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Influence of Transcranial Electrostimulation on the Osseointegration of Dental Implant in the Experiment.

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ABSTRACT

Ratio of nervous, endocrine and immune mechanisms regulating bone formation realized by several biologically active mediators is essential for an adequate bone regeneration in diseases of the maxillofacial area, traumatic lesions, and implantation. They provide regulation in time and volume of growth regenerate and differentiation of osteoblastic, osteoclastic, vascular and connective germs forming eventually a functioning bone. Therefore, a drug-free effect on the opioidergic brain structures is of great interest. A method of transcranial electrostimulation (TES), which is carried out with a weak current of special characteristics through electrodes placed on the scalp, has these features. Transcranial electrostimulation in the therapeutic regimen has reparative, immunomodulatory and vegetotropic effects, which are implemented with the participation of opioid mechanisms.
Keywords: Osseointegration, transcranial electrostimulation, implant.

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INTRODUCTION

Osseointegration is a biological phenomenon, indicating fusion of nonliving (metallic) element with living tissue. Bранемарк defined the osseointegration as "obvious direct connection or attachment of living bone tissue to the implant surface without introducing a layer of connective tissue”.

Bone has a strong potential for regeneration, however, the natural healing process not always leads to the complete restoration of anatomic integrity and functionality of bone tissue [3, 11]. Ratio of nervous, endocrine and immune mechanisms regulating bone formation realized by several biologically active mediators, such as beta-endorphin, serotonin and others, is essential for an adequate bone regeneration in diseases of the maxillofacial area, traumatic lesions, and implantation [1, 2, 3, 7]. They provide regulation in time and volume of growth regenerate and differentiation of osteoblastic, osteoclastic, vascular and connective germs forming eventually a functioning bone [7-9].

Given that biologically active substances have homeostatic effect and influence on the regulation of various physiological functions, including reparative regeneration of tissues damaged, a drug-free effect on the opioidergic brain structures is of great interest.

A method of transcranial electrostimulation (TES), which is carried out with a weak current of special characteristics through electrodes placed on the scalp, has these features. The method was developed at the Pavlov Institute of Physiology RAS in the laboratory of physical methods of anesthesia under the supervision of the State Prize winner Professor Dr. V.P. Lebedev [4, 5]. Many authors revealed in their works central analgesic and peripheral effects of TPP [2, 5, 6], therefore, we have concluded that the occurrence of transcranial electroanalgesia involves the related opioids, serotonergic and cholinergic mechanisms. Transcranial electrostimulation in the therapeutic regimen has reparative, immunomodulatory and vegetotropic effects, which are implemented with the participation of opioid mechanisms.

To study the effect of transcranial electrostimulation on the process of osseointegration of dental implants we have elaborated a scheme of surgery on laboratory animals.

Technique

The study was conducted in the pathology modeling laboratory of Volgograd Medical Science Center. The experiment was performed on 60 white rats - male weighing 250-300 g, kept in vivarium conditions (t ° = 22-24 C °, relative humidity 40-50%), with a natural mode and a standard diet, observing the rules of good laboratory practice when conducting preclinical studies in the Russian Federation and the rules of humane treatment of animals [6, 10], as well as the rules of the international recommendations of the European Convention for the protection of Vertebrate Animals used for experimental studies. Experiments were approved by the ethical review committee.

All animals were divided into 2 groups:

- Main group included animals treated with inclusion of TES therapy in the postoperative period during implantation.
- Control group included animals treated without TES therapy.

All manipulations were performed under general anesthesia in a sterile operating conditions after treating the surgical field. Anesthesia was carried out by administering of 0.1% atropine into the left gastrocnemius muscle in an amount corresponding to the weight of the animal (according to manufacturer's instructions). Then, "Zolitil 100" at a dose of 8 mg/kg was administered into the same muscle (according to manufacturer's instructions). The animal was fixed immediately after ceasing to respond to stimulation. 1.5 cm length skin and subcutaneous fat incision was made on the anterior surface of the left thigh. Edges of the wound were mobilized, muscle layer and the periosteum were longitudinally dissected, anterior surface of the femoral was dissected in the diaphysis zone (part of the metaphysis). Using a drill, a cortical plate and cancellous bone were sawn 8 mm in depth and 0.8 mm in diameter with ball-shaped burr. A sterile specimen 8 mm long, 0.8 mm diameter was implanted into the resulting bone cavity. The wound was sutured in layers with interrupted sutures, completely hiding the implant. Skin wound was sutured with interrupted sutures polyglycolide 4/0. Hemostasis was produced during the operation. Radiographic studies were used as a control technique.

Animals were taken out of the experiment in terms of 14, 30 and 90 days by overdosing with ether anesthesia. Femoral bones were sampled during the autopsy, as well as implantation area was cut out.

Histological processing. Tissue samples were fixed in 10% neutral formalin, decalcification was performed in 25% Trilone B. They further were processed with high-proof alcohols and embedded into paraffin. 5-6 microns thick sections were prepared on a HM 355S rotary microtome of Microm company, and stained with hematoxylin and eosin. Histological preparations were photographed with a digital camera Canon (Japan, 5.0 megapixels) based on Axiostar plus microscope (Carl Zeiss, Germany) using a x50, X100, X400 objective and x10 eyepiece.

Working with the preparations the state of tissue structures around the implant was studied according to the following criteria:

Assessment of the connective tissue layer between the implant and the bone:

- Very broad, high cellular, including admixuters of lympho-macrophage elements and angiomatosis - "-5";
- Medium width with moderate number of cells, mainly fibroblasts, collagen fibers form bundles with a moderately developed fibrillar structures, dissociation areas are presented - "-4";
- Connective tissue layer of medium width, has a coarse fiber structure, low content of cellular elements, constitutes mature fibroblasts - "-3";
- Narrow coarse-tissue layer — “-2”;

- Bundle of elongated connective tissue cells - "-1".
- Connective tissue layer is absent — “+1”.

Assessment of maternal bone structure

- Signs of necrobiosis and necrosis of osteocytes of the marginal zone - "-1";
- No signs of necrobiosis and necrosis of osteocytes of the marginal zone - "+1";
- Sharply expressed rarefication of the maternal bone - "-3";
- Moderately expressed rarefication of the maternal bone - "-2";
- Mildly expressed rarefication of the maternal bone - “-1";
- No rarefication of the maternal bone - "+3";

Assessment of newly formed bone structure

- No new bone formation — 0;
- The predominant formation of osteoid bone tissue - “+1";
- The predominant formation of trabecular bone tissue with fibrous matrix “+2";
- Intensive new bone formation - “+3";
- Compaction of the newly formed bone substance - “+4."

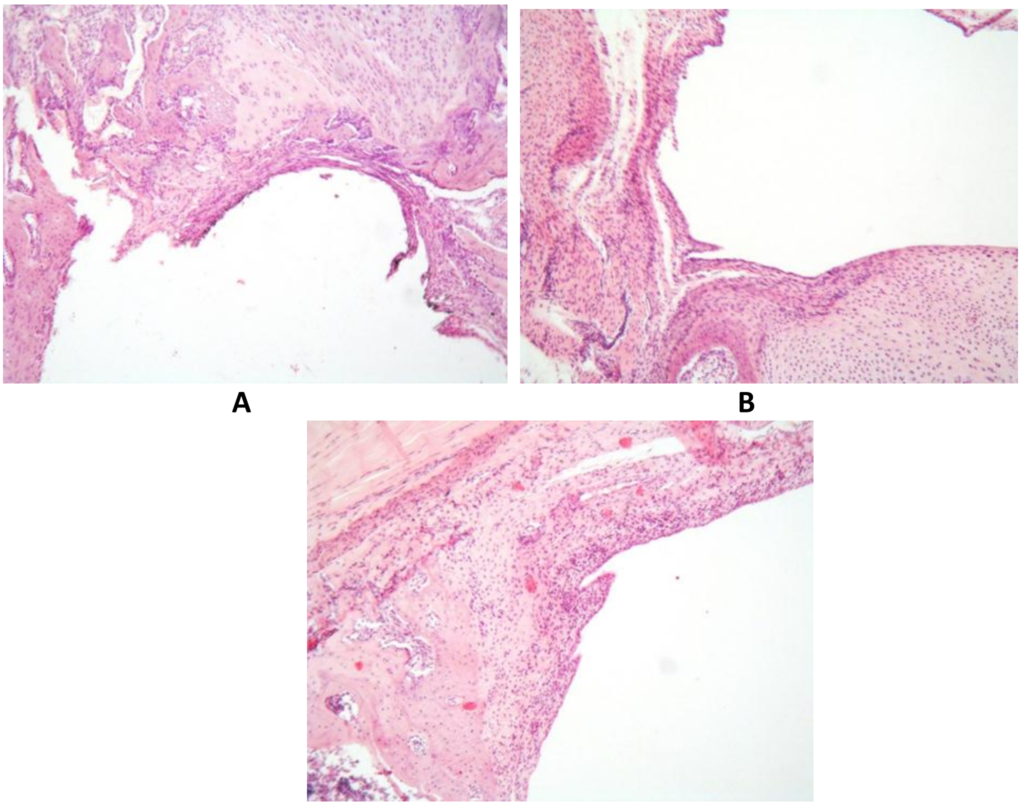
Total 240 radiographs, 264 photos and 70 histological preparations were analyzed during the whole period.

Main Body

Microscopic examination in the first experimental group after 2 weeks reveaked the formation of acellular zone around the titanium implants that separated the implant from zone expressed bone tissue remodeling. Gaps of the osteoclastomic resorption were determined in some of bone trabeculae, as well as newly formed bone and osteoid beams were visualized. Newly formed loose connective tissue and cartilage were located along the perimeter of the implant. The volume ratio of bone, cartilage and connective tissue was 25.1 ± 2.8%, 15.7 ± 4.2% and 59.2 ± 2.3%, respectively (Fig. 1, A).

Histologically, the formation of predominantly coarse fiber and connective hyaline cartilage with the presence of individual sections of the newly formed cancellous bone was noted around titanium implants behind the acellular tissue layer by early fourth week (Fig. 1, B). The volume ratio of bone, cartilage and connective tissue was 38.6±4.1%, 15.3±2.9% and 46.1±4.9%, respectively.

When studying the implantation zone, an acellular zone was slightly determined on the 12th week of experiment, the amount of bone tissue increased, the volume ratio of connective tissue and cartilage decreased (Fig. 1, B).



A B

C

Figure 1: Group 1. Overgrowth of fibrous connective tissue and cartilage around the titanium implant. A - 2 weeks. B - 4 weeks. C - 12 weeks. Hematoxylin and eosin staining. Initial zooming x50.

The volume ratio of bone, cartilage and connective tissue was 47.9±3.3%, 13.1±2.1% and 39±3.5%, respectively. Inflammatory and degenerative-dystrophic changes were also absent in all periods of observation.

Histological examination of the implantation zone in animals of the second group revealed on the 2nd week of the experiment the presence of a homogeneous eosinophilic acellular zone, behind which the loose connective tissue with expressed symptoms of edema was visualized, followed by zone of bone tissue remodeling in the form of osteoclastic resorption of individual beams on par with the formation of osteoid and bone-osteoid beams. Individual sections of cartilage were determined. The volume ratio of bone, cartilage and connective tissue was 18.9±3.3%, 7.2±2.1% and 73.9±4.5%, respectively (Fig. 2, A).

By the 4th week of observation, a narrow homogeneous eosinophilic acellular zone with perifocal formation of coarse connective tissue, which alternated with areas of hyaline cartilage, was determined in the implantation zone. Fragments of cancellous bone tissue with minimal signs of remodeling were determined in some areas (Fig. 2, B). The volume ratio of bone, cartilage and connective tissue was 13.3±2.8%, 34.2±6.1% and 52.5±3.7%, respectively. Microscopically, histological changes by the 12th week of the experiment were similar in nature as compared to the 4th week. Acellular zone, well-formed single bone beams were determined among the mature fibrous tissue and areas of hyaline cartilage (Fig. 2, B). The volume ratio of bone, cartilage and connective tissue was 18.9±3.3%, 31.3±2.1% and 49.8±4.5%, respectively. Inflammatory and degenerative-dystrophic changes were also absent in all periods of the experiment.

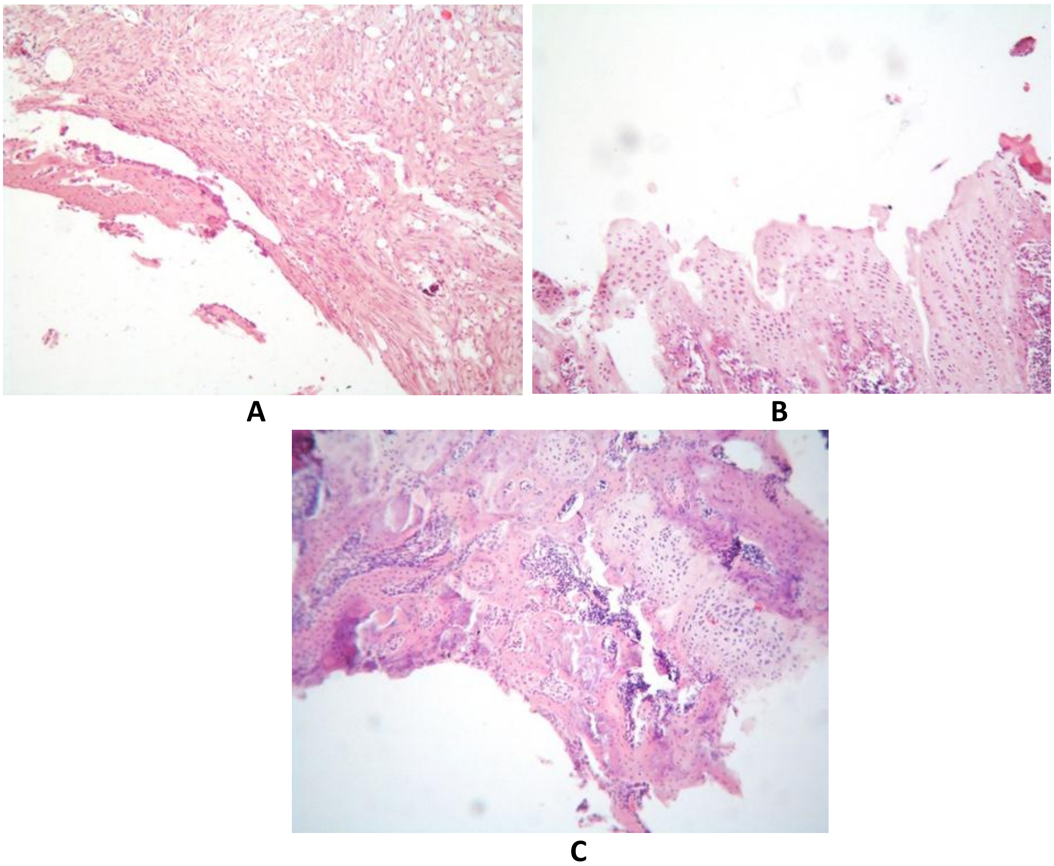


Figure 2: Group 2. Overgrowth of fibrous connective tissue and cartilage around the titanium implant. A - 2 weeks. B - 4 weeks. C - 12 weeks. Hematoxylin and eosin staining. Initial zooming x50.

No morphological changes were observed in bone tissue of the animals from Group 3 (control), since none of animals of this group was subjected to surgery during this experiment and served as the norm in the morphological structure of the jaw bone tissue.

SUMMARY

Summarizing the results, we can say that the proposed method of non-drug effects on opioidergic brain structure has a stimulating effect that affects the processes of reduction of inflammatory reactions, adaptation of the tissues of surgical field after the intervention and the ability to predict the quality and speed of osseointegration. It is extremely important to consider optimal regimes of the impacts proposed.

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