10. NEW CHEMOTHERAPY FOR THE TREATMENT OF INFECTIOUS DISEASES

10.1 doi: 10.15789/2220-7619-2018-4-10.1

STUDY OF CYTOTOXIC AND ANTIPROLIFERATIVE ACTIVITY OF FUNGICIDAL SAPONIN TAUROSID Sx1 ON TRANSFORMED MAMMALIAN CELLS

M.A. Kirshana1, Yu.L. Krivorutchenko1, D.N. Nossik2, O.A. Lobach2

1Medical Academy named after S.I. Georgievsky, V. I. Vernadsky Crimean Federal University, Simferopol, Russia; 2D.I. Ivanovsky Institute of Virology, Research Centre for Epidemiology and Microbiology named after the honorary academician N.F. Gamaleya, Moscow, Russia

Saponins taurosids from the Crimean ivy are capable of enhancing the immune response in mice to HIV surface glycoproteins and influenza virus. It was shown that saponin taurosid Sx1 has a fungicidal activity against Candida spp. The aim of our work was to determine cytotoxic properties of saponin taurosid Sx1 on mammalian transformed cells such as MT-4 lymphoblastoid cell line and Vero fibroblast-like cell line.

The triterpene saponin taurosid Sx1 with the structure 3-O-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside hederagenin isolated from the Crimean ivy Hedera taurica Carr. (Araliaceae), lymphoblastoid tumor cells of MT-4 line and fibroblast-like cells of the Vero line were used in the study. The saponin toxicity was determined with a methylthiazolium test (MTT).

The effects of taurosid Sx1 taken in 0.019–50.0 µg/ml concentrations on MT-4 cells were assessed. The saponin concentration of 3.13 µg/ml was shown not toxic — the number of surviving cells was 81.56%. The marked toxic effects were observed with saponin concentrations 25 and 50 µg/ml — the number of surviving cells were 68.10% and 30.43%, correspondently. For Vero cells the non-toxic saponin concentration was 0.78 µg/ml (the number of surviving cells was 84.14%). Regarding Vero cells, taurosid Sx1 exhibited cytotoxic properties at lower concentrations — at 6.25 µg/ml. The number of surviving cells was 44.15%.

Cytotoxic concentrations of taurosid Sx1 from Hedera taurica Carr. (Araliaceae) are similar to the cytotoxic concentrations of triterpene saponins from plants such as Albizia procera and Lysimachia thyrsiflora L. Saponins from these plants exhibited cytotoxic and anti-proliferative properties for transformed and normal mammal cells at concentrations close to the cytotoxic concentrations of taurosid Sx1. This result allows us to consider taurosid Sx1 as a potent anti-fungal, anti-viral and immunomodulating agent, but also as an anti-proliferative substance possessing potential antitumor effects.

10.2 doi: 10.15789/2220-7619-2018-4-10.2

ANTIMICROBIAL AND IMMUNOMODULATING ACTIVITY OF A TOPICAL GEL CONTAINING ACTIVE PEPTIDE COMPONENTS ON THE MODEL OF EXPERIMENTAL BACTERIAL VAGINITIS

A.A. Kolobov1, G.F. Leontieva2, T.A. Kramskaya3, A.V. Zakrevskaya1, K.B. Grabovskaya1, M.P. Smirnova2, N.F. Gamaleya, Moscow, Russia

Common methods of therapy of bacterial vaginitis are not effective due to the spread of antibiotic resistance, side effects of antibiotics and insufficient immune response. One of modern approaches to the treatment of vaginitis is based on the use synthetic analogues of natural peptides. The objective of the study was to analyze the antibacterial and immunomodulatory effects of gel preparations based on chemically synthesized peptides on the model of experimental vaginitis.

White outbred female mice were infected per vaginum by pathogens: Streptococcus agalactiae and Staphylococcus aureus for 5 days. Then gels containing antimicrobial peptide pentadefenin (P), immunostimulating peptide alferon (A) and both compounds were administered to the animals (groups P, A and PA, respectively) for 5 days. The control group (C) of the infected mice did not receive therapy. During course of therapy, the composition of the vaginal microbiocenosis was assessed using a bacteriological method and quantitative PCR. The concentration of IgA in vaginal lavages and IgM in serum were determined by ELISA.

Experimental vaginitis was accompanied by a change in the vaginal biocontamination: the number of lactobacilli decreased and the content of Gardnerella sp., Prevotella sp., and Porphyromonas sp. increased. Because of the therapy, a gradual decrease in the vaginal contamination of pathogenic bacteria occurred. Infection with S. agalactiae and S. aureus was observed in-group C throughout the observation period. The laboratory signs of bacterial vaginosis in the C group did not disappear, unlike other groups.

The drug P showed maximum antistreptococcal and antistaphylococcal effect only in the course of treatment. However, it acted only bacteriostatically and after its cancellation (day 9), the number of pathogenic bacteria became greater than in the group C. In groups P and PA, pathogenic bacteria practically disappeared, but this occurred only on the 9–14 days of the experiment. The antimicrobial effect of a correlated with an increase in the concentration of bacteriospecific IgA in the vaginal lavages and IgM in serum. Elimination of pathogenic bacteria occurred without the development of bacterial vaginosis, complications after antibiotic therapy or infection. Peptide P, as a bacteriostatic, should be used for a long time. The effect of peptide A is manifested only after the formation of a specific immune response. Thus, the maximum therapeutic effect should be expected in case of A and P mixture application.

10.3 doi: 10.15789/2220-7619-2018-4-10.3

ANTIMICROBIAL ACTIVITY OF SYNTHETIC ANALOGUES OF CAPRINE PEPTIDES BACTENECINS TOWARDS DRUG-RESISTANT BACTERIA

P.M. Kopeykin1, K.V. Cheredova1, M.S. Zharkova1, A.A. Kolobov1, M.P. Smirnova2, D.S. Orlov1, A.G. Afinogenova2, G.E. Afinogenov4, M.S. Sukhareva4, B.L. Milman1, O.V. Shamova1

1Institute of Experimental Medicine, St. Petersburg, Russia; 2State Research Institute of Highly Pure Biopreparations, St. Petersburg, Russia; 3Institute of Experimental Medicine, St. Petersburg, Russia; 4Institute of Experimental Medicine, St. Petersburg, Russia; 5State Research Institute of Highly Pure Biopreparations, St. Petersburg, Russia; 6State Research Institute of Highly Pure Biopreparations, St. Petersburg, Russia

Antimicrobial peptides (AMPs) of the innate immune system are unique molecules, providing human and animals host defense, and prototypes of novel drugs to fight
bacteria, resistant to conventional antibiotics. However, some cytotoxicity of the peptides towards host cells limits their use in medicine and points to the necessity of creation of AMPs analogs with optimized features. Our work is aimed to the analysis of the antimicrobial activity of structural analogs of proline-rich AMPs of the domestic goat Capra hircus leukocytes — bacteriocins ChBac3.4, ChBac5 and ChBac7.5 against drug-resistant clinical isolates of gram-negative bacteria (Pseudomonas aeruginosa MDR 522/17, E. coli ESBL 531/17, Acinetobacter baumanii 7226/16, Klebsiella pneumonia 344/17) and examination of their hemolytic properties towards human erythrocytes. The broth microdilution assay was used to evaluate the minimal inhibitory concentrations (MIC) of chemically synthesized peptides, and it was shown that truncated variants of ChBac5 (1–23 — sequence from the 1st to 23rd amino acid residues) and ChBac3.4 (1–14) exerted a low activity in comparison with that of the full-length peptides, while the peptide ChBac3.4 (1–19) had a significantly higher efficacy against all tested bacteria. We found that adding a fragment Arg-Phe-Arg to the peptides N-termini increased the antibacterial properties of the full length ChBac3.4, and to a much lesser extent of the truncated bacteriocins. A significance of the His-including region (14–18) of ChBac3.4 has been explored: the peptide with modification in this region and a lack of His residue possessed a potent antimicrobial activity. The highest antibacterial effect was observed in the case of a chimeric peptide including N-terminal fragment of ChBac7.5 and a cystein-containing fragment of protegrin 1 (MICs of 0.5–4 microM). Analysis of the hemolytic activity of the studied AMPs revealed that all the peptides do not cause lysis of human erythrocytes in a range of concentrations from 1 to 100 microM, except of the chimeric peptide that induced a significant lysis of red blood cells. The structural-activity analysis of caprine bacteriocins revealed most promising AMPs with potent antibacterial activity and a lack of the cytotoxic effects for human cells (in particular, analogs of ChBac3.4 with modification in 14–18 amino acids region) that point to the prospect of the further investigation of caprine bacteriocins aimed to the creation of novel pharmaceuticals to combat antibiotic-resistant bacteria.

This work was supported with RFBR grant No. 17-04-02177.

10.4 doi: 10.15789/2220-7619-2018-4-10.4

ANTI-INFLAMMATORY EFFECT OF ITRACONAZOLE IN PATIENTS WITH ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

Y.I. Kozlova, E.V. Frolova, A.E. Uchevatkina, L.V. Filippova, N.N. Klimko
North-Western State Medical University named after I.I. Mechnikov, St. Petersburg, Russia

The aim was to study the dynamics of immunological parameters in patients with ABPA on the background of the antifungal therapy.

The study included 11 patients with ABPA at the age from 29 to 78 years (median — 36 years). Allergological examination included skin tests with an allergens A. fumigatus (“Allergopharma”, Germany). The levels of total IgE (“Polinost”, Russia) and specific IgE (sIgE) to fungal allergens (“Alcor Bio”, Russia) in serum were determined by enzyme immunoassay. Spontaneous production of interferon-γ (IFNγ) was determined in the culture supernatant of cells without the addition of inducers. To assess the mitogen-induced production of IFNγ, blood cells were incubated for 24 hours with PHA at a concentration of 50 mg/ml (“Sigma” USA). The production of IFNγ, activated by the allergens A. fumigatus (“Alcor Bio”, Russia) at a concentration of 10 μg/ml, was determined on day 6. The resulting supernatants were used to determine spontaneous and induced IFNγ production by enzyme immunoassay using commercial test systems (“Vector-Best”, Russia). The prick test with A. fumigatus was positive, levels of sIgE to A. fumigatus (Me 1.56 (0.36-10.56) IU/ml) and total IgE (Me 986 (873-1695) IU/ml) were elevated in all ABPA patients. In the analyzed cases, according to the chest CT scans, focal and segmental lung infiltrations were detected in 6 (55%) patients, bronchiectasis — in 5 (45%). During the study, patients with ABPA were treated with itraconazole at a dose of 400 mg per day. In all patients after therapy significant clinical effect was noted: decrease in dyspnea and cough, improvement in the lung function, and positive dynamics in chest CT scans. At a re-examination at 12 weeks, all patients had a statistically significant decrease in the level of sIgE to A. fumigatus (Me 0.01 (0.01-5.24) IU/ml, p = 0.003) and to induction by the A. fumigatus allergen (48.0 (24.0-61.0) vs 19.0 (2.0-34.0) pg/ml, p = 0.001). The absolute number of eosinophils decreased (p = 0.05).

The tendency towards normalization of the immunological profile of patients in association with clinical signs improvement indicates the successful use of antifungal therapy in patients with ABPA.

10.5 doi: 10.15789/2220-7619-2018-4-10.5

SAPONIN TAUROSID Sx1 ADMINISTRATION ENHANCES ANTIBODY PRODUCTION IN MICE, CHALLENGED WITH INFLUENZA VIRUS OR IMMUNIZED WITH INFLUENZA GRIPPOL® VACCINE

V.Yu. Maligina1, I.B. Andronovskaja1, Yu.L. Krivorutchenko1, V.I. Grishkovetz2
1Medical Academy named after S.I. Georgievsky of Vernadsky Crimean Federal University, Simferopol, Russia; 2Taurida Academy of Vernadsky Crimean Federal University, Simferopol, Russia

Several saponins demonstrate antiviral and immune potentiating properties. In this work the influence of saponin Taurosid Sx1 on the anti-hemagglutinin (H) antibodies production has been studied in influenza virus (IV) challenged or GRIPPOL® vaccinated mice.

BALB/c mice were challenged intranasally with 50 μl (10 LD50) of the A/WSN/1/33(H1N1) virulent strain or immunized with polymer-subunit GRIPPOL® vaccine season 2005/2006. A standard vaccine dose contained per 5 μg of H1 and H3, 1 µg of H from the IV type. E. coli hordeosid 540 (73±63) IU/ml, p = 0.003. Was identified increased ability of blood cells to produce IFNγ in response to PHA stimulation of the blood cells (1914 (1294-2232) vs 910 (852-1648) pg/ml, p = 0.004) and to induction by the A. fumigatus allergen (48.0 (24.0-61.0) vs 19.0 (2.0-34.0) pg/ml, p = 0.001). The absolute number of eosinophils decreased (p = 0.05).

The vaccine titers of anti-H antibodies were determined. The tendency towards normalization of the humoral response of cells in association with clinical signs improvement indicates the successful use of antifungal therapy in patients with ABPA.