Conference Paper

Spectral Studies of Rat Bone Tissue in Modeling Osteoporosis and Effectiveness of Treatment By Hydroxyapatite

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Abstract

Presents the result of experiments on the study of the model of osteoporosis in rats using Raman spectroscopy and the effectiveness of its treatment with hydroxyapatite. Were revealed spectral differences between groups of samples (control group, group with the model of osteoporosis and a group with the model of osteoporosis after treatment with hydroxyapatite). In addition, optical coefficients were introduced to evaluate the effectiveness of treatment.

Keywords: Raman spectroscopy, optical coefficients, osteoporosis, hydroxyapatite, collagen matrix

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Received: 28 January 2018
Accepted: 15 March 2018
Published: 25 April 2018

Publishing services provided by Knowledge E

Selection and Peer-review under the responsibility of the PhIO Conference Committee.

1. INTRODUCTION

Osteoporosis is a disease of the musculoskeletal and connective tissue, as a result of which the density of bones decreases and their fragility increases. The importance of studies of osteoporosis is explained by the high prevalence of the disease among the adult population, in the majority of older people [1], as well as its consequences. Osteoporotic fractures significantly impair the quality of life, in some cases leading to fatal outcomes.

Radiography remains the most common method of diagnosing not only osteoporosis, but also virtually all diseases of the musculoskeletal system. However, destructive changes in bone with this method will be noticeable when the amount of bone mass is already reduced by 20-40%. With the help of roentgenography, it is impossible to diagnose osteoporosis in the early stages [2].

To determine the degree of risk of fractures in osteoporosis, densitometric measurement of bone mass is used [3]. Measurement of bone density regardless of localization,

allows you to trace, for example, the dynamics of development of senile (senile) osteoporosis.

There are also biochemical methods based on the study of urine and blood of patients, histomorphological evaluation of bone tissue [4, 5].

However, a high dose of irradiation during radiography, additional surgery in the patient's body during biopsy, and the duration of the analysis in biochemistry require a more accurate technique in the study and treatment of this disease.

The method of Raman spectroscopy is widely used in biomedical research [6-12], in particular in the study of bone tissue and the effectiveness of treatment. Thus, the authors of [13] investigated the distribution of hydroxyapatite in the section of the regenerative material of the parietal bone of the rat skull. The proposed method allows to determine the characteristics of mineralization in bone regenerate. In [14], the analysis of Raman spectroscopy showed stable penetration of hormones through the layers of the skin during a 24-hour period, which served as the basis for the innovative development of transdermal nanoparticles used as a stable and controlled release system for the treatment of menopausal symptoms. These facts indicate the possibility of using Raman spectroscopy for diagnosing diseases and evaluating the treatment.

The aim of this work is spectral studies of the model of osteoporosis in rats with an assessment of the effectiveness of its treatment with hydroxyapatite.

2. MATERIALS AND EXPERIMENTAL SETUP

The experiment was performed on mature female rats aged 6-9 months and weighing 180-230 g. The animals were divided into three groups. The first group (control) is a group of healthy animals. The second group (cortisone) is a group of rats in which a model of osteoporosis was created by the administration of cortisone (a hormonal preparation of a steroid form with pronounced high-speed anti-inflammatory, anti-exsessive, anti-inflammatory, anti-allergic, immunosuppressive, antishock and anti-toxic action). The third group (cortisone + hydroxyapatite) is a group of animals who have performed the model of osteoporosis by administering a cortisone drug followed by a course of treatment with hydroxyapatite powder (hydroxyapatite). The amounts of drugs administered per unit weight of the rat were 10 mg / kg and 40 mg / kg (the second and third groups were divided into two subgroups).

The femoral bones of rats were used as the study materials. Bones were sawn longitudinally. Bony scours were measured in the areas of the head, diaphysis and

epiphysis of bones, but the head of the bone is of greatest interest, in this area the probability of manifestation of osteoporosis is high [15]. The heads of the bones of the samples studied were measured at 17 points.

In addition, the bones were subjected to analysis for mechanical strength for fracture and for deflection.

Spectral characteristics of the bones were investigated using an experimental stand, the scheme of which is shown in Fig. 1. The Raman probe 1 focuses laser radiation at object 2 (at a distance of 7.5 mm from the output window with a focal spot diameter of ~ 0.15 mm) and collects fluorescence radiation and scattered radiation. The built-in broadband filter of probe 1 is designed to extract radiation in the spectral range of 790-1200 nm, which is then routed via optical fiber to spectrometer 3 with built-in camera 4 [16].

To reduce noise, camera 4 is cooled to -60 ° C, providing a spectral resolution of 0.15 nm (~ 1 cm-1). The transporter 8 with a stepper motor made it possible to spatially scan the sample [17].

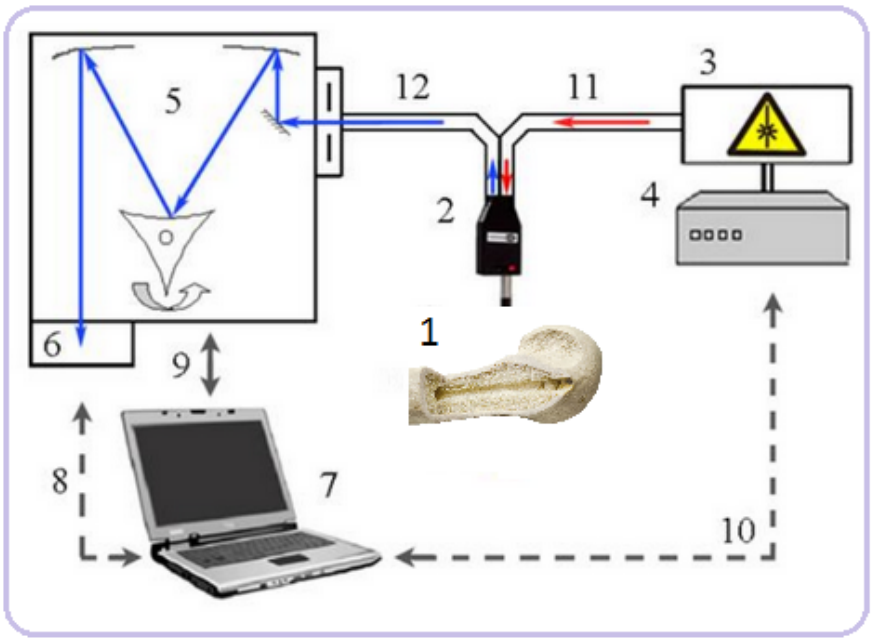

Figure 1: Experimental installation *1-object; 2 - Raman probe RPB785; 3 - laser module LuxxMaster Raman Boxx; 4 - power supply of the laser module; 5 - Sharmrock sr-303i spectrometer; 6 - built-in cooled camera DV420A-OE; 7 - computer 8,9,10 - information electrical cables; 11 - transmitting optical fiber; 12 - receiving optical fiber .*

Spectrum processing was carried out in the software package Wolfram Mathematica 10. The investigated spectrum was cleaned from noise by a smoothing median filter (5 points), it was determined on the selected interval of 400-2200 cm^{-1} by an

iterative algorithm, an approximating line (polynomial of the fifth degree) of the autofluorescent component, and then this component was subtracted, and as a result, a separate Raman spectrum was obtained [18].

3. RESULTS

3.1. Studies of samples by the Raman spectroscopy method

Figure 2 shows the averaged Raman spectra illustrating the changes in the region of the head of the bone in the groups of samples studied. The obtained Raman bands correspond to the vibration modes of the substances and bonds presented in Table 1.

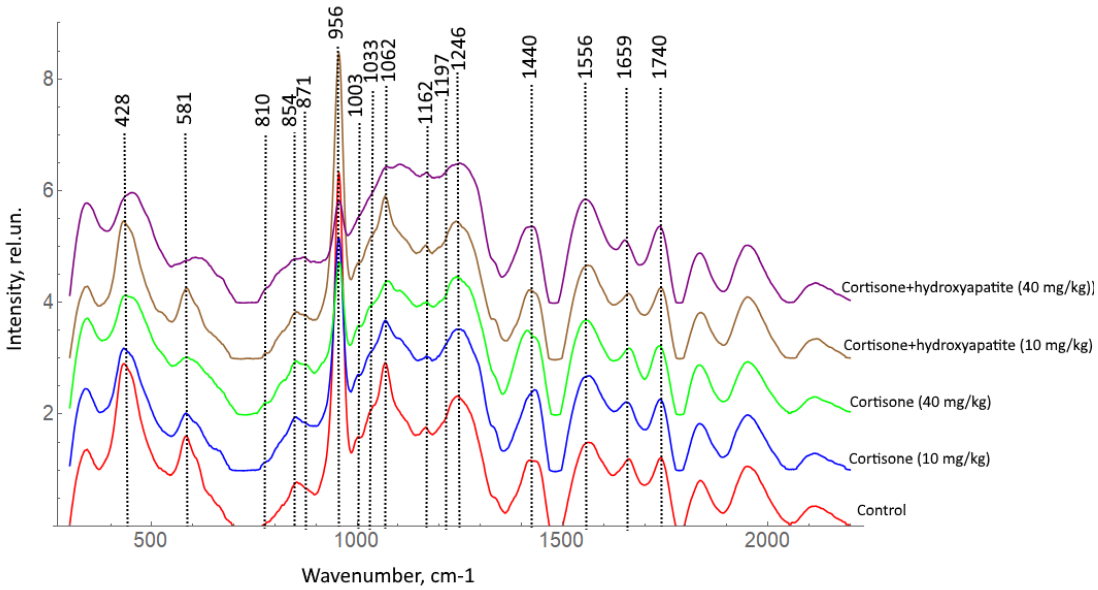

Figure 2: Average Raman spectra of bone samples.

The main spectral differences were revealed at wave numbers of 956 cm^{-1} (phosphate ion $PO_4^{3-}(\nu_1)$ (P-O symmetric valence)), 1033 cm^{-1}(phenylalanine), 1062 $cm^{-1}(CO_3^{2-}(\nu_1)$ substitution of B- type (C-O planar valence)), 1244 - 1271 cm^{-1} (amide III) и 1659 cm^{-1} (amide I).

In the study of osteoporosis, first of all, attention is paid to the change at wave numbers of 956 cm^{-1} и 1062 cm^{-1}, since disorders in the design of bone beams, their thinning and increase in the distance between them, which occurs in osteoporosis, is due to the replacement of phosphate ions PO_4^{3-} carbonates CO_3^{2-} in the hydroxyapatite formula [19]. On the obtained Raman spectra this is illustrated by a decrease in the intensity peaks at a wave number of 956 cm^{-1} (the PO_4^{3-} phosphate ion with

Table 1: Interpretation of Raman spectra of bone tissue.

Wavenumber k, cm^{-1}	Substance, vibration of molecules
428	PO_4^{3-} ($\nu 2$)
581	PO_4^{3-} ($\nu 4$)-(P-O deformation oscillation)
854	Hydroxyproline (C-C oscillation)
871	Hydroxyproline (C-N oscillation)
956	PO_4^{3-} ($\nu 1$) (P-O symmetrical stretching vibration)
1003	mode of the benzene ring of phenylalanine
1033	phenylalanine
1062-1070	CO_3^{2-} (C-O plane valence vibration)
1160	Piranosis (carbohydrate)
1244-1271	Amide III, C-N-H stretching vibration
1440	CH_2 deformation torsional oscillation
1554-1564	Amide II
1655-1660	Amide I, C-C-H stretching vibration
1740	ν (C=O) lipids

respect to intensity peaks at a wave number of 1062 (CO_3^{2-} in the groups "cortisone (40 mg / kg)", "cortisone + hydroxyapatite (40 mg / kg), and cortisone (10 mg / kg)".

The wave number of 1033 cm^{-1} corresponds to the "respiratory" mode of the benzene ring of phenylalanine. This amino acid is important in the study of osteoporosis since it is involved in the collagen synthesis process and determines the properties of collagen fibrils, such as elasticity and elasticity. Reduction of this peak is observed in the groups of samples "cortisone (40 mg / kg)" and "cortisone + hydroxyapatite (40 mg / kg)" compared with other samples most vividly. This peak is associated with the loss of water mass from the collagen structure. The loss of water from collagen leads to a significant change in the structure of collagen filaments (fibrils), which become less elastic, thereby increasing the probability of rupture of fibrils under the influence of tension, which leads to a decrease in the elasticity and elasticity of the bone as a whole [20].

The peak of 1659 cm^{-1} corresponds to amide I, its decrease indicates the violation of hydrocarbon bonds in protein molecules, which also affects the properties of collagen fibrils.

The wave number of 1740 cm^{-1} on the Raman spectra is practically unchanged, so we use it as a denominator in the coefficients introduced below.

On the basis of the described peaks, we introduce the optical coefficients characterizing the component composition of bone samples.

The ratio T is the criterion [21], which determines the degree of destruction of bone beams and a decrease in bone mass, since it characterizes the substitution of bonds in hydroxyapatite by ions CO_3^{2--}:

$$T = \frac{I_{956}}{I_{1062}} \tag{1}$$

To control the relative content of phenylalanine, which determines the water content in the collagen structure, the following coefficient R:

$$R = \frac{I_{1033}}{I_{1740}} \tag{2}$$

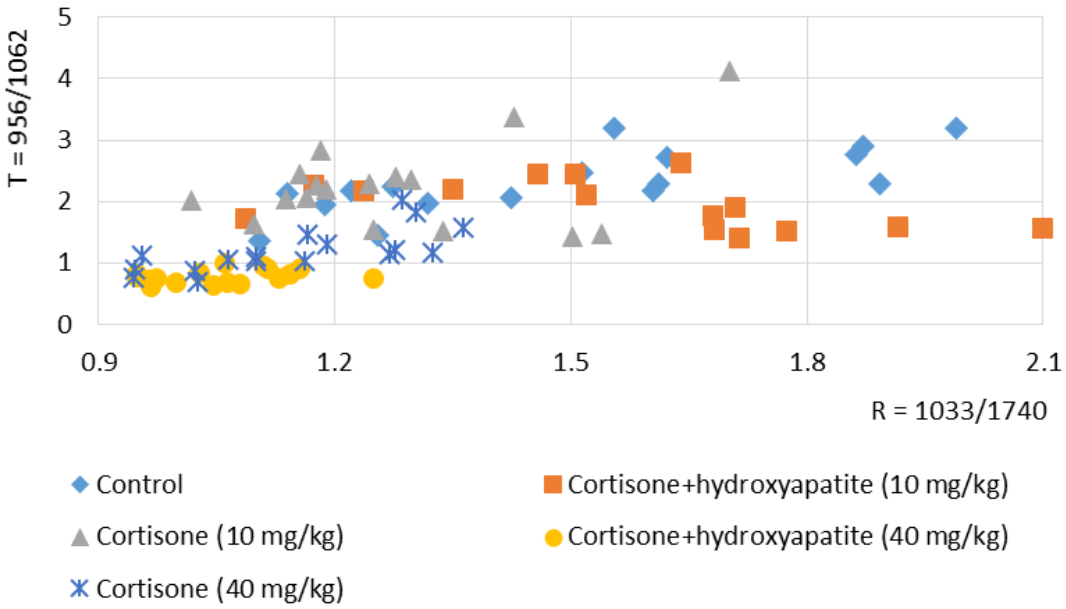

Figure 3: Two-dimensional dependence of the optical coefficients T on R for the analyzed groups of rats.

Figure 3 shows that the groups "cortisone (40 mg / kg)" and "cortisone + hydroxyapatite (40 mg / kg)" are characterized by lower values of the coefficients R and T (0.93-1.35 and 0.55-2, respectively) compared with the control group (1.15-2.00 and 1.9-3.2, respectively), which is a consequence of the development of osteoporosis. However, the differences between these two groups can not be traced, which in this case indicates an ineffective treatment of this model of osteoporosis development. The group "cortisone (10 mg / kg)" due to lower concentrations occupies a transitional position. It should be noted that changes in this concentration cause the initial stage

of osteoporosis development and it can be seen from the figure that changes in the replacement of bonds in hydroxyapatite by CO_3^{2-} ions (coefficient T) have not yet fully manifested, while a decrease in phenylalanine is observed. In the same group, "cortisone + hydroxyapatite (10 mg / kg)" is shifted relative to the group "cortisone (10 mg / kg)" in the region of increased collagen component, which indicates a positive result of treatment with hydroxyapatite under the given conditions. The ratio of T to the full was not restored probably because the hydroxyapatite powder has a T ratio that is lower than that of the control group.

3.2. Studies of bone samples by mechanical testing

In addition to research by the Raman spectroscopy method, all groups of bones underwent a mechanical test for strength and deflection.

Figure 4 a shows the results of mechanical testing of fracture specimens. The diagram illustrates the ultimate strength (F, H) that a bone can withstand. The least resistant model was a model with osteoporosis with a dosage of cortisone 40 mg / kg.

In osteoporosis, there are changes in the collagen matrix - a violation in the spatial structure of the collagen protein, as a result of which it loses its elasticity and the bone becomes less plastic, which also makes it brittle under the action of elastic deformations. Figure 4 b shows a diagram illustrating the results of the deflection tests. The diagram shows the potential for deflection for each sample (l, mm).

The results of mechanical tests confirmed the trend, which was noticed in the analysis of Raman spectra. The least resistant were the samples - "cortisone (40 mg / kg)" and "cortisone + hydroxyapatite (40 mg / kg)", the most durable - the control sample. Treatment with hydroxyapatite in the group "cortisone + hydroxyapatite (10 mg / kg)" gives a positive result, which confirms the test for mechanical strength. However, testing the same sample for deflection indicates an increase in bone hardness compared to the sample "cortisone (10 mg / kg)".

4. CONCLUSIONS

Spectral differences between the study groups of the samples (control group, the group with the model of osteoporosis and the group with the model of osteoporosis after treatment with HAP) were detected at wavenumbers of 428 cm^{-1} (phosphate ion

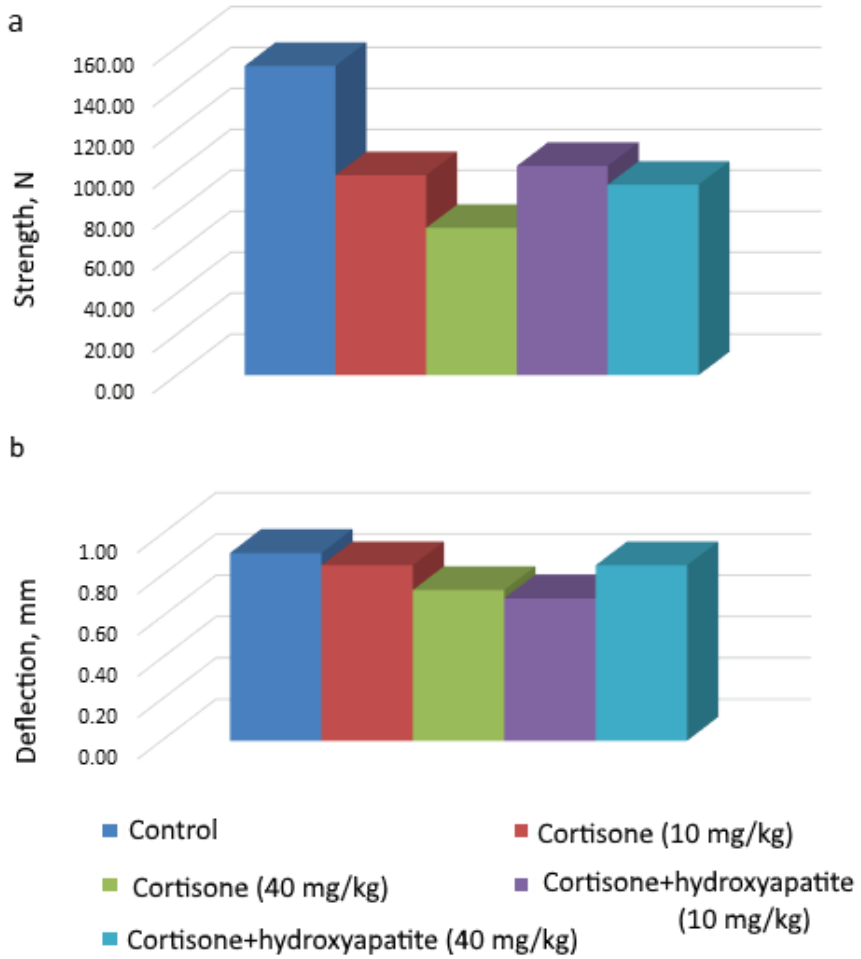

Figure 4: Experimental data on the mechanical strength of bone samples. a) a diagram of the results of mechanical fracture tests; b) a diagram of the results of mechanical deflection tests for each sample.

PO_4^{3-} (v2)), 581 cm^{-1} PO_4^{3-} (v4) - (PO deformation vibration)), 854 cm^{-1} (hydroxyproline, C-C vibration), 956 cm^{-1} (PO_4^{3-} (v1) phosphate ion (P-O symmetric valence)), 1033 cm^{-1} (phenylalanine), 1062 cm^{-1} (CO_3^{2-} (v1) B-type substitution (C-O planar valence)), 1244-1271 cm^{-1} (amide III) and 1659 cm^{-1} (amide I).

The coefficients allowing to estimate the effectiveness of treatment of the model of osteoporosis with cortisone (10 mg / kg) with the help of hydroxyapatite are introduced. For the model with cortisone 40 mg / kg, no changes were observed in the treatment of hydroxyapatite, which in this case indicates ineffective treatment of this model of osteoporosis development.

The results of investigations by the Raman spectroscopy method are confirmed by mechanical tests for strength and fracture.

Acknowledgment

The reported study was funded by RFBR according to the research project №18-32-00004

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