

Original article

Bone Tissue Response to Endomethasone Implanted into the Rat Mandible

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SUMMARY

The aim of the paper was to investigate the tissue response to the implantation of "Endomethasone" (Septodont) in artificially prepared defect in the rat mandible.

Sixteen Wistar male rats were used for the experiment. After anesthesia, a defect between the midline and the mental foramen of the left mandible was made (diameter 1.4 mm, 1.6 mm depth) using sterile steel burs. In the defect of the experimental group (12 animals) "Endomethasone" was implanted while the defect in the control group (4 animals) was left to heal spontaneously. One half of animals of both groups was sacrificed after fifteen days, and the second half after sixty days. The samples consisted of the defect and the surrounding bone. After routine decalcination and processing, the samples were embedded in paraffin, and microscopic preparations were made, on the basis of which a microscopic analysis was performed.

Fifteen-day-old specimens showed the early signs of bone resorption, as well as granulated connective tissue, hyperemic blood vessels, fibrin exudate to a lesser extent, with the difference that inflammatory infiltrates were less present in the experimental group. Sixty-day-old specimens demonstrated the degree of bone healing; tissue filling with newly formed bone was significantly more advanced in the control group. Experimental group showed a sign of delay with significant presence of callus tissue that more in the form of focus showed a deposition of the young bone tissue.

Endomethasone masks the inflammatory reaction which follows operational procedures. In the long run, according to the results of the present research, Endomethasone can interfere with bone healing by causing delayed inflammatory reaction.

Key words: endodontic sealers, healing, bone, Endomethasone

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INTRODUCTION

Obturation is the final phase of the root canal treatment, and as a result it is an object of great interest. It is considered a critical intervention and, at the same time, the most common cause of treatment failure. It is important that the obturating material intimately adheres to the canal walls and thereby ensures adequate coronary, apical and lateral sealing. Sealing should be spread along the canal and within its borders. It is necessary to ensure that the canal is not overfilled or insufficiently filled because each case is unfavorable for the healing of periapical tissues. Even in cases where an ideal filling is only inside the canal system, the material is still in contact with the vital tissue through the apical foramen. Healing after treatment depends directly on the characteristics of the material used for canal sealing. According to the literature, the materials should not cause irritation of the periapical tissue, because this may result in failure of endodontic treatment (1).

Many different materials are used for root canal filling. None of them is ideal so the search for it continues. Materials based on zinc oxide with eugenol are usually used as comparative sealers in studies that examine the biological effects of materials. The results of these studies, however, are not encouraging. As they are highly toxic, some authors do not recommend some of these materials for use (2). Therefore, before recommendations for clinical use, there is a need to provide *in vitro* experiments testing of the influence of endodontic materials and additional experiments on animals (*in vivo*).

One of the commonly used material based on zinc oxide is Endomethasone which due to the presence of dexamethasone affects the uneventful postoperative course (3). Endomethasone powder contains zinc oxide, diioditimidol, barium sulfate, hydrocortisone acetate, dexamethasone and paraformaldehyde. Endomethasone liquid contains eugenol, oil of peppermint and anise oil (4).

Formaldehyde is a component of many sealers based on zinc oxide but is also found as part of other sealers that are based on resin (AH 26, Ribler's past, Top Seal, etc.) (5). Formaldehyde affects irreversible damage to the affected nerve (2). There is a presumption that eugenol, which is being released for long from the materials based on zinc oxide eugenol may have an impact on the persistence of periapical tissue

inflammation. Some studies indicate that the zinc ion can determine the degree of biocompatibility of these materials (2).

The aim of this study was to investigate the tissue response to bone implantation of "Endomethasone" (Septodont, France) in artificially prepared defect in the mandible of rats, using the standard technique of implantation.

MATERIAL AND METHODS

For experiment, 16 Wistar male rats were used, weight 160-180 g (approved by the Ethics Committee of the Faculty of Medicine in Nis No. 01 3797). During the experimental procedure, animals were anesthetized by intraperitoneal injection of ketamine hydrochloride (0.1 ml per 100 g of weight). After preparation procedure, between the midline and the mental foramen of the left mandible of all animals, a defect (diameter of 1.4mm and 1.6mm depth) was made using sterile stainless steel fissure burs.

Animals of the experimental group (n = 12) were divided into two subgroups:

- The first subgroup (n = 6) was sacrificed after 15 days;
- The second subgroup (n = 6) was sacrificed after 60 days.

In the formed bone defects of experimental group animals, "Endomethasone" (Septodont, France) prepared by the manufacturer's instructions was implanted.

Bone defect in the control group (n = 4) was left to heal spontaneously without any implants. Two animals of the experimental group were sacrificed after fifteen days and two animals after sixty days.

After the estimated time the animals were sacrificed by an overdose of anesthetic. The tissue samples were made by resection of the mandible consisting from the defect and the surrounding bone. Tissue samples were fixed in 10% buffered formalin, demineralized in 10% formic acid, dehydrated in a series of graded alcohols and cleared in benzene and embedded in paraffin wax. Cutting was performed in the buccolingual direction on the microtome (HISTORANGE) 2µm thick glass knives. The slides were stained with hematoxylin and eosin. Microscopic analysis was performed by light microscopy.

RESULTS

Fifteen-day experimental period

In the samples from the early experimental and control groups, early signs of bone resorption were observed, as well as the presence of granulation connective tissue, hyperemic blood vessels, fibrin exudate to a lesser extent with the difference that inflammatory infiltrates were less observed in the experimental group.

Experimental group 15 days

Tangential section is visible through a defect in bone tissue, filled with granulation connective tissue with hyperemic blood vessels and diffusely distributed lymphoplasmocytic cell populations inside of it. The specific cell populations (osteoclast and osteoblast type of cell population) were not present at the periphery of granulation tissue that filled the bone defect (Figure 1).

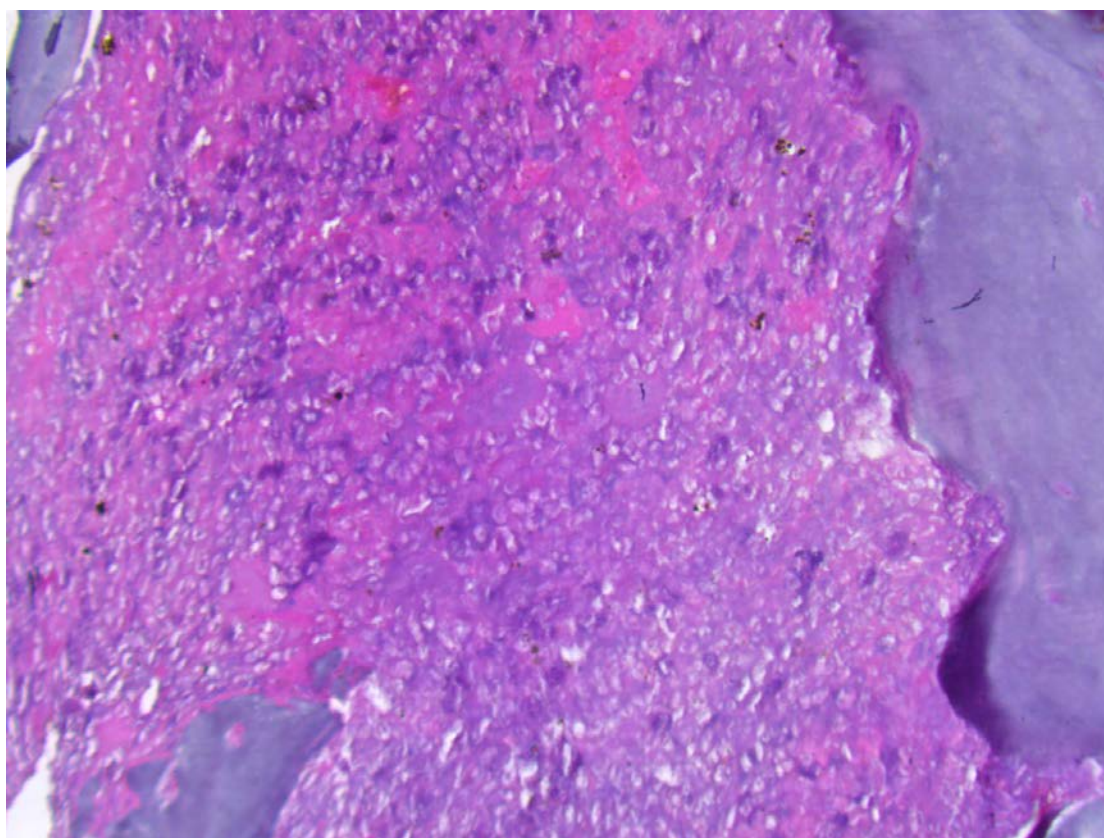


Figure 1. Experimental group 15 days. Granulation tissue; diffuse arrangement of leukocyte infiltration; the absence of specific osteoclast and osteoblast differentiation. (H&E original magnification x 400)

Control group 15 days

Mature granulation tissue present within the bone defect shows signs of follicular lymphocytic infiltrate organization, and at the periphery near the bone tissue the development of resorption cones was

found as well as their early lytic activity. In the bone tissue, normal structure of compact bone was observed with a regular organization of osteons, more prominent cement lines, without osteosynthesis on the border with granulation tissue (Figures 2 and 3).

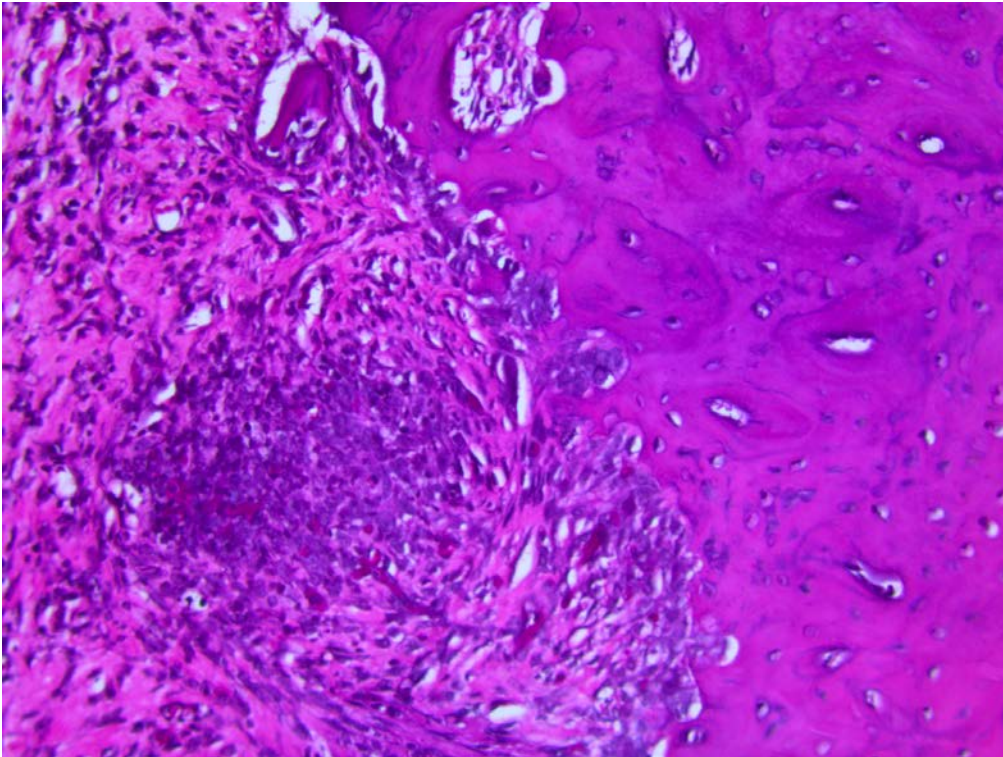


Figure 2. Control group 15 days. Experimental defect filled with granulation tissue (early callus), follicular organization of lymphocytic infiltrate; the normal structure of compact bone with a regular osteon organization. (H&E original magnification x 200)

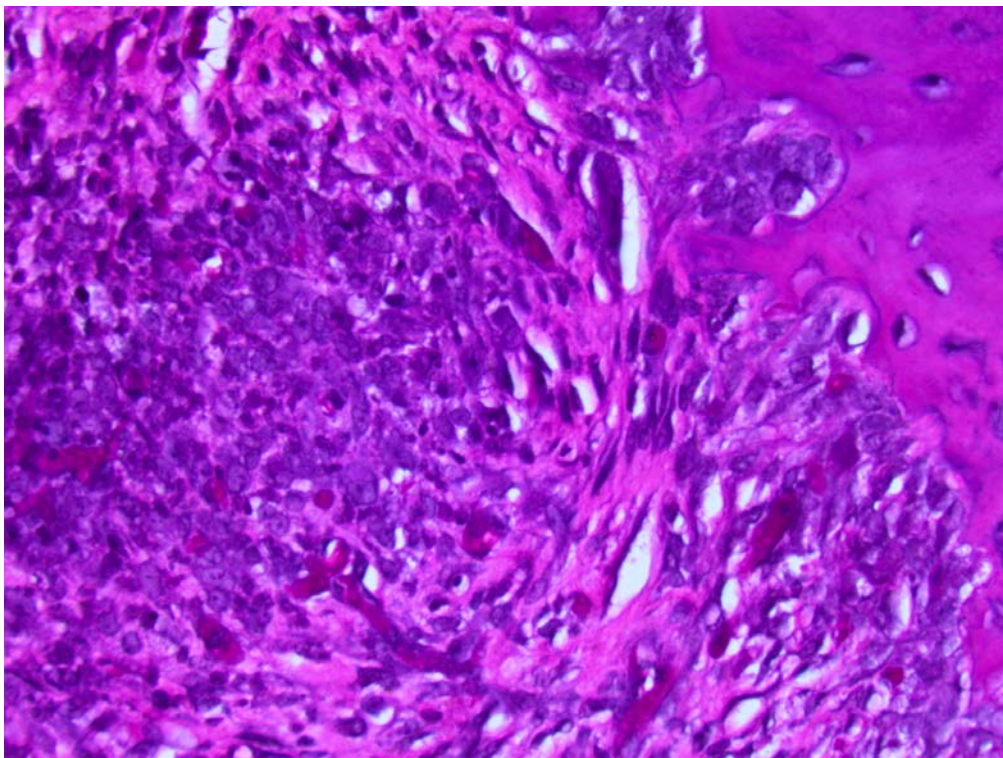


Figure 3. Control group 15 days. Development of resorption cones and their early resorptive activity; (H&E original magnification x 400)

Sixty-day-experimental period

In samples from the experimental group of animals sacrificed after 60 days, a great heterogeneity of connective tissue patterns was observed, whereby the degree of bone healing and closing the defect by the deposition of the newly formed bone were significantly more advanced in the control group. The experimental group showed a sign of delay with significant presence of focally formed callus tissue, with scarce deposition of the young bone tissue.

Experimental group 60 days

In the experimentally made defect of a compact

bone, a mature granulation tissue was detected, in which inflammatory infiltrate was present diffusely in the form of solitary foci. Granulation tissue showed the signs of maturation in the center. At the periphery, few osteoblasts were observed on the border with the bone tissue. In a part of the soft-tissue located in the artificially prepared cavity, the signs of its transformation into the morphology of callus was evidenced, with the appearance of osteoblasts at its periphery. In that region, the bone tissue showed the linear lamellar organization which undulating propagation follows the contour of experimental defect being in contrast to the rest of the bone composed of transversely cut osteons, that are likely representatives of normal bone which was not included in the experimental procedure (Figure 4).

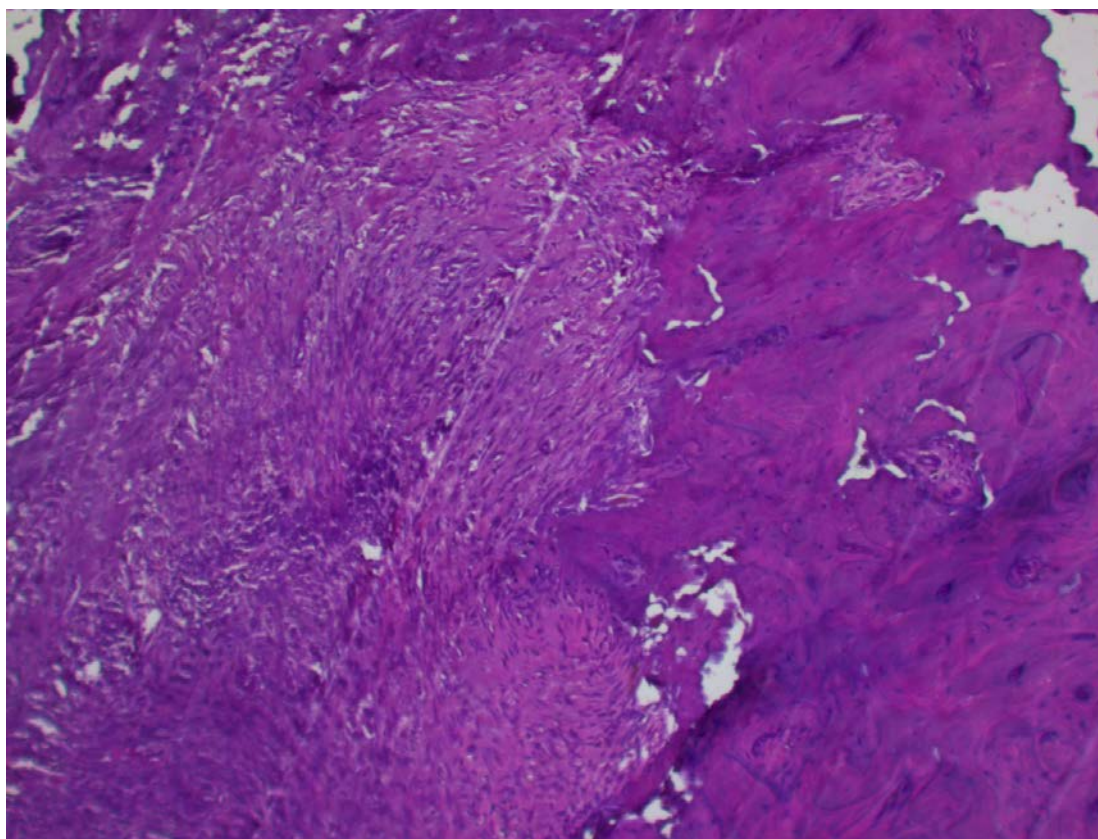


Figure 4. Experimental group 60 days. Mature granulation tissue; group of inflammatory cells in the form of focus; callus structure with osteoblasts present in the periphery. (H&E original magnification x 100)

Control group 60 days

In the region of experimentally made defect of the bone, the structure of the immature bone tissue was present, contrasted by its morphology toward the surrounding uninvolved compact bone tissue. The

immature bone tissue composed of osteons was observed, with centrally positioned wide spaces filled with vascularised connective tissue, peripherally encircled with osteoblasts. Surrounding lamellar organization of this newly formed bone was composed of few lamellas among which the presence

of solitary osteocytes was observed; lamelas were scarcely, unevenly calcified, non-concentrically

parallelly distributed, mainly longitudinal and undulating in shape.

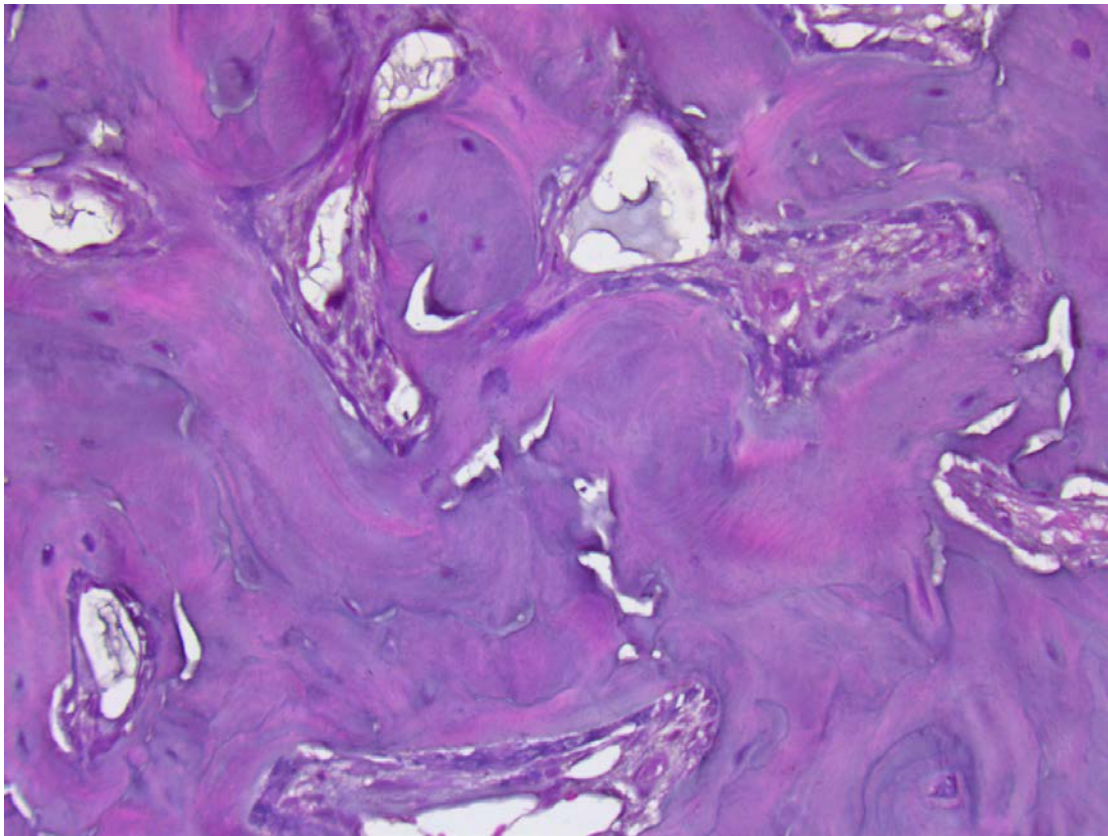


Figure 5. Control group 60 days. Vascularized connective tissue in centers of osteons and their non-concentric arrangement of immature bone lamellas (H&E original magnification x 400)

DISCUSSION

In vitro experiments for testing biocompatibility of endodontic materials have strictly controlled conditions as the advantage, but they do not reflect the response of the living tissue healing. Therefore, the *in vivo* techniques of implantation are considered superior. The bone implantation was selected for this experiment. The materials were implanted in a rat bone prepared defect, immediately after mixing, corresponding to the clinical situation. The results of the present study indicate that the specimens of the experimental group after 15 days show no evidence of inflammation in any of samples, whereas it was present in the specimens of the control group. On the other hand, the samples of the experimental group reveal more remaining defects, in comparison to the control where more connective tissue was present. This can be explained by the influence of

dexamethasone and hydrocortisone acetate in Endomethasone. Corticosteroid components are not typical for all ZnOE-based sealers. Presence of corticosteroids inhibits the production of collagen.³ The defect is visible in the control group, but the beginnings of the healing process are visible, too. The occurrence of mild inflammation in the control group is the natural reaction to the operative procedure itself.

When it comes to healing tissue reaction, the results are not consistent with the results of other authors (4). In a similar study, ZnOE-based sealer challenged early inflammatory response with neutrophil infiltration of the tissue around the defect although the period for early response in this study is significantly shorter (3 days) (5). Other authors have also obtained similar results (4). This inflammatory reaction is considered to be the result of eugenol release from freshly mixed material (6).

Numerous studies suggest a significant

cytotoxic effect of the ZnOE-based sealers, both in cell culture assays as well as assays of implantation. Better results in terms of reduced inflammation and active healing, regardless of the presence of fibrous connective tissue, with the observation of material over a longer period (12 weeks), were attributed to the fact that the release of harmful components sealer decreases in a function of time (5).

In the literature, *in vivo* subcutaneous test of Endomethasone biocompatibility gave different results than obtained in this study. Zafalon et al. have established a strong to moderate inflammatory reaction after 15 days of implantation, and after 30, 60, 90 days, there was no reaction (7). Study used the same material in the same period of time, so the differences can be attributed to various experimental procedures.

Although the methodology is different, the results of the present study are consistent with the results of other authors and suggest that in the long run Endomethasone slows down the process of repair and causes the cytotoxic alterations (8).

The majority of negative Endomethasone components released during the setting reaction can be explained by the large solubility of the material (8, 9). Microleakage of Endomethasone belongs to the most pronounced in comparison with the root canal materials of a different chemical composition (9). Continuous release of toxic components such as eugenol and paraformaldehyde may explain persistent inflammation over a long period of time.

Eugenol (4-allyl-2-methoxyphenol) is an extract of clove oil and is a component of zinc oxide eugenol (ZOE). It has significant potential for periapical toxicity and inhibits the growth and proliferation of human osteoblastic U2OS cell line (10). Since it is difficult to determine how much of eugenol is released from the material through the apical foramen, the cytotoxic effects can be evaluated based on the dose, frequency and duration of exposure. It has been demonstrated that lower concentrations of eugenol can easily reach an effective cytotoxic level on USO2 cells during long-term exposure. Eugenol is capable of inhibiting the function of macrophages and thus affects the inflammation of periapical tissues (11).

The formaldehyde is a disinfectant component in many of the materials, from which it is released over time. It is an allergen, mutagen and carcinogen.

The extent of release of formaldehyde is different for different materials (12).

The presence of the corticosteroid component in Endomethasone, in the clinical sense, can have a positive side. The corticosteroid has an ability to mask the possible postoperative symptoms.³ Corticosteroids inhibit the initial reaction of inflammation and interfere with wound healing by inhibiting osteogenic differentiation. Inflammatory reaction is a defense reaction and the inevitable path to healing.

The effect of dexamethasone on calcium metabolism is not negligible. Glucocorticosteroids inhibit collagen production as well as the activation of osteoprogenitory and osteoblasts cell. This leads to the inhibition of osteogenesis callus maturation (3).

Another anti-inflammatory effect of glucocorticoids is their inhibitory effect on cyclooxygenase (COX), an enzyme that is responsible for the formation of several biological mediators including the prostaglandins (PG) (13).

COX-2 isoenzyme is involved in the initial reaction of osteogenesis, osteoblast maturation and regulation of genes associated with the formation of bone tissue. The healing process of bone tissue includes a large number of cells: osteoblasts, their mature form, osteoclasts etc. Osteoclasts, which are also involved in the process of bone remodeling, have an impact on healing in inflammatory stage when it is necessary to remove necrotic tissue sections from the area of the wound. However, the short-term treatment with corticosteroids has no effect on the activation of osteoclasts in bone healing (3).

Materials for root canal filling are some kind of implants because they permanently remain in the body so that the effect of their corticosteroidal components is not appropriate to compare with the systemic use of similar products in a short period of time. In this study, the control group showed a higher degree of healing in fifteen-day-old samples in comparison to equivalent experimental, which could be explained by the fact that potential glucocorticoid component does not last indefinitely.

There are different views on whether dexamethasone interferes with healing over a longer period of time (3, 13). In the present study, the small defects almost completely healed spontaneously for 60 days (control group), while in the experimental

group two preparations, except for the healing process, presented simultaneous cell response.

CONCLUSION

Endomethasone masks inflammatory reaction that normally occurs after the operating procedure, which is an advantage for clinical conditions. Through a long period of time, according to the results of the present research, Endomethasone can interfere with healing to a certain degree by causing delayed flogistic reactions.

The negative effects on healing lose significance if the biological concept of endodontic treatment is respected (14). Therefore, it is necessary to reduce the possibility of irritation caused by the periapical extrusion of ZOE- based sealer in the clinical treatment.

Some authors have already recommended the removal of ZnO-based sealers from use (2), others concluded that many of sealers are at least irritant (15). However, it is necessary to analyze other biological and physical properties of these materials and to adapt their application to clinical conditions.

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Reakcija koštanog tkiva na endometazon implantiran u mandibulu pacova

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SAŽETAK

Cilj rada bio je ispitivanje tkivnog odgovora na koštanu implantaciju „Endometazona“ (Septodont) u veštački preparisan defekt u mandibuli pacova.

U eksperimentu je korišćeno 16 Wistar pacova muškog pola. Nakon anesteziranja, životinjama je načinjen defekt između medijalne linije i foramena mentale leve strane mandibule (dijametar 1,4 mm, dubina 1,6 mm) pomoću sterilnih čeličnih svrdala. U tako formiran defekt, životinjama eksperimentalne grupe (12 životinja) implantiran je „Endometazon“, dok je defekt kod životinja kontrolne grupe (4 životinje) ostavljen da spontano zarasta. Polovina životinja obe grupe žrtvovana je za petnaest, a polovina za šezdeset dana. Uzorci su se sastojali od defekta i okolne kosti. Rutinskom dekalcinacijom i obradom uzorci su dovedeni do parafinskih kalupa, zatim načinjeni mikroskopski preparati na osnovu kojih je izvršena mikroskopska analiza.

Petnaestodnevni uzorci - zapažaju se rani znaci resorpcije koštanog tkiva, uz prisustvo granulacionog vezivnog tkiva, hiperemičnih krvnih sudova u manjoj meri prisutnog fibrinskog eksudata, sa tom razlikom da je prisustvo zapaljenskog infiltrata slabije prisutno u eksperimentalnoj grupi. Šezdesetodnevni uzorci - stepen zaceljivanja kosti, popunjavanje novonastalim koštanim tkivom značajno uznapredovanije u kontrolnoj grupi. Eksperimentalna grupa pokazuje znak zakašnjenja uz značajno prisustvo tkiva kalusa, koje više u vidu fokusa pokazuje depoziciju mladog koštanog tkiva.

Endometazon maskira reakciju zapaljenja koja prati operativnu proceduru. Kroz duži vremenski period, prema rezultatima ovog istraživanja, Endometazon može u izvesnoj meri ometati zarastanje izazivanjem kasne reakcije zapaljenja.

Ključne reči: endodontski materijali, zarastanje, kost, Endometazon