ISBN 978-5-299-01229-3

ООО «Издательство „СпецЛит“». 190020,
Санкт-Петербург,
10-я Красноармейская ул., 15–17, литер В, пом. 231.
Тел./факс: (812)495-36-09, 495-36-12
http://www.speclit.su

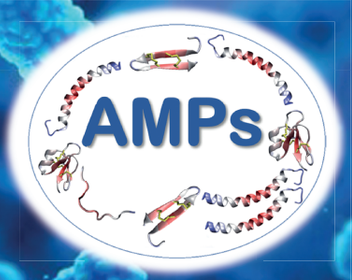
WORLD-CLASS RESEARCH CENTRE

CENTRE FOR PERSONALIZED MEDICINE

IEM

III International Mini-Conference

“Antimicrobial peptides as prototypes of novel antibiotics”



19–20 July 2023
Saint-Petersburg
Russia

ALMAZOV
National Medical Research Centre

World-Class Research Centre
for Personalized Medicine

IEM

III International Mini-Conference

19—20 July 2023

World-Class Research Centre for Personalized Medicine
Institute of Experimental Medicine, Saint Petersburg

Organizing Committee:

Olga V. Shamova — Chairperson
Ilya A. Krenev — Executive secretary
Maria S. Sukhareva
Elizaveta V. Vladimirova
Alexey S. Komlev

The Mini-conference is holding in the frame of realization of the project “Infection diseases and antimicrobial therapy” at the World-Class Research Centre for Personalized Medicine (with the financial support of the Ministry of Science and Higher Education of the Russian Federation, Agreement № 075-15-2022-302).
The Mini-conference is devoted to the prospects of developing novel antibiotics on the base of antimicrobial peptides (AMPs) as tools for combating multidrug resistant bacteria causing severe hospital infections including those arising during or after COVID-19. Varied structural classes of AMPs will be considered as templates for creation of such potent drugs (α-helical, β-hairpin, proline-rich peptides), the pros and cons of these substances will be discussed. An application of AMPs in combination with conventional antibiotics as well as with metal nanoparticles also will be under consideration. Other types of the biological activity of AMPs will be discussed: immunomodulatory, anti-tumor activities, interaction of AMPs (mostly beta-hairpin) with the complement system.
The main aim of the seminar is finding new ways for scientific collaboration.

УДК 615.281

ISBN 978-5-299-01229-3

CONTENTS

SCIENTIFIC PROGRAM

Day 1: 19 July 2023

10:00–10:10	**Olga Shamova (St. Petersburg, Russia)** **Opening the conference**
10:10–10:40	Olga Shamova "What is the most promising way for creation of novel antibiotics based on natural antimicrobial peptides?" *Institute of Experimental Medicine, WCRC for Personalized Medicine, St. Petersburg, Russia*
10:40–11:05	Anastasia Kalganova "Creation of a platform for evaluating the action of antimicrobial peptides and antibiotics against *in vivo* bacterial infection in *C. elegans"* *Alferov National Research Academic University, St. Petersburg, Russia*
11:05–11.30	Anne Maria Thomas***** "Molecular characterization and phylogenetic analysis of a histone-derived antimicrobial peptide from Silver Pompano, *Trachinotus blochii*" *Cochin University of Science and Technology, Cochin, India*
11:30–11:55	K. L. Dhanya Lenin***** "Tissue-wise expression profiling of multiple antimicrobial peptide in Asian sea bass, *Lates calcarifer*" *Cochin University of Science and Technology, Cochin, India*
11:55–12:20	Sheethu Annie Vincent***** "Exploring the antimicrobial peptide (AMP) profile of thymosin derived from *Peneaus monodon*: molecular characerization and *in silico* analysis of Pmthymosin 3" *Cochin University of Science and Technology, Cochin, India*
12:20–12:50	Ilia Krenev "β-Hairpin host defense peptides as complement system modulators" *Institute of Experimental Medicine, St. Petersburg, Russia*
12:50–13.00	**DISCUSSION**
COFFEE BREAK	
13:30–13:55	Ekaterina Umnyakova***** "Factor H binding peptide as a perspective tool for biomaterial protection" *University of Basel, Basel, Switzerland*
13:55–14:20	Arina Kalinina "Fragments of the endogenous antibiotic defensin and their derivatives as possible analgesic substances" *I. P. Pavlov Institute of Physiology of the Russian Academy of Sciences, St. Petersburg, Russia*

14:20–14:45	Sofiya Pipiya***** "Synthetic biology approach for expanded biodiversity design and screening of antimicrobial activity" *Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia*
14:45–15:05	Alexey Chutko "Synergistic effects of the combined treatment of U251 glioma cells with protegrin-1 PG-1 and Etoposide" *Institute of Experimental Medicine, St. Petersburg, Russia*
15:05–15:35	Alexander Yakovlev***** "The role of human antimicrobial peptides and their impact on the frequency of the chronic disease recurrency" **(in Russian)** *CI ImmunoHelp company, Biotechpharm company, Moscow, Russia*
15:35–16:00	**POSTER SESSION (OFF-LINE ONLY)**

* On-line participants.

Day 2: 20 July 2023

10:00–10:10	**Olga Shamova (Saint Petersburg, Russia)** **Introduction to Day 2**
10:10–10:35	Natalia Linkova "Peptides in complex therapy of musculoskeletal pathology: clinical and molecular aspects" *St. Petersburg Institute of Bioregulation and Gerontology, St. Petersburg, Russia*
10:35–11:05	Mikhail Berlov "Antimicrobial and complement-modulating properties of human defensins and structurally related peptides" *Institute of Experimental Medicine, St. Petersburg, Russia*
11:05–11:30	Anastasiya Ilina "The EDR and KED peptides improve the morphofunctional state of neuronal networks in mouse model of Alzheimer's disease" *St. Petersburg Institute of Bioregulation and Gerontology, St. Petersburg, Russia*
11:30–11:55	Maria Khaydukova "Minimal inhibiting concentration predicted *in silico* vs experimental value" *Institute of Experimental Medicine, WCRC for Personalized Medicine, Institute of Human Hygiene, Occupational Pathology and Ecology, St. Petersburg, Russia*
11:55–12:20	Alexander Chernov "The effects of cathelicidin LL-37 towards glioblastoma cells *in vitro* and *in vivo*" *Institute of Experimental Medicine, WCRC for Personalized Medicine, St. Petersburg, Russia*
12:20–12:45	Elizaveta Vladimirova "Comparative analysis of antimicrobial activity of various silver nanoparticles and their combined action with βhairpin peptides" *Institute of Experimental Medicine, WCRC for Personalized Medicine, St. Petersburg, Russia*
12:45–13:10	Mina Adel Botros "Antimicrobial peptides: auspicious tools against the emerging multi-drug resistance" *St. Petersburg State University, St. Petersburg, Russia*

COFFEE BREAK	
13:40—14:10	Vladimir Gusev "Endothelial dysfunction as a cause of infertility and reproductive losses. Restoration of endometrial functionality using a peptide of natural origin — Alloferon" **(in Russian)** *Allokin company, Moscow, Russia*
14:10—14:35	Irina Ryzhova "Functional changes in the activity of the vestibular epithelium afferent synapse under the influence of cytokines and peptides: defensin HNP-1 and interferon α2b — comparative study" **(in Russian)** *I. P. Pavlov Institute of Physiology of the Russian Academy of Sciences, St. Petersburg, Russia*
14:35—15:00	Ilya Bolosov "New analogs of protegrin-1 with improved selectivity of antibacterial action" **(in Russian)** M. M. *Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia*
15:00—15:25	Viktoria Safronova***** "Biodiversity and therapeutic potential of BRICHOS domain-related antimicrobial peptides from marine polychaeta" **(in Russian)** M. M. *Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia*
15:25—15:50	Anna Spiridonova "Time-kill assay: synergy of antibiotics, antiseptics and peptides" **(in Russian)** *I. P. Pavlov St. Petersburg State Medical University, Russia*
15:50—16:15	**POSTER SESSION (OFF-LINE ONLY)**
16:15—16:25	**AWARDING**
16:25—16:30	**Closing conference**

*On-line participants.

ANTIBIOTIC ACTIVITY OF A PEPTIDE OF THE INNATE IMMUNE SYSTEM – PROTEGRIN-1 AND ITS SYNTHETIC STRUCTURAL MODIFICATIONS

Artamonov A. Yu.[1], Orlov S. B.[2], Zheleznikov P. A.[2], Orlov D. S.[1]
[1]Institute of Experimental Medicine, St. Petersburg, Russia
[2]V. I. Razumovsky Saratov State Medical University, Saratov, Russia

ANTIMICROBIAL PEPTIDES AS CANCER DRUGS CANDIDATES

Zharkova M. S., Rudel A. E., Filatenkova T. A.
Institute of Experimental Medicine, St. Petersburg, Russia

PEPTIDES INVOLVED IN THE IMPLEMENTATION OF THE PROTECTIVE FUNCTIONS OF MIXED SALIVA

Sukhareva M. S., Vladimirova E. V.
Institute of Experimental Medicine, WCRC Medicine, St. Petersburg, Russia

APPLICATION OF PALMITOYL HEXAPEPTIDE PEPTIDE AS A WOUND HEALING AGENT

Petrova P. E., Komlev A. S., Sukhareva M. S., Vladimirova E. V., Ivasenko A. A., Yudin D. A., Fedorov I. P, Galianova M. V.
Institute of Experimental Medicine, WCRC for Personalized Medicine, St. Petersburg, Russia

MODIFICATIONOFTHEC-TERMINUSOFAPEPTIDEINSOLUTION THROUGH ESTERIFICATION USING TRIMETHYLCHLOROSILANE[2]

Galianova M. V.[2], Komlev A. S.[2], Petrova P. E.[1], Ivasenko A. A.[1], Fedorv I. P.[1], Yudin D. A.[1], Zabrodskaya Y. A.[1,3]
[1]Institute of Experimental Medicine, WCRC for Personalized Medicine, St. Petersburg, Russian Federation
[2]St Petersburg University, St. Petersburg, Russia
[3]Research Institute of Influenza, St. Petersburg, Russia

CYTOTOXIC ACTIVITY OF PEPTIDES OF INNATE IMMUNITY FROM VARIOUS STRUCTURAL CLASSES ON RAT GLIOMA TUMOR CELLS

Rudel A. E., Filatenkova T. A.
Institute of Experimental Medicine, St. Petersburg, Russia

ALTERED CORTICAL GLUTAMATERGIC GENE EXPRESSION AND GUT MICROBIOTA COMPOSITION LINKED TO COGNITION-ENHANCING EFFECTS OF MEDIUM-CHAIN TRIGLYCERIDE SUPPLEMENTATION

Kim E. A.[1, 2], Shirokov E. A.[1, 3], Nikitina V. A.[1], Schwarz A. P.[4], Krytskaya D. U.[1], Arseniev N. A.[2], Abdurasulova I. N.[1], Klimenko V. M.[1], Shcherbakova K. P.[1], Trofimov A. N.[1]

[1]Institute of Experimental Medicine, St. Petersburg, Russia

[2]St. Petersburg State Chemical Pharmaceutical University, St. Petersburg, Russia

[3]Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia

[4]Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

ACUTE EFFECTS OF MEDIUM-CHAIN TRIGLYCERIDE SUPPLEMENTATION ON GENE EXPRESSION, MONOAMINE LEVELS IN THE BRAIN, AND BLOOD CYTOKINE LEVELS IN RATS

Nikitina V. A.[1], Schwarz A. P.[2], Traktirov D. S.[1], Apryatin S. A.[1], Karpenko M. N.[1], Krytskaya D. U.[1], Klimenko V. M.[1], Shcherbakova K. P.[1], Trofimov A. N.[1]

[1]Institute of Experimental Medicine, St. Petersburg, Russia

[2]Sechenov Institute of Evolutionary Physiology and Biochemistry RAS, St. Petersburg, Russia

CHRONIC FATIGUE SYNDROME AND THERAPEUTIC PERSPECTIVES OF CYTOKINE DRUGS

Filatenkova T. A., Shanin S. N., Fomicheva E. E., Serebryanaya N. B.

Institute of Experimental Medicine, St. Petersburg, Russia

ABSTRACTS

Oral reports

WHAT IS THE MOST PROMISING WAY FOR CREATION OF NOVEL ANTIBIOTICS BASED ON NATURAL ANTIMICROBIAL PEPTIDES?

Shamova O. V., Zharkova M. S., Sukchareva M. S., Vladimirova E. V., Komlev A. S., Protasov E. A., Khaydukova M. M., Filatenkova T. A., Goncharov A. E., Chernov A. N., Klimov N. A., Korneva E. A., Krenev I. A., Artamonov A. Yu., Berlov M. N., Orlov D. S.

Institute of Experimental Medicine, WCRC for Personalized Medicine, St. Petersburg, Russia

Introduction Antimicrobial peptides (AMPs) of human and animals' innate immune system provide the first line of defense against a broad spectrum of pathogens. AMPs have a variety of primary structures and adopt different conformations. Except of marked antimicrobial activity the peptides exert multiple effects towards eukaryotic cells participating in numerous host defense reactions. These features determine the wide prospect of AMPs application in medicine. Special attention for these peptides is paid due to a problem of a rapid growth of bacterial resistance to conventional antibiotics and the urgent need for creation of alternative antimicrobials with principally new mode of antibacterial action. AMPs may serve as such new drugs because of their fast and multitargeting antimicrobial action, and an array of other varied defensive functions. However there are some limitations for introducing of AMPs to medicine: relatively low stability, some toxicity for host cells, susceptibility to host or microbial proteases. There are several ways for overcoming these limitations.

Purpose. The main purpose of our study is examination of varied approaches for developing the prototypes of new antimicrobial agents on a base of natural AMPs: modifying the peptides' structure, composing AMPs with convenient antibiotics or antiseptics, use as immune modulators.

Methods. An array of structural variants of natural AMPs have been designed and chemically synthesized; their antimicrobial activity has been explored by means of the broth microdilution assay (including checkerboard titration for evaluation a combined action) and other appropriate methods; cytotoxicity for the host cell also has been examined.

Results. We have developed a set of structural modifications of proline-rich bactenecin 3.4, cathelicidin buCATHL4D, beta-hairpin peptide arenicin 1 and others. It was shown that the synthetic analogues of natural AMPs exerted

potent antimicrobial activity against multidrug resistant strains of *Klebsiella pneumonia, Acinetobacter baumannii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, etc. Study of the cytotoxicity of these AMPs for the host cells revealed that the best index of selectivity was obtained for an analog of bactenecin 3.4. Another way for increasing the effectiveness of AMPs is their combined use with conventional antibiotics, antiseptics or nanomaterials in the case of synergistic effects of antimicrobial action. The most frequent cases of synergy were observed for combining AMPs with antibiotics erythromycin and amikacin, antiseptics prontosan, dioxidin, etidronic acid and silver nanoparticles (including poviargolum antiseptic). An ability of AMPs to prevent the bacterial biofilm formation has been studied; we have shown that some peptides in sub-microbicidal concentrations effectively prevented forming of biofilms by multidrug resistant *P. aeruginosa* MDR 522/17 and *A. baumannii* 7226/16 and this action can be synergistically increased in combination with prontosan, cocamidopropyl-betain and some other antiseptics**.** The immunomodulatory action of certain AMPs is described in literature. We found several peptides among our studied AMPs with immunomodulatory activity (cationic salivary proline-rich peptides) as well as with wound-healing capability (ChBac5 derivatives).

Conclusion. The data obtained confirm the idea regarding the prospects of a practical use of AMPs of animal origin and provide the successful examples of improving the effectiveness of natural peptides for optimizing their features for medical application. The project is funded by the Ministry of Education and Science of the Russian Federation, Agreement №075-15-2022-302 (20.04.2022).

CREATION OF A PLATFORM FOR EVALUATING THE ACTION OF ANTIMICROBIAL PEPTIDES AND ANTIBIOTICS AGAINST *IN VIVO* BACTERIAL INFECTION IN *C. ELEGANS*

***Kalganova A. I.*[1], *Eliseev I. E.*[1], *Shamova O. V.*[1, 2]**

[1]Alferov National Research Academic University, St. Petersburg, Russia
[2]Institute of Experimental Medicine, St. Petersburg, Russia

Introduction. The crisis of antibiotic resistance is related to the problem of finding and developing new antimicrobial drugs. Currently, there are no effective platforms for screening new antimicrobials that are active against MDR bacteria [1, 2, 3]. In this work, we evaluate the capabilities of the whole-animal *C. elegans* system for screening drugs *in vivo*. This technology will make it possible to study the course of an infection in a living animal and explore the interaction of resistance and virulence factors in bacteria and the formation of biofilms [4, 5].

Purpose. The aim of this work is to develop a platform for testing the efficacy of antimicrobial agents *in vivo* in a model of bacterial infection in *C. elegans*.

Methods. In the course of the work, in addition to SOP for working with nematodes and bacteria, optical microscopy, confocal fluorescence microscopy, bacterial plasmid transformation, and the method of using an old nematode population were used. Statistical data processing was carried out using the construction of Kaplan-Meier survival curves.

Results. A model of lethal bacterial infection inside the *C. elegans* gut was created using the *P. aeruginosa* Ps1 strain. A protocol for infection and treatment of nematodes has been developed, as well as a system for identifying the fact of the death of a single nematode. The use of the old population of *C. elegans* N2 allows the MDR strain *P. aeruginosa* 522/17 to form a lethal infection. However, the wide use of the old population limits the possibilities of the testing system, as it reduces the effect of oxidative stress. Amikacin and colistin, and AMP PG1 were used as test antibiotics for treatment. When colistin, «the antibiotic of last resort», was tested, it was shown to be effective even at a concentration of 0.5 mg/ml. The use of a high concentration of amikacin and PG1 50 μM (10 MIC *in vitro* [6]) has a significant positive effect on the old population. However, a similar treatment did not cause a positive response when using a young population. The use of a combination of AMK and PG1 gave a synergistic effect only at low concentrations, which may be associated with stimulation of the production of native AMK.

Conclusion. The use of *C. elegans* model organisms to simulate bacterial infections inside a well plate is a novel approach that allows one to

study organism-pathogen interactions and identify molecules with new mechanisms of action, including considering the activation of innate immunity.

1. *Lewis K.* At the crossroads of bioenergetics and antibiotic discovery // Biochemistry (Moscow). — 2020. — V. 85. — P. 1469—1483.

2. *Lewis K.* The science of antibiotic discovery // Cell. — 2020. — V. 181. — № 1. — P. 29—45.

3. *Lewis K.* Platforms for antibiotic discovery // Nature reviews Drug discovery. — 2013. — V. 12. — № 5. — P. 371—387.

4. *Moy T. I.* [et al.]. Identification of novel antimicrobials using a live-animal infection model // Proceedings of the National Academy of Sciences. — 2006. — V. 103. — № 27. — P. 10414—10419.

5. *Powell J. R., Ausubel F. M.* Models of Caenorhabditis elegans infection by bacterial and fungal pathogens // Innate immunity. — 2008. — P. 403—427.

6. *Zharkova M. S.* [et al.]. Application of antimicrobial peptides of the innate immune system in combination with conventional antibiotics—a novel way to combat antibiotic resistance? // Frontiers in cellular and infection microbiology. — 2019. — V. 9. — P. 128.

MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF A HISTONE-DERIVED ANTIMICROBIAL PEPTIDE FROM SILVER POMPANO, *TRACHINOTUS BLOCHII*

Anne Maria Thomas, Greeshma T. J., Swapna P. Antony

Cochin University of Science and Technology, Kochi, India

Introduction. The antimicrobial peptides (AMPs) produced by various organisms are crucial for innate immunity against pathogens. These peptides have the potential to replace antibiotics due to their wide range of antibacterial activity, highly selective toxicity, and significantly lower resistance levels compared to antibiotics. Alongside conventional AMPs, there are other molecules with antimicrobial properties that are not traditionally considered part of innate defenses. Histone H2A-derived peptides are unconventional AMPs that serve as ancient defense factors, repurposed in novel ways throughout evolution.

Purpose. This study aims to characterize the histone H2A-derived AMP from the silver pompano fish, *Trachinotus blochii*. The species is popular for mariculture due to its taste, adaptability to cultural conditions, acceptability to formulated diets, high meat quality, and international market demand.

Methods. The experimental organism used in this study was the fish silver pompano, *Trachinotus blochii*. The IAEC approval with reference number 363/Go/Re/S/01/CPCSEA/27 was obtained before the initiation of the study. Tissues from live fish samples were dissected under sterile RNase-free conditions immediately after euthanizing the fish. For RNA extraction, the TRI reagent (Sigma) protocol was followed. The RNA quantity and quality were assessed through spectrophotometry and gel electrophoresis, respectively. cDNA synthesis was carried out using the reverse transcription method. The efficiency of the reverse transcription process was verified by detecting the presence of the β-actin reference gene, utilizing gene-specific primers designed for this purpose. PCR amplification was conducted in a 10 µl reaction volume using the EmeraldAmp® PCR Master Mix (Takara Biomedical Inc., Japan), following the manufacturer's protocol. After that, the amplified PCR products were examined using agarose gel electrophoresis to ensure the presence and size of the amplified fragment. Following PCR amplification, the PCR products were purified using EXO-CIP and sequenced at the Genespec sequencing facility in Kochi, India, using gene-specific primers. The resultant gene sequence was then submitted to the GenBank database.

Results. A histone H2A-derived AMP gene consists of 93 nucleotides encoding 31 amino acids was isolated from silver pompano. Additionally, this study investigates the evolutionary relationship of this peptide with histone H2A-derived peptides from other vertebrates and invertebrates. The peptide

showed close resemblance with histone H2A-derived peptides from other fishes.

Conclusion. This is the first report of a histone H2A-derived antimicrobial peptide from silver pompano, *T. blochii*. Further studies are needed to explore the antimicrobial activities of this peptide as a synthetic or recombinant compound. This research could unveil its potential use in aquaculture or medicine as a therapeutic agent.

TISSUE-WISE EXPRESSION PROFILING OF MULTIPLE ANTIMICROBIAL PEPTIDES IN ASIAN SEA BASS, *LATES CALCARIFER*

K. L. Dhanya Lenin, Swapna P. Antony

Cochin University of Science and Technology, Kochi, India

Introduction. Fish, being an organism thriving in a habitat teeming with pathogenic microbes, are one of the best models to study the immunological implications of antimicrobial peptides (AMPs). AMPs are ubiquitously present in organisms and form an important active component of their innate immune mechanism once an infection sets in. Immunomodulatory properties, the broad spectrum of activity, and tolerance to high salt concentrations are properties that can turn helpful in its use as a therapeutic in aquaculture.

Purpose. The study reveals the constitutive expression pattern of AMPs in various tissues and its possible immunological implications. The data can be tapped for developing health management strategies in aquaculture.

Methods. Different tissues from healthy adult Asian sea bass (*Lates calcarifer*) were extracted under sterile conditions and total RNA was extracted followed by complimentary DNA synthesis. Further, the cDNA was subjected to screening, for peptides of interest using specific forward and reverse primers. Gel electrophoresis was performed and was visualized under a UV transilluminator and the resulting electrophoretogram was subjected to ImageJ semi-quantitative analysis.

Results. The semi-quantitative analysis revealed differential expression patterns of the AMPs analysed in different tissues. As the tissues were extracted from healthy fishes, the expression can be considered a constitutive one and was noticed in all the tissues. Thymosin, hepcidin, β-defensin, and Piscidin 2 were present constitutively in all the tissues analysed and two AMPs, i. e. thymosin and hepcidin were found to dominate the expression analysis. Thymosin was found in high levels in gills, muscles, stomach, intestine, pancreas, ovary, brain, and heart, whereas in tissues such as blood, and skin, hepcidin was found to dominate.

Conclusion. The tissue-specific distribution pattern of an AMP can be linked to the different functions attributed to the tissues and the presence of different AMPs in tissues indicates the closely interlinked network of the innate immune system that plays an important role in fish immunity.

EXPLORING THE ANTIMICROBIAL PEPTIDE (AMP) PROFILE OF THYMOSIN DERIVED FROM *PENEAUS MONODON*: MOLECULAR CHARACTERIZATION AND *IN SILICO* ANALYSIS OF PMTHYMOSIN 3

Sheethu Annie Vincent*, *Swapna P. Antony

Cochin University of Science and Technology, Kochi, India

Introduction. Thymosin β peptides are known for their interaction with actin and involvement in various biological processes such as inflammatory responses, cell migration, wound healing, stem cell differentiation, and angiogenesis. Recently, interest has grown in exploring the antimicrobial properties of these peptides, as they have shown potential in antimicrobial control.

Purpose. To validate the antimicrobial potential of the thymosin beta 3 peptide using *in silico* techniques.

Methods. Thymosin peptides were targeted from the cDNA using gene specific primers followed by TA cloning to extract the complete sequence of thymosin β 3. The sequencing was done using 377AB Prism Sequencer at Agrigenome, Kochi.

Results. In this study, we identified three isoforms of β thymosin (thymosin β 2-4) from *Penaeus monodon*. The isoforms were cloned and analysed, leading to the identification of a 387 base pair nucleotide sequence encoding 128 amino acids, designated as Pmthymosin3. Molecular characterization and *in silico* analysis revealed that Pmthymosin3 belongs to the thymosin β 3 family. Structural analysis indicated that Pmthymosin3 adopts an α-helical structure with random coils. *In silico* functional characterization demonstrated the antimicrobial, anticancer, and antibiofilm activities of the active peptide, Pmthymosin3.

Conclusion. The physicochemical properties and *in silico* analysis suggest that Pmthymosin3 possesses characteristics typical of antimicrobial peptides, warranting further investigation as a potential antimicrobial peptide.

β-HAIRPIN HOST DEFENSE PEPTIDES AS COMPLEMENT SYSTEM MODULATORS

***Krenev I. A.*[1], *Berlov M. N.*[1], *Umnyakova E. S.*[2], *Gorbunov N. P.*[1], *Kostevich V. A.*[1], *Panteleev P. V.*[3], *Komlev A. S.*[1], *Ovchinnikova T. V.*[3], *Shamova O. V.*[1], *Aleshina G. M.*[1]**

[1]Institute of Experimental Medicine, St. Petersburg, Russia
[2]University of Basel, Basel, Switzerland
[3]Ovchinnikov and Shemyakin Institute of Bioorganic Chemistry, Moscow, Russia

Introduction. Complement plays a pivotal role in immune defense but is involved in many pathological processes. Preliminary studies revealed host defense peptides (HDPs) as potential complement inhibitors. HDPs with intrinsic antimicrobial activity may be beneficial in therapeutic complement inhibition.

Purpose. To characterize the action of β-hairpin HDPs on human complement activation *in vitro*.

Methods. Arenicin-1[V8R], ALP-1, arenicin-2, AA139, tachyplesins I/III, gomesin, capitellacin, androctonin, HDP from *Echinostoma caproni*, thanatin were expressed as recombinant peptides; arenicin-1, arenicin-1[C/A], protegrin-1 were obtained by solid-phase synthesis. The models of the classical (CP) and the alternative pathways (AP) of complement contained antibody-sensitized sheep red blood cells (RBCs) or rabbit RBCs, respectively. The model of paroxysmal nocturnal hemoglobinuria (PNH) contained AET-treated human RBCs. Complement activity was estimated by hemolysis in human normal serum and by ELISA (C3a and C5a anaphylatoxins accumulation). Absolute IC_{50} values, AUC values, hierarchical and principal component clustering were used to compare the studied HDPs. Radial diffusion assay was used to evaluate MIC of an elaborated peptide towards 5 bacterial species. Toxicity towards human RBCs was estimated in PBS and in compatible serum.

Results. Many of the β-hairpin peptides exerted bidirectional effect on complement activation. Tachyplesins, ALP-1, capitellacin possessed the most pronounced activating capacity, especially in the CP model. This effect was reduced at high concentrations (80-160 μg/mL) up to complement-inhibiting effect. Arenicin-1[V8R], arenicin-2, HDP from *Echinostoma caproni*, protegrin-1 appeared to be the most potent inhibitors. Arenicin-1[V8R] was a "pure" inhibitor in the CP model (IC_{50} 15, 0.75, 3 μM for inhibition of C3a, C5a accumulation and hemolysis) while arenicin-2 – in the AP model (IC_{50} 27, 9, 18 μM). A novel peptide was synthesized and studied *in vitro*. It was a "pure" inhibitor in the CP model (IC_{50} 8, 2, 5 μM);

it inhibited the AP (IC_{50} 54, 33, 29 μM) with a slight increase in C3a signal. It inhibited C5a production and hemolysis in the PNH model with IC_{50} 32 and 35 μM; C3a generation was inhibited by 37 %. The peptide was a potent antimicrobial with MICs 0.3—0.7 μM and was non-hemolytic.

Conclusion. Study of 15 natural and artificial β-hairpin HDPs revealed different modes of sequence-depending action on human complement. A novel inhibitor was studied and the structure of another was suggested.

The project is supported by the Ministry of Education and Science of the Russian Federation (State assignment № 122020300189-6).

FACTOR H-BINDING PEPTIDES AS A PERSPECTIVE TOOL FOR BIOMATERIAL PROTECTION

***Umnyakova E. S.*[1], *Felsch J.* [1], *Jacquemai S.* [1], *Rabbani S.* [1], *Bechtler C.*[1], *Colombi A.*[1], *Lambris J. D.*[2], *Ricklin D.*[1]**

[1]University of Basel, Basel, Switzerland
[2]University of Pennsylvania, Philadelphia, USA

Introduction. Non-self biomaterials widely used in medical practice (e.g., donor organs, transplants, therapeutic liposomes, etc.) can trigger undesirable immune reactions. In particular, side effects may derive from uncontrolled complement system activation that generates fluid phase anaphylatoxins (C3a, C5a), surface-bound opsonins (C3b/iC3b) and membrane-attack complexes (MAC). These defence reactions are triggered upon blood contact with introduced surfaces that lack natural complement regulators. One option to avoid adverse complement activation is the recruitment of the abundant regulator factor H (FH) to the biomaterial surface using FH-binding peptide coatings developed in our group.

Purpose. In this study, we analysed the ability of such a peptide, termed 5C6, to bind human FH and to diminish complement activation on polystyrene magnetic beads when incubated in normal human serum. Considering the homology of several FH domains with FH-related protein 5 (FHR-5), we explored potential cross-reactivities of 5C6.

Methods. We used N-terminally biotinylated synthesised 5C6 peptides. Peptides with scrambled sequences were utilized as negative controls. To assess the ability of 5C6 to bind FH from normal human serum (NHS) we used flow cytometry by measuring FH recruitment and/or C3b deposition on the surfaces of magnetic beads that were pre-coated with 5C6 peptides. Using Western blot, ELISA and microscale thermophoresis (MST) we evaluated the ability of 5C6 to discriminate between FH and FHR-5.

Results. In contrast to the scrambled analogues, the 5C6 peptide actively recruited FH to the material surface from NHS. Importantly, the active presentation of 5C6 also had a direct impact on preventing C3b deposition in the bead assay. Using a number of methods, we explored potential cross-reactivities of 5C6 with FHR-5 but did not detect any relevant interactions.

Conclusion. Our study confirmed that 5C6-based coatings impair complement mediated C3b/iC3b opsonization by recruiting FH, thereby supporting further development of the promising technology as protective coating for biomaterials to reduce adverse immune reactions and improve their lifetime and functionality when introduced into a patient's body.

FRAGMENTS OF THE ENDOGENOUS ANTIBIOTIC DEFENSIN AND THEIR DERIVATIVES AS POSSIBLE ANALGESIC SUBSTANCES

***Kalinina A. D.*[1], *Plakhova V. B.*[1, 2], *Rogachevskii I. V.*[1]**

[1]Pavlov Institute of Physiology, St. Petersburg, Russia
[2]Institute of Biophysics, National Research Council, Trento, Italy

Introduction. Application of the endogenous antibiotic defensin NP-1 at nanomolar concentrations to the nociceptive neuron membrane decreases the effective charge transfer of the $Na_V1.8$ channel activation gating system (Z_{eff}), which results in the antinociceptive effect. Several short arginine-containing fragments of the defensin NP-1 amino acid sequence, including Ac-RERR-NH_2, also modulate the $Na_V1.8$ channel functioning and can be considered as possible analgesic substances. Their ligand-receptor binding mechanism is based on the formation of intermolecular ionic bonds between positively charged guanidinium groups of the short peptides and appropriate functional groups of the $Na_V1.8$ channel molecule. The distances between the central carbon atoms of the guanidinium groups should be in a certain effective range with the lower threshold of ca. 9 Å.

Purpose. The lysine-containing Ac-KEKK-NH_2 peptide was investigated to elucidate whether the lysine-to-arginine substitution affects the ability of a short peptide to decrease the Z_{eff} value.

Methods. Nociceptive neurons from the dorsal root L_5–S_1 ganglia of newborn *Wistar* rats were studied using the patch-clamp method in the "whole-cell recording" configuration. The TINKER 8.0 program with MMFF94 force field was used for the conformational analysis of the Ac-KEKK-NH_2 molecule.

Results. The Z_{eff} decrease due to application of Ac-KEKK-NH_2 at 100 nM is comparable to that of Ac-RERR-NH_2. The substitution of positively charged arginine guanidinium groups for positively charged lysine amino groups retains the modulating effect of the short peptides on the $Na_V1.8$ channel, which indicates the same electrostatic mechanism of ligand-receptor binding for arginine- and lysine-containing short peptides. The distances between the lysine amino groups are calculated to be 11–12 Å.

Conclusion. Further studies of the discovered ligand-receptor binding mechanism will make it possible to create effective and safe analgesics that can replace opiates for a number of indications.

This study was supported by the State Program GP-47 "Scientific and Technological Development of the Russian Federation" (2019–2030), theme 0134-2019-0001.

SYNTHETIC BIOLOGY APPROACH FOR EXPANDED BIODIVERSITY DESIGN AND SCREENING OF ANTIMICROBIAL ACTIVITY

***Pipiya S. O.*[1], *Mirzoeva N. Z.*[1], *Baranova M. N.*[1], *Eliseev I. E.*[1], *Mokrushina Yu. A.*[1, 2], *Shamova O. V.*[3], *Gabibov A. G.*[1, 2], *Smirnov I. V.*[1, 4], *Terekhov S. S.*[1, 2]**

[1]M.M. Shemyakin and Yu.A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia
[2]M.V. Lomonosov Moscow State University, Moscow, Russia
[3]WCRC for Personalized Medicine, St. Petersburg, Russia
[4]National Medical Research Center of Endocrinology, Moscow, Russia

Introduction. One of the most alarming issues facing modern medicine is antibiotic resistance. Antibiotic overuse promotes the development of multidrug resistant strains. As a result, conventional medical practices are no longer effective, which makes it more challenging to combat infectious diseases and raises mortality. Thus, the problem of antibiotic resistance requires constant attention and the development of new approaches to the search for antibiotics.

Purpose. The use of techniques for large-scale screening of artificial or natural diversity to look for active compounds is a new vector in the development of antimicrobials. Antimicrobial peptides (AMPs) have the potential to be used in the development of new antibiotics, due to their broad range of activity and low rates of bacterial resistance emergence. The genetically encoded nature of AMPs enables the design and screening of cell libraries of their variants, as well as the adaptation of their production to heterologous producers.

Methods. The methylotrophic yeast *Pichia pastoris* was employed in this study as a heterologous AMP producer. AMP library was designed and cloned into yeast expression vector and transformed into yeast cells. Positive clones were screened for antimicrobial activity against target bacteria. Chosen AMP variants were chemically synthetized and further characterized.

Results. Recombinant yeast clones demonstrated strong antimicrobial activity against the target bacterium. A cellular library of the peptide's analogues was developed based on the primary amino acid sequence of the studied peptide. Through the use of large-scale screening techniques, clones with the best activity against the target bacterium were chosen. Further investigation of the selected variants revealed that the isolated AMP analogs had lower hemolytic activity than the original peptide.

Conclusion. Thus, the created platform enables large-scale antimicrobial compound screening and has the potential to facilitate the development of new antimicrobial drugs.

This work was supported by grant 21-14-00357 of the Russian Science Foundation.

SYNERGISTIC EFFECTS OF THE COMBINED TREATMENT OF U251 GLIOMA CELLS WITH PROTEGRIN-1 (PG-1) AND ETOPOSIDE

Chutko A.[1], Sharapov Ya.[1], Landynya S.[1], Vylegzhanina D.[1], Chernov A.[1], Galimova E.[1, 2]

[1]Institute of Experimental Medicine, St. Petersburg, Russia
[2]Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

Introduction. Glioblastoma (GBM) is the most common and most aggressive malignant brain tumor. Despite optimal treatment and evolving standard of care, the median survival of patients diagnosed with GBM is only 12–15 months. Among the several limitations of current standard of care for GBM patients are incomplete tumor resection, peritumoral edema, blood-brain barrier (BBB) disruption, insufficiency of the maximum radiation dose to eradicate the tumor, the toxic side effects of chemo/radio therapy, and drug resistance. AMPs represent a new class of anticancer drugs that lack toxicity and may overcome tumor resistance to conventional chemotherapy. Drug resistance and disease progression are common in GBM patients, underscoring the need for new therapeutic combinations. In the development of anticancer approaches, combined treatments appear to be of great interest. The idea of combined treatments is based on the possibility to obtain the same biological or therapeutic effect with two or more drugs, using lower concentrations of single drugs. In this case, the side-effects of the single drug are expected to be limited. In addition, the combined therapy of cancer may have an impact on acquired resistance.

Purpose. In this study, we examined the effects induced by PG-1 and its potential antitumor activity in U251 cell line. Moreover, another objective is to investigate the synergistic effect of PG-1 to find out the most effective anticancer treatment combination with other standard chemotherapies.

Methods. Human glioma cell line U251 was obtained from Institute of Cytology of the Russian Academy of Sciences (St. Petersburg, Russia) and was authenticated by short tandem repeat assay. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to study cell viability and to determine cytotoxic effects of PG-1 and their combination with chemotherapy in U251 cells. Synergism or antagonism was determined using combination index (CI) method. Caspase-3 activity was evaluated spectrophotometrically using a «Caspase 3 Assay Kit, Colorimetric» (Sigma, USA) according to manufacturer's protocol. The apoptosis analyzed by flow cytometry using propidium iodide (PI) and YO-PRO-1. GBM cells were treated with PG-1 in concentrations 2, 4, 8, 16, 32, and 64 μM. Chemotherapy drugs were

tested in the following concentrations — cisplatin: 1660, 830, 330, 166, 83, 33.2 μM; carboplatin: 26900, 2690, 1350, 673, 269, 134 μM ; doxorubicin: 920, 460, 73.6, 36.8, 18.4, 7.36 μM; temozolomide: 15000, 5200, 1550, 773, 386, 155 μM; etoposide: 27, 13.5, 6.8, 3.4, 1.7, 0.8 μM.

Results. PG-1 showed a strong cytotoxic effect on U251 glioma cells in the MTT test (IC50 26.1 μM) compared to chemotherapy. The combination of PG-1+etoposide had a synergistic effect on apoptosis of U251 glioma cells. It should be noted that cells were in the early and late stages of apoptosis (70.4 ± 11.4 and 11.7 ± 4.7 % of cells in the state of early and late apoptosis, respectively), compared with control cells. The caspase 3 activation analysis revealed that the caspase-3 level was not significantly ($p > 0.05$) increased in U251 cells following PG-1 with etoposide treatment compared with those in the untreated cells, suggesting that the combination of PG-1 and etoposide may induce caspase-independent apoptosis in U251 cells.

Conclusion. PG-1 represent promising drugs candidate as the treatment regimen for GBM. Furthermore, the synergistic efficacy of the combined protocol using PG-1 and etoposide may overcome some typical limitations of conventional therapeutic protocols, thus representing a promising approach for GBM therapy.

The project is supported by the Ministry of Education and Science of the Russian Federation (State assignment № 122112100051-0).

PEPTIDES IN COMPLEX THERAPY OF MUSCOSKELETAL PATHOLOGY: CLINICAL AND MOLECULAR ASPECTS

***Linkova N. S.*[1], *Myakisheva S. N.*[1], *Ryzhak G. A.*[1], *Khavinson V. Kh.*[1, 2]**

[1]St. Petersburg Institute of Bioregulation and Gerontology, St. Petersburg, Russia
[2]Pavlov Institute of Physiology of the Russian Academy of Sciences, St. Petersburg, Russia

Introduction. The search of new methods of therapy of the musculoskeletal system diseases is an urgent task of gerontology and molecular medicine due to the high prevalence of these pathologies. It is particularly necessary to emphasize the medical and social significance of diseases of the musculoskeletal system, since they reduce the quality of life (pronounced pain syndrome), mobility, efficiency and social activity of middle-aged and elderly people.

Purpose. The purpose of the investigation is to analyse polypeptide complex of cartilage and bone tissues (PCC) and AED peptide efficiency in complex treatment of musculoskeletal system diseases.

Methods and results. The reparative effect of PCC on bone tissue was revealed in 2 experimental models of traumatic fracture of old rabbits. The formation of a full-fledged flat spongy bone was observed on 7 days earlier in comparison with the control. PCC restored the structure of cartilage tissue in the model of posttraumatic osteoarthritis in rats. PCC and AED peptide restored the bone mineral density in rats after ovariectomy (model of osteoporosis). AED peptide contributed to an increase of the number of thyroid C cells and the restoration of their function, which indicates an increase in the process of calcium resorption in bone tissue. PCC and AED peptide stimulated PCNA synthesis and reduced p53 synthesis in chondrocytes of young and old rats.

The efficacy of PCC was evaluated on 33 patients with osteochondrosis of the lumbar spine. The severity of pain syndrome decreased in 67.4 % of patients after PCC applying. This is due to the fact that the progression of the disease, accompanied by arthrotic changes in the intervertebral discs on the X-ray, contributes to the development of spondylosis and neurotrophic disorders. The application of PCC and AED peptide in patients with osteoarthritis of the knee joints reduces the severity of pain syndrome and increase joint mobility in 55—69 % of cases. Pain symptoms disappeared most completely with radiologically determined initial stages of the disease.

Conclusion. PPC and AED peptide have shown high efficiency in the complex therapy of diseases of the musculoskeletal system in experiments and in the clinic. AED peptide normalized bone density in osteoporosis by regulating the function of calcitonin-producing thyroid cells. The mechanism of action of PCC and AED peptide is their ability to reduce p53 synthesis and increase PCNA synthesis in chondrocytes.

ANTIMICROBIAL AND COMPLEMENT-MODULATING PROPERTIES OF HUMAN DEFENSINS AND STRUCTURALLY RELATED PEPTIDES

***Berlov M. N.*[1], *Krenev I. A.*[1], *Umnyakova E. S.*[1, 2], *Gorbunov N. P.*[1, 3], *Kostevich V. A.*[1], *Komlev A. S.*[1], *Yakovlev A. Yu.*[4], *Oborin N. N.*[1, 4], *Aleshina G. M.*[1], *Shamova O. V.*[1, 4]**

[1]Institute of Experimental Medicine, St. Petersburg, Russia
[2]University of Basel, Basel, Switzerland
[3]Pasteur Institute, St. Petersburg, Russia
[4]St. Petersburg State University, St. Petersburg, Russia

Introduction. Human α-defensins 1-3, or human neutrophil peptides (HNPs) are among the best known animal antimicrobial peptides. HNPs exhibit appreciable antimicrobial activity only under hypotonic conditions. However, in circulation, HNPs can manifest some immunomodulatory properties, in particular inhibiting the classical pathway of complement activation. In this work, we compared some properties of HNPs and two other peptides, defensin from the blowfly *Calliphora vicina* (CvD) and porcine protegrin-1 (PG-1). Although PG-1 belongs to cathelicidin family, it has moderate homology with HNPs.

Purpose. To compare the complement-modulating properties and antimicrobial activity under different conditions of human α-defensins, *C. vicina* defensin and protegrin-1.

Methods. HNPs and CvD were purified from natural sources, PG-1 was obtained by solid-phase synthesis. The models of the classical (CP) and the alternative pathways (AP) of complement contained antibody-sensitized sheep red blood cells or untreated rabbit red blood cells, respectively. Complement activity in human normal serum was estimated by hemolysis and by ELISA for C3a. Antimicrobial activity against *Listeria monocytogenes* EGD was determined by colony counting assay.

Results. While HNPs behave as a specific CP inhibitor in complement modulation experiments, PG-1 showed the ability to inhibit both pathways. Furthermore, PG-1 at low concentrations enhanced C3a production in the CP model, thus demonstrating a bidirectional effect on complement activation. CvD at high concentrations caused only a moderate increase in C3a accumulation in the CP model without affecting the hemolysis level. PG-1 demonstrated significantly higher efficacy than defensins in antimicrobial activity experiments. Heat-inactivated human serum and C1q complement protein inhibited antimicrobial activity of all the peptides studied to a different extent. NaCl decreased the antimicrobial activity of defensins but, on the other hand, in the range of 0-0.15 M it enhanced the activity of PG-1.

Conclusion. Most likely, in serum, HNPs will behave as complement inhibitors, CvD will behave as an antimicrobial peptide, and PG-1 may exhibit both of these activities. CvD and PG-1 partially retain antimicrobial activity in human serum. C1q can contribute to the inhibition of antimicrobial activity of defensins and protegrin by human serum.

The project is supported by the Ministry of Education and Science of the Russian Federation (State assignment № 122020300189-6).

THE EDR AND KED PEPTIDES IMPROVE THE MORPHOFUNCTIONAL STATE OF NEURONAL NETWORKS IN MOUSE MODEL OF ALZHEIMER'S DISEASE

Ilina A. R.[1, 2]

[1]Institute of Experimental Medicine, St. Petersburg, Russia
[2]Institute of Bioregulation and Gerontology, St. Petersburg, Russia

Introduction. Alzheimer's disease (AD) is a common neurodegenerative disease in the elderly and the most prevalent cause of dementia. AD is characterized by a progressive cognitive impairment. Currently FDA-approved drugs for the treatment of AD don't prevent or slow down the neurodegeneration. Moreover, these drugs are characterized by considerable side effects that aren't incompatible to normal vital activities of patients. EDR and KED peptides are potential therapeutic agents for AD treatment in view of low immunogenicity, lack of side effects, high physiological activity.

Methods. Synaptic loss underlies the cognitive impairments in AD. Impaired neurotransmission reflects changing in the functional state of synapses and mediated by decreased protein synthesis involved in neurotransmission. These changes trigger the decreased number of postsynaptic contacts and changes in the dendritic spine morphology. A 5xFAD-M transgenic line of mice allows to visualize the dendritic spine morphology in mouse brain affected by neurodegeneration. Fixed slices of a mouse hippocampus were taken after course injections of peptides. Prepared fixed hippocampal slices were directly analyzed by means of confocal microscopy followed by obtaining the microphotographs of neuronal dendrites in mouse hippocampus. Morphological analysis of dendritic spines was carried out by NeuronStudio software.

Results. It was established that daily intraperitoneal injection of EDR and KED peptides at the concentration of 400 μg/kg provide statistically significant rising number of mushroom spines by 25 and 27 %, respectively, in 5xFAD-M mice from 3 to 5 months of age. Dendritic spine density in neurons of 5xFAD-M mice was increased by 13 and 22 % in 5xFAD-M mice injected by EDR and KED, respectively. These findings suggest that EDR and KED short peptides improve the functionally state of neuronal networks in hippocampus that underlies the neuroprotective effect of these compounds in in vivo AD model.

Conclusion. In conclusion, it should be noted that EDR and KED peptides as active compounds may be recommended to future investigation in order to develop a safe and effective therapeutic agent for the treatment of Alzheimer's disease.

MINIMAL INHIBITING CONCENTRATION PREDICTED *IN SILICO* VS EXPERIMENTAL VALUE

***Khaydukova M. M.*[1, 2], *Kemp-Nemm E. A.*[2], *Shamova O. V.*[1]**

[1] Institute of Experimental Medicine, WCRC for Personalized Medicine, St. Petersburg, Russia
[2]Institute of Human Hygiene, Occupational Pathology and Ecology, St. Petersburg, Russia

Introduction. Natural and synthetic antimicrobial peptides (AMPs) are considered as prospect molecules for counteraction grooving problem of antibiotic resistivity. One of the approaches for new AMPs creation is application of machine learning techniques. Usually mathematical models were used for class membership identification. In such case response of model is probability of belonging to the class of AMPs. At the same time value of minimal inhibitory concentrations (MICs) of the substances classified as AMP can vary in the wide range. MIC is the lowest concentration of AMP that inhibits the visible growth of a microorganism and it should be as small as possible. In the study partial least squares regression (PLS) was used for the MICs prediction based on the amino acid (AA) sequences. This approach provides an opportunity for quantitative prediction of the parameter *in silico*. However, prediction ability of the model has to be validated with real experimental data.

Purpose. Validation of the PLS models which were build for prediction of MIC towards particular bacteria upon AA sequences.

Methods. Two PLS models were built based on data from DRAMP database. These models suppose to predict MICs of the new peptides towards *Pseudomonas aeruginosa* or *Acinetobacter baumannii*. Six new peptides with predicted MICs near to 5 μM were synthesized by solid phase peptide synthesis technique, purified by RP HPLC. MICs of the obtained products were estimated.

Results. Four peptides were determined with PLS model for estimation of the AMPs against *P. aeruginosa*. Two synthesized products have MICs 4 μM towards laboratory strain of bacteria. Another two peptides had predicted activity against *A. baumannii* and one of them have shown MIC 4 μM towards two studied strains.

Conclusion. The predicted and experimentally obtained MICs were close in half of the cases. It is shown that in order to find peptides active against drug resistant strains, it is necessary to carry out modeling based on the data about peptides activity towards such strains.

THE EFFECTS OF CATHELICIDIN LL-37 TOWARDS GLIOBLASTOMA CELLS *IN VITRO* AND *IN VIVO*

***Chernov A. N.*[1], *Pyurveev S. S.*[1, 2], *Filatenkova T. A.*[1], *Rudel A. E.*[1], *Shamova O. V.*[1, 3]**

[1]Institute of Experimental Medicine, St. Petersburg, Russia
[2]St. Petersburg State Pediatric Medical University, St. Petersburg, Russia
[3]St. Petersburg State University, St. Petersburg, Russia

Introduction. Malignant brain neoplasms in adults include glioblastomas and astrocytomas. The prognosis for these patients is unfavorable. Therapy for central nervous system tumors is commonly carried out using chemotherapy with agents such as temozolomide, cisplatin, carboplatin and bevacizumab. However, the use of chemotherapy is accompanied by several problems: (1) poor permeability of the blood-brain barrier for chemotherapy; (2) high cytotoxicity, causing adverse side reactions in the physiological system; (3) the non-selective action of chemotherapy. More than 5000 AMPs are known to date. Most AMPs are molecules consisting of 12–50 amino acids with a high content of arginine and/or lysine, possessing antimicrobial activity against bacteria, unicellular fungi, protozoa and viruses. AMPs exhibit immunomodulatory, mitogenic, antitumor and less toxic effects on normal tissues. To study the antitumor effects, we selected a cathelicidin of LL-37, with an α-helical structure from azurophilic granules of human neurophils.

Purpose: to study the effects of LL-37 on size, weight of C6 glioma and survival of the rats.

Methods. The experiments were performed on 17 Wistar rats weighing 200—300 g, which are on a standard diet in the vivarium of the Institute of Experimental Medicine. All rats underwent a neurosurgical operation with the introduction of 1 million C6 glioma cells in 10 μl of saline. After that the rats were randomly divided into 3 groups: control (n = 9) and experimental (n = 8). Control animals were intranasally injected 20 μl of saline twice a week. In the experimental groups, 20 μl of LL-37 was intranasally administered at a dose of 35 μM (n = 4) and at a dose of 300 μM (n = 4) twice a week. The weight, size of the tumor, and survival of the animals were assessed.

Results. The results show that the intranasal administration of the LL-37 at a dose of 300 μM increases the survival rate of the rats (49.3 ± 17.61 days) compared with the control (26.22 ± 2.66 days, p = 0.04). At the same time, the size and weight of the tumor are increased (153.8 ± 43.53 mg, and 80.0 ± 16.3 mm^3) relative to the control (49.4 ± 13.3 mg, p = 0.038, 31.0 ± 8.8 mm^3, p = 0.0076). Intranasal administration of LL-37 at a dose of 35 μM also significantly increases the survival rate of rats (66.75 ± 12.6 days) compared with the control (26.2 ± 2.66 days, p = 0.0008).

Conclusion. Thus, the use of LL-37 can increase the lifespan of rats with C6 glioma. LL-37 does not inhibit tumor growth in vivo, but, possibly, protects the tumor from the effects of immune system, making it non-immunogenic.

The project is supported by the Ministry of Education and Science of the Russian Federation (State assignment № 122112100051-0).

COMPARATIVE ANALYSIS OF ANTIMICROBIAL ACTIVITY OF VARIOUS SILVER NANOPARTICLES AND THEIR COMBINED ACTION WITH β-HAIRPIN PEPTIDES

***Vladimirova E. V.*[1], *Vishnevetskii D. V.*[2], *Sukhareva M. S.*[1], *Komlev A. S.*[1]**

[1] Institute of Experimental Medicine, WCR Centre for Personalized Medicine, St. Petersburg, Russia
[2]Tver State University, Tver, Russia

Introduction. Silver nanoparticles (AgNPs) exhibit pronounced antibacterial activity against many Gram-positive and Gram-negative bacteria. The antibacterial potential of AgNPs can be additionally increased by combined use with antimicrobial peptides (AMPs). The most active and stable AMPs under physiological conditions are peptides of animal origin with a β-hairpin structure.

Purpose. To evaluate the antimicrobial activity of AgNPs and their combined antimicrobial activity with β-hairpin peptides.

Methods. Antimicrobial activity of the substances was determined by the method of serial dilutions in a liquid nutrient medium and the joint antimicrobial action — by serial dilutions according to the "checkerboard" method. Spherical silver nanoparticles (NPs) stabilized with sodium oleate (AgNPs/OleNa), polyethylene glycol 6000 (AgNPs/PEG 6000), polyvinylpyrrolidone (AgNPs/PVP), tween 80 (AgNPs/Tw8), low molecular weight sodium hyaluronate (AgNPs/HiLm) and a combination of panthenol with low molecular weight sodium hyaluronate and sodium oleate (AgNPs/DPT) (Nanomaterials & Technologies M9, Russia). Acetylcysteine-silver solutions and hydrogels (AgNPs № 1, AgNPs № 2) were produced in Tver State University in Dmitriy Vishnevetskiy lab. The peptides Shuchin-4 (Shu-4), protegrin-1 (PG-1), two structural modifications of Shuchin 4 (SR and SM) were chemically synthesized in the laboratory of design and synthesis of biologically active peptides of the Department of General Pathology and Pathological Physiology of the Institute of Experimental Medicine.

Results. Minimum inhibitory concentrations (MICs) against *E. coli* ML-35p were 1.95 ppm (μg/mL) for AgNPs/OleNa, AgNPs/DPT, AgNPs/PEG6000, AgNPs/HiLm and 3.9 ppm for AgNPs/PVP and AgNPs /Tw8. AgNPs/OleNa were the most active against *P. aeruginosa* 522/17 MDR and *A. baumannii* 7226/16, for which the MIC was 0.98 and 1.9 ppm, respectively, and for the remaining AgNPs, MIC was 3.9—7.8 ppm. These AgNPs are the least active against gram-positive bacteria MRSA ATCC 33591 and *S. aureus* 1399/17, the range of MIC was 15.6-125 ppm. MICs of AgNPs № 1 and № 2 against *P. aeruginosa* 522/17 MDR were 11.7 and 23.5 ppm, respectively, and

against *A. baumannii* 7226/16 — 23.5—46.9 ppm. AgNPs № 1 and № 2 are also less effective against gram-positive bacteria *S. intermedius* and *S. aureus* ATCC 25923, MIC — 93.8-187.5 ppm. It was found that AgNPs/OleNa, and AgNPs № 2, exhibit synergistic antibacterial action with PG-1 against *P. aeruginosa* 522/17 MDR. Synergistic antimicrobial effects are also observed when SR is combined with AgNPs № 1 and № 2 against *A. baumannii* 7226/16 and Shu-4 is combined with AgNPs № 2 against *E. coli* ML-35p. The combined action of SM with NPs did not reveal any synergistic antimicrobial effects.

Conclusion. The studied silver nanoparticles are more effective against gram-negative bacteria. The combined use of silver nanoparticles with β-hairpin peptides seems to be a promising approach to the development of antimicrobial drugs.

The project is funded by the Ministry of Education and Science of the Russian Federation, Agreement № 075-15-2022-302 (20.04.2022).

ANTIMICROBIAL PEPTIDES: AUSPICIOUS TOOLS AGAINST THE EMERGING MULTU-DRUG RESISTANCE

***Botros M. A. F.*[1, 2], *Sukhareva M. S.*[2], *Vladimirovna E. V.*[2], *Komlev A. S.*[2], *Abelo M.*[2], *Shamova O. V.*[1, 2]**

[1]St. Petersburg State University, St. Petersburg, Russia
[2]Institute of Experimental Medicine, St. Petersburg, Russia

Introduction. Due to abuse of antibiotics in medicine, agriculture and animal husbandry, especially in developing countries, resistance to antimicrobials had become one of the most current dangers to human wellbeing and every year multi-drug resistant microbes are tainting millions of individuals around the world, with numerous passing on as a result. Since their discovery a few decades ago, antimicrobial peptides (AMPs) from innate defenses have been hailed as a potential alternative to ordinary antimicrobials due to their relatively low potential for bacterial resistance. Despite the continued efforts of academia and start-ups, there are currently no AMPs-based drugs in use.

Purpose. Our aim was to determine the minimal inhibitory concentrations (MICs) of some modified bactenecin ChBac3.4 variants which had been modified via adding methyl group (ChBac. 3.4 — OMe), ethyl group (ChBac. 3.4 — OEt) or n-butyl group (ChBac. 3.4 — OBu) in order to enhance their stability and activity compared to the native peptide. Also, our target includes examination the effects of these AMPs on the barrier function of bacterial membranes and check their side effects such as hemolytic activity which can hinder clinical development.

Methods. We studied the antimicrobial activity of the previously mentioned peptides using broth microdilution assay. Human erythrocytes were used for preliminary *in vitro* evaluation of hemolytic activity. The effect of these AMPs on the barrier function of bacterial membranes was examined via assessing their influence on the permeability of the outer and cytoplasmic membranes of *E. coli* ML35p using Nitrocefin and *o*-Nitrophenyl-β-D-galactopyranoside [ONPG] as chromogenic markers.

Results. Generally, preliminary results show that the modified variants have better activity than the native peptide having lower MICs against both Gram-negative and Gram-positive bacteria, including antibiotic resistant bacteria, and lower hemolytic activity. Interestingly, the modified AMPs demonstrated very weak influence on bacterial membranes permeability compared to the native ChaBac. 3.4, which, in turn, can give a hint that the effect exerted by these variants maybe through an intracellular mechanism. We are going to discover their mechanism of action through further studies.

FUNCTIONAL CHANGES IN THE ACTIVITY OF THE VESTIBULAR EPITHELIUM AFFERENT SYNSPSE UNDER THE INFLUENCE OF CYTOKINES AND PEPTIDES: DEFENSIN HNP-1 AND INTERFERON α2b — COMPARATIVE STUDY

***Ryzhova I. V.*[1], *Shamova O. V.*[2], *Tobias T. V.*[1], *Protasov E. A.*[2]**

[1]I.P. Pavlov Institute of Physiology of the Russian Academy of Sciences, St. Petersburg, Russia
[2]Institute of Experimental Medicine, St. Petersburg, Russia

Introduction. The afferent glutamatergic synapse of the vestibular epithelium is extremely sensitive to the influences of exogenous and endogenous factors and some of pharmacological drugs.

Purpose. The aim of this study was to investigate the modulating effect of defensin (HNP-1) and interferon (IFN-α2b) on the function of the afferent glutamatergic synapse.

Methods. The experiments were performed on the isolated vestibular apparatus of the frog using multiunit recording of the action potentials from the semicircular canal afferents under the conditions of drug application to the synaptic zone.

Results. Defensin (0.001–10 nM) had no effect on efferent cholinergic synaptic transmission, but decreased the frequency of the background activity of afferent fibers and the L-Glu evoked responses and its agonists such as AMPA, NMDA and ACPD. In contrast, IFN-α2b (0.2-40 mg/ml) increased the level of resting activity of afferent fibers with a subsequent decrease in the frequency of discharges. Important, that in one quarter of the experiments high concentrations of IFN-α2b (20-40 ng/ml) either did not change or reduced the level of background activity of afferent fibers. The effect of IFN-α2b (10 mg/mL) on the amplitude of L-Glu evoked responses depended on the way of changes in background activity upon exposure to the cytokine. In most experiments, IFN-α2b reduced the amplitude of the response caused by L-Glu. Both HNP1 and IFN-α2b modulated the frequency of impulse activity of afferent fibers, restored by L-Glu, under conditions of blocking the mediator release from hair cells in hyper-Mg^{2+} hypo-Ca^{2+} solution, which suggests their postsynaptic influence.

Conclusion. The presented data indicate the polyfunctional neuroimmunomodulatory effects of HNP-1 and IFN-α2b on the synaptic activity of the afferent synapse of the vestibular apparatus.

NEW ANALOGS OF PROTEGRIN-1 WITH IMPROVED SELECTIVITY OF ANTIBACTERIAL ACTION

Bolosov I. A., Panteleev P. V., Ovchinnikova T. V., Balandin S. V.

M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia

Introduction. Host-defense antimicrobial peptides (AMPs) are key molecular factors of the innate immune system of multicellular species, including humans. They have a unique mechanism of action with a rapid bactericidal effect on pathogenic bacteria. However, many of them can also non-specifically damage host cell membranes due to their strong amphiphilic properties. In some cases, their selectivity can be improved by making minor modifications to the peptide structure using data on structure-activity relationship. One of the most studied vertebrate AMPs are cathelicidins — host-defense peptides synthesized as precursor proteins containing a cathelin-like pro-domain. β-Hairpin cathelicidins are of the greatest interest for use as a drug with a systemic administration due to the rigid structure that provides resistance to proteolytic degradation. This group of peptides includes a family of protegrins, which were first isolated from porcine (*Sus scrofa*) leukocytes.

Purpose. The main goal of the work was to design mutant protegrin-1 (PG-1) analogs with the aim to mitigate its toxic side effects.

Methods. The analogs were produced as thioredoxin fusions in *Escherichia coli*. The activity of the peptides was determined using two-fold serial dilution method against a wide panel of bacterial pathogens and freshly isolated human erythrocytes. The ability of the peptides to damage the bacterial membranes was assessed by measuring their permeability to o-nitrophenyl-β-D-galactopyranoside. Fourier-transform infrared spectroscopy (FTIR) and circular dichroism (CD) spectroscopy were used for structural analysis of the samples and to study of dimerization and oligomerization processes.

Results. Among the cathelicidins identified by us earlier in the genome-wide and transcriptome sequencing databases of Suidae species, new homologs of natural protegrins were found. In this study, we rationally modulated the net charge and hydrophobicity of PG-1. As a result, analogs with reduced toxicity were obtained. Their antimicrobial activity was similar to that of natural PG-1. It was shown that the single amino acid substitution V16R in the *C*-terminal β-strand responsible for PG-1 dimerization could significantly reduce hemolytic activity without loss of antimicrobial activity.

Conclusion. The obtained analogs have an improved therapeutic index and can be considered as leads for the development of new peptide antibiotics.

This study was supported by the Russian Science Foundation, project № 22-25-00496.

BIODIVERSITY AND THERAPEUTIC POTENTIAL OF BRICHOS DOMAIN-RELATED ANTIMICROBIAL PEPTIDES FROM MARINE POLYCHAETA

Safronova V. N., Panteleev P. V., Ovchinnikova T. V.

M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia

Introduction. In recent years, the antimicrobial resistance, particularly among Gram-negative bacteria, is growing rapidly, and new antibacterial agents are required. Antimicrobial peptides (AMPs) also referred to as host-defense peptides are characterized by various mechanisms of antibacterial and antibiofilm action against socially significant pathogens. The role of AMPs is especially important for invertebrates due to the lack of an adaptive immune system. Marine polychaeta is an evolutionarily ancient class of annelid worms, which can produce a wide variety of bioactive molecules. In previous study, we have shown that polychaeta have a universal system of AMPs biosynthesis based on synthesis of precursor proteins containing the so-called BRICHOS domain as their *N*-terminal parts. Therefore, all the above-mentioned AMPs can be designated as BRICHOS domain-related antimicrobial peptides. This work expands the understanding of biological functions of new structural families of host defense peptides in polychaeta and discover the panel of new AMPs with a unique mechanism of action.

Purpose. This investigation focuses on the study of biodiversity of BRICHOS domain-related antimicrobial peptides from marine polychaeta and identification of therapeutically valuable compounds to develop drug candidates against Gram-negative bacteria and, finally, to design novel antibiotics.

Methods. BRICHOS domain-based bioinformatic search in transcriptomes of polychaeta; cloning, sequencing and identification of putative AMPs; recombinant production in bacterial expression systems; antimicrobial, cytotoxicity and antibiofilm assays; ONPG test, induction of resistance and its assessment; rational design of AMPs.

Results. We found 7 new BRICHOS domain-related AMPs with different structures in several polychaeta species and obtained them by heterologous expression in the *E. coli* BL21(DE3) or endotoxin-free ClearColi® BL21(DE3) systems. We investigated the antimicrobial, hemolytic and cytotoxic activity, as well as putative mechanisms of action of the obtained peptides against bacteria. To develop compounds with selective antibacterial activities against Gram-negative ESKAPE pathogens, including multidrug-resistant strains, we designed a number of analogs of β-hairpin AMPs by substitution of individual amino acids residues in the β-turn region. This approach let us get two modified analogs of natural peptides, which retain a high antibacterial activity and

lack toxicity potency toward mammalian cells. In addition to antibacterial potential, improved analogs can act in the early stages of biofilm formation and destroy mature biofilms of *P. aeruginosa*. These AMPs were chosen as lead candidates for further testing in mice models of Gram-negative infections.

Conclusion. In this study the seven novel BRICHOS domain-related AMPs from marine polychaeta were investigated. The ability of modified analog of β-hairpin antimicrobial peptide to effectively kill ESKAPE pathogens allow us to consider BRICHOS domain-related antimicrobial peptides as promising molecular scaffolds for the design of novel antibiotics for systemic administration.

The work was supported by the Russian Science Foundation (Agreement № 22-14-00380, https://rscf.ru/project/22-14-00380/).

TIME-KILL ASSAY: SYNERGY OF ANTIBIOTICS, ANTISEPTICS AND PEPTIDES

***Spiridonova A. A.*[1], *Afinogenova A. G.*[2], *Afinogenov G. E.*[3]**

[1]I. P. Pavlov St. Petersburg State Medical University, St. Petersburg, Russia
[2]Pasteur Institute, St. Petersburg, Russia
[3]St. Petersburg State University, St. Petersburg, Russia

Introduction. Pathogenic bacteria may survive antibiotic attack, exert tolerance and persistence accompanied with the ongoing infectious process. In connection with this, determining dependence between bactericidal effect of antimicrobial agents and exposure time on bacteria at 1, 2, 4, 6, 8, 12 and 24 hours after the onset, a so called time-kill assay, is necessary.

Purpose. The purpose of the study was to evaluate antimicrobial synergistic effect of combinations of antiseptics, antibiotics and peptides at various expositions towards antibiotic-resistant isolates.

Methods. Approved for clinical use antiseptics (sodium hypochlorite, poviargol, dioxidine), antibiotic (gentamicin), polypeptide antibiotic (gramicidine) at sub-bactericidal concentrations as well as their combinations were tested against polyresistant isolates. The dependency of the bactericidal action of the preparations on the time of the exposition to pathogen was evaluated by the microdillution method (Time-kill assay).

Results. Optimal concentrations of antiseptics and peptides were found. The best combinations inhibited bacterial growth after 6 hours of incubation by two $\log_{10}$; after 20 hours they inhibited bacterial growth by four $\log_{10}$ or exerted full bactericidal action. This suggests a synergetic effect of the tested preparations.

Conclusion. Time-kill assay allowed to investigate effective combinations of antiseptics and antibiotics towards resistant hospital strains of microorganisms including combinations with gentamicin to which isolates were resistant. The doses of the components in the elaborated antimicrobial combinations were hundreds or thousand times lower than the permitted doses for clinical topical use.

ANTIMICROBIAL PEPTIDES AS CANCER DRUGS CANDIDATES

Zharkova M. S., Rudel A. E., Filatenkova T. A.

Institute of Experimental Medicine, St. Petersburg, Russia

Introduction. Antimicrobial peptides (AMPs) with anticancer activity are believed to distinguish between normal and cancer cells due to the difference in the membrane composition (primarily, its lipidic part), unlike other anticancer agents. Thus, they are regarded as interesting candidates to broaden anticancer drugs arsenal and overcome common resistance mechanisms of the cancer cells.

Purpose. The study was aimed at analyzing anticancer activity of a number of natural AMPs to select candidates for further development of modified optimized variants.

Methods. AMPs PG-1 (β-hairpin) and ChBac 3.4 (proline-rich) previously reported for anticancer activity against hematologic cancer cells, as well as previously non-characterized for anticancer properties β-hairpin peptides Thanatin and Shu4 and proline- and tryptophan-rich peptide BuCath were tested against a number of monolayer-forming cancer cells lines (human colorectal adenocarcinoma Caco-2, hepatocellular carcinoma Hep G2, immortalized human vascular endothelial cells EA.hy 926, and rat glioma C6) using MTT-test for *in vitro* assessment of their potential against solid tumors. PG-1 and a slightly modified variant ChBac 3.4-1 were also analyzed in Ehrlich ascites carcinoma (EAC) mice model. For the solid form mice were subcutaneously injected with 100 000 EAC cells in the upper back and treated intraperitoneally once per week till the end of experiment; for the ascitic form mice were intraperitoneally injected with 2 000 000 EAC cells and treated intraperitoneally every other day for 6 days (with a total of 3 injections).

Results. Thanatin and Shu4 showed no anticancer activity against the selected cancer cells lines. PG-1 was the most active among the tested peptides (IC_{50} from 4 to 23 μM), followed by BuCath (IC_{50} from 10 to 34 μM) and ChBac 3.4 (IC_{50} from 27 to 73 μM). PG-1 (in 5 μg per mice per injection dose) showed no positive effect on mice survival in EAC models. However, ascites bearing mice treated with PG-1 sacrificed on the 10^{th} day of the experiment demonstrated the statistically significant reduction of the cancer cells density in ascitic fluid compared with the untreated control. Interestingly, ChBac 3.4 variant improved the lifespan of the solid EAC bearing mice similarly at 1 and 100 μg per mice per injection doses. Combined with lower *in vitro* activity it may suggest indirect (e. g. immunomodulatory) *in vivo* mechanism.

Conclusion. PG-1, BuCath and ChBac 3.4 demonstrate quite promising anticancer properties for further designing peptidic anticancer agents based on their structure. Primary optimization goals are enhancing their targeting of cancer cells and stability within the organism.

The project is supported by the Ministry of Education and Science of the Russian Federation (State assignment № 122112100051-0).

PEPTIDES INVOLVED IN THE IMPLEMENTATION OF THE PROTECTIVE FUNCTIONS OF MIXED SALIVA

Sukhareva M. S., Vladimirova E. V.

Institute of Experimental Medicine, WCRC for Personalized Medicine, St. Petersburg, Russia

Introduction. The oral cavity is one of the main barriers to the penetration of pathogens into the internal environment of the body. Saliva plays an important role in the implementation of anti-infective functions of the oral cavity. It contains antimicrobial cationic peptides, but their concentration in the salivary fluid is relatively low, as well as proline-rich proteins and peptides, the functions of which are currently poorly understood and not elucidated. Obtaining new data on the biological activity of saliva PRPs, finding out whether they play a significant role in providing its protective functions, whether they interact with other well-studied proteins involved in the implementation of anti-infective protection of the oral cavity, in particular with AMP, is important for understanding molecular-cellular bases for maintaining homeostasis of the oral cavity.

Purpose. Elucidation of the role of proline-rich peptides of saliva in the implementation of protective reactions in the oral cavity.

Methods. The following methods were used in the work: 1. Method of serial dilutions in a liquid nutrient medium, containing microorganisms. 2. The study of the joint antimicrobial action of the analyzed peptides with other proteins and peptides present in saliva. 3. Study of the effect of proline-rich peptides on bacterial biofilm formation using crystal violet. 4. Wound healing in a model of a full-thickness skin wound in mice, the wound surface of which was treated with the analyzed peptides.

Results. 1. Chemically synthesized fragments of proline-rich human saliva proteins: P-F (43-61), P-H (37-51), IB6 (98-116), p1932 have low antimicrobial activity or practically do not show it against gram-negative and gram-positive bacteria. 2. The antimicrobial activity of some AMPs and proteins present in saliva against planktonic bacteria increases, in the presence of proline-rich peptides. 3. With the combined action of PRPs with LL-37, an increase in inhibition of the formation of bacterial biofilms is observed. 4. The use of various combinations of PRPs with lysozyme, lactoferrin, LL-37 has a positive effect on the dynamics of healing of infected wounds in experimental animals.

Conclusion. Data obtained help to assess the role of proline-rich peptides in the antimicrobial protection of the oral cavity, as well as to suggest that they are able to regulate inflammatory processes and can take part in regenerative processes.

The project is funded by the Ministry of Education and Science of the Russian Federation, Agreement № 075-15-2022-302 (20.04.2022).

APPLICATOPN OF PALMITOYL HEXAPEPTIDE PEPTIDE AS A WOUND HEALING AGENT

Petrova P. E., Komlev A. S., Sukhareva M. S., Vladimirova E. V., Ivasenko A. A., Yudin D. A., Fedorov I. P., Galianova M. V.

Institute of Experimental Medicine, WCRC for Personalized Medicine, St. Petersburg, Russia

Introduction. Healing of wounds is a complex process that involves various cellular and molecular mechanisms. The search for effective therapeutic agents to enhance wound healing has led to the investigation of peptide-based compounds.

Purpose. In this study, we evaluated the wound healing efficacy of palmitoyl hexapeptide (PGP) using an experimental model of infected full-thickness skin wounds.

Methods. Using of SPPS (solid phase peptide synthesis), analyzing by HPLC (high-performance liquid chromatography), modeling of contaminated wounds on laboratory mice's full-layer skin.

Results. Our results demonstrate a significant positive effect of palmitoyl hexapeptide on wound healing. Specifically, the rate of wound area reduction over an 16-day period was remarkably accelerated in wounds treated with palmitoyl hexapeptide compared to those treated with the control of bacteria. Wounds treated with palmitoyl hexapeptide exhibited practically complete healing within the 14 day and its surface has been reduced by 98,1 % while in control group it was only 86,5 %. However, there are no significant differences between groups. That means further research is needed in this direction. These findings highlight the promising potential of palmitoyl hexapeptide as a novel therapeutic agent for enhancing wound healing. Furthermore, the formulation of a gel-based delivery system utilizing palmitoyl hexapeptide shows great prospects for future clinical investigations.

Conclusion. Further studies are warranted to elucidate the underlying mechanisms by which palmitoyl hexapeptide promotes wound healing and to assess its safety and efficacy in real-world clinical settings. The development of innovative wound healing therapies based on palmitoyl hexapeptide could revolutionize the field and provide new strategies for improving patient outcomes in wound care.

The project is funded by the Ministry of Education and Science of the Russian Federation, Agreement № 075-15-2022-302 (20.04.2022).

MODIFICATION OF THE C-TERMINUS OF A PEPTIDE IN SOLUTION THROUGH ESTERIFICATION USING TRIMETHYLCHLOROSILANE

***Galianova M. V.*[2], *Komlev A. S.*[2], *Petrova P. E.*[1], *Ivasenko A. A.*[1], *Fedorv I. P.*[1], *Yudin D. A.*[1], *Zabrodskaya Y. A.*[1, 3]**

[1]Institute of Experimental Medicine, WCRC for Personalized Medicine, St. Petersburg, Russia
[2]St. Petersburg University, St. Petersburg, Russia
[3]Research Institute of Influenza, St. Petersburg, Russia

Introduction. In our research, we decided to modify proline-reach peptides with ethanol, methanol, butanol and got C-terminally modified molecules. Foreign authors (Christine A. Arbour, Lawrence G. Mendoza, and Jennifer L. Stockdill) investigated the increased activity of this modification and the vulnerability to cleavage by endogenous esterases.

Purpose. We tried to compare the characteristics of non-modified and esterificated peptide.

Methods. Peptide RFR 1-14 (a part of bactenicin 3.4) was synthesized with solid-phase peptide synthesis. We used earlier described method of ultrasonication to decrease the duration of reaction and purity of crude peptides. We used the protocol of esterification with trimethylchlorosilane in alcohols during 21 hours. Then we verified the structure of ester with mass-spectrometry and performed the analysis of antimicrobial activity.

Results. The ester of RFR 1-14 showed high antimicrobial activity, but also the tendency to hydrolysis.

Conclusion. We synthesized peptide RFR1-14 and achieved the purity level over 95 % measured by HPLC and analyzed with MS. This modified peptide could be used in the future research as a pro-drug because of high antimicrobial activity. These esters possess tendency to aminolysis and this ability could be used to obtain corresponding C-terminal amidated peptides.

The project is funded by the Ministry of Education and Science of the Russian Federation, Agreement № 075-15-2022-302 (20.04.2022).

CYTOTOXIC ACTIVITY OF PEPTIDES OF INNATE IMMUNITY FROM VARIOUS STRUCTURAL CLASSES ON RAT GLIOMA TUMOR CELLS

Rudel A. E., Filatenkova T. A.

Institute of Experimental Medicine, St. Petersburg, Russia

Introduction. Unfavorable environmental conditions, chronic stress, infections contribute to impaired immune system functions and lead to an increase in the number of various types of pathology. One of the most important problems in medicine is to overcome drug resistance of both microorganisms and tumor cells. A promising basis for creating new generation drugs are cationic peptides of the innate immune system.

Purpose. The aim of the study is to establish the antitumor activity of peptides of various structures against rat brain tumor cell lines.

Materials. Rat glioma cell line C6 was selected as the studied tumor cells. The following peptides were also used: protegrin-1 (PG-1), protegrin-2 (PG-2), indolicidin-2 (Ind-2), buffalo cathelicidin-4 (Bucathl4), bactenecin ChBac 3.4 and its modifications ChBac 3.4-1-COOH, ChBac 3.4-1-NH_2. Four dilution series of 8, 16, 32 and 64 μM were selected as final concentrations.

Methods. 1. Evaluation on cells using the MTT test. Two types of controls were used in the experiments: 1) Positive control; 2) Negative control. 2. Measurement of optical density in the wells of a plate at a wave length of 540 nm using a plate spectrophotometer POLARstar Omega (Labtech, Italy). 3. Statistical calculations were performed using the Prism 4.5 package (GraphPad Software, USA).

Results. The results of the MTT test showed that the peptides PG-1, PG-2, Bucathl4, ChBac3.4 and ChBac3.4-1-COOH suppress cells within the selected peptide concentrations. Fifty percent inhibitory concentrations (IC_{50}) were also calculated for the selected cell line. According to the results of the experiment, IC_{50} for the protegrin family (PG-1 and PG-2) was 15.23 μM and 50.79 μM. Only two of the presented had a cytotoxic effect, namely: ChBac 3.4 (IC_{50} = 36.94 μM) and ChBac 3.4-1-COOH (IC_{50} = 42.51 μM). For cathelicidin Bucathl4 IC_{50} was also high and reached 40.04 μM.

Conclusion. As shown by a number of experiments, the C6 cell line is resistant to the action of ChBac 3.4-1-NH_2 and Ind-2 peptides at concentrations below 64 μM, which may be due to their low electric charge. The protegrin family binds to tumor cell membranes through electrostatic interactions. It is these interactions between the arginine residues of the peptide and the phosphate groups of phospholipids and other membrane components that are decisive for the realization of cytotoxic effects, which is reflected in the threefold difference in inhibitory concentrations.

The project is supported by the Ministry of Education and Science of the Russian Federation (State assignment № 122112100051-0).

ALTERED CORTICAL GLUTAMATERGIC GENE EXPRESSION AND GUT MICROBIOTA COMPOSITION LINKED TO COGNITION-ENHANCING EFFECTS OF MEDIUM-CHAIN TRIGLYCERIDE SUPPLEMENTATION

***Kim E. A.*[1,2], *Shirokov E. A.*[1,3], *Nikitina V. A.*[1], *Schwarz A. P.*[4], *Krytskaya D. U.*[1], *Arseniev N. A.*[2], *Abdurasulova I. N.*[1], *Klimenko V. M.*[1], *Shcherbakova K. P.*[1], *Trofimov A. N.*[1]**

[1]Institute of Experimental Medicine, St. Petersburg, Russia

[2]St. Petersburg State Chemical Pharmaceutical University, St. Petersburg, Russia

[3]Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia

[4]Sechenov Institute of Evolutionary Physiology and Biochemistry, St. Petersburg, Russia

Introduction. The neuroprotective effects of ketosis, induced by a ketogenic diet (KD), are well established. However, prolonged adherence to KD can lead to side effects. The consumption of medium-chain triglycerides (MCTs) offers a potential way to achieve a state of moderate ketosis on a normal diet, correcting cognitive impairments. Both the consumption of a KD and medium-chain triglycerides MCT have been shown to induce alterations in gut microbiota, potentially influencing brain activity via the gut-brain axis.

Purpose. This study aimed to investigate the effects of moderate ketosis, induced by MCTs, on memory performance, gut microbiota composition, and expression of glutamatergic-related genes in the brain cortex.

Methods. Adult male Wistar rats (9 m. o.) on a standard diet were tested in behavioral tests (Y-maze, open field), after which part of the rats had their food removed for 6 hours a day and were orally administered MCTs (2 ml/kg/day) for two weeks, and then repeated the tests, adding Morris Water Maze. After the final MCT administration, the animals were euthanized, blood was collected for biochemical analysis, and the brain was collected for qPCR. The microbiota composition was studied using genomic DNA sequencing isolated from animal feces. Statistical analysis of the results: rm-ANOVA, Student's t-test, and Mann-Whitney U-test, $p < 0.05$.

Results. MCT administration increased ketone body concentration in the blood, improved working memory, suppressed locomotor activity, and improved spatial memory compared to the control group. The expression of genes encoding glutamatergic NMDA and AMPA receptor subunits (*GluN2a*, *GluN2b*, *GluA1*, *GluA2*) was increased in the brain cortex of the MCT group, indicating more active memory consolidation. The MCT group had increased

levels of *Bacteroidota* and decreased levels of *Patescibacteria* in their gut microbiota composition.

Conclusion. The results suggest that MCTs could be a promising approach for further research into the protective effects of a ketogenic diet in cognitive impairment models in laboratory rats.

Supported by Russian Science Foundation, project № 19-75-10076.

ACUTE EFFECTS OF MEDIUM-CHAIN TRIGLYCERIDE SUPPLEMENTATION ON GENE EXPRESSION, MONOAMINE LEVELS IN THE BRAIN, AND BLOOD CYTOKINE LEVELS IN RATS

***Nikitina V. A.*[1], *Schwarz A. P.*[2], *Traktirov D. S.*[1], *Apryatin S. A.*[1], *Karpenko M. N.*[1], *Krytskaya D. U.*[1], *Klimenko V. M.*[1], *Shcherbakova K. P.*[1], *Trofimov A. N.*[1]**

[1]Institute of Experimental Medicine, St. Petersburg, Russia
[2]Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, St. Petersburg, Russia

Introduction. Medium-chain triglycerides (MCT) possess neuroprotective properties, but the underlying molecular mechanisms are insufficiently understood. We previously demonstrated that chronic 3 g/kg/day MCT improved working memory in rats without causing chronic metabolic changes.

Purpose. This study aimed to investigate the acute effects of MCT on brain gene expression and immunomodulatory effects, in order to identify the underlying mechanisms responsible for the cognition-enhancing effects of MCT.

Methods. We administered a single oral dose of 3 g/kg MCT to 2.5 m. o. male Wistar rats and assessed their blood cytokines (by multiplex immunoassay), brain gene expression (by RT-qPCR), and striatal monoamine levels (by HPLC) at 30, 60, 120, and 180 min post-administration.

Results. In the dorsal hippocampus, MCT administration significantly reduced gene expression of the matrix metalloproteinase-9 (*Mmp9*) at 120—180 min and glucose transporter (*Glut3*) at 120 min, increased the fibroblast growth factor-2 (*Fgf2*) mRNA at 180 min, and increased the relative mRNA levels of NMDA receptor subunits (*GluN1* and *GluN2a*, but not *GluN2b*) at 180 min after administration. Blood ketone body levels inversely correlated with the *GluN1* and *GluN2a* hippocampal expression. In the medial prefrontal cortex, the *GluN2b* and *GluA1* expression peaked at 60 min. Striatal levels of dopamine, serotonin, and their metabolites were not affected, while the HVA/DOPAC ratio significantly increased at 30 min compared to baseline. Several cytokines, including IL-1β, IL-10, and LIX, showed a similar pattern of decreasing at 60 min and then returning to baseline levels at 120—180 min. Leptin and RANTES levels also initially decreased at 60 min but increased significantly above baseline at 120-180 min. IL-2 levels decreased at 30 min, remaining low until 180 min. No significant differences were observed for VEGF, TNF-α, MIP-1α, IL-4, IL-13, and IL-17.

Conclusion. Acute MCT administration affects gene expression in the brain and blood cytokine levels, suggesting potential therapeutic effects of MCT in neurological and immunological disorders.

Supported by Russian Science Foundation, project № 19-75-10076

CHRONIC FATIGUE SYNDROME AND THERAPEUTIC PERSPECTIVES OF CYTOKINE DRUGS

Filatenkova T. A., Shanin S. N., Fomicheva E. E., Serebryanaya N. B.

Institute of Experimental Medicine, St. Petersburg, Russia

Introduction. Chronic fatigue syndrome (CFS) is a common disease in modern world affecting the most young hard-working people in the cities with high pace of life. There is a theory of microbial etiology of this syndrome, but the most convincing one is associated with viral infection. IL-2 is a peptide mediating inflammation and immune response. This cytokine has antibacterial, antiviral and immunomodulatory activity. That is why it could be prospective in complex therapy of CFS.

Purpose. The aim of present study was to analyze possible therapeutic effects of recombinant human IL-2 under the CFS experimental model on cytokine gens expression and metabolic index — concentration of lactate.

Methods. Immunologically induced experimental model of CFS on male Wistar rats by single Poly I:C (Sigma) injection was used. Expression of *IL-1*β, *IL-10, INF-*α, *TLR3* and *5HTT* gens in hypothalamus was analyzed by Real Time PCR with reverse transcription. Also lactate concentration was analyzed in blood plasma. Injection of recombinant human IL-2 (rh IL-2) in dose of 30 μg/kg of body weight was presented as a therapy. All material were collected on 7th day after Poly I:C injection.

Results. After the experimental CFS model application a doubling concentration of lactate in blood plasma of experimental animals was detected. After single medication of rh IL-2 there was no effect on lactate level. There was detected a significal increased level of *IL-1*β and *IL-10* gene expression, as well as increased level of *INF-*α, *TLR3* and *5HTT* gene expression. Injection of rh IL-2 normalized expression of all of the analyzed gens except for *TLR3* gene.

Conclusion. Decreasing and normalizing effect of rh IL-2 single injection on *IL-1*β and *IL-10* gene expression prevents excessive inflammation that can be destructive to organism. Though INF-α is a peptide of the first-line antiviral protection, in general interferon-induced inhibition of translation is lethal for both virus and host cell. That is why its overexpression is also unwelcome. Concentration of lactate is connected with muscle strength and energy metabolism of cells. Changes in energy metabolism could cause fatigue symptoms. The effect on lactate concentration was not shown probably because of short period of experiment.

Increased level of *5-HTT* gene expression could indicate the development of depression, that is why decreasing its level could prevent it. According to this data rh IL-2 could be prospective in CFS therapy and requires further complex research.

The project is supported by the Ministry of Education and Science of the Russian Federation (State assignment № 122020300189-6).

ANTIBIOTIC ACTIVITY OF A PEPTIDE OF THE INNATE IMMUNE SYSTEM - PROTEGRIN-1 AND ITS SYNTETIC STRUCTURAL MODIFICATIONS

Artamonov A.Yu., [1] Orlov S.B. [2], Zheleznikov P.A.[2], Orlov D.S.[1],

[1]Institute of Experimental Medicine, St. Petersburg, Russia
[2]Saratov State Medical University named after V.I. Razumovsky, Saratov, Russia

Introduction. In recent years there has been an alarming increase in the number of cases of antibiotic resistant bacterial infections. Porcine protegrins are β–hairpin peptides with high activity against a wide variety of microbial species, but in their native state these peptides are toxic to human cells. Quantifying and predicting the antimicrobial activity and toxicity towards host cells for natural antimicrobial peptides (AMPs) and their structural synthetic modifications is an important goal of AMP related research.

Purpose. Modern strategies include the development of numerous synthetic structural analogues of peptides with optimized properties. We supposed that in vivo antimicrobial activity of certain host defense peptides could be modulated by modification of their structure. The aim of our experimental work was to study the antibiotic activity of protegrin-1 and its synthetic structural variants.

Methods. Peptide were obtained by means of solid phase chemical synthesis. A set of the peptides was synthesized: protegrin-1 (RGGRLCYCRRRFCVCVGR, C6-C15, C8-C13) and the peptides with different arrangement of disulfide bonds: P45 (RRLCYCRRFCCV, C4-C11, C6-C13); P88 (LGGCRRRFCNPSCK, C4-C9), P85 (RRRFCNPCRRRFCK, C4-C7), P90 (LYCADPRCRPCRRRFRCNPCADPRCKH, C3-C8, C11-C16, C19-C24). Antimicrobial activity was explored using the radial diffusion assay and the photometric procedure based on resazurin reduction assay to quantify cell growth. Bacterial membrane permeability was investigated using special chromogenic markers - nitrocefin and ONPG and the model strain of Gram-negative bacteria - E.coli ML-35p (kindly provided by professor R. Lehrer, UCLA, US). Analysis of the hemolytic activity was performed.

Results. The minimal inhibition concentrations (MICs) of the peptides were evaluated. For *E.coli* ML35p we found the following MICs: PG-1 - 0.67μg/ml, P45 - 2,5 μg/ml, P90 - 5 μg/ml; for *Listeria monocytogenes* EGD: PG-1 - 0.7 μg/ml; P45 - 7 μg/ml; P88 - 5 μg/ml; P85 - 5 μg/ml; P90 - 4 μg/ml; for *Candida albicans*: PG-1 - 0.7 μg/ml; P45 - 6 μg/ml; P88 - 29 μg/ml; P85 - 21 μg/ml; P90 - 21 μg/ml. Permeabilization assay revealed changes in optical density (OD) of *E.coli* ML35p suspension in 60 min after adding the peptides, indicating damage of bacterial outer and inner mem-

branes. For the outer membrane the values of OD were the following: control without peptides - 0,1810 ± 0.003; PG-1 - 0.43 ± 0.003; P88 - 0.3 ± 0,006; P45 - 0.44 ± 0,0006; P90 - 0,2363 ± 0,0026; P85 - 0,1800 ± 0,003. Inner membrane: no peptide - 0,2975 ± 0.0033; PG-1 - 1.3 ± 0.12; P88 - 0.29 ± 0.63; P45 - 0.6 ± 0.001; P90 - 0.027 ± 0.0026; P85 - 0.2827 ± 0.0050. All studied peptides (except P45) demonstrated the low activity towards the outer membrane as well as for the inner bacterial membrane in comparison with PG-1 which induced a fast membrane permeabilization. PG-1 exerted a high hemolytic activity whereas the synthetic except P45 had a lack of the hemolytic activity in the range of concentrations from 1.25 μM to 80 μM. The data obtained suggest that the studied variants of PG1 have a different mechanism of antimicrobial activity compared to the highly membranolytic peptide PG-1 and that the location of disulfide bonds plays a crucial role in the realization of the antibiotic activity of this group of AMPs.

Conclusion.

Antimicrobial peptides (AMPs) of the innate immune system are promising candidates for a role of novel antibiotics. However, some cytotoxicity of AMPs toward host cells limits their active implementation in medicine and forces attempts to design numerous ways of usage of the peptides with optimized properties. Our data support the idea that changing the structures of natural peptides is a promising route in this direction.

The project is supported by the Ministry of Education and Science of the Russian Federation (State assignment № 122020300189-6).

ALPHA MELANOCYTE-STIMULATING HORMONE INCREASES IL-10 AND TGF-β GENE EXPRESSION IN RAT MONOCYTES

Aleshina G. M.[1], Chervyakovskaya O. D.[1], Shilova Yu. S.[2]

[1]Institute of Experimental Medicine, St. Petersburg, Russia
[2]St. Petersburg State Institute of Technology, St. Petersburg, Russia

Introduction. Alpha-melanocyte-stimulating hormone (α-MSH) is a neuropeptide formed during the proteolytic cleavage of proopiomelanocortin. To date, a very wide spectrum of biological activities has been established for α-MSH, including neuroprotective, antimicrobial (which gives some reason to attribute it to the family of antimicrobial peptides), and anti-inflammatory effects. In particular, human blood monocytes stimulated with α-MSH increased the production of the cytokine IL-10 and its mRNA, however, the peptide did not alter the release of IL-10 by T-cells. In addition, α-MSH has been shown to inhibit the production of IFN-γ and TNF-α by human peripheral blood mononuclear cells.

Purpose. The purpose of this work was to study the effect of α-MSH at various concentrations on the expression of the anti-inflammatory cytokine genes IL-10 and TGF-β in rat peripheral blood monocytes and lymphocytes.

Methods. Rat peripheral blood mononuclear cells were obtained by centrifugation using Histopaque-1077 density gradient separation medium (Sigma). The obtained cells were loaded into the wells of a plastic plate (Nunc) and incubated for 2 hours at 37 °C in a CO_2 incubator (Flow Laboratories, UK). During this time, monocytes adhered to the plastic surface, and lymphocytes, as non-adhering cells, remained in the supernatant, which was transferred into test tubes. α-MSH was added to monocytes and lymphocytes at final concentrations ranging from 10^{-8} M to 10^{-12} M. After 2 hours of incubation, RNA was isolated from the cells, and the expression of IL-10 and TGF-β genes was assessed by real-time PCR.

Results. It has been shown that α-MSH does not affect the expression of the TGF-β gene in blood lymphocytes and reduces the expression of the IL-10 gene in lymphocytes by 2—3 times in a wide range of concentrations. α-MSH increases IL-10 gene expression by 9—10 times and TGF-β gene expression by 2-3 times in blood monocytes at a final concentration of 10^{-12} M

Conclusion. The data obtained suggest that the ability to stimulate the production of not only IL-10 in monocytes but also such an important immunosuppressive factor as TGF-β may contribute to the anti-inflammatory activity of α-MSH.

The project is supported by the Ministry of Education and Science of the Russian Federation (State assignment № 122020300189-6).

OBTAINING GELS WITH ANTIBACTERIAL, ANTIVIRAL AND REGENERATIVE EFFECTS FOR LOCAL WOUND TREATMENT

Andrusenko E. V.[1], Poloskov A. I.[1], Tovpeko D. V.[1], Petrova P. E.[2], Komlev A. S.[2], Glushakov R. I.[1]

[1]Military Medical Academy named after S. M. Kirov, St. Petersburg, Russia
[2]Institute of Experimental Medicine, WCRC for Personalized Medicine, St. Petersburg, Russia

Introduction. Recently, much attention has been paid to hydrogels due to their natural structure that mimics the extracellular matrix, adjustable mechanical properties, and the ability to easily deliver biologically active substances. They show great potential for wound healing. In this work, we studied the composition and methods for obtaining new hydrogels based on natural components, in particular, phytoextracts and fulvic acids and antimicrobial peptides. Peptides possess antibacterial properties due to their ability to interact with and disrupt bacterial cell membranes. These antimicrobial peptides (AMPs) are small chains of amino acids that can be found in various organisms, including humans, animals, and plants.

Purpose. Obtaining gels based on natural components and peptides with antibacterial, anti-inflammatory and wound healing properties.

Methods. In this study, hydrogel samples were prepared containing low and/or high molecular weight hyaluronic acid, various plant extracts, antimicrobial peptides as well as fulvic acids, and its effectiveness as a wound healing coating was evaluated. Plant extracts were obtained as a result of water-alcohol extraction followed by distillation of the alcohol fraction. Peptides were synthesized by SPPS (solid phase peptide synthesis). Lyophilized peptides were dissolved in water and added to the ultimate substance. Several series of experiments were carried out on 28 outbred rats aged 40 ± 10 days with an initial body weight of 200–250 g, kept under standard vivarium conditions.

Results. Experimental studies of the obtained gels on 28 outbred paints showed that on the 31st day after modeling a dermal burn, the surface of the burn wound was completely epithelialized. Thus, the obtained gels are more effective in comparison with the control and the standard treatment.

Conclusion. Thus, the resulting hydrogels have properties of controlled release of phytoextracts and fulvic acids that promote wound healing. The selection of compositions based on plant extracts and peptides makes it possible to purposefully obtain hydrogels that act on damage of various etiologies, as well as have antimicrobial, anti-inflammatory, and wound healing properties.

REPLACEMENT OF THE TFA COUNTERION IN SOLUTION

***Komlev A. S.*[1], *Petrova P. E*[1], *Ivasenko A. A.*[1], *Fedorv I. P.*[1], *Yudin D. A.*[1,2], *Galianova M. V.*[1,2]**

[1] Institute of Experimental Medicine, WCRC for Personalized Medicine, St. Petersburg, Russia
[2]St. Petersburg State University, St. Petersburg, Russia

Introduction. Antimicrobial peptides are promising objects of investigations aimed at creating new drugs. However, producing of pure natural or synthetic peptides usually requires the use of solutions containing various counterions, and these counterions can interact with the peptides and change their features. Counterions affect the charge of the peptide molecule and can influence its structure, stability, activity, and interaction with other molecules. The replacement of counterions can be used to modify their physicochemical properties and functionality, restore their original characteristics.

Purpose. Development of a method for non-covalent modification of an antimicrobial cationic peptide ChBac RFR1-14 by replacing trifluoroacetic anion (TFA) in solution.

Methods. Sodium deoxycholate and sodium oleate were used as replacements for TFA in the peptide solution. The resulting peptide salts were identified by reverse-phase high performance liquid chromatography (RP-HPLC) and evaluated for their antibacterial activity. The selected peptide was obtained by solid-phase synthesis and identified by RP-HPLC and MALDI-TOF MS.

Results and discussion. The results of the study showed that the replacement of TFA significantly improves the antibacterial properties of the ChBac RFR1-14 peptide. This improvement can be due to the increased lipophilicity and stability of the peptide conferred by deoxycholate and oleate, leading to enhanced efficacy against gram-negative bacteria.

Conclusion. Thus, the replacement of the TFA counterion in the peptide solution represents a promising approach for improving the antibacterial properties of antimicrobial cationic peptides. This methodology can be used in the development of new antimicrobial drugs with improved efficacy and stability. Further research is necessary to gain a deeper understanding of the interaction mechanisms between the substituted counterions and the peptide, as well as to determine their potential in clinical practice.

The project is funded by the Ministry of Education and Science of the Russian Federation, Agreement № 075-15-2022-302 (20.04.2022).

CATIONIC ANTIMICROBIAL PROTEINS AND PEPTIDES OF HUMAN MILK

***Leonova L. E.*[1], *Kurdyumova I. V.*[1, 2], *Grishina T. V.*[1]**

[1]St. Petersburg State University, St. Petersburg, Russia

[2]Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russia

Antimicrobial proteins and peptides are evolutionarily ancient biomolecules whose action is aimed at protecting the organism. They play a complex and multifunctional role in the innate immune system — from counteracting and destroying bacteria, viruses, fungi and parasites, to selective immunomodulatory effects at the molecular level. Of particular interest are low molecular weight antimicrobial peptides with cationic and hydrophobic properties that determine their ability to interact with negatively charged membranes of microorganisms, which leads to membrane destabilization. Many antimicrobial peptides of animal origin have been characterized, which are found in epithelial tissues, phagocytic cells, and biological fluids of many multicellular organisms. Some antimicrobial peptides are synthesized constitutively, others are induced in response to infection or inflammation. The biological value of mammalian milk in the formation of the host defense systems and nutrition from the first days of a newborn's life is currently beyond any doubt.

Mammalian milk is not only a unique food product exclusively for a certain species, the composition of which is diverse and contains proteins, peptides and products of their proteolysis, lipids, carbohydrates and oligosaccharides, macro and microelements, vitamins, microbiome, but it is also a source of biologically active components, such as various growth factors, lysozyme, lactoperoxidase, lactoferrin, immunoglobulins, caseins and products of their proteolysis, etc., as well as low molecular weight cationic antimicrobial peptides of the defensin class and other biologically active components necessary for the growth and development of a newborn, protection from pathogenic microorganisms, formation and development immune system.

In the present study, our attention was directed to the study of cationic fractions of proteins and peptides of mature human milk extracts and their complexes. Preparative polyacrylamide gel electrophoresis in an acidic buffer system in the presence of urea and RP HPLC was used to obtain and characterize the cationic fractions of mature human milk extracts. HNP 1—4, lactoferrin, lactoperoxidase, myeloperoxidase, α-lactalbumin, and

lysozyme were identified in the composition of the obtained high-molecular protein fractions by dot-immunoassay, immunoblotting, MALDI MS, and detection of lysozyme activity.

A more detailed study of antimicrobial proteins and peptides of human milk is an important aspect in understanding the processes of the formation of newborn immunity in an environment containing pathogenic microorganisms, and opens up prospects for creating new generation antibiotics.

A NEW STEP IN THE FIGHT AGAINST TUBERCULOSIS USING ANTIMICROBIAL PEPTIDES

Nazarov J. S. E.

Bukhara State Medical Institute, Bukhara, Republic of Uzbekistan

Introduction. The key problem in the fight against tuberculosis today is the high resistance of pathogenic mycobacteria to existing anti-tuberculosis drugs.

Purpose. Against the background of the increasing resistance of the *M. tuberculosis* to antibiotics, the focus of attention of TB researchers is shifting towards a new group of drugs called antimicrobial peptides.

Methods. Vancomycin is one of the best known antimicrobial peptides. The biosynthesis of this drug was discovered in the soil bacterium *Amycolatopsis orientalis* in the 1950s. Vancomycin has a bactericidal effect in relation to the super bacteria — methicillin-resistant *Staphylococcus aureus*. However, this clinical product is not active against mycobacteria. What cannot be said about defensins. Defensins are highly active against bacteria, fungi, and many enveloped and non-enveloped viruses.

Results. Immune cells use defensins to kill bacteria that have been engulfed by phagocytosis, a classic example of tuberculosis infection. Defensins attach to the cell membrane of mycobacteria and, deepening into the membrane of pathogenic bacteria, form ruptures, leading to the death of the bacterial cell. In addition, defensins can enhance the effectiveness of antibiotics already used in medical practice. Swedish scientists from Lund University have made a discovery regarding the fungus *Pseudoplectania nigrella*. The researchers were able to isolate an antimicrobial peptide from a fungus called plectasin. This antimicrobial peptide is not toxic to human cells and is lethal to *Mycobacterium tuberculosis*, even at low concentrations. Further studies noted that when using Plectasin in the treatment of experimental tuberculosis, the incidence of spontaneous resistance of *M. tuberculosis* strains to Plectasin was very low. Another positive property of plectasin is the anti-inflammatory effect it has on tissues affected by Koch's bacillus.

Conclusion. Considering the importance of the problem of antibiotic resistance of *M. tuberculosis* strains, one can hope that research in the field of treatment and prevention of tuberculosis will continue in search of new and effective solutions to eradicate this socially significant disease. And a new class of drugs known as antimicrobial peptides can play an important role in this.

SPONSORS

LABTECH LLC

LLC “LabTech”
191186, Russia, St. Petersburg, a/z 238
Tel.: +7 (812) 313-02-03
labtech@labtech.su
www.labtech.su

LabTech LLC has been engaged in equipping clinical and scientific laboratories for more than 30 years. The company offers modern solutions to improve the efficiency of laboratory research by supplying equipment and consumables from leading foreign manufacturers.

Equipment for scientific research, including flow cytometers, centrifuges, capillary electrophoresis systems, etc., as well as a wide selection of Sarstedt laboratory plastics: blood collection systems, ready-made test tubes, containers for biological samples, consumables for bacteriology, cell culture, PCR diagnostics, tips for automatic pipettes.

The activity of LabTech LLC is aimed at creating an effectively functioning laboratory complex that meets all the needs of specialists.

The company provides the supply, installation and maintenance of instruments, as well as training of specialists in modern techniques.

For 30 years of work, LabTech has established itself as a solid and reliable partner:

- Geography of activity: almost the entire territory of Russia — from Khabarovsk to Kaliningrad.
- The clients of LabTek LLC are the leading medical institutions in St. Petersburg and other cities.
- State, federal and national projects for equipping laboratories: LabTech has repeatedly participated in equipping federal medical centers and hospitals.

ALLOKIN-ALPHA IS A NEW GENERATION ANTIVIRAL AND IMMUNOMODULATING DRUG

АЛЛОКИН-АЛЬФА
АЛЛОФЕРОН

BRAND-PHARM

The active substance of the drug is the cytokine-like peptide Alloferon. Within European registration studies, the stage of preclinical studies of the drug with the participation of specialized research centers in Europe and the USA was completed. Alloferon is patented in Europe, the USA, Japan, South Korea, Russia, Ukraine and the countries of the Eurasian Union. It has been registered and selling in 10 countries since 2003 for the treatment of HPV-induced chronic viral infection, herpes and acute hepatitis B. The drug in the form of a lyophilisate is available in 1 mg ampoules.

Therapeutic potency:

— According to post-registration studies, when Allokin-alpha is used in monotherapy, HPV is completely eliminated in more than 90 % of cases, and in treatment of chronic genital herpes (CGH), a pronounced therapeutic effect was achieved in 85 % of patients.

— The high efficacy of therapy with Allokin-alpha was noted in case of a combination of genital herpesvirus infection and CIN I in patients. Allokin-alpha therapy of these patients, along with significant reduction in the frequency, intensity and duration of herpesvirus infection recurrences results in a regression of CIN I in 90 % of cases.

— Almost 100 % effectiveness of Allokin-alpha was obtained in pre-conception preparation in women with chronic herpes virus infection, while in the group of these women the risk of intrauterine infection of the fetus and actualization of herpes virus infection is 22 times less compared to the group of patients who used systemic chemotherapy with Acyclovir and by 40 times less compared to a group patients who did not receive the standard therapy.

— In studies of treatment of locally advanced cervical cancer, satisfactory evidence has been obtained of increase in efficacy of standard chemotherapy when combined with Allokin-alpha. Thus, the five-year survival rate in the Allokin-alpha group increased by 32 %.

Properties:

Allokin-alpha does not have general toxicity, allergenic properties, mutagenic and carcinogenic effects, does not have an embryotoxic effect and does not affect the reproductive function.

Allokin-alpha is included in the Russian state clinical guidelines: “Benign and precancerous diseases of the cervix from the standpoint of cancer prevention”.

Allokin-alpha is included in the Russian state clinical guidelines: “Pre-gravidar training of women with miscarriage and chronic endometritis”.

The fundamental difference between the drug and immunomodulators is that it does not have a general effect on the immune system. It causes a cytokine reaction only in the affected area.

Mechanism of action:

1 — activates NK cells and TOLL receptors;

2 — causes intense synthesis in cytokines affected areas: INF-γ (on the 9th day of treatment, the concentration increases by 37 times); IL-1 (increases by 12.5 times), IL-2;

3 — causes activation of various signaling pathways, including Ras/MAPK, which activates the suppressor of malignant tumors — protein p-53;

4 — reduces by 2 times the level of cells — immunity suppressors (T-reg) and transforming growth factor beta1 (TGFβ1) receptors.