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FOLIA MEDICA CASSOVIENSIA

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Program of ISMCK'11

Tuesday 21.6.2011

10.00 - 15.30	Registration
16.15 - 17.30	Opening Ceremony
17.30 - 18.30	Welcome reception
19.30 - ...	Social Program

Wednesday 22.6.2011

8.30 - 9.00	Breakfast	
9.00 - 12.00	Sessions:	Basic Science 1
		Public Health
10:00 -11.00	Workshop dentisty: Aesthetic restorations	
12.00 - 13.00	Workshops:	Ultrasound examination in sports medicine and orthopedics
		Spirotmetry
12.00 - 14.00	Luch time	
14.00 - 17.00	Sessions:	Clinical Medicine - Surgery, Oncology, Varia
		PhD Student Works - Teoretical Part
17.30 - 18.30	Workshop:	Behavioral risk factors of cardiovascular diseases among youths
		Ultrasound examination in sports medicine and orthopedics
	Training:	Motivation
18.30 - 19.30	Dinner	
20.30 - ...	Social program	

Thursday 23.6.2011

8.30 - 9.00	Breakfast	
9.00 - 12.00	Workshop on Biostatistics	
9.00 - 12.00	Projection of cardiosurgical operation	
	Projection of transplantological operation	
13.00 - 14.00	Workshop: Laryngeal tube - an alternative supraglottic device	
12.00 - 14.00	Luch time	
14.00 - 17.00	Sessions:	Basic Science 2
		Clinical Medicie - Internal Medicine, Pediatrics,
		PhD Student Work - Clinical Part
17.30 - 18.30	Workshops:	How to survive PhD study
		Ensembl database meets your needs
	Training:	Leadership
18.30 - 19.30	Dinner	
20.30 - ...	Social program	

Basic Science I

Threshold of PTZ-induced seizure was assessed by inserting a 30-gauge dental needle into the tail vein of mice and infusion of PTZ (1%) with constant rate of 1 ml/min to unrestrained animals. An index of seizure threshold defined as minimal dose of PTZ (mg/kg of mice weight) needed to induce clonic seizure

Results: Sildenafil (1, 5, 10 and 20 mg/kg, i.p.) significantly decreased the seizure threshold in a dose-dependent manner, whereas morphine had both anticonvulsant and proconvulsant effects at low (0.5, 1, and 3 mg/kg, s.c.) and high (60 mg/kg, s.c.) doses. A sub-effective dose of sildenafil (5 mg/kg) combined with a dose of morphine (7.5 mg/kg) which was sub-effective for its proconvulsant effects significantly decreased the seizure threshold. Although naltrexone at 0.5 and 1 mg/kg had no effect on the seizure threshold, it significantly prevented both the proconvulsant effects of sildenafil as well as the anticonvulsant and proconvulsant effects of morphine on the PTZ-induced seizure thresholds.

Conclusion: Our data suggested a role for opioidergic system in the proconvulsant effects of sildenafil on the PTZ-induced clonic seizures in mice.

Abstract ID: 9

Silica nanoparticles - Hepatits B virus like particles in immunomodulation

Presenting Author: ***Marina Romanova***

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Abstract Keywords: *Hepatitis B, virus like particles, silica nanoparticles, immunomodulation*

Introduction: Immunomodulation is a new kind of viral infection treatment where molecular agents injected inside a human organism stimulate immune system to respond effectively to a viral infection, Virus like particles (VLP) can be used as immune response-modulating agents. VLP are injected into blood vessels and delivered to specific cells where they stimulate antibody production. Treatment efficiency is higher when concentration of VLP near the specific cells increases. However, high concentrations of VLP in the human organism might result in side effects. To eliminate this, high concentration of VLP can be provided only in a vicinity of the specific cells.

Aim: In order to reduce the overall concentration of VLP simultaneously increasing the local concentration near the target cells, a number of VLP can be attached to a nanoparticle that will act as the nanolorry and deliver VLP to the cells. Taking into account that the electrical charge is localized at the surface of VLP [1], the latter could be attached to the nanoparticle due to the electrostatic (Coulomb) interaction if the nanoparticle has an opposite charge. Thus, the nanoparticle must have the ability for polarization and must be harmless in respect to the human organism. Both conditions are satisfied by SiO2 nanoparticles [2,3].

The aim of the study is to verify capability of the SiO2 nanoparticles to attach VLP, the Hepatitis B viral capsids [1] being in use as a model.

Material and Methods: VLP (hepatitis B viral capsids) were synthesized by the Latvian Biomedical Research and Study Centre. Certified SiO2 nanoparticles were bought from the Sigma-Aldrich. Size of the nanoparticles was equal to 10 – 20 nm.

To study the capability of the SiO2 nanoparticles to attach VLP, the optical absorbance spectra of VLP, SiO2 nanoparticles and VLP+SiO2 mixture in buffered solutions were recorded and compared. The Thermo Spectronic Heλios Gamma spectrophotometer was in use to record absorbance spectra at wavelengths 200 – 1090 nm. The buffered solution was prepared from 20 mM Tris-HCl pH 7.8, 5 mM EDTA, 150 mM NaCl, and 1 litre distilled water. NaCl concentration was equal to the concentration in the physiological solution. The concentration 1 mg of the SiO2 nanoparticles was mixed with 1 ml of the buffer solution. The concentration of VLP was 12 μl in 1 ml of the solution that corresponded to optical absorbance value 1 (+- 5%) at wavelength 260 nm. The value 12 μl was chosen after the calibration procedure. To make the calibration, optical absorbance for different concentrations of VLP was measured and the concentration where optical absorbance equaled to 1 at 260 nm was chosen.

To verify VLP+SiO2 coupling, transmission electron microscopy (TEM) and fluorescence microscopy (FM) were employed. JEOL JEM-1200EX microscope was in use for TEM, and Leica DMI 3000 B microscope for FM.

To record the fluorescence, VLP were marked with the green FITC agent, which forms covalent bonds with VLP amino acids. Fluorescence was excited at 490 nm and detected at 515 nm.

A preliminary immunological experiment was performed to study the humoral response of Balb/c mice after the immunization with the VLP+SiO2 mixture. The immunization was made on days 0, 14 and 28. Mice from the control group were immunized with VLP only diluted into sterile phosphate-buffered saline. Two weeks after the 3rd immunization (on the day 42) all animals were bled and anti-HBc antibody response was detected using the direct ELISA test.

Results: Optical absorbance spectra of the solutions (SiO2, VLP, VLP+SiO2) were tested on time stability. The results showed that optical absorbance of the SiO2 and VLP+SiO2 solutions decreased after 24 hours (the wavelength 260 nm taken as a reference) and precipitations formed at the bottom of the test-tube. Precipitations could form due to the gravitation forces which move the nanoparticles towards the bottom of the test-tube. However, the absorbance of the VLP solution did not change after 24 hours and no precipitations formed.

The optical absorbance of the VLP+SiO2 solution measured in the experiment was compared with the theoretical absorbance value in order to see if VLP interact with the SiO2 nanoparticles. According to the spectrophotometry laws, these two values must be equal if no interaction between the particles exists. To calculate the theoretical value, the absorbance of the VLP solution at 260 nm was summed up with the absorbance of the SiO2 solution at 260 nm. Results show significant difference between the theoretical and the experimental values. The difference becomes more pronounced when the time given for VLP and SiO2 interaction increases. That proves that VLP adhere to the SiO2 nanoparticles.

Both TEM and FM show the attachment of VLP to the SiO2 nanoparticles. In case of FM the VLP solution without the nanoparticles has homogeneous fluorescence. When the SiO2 nanoparticles are added, VLP are attached to them and the fluorescence exists only in the areas where VLP adhere to the SiO2 nanoparticles.

The results of the preliminary immunological experiment showed that the amount of antibodies produced in Balb/c mice blood depends directly on the concentration of the SiO2 nanoparticles in the VLP+SiO2 mixture. The dose of VLP in the mixtures was kept constant and equal to 25 μg but the concentration of SiO2 varied thus producing different amount of the VLP+SiO2 complexes. VLP without SiO2 induce lower antibody response than the VLP+SiO2 mixtures.

Conclusion: 1. Spectrophotometry, TEM and FM give evidence that hepatitis B VLP adhere to the SiO2 nanoparticles, the VLP+SiO2 complexes are formed.

2. The correlation exists between the increase of the concentration of SiO2 nanoparticles in the hepatitis B VLP+SiO2 mixture and enhancement of the immune response in blood of Balb/c mice.

3. The hepatitis B VLP+SiO2 mixtures could perhaps be used for immumodulation purposed in treatment of hepatitis B but further studies are required.

Abstract ID: 10

Morphological analysis of muscles from patient with peripheral arterial disease after two repeated peripheral blood stem cells transplantation

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Abstract Keywords: *stem cells, PAD, byopsy, CD34, neovascularization*

Introduction: Searching for new methods of treatment of peripheral arterial diseases (PAD) is the important problem of recent years (1).
Aim: The aim of work was to study morphological changes in capillaries and skeletal muscles in patient with PAD after two repeated autologous PBSC transplantations.
Material and Methods: Study was performed on paraffin-embedded slices of m.gastrocnemius biopsies obtained before and 3 months after intramuscular injections of PBSC (transplantations were made 12 months apart). Immunohistochemical staining of biopsies was performed with antibodies against CD34 (endothelial marker) and myogenin (marker of activated myosatellites). We analyzed morphologically stained slices, estimated capillary density. Statistical analysis was performed by using Kolmogorov-Smirnov-test.
Results: Results of staining with antibodies against CD34 showed increase of «capillaries/muscle fibers» ratio 3 months after 1st transplantation (from 1,34±0,131 to 1,72±0,173), 12 months after 1st transplantation it changed insignificantly (1,94±0,308). 3 months after 2nd transplantation (15 months after 1st transplantation) we revealed significantly higher capillaries/muscle fibers» ratio (up to 2,5±0,634; p=0,01). Staining with antibodies against myogenin identified regeneration of skeletal muscles by myosatellites activation, formation of myotubes and new muscle fibers. Muscular regeneration was extended between two transplantations and intensified after 2nd transplantation.
Conclusion: So, single SC transplantation has long-term effect and described procedure is perspective for prolonged treatment of PAD. Subsequent autologous transplantation leads to increase of capillary density and activates regeneration involving myosatellites.