Investigation of the Features of the Composition of Human Bone Tissues

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Abstract

Results of the investigation of bone tissue by means of XPA, IR spectroscopy, thermal analysis, mass spectrometry are presented. It is shown that the crystal basis of the bone tissue is poorly crystallized nonstoichiometric apatite. It is established that in the case of coxarthrosis the amount of PO_4^{3-} in bone tissue decreases, the content of CO_3^{2-} and the organic component increases, therefore, the degree of crystallinity and the stoichiometry of bone apatite decrease. The differences between pathogenic and reference samples in the concentrations of manganese, tin, iron, copper and chromium ions were revealed.

Key words: XPA, IR spectroscopy, thermal analysis, mass spectrometry, bone tissue, biomineral, hydroxyapatite, stoichiometry

INTRODUCTION

At present, investigation of biogenic and abiogenic minerals is going on intensively, including the processes of their formation, changes of the composition, structure and properties, which is necessary to solve a number of ecological and medical problems [1, 2]. The biominerals of interest include the bone tissue. Its detailed investigation under the conditions of normal and pathogenic bone mineralization allows one to reveal the features of the course of some diseases, in particular coxarthrosis.

This disease occupies the leading position among the pathologies of large joints and has a substantial social and economic importance [2, 3]. For instance, the number of operations performed in the Omsk Region during the years 2005–2007 was 358; among the patients, 21 % belongs to the able-bodied citizens 30–49 years old. Nevertheless, the aspects related to the pathogenic changes in bone tissues are poorly considered in literature. For example, some scarce data on the composition and properties of physiogenic bone are reported in [2, 4], but even these data are often superficial.

To obtain complete information on the problem of pathogenic changes of bone tissue in case of pathology, it is necessary to carry out its investigation using the complementary physicochemical methods.

So, the goal of our work was to study the composition of human bone tissues in the normal state and in the case of pathology (for coxarthrosis as example) with the help of X-ray phase analysis (XPA), IR spectroscopy, thermal analysis, mass spectrometry with inductively coupled plasma (MS-ICP).

MATERIAL AND METHODS OF INVESTIGATION

The objects of investigation were normal (reference, 4 species) and coxarthrosis affected (108 species) femoral head bones from patients

at the age of 30–49, 50–59 years after operative extraction during endoprosthesis replacement at the Clinical Medical Surgery Center (Omsk) and submitted by the Chair of Pathological Anatomy and Clinical Pathology of the Omsk State Medical Academy. All the samples differed from each other in size and had a high hardness. On the basis of bone collection, a database containing the complete information about the samples (age, sex of patients, accompanying diseases) was organized.

Samples for investigation were prepared as follows: three horizontal sections were made from each femoral head: upper, medium, lower (the order of alternation is presented in the direction hyaline cartilage – femoral bone), thus obtained plates were ground and sampled dry for analysis using the quartering method. The dynamics of the disease was evaluated through the comparative characterization of the composition of pathogenic sections with each other and with the reference samples.

Diffraction patterns were recorded with DRON-3 diffractometer. Operating mode: for Co and CuK_α radiation – U = 35 kV, I = 15 mA, for MoK_α – U = 38 kV, I = 10 mA, the speed of detector rotation 1 deg/min, the rate of chart strip movement 720 mm/h, working scale of recorder $4 \cdot 10^2$ pulses/s, aligning slits 1 mm, 0.25 mm. Sensitivity of measurements was 3 %.

The IR spectra of bone samples were recorded with the Fourier Transform spectrometer Spectrum One FT-IR, Perkin Elmer, with the samples pressed in tablets with KBr. The source of radiation was a laser tube, the spectral range was 4000–400 cm^{-1}, rate 0.2 cm/s. Detection limit: ~10^{-9} %, error of determination: 2–5 rel. %. The data of IR spectra were interpreted through qualitative identification of the IR spectra recorded under identical conditions, their semi-quantitative analysis with respect to the ratios of integral intensities for bonds: ν_3 C–O carbonate ions to ν_3 P–O phosphate ions and ν_3 P–O PO_4^{3-} to C=O of the organic component. The ratio of CO_3^{2-}/PO_4^{3-} was the basis for evaluating the ordering of the mineral bone structure (the normal value is 1 : 7 [3]).

Mathematical processing of the spectra was carried out using the PeakFit_v 4.11 software package [5]. The essence of the procedure is decomposition of the spectral regions 400–500 and 1350–1800 cm^{-1} into three and six elementary absorption bands (Lorentz distribution, P = 0.99). The parameter of the infrared splitting of the antisymmetric bending vibration ν_4 of O–P–O bond (spectral region) was calculated from the results of the first decomposition as the ratio of the intensities of two peaks to the intensity of the "hollow" between them: IRSF = I(564 cm^{-1}) + I(604 cm^{-1})/I(584 cm^{-1}). The crystallinity degree of the mineral under investigation was estimated on the basis of the value obtained. Using the results of the second decomposition, we calculated the coefficient A/B that characterizes CO_3^{2-} content and determines the type of the substitution of PO_4^{3-} with CO_3^{2-} in the structure of bone apatite (B-type substitution) and the substitution of OH^- (A-type substitution), as the ratio of the sum of peaks corresponding to A-substitution (1452–1456, 1495–1501, 1547–1451 cm^{-1}) and B-substitution (1412–1414, 1469–1472 cm^{-1}) ν_3 of antisymmetric vibrations of C–O bond.

Thermal analysis was carried out with SII Diamong TG/DTA derivatograph, Perkin Elmer. Temperature range under investigation: 25–1000 °C; heating rate: 20 °C/min; sample mass: 25–30 mg; sensitivity of mass measurement $2 \cdot 10^{-3}$ %, error ~0.1 %. The sensitivity of measuring thermal effects was $6 \cdot 10^{-4}$ V, error was approximately ±6 %. The quantitative data on mass losses during annealing were obtained in course of mathematical processing of the thermogravimetric curve (TG) and its derivative (DTG). The character of energy processes was estimated on the basis of the appearance of differential-gravimetric curve (DTA).

The elemental composition was determined by means of mass spectrometry with inductively coupled plasma (ICP-MS, ELAN 9000, Perkin Elmer SCIEX ICp). Bone powder was subjected to triple thermal treatment with the solution of 16 M HNO_3 and 30 % H_2O_2 (80 °C). The sensitivity of measurements was ~10^{-9}–10^{-13} %, error ~0.5 %. Concentrations of elements were calculated using the calibration curves obtained with the multielement standard solutions (Perkin Elmer Instruments, software ISO 9001).

All the results were processed statistically with the help of Statistic Soft 2006 software.

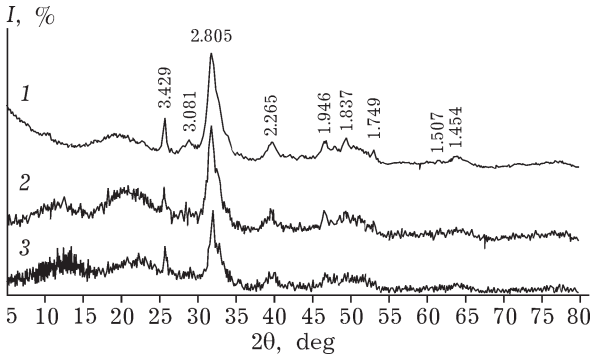

Fig. 1. X-ray diffraction patterns of affected upper (*1*) and lower (*2*) sections and the normal (*3*) human bone tissues.

RESULTS AND DISCUSSION

According to the XPA data, the basis of the crystal phase of bone tissue is poorly crystallized hydroxyapatite (Fig. 1). Such a type of crystallinity is due to the presence of the amorphous component in the form of collagen, other organic substances and calcium phosphates [6]. Diffraction patterns of the samples affected by the disease differ from those of the normal bone tissue by less resolved and broadened reflections, which points to smaller crystallinity of the mineral basis of bones in the case of pathology.

The IR spectroscopic investigation of bone samples confirmed this assumption. The following absorption bands of the vibrations of bonds in inorganic and organic groups were identified in the IR spectra of bone tissue (Fig. 2), cm^{-1}: 1) vibrations of bonds in phosphate ions: 1100–1090 – asymmetric stretching vibration ν_3 P–O; 968–962 – symmetric stretching ν_1 P–O; 610–560 – bending ν_4 O–P–O; 471 – bending ν_2 O–P–O; 2) vibrations of the bonds in CO_3^{2-} ions: 1550 and 1460–1420 – asymmetric stretching ν_3 C–O, corresponding to A- and B-type substitutions of phosphate tetrahedrons; 872–879 – bending ν_2 O–C–O; 3) 640–670 – libration OH^-; 4) 1242 – N–H bonds; 5) 1710 – C=O; 6) 2950–2850 – C–H; 7) vibrations of bonds in water: 3570–3540 – stretching OH^-, 1650 – bending H–O–H [7].

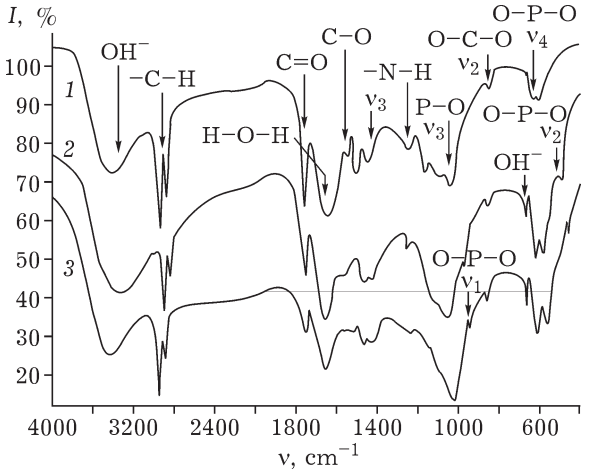

Fig. 2. IR spectra of affected upper (*1*) and lower (*2*) sections and the normal (*3*) human bone tissue.

All the spectra are characterized by small diffuseness of peaks, which provides evidence of the low crystallinity, degree of ordering, stoichiometry of the crystals of bone apatite [8, 9].

The ideas about the dynamics of changes in bones accompanying the disease were obtained by comparing the IR spectra of different sections of one articular femoral head (see Fig. 2); the upper (more affected)a lower (less affected) with each other and with the normal situation. It was established that the intensity of absorption bands of the vibrations of ν_3 P–O bonds in phosphate ions decreases, while the ν_3 C–O of carbonate ions increases passing from the lower section to the upper one. Within the studied region of the IR spectra of lower bone plates, there is an absorption band of the vibrations of OH groups (640–670 cm^{-1}) which is absent from most severely affected upper section. Deficit of OH^- ions in the structure and a decrease in the frequency of phosphate vibrations point to a lesser ordering (stoichiometricity) of bone apatite. Also the intensity of absorption band of the vibrations of ν_4 O–P–O bond decreases in the sequence upper a lower plate; the intensities of the peaks related to water and organic functional groups (N–H, C–H, C=O) increase. This fact provides evidence of a decrease in bone crystallinity.

So, it was established that the affected bone tissue sample contains prevailing carbonate ions (ν_3), organic substances and water. This pathogenic bone apatite is distinguished by lesser crystallinity and stoichiometricity.

Analysis of the results of mathematical description of the spectra (Table 1) shows that the ratios CO_3^{2-}/PO_4^{3-}, PO_4^{3-}/C=O, A/B coefficient, IRSF of the lower section are close to the normal values, therefore, the development of affection proceeds in the direction from the lower bone lamella to the higher one.

TABLE 1

Quantitative description of IR spectra of normal and pathogenic female bone tissue (30–49 years) in the case of coxarthrosis ($n = 4$, $P = 0.95$)

Bone section	Ratio of the intensities of absorption bands of vibrations		Procedure: J. Shi [4]			
	CO_3^{2-} / PO_4^{3-} *	PO_4^{3-} /C=O	A/B		IRSF	
	Pathogenic		Normal	Pathogenic	Normal	Pathogenic
Upper	1.33 : 1	1 : 5	0.67±0.12	1.29±0.01	3.16±0.65	1.17±0.02
Medium	1 : 1	1 : 4	0.73±0.10	0.92±0.02	3.16±0.65	1.12±0.10
Lower	1 : 6	1 : 3	0.79±0.08	0.79±0.05	4.16±0.21	4.10±0.01

*Normal: 1 : 7 [3].

Comparing the composition of consecutive bone sections we revealed that the content of catbonate ions increases in the upper section by a factor of 6–7 with respect to the lower one and to the normal value, that is, the stoichiometry of bone apatite changes. The change of the relations between A- and B-type substitutions with carbonate ions is the evidence of the prevalence of B-type hydroxyapatite in normal bone tissue (substitution of CO_3^{2-} positions by PO_4^{3-}), and domination of A-type (substitution of CO_3^{2-} positions by OH^-) in pathogenic tissue [8, 10]. This conclusion is confirmed by the presence of the vibrations at 640 cm^{-1} related to OH^- groups in the IR spectra of reference samples and the absence of these vibrations from the spectra of affected tissues, as well as by enhancement of the intensity of absorption bands of the vibrations of C–O bonds in carbonate ions at 1550 cm^{-1} in the spectra of the most strongly affected upper section (see Fig. 2). In addition, the amount of organic groups (C=O) increases in the upper section by a factor of 2, and the parameter of IR splitting increases by a factor of 3–4. This causes a decrease in crystallinity of bone apatite in course of pathology.

The results of XPA and IR spectroscopic studies of the normal and coxarthrosis affected bone tissue are confirmed by the data of thermal analysis. Derivatograms exhibit mass losses within temperature ranges (Fig. 3): 25–270 °C – the loss of adsorption water (I); 270–430 °C – removal of structural water and low-molecular organic compounds (LOC), non-collagen proteins LOC), non-collagen proteins with small molecular masses (II); 430–600 °C – decomposition of high-molecular organic compounds (HOC), collagen (III); 700–900 °C – evolution of volatile compounds, mainly carbon dioxide, of the mineral bone component during the transition from nonstoichiometric car-

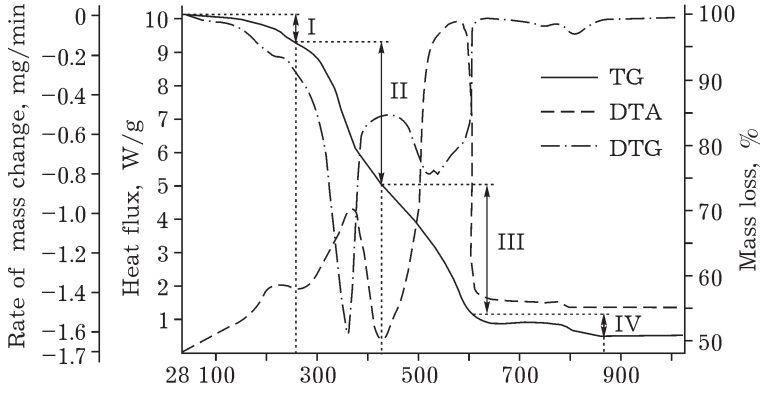

Fig. 3. Derivatogram of the normal human bone tissue. Design. see text.

bonate hydropxyapatite into the stoichiometric one (IV) [11, 12].

The obtained thermal characteristics of the pathogenic bone tissue and detailed investigation of the TG curves showed that the mass loss values are determined by the degree of affection for the samples under investigation. For example, in the bone sections taken from men and women of the given age groups (30–59 years), we observe a decrease in the loss of high-molecular substances while the disease develops (Fig. 4). This may be connected with their destruction into low-molecular products or with denser packing of deformed collagen fibres. The latter assumption may be an explanation of the increased hardness of pathogenic bone samples. The mass losses (content) of low-molecular organics participating in bone exchange increase in the affected bone samples of men.

Increased amounts of adsorbed water, volatile components (CO_2) were also revealed in affected samples with respect to the normal ones (see Fig. 4), which explains prevalence of carbonate ions in the structure of pathogenic apatite.

So, the revealed regularities provide evidence of the smaller crystallinity and stoichiometry of the mineral component in affected bone tissue.

According to the data of MS-ICP, 66 elements were determined in the samples under investigation. It is known that the main reason of their accumulation in bone tissue is the ability of elements both to substitute calcium ions and phosphate tetrahedrons in the structure of bone apatite, and to get adsorbed on its surface [13].

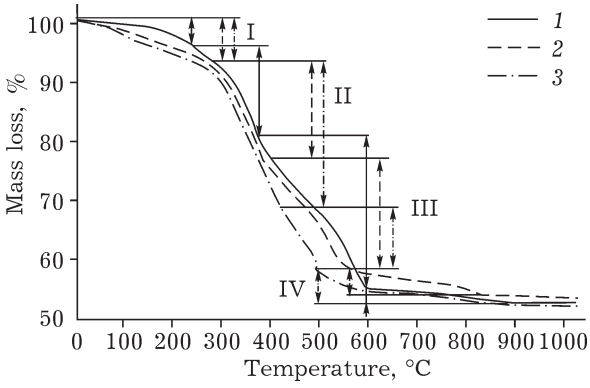

Fig. 4. Curves of mass losses from bone tissue with adsorber water (I), low-molecular (II), high-molecular (III) substances, volatile compounds (IV): *1* – normal; *2*, *3* – bone tissue samples of women and men, respectively.

On the basis of an increase in element concentrations in bone samples for the range 10^{-6}–0.5 mass %, we ranged the elements and composed the sequence: Na, Mg, K (10^{-2}–0.5 mass %), Zn, Si, Fe, Sr (10^{-3}–10^{-2} mass %), Ni, Al, Cr, Ba, Ti, Cu, Co, Mn, Sn (10^{-6}–10^{-3} mass %); other elements were detected in the samples under investigation in concentrations below 10^{-6} mass %.

It was demonstrated that the order of elements in the sequence is almost the same in all the normal sections Na > Mg > K > Fe > Zn > Sr > Si > Cr > Ni > Al > Ba > Cu > Mn > Ti > Co > Sn) and affected ones (Na > Mg > K > Zn > Sr > Si > Fe > Ni > Al > Ba > Cr > Ti > Cu > Co > Mn > Sn). It was stressed that the affected tissues in comparison with normal ones exhibit differences in chromium and iron content; this difference points to variation of concentrations in the case of pathology.

The absolute content of the elements in all the sections of normal bone tissue is approximately the same and close to that for lower affected sections, which coincides with the data on the character of disease development from the upper bone lamella to the lower one. An increase in

TABLE 2

Element content of normal state and pathogenic upper sections of bone tissues of men and women 30–49, 50–59 years (n = 4, P = 0.95)

Element	Mass concentration of elements in bone tissue, %	
	Upper section	Normal value
Na	0.46±0.14	0.44±0.02
Mg	0.22±0.01	0.19±0.007
K	$(0.28\pm0.13)\cdot10^{-1}$	$(0.58\pm0.13)\cdot10^{-1}$
Zn	$(0.81\pm0.23)\cdot10^{-2}$	$(0.77\pm0.01)\cdot10^{-2}$
Si	$(0.27\pm0.05)\cdot10^{-2}$	$(0.19\pm0.03)\cdot10^{-2}$
Fe	$(0.17\pm0.11)\cdot10^{-1}$	$(0.15\pm0.01)\cdot10^{-2}$
Sr	$(0.61\pm0.10)\cdot10^{-2}$	$(0.72\pm0.43)\cdot10^{-2}$
Ni	$(0.46\pm0.10)\cdot10^{-3}$	$(0.44\pm0.15)\cdot10^{-3}$
Al	$(0.44\pm0.14)\cdot10^{-3}$	$(0.29\pm0.11)\cdot10^{-3}$
Cr	$0.18\cdot10^{-2}$	$(0.10\pm0.13)\cdot10^{-3}$
Ba	$(0.37\pm0.08)\cdot10^{-3}$	$(0.21\pm0.14)\cdot10^{-3}$
Ti	$(0.98\pm0.42)\cdot10^{-4}$	$(0.99\pm0.14)\cdot10^{-4}$
Cu	$(0.16\pm0.15)\cdot10^{-3}$	$(0.49\pm0.13)\cdot10^{-4}$
Co	$(0.30\pm0.21)\cdot10^{-4}$	$(0.18\pm0.02)\cdot10^{-4}$
Mn	$(0.14\pm0.02)\cdot10^{-3}$	$(0.82\pm0.47)\cdot10^{-5}$
Sn	$(0.19\pm0.10)\cdot10^{-4}$	$(0.40\pm0.04)\cdot10^{-5}$

the content of iron, manganese, copper and tin ions is observed in affected upper sections (Table 2). Separate pathogenic samples exhibit increased chromium content. The physiological role of these elements may to a definite extent point to the character of pathogenic processes.

It follows from the data shown in Table 2 that the affected upper sections, in comparison with reference samples, exhibit higher content of copper – by a factor of 3, tin – 4, iron – 11, manganese – 16, chromium – 18. The excessive amount of copper, manganese, iron provides evidence of distortions in bone tissue mineralization processes [13]. Increased chromium concentrations in a series of samples also point to the destructive (degenerative) type of metabolism in the course of this disease. The role of tin in bone exchange has not been investigated yet.

CONCLUSIONS

Thus, investigation of the normal and coxarthrosis affected bone tissue of human femoral head with the help of physicochemical methods allows us to make the following conclusions:

1. It is established that the stoichiometry and the degree of crystallinity of bone apatite decrease in the course of the disease.

2. It is revealed that the elemental composition of bone samples changes towards an increase in the content of manganese, tin, iron, copper and chromium in the case of pathology.

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