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POSSIBILITIES FOR ASSESSING STIMULATION OF BONE TISSUE REGENERATION IN THE BACKGROUND OF EXPERIMENTAL STRESS

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ВОЗМОЖНОСТИ ОЦЕНКИ СТИМУЛЯЦИИ РЕГЕНЕРАЦИИ КОСТНОЙ ТКАНИ НА ФОНЕ ЭКСПЕРИМЕНТАЛЬНОГО СТРЕССА

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The results of an X-ray assessment of the dynamics of bone regeneration of postoperative defects in an experiment on rats using a stress-inducing device to form non-physiological occlusion of the jaws are presented. More effective and complete restoration of the alveolar ridge after tooth extraction was shown in animals treated intraperitoneally with ethylmethylhydroxypyridine succinate under chronic stress conditions. X-ray analysis of the bone regeneration confirmed the stimulation of bone healing by the drug in terms of 30 and 60 days in the form of filling the defect with trabecular bone, which, in terms of quantitative and qualitative composition, can provide the function of a scaffold for newly formed vessels and nerves.

Keywords: radiography, experiment, osseointegration, chronic stress

Представлены результаты рентгенологической оценки динамики костной регенерации послеоперационных дефектов в эксперименте на крысах с использованием стресс-индуцирующего устройства для формирования нефизиологической окклюзии челюстей. Показано более эффективное и полноценное восстановление альвеолярного гребня после удаления зубов у животных, получавших внутривенно этилметилгидроксипиридина сукцинат в условиях хронического стресса. Рентгенологический анализ костного регенерата подтвердил стимуляцию препаратом заживления кости в сроки 30 и 60 суток в виде заполнения дефекта трабекулярной костью, способной по количественному и качественному составу обеспечить функцию каркаса для новообразованных сосудов и нервов.

Ключевые слова: рентгенография, эксперимент, остеointеграция, хронический стресс

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SID – stress-inducing device

The emergence of new imaging techniques in clinical and experimental practice has significantly expanded the possibilities for studying the mechanisms of osseointegration [1, 2]. At the

same time, the possibilities of reparative bone formation of postoperative jaw defects, including modeling of bone loss processes under conditions of chronic stress or osteoporosis, are far from being

exhausted [3, 4]. Considering the current practice of using implant treatment in conditions of increasing life expectancy of the population, the comorbid component and financial capabilities of patients, it seems essential to comprehensively study the effectiveness of osseointegrating processes when using various options for metabolic therapy [5, 6].

The study aimed to evaluate radiographic stimulation of the metabolic stimulation of bone regeneration of postoperative defects after tooth extraction in animals under conditions of experimental chronic stress.

Material and Methods. The experiment included 78 mature laboratory rats weighing 180–200 g, in 60 of which the author's stress-inducing device (SID) (patent RU 182498 U1, August 21, 2018) was used for 30 consecutive days, which is a kappa for the formation of non-physiological occlusion of the jaws. All animals are divided into three groups. The first group consisted of animals of the comparison group without a stress-inducing device (terms of withdrawal from the experiment here and below, in all groups are the same – 15, 30, 60 and 90 days, $n=18$); the second (control) group – animals with a (SID), which were injected daily with saline intraperitoneally, $n=40$; the third (main) group – animals with SID, which were daily intraperitoneally injected with ethylmethylhydroxypyridine succinate (Mexidol (Pharmasoft, Russia), 50 mg/kg of body weight), $n=20$. During the experiment, all rats were under standardized conditions with a standard diet, a natural light/dark cycle (with a light duration of at least 16 hours) and had free access to water and food at a room temperature of 22–23 °C.

X-ray studies were performed immediately after surgery to remove the first molar. Then, after 15, 30, 60 and 90 days, the X-ray picture of the complete healing of the postoperative defect was obtained using the PLANMECA 5000 X-ray machine (DIO RT, USA) with different modes and exposure times. The intensity of osteogenesis was studied by assessing the average volume of newly formed bone expressed in mm^3 and bone mineral density (mg/cm^3) at the defect site [7].

The choice of the extraoral method of taking pictures is because it is quite difficult to perform intraoral radiography of the area under study in small rodents and, most importantly, it does not make it possible to observe the entire area of the postoperative defect, as well as the surrounding areas of the jaw.

All digital data are presented as arithmetic mean and standard deviation. Changes in parameters between the main group of animals treated with intraperitoneal ethylmethylhydroxypyridine succinate and the control group were assessed at each time point (15, 30, 60 and 90 days) using an unpaired Student's t-test. Statistical analysis of the measurement results was conducted using STATISTICA 10.0 (StatSoft Inc., USA). A $p<0.05$ value was considered statistically significant.

Results and Discussion. In studying the data obtained, it can be seen that the beginning of reparative changes in wounds in the control group occurs somewhat later than in animals in the main group (where succinate ethylmethylhydroxypyridine was injected intraperitoneally) and the comparative group. So, if the initial radiographic signs of bone tissue regeneration in the outlets of the torn teeth in the main group of animals appeared on the 15th day in 35 % of cases and after 30 days in 47.5 % of cases, then in the second group of animals these traits were determined on 15th day is only 22.8 %, day 30 is 11.4 %, and day 60 is still 10.2 %. In other control animals with SID, these signs appeared later (90 days later).

The X-rays taken immediately after the operation, in the area corresponding to the vestibular wall of the alveolus and the tooth extracted, clearly show a defect of the marginal bone. The contours of this defect are sometimes limited to a compact plate visible on the radio. When compact lamina breaks down due to postoperative inflammation or during surgery, the outlines of the defect seem uneven but clearly defined. In the future, as the bone edge resorption in comparative group animals, the defect contour is smoothed out. By the end of the first month after the radiograph operation, it always looks even.

By the 15th day of the experiment, in all groups, against the background of a bone defect, the inner (lingual) wall of the socket of the extracted tooth is visible (Fig. 1A). By the 30th day after the operation, in most cases, the process of bone rarefaction is determined on radiographs (Fig. 1B). However, in the control group with SID, the alveolar wall appears to be relatively less dense than in the main group. Their osteopenia has a pronounced small-focal character. By the end of the 60th day after the operation, the areas of the newly formed bone tissue in the main group become more pronounced (Fig. 1C), and the rarefaction foci decrease and completely disappear on the 90th day (Fig. 1D).

Radiologically, the beginning of the formation of reparative osteoid tissue has not been determined. The first signs of activation of the post-operative bone defect replacement process were determined radiologically only 15 days after the operation. Among the first two signs of reparative bone formation detected by the end of the 30th day in 25 % of third-group animals and 15.8 % of control group animals by the 60th day, a decrease in the clarity of the bone defect boundaries should be distinguished (54.6 % of cases) and the appearance of a thin shading strip along the edge of the defect (45.4 % of cases). At the same time, osteoidal shadow develops most intensively on the side of the rear and lower edges of the fault. As osteoid tissue grows and matures, a bone structure appears in the form of a thin network of bone bundles, first detected only at the periphery of the defect. Subsequent X-ray studies reveal how the thin structure of the sponge fills the edge of the defect. However, the skeletal pattern is also amplified in the center due to reparative bone formation from the preserved inner (linguistic) wall of the alveolus.

It should be noted that in the main group, at the end of the 30th day after the beginning of the experiment, the edges of the defect on radiographs appeared to thicken; by the 60th day, this type of thickening increased in the form of a linear shadow bordering the defect contour. The width of the sclerosis band gradually increased (up to 30–60 days after surgery) and then changed and progressively became thinner and disappeared. A similar process in the control group proceeded for a longer time, and by the end of the 90th day, the bone defect was not filled with bone tissue; on radiographs, a narrow strip of sclerosis is still visible against the background of an unchanged bone pattern in the adjacent areas.

Analyzing the ratio of the average volume of newly formed bone (mm^3) and mineral bone density (mg/cm^3) [7] in the control and main groups 15, 30, 60 and 90 days after surgery, it was found that in the main group regeneration increases the volume and mineral density of the newly formed bone (Fig. 2) more than in the control groups, acquiring the most considerable statistically significant differences ($p<0.05$) by the 30th and 60th days (by the volume of newly formed bone) and by the 90th day (by mineral density of regeneration).

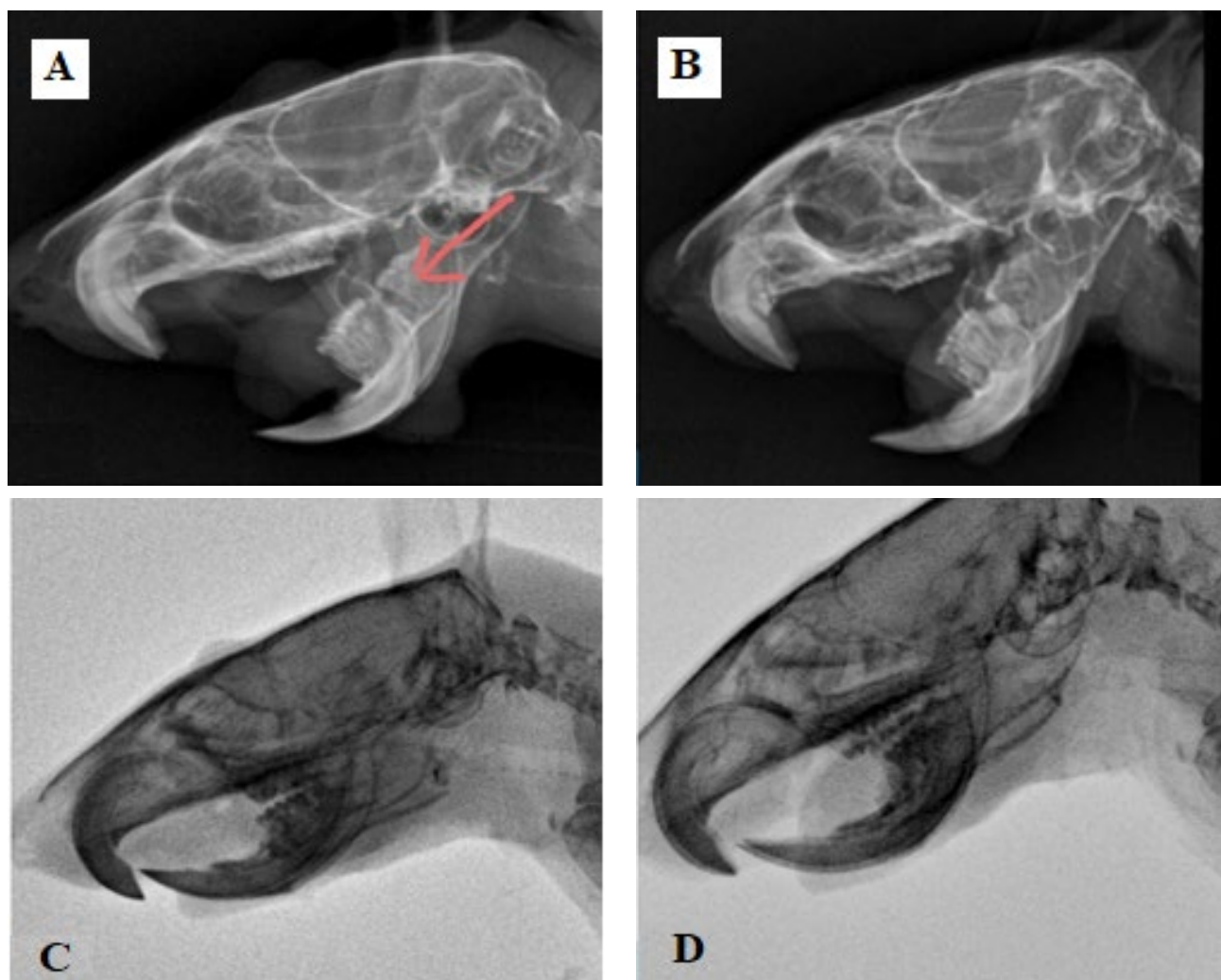


Fig. 1. Changes in the X-ray examination of the processes of bone tissue regeneration in the socket of the removed molar

The fate of the cortical plate of the rosette walls, preserved during the operation, is different. Most often, its complete restructuring was observed during the process of reparative bone formation, which was confirmed radiologically by the disappearance of the shadow. In these cases, an uneven thinning was observed 15 and 30 days after the X-ray operation, followed by fragmentation of the cortical plate and its gradual disappearance. Some

fragments in the form of linear shadows were visualized on the 90th day, but most often, the restructuring process took place within two months of the first signs of these changes. In other cases, the compact well plate was not restored.

In both control and primary groups, with full healing of the postoperative defects by the 90th day, in most cases, the altitude of the alveolar process did not decrease,

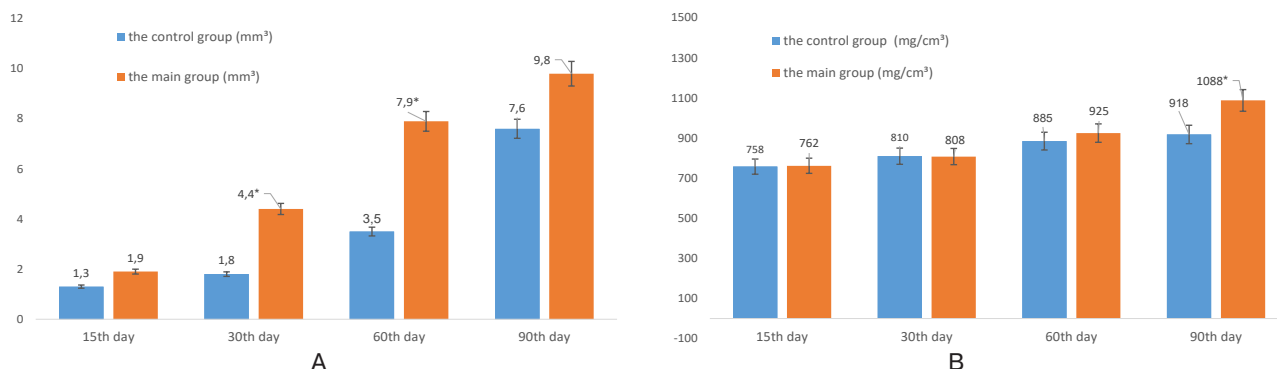


Fig. 2. The ratio of the average volume of newly formed bone tissue (A, mm³) and the average values of the mineral density of the newly formed bone tissue (B, mg/cm³) at different periods of observation in the control (second) group and the main (third) group. * – differences between groups are statistically significant, p<0.05

only in 15 % of the control group after healing, a marginal bone defect due to the resorption of the top of the intact orifice wall. At the same time, the cortical plate of the nest of the adjacent intact tooth (the second molar) began to move from the tooth neck to the healing defect, forming a funnel-shaped extension of the perica line. Externally, this extension resembled a pathological bone pocket in generalized periodontitis [8, 9]. However, neither radiographic nor clinical signs of periodontitis were observed, and the described changes could occur only as a result of a decrease in the volume of the newly formed bone in a certain period of its maturation.

Conclusions. Thus, the X-ray pattern of bone regeneration of postoperative defects in the SID experiment indicates a more effective and complete recovery of the alveolar spine after tooth extraction in animals, intraperitoneal succinate with ethylmethylhydroxypyridine. X-ray analysis of bone regeneration confirmed complete bone

healing within 30 and 60 days, filling of trabecular bone defect, quantitative (in mm³) and qualitative (mg/cm³) composition to provide forest function for newly formed vessels and nerves antihypoxic membrane protection activity [5, 6]. In the control groups, the wells were filled with pores and microspecies with fewer trabecular bones capable of supporting angiogenesis and neogenesis much later, only on the 90th day of observation.

Informed consent: the study was conducted in full compliance with the requirements of the Helsinki Declaration of the World Medical Association (1964), the «International Recommendations for Conducting Biomedical Research using Animals» (1985), the Rules of Laboratory Practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 267 of 19.06.2003) and the positive conclusion of the local ethics committee.

Disclosures: The authors declare no conflict of interest.

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