SMJ Saratov Medical Journal
ISSN 2712-8253, Saratov Medical Journal
2023. Volume 4. Issue 4 Article CID e0403
DOI: 10.15275/sarmj.2023.0403

Original article

Reprint

Effect of metronidazole microcapsules with silver nanoparticles on the efficacy of alginate gel in the correction of structural and functional periodontium disorders in rats with periodontitis

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Received 17 August 2023, Accepted 24 November 2023

Abstract:
Objective: To evaluate the effect of metronidazole loaded into alginate microcapsules impregnated with silver on structural and functional periodontium disorders in periodontitis.
Materials and Methods. Our research was conducted on 30 white male rats, randomly distributed among two groups. The comparison group consisted of 15 animals with experimental periodontitis, which received applications of a gel containing microcapsules without additional loading of active substances. The experimental group included 15 animals with periodontitis, which received applications of gel with microcapsules containing metronidazole to the inflamed gums. To assess the morphological changes in periodontium, the mandible was collected followed by its histological examination.
Results. Application of a gel containing microcapsules with silver nanoparticles loaded with metronidazole on the gums of animals with periodontitis led to a partial reduction of structural disorders. E.g., out of 15 animals, local disorganization of the periodontal ligament fibers in the experimental group was observed only in 5 cases and signs of limited bone loss were observed in 2 cases. In the comparison group, bone resorption was noted in all 15 animals, while diffuse alteration of fibers throughout the periodontal ligament was confirmed in 13 of 15 cases.
Conclusion.The inclusion of metronidazole in alginate microcapsules with silver nanoparticles leads to a significant reduction in the destructive processes occurring in the periodontal tissues during periodontitis.

Keywords: periodontitis, nanoparticles, bone loss.

Cite as: Savkina AA, Lengert EV, Ermakov AV, Stepanova TV, Ivanov AN. Effect of metronidazole microcapsules with silver nanoparticles on the efficacy of alginate gel in the correction of structural and functional periodontium disorders in rats with periodontitis. *Saratov Medical Journal* 2023; 4 (4): e0403. https://doi.org/10.15275/sarmj.2023.0403

Introduction

Inflammatory periodontal diseases currently remain one of the most common dental pathological conditions. This may be due to multiple etiological factors causing these diseases, along with complex mechanisms of their pathogenesis. Pathogenic microflora of the oral cavity has primary importance in the occurrence of periodontal diseases. Among the most pathogenic bacteria inhabiting periodontal pockets are gram-negative bacilli of the *Porphyromonas*, *Prevotella*, *Fusobacterium* and *Actinobacillus genera*. These pathogenic microorganisms influence the destructive processes in periodontal tissues directly via the effect of toxins secreted by them or indirectly through the initiation of the inflammatory process [1]. An inflammatory responsein the host organism to the action of bacteria occurs, which, on the one hand, is a manifestation of internal protective mechanisms, and on the other hand, has a destructive effect on the periodontium. The microbial factor is fully realizedsolely with an inadequate protective response of the host(or its absence) in addition to negative environmental factors (specifically, the unsatisfactory hygienic status of the oral cavity, bad habits, inadequate restoration and the presence of dentures.

By now, the methods of treating inflammatory periodontal diseases have undergone a number of successful transformations, but the solution to this problem still remains relevant. Considering that periodontopathogenic flora plays a crucial role in the etiology of periodontitis, the development of means for coherent antibacterial therapy is of particular importance.

Various studies demonstrated that for the successful treatment of periodontitis, surgical treatment of the periodontal pocket alone is not sufficient.It is also necessary to suppress the impact of the microbial community. At present, metronidazole is a medicinewith a pronounced bacteriostatic effect against a wide range of periodontal pathogens [2, 3].

Objective – to evaluate the effect of metronidazole loaded into alginate microcapsules impregnated with silver on structural and functional periodontium disorders in periodontitis.

Materials and Methods

The experiment was performed on 30 white male rats, which were distributed among comparison and experimental groups using simple randomization. The comparison group comprised 15 animals with an experimental model of periodontitis. These rats received applications of a gel containing microcapsules without additional loading of active agents. The experimental group included 15 animals with periodontitis thatwere subjected to applications of gel with metronidazole microcapsules to the inflamed gums. The total duration of the experiment was five weeks, during which all animals were kept in the same vivarium conditions without restrictions on access to water, food andnatural light. All manipulations with animals were performed under injection anesthesia with a combination of Xylanite (Nita-Pharm LLC, Russia) and Telazol (Zoetis Inc., Spain) in dosages of 1 mg/kg and 0.1 ml/kg, respectively.

To develop experimental periodontitis, a nonabsorbable multifilament surgical suture was sewn into the gums of the central incisors in the lower jaw similarly to the procedure described in Ionel et al. (2015) [4]. On Day 14 after modeling, the ligature was removed.

To correct structural and functional disorders of the periodontium in animals with periodontitis, we employed specially developed hydrogels. The hydrogels included hollow microcapsules 4.3±0.5 μm in size, the shell of which contained silver nanoparticles (AgNP) formed in an alginate matrix by adding 0.1 M silver nitrate solution [5]. To load the capsules, 5 mg/ml metronidazole solution was used. Animals in the experimental group were treated with a gel containing metronidazole, while animals in the comparison group received gel without metronidazole. All animals were applied the gel three times: on days 14, 16, and 18 of the experiment.

The animals were removed from the experiment after five weeks from its onset with an overdose of anesthetic drugs. Preparations of their mandibles were fixed with a 10% neutral formalin solution. Demineralization of the tissues of the lower jaw was performed with a decalcifying electrolyte solution (ErgoProduction LLC, Russia) for 24 hours. After demineralization of the mandible, the sampleswere cut out via removing the periodontium tissues of central incisors. To make the blocks, the material was subjected to ordinary alcohol treatment with a subsequentembedment in paraffin. Horizontal sections (transverse to the incisor axis) with a thickness of 5–7 μm were prepared using a semi-automated microtome RMD 3000 (MtPoint®, Russia–Australia) and stained with Mayer's hematoxylin and eosin (Biovitrum LLC, Russia). BioClear clearing reagent (BioOptica, Italy) was used to clear the sections, and Bio-Monht (BioOptica, Italy) was used to cover the slides.

Examination and microphotographs of periodontiumpreparations were performed in transmitted light on the μVizo-103 video microscope (LOMO PHOTONIKA LLC, Russia). Analysis of morphological changes included assessment of the periodontal ligament structure and identification of signs of alveolar bone resorption.

Results

Morphological analysis of preparations of the periodontium indicated that in all animals with periodontitis, after application of a gel containing solely microcapsules with AgNP, swelling of the connective tissue in the lamina propria, as well as uneven filling of blood vessels, was observed. In 7 out of 15 cases, there was an increase in blood supply in the venous section of the microvasculature. In the connective tissue, infiltration of the lamina propria of the gums with leukocytes (mainly lymphocytes) was noted. In 3 out of 15 cases, lymphocytic infiltration was mild, while in 12 cases it was moderate.

In animals from the comparison group, structural changes in the periodontal ligament were noted, which were characterized by pronounced interstitial and perivascular edema, and disorders in the structure and location of the fibers (*Figure, B*). Structural disorganization of fibers in 13 animals was found diffusely throughout the entire periodontal ligament, while in 2 animals it was local in nature. In 14 out of 15 animals in this group, the phenomenon of blood separation was observed in individual vessels.

In the bone tissue of the alveoliof all ratsin the comparison group, we observed a rarefaction of bone tissue classified as lacunar resorption. In areas of alveolar bone resorption, we noted a proliferation of connective tissue (*Figure, E*).

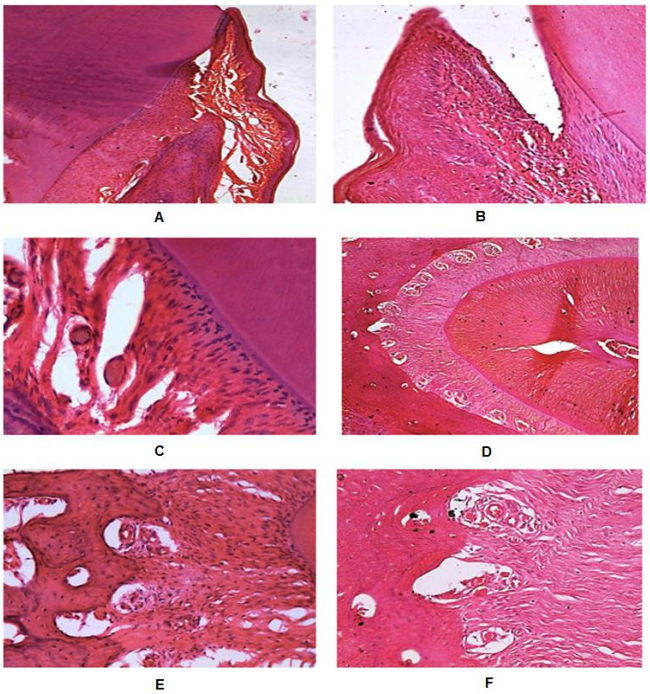

Figure. Structural and functional disorders of periodontium in rats with experimental periodontitis during the use of gels containing microcapsules with AgNP without active components (A, C, E) and with metronidazole(B, D, F): A, B – lamina propria; C, D, E, F – periodontal ligament

Morphological examination of sagittal section preparations of the experimental group rats demonstrated that after the application of a gel containing microcapsules loaded with metronidazole to the lamina propria, signs of uneven blood filling in the vessels and swelling of the connective tissue found in all animals of this group remained, as well as when using an analogue without active components (*Figure, A* and *B*). Signs of congestion of the venous microvasculature were noted in only 2 out of 15 cases. A few leukocytes (mainly lymphocytes) were found in the lamina propria of the gingiva. Minor leukocyte infiltration was detected in 7 out of 15 cases; in most animals, only single lymphocytes were observed in the lamina propria.

In the periodontal ligament in the experimental animals, we observed an ordered course of fibers (in contrast to the animals of the comparison group) (*Figure, C* and *D*). Disorganization of the fibers of the periodontal ligament was strictly local in nature (i.e., in individual areas of the ligament) and was detected in 5 cases out of 15. The bone tissue of the alveoliwas dense, the resorptive phenomena of the alveolar bone were not expressed, and individual areas of rarefaction were recorded in only 2 out of 15 animals, in contrast to rats from the comparison group (*Figure, E* and *F*).

Hence, in white rats of the experimental group, after application of a gel containing microcapsules with AgNP and metronidazole, we observed a marked decrease in destructive processes compared with animals of the comparison group, which received an analogue without active components.

Discussion

The results obtained during the experiment confirmed that gels containing microcapsules with AgNP without additional loading and with metronidazole were able to correct structural damage to the tissues of the periodontium in rats with periodontitis. This may be due to the antibacterial effect of AgNP, which is believed to be implemented through the following effects: the release of silver ions destroying the cellwall and cytoplasmic membrane of bacterial cells; an increase in the generation of reactive oxygen species in bacterial cells, which destroy cell membranes and cause DNA modification, thereby preventing their replication; and inhibition of protein synthesis due to modification of ribosomes [6]. In addition, the osteoinductive properties of AgNP were demonstrated in some studies [7]. It was previously shown that AgNP in silk fibroin-based coatings accelerate the maturation of osteoblasts, which was accompanied by the production of alkaline phosphatase, matrix secretion, and calcification [8]. Our data suggested that the effect of the proposed gel on disorders occurring during experimental periodontitis in white rats depended on the content of AgNP microcapsules in its composition. A gel with a low AgNP content lowered increased gingival perfusion in rats with experimental periodontitis more significantly [5], probably due to the lack of irritation. However, it was inferior to its analogue with a high AgNP content regarding the efficacy of preventing destructive changes in periodontal tissue, including alveolar bone resorption [9], probably due to a decrease in the antibacterial effect and osteoinductive properties. These findings necessitate the loading of active components with antibacterial action into a prolonged-release system based on microcapsules with AgNP.

Loading metronidazole into microcapsules with AgNP significantly reduced the alteration of the periodontal ligament and bone tissue resorption compared with its analogue without active components. The obtained data were consistent with the results of recent studies by other authors demonstrating that metronidazole compounds with PEGylated AgNP significantly reduce the production of matrix metalloproteinases 3 and 8 (enzymes involved in the destruction of periodontal tissues) [10].

Conclusion

In our experiment, the inclusion of metronidazole in alginate microcapsules with silver nanoparticles has led to a significant reduction in the destructive processes occurring in the periodontal tissues during periodontitis.

Author contributions: all authors contributed equally to the preparation of the manuscript.

Conflict of interest. None declared by the authors. The study was carried out within the framework of the Government Procurement toRazumovsky State Medical University of Saratov, Russian Federation Ministry of Healthcare, Development and Pathogenetic Substantiation of UseUsing theProlonged-Release System of Antibacterial and Anti-Inflammatory Agentsfor the Correction of Microcirculatory Disorders in Experimental Periodontitis (registration number 121032500024-2).

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