CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1324403

Available online at: http://www.iajps.com Research Article

ULTRASTRUCTURE OF REGENERATE'S BIOMINERAL AFTER 60-DAY OF TARTAZINE EXPOSURE

Vladyslav V. Luzin[1], Olga N. Fastova[1], Aleksei V. Tverskoi[2], Vitaliy N. Morozov[2], Elena N. Morozova[2]

[1]State Institution «Lugansk State Medical University» 91045, People's Republic of Lugansk, 50-letya Oborony Luganska qr. 1g

[2]Belgorod State University 308015, Russia, Belgorod, Pobeda St. 85

morozov_v@bsu.edu.ru

Abstract:

The ultrastructure of regenerate's biomineral formed in the tibia after a 60-day tartrazine exposure at concentrations 750 and 1500 mg/kg/day was studied in an experiment on 140 mature rats. X-ray diffraction study of regenerate's biomineral was carried out on the DRON-2.0 apparatus with goniometer attachment GUR-5. 60-day tartrazine exposure at concentration 750 mg/kg/day is accompanied by delay in the formation of the crystalline lattice of regenerate's biomineral in tibial defect applied from 3 to 45 days of the experiment. It followed from an increase in the size of the elementary cells of crystalline lattice along the axis c from 10 to 45 days by 0.20%, 0.27%, 0.24% and 0.14%; the size of elementary cells along the axis a from 15 to 45 days - by 0.31%, 0.14% and 0.10%, and the sizes of crystallites from 15 to 45 days - by 14.14%, 8.40% and 3.45%. The coefficient of microtexturing of regenerate's biomineral is decreased at 3rd, 10th, 15th, 24th and 45th days of the experiment by 3.49%, 6.24%, 6.50%, 8.19% and 6.33% respectively compared to the group without tartrazine exposure. In the group of rats with tartrazine exposure at concentration 1500 mg/kg/day there was an impairment of the slowing down of the formation of the crystalline lattice of the regenerate's biomineral in whole observation period. Thus, the size of the elementary cells along the axis c were larger from 3 to 45 days by 0.15%, 0.24%, 0.30%, 0.28% and 0.17%, and the coefficient of microtexturing was less by 4.49%, 7.36%, 7.95%, 8.53% and 7.55 %. The size of the elementary cells along the axis a and the size of the crystallites were larger by 0.13%, 0.33%, 0.18%, 0.17%, and by 5.29%, 20.36%, 11.46%, 9.16% respectively from the 10th to the 45th day of experiment. Thus, 60-day tartrazine exposure is accompanied by a violation of the formation of the crystalline lattice of the regenerate's biomineral of tibia in both groups more pronounced in the group using a dose of 1500 mg/kg/day.

Key words: *rats, tibial defect, regenerate's biomineral, tartrazine, X-ray diffraction analysis, crystalline lattice.*

*** Corresponding author:**

Vitaliy N. Morozov,

State Institution «Lugansk State Medical University» 91045,
People's Republic of Lugansk, 50-letya Oborony Luganska qr. 1g
E-Mail:morozov_v@bsu.edu.ru

QR code

Please cite this article in press Vitaliy N. Morozov et al., ***Ultrastructure of Regenerate's Biomineral after 60-Day of Tartazine Exposure.,*** *Indo Am. J. P. Sci, 2018; 05(07).*

INTRODUCTION:

Tartrazine (E 102) is a food additive which [1] widely used in food industry as yellow dye. The previous experimental studies show the adverse effects of 90-days tartrazine exposure on functional state of liver and kidneys [2, 3]. There is also data that the 60-days intragastric tartrazine administation an increase of amorphousness of bone biomineral in rats [4]. However, information on the regeneration of the bones after a long use of tartrazine for food in the available literature is almost absent.

Purpose

To study the ultrastructure of regenerate's biomineral after 60-day of tartazine exposure.

Methods

The 140 white mature male rats (200-210 g) were taken for experiment. 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 rats were distributed into 4 groups:

Group I – control animals which received intragastrically 1 ml of 0.9% isotonic sodium chloride solution for 60 days.

Group II – rats which applied through defect in the proximal end of both tibia after 60-days from the beginning of the experiment.

Group III – animals which received intragastrically 1 ml of tartrazine at the dose of 750 mg/kg/day for 60 days (manufacturer ROHA DYECHEM PVT LTD). The through defect in the proximal end of both tibia was applied after 60-days tartrazine exposure.

Group IV – rats which received intragastrically 1 ml of tartrazine at the dose of 1500 mg/kg/day for 60 days. The through defect in the proximal end of both tibia was applied after 60-days tartrazine exposure.

The dose of tartrazine administrated to rats was calculated according to coefficient of biological stability [7]. The periods of the experiment were 3, 10, 15, 24 and 45 days after the end of tartrazine administration which correspond to traditional stages of bone regeneration [8]. X-ray examination was carry out on DRON-2.0 device with a goniometetric attachment GUR-5. K$\angle$ 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 x-rays diffracted were recorded in the angular range from 2° to 37° with a recording rate of 1° per minute. The sizes of the elementary cells of regenerate's biomineral as well as crystallites and coefficient of microtexturing were determined on diffractograms obtained. The methods of variation statistics were used for processing of data obtained [9].

Main part

The changes of ultrastructure of regenerate's biomineral had two-phase manner in rats of Group II (Fig. 1).

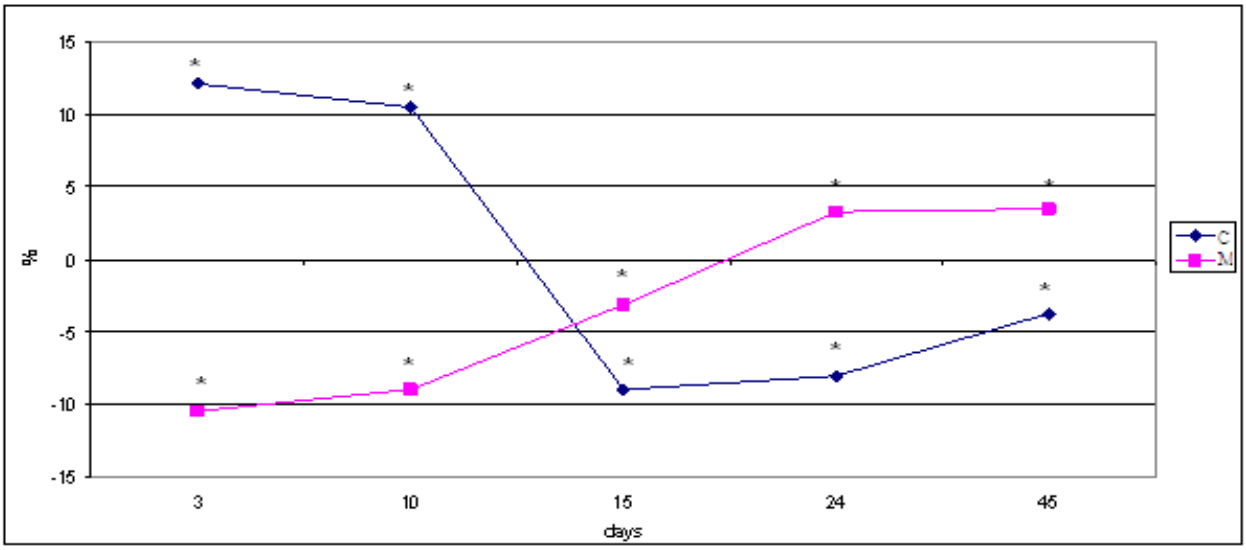

Figure 1. Dynamics of crystallite's sizes (C) and coefficient of microtexturing (M) changes of regenerate's biomineral in Group II compared with same parameters of Group I (in percentages) during experimental period.

Note: * - here and in the following figure shows a significant difference from the parameters of the control group ($p<0.05$).

Due to resorption of bone fragments, the first phase was characterized by the signs of destabilization and destruction of the crystalline lattice of bone biomineral. Thus, the sizes the elementary cells along the axis *a* and sizes of crystallites were larger than similar parameters of Group I by 0.15%, 0.13%, and by 12.08%, 10.45% from 3rd to 10th days of experiment (here and below, all the above differences are reliable, $p \leq 0.05$). The coefficient of mixrotexturing of regenerate's biomineral was less by 10.46%, 9.01%, and 3.12% from 3rd to 15th days of experiment (Table 1).

Table 1:The parameters of the crystalline lattice of regenerate's biomineral of the tibiae (X +/- Sx)

Group	Periods of experiment	Size of elementary cells along the axis *a*, 10^{-10} M	Size of elementary cells along the axis *c*, 10^{-10} M	Size of crystallites, nM	Coefficient of microstructuring, c.u.
I	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
II	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*
III	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^
IV	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*^

Note: * - shows a significant difference from the parameters of Group I ($p<0.05$);
^ - shows a significant difference from the parameters of Group II ($p<0.05$).

The signs of predominance of the growth processes for newly formed elementary cells of the bone biomineral and stabilization of its crystal lattice were observed in the second phase of bone regeneration. Thus, the sizes of the crystallites were less than similar parameters of Group I by 0.12%, 0.13% and 0.10% and by 9.00%, 8.04% and 3.74%, respectively from 15th to 45th days of experiment and sizes of the elementary cells along the axis *c* - by 0.24% and 0.17% from 15th to 24th days. At the same time, the coefficient of microtexturing of regenerate's biomineral was higher by 3.24% and 3.49% from 24th to 45th days of experiment.

The data obtained in rats of the Group II generally correspond those described in the literature and our previous studies [10].

The delay in the development of the crystalline lattice of regenerate's biomineral was observed in the Group III throughout the entire period of experiment. The coefficient of microtexturing of the regenerate's biomineral was less than similar parameter of Group II by 3.49%, 6.24%, 6.50%, 8.19%, and 6.33% from 3rd to 45th days of experiment (Fig. 2).

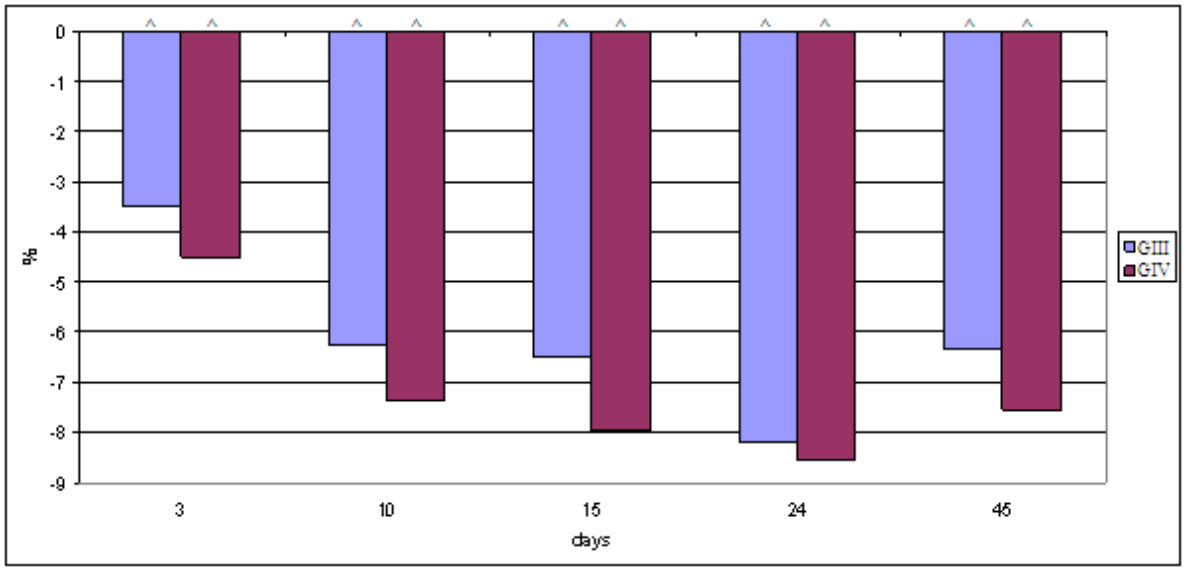

Figure 2. The dynamics of changes of coefficient of microtexturing in the Groups III, IV (GIII, GIV) compared with similar parameter of Group II (in percentages) during experimental period.

At the same time, the sizes of the elementary cells of regenerate's biomineral along the axis *c* were higher than the parameters of Group II by 0.20%, 0.27%, 0.24% and 0.14%, respectively from 10th to 45th days of experiment, the sizes of elementary cell along the axis *a* - by 0.31%, 0.14% and 0.10%, and the sizes of crystallites - by 14.14%, 8.40% and 3.45% from 15th to 45th days.

In the group IV where rats received intragastrically 1 ml of tartrazine at the dose 1500 mg/kg/day for 60 days and then the through defect in the proximal end of both tibia was applied the impairment of crystal lattice development of regenerate's biomineral was observed throughout the entire period of experiment.

The sizes of the elementary cells along the axis *c* of regenerate's biomineral were higher than the similar parameter of Group II by 0.15%, 0.24%, 0.30%, 0.28% and 0.17%, and the coefficient of microtexturing was less by 4.49%, 7.36%, 7.95%, 8.53% and 7.55% from 3rd to 45th days of experiment. Also, the sizes of the elementary cells along axis *a* 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 from 10th to 45th days of experiment.

DISCUSSION:

The results can be explained 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 abovementioned 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 bones.

CONCLUSIONS:

1. The rats of Group II shows biphasic character of changes of the regenerate's biomineral ultrastructure. The 1st phase of bone regeneration process (3rd to 15th days) is accompanied by the signs of destabilization and destruction of the crystalline lattice, probably due to prevalence of resorption bone fragments in the area of defect applied. The signs of stabilization of crystal lattice as well as increase of growth processes of newly formed elementary cells were observed during the 2nd stage of bone regeneration process (15th to 45th days).
2. The animals of Group III-IV which received tartrazine intragastrically for 60 days at the doses 750 and 1500 mg/kg/day and then the through defect was applied in the proximal end of both tibia shows delay in the development of regenerate's biomineral crystalline lattice. The dynamics of changes of parameters of the crystalline lattice had the dose-dependent character.

3. The most pronounced changes of the parameters studied were registered in rats of Groups III-IV up to 15th day after application of defect in the period of prevalence of elementary cells growth and nucleation of crystalline lattice of regenerate's biomineral. The somewhat later (up to 24th day) the maximum changes of coefficient of microtexturing was determined in period of crystallization of newly formed crystalline lattice.

REFERENCES:

1. Buldakov, A.S. Nutritional supplements. Reference book. Sankt-Petersburg. 1996 [in Russian].
2. Golovacheva, V.A., 2012. The influence of food additives on the development of kidney disease among children (clinical and experimental research). Bulletin of medical Internet conferences, 2(1): 7-14 [in Russian]. URL: https://medconfer.com/node/1506.
3. Meyer, S.K., Probert, P.M.E., Lakey, A.F., 2017. Hepatic effects of tartrazine (E 102) after systemic exposure are independent of oestrogen receptor interactions in the mouse. Toxicol Lett., 5(273): 55-68. doi: 10.1016/j.toxlet.2017.03.024.
4. Luk'yantseva, G.V., Luzin, V.I., 2013. The phase composition of the pelvic bone biomineral among white rats after a 60-day intragastric administration of tartrazine at various concentrations. Ukrainian Medical Almanac, 11(3): 85-87 [in Russian]. URL: www.umorpha.inf.ua/UMorphA_2013/UMorphA_2013_3/ru/22.pdf.
5. European convention for the protection of vertebrate animals used for experimental and other scientific purpose: Council of Europe, Strasbourg 1986 URL: https://rm.coe.int/168007a67b
6. Luzin, V.I., Ivchenko, D.V., Pankrat'ev, A.A., 2005. Bone defect modeling among laboratory animals. Ukrainian Medical Almanac. 8(2): 162 [in Russian].
7. Rybolovlev, Y.R., Rybolovlev, R.S., 1979 Dosage of substances for mammals according to the constant of biological activity. Reports of the USSR Academy of Sciences, 247(6): 1513-1516 [in Russian]. URL: medved.kiev.ua/arhiv_mg/st_2003/03_3_15.htm.
8. Ponomarev, V.V. X-ray structural research methods in engineering geology, Nedra, Moskva 1981 [in Russian].
9. Lapach, S.N., Chubenko, A.V., Babich, P.N. Statistical methods in biomedical research using Excel, Morion, Kiev 2000 [in Russian]. https://www.bsmu.by/downloads/kafedri/k_fiziki/2013.../20120917110323vexcel.pdf
10. Luzin, V.I., Vereskun, R.V., Miroshnichenko, P.V. 2013. Age dynamics of bone biomineral ultrastructure among white rats. Modern medicine: topical issues. 9(23): 152-158 [in Russian]. URL: https://elibrary.ru/download/elibrary_20282447_18151528.pdf
11. Visweswaran, B., Krishnamoorthy, G., 2012. Oxidative Stress by Tartrazine in the Testis of Wistar Rats. Journal of Pharmacy and Biological Sciences, 2(3): 44-49 [PubMed].
12. Elhkim, M.O., Heraud F., Bemrah, N., 2007. New considerations regarding the risk assessment on Tartrazine. An update toxicological assessment, intolerance reactions and maximum theoretical daily intake in France. Regul. Toxicol. Pharmacol., 47(3): 308-316 [PubMed].
13. Khayyat, L., Essawy, A., Sorour, J., 2017. Tartrazine induces structural and functional aberrations and genotoxic effects in vivo. Peer J., 23(5): e3041. doi: 10.7717/peerj.3041.