Research Article

Influence of 60-day introduction of tartrazine in various concentrations on the ultrastructure of tibia graft biomineral

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ABSTRACT

Aim: The aim of the research was to study the effect of 60-day intake of tartrazine with food at various concentrations on the ultrastructure of the biomineral crystalline lattice of the tibia regenerates among sexually mature white rats. **Material and Method:** The experiment was carried out on 140 rats distributed into four groups: 1st group - control, 2nd - the rats with an applied 2.0 mm defect in both tibia, and the group 3, 4 - the rats with tartrazine administered intragastrically for 60 days in the dose of 750 and 1500 mg/kg/day, after which the defect for the tibia was applied. X-ray structural study was carried out using DRON-2.0 apparatus with a goniometric attachment GUR-5. Kαa radiation of copper with the wavelength of 0.1542 nM was used; the voltage and the strength of the anode current were 30 kV and 20 A, respectively. **Results and Discussion:** The results of the study showed that the microtexturing ratio of the regenerate biomineral was less than the 2nd group values at 3rd, 10th, 15th, 24th, and 45th day after the administration of tartrazine at the dose of 750 mg/kg/day by 3.49%, 6.24%, 6.50%, 8.19%, and 6.33%, and the sizes of crystallites were more by 14.14%, 8.40%, and 3.45% on 15th, 24th, and 45th day. With the dose increase of preliminary introduced tartrazine up to 1500 mg/kg/day, the regeneration biomineral development slowed more significantly: The microtexturing coefficient was less than the values of the 4th group in all terms by 4.49%, 7.36%, 7.95%, 8.53%, and 7.55% and crystallite sizes increased by 5.30%, 20.36%, 11.47%, and 9.16% during 10–45th day. **Conclusion:** Thus, intragastric administration of tartrazine for 60 days is accompanied by the delay in the development of the biomineral crystalline lattice of bone regenerate, and the intensity of the changes depends on tartrazine dose.

KEY WORDS: Bone biomineral, Rats, Regeneration, Tartrazine, X-ray diffraction analysis

INTRODUCTION

Tartrazine (E102) - a yellow synthetic dye[1] - is widely used in food industry nowadays. The hepatotoxic and nephrotoxic effects of tartrazine were revealed in experimental studies after eating it for 90 days.[2,3] There is also evidence that the use of tartrazine for 60 days is accompanied by an increase of bone biomineral amorphous structure degree among rats.[4] At the same time, we were not able to find information on the regeneration of skeleton bones after long-term use of tartrazine for food.

Purpose

This study aims to study the effect of 60-day intake of tartrazine at various concentrations on the ultrastructure of tibia regenerates crystalline lattice among adult white rats.

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METHODS

The study was carried out on 140 white male rats of the reproductive period of ontogeny with an initial body weight of 200–210 g. All manipulations with the experimental animals were carried out in accordance with the rules established by the "European Convention for the protection of vertebrates used for experimental and other scientific purposes" (Strasbourg, 1986).[5] The animals were divided into four groups: The first group included control animals, for which 1 ml of 0.9% isotonic sodium chloride solution (group K) was injected intragastrically for 60 days, the second group was represented by rats, which had a through defect within 3–4 groups with the diameter of 2.0 mm in the proximal metaphysis of both tibia (D) during the period corresponding to

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the end of tartrazine introduction.[6] The 3rd and the 4th groups were rats that received 1 ml of tartrazine at the dose of 750 mg/kg/day and 1500 mg/kg/day of body weight, respectively, during a 60-day period with a gastric probe (manufacturer Roha Dyechem Pvt. Ltd.), after which the tibia defect (TD1 and TD2) was applied. The calculation of administered drug dose was carried out taking into account biological stability.[7] At the end of the experiment (3rd, 10th, 15th, 24th and 45th day after the end of tartrazine administration, the animals were decapitated under ether anesthesia, the tibia were skeletonized, the proximal metaphysis area was separated and examined by X-ray analysis which corresponds to the traditionally isolated stages of reparative bone regeneration process).[8] Bone powder obtained in an agate mortar was examined using DRON-2.0 device with a goniometric attachment GUR-5. Kα radiation of copper was used with the wavelength of 0.1542 nM; the voltage and the strength of the anode current were 30 kV and 20 A, respectively. The diffracted X-rays were recorded in the angular range from 2° to 37° with a recording rate of 1° per minute. The obtained diffractograms studied the most pronounced diffraction peaks, according to the angular position of which the parameters of the elementary cells of the bone biomineral were calculated, the dimensions of the coherent scattering blocks according to the Tselyakov-Scherrer equation, and the coefficient of microtexturing by the method of reflex ratio. The obtained digital data were processed by variational statistics methods using standard application programs.[9]

Main part

The animals with a perforated hole defect of 2.0 mm in diameter within the proximal metaphyseal area of the tibia had the dynamics of ultrastructure changes among newly formed bone biomineral of the regenerate with a pronounced two-phase character [Figure 1].

The first phase was characterized by the signs of destabilization and destruction concerning the crystalline lattice of bone biomineral, which is related to the prevalence of bone fragment resorption processes. At that, on the 3rd and the 10th day of observation, the dimensions of bone biomineral elementary cells along the a axis and the sizes of coherent scattering units were larger than similar indicators of control group by 0.15% and 0.13%, and by 12.08% and 10.45% (all numerical differences are reliable ones, $P \leq 0.05$). Under these conditions, the coefficient of the regenerate bone mineral microtexturing since day 3 until day 15 was less than the values of the K group by 10.46%, 9.01%, and 3.12% [Table 1].

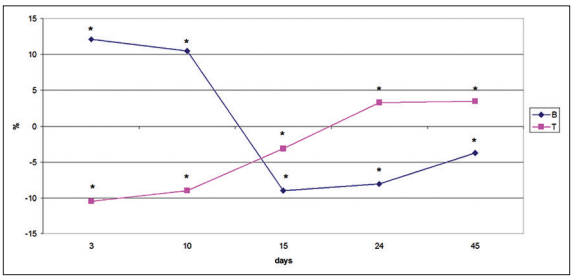

Figure 1: Dynamics of changes concerning coherent scattering unit size and the microtexturing coefficient of biomineral regenerate in group D, depending on the period after the tibia defect application (in % according to group K). *Indicates a significant difference from the parameters of the control group ($P < 0.05$) in Figures 1 and 2

The second phase of biomineral regenerates development was characterized by the predominance of the growth processes for newly formed elementary cells of the bone biomineral and by the stabilization of its crystal lattice. In this case, the sizes of the coherent scattering units since the 15th until the 45th day of observation were less by 0.12%, 0.13%, and 0.10% and by 9.00%, 8.04%, and 3.74%, respectively, and the sizes of the elementary cells along the c axis on the 15th and 24th day - by 0.24% and 0.17%. Under these conditions, the coefficient of bone biomineral microtexturing was higher by 3.24% and 3.49% on the 24th and the 45th day of the observation group.

The obtained results on the orientation generally correspond to the stepwise dynamics of the biomineral crystal ultrastructure formed in the region of the bone defect described in the literature and our previous studies.[10]

In the case, when the defects of the tibia were applied to experimental animals that received tartrazine at the dose of 750 mg/kg/day for 60-day intragastrically, the delay in the development of the regenerate biomineral crystalline lattice was observed, expressed during the entire observation.

At that, the coefficient of the regenerate biomineral microtexturing was lower by 3.49%, 6.24%, 6.50%, 8.19%, and 6.33%, respectively, in the group D during the entire observation period [Figure 2].

At the same time, the dimensions of the elementary cells of biomineral regenerate along the c axis were more than the values of group D since the 10th until the 45th day of observation, by 0.20%, 0.27%, 0.24%, and 0.14%, respectively, the sizes of elementary cell along the axis a since the 15th until the 45th day - by 0.31%, 0.14%, and 0.10%, and the sizes of crystallites since the 15th until the 45th day - by 14.14%, 8.40%, and 3.45%.

Thus, the application of a defect in tibia on the background of preliminary 60-day administration of tartrazine at the dose of 750 mg/kg/day is accompanied

Table 1: Crystallographic parameters of tibia regenerate biomineral (X±Sx)

Group	Terms	Elementary cell dimension along the axis *a*, 10^{-10} M	Elementary cell dimension along the axis *c*, 10^{-10} M	The size of coherent scattering units, nM	Microstructuring coefficient, c.u.
К	3	9.378±0.002	6.842±0.003	42.97±0.54	0.3923±0.0041
	10	9.377±0.003	6.843±0.003	42.94±0.49	0.3938±0.0038
	15	9.381±0.004	6.845±0.003	43.15±0.52	0.3962±0.0038
	24	9.383±0.003	6.843±0.003	43.29±0.41	0.3965±0.0042
	45	9.385±0.003	6.846±0.003	43.07±0.54	0.3980±0.0043
Д	3	9.392±0.003*	6.851±0.003	48.16±0.58*	0.3512±0.0036*
	10	9.389±0.003*	6.847±0.003	47.43±0.54*	0.3583±0.0038*
	15	9.370±0.003*	6.828±0.003*	39.26±0.46*	0.3838±0.0033*
	24	9.371±0.003*	6.832±0.003*	39.81±0.61*	0.4093±0.0039*
	45	9.376±0.003*	6.842±0.003	41.46±0.35*	0.4119±0.0035*
Т1Д	3	9.396±0.003*	6.858±0.002	47.95±0.62*	0.3390±0.0041*^
	10	9.397±0.003*	6.861±0.003*^	49.10±0.60*	0.3360±0.0043*^
	15	9.398±0.003*^	6.846±0.003^	44.82±0.40*^	0.3588±0.0049*^
	24	9.384±0.003^	6.848±0.002^	43.16±0.42^	0.3758±0.0043*^
	45	9.385±0.003^	6.851±0.003^	42.89±0.40^	0.3858±0.0044^
Т2Д	3	9.398±0.003*	6.861±0.003*^	49.15±0.41*	0.3355±0.0043*^
	10	9.402±0.003*^	6.864±0.002*^	49.94±0.57*^	0.3320±0.0036*^
	15	9.400±0.003*^	6.849±0.003^	47.26±0.43*^	0.3533±0.0041*^
	24	9.388±0.003^	6.851±0.003^	44.37±0.45^	0.3744±0.044*^
	45	9.391±0.003^	6.853±0.002^	45.25±0.51*^	0.3808±0.0033*^

*Indicates a significant difference from the group of control (K) animals (P<0.05); ^Indicates a significant difference from the group of animals with an applied defect (D) (P<0.05)

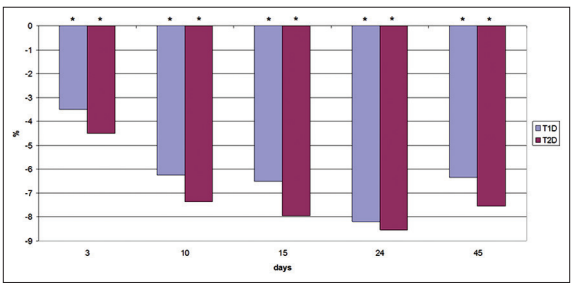

Figure 2: The dynamics of microtexturing coefficient change in respect of regenerate biomineral depending on a period after a defect application and the concentration of the preliminary introduced tartrazine (in % with respect to group D)

by the slowing of biomineral regenerate crystal lattice development, the features of which are expressed since the 3rd until the 45th day of observation, and the maximum amplitude deviations are registered usually on the 15th day after a defect application.

In the case, where the tibia defects were applied to experimental animals, which at first took tartrazine for 60 days in the dose of 1500 mg/kg/day the worsening of biomineral regenerate crystal lattice development was noted expressed during the entire observation period.

At that, the dimensions of the regenerate biomineral unit cells in TD2 group along the c axis were larger than the similar values of the D group since the 3rd until the 45th day of observation by 0.15%, 0.24%, 0.30%, 0.28%, and 0.17%, and the microtexturing coefficient was less by 4.49%, 7.36%, 7.95%, 8.53%, and 7.55%. Furthermore, since the 10th until the 45th day of observation, the dimensions of the regenerate biomineral elementary cells along "a" axis and the sizes of the crystallites were higher by 0.13%, 0.33%, 0.18%, and 0.17% and by 5.29%, 20.36%, 11.46%, and 9.16 %, respectively.

Thus, the application of a defect in the tibia is accompanied by the delay in the development of the biomineral regenerate crystalline lattice, the signs of which are expressed since the 3rd until the 45th day of the observation, and the maximum deviations for the majority of the studied parameters are recorded on the 15th day after a defect application. The deviations of the microtexturing coefficient reach its maximum on the 24th day; probably, this is due to the fact that the processes of bone biomineral crystallization are carried out somewhat later than the processes of nucleation and elementary cell development.

DISCUSSION

The obtained results can be explained presumably as follows. It has been proved that the use of tartrazine is accompanied by the activation of lipid peroxidation processes and the decrease of the antioxidant system activity in kidney.[11] This leads to the development increase of free radicals and causes the damage to biological membranes, proteins, cell nucleus chromatin, and the violation of specific ion channels and receptors stability. However, in addition to this, tartrazine contains "pyridine nitrogen" in its structure, which has a strong, complex effect.[12] Thus, tartrazine acts as a chelating agent with the molecules of copper, zinc, and manganese.[13] It is known that the above-mentioned microelements act as the cofactors of various enzymes and energy cycles. Consequently, their lack can affect the processes of mineralization in

both physiological and reparative regeneration of the skeleton bones.

CONCLUSIONS

1. The application of a perforated hole defect with the diameter of 2.0 mm in the region of the proximal metaphysis of the tibia is accompanied by the changes in the ultrastructure of the regenerate biomineral that has a biphasic character.
2. Since the 3rd until the 15th day after a defect application, the first phase is characterized by the signs of destabilization and destruction of the crystalline lattice of bone biomineral (elementary cell and crystallite size increase, the decrease of crystalline orientation homogeneity in a crystal lattice), which is associated with the prevalence of bone fragment resorption processes. Since the 15th until the 45th day after a defect application, the second phase is characterized by the predominance of the growth processes of newly formed elementary cells in the bone biomineral and the stabilization of its crystal lattice (the reduction of elementary cell and crystallite sizes, the increase of the microtexturing coefficient).
3. In the case, when the defects of the tibia were applied to the animals that took tartrazine intragastrically for 60 days, the delay in the development of biomineral regenerate crystalline lattice is noted, the severity of which depends on tartrazine dose. When tartrazine was administered at the dose of 1500 mg/kg/day, the severity of the revealed abnormalities was higher than with the use of tartrazine at the dose of 750 mg/kg/day.
4. For the majority of the investigated parameters, the maximum deviations are recorded on the 15th day after the defect application. At that, the deviations of the microtexturing coefficient reach its maximum by the 24th day, which may be related to the fact that the processes of bone biomineral crystallization are carried out somewhat later than the processes of elementary cell nucleation and growth.

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