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Anti-influenza Action of Multinutrient Functional Peptide Complex (MFPC) Grinization®

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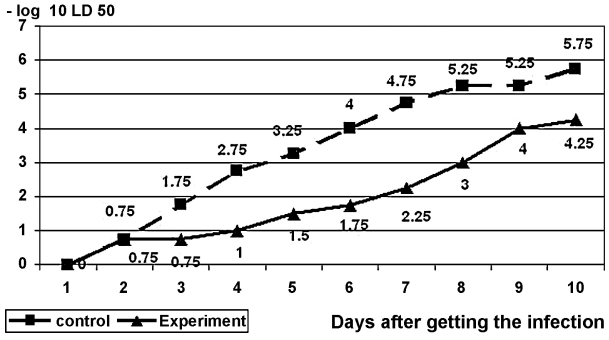
MFPC GRINIZATION® is represented by two forms, the liquid GRIN MIX, and the dry GRIN PRO. It contains natural food factors (nutrients) for special diet food, and is a unique "repair-restorative" complex for practically all systems of organism. Both MFPC compositions are processed by special GRINIZATION technology. It was shown that usage of MFPC GRINIZATION® resulted an immunoenhancement, organism resistance amplify and cell membranes stabilization. Therefore we had studied protective and antiviral actions of MFPC GRINIZATION® on the model of influenza infection in mice. Mice of experimental group received 15 mg/kg of GRIN MIX and 15 mg/kg of GRIN PRO daily during 7 days before infection with influenza virus A/PR/8/34 (H1N1) and 14 days subsequently. The results are demonstrated in figure. Infectious titers of virus in lungs of experimental mice were significantly lower than in control group during observation. Pathomorphological study demonstrated that usage of MFPC GRINIZATION® resulted in considerable reduction of lung injuries, such as reduction of volume and density of inflammatory lesions, number and size of haemorrhages, decreased manifestation of interstitial edema, distelectases, emphysema. More distinct demarcation of the lesions was noticed though the character of inflammatory reaction was preserved. State of myocardium and liver was related to normal whereas myocardium and liver of mice from control group showed diffuse microfocal inflammatory and degenerative changes. The results of this study show anti-influenza action of MFPC GRINIZATION®.



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Antiviral Action of Artificial Ribonucleases against Avian & Human Influenza Viruses

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Background: Ortho-myxoviruses are the cause of the most mass acute infections. They make an enormous harm to the population health and are the cause of significant economic losses. Influenza held the first place as the cause of viral infections' lethality. That's why the elaboration of the methods and means for such infections prevention and treatment is extremely actual task. Artificial ribonucleases (AR) hold promise as reactive groups in conjugates intended for cleavage of particular RNAs, as therapeuticals inactivating virus genome RNAs or certain mRNAs, and as a promising antiviral agents.

Methods: AR are peptidomimetics containing the following amino acids in different combinations: Lys, Glu, Arg, Ser, His and unnatural 6-aminohexanoic acid. The solution synthesis of peptidomimetics was carried out using the method of activated esters and the Boc-strategy. Antiviral activity of 9 AR was studied on the model of influenza viruses strains H_3N_2&H_5N_3 in the tissue culture of 11–14-days chicken embryos' choryoallantoic membranes (CAM).

Results: Anti-influenza activity of AR has presented in table.

Six AR (II, III, IV, V, VI and VII) have antiviral activity toward both researched strains. AR VIII does not display anti-influenza activity. I and IX AR show antiviral activity only against H_5N_3 strain.

Conclusions: The results of the present study are evidence of anti-influenza activity AR. Thus, these substances should regard as candidates for anti-influenza preparations.

Keywords: Antiviral action; Anti-influenza activity

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Table

Code of substance	AR	Anti-influenza activity (in log_{10} TID_{50}) against	
		strain H_3N_2	strain H_5N_3
I	Lys-L1	0,42	2,67
II	Ser-L1	2,75	1,08
III	Glu-AHA-Ser-L1	3,91	3,42
IV	Arg-L1	4,34	3,75
V	Lys-AHA-Ser-1	3,83	2,92
VI	Lys-L2	2,84	4,0
VII	Glu-L2	2,25	1,67
VIII	His-L1	0,16	0,58
IX	His-L2	0,08	2,75

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Efficient Suppression of Human Immunodeficiency Virus in Macrophages by Nano-NRTIs

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Macrophages serve as natural HIV-1 reservoir in the central nervous system. To efficiently target macrophages, we developed Nano-NRTIs, nanoformulations of 5′-triphosphates of nucleoside reverse transcriptase inhibitors, zidovudine (AZTTP), didanosine (ddITP), or their 5′,5′-tetraphosphate dimer. Cationic nanogels consisting of PEG- or Poloxamer-PEI biodegradable networks, star PEG-PEI or PAMAM-PEI-PEG dendritic networks were synthesized and fractionated to isolate nanocarriers with hydrodynamic diameters below 220 nm. Brain-targeted nanogels were obtained by decoration with PEG-linked peptides binding an apolipoprotein E receptor highly expressed in the blood–brain barrier. Nano-NRTIs were obtained by mixing aqueous solutions of AZTTP, ddITP, or dimer with nanogels and freeze-drying. Human monocyte-derived macrophages (MDM) were used for evaluation of intracellular

accumulation, cytotoxicity and antiviral activity of Nano-NRTIs. HIV-1 RT activity was measured following the 2–4 h preincubation of MDM with nanoformulations and viral infection. Mitochondrial DNA levels were determined by SYBR Green real-time PCR after multiple treatments of HepG2 cells used for evaluation of mitochondrial toxicity by Nano-NRTI. Nanogels were efficiently captured by MDM, demonstrated low cytotoxicity, and had no effect on viral infection without drugs. Nanoformulations with the highest inhibition of HIV-1 activity and the lowest toxicity were selected, and up to 12-fold reduction in efficient drug concentrations (EC90) was observed for Nano-NRTIs as compared to free drugs. Cytotoxicity (IC50) of Nano-NRTIs began at 200-fold higher concentrations. Antiviral activity of the nanoencapsulated dimer was the same as the one observed for both AZTTP and ddITP. Peptide modification of Nano-NRTIs did not affect their antiviral efficacy. The loss of mitochondrial DNA, a major cause of neurotoxicity, was reduced 2-fold in comparison to free drugs at application of selected Nano-NRTIs. Nano-NRTIs demonstrated important advantages over free nucleoside analogs and therefore held a great promise in the development of potent and low neurotoxic antiviral drug formulations for systemic targeting of HIV-1 infected macrophages.

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Oseltamivir Protection of Oxidative Damages in Mice Experimentally Infected by Influenza Virus

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Oseltamivir is a neuraminidase inhibitor with a specific action against influenza A and B viral infection. As a structural analogue of neuraminic acid oseltamivir competitively binds the active site of the enzyme neuraminidase on the influenza virus surface. The present study was designed to investigate the effect of oseltamivir on the oxidative damages in lung and liver of influenza virus infected mice. It was established that supplementation of mice with oseltamivir leads to protection against the oxidative stress in lung and liver of mice experimentally infected with influenza virus A/Aichi/2/68 (H3N2) (1.5 LD 50). As markers of oxidative damages we use two products of lipid peroxidation—malondialdehyd, and fluorescent lipofuscine-like products, as well as the levels of natural antioxidants vitamin E and glutathione on the 5th and 7th day after virus inoculation. The results showed that influenza virus infection A/Aichi/2/68 (H3N2) was accompanied by a significant increase of the markers of lipid peroxidation and decrease of natural antioxidants (vitamin E, glutathione). The changes of CYP system are as follows—decrease in cytochrome P-450, NADP.H-cytochrome *c*-reductase activities, and liver monooxygenases (aniline hydroxylase, ethylmorphine-*N*-demethylase, analgin-*N*-demethylase and amidopyrine-*N*-demethylase) as compared to the controls. We find out that oseltamivir treatment led to decrease of the products of lipid peroxidation on days 5 and 7 after the inoculation as well as on the positive changes on the compounds of CYP system. The antioxidant properties of oseltamivir were investigated by measuring the ability of the drug to influence the lipid peroxidation and to scavenge superoxide radicals in some model system. From these experiments we could conclude that oseltamivir does not show scavenging properties and does not influence lipid peroxidation.

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Discovery and Treatment of Respiratory Neurological Sequelae in West Nile Virus Infected Hamsters

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Based upon respiratory distress observed in WNV-infected human patients, we addressed the hypothesis that respiratory distress is caused by lesions in the central nervous system. In rodents, arterial oxygen hemoglobin saturation (SaO2) was slightly suppressed in alert WNV-infected C57BL/6 mice and anesthetized golden Syrian hamsters. To determine if the cause was neurological, electromyographs (EMGs) were measured longitudinally from the diaphragms of alert WNV-infected hamsters. The amplitudes of EMGs in hamsters injected subcutaneously (s.c.) were significantly less than sham-infected animals, beginning with suppression at day 3 and continuing to beyond day 17 after viral challenge. To further confirm the neurological cause, immunohistochemistry (IHC) was performed on hamster tissues known to control respiration, i.e., lung, diaphragm, cervical spinal cord, brain stem, and the carotid or aortic bodies sensing pH, O_2, or CO_2. At various times after viral challenge, viral foci in some animals with EMG suppression were detected in the medulla oblongata, but not in the spinal cord, or the carotid or aortic bodies, which suggested that the offending lesions were primarily located in the medulla, which contains areas of respiratory function. WNV injected directly into the ventral medulla or the cervical cord suppressed EMG amplitude. EMG, SaO2 and IHC data indicated that lesions in the ventral medulla, and possibly the cervical cord, can cause respiratory dysregulation. These markers for respiratory function were improved upon treatment with a therapeutic antibody, MGAWN1 (hE16) or cyclosporine A administered intraperitonally after the virus had infected the central nervous system (>5 days). Moreover, these data demonstrated that WNV infection in the medulla, and possibly the cervical cord, results in EMG dysregulation in WNV-infected hamsters.

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Breaking Tolerance with CLDC-HBsAg in HBV Transgenic Mice

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Immune tolerance to hepatitis B virus (HBV) is thought to play a role in the maintenance of chronic hepatitis. This study tested the hypothesis that CLDC/antigen complexes can break immune tolerance in transgenic mice expressing HBV. Previous *in Vivo* studies suggest that administration of CLDC/antigen complexes induce robust antibody and T-cell responses versus the target antigen. These adaptive immune responses have been shown to be therapeutic in a wide variety of viral, bacterial, and cancer model systems. In this study, male and female transgenic mice expressing HBV were block-randomized across groups and administered with combinations of HBV antigen (HBsAg) and CLDC-adjuvant (JVRS100) at days 1, 22, and 43. At the end of the