ORIGINAL ARTICLE

EFFECTS OF STRONTIUM RANELATE ON ALVEOLAR BONE IN RATS WITH EXPERIMENTAL DIABETES MELLITUS

DOI: 10.36740/WLek202201201

Lubov M. Matvieienko, Rostyslav Y. Matvieienko, Olena O. Fastovets
DNIPROPETROVSK MEDICAL ACADEMY OF THE MINISTRY OF HEALTH OF UKRAINE, DNIPRO, UKRAINE

ABSTRACT

The aim: To investigate effects of strontium ranelate on alveolar bone loss in rats with experimental diabetes.

Materials and methods: Histological examination of bone tissue was carried out for 24 white male rats, divided into three identical groups of 8 animals (the first group included animals with experimental type-2 diabetes, based on the use of Streptozotocin; in the second group, it was additionally reproduced periodontitis by the introduction of Penicillamine; and in the third group, in addition, it was used strontium ranelate) and 6 intact rats.

Results: In the second group, osteoporosis phenomena were most significant, while in the third group the average specific area of the inter-root trabecular bone differed a little from the control. In the control group, the number of osteoclasts was 2.24 ± 1.41 cells per mm^2, in the first group – 4.34 ± 1.37 cells per mm^2, in the second group – 2.96 ± 1.26 cells per mm^2 and in the third group – 2.24 ± 1.41 cells per mm^2 ($p>0.05$). The samples of the third group have the most expressive manifestations of osteogenesis and the most intense expression of osteopontin, both in trabecular and compact bone tissue.

Conclusions: The use of strontium drugs reliably slows down the processes of bone resorption due to both inhibition of the function of osteoclasts, and by activating osteoblasts, thus stimulating osteogenesis.

KEY WORDS: osteoblasts, osteoclasts, osteopontin, periodontitis, treatment

Wiad Lek. 2022;75(1 p.2):151-155

INTRODUCTION

General periodontitis is a chronic inflammatory disease of tooth-supporting tissues, defined by pathologic loss of periodontal ligament and alveolar bone, which affects more than in 60% of adults. Moreover, in 10% cases these patients have aggressive forms, which become the reason of a quick progression of the periodontal tissue damage [1].

At the same time, epidemiological data demonstrated that diabetes is associated with increased risk of periodontitis onset and progression in adults. Thirteen studies matched the inclusion criteria, comprising 49 262 individuals, including 3 197 diagnosed with diabetes. Meta-analyses of adjusted estimates showed that diabetes increased the risk of incidence or progression of periodontitis in 86.0% [2]. The most important long-term complications of periodontitis, associated with diabetes, are changes in bone metabolism [3].

In turn, the main aim of periodontal therapy is regeneration of periodontal tissues that has been destroyed by periodontal disease. Current conventional techniques for the treatment of periodontitis, which aim is to decrease microbial levels and modify the local environment to reduce inflammation, have shown a limited potential for complete periodontal regeneration. The perspective direction to enhance periodontal tissue reconstruction and its biomechanical integration is represented by the use of specific bioactive molecules that can drive and promote the complete process of regeneration. Strontium ion can be an attractive candidate to improve osteogenic activity in periodontal diseases treatment [4].

Strontium ranelate is a medication indicated for the treatment of osteoporosis that presents concomitant anti-resorptive and osteoanabolic dual biological activity. However, the effects of strontium ranelate on alveolar bone have been poorly explored [5].

THE AIM

The aim of this study is to investigate effects of strontium ranelate on alveolar bone loss in rats with experimental diabetes.

MATERIALS AND METHODS

Experimental studies were conducted on 24 white male rats of Wistar line, aged 4 months, whose weight was 230-250 g, with experimental diabetes and 6 intact rats of the same mass and sex. Experimental rats were divided into three identical groups of 8 animals (the first group included animals with experimental diabetes; in the second group, it was additionally reproduced periodontitis by the introduction of Penicillamine (Cuprenil®, Teva, Poland); and in the third group, in addition, it was administered strontium ranelate (Bivalos®, Les Laboratoires Servier, France).

To reproduce the experimental equivalent of type-2 diabetes in the animals, after the previous 24-hour food deprecation (with free access to water), it was used a single intra-abdominal aqueous solution of Nicotinamide (Sigma-Aldrich, USA) (230 mg/kg) and 15 minutes after that – Streptozotocin (Sigma-Aldrich, USA) at a dose of 65 mg/kg as a 5% solution in the citrate buffer, pH 4.5 [6].

The determination of glucose concentration was carried out using the Optium Omega glucometer (Abbott Diabetes Care Inc., USA) in blood obtained by scaring the tip of the animal's tail. Blood glucose levels were determined on the third day after introduction of drugs and 6-hour food disruption with free access to water. Only animals with elevated glucose (8-14 mmol/l) were used for further research.

On the 14th day, a re-measurement of glucose content was carried out to identify glucose tolerance disorders. Before the test, all animals of the control and research groups were subjected to 12-hour food depreciation with free access to water (glucose concentration was determined on an empty stomach), so this sample was considered as original (control). Glucose was injected once orally in the form of 40.0% aqueous solution, at a level of 2.0 g/kg of body weight. The dynamics of changes in blood glucose levels were estimated in the original state and 30, 60, 90 and 120 minutes after glucose intake. The results of the study of blood glucose levels was represented graphically in the form of the so-called "glycemic curve", assessed the levels of lifting of the glycemic curve at temporary intervals and its shape [7].

In 16 rats of the second and third research groups, the periodontitis model was reproduced by drinking water with Penicyllamine (Cuprenil®, Teva, Poland, No series 16525016) at a dose of 20 mg/kg of rat body weight, 7 days a week for 55 days, starting from 6 days after the reproduction of type-2 diabetes [8].

In 8 rats of the third research group, Strontium Ranelate (Bivalos®, Les Laboratoires Servier, France, No series 617687) was introduced intravenously once a day during the last 28 days of the experiment. The dose of the drug, 250 mg/kg (intravenously, once daily), was in the range of therapeutic (ED_{50}), which are recommended in experimental studies in accordance with the recalculation formula. It was in the range of doses used in other experimental studies [9].

The control group of animals received drinking water in the appropriate period of the experiment.

On the 60th day of observation, the animals were withdrawn from the experiment by decapitation. Bone tissues of the mandibles were fixed in 10% of the formalin solution on the phosphate buffer (pH 7.0).

In histological examination of bone tissue, it was carried out a morphological assessment of bone density according the index of the ratio of bone tissue area (BA) to the total area of all tissues (TA) at the location of the first lower molar (BA/TA); according the index of qualitative and quantitative changes in expression of noncollagenic protein of bone matrix – osteopontin; according the presence of leukocyte infiltration of tissues and cellular composition of inflammatory infiltrate; according the index of cortical plate area of the mandible bone in the area of alveolar ridge.

The obtained data were processed by method of variation statistics using the software MS Excel 2003 (license disk No 74017–641–9475201–57075).

RESULTS

The general histological structure of bone tissue of the mandible in rats of the control group was marked by typical features of the bones of the frontal part of the skull (a dense outer layer of compact bone and an inner, spongy part) (Fig. 1).

When comparing the structure of the cortical plate of the mandible bone of animals of the first, third and control groups, no significant differences in the structure of bone tissue were found. In contrast, animals of the second group demonstrated uneven areas of thickening of the cortical layer, which was especially noticeable in areas of alveolar bone, where there were signs of chronic periodontitis with the presence of diffuse lymphocytic infiltrate (Fig. 2).

Bone trabecula was formed along one surface by osteoblasts, and along another one it was resorbed with osteoclasts (Fig.3). The specific areas of the inter-root trabecular bone were different between the animals of the research groups. Thus, in the control group, the average BA/TA was 53.4±3.1%. In the first group it was 47.1±1.9%. In the second group, osteoporosis phenomena were most significant (BA/TA was 44.2±2.9%), while in the third group the average specific area of the inter-root trabecular bone differed a little from the control group (49.0±3.2%). Statistically significant differences were observed only between the control and second groups (p=0.00034).

Single osteoclasts and groups of osteoclasts were located on the surface of bone trabecules and in recessed depressions - Housip lacunah (Fig. 3, 4). All the groups showed relatively small osteoclasts content (no more than 3 cells per mm^2). This reduction in the specific area of trabecular bone in the first and second groups was corroquirated with the increase in the number of osteoclasts. Thus, in the control group, this value was 2.24 ± 1.41 cells per mm^2, in the first group – 4.34 ± 1.37 cells per mm^2, in the second group – 2.96 ± 1.26 cells per mm^2 and in the third group – 2.24 ± 1.41 cells per mm^2 (p>0.05).

Among the significant differences in the research groups, it was seen the increase of number of active osteoblasts in the third group (Fig. 2, 5).

However, the most objective indicator of osteoblasts activity in this study was osteopontin, namely its expression in the bone matrix. It was not distributed evenly throughout the calcified bone matrix; osteopontin was concentrated in cement lines between the old and the new bone (Fig. 6).

According the results of the immunohistochemical detection of osteopontin expression and its distribution in bone tissue samples of the mandible among the research groups it was established that in the samples of the first and control groups, there was a similar situation: reduced expression of osteopontin in the alveolar bone and active expression of osteopontin in the bone tissue between roots (Fig. 7).

In the second group, there was the emergence of uneven foci of osteopontin expression in the compact bone tissue of alveolar ridge of the mandible, which coincided with the areas of its thickening, which were located near to the zones of chronic inflammation of the periodontal tissues (Fig. 8).

The samples of the third group had the most expressive manifestations of osteogenesis and, at the same time, showed the most intense expression of osteopontin, both in trabecular and compact bone tissue. Unlike the second group, in expression

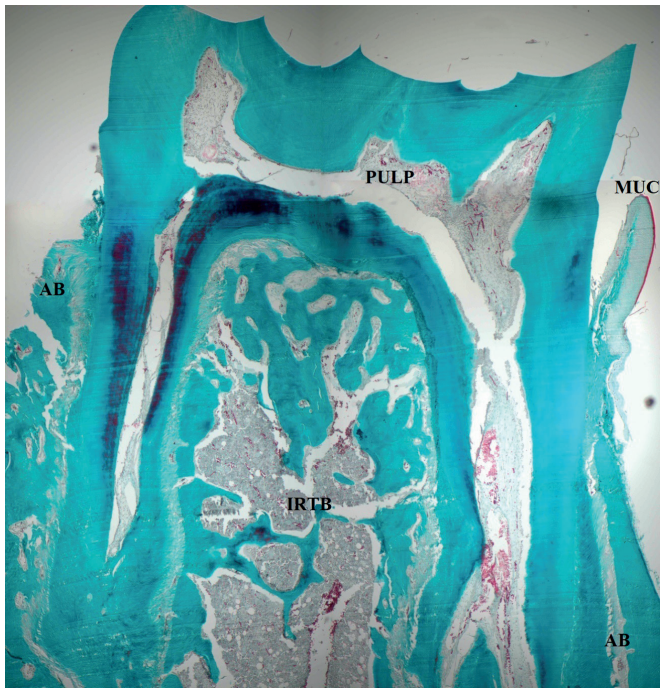

Fig. 1. Overview photo of tooth tissue (first lower molar) and surrounding bone tissue: PULP – tooth pulp, MUC – gingival mucosa, AB – alveolar bone, IRTB – inter-root bone membrane. The second research group. Gluing of 4 photos. Increase ×50. Coloring three-chrome by Masson-Goldner

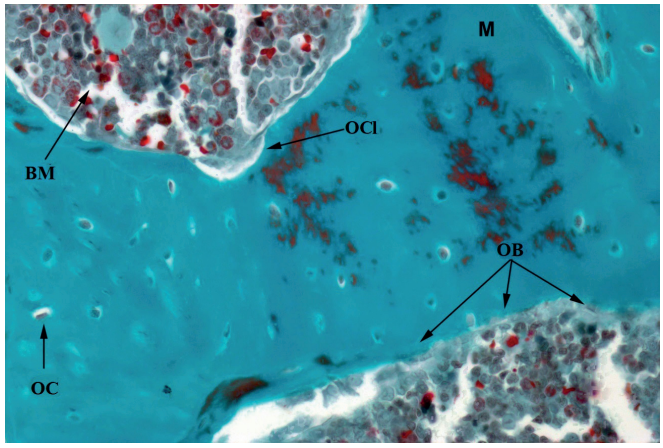

Fig. 3. The structure of the trabecular bone. Inter-root membrane of the first molar of the animal from the control group. Bone tissue consists of a mineralized collagen matrix (M) of green color and several cellular populations: osteoblasts (OB), osteocytes (OC), osteoclasts (OCl). Bone lacuna is filled with red bone marrow (BM). Increase ×400. Coloring three-chrome by Masson-Goldner

intensity this increase had a more uniform nature, distributed almost parallel along the bone plates. Just as in previous groups osteopontin was actively expressed between the root crossing and the dentine (Fig. 9).

DISCUSSION

The elevated pro-inflammatory factors in the gingiva of patients with poorly controlled diabetes suggest a biological

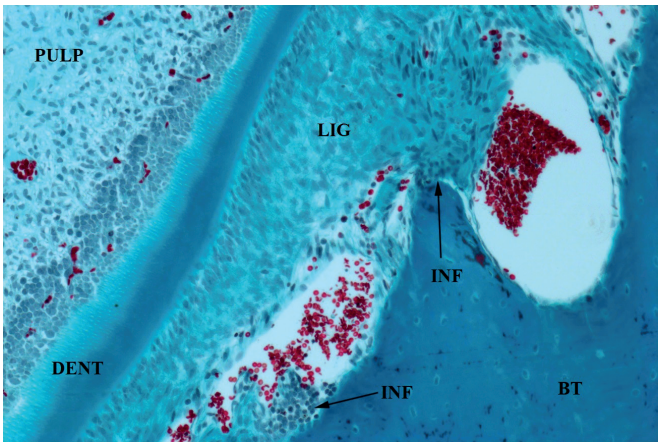

Fig. 2. Bone tissue (BT) of the alveolar ridge and the lower molar (dentin (DENT) with pulp (PULP)). The second research group. In the periodontal ligament (LIG) the chronic inflammation (INF) is observed, hemopapillars are significantly expanded.
Increase ×200. Coloring three-chrome by Masson-Goldner

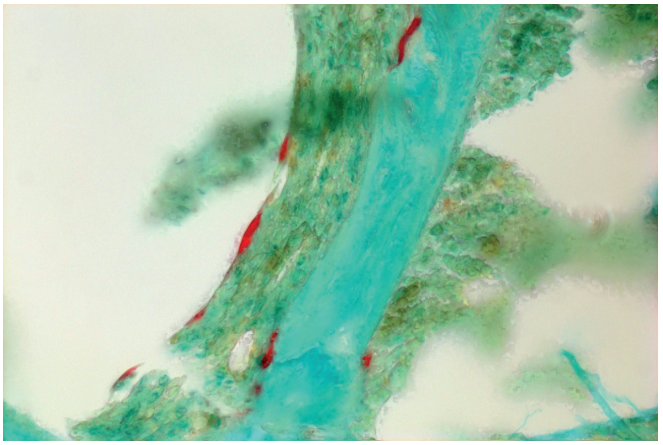

Fig. 4. TRAP-positive cells, osteoclasts (raspberry color), are arranged in groups along the bone trabecula. Inter-root bone membrane in the area of the first lower molar. The second research group. Increase ×400. Histochemical color on TRAP. The background dye is strong green

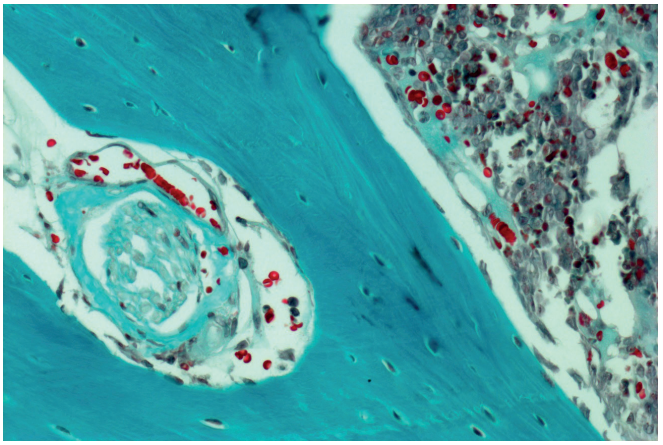

Fig. 5. Fragment of trabecular bone. Inter-root membrane of the first lower molar, the first research group. Stable bone tissue: osteoblasts are located along the trabeculaes, they have a flattened appearance. Increase ×200. Coloring three-chrome by Masson-Goldner

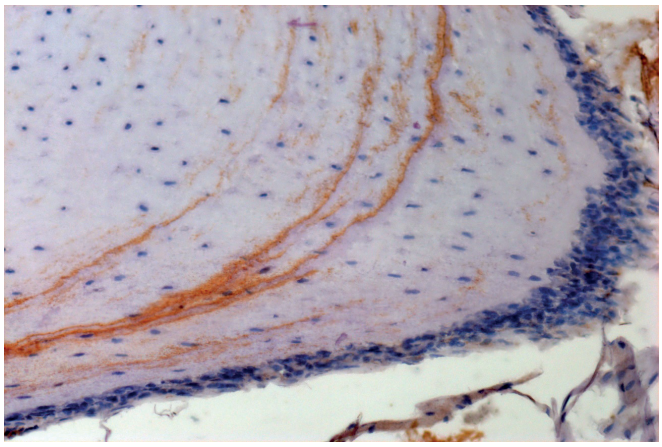

Fig. 6. Compact bone of the alveolar ridge in the area of the first molar of the rat's mandible. Uniform expression of osteopontin is along the mineralization lines. The control group. Immunoperoxidase method. Increase ×200

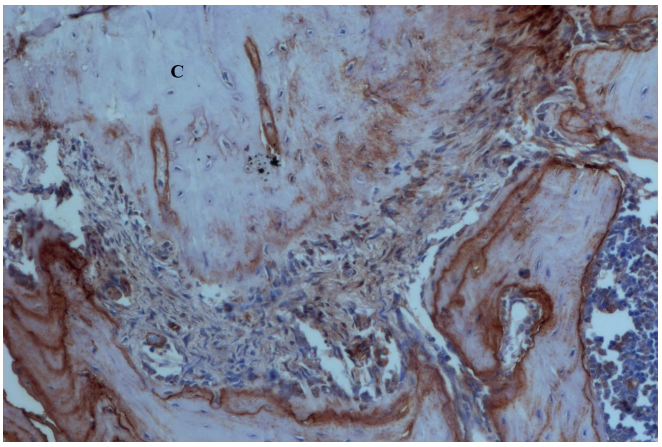

Fig. 7. Compact bone of the alveolar ridge in the area of the first molar of the rat's lower jaw. Active expression of osteopontin is in the zone of intensive osteogenesis. In addition, osteopontin expression is observed in enslaved cement (C). The first research group. Immunoperoxidase method. Increase ×200

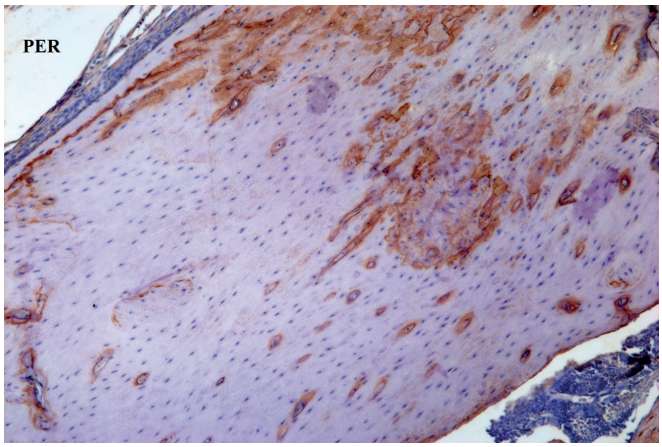

Fig. 8. Compact bone of the alveolar ridge in the area of the first molar of the rat's mandible. Expressive and uneven expression of osteopontin is in areas of bone mineralization. The second research group. Per – periodontal tissue. Immunoperoxidase method. Increase ×100

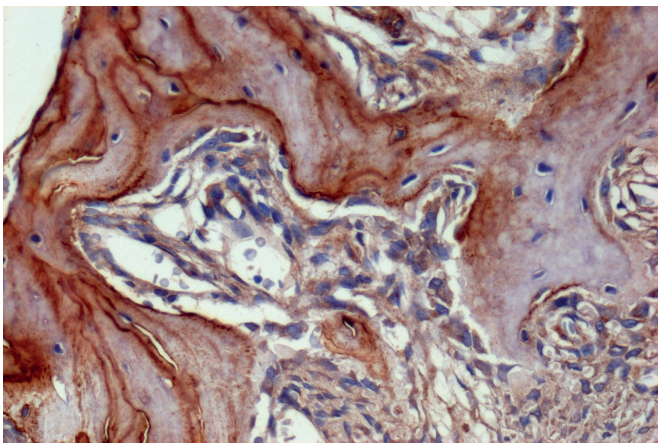

Fig. 9. Trabecular bone tissue of the inter-root bone of the first molar of the rat's mandible. The third research group. Expressive, uniform expression of osteopontin is in areas of bone mineralization. Immunoperoxidase method. Increase ×400

pathway that may aggravate periodontitis [10]. It was confirmed by the results of our histological study of the cortical plate of the mandible bone. In rats with experimental diabetes in the first and second groups there were signs of chronic periodontitis with the presence of diffuse lymphocytic infiltrate. In our study in the first and second groups it was established the increase in the number of osteoclasts in trabecular bone, reduced expression of osteopontin in the alveolar bone. But the use of Streptozotocin in the first group caused a slight resorption of alveolar bone, which was increased in the second group by means of Penicillamine as additional pro-inflammatory stimuli.

The results of experimental research proved that strontium ion attenuates lipopolysaccharide-stimulated proinflammatory cytokine expression and lipopolysaccharide-inhibited early osteogenic differentiation of human periodontal ligament cells [11]. According to the data [3] bone remodelling begins with resorption of bone by osteoclasts, follows by new bone formation by osteoblasts in the resorption lacunae. Thus, the potential mechanism of diabetes-enhanced bone loss in relation to osteoblasts and osteoclasts has been learned. Really, in this study in rats of the third group we observed that strontium ranelate eliminated inflammation, inhibited osteoclasts, activated osteoblasts, thus stimulated osteogenesis.

The use of strontium ranelate helped to reduce the number of osteoclasts to the index of the control group. This is quite consistent the data that in rats with experimental periodontitis strontium ranelate can reduce receptor activator of nuclear factor-kappa B ligand (RANKL) activity and osteoclast numbers, as well as alveolar bone loss [12].

In the third group it was established the increase of number of active osteoblasts and the most intense expression of osteopontin, both in trabecular and compact bone tissue. That proved activating osteoblasts and stimulating osteogenesis by strontium ranelate. This can be explained by its effect to prevent ligature-induced alveolar bone loss, increased the expression of bone markers, producing by osteoblasts as an anti-resorptive agent [5].

In the work [13] it was proved, that Strontium-mesoporous bioactive glass demonstrated their ability to promote periodontal regeneration when compared to mesoporous bioactive glass alone. So the release of strontium ions by strontium ranelate have a direct effect on preventing osteoclasts activation and promoting osteoblast differentiation. Thus, in our study we had got the same results.

CONCLUSIONS

The experimental model of type-2 diabetes, based on the use of Streptozotocin, demonstrates a slight resorption of alveolar bone, which can be exacerbated by pro-inflammatory stimuli, in particular the use of Penicillamine. In this case, there is a reliable acceleration of bone resorption associated with the activation of osteoclasts. The consistent use of strontium drugs reliably slows down the processes of bone resorption due to both inhibition of the function of osteoclasts, and by activating osteoblasts, thus stimulating osteogenesis. The obtained results allow for the clinical approbation of strontium drugs in patients with generalized periodontitis against the background of type-2 diabetes.

REFERENCES

1. Slots J. Periodontitis: facts, fallacies and the future. Periodontol 2000. 2017;75(1):7-23.
2. Nascimento G.G., Leite F.R.M., Vestergaard P. et al. Does diabetes increase the risk of periodontitis? A systematic review and meta-regression analysis of longitudinal prospective studies. Acta Diabetol. 2018;55(7):653-667.
3. Wu Y.Y., Xiao E., Graves D.T. Diabetes mellitus related bone metabolism and periodontal disease. International Journal of Oral Science. 2015; 7: 63-72.
4. Romagnoli C., Zonefrati R., Galli G. et al.The effect of strontium chloride on human periodontal ligament stem cells. Clinical Cases in Mineral and Bone Metabolism. 2017; 14(3): 283-293.
5. Marins L.M., Napimoga M.H., Malta F.S. et al. Effects of strontium ranelate on ligature-induced periodontitis in estrogen-deficient and estrogen-sufficient rats. J Periodontal Res. 2020; 55(1): 141-151.
6. Spasov A.A., Voronkova M.P., Snigur G.L. et al. Eksperimentalnaya model sakharnogo diabeta 2-go tipa [Experimental model of type 2 diabetes]. Biomeditsina. 2011; 3: 12-18. (in Russian).
7. Kamyshnikov V.S. Spravochnik po kliniko-biokhimicheskim issledovaniyam i laboratornoy diagnostike [Reference book on clinical and biochemical research and laboratory diagnostics]. Moskva: MEDpress-inform. 2009. 896 p. (in Russian).
8. Nikolayeva A.V., Tkachenko Y.K., Breus V.Y. Tsitomorfologicheskoye issledovaniye vliyaniya preparata polifenolov travi Hypericum perforatum L. na sostoyaniye slizistoy obolochki shcheki krys v usloviyakh modelirvoaniya parodontita [Cytomorphological study effects of Hypericum perforatum L. Polyphenol on buccal mucosa of rats in the conditions of periodontitis modeling]. Innovacii v stomatologii. 2013; 2: 2-4. (in Russian).
9. Rodrigues T.A., de Oliveira Freire A., Carvalho H.C.O. et al. Prophylactic and therapeutic use of strontium ranelate reduces the progression of experimental osteoarthritis. Front Pharmacol. 2018; 9: 975. doi: 10.3389/fphar.2018.00975.
10. Polak D., Shapira L. An update on the evidence for pathogenic mechanisms that may link periodontitis and diabetes. J Clin Periodontol. 2018; 45(2):150-166.
11. Wei L., Jiang Y., Zhou W. et al. Strontium ion attenuates lipopolysaccharide-stimulated proinflammatory cytokine expression and lipopolysaccharide-inhibited early osteogenic differentiation of human periodontal ligament cells. J Periodontal Res. 2018; 53(6): 999-1008.
12. Karakan N.C., Akpinar A., Göze F. et al. Investigating the effects of systemically administered strontium ranelate on alveolar bone loss histomorphometrically and histopathologically on experimental periodontitis in rats. J Periodontol. 2017; 88(2): 24-31.
13. Jia X., Miron R.J., Yin C. et al. HnRNPL inhibits the osteogenic differentiation of PDLCs stimulated by SrCl 2 through repressing Setd2 J. Cell Mol Med. 2019; 23(4): 2667-2677.

The study is an independent fragment of the research work of Department of Therapeutic Stomatology of State Institution "Dnipropetrovsk Medical Academy of the Ministry of Health of Ukraine" "Improvement of new methods of diagnostics, treatment and prevention of main stomatological diseases" state registration number 0117 U 004731.

ORCID and contributionship:
Liubov M. Matvieienko: 0000-0001-7615-1065 [B-E]
Rostyslav Y. Matvieienko: 0000-0003-0888-3226 [B]
Olena O. Fastovets: 0000-0002-2769-3244 [A, F]

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR

Olena O. Fastovets
Dnipropetrovsk Medical Academy of the
Ministry of Health of Ukraine
9 Vernadsky St., 49027 Dnipro, Ukraine
tel : +380979971124
e-mail: fastovets.e@ex.ua

Received: 01.02.2021
Accepted: 28.08.2021

A - Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article