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Research paper

The Evaluation of Effect of 60-Days Sodium Benzoate Administration on Phase Composition of The Tibia' Regenerate by Two-Way Anova

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

[1]Belgorod State University 308015, Russia, Belgorod, Pobeda St. 85
[2]State Establishment LPR "Sent Luke Lugansk State Medical University"91045, People's Republic of Lugansk, 50-letya Oborony Luganska qr. 1g
**Corresponding author E-mail morozov_v@bsu.edu.ru*

Abstract

Currently, sodium benzoate is widely used in the food industry, cosmetology and medicine. The literature provides information about the effect of its introduction on the growth and shaping of bones, the parameters of the crystal lattice of the regenerate formed therein. However, information about the effect of this food additive on the phase composition of the bone regenerate being formed and the degree of this influence is practically absent, which was the purpose of the study. The experiment was conducted on 210 white male rats, divided into 6 groups: 1st - control animals, 2nd and 3rd - rats, daily receiving intragastrically 1 ml of sodium benzoate 500 and 1000 mg/kg/day, respectively for 60 days. The 4th group - rats being inflicted a defect in both tibial bones, and the 5th and 6th - rats being inflicted a defect after the end of the 60-day injection of sodium benzoate. The timing of the experiment after the introduction of sodium benzoate was 3, 10, 15, 24 and 45 days. The area of the forming regenerate of tibial bones was investigated by X-ray diffraction analysis: the percentage content in the mineral of the regenerate of whitlockite, hydroxylapatite and calcite was calculated by the method of internal control. The impact of the introduction of sodium benzoate and the application of the defect on the above indicators was evaluated by two-factor analysis of variance. The results of the study showed that 60-day administration of sodium benzoate leads to an increase in the amorphism of the biomineral of the forming regenerate of tibial bones, the severity and recovery rate of which depends on its administered dose. Thus, the group being inflicted the defect of the tibial bones after the completion of the 60-day administration of sodium benzoate at a dose of 500 mg/kg/day had greater calcite content on day 10 to 45 by 17.88%, 12.88% and 6.63%, and whitlockite - on day 3, 24 and 45 by 14.30%, 4.52% and 6.20%, and hydroxylapatite - less on day 15 to 45 by 3.11%, 3.31% and 2.14%, compared with the defect-inflicted group without the introduction of sodium benzoate. An increase in the administered dose of sodium benzoate up to 1000 mg/kg/day led to aggravation of the identified deviations: the whitlockite content on day 3, 24, and 45 was higher by 16.15%, 6.21% and 9.54%, and the hydroxylapatite content on day 15 to 45 was lower by 3.98%, 4.16% and 3.34%. The conducted two-factor analysis of variance showed that the introduction of sodium benzoate had a significant effect on the change in the content of calcite, whitlockite and hydroxylapatite in the regenerate, the strength and duration of which depends on its administered dose. The maximum power of influence was registered for the increased content of calcite and the decreased content of hydroxylapatite in the mineral of the regenerate on day 45 of the experiment, and for the increased content of whitlockite - on day 3. When a defect was inflicted, the power of influence was maximal for the increased content of calcite and decreased content of hydroxylapatite on day 10, and the increased content of whitlockite - on day 15. In this case, a reverse dose-dependent effect was observed in all periods of the experiment.

Key words: *rats, tibial defect, regenerate's biomineral, sodium benzoate, phase composition, two-way analysis of variance.*

1.Introduction

Sodium benzoate (E211) is widely used in the food industry as a preservative, in the manufacture of a wide range of products and cosmetics [1, 2], as well as in medicine for the treatment of schizophrenia and hepatic encephalopathy [3, 4, 5]. The ability of sodium benzoate to cause damage to the mitochondrial DNA molecule of eukaryotic cells and to initiate the production of reactive oxygen species and, accordingly, free radicals, has been proven [6, 7]. There is also information that the prolonged use of sodium benzoate inhibits the functional activity of the reactive parts of the skeleton bones [8].

Previous studies have shown that intragastric administration of sodium benzoate for 60 days is accompanied by a slower formation of the crystal lattice and destabilization of the regenerate phase composition resulting from the inflicted defect in the proximal metadiaphysis of the tibial bones [9, 10]. However, the available literature provides no information on the quantitative assessment of the extent of the effect of prolonged use of sodium benzoate and the inflicted defect in the tibial bones on the change in the phase composition of the mineral of the regenerate.

2.Objective

To study the dynamics of the phase composition of the tibial regenerate in adult white rats, forming on the background of 60-day administration of sodium benzoate in various concentrations, as well as to assess the degree of influence of controlled factors (introduction of sodium benzoate, the defect of the tibial bones) on the changes in the phase composition of the regenerate.

3.Methods

The study was conducted on 210 white outbred male rats with an initial body weight of 200-210 g. The management of and manipulations with the laboratory animals were carried out in accordance with the "European Convention on the Protection of Vertebrate Animals used for experimental and other scientific purposes" (Strasbourg, 1986) [11].

All animals were divided into into 6 groups: 1st - control animals, 2nd and 3rd - rats, daily receiving intragastrically 1 ml of sodium benzoate 500 and 1000 mg/kg/day, respectively for 60 days (manufacturer Eastman Chemical BV, the Netherlands). The 4th group consisted of rats, which in time, corresponding to the end of the introduction of sodium benzoate in the 2-3rd groups, were inflicted a defect with a diameter of 2.0 mm in the proximal metaphysis of both tibial bones. The rats of the 5-6th groups, on the 1st day after the introduction of sodium benzoate, were inflicted a tibial defect. Calculation of the dose of injected drugs was made subject to the recommendations by Iu.R. and R.S. Rybolovlev [12].

After the end of the experiment (3, 10, 15, 24 and 45 days after the end of the administration of sodium benzoate, which corresponds to the traditionally distinguished stages of the process of reparative bone regeneration [13]), the animals were decapitated under ether anesthesia, the tibial bones were skeletonized, the proximal metaphysis area was separated and examined by X-ray diffraction analysis [14]. The bone powder obtained in an agate mortar was examined on a DRON-2.0 device with a GUR-5 goniometric prefix. The Kα radiation of copper with a wavelength of 0.1542 nM was used; the anode current and power were 30 kV and 20 A, respectively. Diffracted X-rays were recorded in the angular range from 2° to 37° at a recording rate of 1° in 1 minute. The percentage of regenerated amorphous calcium phosphate (whitlockite), crystalline calcium phosphate (hydroxylapatite) and calcium carbonate (calcite) in the mineral was calculated by the internal control method [15].

The obtained digital data was processed by methods of variation statistics using standard application programs [16]. In order to identify the strength of the influence of two controlled factors (the inflicted tibial bone defect and the introduction of sodium benzoate) on the resulting indicators of the phase composition of the regenerate, two-factor analysis of variance was also performed. The square of the correlation ratio (η2) (coefficient of determination) was calculated, which, when multiplied by 100%, showed the power (degree) of influence of the controlled factor on the resultant feature [17].

4.Main part

The 60-day administration of sodium benzoate was accompanied by an increase in the degree of amorphism of bone biomineral, the severity and recovery rate of which depended on the dose of sodium benzoate administered.

In the 2nd group, the content of calcite in the biomineral of the proximal metadiaphysis of the tibial bones on day 3 to 24 of observation was higher than the values of the control group by 5.98%, 4.91%, 5.61% and 3.11%, and the whitlockite content - by 9.53%, 10.20%, 8.17% and 9.07%. At the same time, the proportion of hydroxylaptite was less than in the control by 3.02%, 3.00%, 2.67% and 2.46%.

An increase in the dose of sodium benzoate in the 3rd group led to the aggravation of the identified deviations: from day 3 to 45 of observation, the calcite content remained higher than in the control group by 9.69%, 8.62%, 8.40%, 6.14% and 4.43%, and the content of whitlockite - by 11.98%, 12.05%, 11.03%, 12.02% and 6.60%. At the same time, during the whole period of observation, the proportion of hydroxylapatite was lower by 4.12%, 3.97%, 3.72%, 3.55% and 2.06%.

Defects in the tibial bones, in comparison with the 1st group, were accompanied by an increase in the percentage of calcite in the regenerate from day 3 to 24 of observation, respectively, by 50.73%, 42.71%, 15.45% and 12.30%, and whitlockite from day 10 to 24 - by 7.48 %, 14.26% and 9.07%. Also, from day 3 to 24, the percentage of hydroxyapatite in the regenerate was lower than the values of the 1st group by 7.27%, 7.98%, 5.49% and 3.79%.

On day 45 of observation, the situation changed: the content of calcite and whitlockite was lower than the values of the 1st group by 3.41% and 5.84%, and the content of hydroxylapatite was higher than 1.75%. Such changes are explained by a higher degree of crystallization of the newly formed bone tissue in the area of the inflicted defect.

In the case when the defect was inflicted against the background of the preliminary introduction of sodium benzoate, the phase composition of the regenerate changed towards its increasing amorphism.

In the 5th group, the content of calcite in the regenerate on day 3 of observation was lower than the values of the 4th group by 12.42%, and on day 10 to 24 - by 17.88%, 12.88% and 6.63% higher. At the same time, the content of whitlockite was higher on day 3, 24 and 45 by 14.30%, 4.52% and 6.20%, and the content of hydroxyapatite on day 15 to 45 was lower by 3.11%, 3.31% and 2.14%.

An increase in the dose of sodium benzoate in the 6th group led to the aggravation of the identified deviations: the whitlockite content on day 3, 24 and 45 was higher than in the 4th group by 16.15%, 6.21% and 9.54%, and the hydroxylapatite content on day 15 to 45 was lower by 3.98%, 4.16% and 3.34%. At the same time, the calcite content on day 3 of observation was lower by 13.04%, and on day 15 to 45 exceeded them by 19.49%, 15.40% and 10.58%.

A two-factor analysis of variance showed that intragastric administration of sodium benzoate at a dose of 500 mg/kg/day had a significant effect on changes in the calcite content in the regenerate from day 10 to 45 of observation, while the determination coefficient was 0.007, 0.219, 0.170 and 0.310. An increase in the dose of sodium benzoate up to 1000 mg/kg/day resulted in a significant effect recorded from day 10 to 45 of observation, but its severity was higher (the coefficient of determination was 0.026, 0.288, 0.262 and 0.594).

Introduction of sodium benzoate at a dose of 500 mg/kg/day had a significant effect on reducing the hydroxyapatite content in the regenerate from day 3 to 45 of observation, while the determination coefficient was 0.071, 0.038, 0.199, 0.305 and 0.450 (Fig. 1). An increase in the dose of sodium benzoate resulted in a significant effect of this controlled factor on the change in the content of hydroxylapatite in the regenerate recorded during all periods of observation, but its severity was also higher (the coefficient of determination was 0.138, 0.099, 0.297, 0.452 and 0.722).

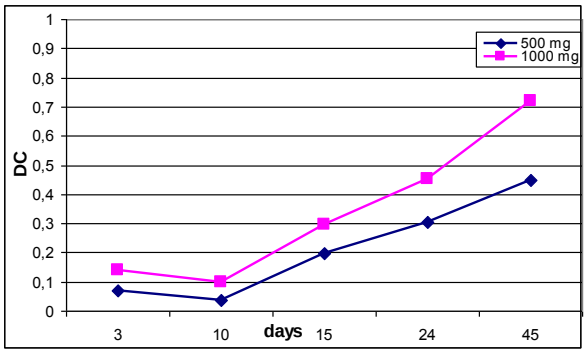

Figure 1. Dynamics of changes in the effect of the introduction of sodium benzoate in various concentrations on the percentage of

hydroxylapatite in the regenerate of tibial bones depending on the observation period.

Note: DC is the determination coefficient; F - the designed value in all cases is higher than F critical.

Finally, the introduction of sodium benzoate at a dose of 500 mg/kg/day had a significant effect on increase in the whitlockite content in the regenerate on day 3, 15, 24 and 45 of observation, while the determination coefficient was 0.834, 0.114, 0.411 и 0.297. An increase in the dose of sodium benzoate resulted in a significant effect of this controlled factor on the change in the content of whitlockite in the regenerate recorded during all periods of observation, and its severity was higher (the coefficient of determination was 0.899, 0.290, 0.238, 0.582 и 0.576).

The infliction of a defect in the tibial bones with the introduction of sodium benzoate at a dose of 500 mg/kg/day had a significant effect on the increase in the content of whitlockite in the regenerate from day 3 to 24 of observation, and the determination coefficient was 0.882, 0.984, 0.696 and 0.728. At the same time, the infliction of a defect with the introduction of sodium benzoate at a dose of 1000 mg/kg/day had a significant effect on the increase in the content of whitlockitein the regenerate also from day 3 to 24 of observation, but its severity was lower (the determination coefficient was 0.835, 0.963, 0.631 and 0.661).

Introduction of sodium benzoate at a dose of 500 mg/kg/day had a significant effect on reducing the hydroxyapatite content in the regenerate from day 3 to 45 of observation, while the determination coefficient was 0.873, 0.882, 0.786, 0.675 and 0.407 (Fig. 2). The infliction of a defect after the introduction of sodium benzoate at a dose of 1000 mg/kg/day led to decrease in the duration and strength of the influence of the controlled factor on the change in the hydroxyapatite content in the regenerate: from day 3 to 24 of observation, the determination coefficient was 0.778, 0.820, 0.687 and 0.523.

Finally, the second controlled factor after the administration of sodium benzoate at a dose of 500 mg/kg/day had a significant effect on the increase in the content of whitlockite from day 15 to 45 of observation, and the coefficient of determination was 0.733, 0.492 and 0.506. The infliction of a defect after the introduction of sodium benzoate at a dose of 1000 mg/kg/day led to decrease in the duration and strength of the influence of the controlled factor on the change in the whitlockite content in the regenerate: from day 15 to 45 of observation, the determination coefficient was 0.632, 0.313 и 0.334.

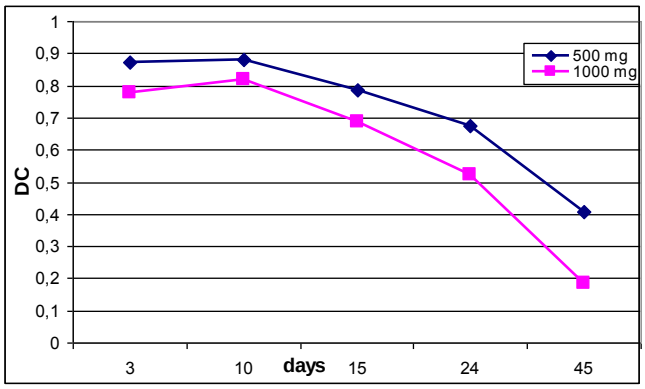

Figure 2. Dynamics of changes in the influence of reparative regeneration processes on the percentage of hydroxylapatite in the regeneration of tibial bones after the introduction of sodium benzoate in various concentrations depending on the period of observation.

Note: DC is the determination coefficient; F - the designed value in all cases is higher than F critical.

Thus, the change in the calcite content in the regeneration of the tibial bones by intragastric administration of sodium benzoate had a significant effect on day 10 to 45 of observation with the maximum degree of influence on day 45 (stage 5 of the regenerate formation). The intragastric administration of sodium benzoate had a significant effect on reducing the hydroxylapatite content in the regenerate from day 3 to 45 of observation with the maximum degree of influence recorded on day 45 (stage 5 of the regenerate formation). The intragastric administration of sodium benzoate had a significant effect on the change in the whitlockite content in the regenerate from day 3 to 45 of observation with the maximum degree of influence on day 3 (stage 1 of regenerate formation). In all cases, a pronounced dose-dependent effect was observed.

The processes of reparative regeneration (infliction of a defect) had a significant effect on the change in the calcite content in the bone regenerate from day 3 to 24 of observation, and the power of influence reached its maximum on day 10 (stage 2 of regenerate formation). The processes of reparative regeneration had a significant effect on the change in the content of hydroxylapatite in the bone regenerate from day 3 to 45 of observation, and the force of influence was also maximum on day 10 (stage 2 of regenerate formation). Finally, the reparative regeneration processes had a significant impact on the change in the content of whitlockite in the regenerate from day 15 to 45 of observation, and the power of the effect was maximum on day 15 (stage 3 of regenerate formation). At the same time, in all cases a reverse dose-dependent effect was observed.

5.Conclusion

The intragastric administration of sodium benzoate for 60 days is accompanied by a slowdown in the formation of tibial regenerate, the severity of which depends on the administration dose. The processes of reparative regeneration have a significant effect on the change in the phase composition of the formed regenerate to a lesser extent than the preliminary introduction of sodium benzoate and with the reverse dose-dependent effect. It also confirms the leading role of the introduction of sodium benzoate in slowing the formation of the phase composition of the tibial regenerate.

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Information about the authors

Krikun Evgeniy Nikolaevich – doctor of medical sciences, full professor of Human anatomy and histology department, Belgorod State University, krikun@bsu.edu.ru

Luzin Vladyslav Igorevich – doctor of medical sciences, full professor of department of Human anatomy, operative surgery and topographical anatomy, State Establishment LPR "Sent Luke Lugansk State Medical University, vlad_luzin@i.ua

Fastova Olga Nikolaevna – candidate of medical sciences, associate professor of department of Human anatomy, operative surgery and topographical anatomy, State Establishment LPR "Sent Luke Lugansk State Medical University, faster-and-faster@mail.ru

Morozov Vitaliy Nikolaevich – candidate of medical sciences, associate professor of Human anatomy and histology department, Belgorod State University, morozov_v@bsu.edu.ru

Morozova Elena Nikolaevna – candidate of medical sciences, associate professor of Human anatomy and histology department, Belgorod State University, morozova_en@bsu.edu.ru