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Method of Raman scattering spectroscopy to evaluate bone tissue of animals with single and double administration of mineral bone component

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Results of application of the method of Raman scattering spectroscopy to evaluate the functional state of bone tissue of animals with single and double administration of mineral bone component (MBC), used during treatment of osteoporosis and stimulation of osseointegration. Bone tissue and pig blood serum were used as the study objects. The blood biochemical test was used as additional method of analysis. It was identified that MBC does not have negative effect on the bone tissue with either single or double administration.

Keywords: Raman scattering spectroscopy, spectral analysis, mineral bone component (MBC), bone tissue, biochemical analysis.

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The problem of prevention and treatment of the osteoresorption is of the actual problems in experimental biology and medicine [1]. The actual problem is search of new biotechnological solutions aimed to correct exchange processes and restoration of changes structure-functional integrity of the bone tissue. When developing biocompatible materials of allogenic origin first of all their safety, ability for osteointegration and assurance of physiological regeneration. One of perspective materials having large potential for application in traumatology, dentistry and maxillofacial surgery is mineral component of bone (MBC), made as per technology „LIOPLAST“ (TU-9398-001-01963143-2004, RF patent № 2366173 dated 15.05.2008; Certificate of compliance ISO 13485:2016, reg. № RU CMS-RU.PT02.00115; certificate ISO 9001:2015, reg. № TIC 15 100 159171) and produced by demineralization of bone tissues of human and animals. It can be used to treat osteoporosis and to improve processes of osteointegration during implantations. But there is need to study its secondary action on the organism, it results in excessive accumulation in bones and causes changes of key biochemical parameters of blood.

Currently the optical methods of study are widely used in the biomedical tasks [2,3]. Our previous studies showed that the Raman scattering spectroscopy ensures quality evaluation of MBC during its manufacturing [4].

The biochemical analysis is performed during extended study of toxicity during administration of the drug in order to assess its safety when used in vivo [5]. Selection of such animals in the current study was determined by suppositions that pig organism is an adequate model when performing different biological experiments and preclinical safety assessment of new drugs.

The study objective is use of the Raman scattering spectroscopy for state evaluation of bone tissue of pigs (Susscrofa) after single and double intramuscular administration of aqueous suspension of mineral bone component.

The experiments were performed on pigs of breed „Livenskaya“ with weight 13–15 kg. Three groups: 1) reference (5 animals), 2) group 2 (12 animals), group 3 (13 animals). The reference animals (group 1) were subjected to single intramuscular administration of sterile saline. Pigs of the experimental group 2 were subjected to intramuscular single administration of suspension of allogeneic mineral bone component in dose of 100 mg/kg on the first day of the experiment. In the third group intramuscular single administrations of suspension of mineral bone component were performed two times in dose of 100 mg/kg on the first and fourteenth days of the experiment. Total duration of the experiment was 28 days. The blood sampling for biochemical analysis was performed three times: during first days prior to administration of the mineral bone component, at 14-th and 28-th days of the experiment.

After animals removal from the experiment the fragments of shoulder bones (diaphyseal and metaphyseal areas) and of shield bones were taken.

Further studies were performed *in vitro* using the Raman scattering spectroscopy (RSS) (main method). RSS was implemented using the experimental set-up described in paper [6]. Use of the spectrograph ANDOR Sharmrock 303i

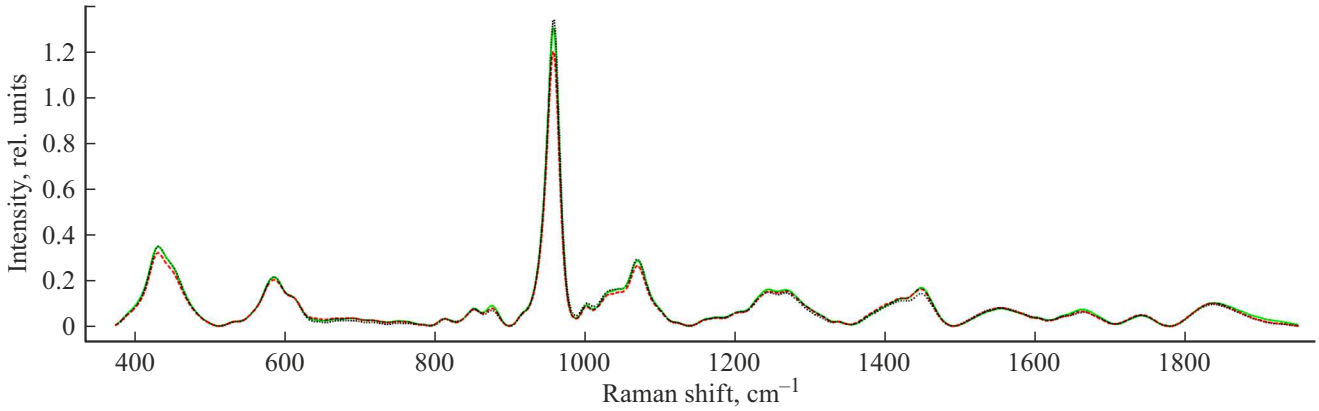

Figure 1. Averaged RS spectra of bone tissue of studied groups: first group — black dashed line, second group — green solid line, third group — red dashed line.

with cooling chamber DV-420A-OE ensured resolution 0.15 nm ($\sim 1.5\,\text{cm}^{-1}$). For excitation the laser 785 nm with rated power below 450 mW with fiber output was used. Raman scattering (RS) spectra were recorded using an optical probe placed above the object under study at a distance of 7 mm. The spectra were normalized by method of *standard normal variate* (SNV). For each animal 3 to 5 spectra were taken (in group spectra were averaged, and during statistical analysis were used as individual points). The spectra were smoothed using the *Maximum Likelihood Estimation Savitzky-Golay filter* (MLE-SG) method with the $\sigma = 4$ parameter. To exclude the contribution of autofluorescence in RS spectrum, a modified method of subtracting the fluorescent component by polynomial approximation *Extended Modified Multi-Polynomial Fitting* (Ex-ModPoly) with a polynomial degree of 10 was used.

The blood biochemical analysis was used as additional method of study.

Fig. 1 shows the averaged RS spectra of studied groups of samples. The Figure shows that main spectrum maximums of studied groups on lines $\sim 958-960\,\text{cm}^{-1}$ (hydroxyapatite, PO_4^{3-} (ν_1) (P–O symmetric stretch)), $\sim 1070\,\text{cm}^{-1}$ (carbonate-containing hydroxyapatite, CO_3^{2-} (ν_1) *B-type substitution* (C–O *in-plane stretch*)), which correspond to mineral components of the bone tissue, and on lines $\sim 1229-1242\,\text{cm}^{-1}$ (Amide III), $1445\,\text{cm}^{-1}$ ((CH_2), (CH_3), *scissoring, phospholipids* (*lipid assignment*), $\sim 1537-1587\,\text{cm}^{-1}$ (Amide II) and $\sim 1651\,\text{cm}^{-1}$ (Amide I) and $\sim 1745\,\text{cm}^{-1}$ (υ(C=O), *phospholipids* (*lipid assignment*) correspond yo organic components of the bone tissue. The Figure shows that no significant spectral differences are observed between studied groups in the entire studied spectrum range.

For further analysis of the decomposed lines PLS-DA-discriminant analysis by the method of partial least squares was selected (Fig. 2).

The graph shows that no significant differences in the spectral components of the reference group, as well as groups with single and double administration.

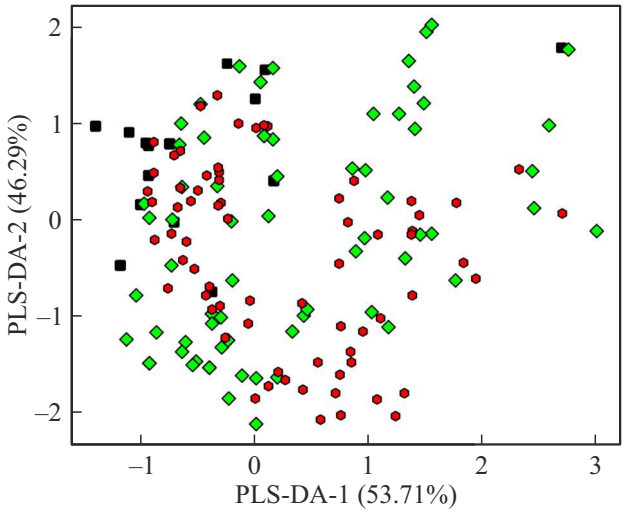

Figure 2. Two-dimensional graph of values of linear discriminant function LDA: first group — black square, second group — green diamond, third group — red circle.

The data of the blood biochemical analysis showed that the activity of enzymes, the concentration of creatinine, glucose, total protein, total calcium, phosphates, IgE, C-reactive protein, urea in the initial state in the experimental groups correspond to the norm typical for this species of animals. From the experimental results it follows that MBC does not have significant effect on the main biochemical parameters of the blood. So, we can conclude about its relative safety for use in medical purposes.

As a result of the conducted studies on the effect of the administered dose of MBC on the composition of bone tissue in pigs using Raman scattering spectroscopy, it was established that the administered dose of MBC (100 mg/kg of weight), both with single and double administration of the drug, does not affect the composition of bone tissue, which is clearly visible in the RS spectra. It is identified that in the entire studied spectral range $380-1900\,\text{cm}^{-1}$

no significant spectral differences between the groups are observed.

The mineral bone component with single and double intramuscular administration does not affect the major biochemical components of blood. The established changes in biochemical parameters were within the normal values typical for this species.

The obtained results can be used further as pre-requisites for this drug testing on larger animals and for transition to clinical studies.

Ethics committee

All applicable international, national, and/or institutional guidelines for animal care and management were observed. Extract from the Minutes of meeting № 200 of the Bioethics Committee at Samara State Medical University dated 22.05.2019.

Conflict of interest

The authors declare that they have no conflict of interest.

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