THE EFFECT OF 60-DAY EXPOSURE TO SODIUM BENZOATE ON THE CHEMICAL COMPOSITION OF THE REGENERATE FORMED IN THE TIBIA

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ABSTRACT

Background

Sodium benzoate is a preservative widely used in food and pharmaceutical industries.

There is some evidence of its effect on the morphofunctional condition of metaepiphyseal and condylar cartilages, the chemical composition of bones, and their ultrastructure.

However, there is virtually no data on the chemical composition of the tubular bone regenerate formed therein.

Aim

To study the effect of 60-day exposure to sodium benzoate on the chemical composition of the regenerate formed in the tibia during key stages of reparative osteogenesis.

Material and methods

The experiment was conducted on 90 mature white male rats divided into 3 groups as follows: a control group (60-day administration of saline followed by a tibial fracture modeling), the first and the second experimental groups (sodium benzoate administration at a dose of 500 and 1000 mg/kg respectively instead of normal saline).

The content of water, organic, and mineral matters in the tibial regenerate was determined gravimetrically on days 3, 10, 15, 24, and 45 after the fracture modeling.

Results

On days 3, 10 of the experiment, the first and second experimental groups showed water content decrease in the regenerate compared to the control value by 3.27% ($p=0.073$) and 0.77% ($p=0.383$), 4.18% ($p=0.017$) and 1.60% ($p=0.383$), respectively; a decrease in organic matter by 2.48% ($p=0.383$) and 3.45% ($p=0.128$), 1.04% ($p=0.620$) and 3.08% ($p=0.165$); and an increase in mineral content by 5.03% ($p=0.011$) and 3.24% ($p=0.053$), 5.30% ($p=0.002$) and 3.98% ($p=0.053$).

On days 15, 24, 45, water content increased by 0.52% ($p=0.535$) and 3.59% ($p=0.038$), 7.58% ($p<0.001$) and 11.49% ($p<0.001$), 6.50% ($p=0.017$) and 10.86% ($p<0.001$), while organic matter content, on the contrary, decreased by 4.36% ($p=0.053$) and 6.19% ($p=0.011$), 4.95% ($p=0.038$), 6.92% ($p=0.007$), 2.78% ($p=0.259$) and 4.43% ($p=0.038$).

On day 15, the mineral content remained higher than the control value by 2.69% ($p=0.165$) and 1.04% ($p=0.710$), while on days 24 and 45, it was lower by 2.18% ($p=0.209$) and 3.76% ($p=0.073$), 2.88% ($p=0.165$) and 4.98% ($p=0.025$).

Conclusion

Sixty-day exposure to sodium benzoate affects the chemical composition of the regenerate formed in the tibia at various terms, with the percentage of deviations and the duration of changes depending on the dose of sodium benzoate.

Key words: tibia, bone regenerate, sodium benzoate, chemical composition.

RELEVANCE

Sodium benzoate currently remains one of the most widely used food additives – a preservative to extend the shelf life of food products and facilitate transportation. This is due to its effectiveness in suppressing the activity of fungi and bacteria. Sodium benzoate also finds application in the pharmaceutical industry as a component of medications, cosmetics, and personal hygiene products [1].

At the same time, attention should be paid to emerging scientific data on side effects of this food additive, such as genotoxicity, nephrotoxicity and hepatotoxicity, and sensitization [2, 3].

Therefore, further study of its properties will expand existing knowledge and help develop measures to mitigate its impact on the body. Regarding the skeletal system, there is some evidence of the effect of sodium benzoate at various doses on growth processes, the morphofunctional condition of the metaepiphyseal and condylar cartilage, the chemical composition of bones, and the ultrastructure of their biomineral [4-6].

However, the literature lacks data on the long-term effects of sodium benzoate on similar aspects of the bone under conditions of regenerate formation at various stages of reparative osteogenesis.

AIM

To study the effect of 60-day exposure to sodium benzoate at various doses on the chemical composition of the regenerate formed in the tibia during key stages of reparative osteogenesis.

MATERIAL AND METHODS

90 mature white male rats weighing 200-210 g were divided into 3 groups (see the Table below).

***Table.* Rats Distribution into Groups**

Group Name	Exposure
Control group (30 rats)	In this group, rats received 1 ml saline daily for 60 days via gastric gavage. On day 61, a fracture was modeled at the border of the proximal epiphysis and diaphysis of the tibia by a through perforation according to the method developed by V.I. Luzin et al. (2005) [7].
The first experimental group (30 rats)	Conditions were similar to the control group, but rats received an equivalent amount of sodium benzoate (500 mg/kg body weight).
The second experimental group (30 rats)	In this group, the dose of the administered sodium benzoate was increased to 1000 mg/kg body weight.

The care and handling of animals were conducted in accordance with the guidelines for experimental animal care established by Directive 2010/63/EU of the European Parliament and the Council of the European Union [8].

The animals were mortified with a lethal dose of diethyl ether on days 3, 10, 15, 24, and 45 upon completion of the 60-day administration of sodium benzoate. The regenerate forming in the perforated defect was subjected to chemical analysis during key stages of reparative osteogenesis according to N.A. Korzh and N.V. Dedukh (2006) [9].

The first step of the sample processing was drying in a dry heat oven (105°C, 12 hours), and the second step was ashing in a muffle furnace (450-500°C, 6 hours). The percentage of water, organic, and mineral substances in the bone regenerate was determined gravimetrically [10], that is, by sequential measuring the sample mass before drying, after drying, and after ashing.

The data obtained were imported into the licensed computer program "JASP" (The JASP Team, Amsterdam) for descriptive statistics (calculation of mean values, standard error), checking the data distribution using the Shapiro-Wilk test, and determining the reliability of differences in the parameters under study between groups using the Mann-Whitney U-test. The differences were considered statistically significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

On day 3 of the experiment (corresponding to the inflammation stage of reparative osteogenesis), the first and second experimental groups showed a decrease in the percentage of water in the tibial regenerate compared to the control value by 3.27% (p=0.073) and 0.77% (p=0.383), a decrease in organic matter by 2.48% (p=0.383) and 3.45% (p=0.128), and an increase in mineral content by 5.03% (p=0.011) and 3.24% (p=0.053).

The absolute values of water, organic, and mineral content in the first and second experimental groups were 35.53±0.35% and 36.44±0.16%, 25.76±0.38% and 25.50±0.37%, 38.72±0.40% and 38.06±0.37%, respectively.

On day 10 of the experiment (the stage of cell differentiation and tissue-specific structures formation in the bone defect application area), the trends observed during the inflammation phase persisted. The percentage of water in the tibial regenerate was lower than the control value by 4.18% (p=0.017) and 1.60% (p=0.383), organic matter content was lower by 1.04% (p=0.620) and 3.08% (p=0.165), while mineral content was higher by 5.30% (p=0.002) and 3.98% (p=0.053) in the first and second experimental groups. The absolute values of water, organic, and mineral content in the first and second experimental groups were 37.00±0.41% and 37.99±0.57%, 25.58±0.40% and 25.06±0.33%, 37.42±0.41% and 36.95±0.50%, respectively.

On day 15 of the experiment (the stage of tissue reorganization and mineralization), the trend in the chemical composition of the bone regenerate changed in the second and the third groups: the percentage of water was higher than the control value by 0.52% (p=0.535) and 3.59% (p=0.038), mineral content – by 2.69% (p=0.165) and 1.04% (p=0.710), while organic matter remained lower than the control value by 4.36% (p=0.053) and 6.19% (p=0.011). Absolute values of water, organic, and mineral content in the first and second experimental groups were 36.19±0.47% and 37.29±0.44%, 25.88±0.35% and 25.39±0.40%, 37.93±0.46% and 37.32±0.42%, respectively.

On day 24 of the experiment (the remodeling stage), the second and the third groups showed not only a decrease in the percentage of organic matter in the bone regenerate but also a decrease in mineral content, while the percentage of water continued to increase relative to the control values.

Specifically, the percentage of water was higher than the control values by 7.58% (p<0.001) and 11.49% (p<0.001), the content of organic, mineral matters was lower by 4.95% (p=0.038) and 2.18% (p=0.209), by 6.92% (p=0.007) and 3.76% (p=0.073) respectively. The absolute values of water, organic, and mineral content in the first and second experimental groups were 33.32±0.37% and 34.53±0.65%, 28.93±0.39% and 28.33±0.45%, 37.75±0.29% and 37.14±0.51%, respectively.

On day 45 of the experiment (the outcome stage), the trends observed on day 24 persisted in the first and second experimental groups: the percentage of water was increasing compared to the control value by 6.50% (p=0.017) and 10.86% (p<0.001), the content of organic matter decreased by 2.78% (p=0.259) and 2.88% (p=0.165), and mineral content decreased by 4.43% (p=0.038) and 4.98% (p=0.025). The absolute values of water, organic, and mineral content in the first and second experimental groups were 32.36±0.51% and 33.69±0.38%, 29.33±0.42% and 28.83±0.29%, 38.31±0.45% and 37.48±0.42%, respectively.

Thus, by day 45 of the experiment, the group exposed to sodium benzoate at 1000 mg/kg still exhibited significant changes in all parameters of the chemical composition under study – an increase in water content and a decrease in organic and mineral matter content, unlike the group exposed to the additive at 500 mg/kg.

In the latter group, only a significant increase in water content in the bone regenerate persisted by the outcome stage, indicating that the processes of chemical composition restoration proceed more intensively than in the group exposed to the higher dose of sodium benzoate.

Therefore, the dynamics of changes in the chemical composition of the tibial regenerate at different stages of reparative osteogenesis after exposure to both concentrations of sodium benzoate exhibit a biphasic pattern. In the first phase – on days 3 and 10 of the experiment – the chemical composition of the bone regenerate is characterized by hypohydration, a decrease in organic matter content, and an increase in the mineral content. In the second phase – on days 15, 24, and 45 – the regenerate hypohydration is replaced by hyperhydration, the decrease in organic matter persists, and the trend in mineral content changes, showing a decrease from day 15 to day 45. According to our own data and literature, the opposite pattern is observed in the bone regenerate of intact rats: in the early stages of reparative osteogenesis, up to day 15, water content increases while organic and mineral matter decrease, and in later stages – after day 15, the degree of hyperhydration decreases, and the content of organic and mineral matter proportionally increases [11].

This indicates that the 60-day exposure to sodium benzoate prior to modeling a tibial fracture affects its morphogenesis in mature rats, which subsequently manifests as impaired osteoid synthesis, its mineralization, and chemical composition at various stages of reparative osteogenesis.

This may be due to direct effect of sodium benzoate on the genetic material of cells involved in bone remodeling during prolonged exposure, as well as its ability to induce oxidative stress in cells by triggering the formation of reactive oxygen species [12, 13].

CONCLUSION

Sixty-day exposure to sodium benzoate is accompanied by changes in the chemical composition of the regenerate formed in the tibia at different stages after surgery. In the early stages of reparative osteogenesis (days 3 and 10), this manifests as hypohydration, a decrease in organic matter content, and an increase in mineral content, while in later stages (days 15, 24, and 45), it manifests as hyperhydration, a decrease in organic and mineral matter content.

The percentage of deviations and their duration depend on the dose of sodium benzoate.

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