ORGANOMETRIC PARAMETERS OF THE TIBIA DURING THE FORMATION OF REGENERATE IN IT AFTER 60-DAY EXPOSURE TO TARTRAZINE

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ABSTRACT

ORGANOMETRIC PARAMETERS OF THE TIBIA DURING THE FORMATION OF REGENERATE IN IT AFTER 60-DAY EXPOSURE TO TARTRAZINE

Background

Tartrazine is a food additive that is widely used in the food, textile, and pharmaceutical industries due to its persistent coloring properties. The potential toxic effects of its metabolites on cells in various tissues and organs have been described, but information about changes in bone structure and organization during the process of regenerative osteogenesis under the influence of tartrazine remains unclear.

The aim of this study is to investigate changes in organometric parameters of the tibia during reparative osteogenesis after 60 days of tartrazine administration.

Material and methods

Sixty male rats weighing 200-210 grams were used and divided into two groups. A control group received saline solution via gavage for 60 days. An experimental group was exposed to tartrazine at an equivalent volume and dose of 750 mg/kg body weight over the same period. On day 61 surgery was performed to perforate the proximal metaphysis of the tibia for both groups. Animals were withdrawn from the experiment at 63, 70, 75, 84, and 105 days after surgery. The tibias were examined macroscopically for linear dimensions, absolute mass, and Simon index calculation.

Results

On days 63 and 70 of the experiment, the experimental group showed a decrease in the absolute mass of the tibia compared to the control value by 7,30% and 8,45%, the maximum length by 6,16% and 7,17%, the width of the proximal epiphysis by 6,13% and 5,68%, the width of the diaphysis by 5,94% and 14,75%, and the thickness of the diaphysis by 8,14% and 9,85%, the width of the distal epiphysis by 4,76% and 2,78%, and the Simon index by 3,76% and 5,42%. On days 75, 84, 105, the values of the organometric parameters of the bone continued to lag behind the control ones, but to a lesser extent than in previous periods. The absolute mass was in the range of 8,06%-8,15%, the maximum length – 7,70%-2,82%, the width of the proximal epiphysis – 4,46%-2,62%, the width of the diaphysis – 9,00%-6,51%, the thickness of the diaphysis – 8,52%-4,12%, the width of the distal epiphysis – 1,52%-2,48%.

Conclusion

Exposure to tartrazine at a dose of 750 mg/kg body weight for 60 days is accompanied by an inhibition of the rate of longitudinal, appositional growth, and weight gain of the tibia at various times of its regenerate formation. In the early stages (days 3, 10), the percentage of deviations of organometric parameters from the control is the highest, and from 15 to 45 days the severity of their changes gradually decreases.

Key words: long bone, fracture, bone healing, tartrazine, organometry.

REVELANCE

At present, human food not only provides nutritive and energy-rich materials. for our bodies, but also contains substances that lack nutritional value and are artificially added to our food. These substances are known as food additives. One of the most widely used food additives in the food and pharmaceutical industries is synthetic yellow azo dye tartrazine (E102). Its persistent coloring properties make it ideal for use in the production of confectionery, carbonated beverages, condiments, and drug coatings [2].

The scientific literature has described potential toxic effects from tartrazine metabolites, as they stimulate the production of reactive oxygen species that cause oxidative stress and disrupt biochemical processes in cells. This can lead to damage to various tissues and organs [3].

Data have been collected on the effects of long-term exposure to tartrazine on the histostructure, chemical properties, and biomechanical characteristics of the skeletal system [4, 5, 6, 7].

However, issues related to the morphofunctional organization of long tubular bones during the process of reparative osteogenesis following prolonged exposure to tartrazine have not been extensively studied.

AIM

To study the features of changes in the organometric parameters of the tibia during the course of reparative osteogenesis following 60-day administration of tartrazine.

MATERIAL AND METHODS

The study was carried out on sixty white mature male rats weighing 200–210 g. From the first thirty animals, a control group was formed, which received 1 ml of saline solution intragastrically for 60 days. On day 61, the animals underwent a fracture of the proximal metaphysis of the tibia by means of a through-hole perforation uncomplicated by a transverse fracture and maintaining support on the limb [8].

The animals were removed from the experiment by overdose of anesthesia with diethyl ether on the 63rd, 70th, 75th, 84th, and 105th days after surgery, which corresponds in time to the key stages of osteogenesis [9].

From the following thirty rats, a group TZ750+Def was formed, in which the animals were exposed to tartrazine (manufacturer Roha Dyechem Pvt Ltd, India) at a dose of 750 mg/kg body weight. The animals were kept in a vivarium and manipulated in accordance with established regulations [10].

The tibiae were skeletonized and macroscopically examined. The absolute mass of the tibia was determined on a WT-1000 torsion scale. The linear dimensions of its epiphyses and diaphysis were determined using an electronic caliper (accuracy up to 0,01 mm). Additionally, the Simon index, as an integral indicator of the macroscopic examination of a bone organ that characterizes the rate of longitudinal bone growth in relation to its weight gain, was calculated. The Simon index was calculated as the ratio of bone length to the cube root of mass. The measurement results were entered into the JASP computer program ("The JASP Team", Amsterdam), where the "Descriptive Statistics" function was used to calculate the mean values of the parameters, the standard error, and the Shapiro-Wilk test [11]. Using the function of the program "Classical analysis. T-test for independent samples (Mann-Whitney)", data from the control group and the TZ750+Def groups were compared. The threshold value of the confidence interval was set at at least 95%.

RESULTS AND THEIR DISCUSSION

In the early follow-up periods (days 63 and 70) in the TZ750+Def group, the absolute mass of the tibia was 475,43±8,66 and 465,71±7,11 mg, the maximum length was 36,34±0,36 and 36,46±0,41 mm, the width of the proximal epiphysis was 6,56±0,08 and 6,64±0,06 mm, the width of the diaphysis was 2,71±0,04 and 2,64±0,04 mm, the thickness of the diaphysis was 3,39±0,05 and 3,40±0,06 mm, the width of the distal epiphysis was 5,46±0,06 and 5,50±0,06 mm, and the Simon index was 4,66±0,04 and 4,70±0,04 conventional units. The comparison of the established organometric parameters of the tibia with those in the control group during the same follow-up period showed that absolute mass decreased by 7,30% and 8,45%, maximum length – by 6,16% and 7,17%, the width of the proximal epiphysis – by 6,13% and 5,68%, the width of the diaphysis – by 5,94% and 14,75%, the thickness of the diaphysis – by 8,14% and 9,85%, the width of the distal epiphysis – by 4,76% and 2,78%, and the Simon index – by 3,76% and 5,42% ($p<0,05$ for all cases, except for the width of the distal epiphysis on day 10 ($p>0,05$)).

From this, it can be concluded that the TZ750+Def group, in comparison with the control group, slows down both longitudinal and oppositional growth in the tibia, as well as bone mass gain in the early stages of bone healing.

On days 75 and 84 of follow-up in the TZ750+Def group, absolute mass was 472,29±7,63 and 474,71±7,81 mg, maximum length was 36,99±0,31 and 37,71±0,36 mm, the width of the proximal epiphysis was 6,73±0,09 and 6,83±0,07 mm, the width of the diaphysis was 2,74±0,04 and 2,83±0,04 mm, the thickness of the diaphysis was 3,53±0,04 and 3,60±0,05 mm, the width of the distal epiphysis was 5,56±0,07 and 5,59±0,08 mm, Simon index was 4,75±0,03 and 4,84±0,05 conventional units. In percentage terms, this was lower by 8,06% and 7,87%, 7,70% and 5,85% ($p<0,05$ for all cases), 4,46% ($p<0,05$) and 2,45% ($p>0,05$), 9,00% and 7,48%, 8,52% and 8,36% ($p<0,05$ for all cases), 1,52% ($p>0,05$) and 1,26% ($p>0,05$), 5,08% ($p<0,05$) and 3,25% ($p>0,05$) respectively, compared to the control values.

Based on the results at these follow-up periods, it can be concluded that the dynamics of slowing down the longitudinal and appositional growth and loss of tibial mass in rats in the TZ750+Def group remain, but it becomes less pronounced than in the early follow-up periods.

On day 105, in the TZ750+Def group, absolute mass was 491,29±9,99 mg, maximum length was 38,91±0,38 mm, the width of the proximal epiphysis was 6,91±0,08 mm, the width of the diaphysis was 2,87±0,04 mm, the thickness of the diaphysis was 3,66±0,07 mm, the width of the distal epiphysis was 5,61±0,06 mm, and the Simon index was 4,93±0,04 conventional units. In percentage terms, relative to the control, it was lower by 8,15% ($p<0,05$), 2,82%, 2,62%, 6,51%, 4,12%, and 2,48% ($p>0,05$ for all cases, except for the width of the diaphysis ($p<0,05$)). The Simon index corresponded to the value of the control group.

Thus, by the last studied period of bone healing (day 45 after the surgery), the values of most tibial organometric parameters approached the control values. This indicates the reversible nature of changes in the tibia after 60-day administration of tartrazine at a dose of 750 mg/kg body weight.

According to the literature data, mature animals underwent a fracture of the proximal metaphysis of the tibia the values of the parameters characterizing longitudinal growth of the whole tibia, as well as the longitudinal and appositional growth of its diaphysis were statistically significantly higher than those in intact rats during the bone healing process.

The width of the tibial epiphyses did not significantly lag behind the data of intact rats. This is due to increased blood circulation in the area of surgery, the active intake of systemic and local factors and, as a result, the activation of the function of the metaepiphyseal cartilage, as well as the reactivity of the periosteum to damage [12].

In the works of G.V. Lukyantseva and V.I. Luzin (2013, 2014), it was shown that two-month exposure to tartrazine causes a violation of the growth processes of various types of bones – tubular, spongy and mixed [13], a decrease in the morphofunctional activity of metaepiphyseal cartilage [14].

These effects of tartrazine on the bone system are explained by its ability to cause imbalance in the oxidant-antioxidant system by initiating the production of reactive oxygen species by its metabolites, which have a damaging effect on the genetic material of cells, the lipid bilayer of cell membranes, as well as its complexing properties when broken down in the intestine (zinc binding) [15, 16].

CONCLUSION

Exposure to tartrazine at a dose of 750 mg/kg body weight for 60 days is accompanied by an inhibition of the rate of longitudinal and appositional growth, and weight gain of the tibia at various times during its regenerate formation.

In the early stages (days 3, 10), the percentage of deviations of organometric parameters from the control is the highest, and from 15 to 45 days the severity of their changes gradually decreases.

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