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Eliška Kubíková
Peter Weismann
Jana Jakimová

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AGE-RELATED FEATURES OF HYDROXYAPATITE STRUCTURE IN A SPONGY BONE TISSUE

***Gordienko O. V.*[1]*, Yarmolenko O. S.* [1]*, El Falougy H.*[2]**

[1]*Sumy State University, Sumy, Ukraine, 2. Rymskogo-Korsakova st.,40007*
o.yarmolenko@med.sumdu.edu.ua
[2]*Comenius University, Bratislava, Slovakia*

Abstract
The structure and size of hydroxyapatite depend on age. X-ray diffraction analysis helps to determine age-related features of hydroxyapatite structure in a spongy bone tissue.

Keywords
spongy bone, x-ray diffraction analysis, age

Bone tissue has a complex multi-level structure. It comprises mineral (apatite crystals) and organic (collagen) phases (1, 2, 3).
Bone contains about 60–70% w/w of calcium phosphate mineral (apatites), about 20–30% w/w of organic matrix and 10% of water. The major organic component of bone is represented with fibrillar collagen type I (4).
Water is not less significant inorganic component of the bone matrix. It provides a link between the internal and external environment and the transport of substances between cells and the extracellular matrix.
There are several hierarchical levels representing bone structure. The main components of bone tissue are mineralized collagen fibers of type I. The ratio of hydroxyapatite and collagen determines the mechanical properties of the bone.
However, the structure and size of hydroxyapatite undergo significant changes with age, which have not been well studied, especially in spongy bone.

Objectives
To investigate age-related features of the hydroxyapatite structure in the spongy bone tissue by x-ray diffraction analysis.

Methods and materials
We performed the experiment on 12 laboratory male rats for the investigation of spongy bone hydroxyapatite structure. Animals were divided into three age groups. The first group includes 4 immature rats of two months of age, the second group - 4 mature rats of five months of age, and the third – 4 old animals at the age of 14 months.
The experiment was conducted in accordance with the requirements of the “European Convention of the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (Strasbourg, 1986) and "General ethics of animal experimentation", approved by the I National Congress on Bioethics (Kyiv, 2001).
Twenty four calcaneal bones were extracted in animals of all age groups. They were burnt in a muffle furnace at a temperature of 900°C. The ash was investigated on a DRON-4-07

apparatus with the goniometric prefix GUR-9, $K\alpha$ radiation from copper with a wavelength of 0.154 nm was used. The voltage and current on the X-ray tube were 20 mA and 40 kV.
Diffracted X-rays were recorded in the angular range of 2θ from 10° to 60° with a speed of 1°/min (5). On the obtained diffraction patterns, we studied the crystallographic characteristics of hydroxyapatite. We investigated the most pronounced diffraction peak and determined its amplitude.
Also, we calculated interplanar distances in hydroxyapatite crystals on the angular position of the diffraction peaks.
Moreover, we calculated the sizes of coregent scattering blocks (crystallites), using the Scherrer formula4, and the microtexturing coefficient by the reflex ratio method.
Furthermore, we determined the lattice parameters of hydroxyapatite taking into account the hexagonal crystal system (6, 7, 8).

Results and discussion
In the course of study, we found that the maximum amplitude of diffraction peak of the mineral component of the bone is recorded in the range from 30° to 32°. It corresponds to the crystallographic characteristics of hydroxyapatite - the predominant component of the crystal lattice of bone mineral.
The size of the unit cell along the C axis increases from 6.900-6.913 (10^{-9} m) in young to 6.913-6.916 (10^{-9} m) in old rats. The dimensions of the unit cell of hydroxyapatite along axis A also increase from 9.426-9.434 (10^{-9} m) in young animals to 9.420-9.456 (10^{-9} m) in old animals (Tab.1).
The C/A cell size ratio, which characterizes the degree of symmetry of the elementary cells of crystals, also slightly increases from 73.14-73.35 (10^{-4}m) in young animals to 73.26-73.62 (10^{-4}m) in old ones. This indicates a decrease in the interplanar distances in the crystal lattice of bone mineral with age.
Coregent block sizes (i.e., conglomerates of unit cells or crystallites) increase from 35.01–39.43 nm in immature rats to 41.10–48.06 nm in old rats. This indicates a decrease in the total exchange surface of the bone mineral, as well as the ordering of the crystal lattice of hydroxyapatite with age, which is the cause of increased bone fragility in senile age. In animals of different age groups, this fact is also confirmed by changes in the microtexturing coefficient, which testifies to the unidirectionality of the crystals orientation: during the observation, it was the highest in young animals 0.755-1.108 cu, and it was only 0.518- 0.593 cu in the old ones.
Collagen molecules have a very complex configuration. In order to simplify the calculation of the sizes of coregent blocks only the gallo area in the region of the basic reflexes of the organic matrix was calculated. In general, this is a reflection of physical essence of the Scherer formula, which is used to study the mineral phase.
In young animals, the area of reflex D ranges from 127.68 ± 10.51 mm^2 to 142.86 ± 3.60 mm^2, the reflex E increases from 365.18 ± 7.91 mm^2 to 408.93 ± 9 , 87 mm^2, and the reflex F - from 66.96 ± 6.70 mm^2 to 90.18 mm^2. This indicates an active formation of matrices for the nucleation of mineral phase of the bone.
In reproductive-aged rats, the gallo areas reflections remain almost unchanged in the region of D, E and F, which indicates the stability of an organic matrix synthesis.

Age group	Block size, nm	Microtexturing coefficient	Unit cell size along axis A, 10^{-9} m	Unit cell size along axis C, 10^{-9} m	C/A cell size ratio, 10^{-4}
Immature rats	37,01±1,37	0.867±0,076	9,431±0,003	6,906± 0,008	73,22±0, 07
Mature rats	40,286±1,23	0,766±0,037	9,415±0,004	6,907± 0,006	73,35±0, 04
Old rats	43,98±0,75	0,577±0,053	9,426±0,004	6,911± 0,004	73,33±0, 05

Table 1 Ultrastructural indicators of calcaneal bone hydroxyapatite of different-aged intact laboratory rats

In old rats, the gallo areas reflexes are slightly less than in mature-aged animals in the region of D, E, and F. This fact indicates an age-related decline in the synthesis of bone organic matrix.

Conclusions

Comparing the obtained data with literature data (6,7,8), the ontogenetic transformations in spongy bone tissue can be divided into several periods:

1) period of intensive osteogenesis - in immature age;
2) stabilization period is in mature animals;
3) period of regressive changes with the predominance of resorption processes in old rats.

The mineral component of the calcaneus matrix in laboratory rats of different age groups is characterized by changes at the microstructural level. An increasing in crystallite size rises the level of microdeformation of the crystal lattice. Defective increase, i.e. structural imperfection of biominerals, indicates violations of normal metabolic processes in the calcaneus, which in turn leads to a decrease in its strength.

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