japan) was used as the stabilizing component. Papaverine hydrochloride (1-[(3,4-dimethoxyphenyl) methyl]-6,7 dimethoxyiso-quinoline was used as the medicament). The synthesis of emulsions occurred in a cylindrical cell reactor (type Amicon 8050, Milipore, USA). Mixing of the emulsions is carried out using an IKA® C-MAG HS7 magnetic stirrer. Weighing of components was carried out on electronic laboratory scales (LLC Technoagy, Ukraine). The solution was heated in a water bath, the pH was determined using a pH meter, the concentration was determined on a LABIntech UV spectrophotometer (China) at a wavelength of 310 nm.

Results and conclusions

The optimal parameters of membrane emulsification were determined. The rotational speed of the stirrer was 230 rpm, the volume of the dispersion medium was 15 ml, the volume of the dispersion phase was from 2 to 3 ml, the Tween 80 concentration was 5%, the concentration of the dispersion phase — 0.5%, pressure — 20 kPa. It has been experimentally found that the optimal ratio of polymer systems in which a stable suspension of microcapsules is formed is chitosan-carboxymethylcellulose — 2:1 and 1:1. The DSR method determines the sizes of microcapsules based on the chitosan-CMC complex. An increase in the amount of chitosan has been shown to increase the size of microcapsules from 800 nm to 2000 nm. The release kinetics of papaverine hydrochloride from chitosan-carboxymethyl cellulose microcapsules at 17 °C and 37 °C were investigated.

It is shown that the degree of release of papaverine hydrochloride at room temperature for both samples is low and does not exceed 7%. At a temperature of 37 °C release rate increases and is 70% for the complex chitosan-carboxymethylcellulose =2:1. The activation energies of the release process were calculated and papaverine hydrochloride was found to bind to chitosan-carboxymethyl cellulose, both due to physical and chemisorption. The effect of pH on the release kinetics was investigated. It is shown that for the chitosan-carboxymethylcellulose complex 1:1 pH has little effect on the kinetics of drug release, the degree of release does not exceed 44%. Increasing the amount of chitosan pH significantly affects the degree of release and is 72% at pH 7.

Schröder V., Behrend O., Schubert H. (2002) Effect of dynamic interfacial tension on the emulsification process using microporous, ceramic membranes. J. Colloid Interface Sci., 202(2): 334–340. doi: 10.1006/jcis.1998.5429

BIOGENIC SILVER NANOPARTICLES AS POTENTIAL ANTIMICROBIC AGENTS

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Introduction

In recent years, the biosynthesis of metal nanoparticles (MeNP) has been very popular. This method applies to new green generation processes and is considered environmentally friendly. The biosynthesis of nanoparticles is an alternative to chemical and physical methods.

The aim of the study

Presentation of the latest literature data on the study of synthesis and properties of biogenic nanoparticles. Demonstration of opportunities, feasibility and perspectives for use biogenic silver nanoparticles (AgNPs) in biotechnology, medicine and pharmacy. Actualization of the direction of industrial synthesis of biogenic silver nanoparticles and their practical implementation in these areas.

Materials and methods

The analysis of literary data and scientific-experimental articles on the topic over the last 10 years is carried out. Information on the prospects of using silver nanoparticles as an antimicrobial agent was selected and presented.

Results and conclusions

Nanoparticle biocompatibility, such as reduced metal cytotoxicity, is required for nanoparticles with biomedical applications. Compared to the nanoparticles obtained by physicochemical means, the nanoparticles obtained by biogenic pathways are free from toxic contamination by by-products that attach to the nanoparticles during physicochemical synthesis, which in turn limits the biomedical application of the obtained nanoparticles. The biological synthesis of nanoparticles has several advantages, including rapid and environmentally friendly production methods and the cost-effective and biocompatible nature of synthesized nanoparticles. In addition, there is no need for further stabilizing agents, since the components of plants and microorganisms themselves act as restraining and stabilizing agents (Makarov V.V. et al., 2014).

There are many studies, in which for the biological synthesis of silver nanoparticles are used a completely different organisms, from plants and fungi to algae and bacteria. Many studies in the field of nanoparticle biosynthesis have focused on prokaryotic producers. Due to their enormous amount in the environment and the ability to adapt to extreme conditions, high growth rate, inexpensive cultivation and ease of manipulation, the bacterium is a good choice for research as a promising producer. Growth conditions such as temperature, oxygenation and incubation time are relatively easy to control. An indication of the formation of AgNPs assumed the change in the color of the culture fluid — a dark brown color appears (due to the recovery of Ag^+ ions) (Elbeshehy E.K.F. et al., 2015).

Among the promising producers of biogenic silver nanoparticles are bacteria *Bacillus licheniformis* (Kalimuthu K. et al., 2008), *Bacillus subtillis* (Płaza G.A. et al., 2015), *Acinetobacter calcoaceticus* (Singh R. et al., 2013), *Rhodococcus sp.* (Otari S.V. et al., 2012).

In all cases, AgNPs are formed in the culture medium in the presence of an $AgNO_3$ salt. The cultivation time is within 24–48 hours. Some areas of research involve intracellular synthesis of nanoparticles, which provides an additional stage of extraction. At the same time, despite the additional extraction step, the proposed method is industrially significant since it took only 24 hours to form AgNPs with *B. licheniformis* or *A. calcoaceticus*. However, the approach with the highest potential for industrial bioproduction of AgNPs involves the centrifugation of the live culture and the separation of the supernatant, which is then used for the synthesis of metal nanoparticles by the ion reduction method. The results of the experiments showed the ability to form AgNPs within 5 min, without the need for the stage of cell lysis by using the culture supernatant. This type of extracellular formation is more desirable not only because of the ease of purification, but also because there is an increase in production speed (Das V. et al., 2014).

The results of many experimental studies indicate a higher efficiency of biological nanoparticles compared to synthesized with physicochemical methods. There is evidence of the use of biological AgNPs in many biomedical fields, including anticancer and antimicrobial. For example, Mukherjee and colleagues have demonstrated higher anticancer activity and biocompatibility for drug delivery of biological silver nanoparticles obtained from *Olax scandens* leaves compared to chemically synthesized. In addition, biogenic nanoparticles exhibited high anticancer activity in cancer cell lines A549 (human lung cancer), B16 (mouse melanoma), and MCF7 (human breast cancer). It is also indicated that biogenic AgNPs have greater biocompatibility with the normal cell line of rat cardiomyoblasts (H9C2), human umbilical vein endothelial cells (HUVEC) and Chinese hamster ovary cells (CHO) compared to chemically synthesized nanoparticles, which further indicates the possibility of future use of biological nanoparticles as carriers for targeted drug delivery. Moreover, biological nanoparticles exhibit bright red fluorescence within cells, which can be used to detect the localization of drug molecules within cancer cells (Mukherjee S. et al., 2014).

An interesting and revealing study is the use of a diffusion method that examined the individual and combined effects of AgNPs with 14 antibiotics of seven different classes against seven pathogenic bacteria. AgNPs exhibited antibacterial activity against *P. aeruginosa* and *A. baumannii* (gram-negative) compared to *S. aureus* and *S. mutans* (gram-positive), which was observed in the inhibition zone. The explanation for this is the structural difference in the cell wall composition of gram-positive and gram-negative bacteria. Gram-positive bacteria have a thick layer of peptidoglycan (20–80 nm), which impedes the penetration of AgNPs. There was a wide variation in the activity of antibiotics in the presence and absence of AgNPs, which is interpreted as an increase in the area of inhibition zones. Thus, aminoglycosides showed a slight increase in the range of 0.0–0.8 times, with the exception of gentamicin against *A. baumannii* and kanamycin against *P. aeruginosa*, where an increase of 1.8 times was observed. A significant increase in antibacterial activity was observed for amoxicillin in the presence of AgNPs against *P. aeruginosa*, where a 1.8-fold increase was observed. Penicillin showed a 3.0-fold increase in activity against *S. mutans* in the presence of AgNPs. Vancomycin has been found to have the highest synergistic activity in combination with AgNPs compared to all other antibiotics. For *E. aerogenes*, a 3.8-fold increase in the inhibition zone was observed when vancomycin and AgNPs were combined (Singh R. et al., 2013).

A significant synergistic effect of AgNPs was observed against *A. baumannii*, which showed resistance to seven of ten antibiotics. When exposed to AgNPs in combination with antibiotics, the minimum inhibitory concentration (MIC) was significantly reduced, and bacteria were found to be sensitive to all tested antibiotics except cephalosporins.

Although gram-positive bacteria showed resistance to vancomycin, the addition of AgNPs not only reduced MIC but also made *S. mutans* susceptible to antibiotic treatment. The authors not only demonstrated the synergistic effect of AgNPs on the activity of antibiotics, but also determined its value using the MIC checkpoints provided in the Clinical and Laboratory Standards Institute (CLSI) guidelines. This shows that the administration of a small amount of AgNPs in combination with antibiotics can reduce the required dose of antibiotics by up to 1000 times. In addition, there are real prospects for combating multiple drug resistance among pathogenic bacteria (Singh R. et al, 2013).

To explain the antibacterial activity of AgNPs several mechanisms have been proposed. Such as the release of silver ions from AgNPs, the formation of reactive forms of oxygen, impaired cell morphology, inactivation of vital enzymes, DNA condensation, and loss of DNA replication. The resistance that occurs among some pathogenic bacteria makes the available antibiotics ineffective. The synergistic action of AgNPs and antibiotics increased the antibacterial effect. In addition, the simultaneous action of antibiotics and AgNPs will complicate the development of resistance in pathogenic bacteria, and therefore, this combination therapy can be further explored to develop new formulations of AgNPs in combination with antibiotics.

Given the relevance and perspective of biosynthesis of metal nanoparticles, we plan to begin the study of the biosynthesis of silver nanoparticles by bacteria and to study their biological properties.

DEVELOPMENT OF COMPOSITION AND TECHNOLOGY OF REGENERATING ACTION COSMETIC CREAM

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Introduction

Cosmetic skin care products are of considerable interest not only in cosmetic practice, but also in medical, especially in pathological processes related to dry skin. Moisturizing therapy is a complementary aspect of basic therapy in the treatment of skin diseases accompanied by keratinization of epidermal cells. As a rule, these are severe forms of dermatological diseases (psoriasis, etc.), the treatment of which is long, requires the prescription of drugs of different pharmacotherapeutic groups and which are prone to frequent relapses. In this regard, it is important to create curative and preventive means of moisturizing and regenerating action. Today, medicines in the form of emulsions are widely used in medical practice. The considerable development of scientific research and the high level of achievements allows expanding the range of pharmaceutical emulsions for both oral and topical application.

Research objective

Theoretical and experimental justification of the optimal ratio of the first and second kind emulsifiers for the manufacture of the basis of regenerating and moisturizing action cream with the content of bee products (honey, propolis, royal jelly, drone brood).

Materials and methods

In the development of the composition of the cream used auxiliary substances, which by functional purpose can be divided into 4 groups: components of the oil phase — isopropyl myristate, grape seed oil, olive oil, dimethicone; surfactants — emulsifier of the first kind — PEG-100-stearate; second type emulsifiers — cetostearyl alcohol, glyceryl monostearate, sorbitol stearate; the moisturizing component is glycerol; dispersion medium — water purified.

Samples of the cream base were prepared by mixing both heated phases. The oil and water phases were separately heated to a temperature of 60–65 °C and homogenized at a speed of 3000 rpm.

Results

Emulsions, as complex heterogeneous systems, require the use of surfactants to stabilize the system. Such properties of emulsions as dispersion, structural viscosity, thixotropy, elasticity and plasticity are directly dependent on the type of surfactants, their concentration and manufacturing technology.

To substantiate the composition of the cream base, 3 rows of samples were developed, which were characterized by the presence of different emulsifiers of the second kind. Cetostearyl alcohol was added to the bases of the first row, glyceryl monostearate to the second row and sorbitol stearate to the third row. In each row, the cream bases contained the same amount of isopromyl myristate, grape seed oil, olive oil, dimethicone, glycerol, and purified water. However, the ratio of the concentration of emulsifiers of the first and second kind ranged from 3 to 7%. In total, 15 samples of cream bases were obtained, for which the quality of emulsions was evaluated according to the following indicators: colloidal and thermal stability, dispersion analysis, flow type, thixotropy.

After centrifugation of the first and second sample of the first row stratification of the oil and water phases was observed. All samples were tested for thermal stability at different temperatures. Microscopic examination of the specimens has shown that, in general, the particles of the dispersed phase do not exceed 10 microns in size, but single particles of 15–20 microns are encountered. In the second-row samples, the dispersed phase is unevenly distributed over the volume of the emulsion. In the first and third row samples, the oil phase droplets are homogenous in size and are uniformly distributed. A rheological behaviour study was performed for all samples. The type of emulsifier of the second kind influences the profile of rheological behaviour and consistent properties. All samples have a plastic flow type. Second row specimens are most susceptible to mechanical deformation. The most resistant to deformation are the first row specimens.

Conclusion

For further research on the development of the composition of the cream one sample was taken from each row containing 5% emulsifier of the first and second kind.

STUDY OF THE PHARMACOTECHNOLOGICAL PROPERTIES OF HEALTH IN GUM CHEWING BASE AND ITS MIXTURE WITH GLYCINE AND MAGNESIUM CITRATE

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Introduction

Workplace stress attributed to the 21st Century major diseases by International Health Organization and has become the topic of a report by the International Labor Organization in 2016. Moreover, depression and anxiety affect the course and severity of bowel disease. K. Brajovic's research has identified the importance of stresses caused by acute and chronic life events, its impact on coronary heart disease. According to the results of previous marketing studies, the development of a sedative medical chewing gum is very perspective.

Aim

To study the pharmacotechnological properties of chewing gum base and mixtures of active pharmaceutical ingredients (API) in order to develop a rational sedative medicated chewing gum production technology.

Materials and methods

A mixture of magnesium citrate and glycine, chewing basic Healthingum-01 and was chosen as the studied substances.

Results

It is established that the basis of Healthingum-01 requires the use of vibration for fluidity studies and has a result of 8.15 s/100 g. The angle of natural repose is 29 degrees, the bulk density before shrinkage is 0.625, and after shrinkage is 0.694. The mixture of API has a fluidity of 26.15 s/100 g, the angle of natural repose 44 degrees, the bulk density before shrinkage 0.625, and after shrinkage 0.820. The compressibility rate for the Healthingum-01 base and API mixture is 11% and 31%, respectively.

Conclusion

The obtained results do not allow us to conclude on the most effective technology for the production of medicated chewing gum. Therefore, the next stage of our work will be to conduct additional studies mixture of API with base.

QUANTIFICATION OF ASARONE IN ACORUS CALAMUS BY RP-HPLC

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Introduction

One of the main requirements for medicines is their safety. The absence of serious and unpredictable side effects or side effects is often considered one of the benefits of using herbal remedies. However, not all herbs and preparations based on them are always safe. Often, the biologically active components of a plant may include sub-

stances that, when accumulated at a high concentration, can lead to severe side effects. One such substance is asarone. Studies have shown that at high concentrations, azaron, and especially its β-isomer, have mutagenic and teratogenic effects. Asarone is considered one of the main components of the essential oil of the rhizomes of the *Acorus calamus* L. Due to the toxicity, the Committee for Medicinal Herbs of the European Medicines Agency recommends that you use raw materials that are devoid of asarone (*Acorus calamus var. Americanus*), or that it contains in small quantities (*Acorus calamus var. Calamus*), subject to a maximum concentration limit. Also in the monographs «Kalmuswurzelstock» DAC and the monograph «Kalmuswurzel» in the Austrian Pharmacopoeia regulates a maximum β-asarone content of not more than 0.5%. According to the requirements of the State Pharmacopeia of Ukraine (SPhU) 2.3 monograph «Acorus Calamus rhizomes», the content of asarone should also be no more than 0.5%.

Research objective

Determination of the quantitative content of asarone in the raw material of acorus calamus by high-performance liquid chromatography and validation of the method for introduction in SPhU.

Materials and methods

Plant materials of calamus were collected in summer from Kharkiv region in 2018 years. All collected samples were dried at 25 °C. Before analysis the material was grounded to obtain a powder.

Apparatus. The chromatographic determination was carried out on a ProStar (Varian, USA) chromatograph under the following conditions:

Test solution. Place approximately 0.2 g (accurate weight) of the powdered of samples of calamus in a 100 ml flask, add 60 ml of methanol R, sonicate for 10 min. Filter the solution through a «blue tape» paper filterto into the 100 ml volumetric flask and filled up to the mark with methanol. Filter the solution obtained through a 0.45 µmmembrane filter.

Standard solution. Place approximately 10 mg (accurate weight) α-asarone RS in a 100 ml volumetric flask, dissolve in methanol R, dilute the solution to the volume with the same solvent and mix. 10 ml of the resulting solution add to 100 ml volumetric flask and filled up to the mark with methanol. Filter the solution obtained through a 0.45 µm membrane filter.

Column: Purospher® STAR RT-18e, 0.25 m × 4.0 mm with octadecyl silica gel for chromatography with the sorbent particle size of 5 µm. Column temperature: 25 °C.

Mobile phase: water for chromatography *R* — acetonitrile R (40:60). The flow rate of the mobile phase: 1.0 ml/min. *Detection*: spectrophotometry at the wavelength of 303 nm. The volume of injection: 20 µL. The run time: 15 min.

The chromatographic system is considered to be suitable if the following requirements of the system suitability test are met:

- the symmetry coefficient calculated for the main peak is not less than 2.0;
- the chromatography resolution of α-asarone and ß-asarone is not less than 1.2.

In order to meet the requirements of the chromatographic system suitability the changes in the chromatographic conditions within the limits are permitted by the SPhU, 2.2.46, «Methods of chromatographic separation».

Results and conclusions

Phytochemical analysis of the quantitative content of asarone in the raw material of acorus calamus by high-performance liquid chromatography have been performed. It was determined that the content of asarone in terms of α-asarone in the rhizomes and leaves was almost at the same level (0.31% and 0.33%, respectively).

The method has been validated, according to requirements of ICH and State Pharmacopoeia of Ukraine in terms of linearity, accuracy, specificity, and repeatability.

SYNTHESIS OF ACETAMIDE DERIVATIVES OF 2-(2,4-DIOXO-1,4-DIHYDROQUINAZOLINE AS PERSPECTIVE ANTICONVULSANTS

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Introduction

Epilepsy takes one of the leading places among neurological diseases, which greatly affects life quality and life expectancy. Mortality from epilepsy today is 0.22% of global mortality. Disability-adjusted life years for epilepsy is 0.5% of total, and this disease is in the top 5 of neurological diseases for years of healthy life lost as a result of disability. That is why the expansion of the range of antiepileptic drugs is necessary for improving this situation. One of the most promising directions in modern medical chemistry used for purposeful synthesis of potential new APIs is the structural modification of known drugs in order to improve their pharmacological properties. In view of this we decided to modify the structure of methaqualone, has previously been used in medical practice as a hypnotic and anticonvulsant drug, replacing the aryl substituent on the acetic acid residue, thus combining quinazoline scaffold with one of the major neurotropic mediators, glycine.

Research objective

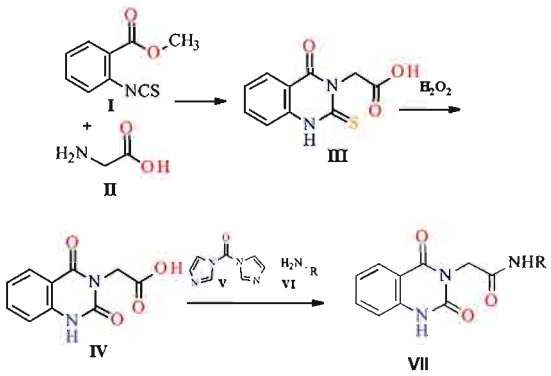
The synthesis of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl)-N-R-acetamides as promising anticonvulsants.

Materials and methods

Reagents manufactured by Sigma-Aldrich, USA were used in this work. The required reagents were purified using standard techniques. The structure and purity of the resulting substances was confirmed by elemental analysis, ^{1}H NMR, ^{13}C NMR spectroscopy and LC/MS. The docking simulations were performed with the SCIGRESS software package (Fujitsu, Fukuoka, Japan (license 742F6852C191)). To carry out the docking studies the proteins type-A γ-ami-nobutyric acid receptors (GABAARs) (code 4COF), carbonic anhydrase II (gene name CA2) (codes 3F8E and 1OEU) domains were chosen as a targets for anticonvulsant activity.

Results

A starting material for the synthesis of a 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)-*N*-R-acetamide ***VII*** systematic series was used 2-(2,4-dioxo-1,4-dihydro-quinazolin-3(2H)-yl)acetic acid ***IV*** which was prepared by oxidation of 2-(4-oxo-2-thioxo-1,4-dihydroquinazolin-3(2*H*)-yl) acetic acid ***III*** hydrogen peroxide heating to 70 °C for 30 min. 2-(4-Oxo-2-thioxo-1,4-dihydroquinazolin-3(2*H*)-yl) acetic acid ***III*** was synthesized by adding methyl 2- isothiocyanatobenzoate ***I*** glycine ***II*** in the presence of triethylamine in a medium of 2-propanol during boiling for 30 min. New derivatives of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl) acetamide ***VII*** were prepared by sequential interaction of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2*H*)-yl)acetic acid ***IV*** with *N,N'*-carbonyldiimidazole ***V*** and the corresponding amines ***VI*** in dioxane.



The target protein structures of 4COF, 3F8E and 1EOU were docked with new quinazoline derivatives, which provided excellent results — the least values of the binding energy.

Conclusion

A synthesis of new derivatives of 2-(2,4-dioxo-1,4-dihydroquinazolin-3(2H)-yl) acetamide was performed with good yields by amination of 2-(2,4-dioxo-1,4-dihydro-quinazolin-3(2H)-yl)acetic acid in anhydrous dioxane at CDI presence. A novel approach for synthesis key intermediate — 2-(2,4-dioxo-1,4-dihydro-quinazolin-3(2H)-yl) acetic acid has been developed. Docking studies confirmed the potential of screening for anticonvulsant activity.

MODERN DESIGN OF MANUFACTURING AND DESIGN OF EXPERIMENTAL RESEARCH ON SELECTION OF THE COMPOSITION AND TECHNOLOGY OF TABLETS MEDICINAL FORMS

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Introduction

The article deals with the aspects of substantiation and design of choice of excipients in the industrial production of tablets dosage forms. The basic methods and technologies of industrial production of tablets are determined. Examples of innovative tablets forms in the industrial pharmaceutical production.

The tablet formulations are solid dosage forms made by extrusion of medicinal substances, mixtures of medicinal and auxiliary substances or moulding of special masses, granulate production and subsequent pressing intended for internal, sublingual, external or parenteral use (Shipar A.H., 2015).

The first information on the pressing of powders dates to the middle of the XIX century. In 1844, in England, was patented for the preparation of potassium hydrocarbon tablets by pressing. Two years later, tablet production was established in the United States, France, Switzerland and Germany. In Russia, the first tablet production was opened in 1895 at a military-drug factory harvested in St. Petersburg. For the first time, tablets as a dosage form were included in the Swedish Pharmacopoeia in 1901. Currently, tablets are widespread and make up about 80% of the total dosage forms (Kotvitska A.A., Kostiuk V.H., 2016).

The vast majority of tablets are obtained by compression and only 1–2% of the total production of the tableted preparations is prepared by the method of moulding. Trituration tablets are made when it is necessary to obtain micro tablets (diameter 1–6 mm) from an explosive drug that cannot be pressed (for example, nitroglycerin tablets).

Tablets made by pressing method have different shape, size and weight. The most common in tablet manufacturing design is a circular shape with a flat or biconvex end face. The size of the tablets ranges from 3 to 25 mm in diameter (most often produce tablets with a diameter of 7 to 14 mm). Tablets over 9 mm in diameter have a single bar or two bars perpendicular to each other, allowing the tablet to be divided into two or four parts, and thus to vary the dosage of the drug. Tablets over 25 mm in diameter are called briquettes. The weight of the tablets (from 0.05 to 0.6 g) is determined mainly by the dosage of the drug substance, but its excipients, which are usually included in the composition, also exert some influence on it.

In the modern pharmaceutical industry, multilayer prolonged and modified release tablets are distinguished as an innovative form, allowing to combine substances that are incompatible with physicochemical properties, to prolong the action of medicinal substances, to regulate the sequence of their absorption in time. The manufacture of multilayer tablets, each layer of which contains the specified medicinal substances, occurs on tablet machines with multiple loading of the granulate.

Retard tablets — tablets with prolonged (periodic) drug release. Typically, they are micro granules with a drug substance, surrounded by a biopolymer matrix (base); Layer or micro granules dissolve layer by layer, releasing another portion of the drug. Rapid Retard Tablets are two-phase release tablets containing a rapid-release, sustained-release micro granules mixture.

Also an innovative form are frame tablets, which consist of excipients that form a continuous mesh structure (matrix) into which the drug substance is incorporated. Frame tablets are prolonged drugs, they do not break down in the gastrointestinal tract. The drug substance is released by washing them. Depending on the nature of the matrix, it can swell and slowly dissolve or retain its geometric shape throughout its stay in the body and be excreted unchanged in the form of a porous mass in which the pores are filled with fluid.

By pressing or coating, pellets are obtained, which cover the sweat with a shell.

Coated and uncoated tablets contain special excipients or obtained with a special technology that allows you to program the rate or place of release of the drug.

Tablets coated with a coating of one or more layers of excipients of natural or synthetic origin, sometimes with the addition of substances that form the coating of medicinal or surfactants. Depending on the composition and method of application, there are coatings: drained, film, extruded; depending on the environment in which the coating should dissolve: gastro-soluble and enteric.

Intestinal tablets (gastrointestinal tablets) — tablets that are resistant to gastric juice and release medicinal substance or substances in intestinal juice. Obtained by coating the tablets with a gastro-resistant coating (enteric-soluble tablets) or by pressing granules and particles pre-coated with a gastro-resistant coating or pressing drugs in admixture with a gastro-resistant filler (duruls).

Tablets that are stable in gastric juice and release drug or substance in intestinal juice. Intestinal tablets are obtained by coating the tablets with the enteric coating or pressing the granules and particles coated with the enteric coating or pressing the drugs in admixture with an acid-resistant excipient.

Tablets coated with film — tablets coated with a thin film (film), which is less than 10% by weight of the tablet. The film coatings can be soluble in water (from solutions of natural cellulose, polyethylene glycols, gelatin and gummiarabic, etc.) or insoluble in water, for example, varnishes (from some high molecular weight compounds) (Shah K.R. et al., 2008; Popova M.E., Leshchishin M.M., 2019).

Depending on the qualitative composition of the active pharmaceutical ingredients (AFI) and the purpose of the application, the selection of excipients and create a tablet form design, scientifically substantiate the manufacturing technology and process steps to obtain a quality pharmaceutical product (Fini A. et al., 2019).

Of particular importance is the design of the package of tableted drugs. The packaging of the tablets should provide ease of use, protection of the tablets from the effects of light, atmospheric moisture, oxygen, microbial contamination, mechanical damage during transportation and storage, as well as effectively combine the informative and aesthetic components of the packaging.

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DRUG DEVELOPMENT MODEL WITH ANTI-BACTERIAL AND IMMUNE PROTECTIVE EFFECT FOR THE TREATMENT OF PURULENT AND SEPTIC DISEASES

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Introduction

Today it is relevant to create pharmaceuticals and technologies to struggle against purulent-inflammatory processes in conditions of immunodeficiency and immunosuppression of the organism. Also, due to development of the industry and the need to provide the market with new pharmaceuticals, it is relevant to study the development of the production of the new drugs, including antibiotics combined with other substances for the strengthening of antibacterial action and to ensure the stability of the body.

Staphylococcus aureus is a widespread and persistent human pathogen that causes a remarkable range of community-acquired and nosocomial diseases in humans and animals.

The extracellular adherence protein (Eap) of *Staphylococcus aureus* participates in a wide range of protein–protein interactions that facilitate the initiation and dissemination of *Staphylococcal* disease. Depending on the *S. aureus* strain, the mature Eap molecule is ≈50–70 kDa. The SERAM extracellular adherence protein (Eap) is nearly ubiquitously distributed among *S. aureus* strains and appears to function as a virulence determinant in animal models of chronic *S. aureus* infection. In addition to it's well-established roles in promoting adhesion-based processes such as bacterial aggregation and invasion of eukaryotic cells, Eap has also been shown to interfere directly with complex, signaling-dependent events such as leukocyte recruitment and both wound healing and angiogenesis. However, the structural adaptations and biochemical features of Eap that allow specific interactions with so many different ligands remain largely unexplored.

It is important to establish previously discovered signaling mechanisms of molecular interaction of EAP with cells of the immune system and launching the production of protective cytokines (Liubchenko G.A., 2012).

Nowadays are widely used antibacterials with unique properties — vancomycin and gentamicin. Vancomycin is a tricyclic glycopeptide natural antibiotic obtained from *Amycolatopsis orientalis* the spectrum of action of which applies to gram positive bacteria: *Staphylococcus spp.*, including *Staphylococcus aureus* and *Staphylococcus epidermidis*, incl. methicillin-resistant strains; *Streptococcus spp.*, incl. *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus agalactiae*; *Enterococcus faecalis*, *Listeria spp.*, *Clostridium spp.*, *Corynebacterium diphtheriae*, *Actinomyces spp.*, *Bacillus spp.*, *Lactobacillus spp.* Vancomycin doesn't inhibit gram negative bacteria, mycobacterium, fungi, viruses, protozoa. The bactericidal action is to inhibit bacterial wall synthesis by inhibiting the polymerization of glycopeptides and selectively inhibiting bacterial RNA synthesis. The drug is effective as antibacterial therapy in the surgical treatment of purulent processes caused by *Staphylococci*. Vancomycin is successfully used for the treatment of patients with diphtheroid endocarditis, and in combination with aminoglycosides or rifampicin — for the treatment of early endocarditis caused by *S. epidermidis* or diphtheroids, after prosthetic heart valve. In combination with aminoglycoside antibiotics, vancomycin exhibits a synergistic effect against many *S. aureus* strains. Also used in the treatment of sepsis, bone and joint infections, lower respiratory tract infections, skin and soft tissue infections. Gentamicin is a aminoglycoside with broad bacteriocidal activity against many aerobic gram negative and some aerobic gram positive organisms: *Escherichia coli*, *Proteus spp.* (indolpositive and indolnegative), *Pseudomonas aeruginosa*, *Klebsiella spp.*, *Enterobacter spp.*, *Serratia spp.*, *Citrobacter spp.*, *Salmonella spp.*, *Shigella spp.*, *Staphylococcus spp.* (including penicillin- and meticilin-resistant strains). The following microorganisms are generally resistant to gentamicin: *Streptococcus pneumoniae*, most other types of streptococci, enterococci, *Neisseria meningitidis*, *Treponema pallidum* and anaerobic microorganisms such as *Bacteroides spp.* or *Clostridium spp.* Like other aminoglycosides, gentamicin is thought to act by binding to bacterial ribosomes (30S) and inhibiting protein synthesis. Nevertheless, gentamicin is considered bacteriocidal as well as bacteriostatic. Gentamycin sulfate is used to treat infections caused by sensitive pathogens, such as lower respiratory tract infections, complicated urogenital infections, bone and joint infections, including osteomyelitis, skin and soft tissue infections, infected burm wounds, abdominal infections (peritonitis), central nervous system infections, including meningitis in combination with β-lactam antibiotics, septicemia, bacterial endocarditis, pelvic inflammatory disease and pneumonia.

Research objective

The combined effect of vancomycin or gentamicin antibiotic with the inducer of the production of protective cytokines by cells of the immune system was studied. Potential effects were carried out using a biopolymer of natural origin (EAP), highly purified by ion exchange chromatography and gel filtration and a biochemically characterized staphylococcal adhesive protein EAP (mM 70 kDa), as described by G.A. Liubchenko (Taras Shevchenko Kyiv National University).

Materials and methods

The studies were conducted on mice of the CBA line (weighing 18–22 g), which were kept under standard vivarium conditions. Experimental animals were divided into groups depending on the active ingredient used. Antibiotics were injected to animals according to the instructions, and the EAP was administered intramuscularly at a dose of 1 mg/kg. The bactericidal activity of the antibioticogram was evaluated by diffusion method using standard disks. Microorganisms were grown on meat-peptone agar (MPA) and after 20–24 hours the diameter of the staphylococcal growth suppression zones was determined. Phagocytic activity (FA), cytotoxicity index (IC) of phagocytic cells was also used, using methicillin-resistant *Staphylococcus aureus* (MRSA) Cowan-1 as the object of phagocytosis, producing the main pathogenicity factor of staphylococcal protein A and *Staphylococcus aureus* strain 209 (Collection of microorganisms of the Department of Microbiology and Immunology, Taras Shevchenko Kyiv National University).

Results

Combination of vancomycin or gentamicin with biopolymer significantly increased the bactericidal effect of antibiotics in relation to the isolated *Staphylococcus*. By assessing bactericidal activity by antibioticogram by diffusion method, by determining the surrounding growth lag delays, dose-dependent growth inhibition of both strains of staphylococci was investigated, fluctuating between 10–25 mm. Increasing the area of inhibition of growth requires further detailed study. The dynamics of changes in phagocytic activity (FA) and the index of cytotoxicity (IC) of macrophages and neutrophils in animal groups after the introduction of combined medications was also studied. There was observed a dose-proportional increase in FA and IC in the groups of animals receiving an antibiotic in combination with protective cytokine inducer. Such a dosing effect is obviously due to the effect of investigated cytokines on membrane processes in lymphocytes and phagocytic cells, which leads to their activation, signaling of cytokine production, as shown in earlier studies. When combined introduction of antibiotic and staphylococcus biopolymer EAP intramuscularly, the results were also evaluated by FA and IC. The dose-dependent changes of the investigated parameters, indicating the potentiating effects of this biopolymer, the launch of mechanisms of immune-regulating influence, stimulation of FA and its regulation by immunocompetent cells, have been established, which leads to immunocorrectional effects.

Conclusion

The conducted studies testify the synergy of action in the combined antibiotics with the inducer of production of protective cytokines — adherence protein. The obtained results may form the basis of the development of combined antibacterial drugs with adherence protein and immunocorrective effects that can be used to treat purulent and septic diseases.

EFFECT OF ULTRASOUND ON EXTRACTION OF INULIN FROM INULA

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Introduction

Nowadays rapid development of pharmacy require the constant researches and investigation of new sources of biologically active com-

pounds as well as development of modern tools for conventional procedures of extraction optimization. In last decade the demand on herbal medicines based on biologically active compound's has highly increased, so the urgent task of scientists is to find a suitable raw materials and optimize the extraction methods for compliance with the requirements.

One of the promising plants possessing a rich source of biologically active compounds and corresponding high spectrum of pharmacological effects are plants of the genus *Inula*. In medicine, the root and rhizomatous of plant are used extensively as diuretic, anti-inflammatory, antimicrobial, antitumor spasmolytic, expectorant, antitussive and digestion improving aids. Such broad spectrum of therapeutic activities is related to the presence of various biological active compounds like inulin (up to 44%) coumarins, flavonoids, fatty acids and saponins, essential oils, etc.

Inulin is natural polysaccharide that is considered as soluble dietary fiber having broad spectrum of activity including prebiotic and immunomodulation properties. It can be used as substitute for sugar or fat, as texture modifier in food products, as vaccine adjuvant in pharmacy as well as multifunctional drug. Application of inulin can results in benefits for metabolism connected with reduction of cholesterol and blood serum triglycerides, it also assist in maintaining of blood sugar levels by reducing lipogenesis, that is of great importance for persons suffering from diabetes. Moreover, inulin may be used as prebiotic due its ability to stimulate the growth of *Bifidobacterium* and *Lactobacillus spp*. in the human colon that results in digestion improving by means of increasing the absorption of mineral ions (Ca^{2+} and Mg^{2+}) as well as other nutrients necessary for human organism. Thus due to such spectrum of health benefits, strategies of inulin extraction from natural source has become a subject of modern pharmacy.

Research objective

The aim of the current research was to optimize the inulin extraction process from *Inula* roots, applying ultrasound and compare efficiency of ultrasound-assisted extraction with traditional method, by inulin yield.

Materials and methods

The roots of *Inula helenium* were used as the raw material for experiment. The roots of *Inula helenium* have been gathered and stored in dry dark place to decrease the moisture content. After that the roots have been additionally dried and grounded using mortar and pestle. The aqueous extraction was carried out as distilled water is considered the best extracting agent for inulin. Thus in experiment the raw material and distilled water were taken in ratio 1:20 correspondingly. After mixing the obtained mixture was given to stand for 30 min, for conventional extraction process. Than the filtration procedure of infusion was carried out through a cloth to remove large root pieces and then through a paper filter to remove smaller particles.

Determination of inulin content in water extracts was carried out by simple and rapid method based on measurement the optical density of the control solution at a wavelength of 670 nm relative to the test solution after coloring reaction with Fehling reagent.

The extraction process was carried out by two methods including conventional one and under ultrasonic irradiation in ultrasonic bath. In ultrasound-assisted extraction at frequency level 800–870 kHz various time of ultrasound treatment of materials were tested to choose the optimal. The ultrasound treatment was carried out in 5, 10, 15, 20, 25 and 30 min, with corresponding measurements of optical density of samples to evaluate the inulin yield.

Results and conclusions

Conventional and ultrasound-assisted extraction of inulin from *Inula* roots was performed and its efficiency has been analyzed. The new approach is application of ultrasound for optimization of extraction process and increasing the yield of inulin. It is well known that during the extraction usually process of a mass transfer takes place, which is characterized by the transition of substances from one phase to another. In case of inulin extraction, the complex process of mass transfer takes place. It involve transfer of substances within particles of raw materials, the transfer within the directly diffusive adjacent layer and the transfer of the substance with a moving extracting agent.

During experiment various time of material treating with ultrasound was carried out and resulted data showed that treatment during 5 min results in increasing of inulin yield by 7.6% in comparison with conventional methods. For 10 min yield increased by 10.5%, for 15 min by 15.4%, for 20 min by 15.7%, for 25 min by 27.9%, for 30 min by 15%. Thus, experiments have showed that applying ultrasound significantly increases the yield of inulin in comparison to conventional methods, however increasing ultrasound-assisted extraction time after 25 min leads to the decrease of inulin yield. Therefore, the optimal regime for ultrasound-assisted extraction to obtain the highest yield of inulin was 25 min at a frequency of 800–870 kHz.

Such results may be explained in terms of ultrasound impact on materials and extraction kinetics. It is well known that ultrasound is low frequency sound and high power that allows transforming electrical energy into mechanical vibrations. Ultrasound-assisted extraction influences primarily on the kinetic of the extraction process; however it also may have an impact on surface area of material, as it may lead to eventual disintegration of larger particles. The ultrasound-assisted extraction is a good choice for extraction processes within biological materials, as it causes the disruption of biological cell walls that correspondingly allows to facilitate the release of target substances.

The base of ultrasound-assisted extraction lays in mechanical effects that intensify the penetration of solvent into cellular materials and due to this highly improve mass transfer. Such effect of ultrasound extraction also may be explained in terms of direct influence on target product- by decreasing the degree of polymerization of inulin under effect of ultrasound that as well lead to intensification of mass transfer. Thus, ultrasound application causes the increase of the local turbulences, and mixing rate of solvent and raw material that highly increase the rate of mass transfer.

To the advantages of ultrasound-assisted extraction belongs the higher yield of inulin in comparison to conventional process. Application of ultrasound also allows to decrease the time of extraction as well as allows to operate at lower temperature than in conventional methods, so it is applicable for thermolabile compounds as well. This procedure is also environmentally safety and non-hazardous for human as well as workers, who perform extraction. It is also important that it is an inexpensive and quite simple process so it may serve as efficient alternative to conventional extraction technique.

However, as any method the ultrasound-assisted extraction also has several limitations that must be evaluated when applying this strategy. Therefore, the main drawback is the difficulties in scaling-up, as it is necessary to choose strictly the mode and time of operation to avoid the danger of overexposure, which may results in destroying of target material. The process is also dependent on particle size. It also may be more energy consuming in comparison with traditional methods. Sometimes in cases of overexposure, active constituents of medicinal plants may be damaged through formation of free radicals and consequently undesirable changes in the drug molecules. Which potentially may cause the violation of therapeutic activities and even emerging of certain adverse effects. Therefore, all advantages and limitations of ultrasound-assisted extraction should be analyzed before applying the process to obtain the best results.

DETERMINATION OF PHYTOCHEMICAL CONSTITUENTS IN HEDERA HELIX L. PLANT RAW MATERIAL AND IT'S THE ANTIRADICAL ACTIVITY DEPENDING FROM PLACE OF GROWING

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Introduction

Hedera Helix L. (English ivy) is widely grown in European countries. Medicines contain ivy leaf extract can be used for treating cold or acute and chronic inflammatory bronchitis accompanied by cough and sputum. Many clinical studies have demonstrated the efficacy and safety of the dosage forms with ivy leaf.

The efficacy of herbal drugs directly depends on the quality of raw materials. Chemical constituents of herbs can be represented by

various groups of components, their determination and quantification become essential to produce effective medicines. According to the fact, that different environmental factors have a great impact on the composition of biologically active substances in the plant, investigation of such influence should be carried out. Oxidative stress is one of the general contributors of pathogenesis caused by chronic obstructive pulmonary disease, due to the imbalance between oxidant and antioxidant systems, which leads to the accumulation of reactive oxygen species, resulting in organ tissue injury. Thus, cough medicines with antioxidant properties may have a significant role in the treatment of airway disease.

The aim of this study is an investigation of composition in ivy leaves collected from different European countries and the estimation of their antioxidant activity *in vitro*.

Research objective

Plant materials of ivy leaf were collected in spring to early summer from different European countries such as Lithuania, Ukraine, Poland, Czech Republic, Austria, Slovakia, Hungary, Greece, and Italy. All collected samples were dried at 25° C after the material was grounded to obtain a powder.

Materials and methods

1.0 g of ivy leaf powder was transferred to a conical flask with 15 mL of methanol and sonicated for 15 min. Extraction was repeated two more times. The supernatant of each extraction was placed into a 50 mL volumetric flask and filled up to the mark with methanol.

The phytochemical investigation has been performed using Shimadzu HPLC system, with a C18 reversed-phase column (250 mmL x 4.6 mml with particle size 5 µm), the temperature was set at 25 °C. For separation 0.1% acetic acid in water as mobile phase A and acetonitrile as a mobile phase B at the flow rate 1.0 ml/min was used, elution has been carried out with following gradient program: 0–8 min, 5–15% B; 8–30 min, 15–20% B; 30–48 min, 40–50% B; 58–65 min, 50% B; 65–66 min, 50–95% B. Identification of components has been done by comparison with retention times and UV-spectrums of standards. The developed method has been validated, according to requirements of ICH and State Pharmacopoeia of Ukraine in terms of linearity, accuracy, specificity, and repeatability.

Estimation of antioxidant (antiradical) activity has been investigated using Waters 2695 chromatography system equipped with Waters 996 PDA Detector. For separation of substances ACE 5 C_{18} (250 mmL x 4.6 mm) was used. Conditions for determination of Hedera Helix L. antioxidant activity was as follows: the solvent A (0.1% acetic acid) and solvent B (acetonitrile), The following evaluation profile was used: 95% A/5% B — 0 min, 85% A/15% B — 8 min, 80% A/20% B — 30 min, 60% A/40% B — 48 min, 50% A/50% B — 58 min, 50% A/50% B — 65 min, 5% A/95% B — 66 min. After applying the postcolumn HPLC-PDA detection system, the mobile phase was entered into a reaction coil through a mixing tee where the reagent (ABTS) was supplied at the same time by a Gilson pump 305. Reaction coils made of TFE (Teflon) of 3 m length, 0.25 mm i.d. was used. The system with the ABTS solution was monitored as follows: temperature set at 50 °C and the flow rate of the reagent was set 0.5 ml/min.

Results

During analysis, various groups of biologically active compounds were found among them polyphenols, flavonoids, and triterpene saponins. As expected the general components for ivy leaf were hederacoside C and α-hederine, although their contents were diverse. Some pharmacological studies have shown that these components are responsible for the ivy leaf usage in pharmacy. Also, chlorogenic acid, neochlorogenic acid, and hyperoside have been detected in all samples also other components were detected, such as gallic, t-cinnamic, caffeic acids, rutin, isoquercitrin, apigenin 7-glucoside, apigenin, quercetin, luteolin, kaempherol, and 6–7 dihyroxyisoflavone.

The HPLC-ABTS assay has been developed and applied for rapid screening and identification of components that have antioxidant activity from the extracts of herbs. The results of the ABTS postcolumn assay in terms of the TEAC values (mg/g) for the measured compounds showed statistically significant differences in antiradical response. Results showed differences in the antiradical activity of the compounds between 21 different places of ivy leaf collecting. The highest value of chlorogenic acid was measured in sample from Ukraine (Kharkiv) 3.81 mg/g in terms of TEAC values, for hyperoside — Hungary (Budapest) 1.49 mg/g, apigenin 7-glucoside — Lithuania (Naujoji Akmene) 6.03 mg/g, also the highest total amount was measured in Ukraine (Kharkiv) 12.42 mg/g.

For estimation of components similarity between all samples hierarchical cluster analysis has been performed. Thus, all 21 samples were separated into 4 groups, since statistically significant differences were inferential. Therefore, the impact of different environmental factors on the variety of phytochemical constituents has been determined. Duration of sunlight, soil, and precipitation have been found as the most significant.

Conclusion

Phytochemical analysis and antiradical estimation of ivy leaf have been performed. The profile of biologically active components varies significantly depending on the place of growth. Such an investigation covering the compositions of bioactive compounds under the influence of environmental factors could be used to predict the amounts of main compounds and obtain effective cough medicines to deliver satisfactory therapy for patients.

SILVER NANOPARTICLES AS MULTI-FUNCTIONAL DRUG DELIVERY SYSTEMS

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Introduction

Nanoscale delivery vehicles can improve the therapeutic efficiency and minimize side effects associated with available drugs, enable new classes of therapeutics and encourage to renew investigations of new biologically active molecules that were previously considered as undevelopable.

Metal-based drug nanocarriers are considered to be efficient therapeutic systems with diverse potentials. For instance, metal nanoparticles including gold (Au), silver (Ag), iron oxide (Fe_2O_3 and Fe_3O_4) and zinc oxide (ZnO) are perspective in therapeutics (antibacterial, anti-fungal and anti-cancer) and diagnostics (MRI, PET, SPECT, fluorescent NCs, quantum dots) application. Among these nanomaterials, nanoparticles and complexes of Ag are known for having antimicrobial and cytotoxic effects. These properties of Ag-NPs have enabled their use in the fields of nanomedicine, pharmacy, biosensing, and biomedical engineering. Ag-NPs prepared for drug delivery are mostly greater than 100 nm to accommodate the quantity of the drug to be delivered. Ag nanoparticles could not be used therapeutically yet, because of high nonspecific cytotoxicity and lack of delivery strategies, but compounding Ag-NPs with other materials may be a good way to overcome these disadvantages.

Research objective

Ag-NPs at drug-delivery systems.

Materials and methods

An overview of recent study researches of silver nanoparticles application and functions at drug-delivery systems.

Results and conclusions

There are numerous reports about researches in the field of medical and pharmaceutical applications of silver nanoparticles. Below some examples are given.

Recently, a group of researchers from Heilongjiang University, Harbin in China designed and tested a vaccine delivery system. The $Ag@SiO_2$ hollow nanoparticles prepared in that study were used as the delivery carrier of Newcastle disease virus DNA vaccine with plasmid DNA containing the F gene designated as pFDNA-$Ag@SiO_2$-NPs. Silver nanoparticles as a delivery carrier cause lower cytotoxicity and can provide full protection for the loaded plasmid DNA, while hollow mesoporous silica nanoparticles as a vaccine carrier can improve both cellular and humoral immune responses. Furthermore, core-shell nanoparticles also have numerous merits, including the lower cytotoxicity, the increased dispersibility, bio- and cytocompatibility, and better conjugation with other bioactive molecules, etc. According to the research, $Ag@SiO_2$ hollow nanoparticles as a carrier for DNA vaccines are not only capable of delivering DNA into 293 T cells, but also are capable of enhancing mucosal immune responses. The results demonstrated that the $Ag@SiO_2$ hollow nanoparticles could be used as a delivery vehicle for the NDV DNA vaccine containing the F gene plasmid DNA. Intranasal immunization of pFDNA-$Ag@SiO_2$-NPs induced stronger humoral, cellular and mucosal immunities and achieved sustained release of vaccine. Future studies may yield more promising results in the use of nanoparticles as carriers of DNA vaccine.

The researchers from Louisiana State University and LSU AgCenter, Baton Rouge, United States demonstrated the potential of silver nanoparticles as photoactivated drug-delivery vectors. Nanoparticles were decorated with thiol-terminated photolabile DNA oligonucleotides. *In vitro* assays and fluorescent confocal microscopy of treated cell cultures show efficient UV-wavelength photoactivation of surface-tethered caged ISIS2302 antisense oligonucleotides possessing internal photocleavable linkers. The results suggest means to achieve light-triggered, spatiotemporally controlled gene silencing via silver nanocarriers, which hold promise as tailorable platforms for nanomedicine, gene expression studies, and genetic therapies.

Ag-NPs can be an alternative therapeutic strategy as drug-delivery systems because they can provide targeting to tumor tissue and accumulation of drugs at desired sites which increases the efficacy of the therapy. In 2018 a group of researchers from Pakistan reported about developing a silver-containing nanocarrier for docetaxel (DTX), which could enable drug's oral administration. DTX is a clinically proven drug against numerous cancers including breast neoplastic malignancy. Commercially available DTX (Taxotere®) administrates only intravenously and has infusion-related side effects and high cost. Nanoparticles based oral delivery may improve the oral bioavailability of DTX. In that study, a polymeric scaffold was first synthesized by grafting folic acid and thiol groups to chitosan (CS) for cancer cell targeting and improved gastric permeation. Silver nanocluster (Ag-NCs) were synthesized *in situ*, within CS scaffold by microwave irradiation. Core-shell nanocapsules (NCPs) were prepared with hydrophobic DTX in the core and Ag-NCs embedded CS in the shell. A significant cytotoxicity synergism was observed for DTX with co-delivery of Ag-NCs against breast cancer MDA-MB-231 cells. Following oral administration, the DTX-Ag-NCPs increased bioavailability due to enhanced drug transport across gut, circulation half-life and mean residence time, as compared to the control DTX suspension. Moreover, 14-days acute oral toxicity of the DTX-Ag-NCPs was performed in mice and revealed no significant evidence of toxicity suggesting the safety and efficiency of the DTX-Ag-NCPs as a hybrid nanocarrier for biocompatible delivery of metal NCs.

The Chinese research team was studying alternative chemotherapeutics for glioma. They used co-delivery of albendazole (Abz), nano-silver and conjugated to bovine serum albumin menthol (MeB). In that study, Abz-loaded MeB-silver nanoparticles (MBS-Abz) were developed by self-assembly of MeB, albendazole, and nanosilver for glioma targeting therapy. According to the study, the nanoparticle entered the brain across the blood-brain barrier (BBB) and specifically accumulated in the glioma region. The study demonstrates that by combining the energy restriction effect of albendazole and nanosilver, as well as the BBB penetration ability of menthol, MBS-Abz achieves superior anti-glioma efficacy and can be an effective strategy for glioma therapy.

A research group that included scientists from the University of Oslo, Oslo, Norway, and the University of Tehran, Tehran, Iran, investigated the anti-cancerous effect of albumin-coated silver nanoparticles. To make a specific targeting of silver nanoparticles as a drug for tumor cells and develop new anticancer agents, this novel nanocomposite was developed. In that study, albumin coated silver nanoparticles (ASNPs) were synthesized, and their anti-cancerous effects were evaluated against MDA-MB 231, a human breast cancer cell line. The cytotoxic properties of ASNPs and their anti-cancerous effects were investigated on the most invasive cell line of human breast cancer and white blood cells as normal cell control. It seems that ASNPs make the specific targeting of SNPs to tumor cells possible, which leads to less toxic effects on non-cancerous cells. The results show higher cytotoxicity against cancer cells over normal cells and the cell death based on apoptosis, and the observation that ASNPs can be used as a chemotherapeutic drug.

In therapeutics outside oncology, researchers from Malaysia have described AgNPs conjugated with anti-seizure drugs (as a drug carrier) against brain-eating amoebae (*Naegleria flowleri*) to treat central nervous system (CNS) infection. Anti-seizure drugs which are known to cross the BBB were attached to the surface Ag-NPs as capping agents. Ag-NPs conjugated with drugs, such as diazepam, phenobarbitone, and phenytoin exhibited overall anti-amoebic activities against both trophozoite and cyst stages. Moreover, significant enhancement of fungicidal activities was shown against both trophozoite and cyst amoebic stages compared to those of the drugs alone. The researchers suggested that a feasible mechanism of AgNPs-based drugs that can penetrate BBB might lie in their ability to bind to the receptors and ion channels on the cell membrane of amoebae.

Silver nanoparticles can be used in drug-delivery as vaccine carriers, photoactivated vectors, to address and enhance the efficiency of antineoplastic drugs. Apart from that, Ag-NPs exhibit anti-amoebic and fungicidal activities.

GENETIC DIAGNOSTIC SYSTEM FOR DETECTION OF BREAST CANCER IN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Breast cancer is the most common cancer among women worldwide. Detection of breast cancer plays a key role in successful treatment and patient survival. Mammographic screening is the main modalities for breast cancer detection today. However, rapid growing of tumors, dense breast tissue or menopausal hormone therapy can reduce the efficiency of breast cancer detection by mammography. So precise diagnosis of breast cancer and evaluation of efficiency treatment of this disease is required.

In the interaction between cancer cells and immune cells, the presence of cancer cells causes immune cells to undergo various phenotypic and functional changes and the affected immune cells kill cancer cells or promote proliferation and metastasis of cancer cells. Thus the analysis of peripheral immune cells might be appropriate to evaluate host immune reaction against cancer cells in addition to analyzing tumor-infiltrated immune cells. Using peripheral blood cells (PBCs) for gene expression analysis is valuable to evaluate disease-associated and drug-response related genes.

Blood samples are easily available, minimally invasive and can be collected at low cost making them an attractive alternative modality for diagnostic purposes. The rationale for using blood as a clinical sample is that breast cancer triggers a response in circulating blood cells, leading to a traceable change genes expression in the whole blood.

Research objective of this study was exploring of the innate immune gene expression profile of PBCs in patients with breast cancer and based on genes expression changes to develop a genetic diagnostic system for early detection of breast cancer in whole blood samples.

Whole blood samples were collected from 30 breast cancer patients and 42 healthy volunteers. The gene expression of PBCs was determined by RT-qPCR.

Using whole blood samples of breast cancer patients and healthy volunteers, we studied expression of reference genes and selected *TBP* gene as a control for normalization relative expression in PBCs. A healthy sample was created using the total RNA of healthy volunteers. We investigated the expression of receptors and its ligands, transcription factors, cytokines, chemokines, interferon-stimulated and prooxidation genes related to the innate immune system in breast cancer and healthy samples. Deregulated expression of a few genes was found out in the blood of breast cancer patients compared to the healthy sample. These results suggested that the expression of innate immune genes in PBCs can be used for diagnostic of breast cancer and evaluation of efficiency treatment of this disease. We developed the molecular-genetic test system for diagnostic of breast cancer that involves the following stages: a sampling of whole blood, PBCs isolation, RNA isolation, RNA analysis, cDNA synthesis, RT-qPCR, data interpretation, diagnosis. For RT-qPCR stage, we developed a genetic testing panel consist of the innate immune genes, are deregulated by breast cancer in PBCs.

We have identified the innate immune genes in whole blood, that classifies breast cancer patients and healthy women with good accuracy. Based on our results, we developed the molecular-genetic test system for diagnosis of breast cancer and evaluation of efficiency treatment of this disease.

ANALYSIS OF AMYLOIDOGENIC POTENTIAL, STABILITY AND FLUORESCENCE PROPERTIES OF INSULINS AND INTERFERONS OF DIFFERENT PURITY

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Introduction

Among medicines with a well-studied spectrum of action, a special place belongs to preparations based on biologically active proteins. A distinctive feature of the compounds of this group is the dependence on the functional activity of the conformational structure molecule. Ensuring the proper assembly of protein, synthesized de novo, and protecting the native structure from the denaturing effect of various physicochemical factors in the process of purification and isolation is a complex and far from solving the problem of protein drug technology. Formation of the native structure and support of the protein molecule in a functionally active form is an extremely complex and dynamic process, mediated by a complex of intra- and inter-molecular interactions. Maintaining the molecular environment of the protein molecule to the conditions of the original biological environment is an essential prerequisite for maintaining an active conformational state. Naturally, changing these conditions leads to a more or less significant shift in the dynamic equilibrium of the conformational states of the protein toward the formation of altered molecular forms that are more appropriate to the changing environment.

Amyloid is a protein with an abnormal structure that is insoluble, capable of aggregation and accumulation in the body, poorly or not performing its functions. Nasal forms of protein drugs can accumulate in the brain, causing Alzheimer's disease or prion damage, while injections can accumulate in the kidneys and liver, disrupting their function. Also, preparations containing protein isoforms can produce a healthy immune response. The development of an immune response to injected drugs not only reduces the effectiveness of their therapeutic action but also threatens the development of unpredictable consequences of autoimmune, allergic, and anaphylactic. Such anomalies attributed to improper protein folding, impaired protein structure upon receipt and storage, replacement of Ser on Pro, Asn on Thr, Ala, and Leu on Tyr in the protein sequence, and interaction with impurities upon insufficient protein purification.

Research objective

We studied recombinant insulins and interferons of different origination and purity.

Materials and methods

The amyloidogenic potential determined by fluorescence detection of amyloid fibrils in association with thioflavin-T. Fluorescence spectra were registered using fluorescent spectrophotometer Jasco-8200. Spectral-luminescent characteristics of free dyes in aqueous buffer studied at room temperature. Fluorescence emission was excited a the maximum wavelength of the excitation spectrum of the corresponding dye solution. Interferons and insulins stability were studied using it's analyzing the thermal stability of the fluorescence quenching — the purity of the samples determined by the ratio of 280/320 nm on the absorption spectra. Fluorescence characteristics determined by measuring the 3D fluorescence spectra.

Results and conclusions

The data obtained indicate that insulins and interferons of different origin, different series, in pure form and in the presence of impurities differ in the content of alpha and beta structural components, thermal stability, the presence of amyloid inclusions, changes in intensity and the ratio of fluorescence maxima at 230 and 275 nm. We have inserted that the addition of auxiliary components and alteration of its amino acid sequence of the protein preparation changes its thermal stability, leads to the appearance of amyloid fibrils, reduces the intensity of fluorescence on the 3D graph. The latter may be related to tyrosine oxidation. From the data obtained, it can be concluded that the addition of auxiliary components and unsatisfactory purification are associated with the risk of forming stable mono and oligomeric isoforms, indeed different from the original molecular form.

BIOLOGICAL ACTIVITY OF SURFACTANTS OF RHODOCOCCUS ERYTHROPOLIS IMV AC-5017

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Introduction

Microbial surfactants (MS) are multifunctional preparations that are synthesized as a complex of compounds. MS due to a complex of unique biological properties (antimicrobial, anti-adhesive activity, including the ability to destroy microbial biofilms) are promising for use in many branches of industries, agriculture, medicine, and environmental technologies.

Producers of glycolipids (trehalosomycolates) are actinobacteria of the genus *Rhodococcus*. Despite the fact that the ability of representatives of the genus *Rhodococcus* to synthesize glycolipids was established in the 70's and 80's of the twentieth century, so far, the main focus is on the study of these surfactants as destroyers of xenobiotics for use in environmental technologies. Currently, there is little information in the literature on the biological properties of surfactants of Rhodococcus, in particular, their antimicrobial, anti-adhesive activity, their ability to destroy biofilms and immunomodulatory properties.

In previous studies, we isolated the *Rhodococcus erythropolis* strain IMV Ac-5017 and developed technologies of surfactant synthesis on a variety of carbon substrates, including industrial and

food waste (technical glycerol and waste oil), but the biological properties of such surfactants have not been investigated.

Research objective

The purpose of this work is to study the antimicrobial and anti-adhesive activity of surfactants synthesized by *Rhodococcus erythropolis* IMV Ac-5017 on different carbon substrates, including industrial and food waste (technical glycerol and waste oil).

Materials and methods

The strain *R. erythropolis* IMV Ac-5017 is the main research object isolated from oil-contaminated soil samples and is registered in the Microorganism Depository of the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

As test cultures in determining the biological properties of MS (antimicrobial, anti-adhesive activity and ability to destroy microbial biofilms) were used bacterial strains (*Escherichia coli* IEM-1, *Bacillus subtilis* BT-2, *Pseudomonas spp*. MI-2) and yeasts (*Candida albicans* D-6, *Candida utilis* BVS-65, *Candida tropicalis* RE-2) from the collection of live cultures of the Department of Biotechnology and Microbiology of the National University of Food Technology.

The strain *R. erythropolis* IMV Ac-5017 was grown in the liquid mineral medium. As carbon source was used: refined glycerol, waste from biodiesel production (technical glycerol), ethanol, refined sunflower oil, waste sunflower oil after frying potatoes, waste mixed sunflower oil after frying meat, potatoes, onions, cheese.

The surfactant concentration in the culture liquid (g/l) was determined gravimetrically after extraction with a modified Folch mixture (chloroform — methanol — 1 M HCl, 4:3:2) from the supernatant.

Antimicrobial properties against bacteria and yeast were determined in liquid medium (suspension culture) monitoring the value of the minimum inhibitory concentration (MIC).

The level of adhesion of test culture cells to abiotic surfaces and the degree of destruction of microbial biofilms were determined by a spectrophotometric method

Results

It was found that the surfactants synthesized on all substrates were characterized by high antimicrobial activity against bacteria and yeast of the genus *Candida* (MIC from 2 to 500 μg/ml). The surfactants synthesized on all substrates showed antibacterial activity, but the level of activity depended on the nature of the carbon source in the culture medium, its concentration and the type of test culture. Thus, the most active against *E. coli* IEM-1 were MS synthesized on ethanol (MIC was 2 μg/ml). A sufficiently high antimicrobial activity against *B. subtilis* BT-2 and *Pseudomonas spp*. MI-2 is established for surfactants synthesized on glycerol (MIC was 62.5 and 31 μg/ml, respectively). With an increase in the concentration of biodiesel production wastes in the culture medium of *R. erythropolis* IMV Ac-5017, the antimicrobial activity of the synthesized MS decreased against all bacterial test cultures.

The antimicrobial activity against the yeast of the genus *Candida* depends on the nature of the carbon source in the culture medium, its concentration and the type of test culture. In this case, the most effective antimicrobial agents were surfactants synthesized on all oil-containing substrates (MIC was 20–160 μg/ml). Also, the replacement of refined oil in the culture medium of the strain IMV Ac-5017 for waste oil was accompanied by the synthesis of surfactants, whose antimicrobial activity increased by 2–8 times. Similarly, in the case of replacement of refined glycerol for biodiesel production wastes in the culture medium of *R. erythropolis* IMV Ac-5017, an increase of the antimicrobial activity of the synthesized surfactants was observed against the yeast.

Experiments have shown that the anti-adhesive activity of the surfactant of the strain IMV Ac-5017, as well as the antimicrobial, depended on the nature of the carbon source in the culture medium, its concentration, the type of test culture and the surface.

The most effective anti-adhesive agents were the surfactants synthesized during the cultivation of *R. erythropolis* IMV Ac-5017 on all oil-containing substrates. Treatment of tile, steel and glass with such surfactant in low concentrations (only 3 μl/ml) made it possible to reduce the adhesion of the test cultures by more than 50%. The increase in the concentration of refined glycerol in the medium was accompanied by the synthesis of surfactants with lower anti-adhesive activity, while in the case of increasing the concentration of waste from biodiesel production in the culture medium cause the formation of surfactants, after treatment of materials with these MS the adhesion of bacterial test cultures decreased (and did not exceed) by 32–55%.

The surfactants synthesized during the cultivation of *R. erythropolis* IMV Ac-5017 on all carbon substrates are capable of degrading biofilms, the extent of which depended on the conditions of cultivation of the producer and the type of test culture.

The most effective preparations capable of destroying *B. subtilis* BT-2 biofilm were MS synthesized on technical glycerol and oil-containing substrates, against *E. coli* IEM-1 biofilm, were surfactants obtained on refined glycerol (destruction of biofilm was 54%) and against *Pseudomonas spp*. MI-2 biofilm were MS synthesized on technical glycerol and waste mixed sunflower oil (destruction of biofilm was 72 and 63%, respectively). It should be noted that the ability to destroy bacterial biofilms was achieved at low concentrations (12–30 μg/ml) of surfactants of *R. erythropolis* IMV Ac-5017.

Further studies have shown that the surfactants of *R. erythropolis* IMV Ac-5017 are also capable of effectively destroy yeast biofilms, although at a higher concentration (500 μg/ml) than bacterial (12–30 μg/ml). It should be noted that the destruction of the biofilms of yeast of the genus *Candida* is an urgent problem of today, as most modern biocides, including some surfactants, are not effective enough.

Conclusion

Therefore, the above data indicate that the surfactants synthesized by *R. erythropolis* IMV Ac-5017 on various carbon substrates, including industrial wastes, are characterized by high antimicrobial and anti-adhesive activity. This combination of biological properties makes MS of the strain IMV Ac-5017 promising for practical use.

From the described in the literature representatives of the genus *Rhodococcus* the researched strain *R. erythropolis* IMV Ac-5017 compares favorably and has the following advantages: in addition to the ability to degrade oil pollution, surfactant inherents biological activity, compared with other known surfactants (rhamno-, amino-, sophorolipids); also, the strain is able to synthesize surfactants on a wide range of substrates, including toxic industrial and food waste.

RESEARCH OF MICROCYSTIN INHIBITORY PROPERTIES FOR THE PREVENTION OF LATE BLIGHT

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The relevance of this problem is due to the massive cyanobacteria development, namely Microcystis aeruginosa, in the Dnipro cascade reservoirs, it is a source of fresh water for 80% of Ukraine population, the solution of which is possible, including by collecting excess biomass and its subsequent use in various national economy sectors: energy (processing for biogas), pharmaceutical (as a source of biologically active substances) and agricultural (as biofertilizers and drugs for the prevention of late blight). Processing of excess cyanobacteria biomass collected during their mass development («flowering») has the following environmental effects:

- compliance with the Kyoto Protocol to the UN Framework Convention on Climate Change (Rio de Janeiro, 1992);
- accession to the Directive 2000/60/EU of the European Parliament and of the Council «On Establishing the Framework for Community Activities in the Field of Water Policy» of 10/23/2000;
- the use of environmentally safe, without significant energy costs, collecting seston methods;
- restoration of the disturbed structural and functional organization of the littoral ecosystems of reservoirs Dnieper cascade (gas balance, hydrochemical regime, reduction of water toxicity, spawning of ichthyofauna, etc.);
- environmental improvement and the population by improving quality the natural, including drinking water.

The object of our research is cyanobacteria (Oxyphotobacteriobionta) or blue-green algae (Cyanophyta) as producers of algotoxins, because it is cyanobacteria that are the dominant agents of the water «flowering», cause massive fish killing and poisoning of animals and humans.

The subject of our research is the inhibitory microcystin properties (cyanobacteria algotoxins), due to which it can be used to prevent late blight of cultivated plants, which are the most common in Ukraine. The subject of development is also the search and study of methods for utilizing cyanides collected during flowering from the reservoir water of the Dnieper cascade and aimed at using their biomass as an additional source of other biologically active compounds: cyanolipid, chromoproteins, in particular phycobiliprotein (red and blue pigments), from which hemoproteins, flavoproteins and phycobilins can be isolated — pigments added to cosmetic compositions improve skin tissue respiration. A separate component of the original research will be devoted to the relevant elements development of industrial technological processes for medical, pharmacological, cosmetic biotechnology.

The purpose of this study is a comprehensive possibility research of using microcystins for the late blight prevention. To accomplish this, we have solved the following tasks: a) a literary review has been carried out; b) material collection, processing and preparation; c) the cells number is determined; g) conducted appropriate microbiological studies. Among the research methods used in the study, the main ones are mathematical (statistical and computer methods), physical (optical and electron microscopy) and microbiological (inoculation, cultivation, transfection, etc.).

The scientific novelty lies in the fact that for the first time it was proposed to use algotoxins (for example, microcystins) for the late blight prevention and treatment of cultivated plants.

Innovative methods, approaches, ideas and hypotheses:

- the idea of using excess aquatic organisms biomass in general and blue-green algae in particular as a biologically active substances source; the idea of using environmentally safety and cost-effective collecting seston ways (free raw materials);
- approach to environmental and the population improvement by improving the quality of natural, including drinking water, due to the BGA removal from the water reservoirs of the Dnieper cascade.

The practical value is due to using of cyanobacteria suspension during their mass development for the production of anti-phytophthora drugs.

Conclusion

1. The expediency of using cyanobacteria suspension, which are algotoxins producers, in particular microcystin, for the late blight prevention and treatment on the example of tomato.

2. The inhibitory effect of microcystins on the Phytophtora infestans fungi colony was proved, and its immunomodulating effect on adult tomato plants was also confirmed. For the first time, it is proposed to use a cyanide suspension during their mass development for the production of anti-phytophthora drugs.

3. A mathematical process model has been created and the time changes of such dependencies have been calculated: substrate concentration from the consumption of micro fungi and degradation of phytophthora colonies under the microcystin influence.

DESIGN AND PERFORMANCE MANAGEMENT OF PHARMACOTECHNOLOGICAL RESEARCH ON THE BASIS OF MATHEMATICAL MODELING

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At the stage of studying the modern design of pharmaceutical development, preclinical and clinical trials of new pharmaceuticals with known and new active pharmaceutical ingredients (AFI) in equivalent doses, in the original, innovative drugs and dosage forms is important to use the method of mathematical modeling of experiments. The role of applying experience of system researches of evidence-based medicine; a designing of research plan in accordance with GLP guidelines for creation of human medicines, and veterinary practice are also enhanced.

The use of modern information content of leading scientific metric databases in the field of medicine and pharmacy contributes to the formation of the management skills of young scientist's justification and management of theoretical and experimental research on the development of innovative and generic drugs and their dosage forms. The basis of applying the methodology of evidence-based medicine in the experimental research of pharmaceutical development is the scientific search for evidence-based information about medicines, its analytical review, selection and systematization, creation of own information resources and content and their transformation into the implementation of the laboratory stage of pharmaceutical development.

The mathematical modeling method is a modern powerful cognitive method and an effective means of solving applied problems. It is based on the application of a mathematical model as a means of exploring real objects, processes or phenomena, and consists in the implementation of a certain sequence of stages. The stages of mathematical modeling are essentially similar to all researchers and are widely covered in the scientific literature. The method of mathematical modeling eliminates the need to make cumbersome physical models associated with material costs; to reduce the time of defining characteristics (especially when calculating mathematical models using computer technologies and efficient computational methods and algorithms).

1. Classification of mathematical models by modeling purposes according to the purposes: descriptive; optimization; managerial.

2. Classification of mathematical models by implementation methods: analytical; algorithmic; linear; nonlinear.

3. In general, the parameters that describe the state and behavior of a simulation object can be divided into the following sets: set of input (managed) effects on the object; set of environmental influences (uncontrolled); set of initial characteristics.

4. Classification of mathematical models by model parameters, depending on the type of used sets, model parameters: qualitative and quantitative; discrete and continuous; mixed.

5. Building models, you often have to deal with a lack of information. In this case the descriptive options of uncertainty of the parameters are possible.

6. Determined — the value of all the parameters of the model is determined by the determined values (ie each parameter corresponds to a specific number or function). This method corresponds to the full definition of the parameters

7. Stochastic — the values of all or some of the model parameters are determined by random variables, given by the probability densities

8. Random — the values of all or some of the model parameters are set at random values given by the estimates of the probability densities obtained as a result of processing a limited experimental sample of these parameters.

9. Interval — the values of all or individual parameters of the model describe the interval values given by the interval formed by the minimum and maximum possible values of the parameter

10. Fuzzy — the values of all or some of the model parameters are described by the function of belonging to the corresponding set.

Statistical models are based on the assumption that the simulated process is random and investigated by statistical methods, in particular Monte Carlo methods. The Monte Carlo method is a numerical method, the basis of which is to obtain a large number of realizations of a random process, which is formed so that probabilistic characteristics (mathematical expectations, probability of some events, probability of falling into the trajectory of a process in some region, etc.) are equal to certain values of the problem (Kornilo I.M., 2015). One of the main features of Monte Carlo simulation is the use of special

computer programs. This is because during the fifth stage of generating random project scenarios, they are repeated 500–1000 times (Ulyanchenko O.V., 2002).

One method for investigating such poorly structured systems is the T.L. Saati (1993) method of hierarchy analysis, which allows the investigation of even very complex systems to a sequence of paired comparisons of appropriately defined components. The analysis method is a closed logical design that provides simple rules to analyze complex problems in all their variety.

It was established (Khomchenko A.N. et al., 2004) that ideas of averaging are present in many numerical methods. In particular, a calculation formula, such a popular method as the finite difference method (MCP), is the arithmetic mean in the case of a square grid, the weighted average in the case of a rectangular grid and an adapted template. The finite element method (ITU) also uses averaging to construct an interpolation polynomial on a single element. In Monte Carlo random walk schemes, we also use weighted averaging. It gives the opportunity to create new models and methods.

It should be generalized that deterministic models imply rigid functional relationships between model variables, and stochastic ones imply random effects on the studied parameters (Leshchinsky O.L. et al., 2003).

The using of information, cloud technologies is effective for systematization of experimental research results in accordance with the design and application of the model in the design of the experiment.

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ANTI-SMOKING LIPSTICK – NEW OPTION INNICOTINE REPLACEMENT THERAPY

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Introduction

Tobacco addiction is a dangerous disease. Nicotine, which is located in tobacco leaves, is a psychostimulant, that causes high level of addiction. Thus, smoking cessation is a long and difficult process, always with withdrawal syndrome.

There are various methods of getting rid of tobacco addiction, such as: psychotherapy, hypnosis, diet, massage and others. Pharmacotherapy is a key point in helping patients. A function of nicotine replacement therapy is to give to human organism a dose of nicotine, without inhaling toxic substances. The main problem in the treatment is the maintenance of the compliance. That is why global pharmaceutical companies are interested in a development of convenient dosage forms. Among medicines containing nicotine and its derivativesare known patches, tablets, lollipops, chewing gums, inhalators. Smoking is often combined with other addictions (drinks, work, computer games, television) to create a «pleasant time spending» scheme. Lipstick is a convenient dosage form that fits perfectly into this system, replacing smoking.

The scientists of Department of Technology of Biologically Active Substances, Pharmacy and Biotechnology of Lviv Polytechnic National University have created a remedy against tobacco smoking in the form of lipstick. Anti-smoking lipstick contain nicotine and it may have aversive effect and form a resistance to smoking.

Research objective

The purpose of this research is to study the physicochemical and pharmacotherapeutic properties ofanti-smoking lipstick, find the ways to improve them, and thus improve the product.

Materials and methods

The used materials were: samples of anti-smoking lipsticks, components of lipstick (waxes, oils, butters, vitamins), extract from nicotine manufacturing, Methods, that were chosen, are the following: metanalysis, organoleptic analysis, physicochemical analysis, biological analysis.

Anti-smoking lipstick (ASL) — is a hygienic productand semisolid preparations for cutaneous application (SSP). SSP are intended for application to skin and mucous membranes for local therapeutic action and/or for penetration of an active compound (AC) through the skin and mucous membranes, and for moisturizing, softening, protective action. The composition of ASL provides the penetration of the AC — nicotine through the skin of the lips (transdermal action) and the mucous membrane of the oral cavity. Therefore, ASL is a soft medicine remedy and has to meet all requirements as for SSP.

According to State Pharmacopoeia of Ukraine (SPhU), Edition 2. Volume 1, general requirements for SSP are relative viscosity (2.2.10), uniformity in dosage forms (2.9.40), microbial purity (5.1.4). The requirements for hygienic lipsticks are given in DSTU 4774: 2007: organoleptic (physical), to quality and quantity of components (chemical). Organoleptic properties are represented by the following indicators: structure, uniform color of the surface, odor, coating ability, pencil condition.

Results

The organoleptic properties of all ASL samples are the following: the samples have a homogeneous, smooth, evenly colored surface; a notable scent of tobacco; easy-to-apply structure; pencil — dense, does not crumble. The amount of nicotine in the sample affects its toxicity and pharmacological activity. Consequently, it is important to accomplish nicotine qualitative and quantitative reactions.

The qualitative and quantitative determination of nicotine was made with the finished extract. The presence of nicotine in the samples was proved by qualitative reactions, according to the requirements: in the reaction with formaldehyde intense dark red color was observed; with a solution of iodine in diethyl ether ruby-red crystals with a dark blue glitter were formed; in the reaction with vanillin appeared intense dark cherry color; with a Dragendorff' sreagent pink crystals were formed. To quantify nicotine in the samples, the classic Guben-Weil method (1967) was chosen.In accordance with this method, distillation of the extract with steam was carried out; to the neutralized (indicator — methyl red) picric acid was added, the picrate was filtrated on a Buchner funnel, washed with picric acid and titrated with alkali (indicator — phenolphthalein), the amount of nicotine in the sample was calculated.

According to the performed experiments, one of the best samples of ASL contain: 1% mass nicotine and,additionally, Shea butter, Coconut butter, Castor oil, Wheat seeds oil and vitamins. Due to such components the ASL is nicotine replacement therapy's remedy and has moisturizing, softening, protective action. The study of the most aggressive sample of ASLfor the determination of acute and subacute toxicity was conducted at the SCIVP of Veterinary Drugs and Feed Additives according to OECD № 402 and OECD № 410. It is shown that single use of ASL did not cause the death of animals, the appearance of toxic phenomena, redness at the site of the drug. So, according to the GHS, ASL belongs to category 5 (not classified). However, it should be noted that 28 days of cutaneous use in the animals of the experimental groups caused a decrease in body weight, decreased hematological parameters, impaired liver and kidney function. Therefore, this sample of ASLcannot be used for a long time.

Initiated under the supervision of anexperienced narcologist, experiments on volunteer patients have shown that composition of the most aggressive sample of ASLhas aversive effect and forms a resistance to smoking.

Conclusion

Our further work is to improve the composition of the lipstick to give it the bestphysicochemical properties, to improve the convenience of ASL in the application, to provide a more intense moisturizing, softening, protective effect on the skin and most importantly to reduce the symptoms of nicotine withdrawal syndrome even more.

BIOMASS OF DELPHINIUM ELATUM – A SOURCE OF ANTITUMOR-ACTIVE DITERPENE ALKALOIDS

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Introduction

Delphinium elatum belongs Ranunculaceae family, wich has more than 50 genus. Many of them contain biologically active substances

and have biological activity. Members of the genus Delphinium are being investigated, as they contain diterpene alkaloids, glycosides and phenolic substances. For example, there are articles about research of D. albocoeruleum, D. chrysotrichum, D. nuttallianu extracts and isolated diterpene alkaloids (elatine, delsemin, delartin, condelphine, camphrol) and flavonoids (quercetin, quercetin3-O-b-D-glycopyranosyl, quercetin 3-O-b-D-glucopyranoside-7-O-a-L-arabinopyranoside).

Some diterpene alkaloids of Delphinium elatum and its derivatives have antitumor activity against cell lines of cancer of the nasopharynx, as well as to resistant cell lines of lung cancer, prostate, nasopharynx. As Delphinium elatum grows in Ukraine, and have medicinal properties, it is a rare species included in the Red Data Book, cultivation of this plant *in vitro* and to study biomass for BAS content, namely diterpene alkaloids and biology activity, is actual.

Research objective

Obtaining biomass of plant Delphinium elatum and identification of biologically active substances in it.

Materials and methods

The cell and tissue culture method was used to produce biomass *in vitro*. Qualitative biomass analysis was performed using a colorimetric test using Dragendorf and Lieberman — Berhad reagents. Quantitative determination of total phenolic compounds and flavonoids was performed using a spectrophotometer.

Results

Delphinium elatum seeds that were stratified in water for 24 hours were used. Sterilization was carried out with H_2O_2 (30%) and ethanol (70%) and washed three times with distilled sterile water. The seeds were introduced *in vitro* and sterile plants were obtained. Parallel grown from plant seeds (10 seeds of each of the two varieties) in the soil. The biotechnological method of plant introduction into culture was used and callus biomass was obtained at t=26 °C, photoperiod — 16/8 hours, illumination — 2000 lux. Murasige-Skuga medium is supplemented with auxins (indolylacetic acid, α-naphthyl-1-acetic acid, 2,4-dichlorophenoxyacetic acid) at a concentration of 0.1 to 3.0 mg/l and cytokinin (6-furfurylaminopurine) at a concentration of 0.02 to 1 mg/l. Duration of cultivation was 45 days. All experiments were performed in 3 replicates and the results were statistically analyzed.

Leaves and stems of plants have been used as explants for the induction of callusogenesis in Murasige Skuga medium with phytohormones. Plants from the soil were used for extraction and further study for the presence of biologically active substances. The cut plants were washed, weighed and dried in a drying cabin for 26 hours at a temperature of 35 °C. Obtained extract of the plant in ethanol-water solution (70/30, v/v) in the ratio of extractant: raw material 1:10. After 7 days extract was filtrated and the presence of common extractives, alkaloids and terpenoids that gave a positive result were identified. The total amount of extractives is 0.28 mg/ml, alkaloids — 1.75%, terpenoids — 2.58%. The determination was performed in a 3x repetition.

A colorimetric test showed that there was a significant amount of both terpenoid and alkaloid compounds in alcoholic biomass extracts. From the literature it is known that in Delphinium elatum the basic alkaloid is 3-methyl-1-oxo-2,3-dihydro-1H-pyrazolofenantrolin, and the main terpenoids are 2H-1-benzopyran-2-one, 3-phenol (coumarin derivative) and stigmasterol. These substances have different biological roles, so knowledge of the active compounds in the plant Delphinium elatum will lead to more effective and wider use of them as antitumor agents.

Conclusion

Phytochemical studies of callus biomass of Delphinium elatum confirm the presence of flavonoids and diterpene alkaloids, so this biomass will be tested for antitumor activity in the future.

EFFECTIVENESS OF PLANT EXTRACTS ON HYDROGEL CARRIERS COMPLEX PREPARATIONS

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Introduction

The use of polymer hydrogels in medicine and pharmacy is a promising and dynamic area. Due to the crosslinked hydrophilic polymer structure, they are an alternative to existing drug delivery systems on the market, as they can contain a large amount of water/biological substances/drug solutions. Hydrogels possessing high degree of flexibility due to large water content. The use as a filler of a plant extracts for hydrogel films has several advantages, namely a wide range of pharmacological activity, low toxicity, fewer contraindications, less side effects. The suggested technology of obtaining a complex of hydrogels with plant extracts allows prolonged use of the dosage form and increase the biological effect.

Research objective

Identification of biologically active substances in the extracts of Calendula officinalis and Arnica montana and in the hydrogel-extract compositions.

Materials and methods

The hydrogels in the form of films were obtained by block polymerization of HEMA and PVP compositions in aqueous medium. Hydrogels based on the following compositions were used for the investigation: HEMA:PVP:water — 10:0:10 and 8:2:10, that are characterized by water absorption (swelling) of 42 and 48% and an osmotic permeability coefficient for water of 0.51 and $5.23 \cdot 10^{-3}$ $m^3/(m^2 \cdot hour)$. Extracts of plants (Calendula officinalis and Arnica montana) were obtained in ethanol/water solutions (70:30 v/v) and extracted for 7 days at room temperature in a dark place. The extracts were filtered off and evaporated in a vacuum evaporator. The hydrogel films were dried at room temperature for 1 day and placed in plant extracts for 2 hours: extract ratio of 1:5 (by weight).

The content of total phenols and flavonoids in plant extracts was determined by a method — spectrophotometrically with a solution of aluminum chloride at 360 nm. The content of flavonoids was compared with routine.

Antioxidant activity was determined using DPPH solution (2,2-diphenyl-1-picrylhydrazyl). The concentration of the test extract providing 50% inhibition (IC50, expressed in mg/mL) was calculated from the graph plotted with inhibition percentage against the extract concentration. The synthetic antioxidant reagent butylated hydroxytoluene (BHT) was used as positive control and all tests were carried out in triplicate.

Antibacterial and antifungal activity of water-ethanol plant extracts was determined by agar-well diffusion method for extracts and the disk-diffusion method for hydrogel film-extract complexes. The microorganisms used for the experiments were bacteria *Escherichia coli, Staphylococcus aureus, Mycobacterium spp.* and fungi *Candida tenuis, Aspergilus niger*. The incubation time and temperature for bacterial and fungal strains were 24 and 48 h, 30 and 37 °C.

Results

Calendula officinalis and Arnica montana extracts have been investigated for phenolic compounds, flavonoids and antioxidant activity. The residues of the extracts were also investigated after the hydrogel film-extract compositions were created. The total phenolic content is 110.81 mg GAE/g of dry weight for the extract for C. officinalis flowers and 128.22 mg GAE/g of dry weight for the extract of A. montana flowers. The highest flavonoids content was 82.63 mg OE/g of dry weight extract for C.officinalis flowers and 105.27 mg OE/g of dry weight extract for A. montana flowers.

The flower and leaf extracts of Calendula officinalis and Arnica montana were subjected to *in vitro* tests to evaluate their antioxidant activities. The restorative radical capacity of DPPH was determined by the decrease in absorption that was induced by plant antioxidants. The scavenging effect of aqueous-ethanol extracts and standard on the DPPH radical expressed as IC50 values was in the following order: flowers C.officinalis (0.42 mg/L), leaves C. officinalis (0.58 mg/L), flowers A. montana (0.84 mg/L), leaves A. montana (0.52 mg/L).

Samples of the hydrogel film (in the form of disks d=1 cm) were saturated in aqueous-ethanol extracts (C. officinalis 70%, A. montana 70%, as control — ethanol 70%) for 2 hours. The bacteriostatic effect was investigated by using the agar disk-diffusion method, where saturated hydrogel films were used as the disks. And for comparison, the bacteriostatic effect of the same extracts was determined by the well-daiffusion method in agar medium. It has been established that hydrogel samples that are saturated with plant extracts have higher bacteriostatic properties, rather than using only extracts. Namely, we observed

zones of growth inhibition of bacteria *Staphylococcus aureus*, *Escherichia coli* with the samples saturated with A. montana. extract, somewhat less observable with the sample of the C. officinalis extract; and growth inhibition of fungi *Candida tenuis*. The most notable effect was observed for bacteria of the genus *Mycobacterium*, with significant growth inhibition zones (d=10–15 mm around the disk for A. montana and d=5 mm for C. officinalis). In comparison, for extracts that were not included in the complex with the hydrogel, we obtained only a slight growth inhibition of *E. coli* and *S. aureus* (for A. montana 70% extract d=0.8 mm).

Conclusions

The presence of phenolic compounds, flavonoids and antioxidant activity of the C. officinalis and A. montana extracts was confirmed. The hydrogel polymer-plant extracts complexes were obtained, the conditions of their production were studied. Microbiological investigation of plant extracts and hydrogel complexes with extracts were performed. Higher results of microbiological activity of plant extract-hydrogel complexes in compare to the extracts themselves were obtained.

PERSPECTIVES OF IMPLEMENTATION OF ANTIVIRUS MEDICINES PRODUCTION IN UKRAINE

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According to state statistics, the vaccination rate in Ukraine does not match the recommended (more than 95% population) within the framework of the generally accepted approach to healthcare organization. Under-vaccination can lead to outbreaks of dangerous vaccine-preventable diseases. The tragic nationwide drop in immunization coverage left an increasing number of children in Ukraine susceptible to dangerous, preventable diseases. Influenza vaccination coverage in Ukraine is also far from satisfactory. Influenza A(H1N1), widely known as the cause of the 2009 pandemic, was the predominant flu virus also during 2015/2016 winter season. The epidemiological situation in the country was unfavorable during both these seasons of influenza.

As is known, the mutation and re-assortment of the Influenza A viruses genome are susceptible to forming new subtypes of influenza virus that may result in widely propagated and destructive pandemics due to the lack of immunity to the emerging pathogen (Reid A.H., Taubenberger J.K., 2003). Influenza virus, which belongs to the family of Orthomyxoviridae, that further divided into several genera, including influenza A virus (IAV), influenza B virus and influenza C virus (Forrest H.L., Webster R.G, 2010). It is stated that the gene of viruses A and B consists of eight segments of negative-sense single-stranded Ribonucleic acid (RNA), and virus C has seven fragments (Shao W. et al., 2017). Actually, S — antigen, created with RNA and general protein shell, determines the type of viruses: A, B, or C. Each gene code the synthesis of a concrete protein and each RNA molecule can direct the synthesis of many identical protein molecules. Genes and proteins of virus have the same names and are depicted with the Latin letters, so the fiction specializing literature operates by such symbols as PB1, PB2, PA, HA, NP, NA, M and NS genes.

One way influenza viruses change is called «antigenic drift». It is based on accidental accumulation of mutations in the genes of influenza viruses that can lead to changes in the surface proteins of the virus: HA (hemagglutinin) and (less) NA (neuraminidase), The HA and NA surface proteins of influenza viruses are «antigens», which means they are recognized by the immune system. When antigenic drift occurs, the body's immune system may not recognize and prevent sickness caused by the newer influenza viruses.

The source of infection is infected person, sometimes sick animals. The infectious doze is very low and costs 0.0001 ml of nasal secrecy, so the level of catarrhal syndrome depicts the epidemic danger of a sick person. On these days there is a stable possibility of flu virus persistence. Ill people with a low intoxication are defined as the most dangerous epidemic group, because they continue to live an active lifestyle, influencing others. Airborne route (when someone inhales the aerosols produced by an infected person coughing, sneezing or spitting) is one of the most significant modes of transmission.

At the beginning of February 2019 31 state representatives among 50 announced the epidemic data on severe acute respiratory infection (SARI) and noted the dramatic increase of morbidity. 34 countries said that the flu virus was detected in clinic material examples, received within the last week of January this year, and it proves the active increase of flu in the European region of WHO. Belarus, Greece, Ireland and Malta point at the flu activity of high intensity Ukraine, Finland and Russia pointed at the high activity in general.

There are only few antiviral medicines for therapy of influenza from several national and foreign producers are registered in Ukraine: zanamivir, oseltamivir,enisamium (Amizon), rimantadine, inosine pranobex, umifenovir and others in forms oftabletes, capsules,

powder for inhalation and syrup. In year 2013, 14 foreign and 7 domestic trademarks of medicines for the treatment and prevention of influenza were present on the market, and 12 and 16 — in 2019, respectively.

Those medicines can be divided to three groups.The first group includes neuraminidase inhibitors zanamivir and oseltamivir (original brands are Relenza, Glaxo Smith Kline and Tamiflu, Hoffmann La Roche Ltd.).The second group consists of inhibitors virus M2 protein (rimantadine, amantadine).The third Group includes other antivirus medicine for treating the flu (inosine pranobex, umifenovir cagocel, tilorone etc.). Among the release forms of these drugs, predominate solid ones (tablets, capsules, powders for inhalation use, etc.).

Conclusions

The number of medicines for treating the flu (domestic and foreign production) registered in Ukraine is constantly increasing. Among the dosage forms of these drugs, tablets and capsules for oral administration predominate. By the end of 2018, Farmak increased retail and hospital sales by 18,9% compared to 2017. It is the Ukrainian manufacturer of the European level. Thanks to the high quality and innovations, Farmak is the leader of the Ukrainian pharmacy field. Such a success was reached thanking to the quality and innovations. Within the last 7 years Farmak aimed 2.8 bln hrv to the development and modernization. Nowadays all the equipment of the company is modern and highly-technic.

Farmak is a leading manufacturer providing an «Affordable medicines» reimbursement program. These are 26 medicines, 11 of which are free, 15 are partially paid.

Due to the version of «Economy truth» portal, in 2017 Farmak was in the top of 5 best innovative companies of Ukraine. Journals like Bussiness, Focus etc. named Farmak as one of the best employment organization among other Ukrainian corporations. Actually, Farmak is the only manufacturer which was in the top of 100 tax payers in 2017.

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See you in Ukraine again!