10.8 doi: 10.15789/2220-7619-2018-4-10.8

A STUDY OF THE POTENTIAL OF SPIDER SILK USE FOR THE DEVELOPMENT OF ANTIBACTERIAL DRUGS

V.I. Rumyantceva, E.I. Koshel

St. Petersburg National Research University of Information Technologies, Mechanics and Optics, St. Petersburg, Russia

Spidroin is the natural material possessed unique properties such as biocompatibility, noninflammatory property, controllable biodegradation, hydrophobicity. It can be used as functional biomaterial after processing and post-processing. In recent years the functional hybrid materials based on spidroin have been developed for the various biomedical and nanotechnological applications including drug delivery, tissue engineering, nanostructured optics, nanoelectronics, sensors, filtration, surface modification. In this study a hybrid consisting of spider silk and metal oxides that can generate active molecular forms as a result of interaction with peptides was investigated. Their biological properties were studied.

Hybrid materials were obtained by precipitation of nanoparticles of tungsten and molybdenum oxides from hydrosol. The natural silk was obtained in insectarium where the spiders *Linothele fallax* are grown. A preliminary study was conducted on the activation of the web hybrids in a contact with two types of the test-microorganisms: gram-positive *Staphylococcus aureus* 209 R and gram-negative *Escherichia coli* XL-1. The study of antibacterial properties of hybrid silk-based material was carried out by agar diffusion method.

The spider silk and their composites had a greater impact on the gram-positive type of bacteria itself. The results obtained for the hybrid material are comparable to the concentration dependence of the effect of nanoparticle solutions on bacterial cells. Moreover, a tendency for the synergistic effect of spider silk with deposited composite metal oxides on it was observed. In addition, there was a color change zone the dense medium. The colored area is considered as the result of interaction of a silk-based composite with the peptides of the medium and the exogenous proteins secreted by bacteria.

The results show a positive trend that requires further study to verify the possibility of creating a new biomaterial as effective antibacterial complex.

10.9 doi: 10.15789/2220-7619-2018-4-10.9

CAPSULE SPECIFIC POLYSACCHARIDE DEPOLYMERASES OF *KLEBSIELLA PNEUMONIAE* BACTERIOPHAGES: IMPLICATION IN TYPING AND TREATMENT

N.V. Volozhantsev[1], E.V. Solovieva[1], V.M. Krasilnikova[1], V.P. Myakinina[1], V.V. Verevkin[1], A.I. Borzilov[1], A.M. Shpirt[2], Y.A. Knirel[2]

[1]State Research Center for Applied Microbiology and Biotechnology, Obolensk, Russia; [2]N.D. Zelinsky Institute of Organic Chemistry, Moscow, Russia

To overcome the carbohydrate barrier of bacteria, many bacteriophages use specific enzymes, polysaccharide-depolymerases (PS-dep), which destroy bacterial polysaccharide capsules, thereby ensuring the adsorption of the phage at the outer membrane receptors, the penetration of phage DNA, and the lysis of the bacterial cell. Phage depolymerases are an attractive and promising means for controlling pathogenic bacteria, such as *K. pneumoniae*, whose main virulence factor is a pronounced polysaccharide capsule.

The aim of the work is to characterize the specificity and anti-bacterial (anti-virulence) potential of polysaccharide depolymerases encoded by capsule specific *K. pneumoniae* bacteriophages.

We cloned and expressed genes PS-dep of the phages KpV71, KpV74 and KpV79, lytic for *K. pneumoniae* of capsule types K1, K2 and K57, respectively, into the *E. coli* cells. The recombinant proteins Dep_kpv71, Dep_kpv74, and Dep_kpv79 were isolated and purified and the PS-degrading activity of the recombinant proteins was demonstrated. The spectrum of activity of PS depolymerases against to *K. pneumoniae* strains of different phenotypes and genotypes was determined. It was shown that recombinant proteins are more specific to polysaccharides of the corresponding types than "parent" phages.

It was found that the depolymerases Dep_kpv74 and Dep_kpv79 are specific glycosidases that cleave the *K. pneumoniae* polysaccharides of capsular types K2 and K57 by β-glucoside and β-galactoside bonds, respectively, to form monomers and dimers of the tetrasaccharide repeating unit of the polysaccharide. Protein Dep_kpv74 is a bifunctional protein and, in addition to β-glucosidase activity, determines, as assumed, the phage binding with the primary bacterial receptors, the capsular polysaccharides.

In vitro and *in vivo* experiments showed that treatment of virulent hypermucoid strains of K2- or K57-type *K. pneumoniae* with Dep_kpv74 or Dep_kpv79, respectively, leads to a significant decrease in *K. pneumoniae* strain virulence in mice and ensures the survival of animals in the development of *K. pneumoniae*-sepsis.

In conclusion, the obtained data testify to the perspectives of using of phage PS depolymerases for *K. pneumoniae* capsular typing, as well as for treatment of *K. pneumoniae*-infections.

The work was supported by the Russian Science Foundation (grant 15-15-00058-P).

10.10 doi: 10.15789/2220-7619-2018-4-10.10

DEVELOPMENT OF A NEW ANTI-INFLUENZA COMPOUND BASED ON CAMPHOR SCAFFOLD

V.V. Zarubaev[1], I.N. Lavrentieva[1], O.I. Yarovaya[2], A.S. Sokolova[2], N.F. Salakhutdinov[2]

[1]St. Petersburg Pasteur Institute, St. Petersburg, Russia; [2]N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, SB RAS, Novosibirsk, Russia

Despite success in chemotherapy and vaccine development, influenza remains a hard-to-control infection due to high genetic variability and long-term complications after the acute stage leading to a "hidden" or secondary mortality caused not by the influenza virus itself but by virus-induced secondary processes. The use of antiviral compounds leads to the rapid emergence of resistant strains. Therefore, the development of new anti-influenza drugs with new targets and other mechanisms of action is an important task of medical science and practical public health worldwide.

We identified a group of derivatives of natural terpenoids that exhibit a high level and a wide spectrum of activity against influenza viruses. Among them, camphecene (1.7,7-trimethylbicyclo [2.2.1] heptane-2-ylidene-aminoethanol) is one of the most active, possessing virus-inhibiting properties against influenza viruses A and B, both *in vitro* and in experiments on laboratory animals. The selectivity index (chemotherapeutic index) for influenza virus was 74–661, depending on the type and subtype of the virus, the protection index in animal experiments was 67% for influenza A and 89% for influenza B. The mechanism of camphecene activity was the suppression of fusogenic activity of the viral hemagglutinin,

which prevents the process of fusion of the viral and cell membranes. Using sequential passages, a laboratory selection of camphecene-resistant strains was carried out and the resistance-conferring mutation V458L located in the HA2 subunit was localized. When comparing the properties of the control and camphecene-resistant strains, it was shown that the pathogenicity of the latter for animals was at least 50 times lower than for a strain passaged without camphecene. No mortality was observed in group of animals inoculated with the resistant virus regardless of the infectious dose of the virus.

Thus, camfecine is a new promising anti-viral compound with a different mechanism of action and a different target as compared to those already used in the clinic.

10.11 doi: 10.15789/2220-7619-2018-4-10.11

COMBINED ANTIBACTERIAL ACTIVITY OF ANTIMICROBIAL PEPTIDES AND ANTISEPTIC AGENTS

M.S. Zharkova[1], E.S. Umnyakova[1], G.E. Afinogenov[2], A.G. Afinogenova[2,3], O.V. Shamova[1,2]

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

Antimicrobial peptides (AMPs) are potent bactericidal molecules of innate immune system effective against antibiotic-resistant bacteria.

In this study we investigated the action of highly membranolytic AMP protegrin-1 (PG1) and of a goat bactenecin 3.4 kDa (ChBac3.4) from bactenecins' family, which members possess also intracellular targets, in combinations with a number of antiseptic agents against antibiotic-resistant clinically isolated bacteria *Escherichia coli* ESBL 521/17 (resistant to ampicillin, amoxicillin/clavulonic acid, cefotaxime, ceftazidime, cefixime, aztreonam, netilmicin, ciprofloxacin, trimethoprim/sulfamethoxazole), *Pseudomonas aeruginosa* MDR 522/17 (meropenem, ceftazidime, cefixime, amikacin, gentamycin, netilmicin, ciprofloxacin, colistin), *Klebsiella pneumoniae* ESBL 344/17 (ampicillin) and *Acinetobacter baumannii* 7226/16 (imipenem, gentamicin, tobramycin, ciprofloxacin, trimethoprim/sulfamethoxazole), *Staphylococcus aureus* 1399/17 (ampicillin, oxacillin, gentamicin, amikacin, ofloxacin), *Staphylococcus epidermidis* isolates 9/17, 10/17, 24/17, 33/17 (various fluoroquinolones), and against laboratory ampicillin-resistant strain *Escherichia coli* ML-35p.

Based on fractional inhibitory concentration indices (FICI) assessed by checkerboard titration (FICI ≤ 0.5 synergy; 0.5 < FICI ≤ 1 additivity; 1 < FICI ≤ 2 independent action; FICI > 2 antagonism), AMPs and sodium hypochlorite were found to be antagonistic. Most numerous and prominent cases of synergy were revealed in combinations of AMPs with poviargolum (a colloidal silver preparation), that corresponds with previous studies on AMPs and silver nanoparticles interaction. In combination with dioxidin PG1 showed synergy against gram-positive bacteria and ChBac3.4 against *A. baumannii* 7226/16 and *K. pneumoniae* ESBL 344/17. Prontosan was synergistic with AMPs against gram-positive bacteria and with PG1 also against *E. coli* ML-35p. Etidronic acid, that was shown to inhibit β-lactamases, acted synergistically with AMPs against *E. coli* strains and *S. aureus* 1399/17 and in case of ChBac3.4 also against *A. baumannii* 7226/16 and *S. epidermidis* 33/17. Besides sodium hypochlorite other cases was mostly additive. Using resazurin metabolic marker we found that dioxidin and prontosan significantly hasten the effect of PG1, and poviargolum of both AMPs, on the metabolic activity of bacteria. Thus, combined use of AMPs with antiseptics has perspectives.

Supported by RFBR grant No. 18-315-00333.

10.12 doi: 10.15789/2220-7619-2018-4-10.12

EVALUATION OF THE EFFECTIVENESS OF PNEUMOCOCCAL VACCINES FOR PREVENTION OF COMMUNITY-ACQUIRED PNEUMONIA IN SERVICEMEN

S.D. Zhogolev, P.V. Kulikov, K.D. Zhogolev, R.M. Aminev, A.A. Kuzin

S.M. Kirov Military Medical Academy, St. Petersburg, Russia

In 2011 13-valent pneumococcal conjugate vaccine "Prevenar 13" (PCV13) was licensed in our country. It is more immunogenic, induces stronger immunity than non-conjugate polysaccharide vaccines Pneumo 23 and Pneumovax 23 (PPV23). In this regard, PCV13 appears to be preferable for the prevention of pneumonia in military personnel than PPV23 which is used now.

The aim of the study was to conduct testing of PCV13 for the prevention of pneumonia in military teams, to compare the epidemiological effectiveness of conjugate and non-conjugate polysaccharide pneumococcal vaccines.

The study was conducted in 3 groups of conscripts. In the first group of soldiers of 571 people, 407 (71.3%) were vaccinated with PCV13. In the second group of 663 people, 472 (71.2%) were vaccinated with pneumococcal polysaccharide vaccines (PPV23). The third group, which included 493 unvaccinated soldiers, formed a comparison group. All military personnel were recruits of the same age (18–22 years) and were in comparable conditions of service and life.

During 5 months of follow-up, the incidence of community-acquired pneumonia among vaccinated by PCV13 was 4.5 times less than in the comparison group, and among vaccinated by PPV23 — 2.8 times less ($p < 0.001$). The index of effectiveness of PCV13 (4.5) was 1.6 times higher than that of PPV23 (2.8). The indicator of the effectiveness of PCV13 made up of 77.7%, PPV23 — 64.3%. Thus, the epidemiological efficiency of PCV13 was 1.2 times higher than that of PPV23.

In the first and second groups of servicemen, the incidence of pneumonia was lower than in the comparison group, not only among vaccinated, but also among unvaccinated. Moreover, the incidence of pneumonia among those not covered by vaccination in the group where PCV13 was used was 1.6 times lower than in the group where PPV23 was used ($p < 0.001$), which is explained by the formation of a stronger collective immunity preventing the circulation of pneumococci during vaccination with conjugated vaccine.

On the basis of the data obtained, it is preferable to vaccinate recruits a month before the call and conscripts, not vaccinated before the call to the armed forces of the RF, by pneumococcal conjugate vaccine.