PATHOHISTOLOGICAL INVESTIGATION ON THE INFLUENCE OF INTRACANAL MEDICATION ON THE REGENERATION OF JAW BONE

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Abstract

The results of histological investigation on the influence of the proposed drug composition (metronidazole, enterosgel (Sigma), alflutop (Biotehnos S.A., Romania) for experimental mandible bone defect regeneration in rats were presented. The high efficiency and osteoregenerative properties of this paste were shown, and its significant clinical efficiency for temporary placement into the root canals in the treatment of chronic apical periodontitis, for stimulating regeneration of the damaged periapical tissues, was assessed.

Keywords: temporary intracanal dressing, metronidazole, enterosgel, alflutop, histological investigation.

INTRODUCTION

One of the major tasks in the treatment of chronic apical periodontitis is regeneration of the pathologically changed periapical tissues [1,2], which might be achieved by means of different remedies introduced into the periapical tissues, stimulating regeneration of the damaged ones [3-5]. One of these approaches recommends irrigation of the root canal with a 0.1% lysozyme solution and placement into the root canal of a lysozyme-vitamin paste as temporary dressing [6]. Remedies in the form of solution or paste are introduced into the root canal using a root filler or a pad. The ingredients of the paste are lysozyme, oil solution of vitamins A and E, excipient [6]; calcitonin and sorbents can be also used [7]. The following biologically active medications in the form of paste have also been used for treating the damaged periapical tissues: Chonsurid, Methyluracylum, Heparin, together with Furazolidone and Dimethyl Sulfoxide [2, 8-11]. Embryoplast (allogeneic embryonic tissues in different development stages) was proved to be effective for stimulating the processes of reparative osteogenesis in the destruction area [12]. Several studies attempted to increase organism’s resistance by introducing immunomodulators into the periapical area of teeth with chronic apical periodontitis [13-15]. The most frequently used drug in the consistency of paste is Levamisole (Decaris), and also a combination of Thyromogenum, Levamisole and Hydrocortisone [6]. The use of plant remedies, such as a 10-40% solution of Erakond, introduced into the periapical tissues and left in the root canal for 24 hours, was proved to be effective [16, 17]. However, it would be premature to consider the problem solved.

In order to stimulate regeneration of periapical tissues in the treatment of chronic apical periodontitis, a paste for temporary placement into root canals was proposed. This material contains an antibacterial drug that effectively inhibits anaerobic microflora – metronidazole, a drug that removes the exudate from the root canal and periodontium – enterosgel, and another one, which stimulates regeneration of bone tissue – alflutop. These drugs were mixed in an ex tempore selected composition to the consistency of paste.

MATERIALS AND METHOD

Investigations on the stimulation effect of the proposed drug composition on the regeneration of bone tissue included experiments on rats. In particular, the influence of the proposed drug composition on the experimental mandible bone defect regeneration in rats was investigated.

The drug Collapan (“Intermedapatit”, Russia) was used as a comparative remedy. Collapan,
belonging to the group of osteoplastic materials applied for bone tissue regeneration and suppurative complications treatment, contains artificial (synthetic) hydroxyapatite, Collagen and Lincomycin.

Experimental investigations were conducted on 35 white rats of the Vistar line (males, aged 13-14 months), divided into 7 groups, as follows:

- Group 1 – rats with intact bone (non damaged mandibular bone (normal, standard);
- Groups 2, 3 – rats with an nontreated mandible bone defect (euthanasia on the 10th day for group 2, and on the 30th day for group 3);
- Groups 4, 5 – rats with mandible bone defect filled with the proposed composition of 15 milligrams per 1 rat (euthanasia on the 10th day for group 4, and on the 30th day for group 5);
- Groups 6, 7 – rats with mandible bone defect filled with Collapan – 2.5 milligrams per 1 rat (euthanasia on the 10th day for group 6 and on the 30th day for group 7).

Bone defect was reproduced under thiopental anesthesia (20 mg /kg). Before incision, the skin on the mandible was treated with a 3-% iodine solution. The 2.5 cm incision involved cutting through the skin, the hypodermic tissue, fascia, at a 0.5 cm distance from the mandible margin. The body and angle of rats’ mandible were opened up to the bone, and the periosteum was removed. With the help of a physiodispenser, in the area of the thickest mandibular angle, a round bone defect was performed in the thickest part of the mandible angle (0.3 - 0.5 cm in diameter), with a spherical burr and inverted cone. The wound was rinsed with a stream of water and dried with cotton balls.

The proposed composition (paste consistency) was introduced into the bone defect of the rats of groups 4, 5 with a spatula; Collapan was introduced into the bone defect of the rats of groups 6, 7.

After placement of drugs into the cavity of bone defect, the piece of periosteum was replaced in order to close the defect opening, and the skin was sewed up with a Vicryl suture material.

On the 10th and 30th days of the experiment, rats’ euthanasia was implemented under thiopental anesthesia by means of total phlebotomization, and the bone tissue in the area of defect was incised.

The biological material was stored at a temperature of –30°C. 3-4 specimens of bone tissue from each group of rats were stored in 10% neutral formalin for further histological investigation.

Histological investigation of the specimens taken from the animals of all groups was carried out. After decalcination, the bone tissues, together with the surrounding soft tissues, were embedded into paraffin. The received microscopic sections were stained with hematoxylin and eosin by a standard technique [18, 19]. Microscopy of the received stained sections was conducted on a Jenamed – 2 microscope. Photoregistration was carried out with a digital camera (Canon 5D).

**RESULTS AND DISCUSSION**

The mandible bone tissue of the 1st group rats (standard) in the histological specimen had a normal structure (immature bone tissue of trabecular structure).

In the histological specimens of the mandible, bone defects in the 2nd group of rats (bone defect without treatment, 10th day) and zones of bone tissue in a state of fragmentation were revealed. Separate bone fragments were surrounded by fragments of connective tissues and blood. Bone lamellae underwent resorption, with increased level of their basophillicity. Degeneration of osteocytes was observed, as a result of which hollows appeared in the bone (Fig. 1). Along a 30-day period, the fragments of bone lamellae were deformed, undergoing local decalcination and being surrounded by necrotic, altered connective tissues. Accumulation of small dispersed basophillic material, hemolytic blood and clots was revealed (Fig. 2). No signs of beginning of bone tissue regeneration and bone tissue formation in the defect zone were observed.

In the histological specimens of the mandible, the bone defects in the 4th group rats (the bone defect was filled with the proposed composition) along 10 days did not considerably differ from the histological picture observed in the rats of the 2nd group. However, resorption of bone lamellae fragments in rats with bone defect filled with the proposed composition was expressed to
a lesser extent. Also, no lysis of connective tissues formations was observed.

Along a 30-day period, in the histological specimens with mandible bone defects of the 5th group rats (bone defect was filled with the proposed composition) signs of repair were observed, expressed by the formation of a large number of new form blood vessels. Moreover, connective tissues formations were closely attached to the bone tissue (Fig. 3). Between the connective tissue and the fragments of the new bone tissue, osteoblasts were active. Small-sized and different from the osteogenesis areas were revealed directly near the bone lamellae surrounded by connective vascularized tissue.

In the histological specimens of the mandible bone defects from the 6th and 7th groups of rats (bone defect being filled with Collapan), the histological picture of bone defects was significantly similar to the condition of bone tissue of the 4th and 5th groups of rats (Fig. 4).
CONCLUSIONS

Our histological investigations have proved the osteoregenerative abilities of the proposed drug composition, similar to those of the material used as a control – Collapan. This supports the idea that the proposed drug composition possesses significant clinical efficiency for temporary placement into the root canals in the treatment of chronic apical periodontitis, for stimulating regeneration of the damaged periapical tissues.

References