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MORPHOLOGICAL CHANGES IN TONGUE TISSUES IN EXPERIMENTAL ANIMALS WITH SIMULATING RHEUMATOID ARTHRITIS

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In order to identify the nature of morphological changes in the mucous membrane and muscles of the tongue in the development of rheumatoid arthritis, we conducted a simulation of autoimmune disease – adjuvant arthritis in 15 rats according to A.G. Kurygin's method. It is represented by single intradermal injection into the hind leg pads of a complete Freund's adjuvant, followed by daily reproduction of immobilization stress from 14 to 28 days of the experiment. Adjuvant arthritis development was accompanied by deterioration of the general condition of rats and clinical signs of local and generalized inflammatory response. Histological examination revealed changes in the epithelium (dilation of intercellular spaces, vacuolar degeneration and necrosis of epithelial cells), systemic connective tissue disease (destruction, edema, stratification and loosening of collagen fibers, histio-lymphocytic infiltration) and generalized vascular pathology (edema of interstitial tissue, perivascular hemorrhages, impaired vascular wall integrity, plethora and hemorrhage). The detected changes indicated a possible negative impact of rheumatoid arthritis on the condition of tongue tissues, deepening and burdening of the pathological process in the oral mucosa with the development of comorbid conditions.

Key words: experimental rheumatoid arthritis, rats, Freund's adjuvant, morphological changes, mucous membrane, tongue muscles.

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МОРФОЛОГІЧНІ ЗМІНИ ТКАНИН ЯЗИКА У ЕКСПЕРИМЕНТАЛЬНИХ ТВАРИН З МОДЕЛЬОВАНИМ РЕВМАТОЇДНИМ АРТРИТОМ

З метою виявлення характеру морфологічних змін слизової оболонки та м'язів язика при розвитку ревматоїдного артриту нами проведено моделювання аутоімунного захворювання – ад'ювантного артриту у 15 щурів за методикою А.Г. Куригіна. Воно складається з одноразового внутрішньошкірного введення в подушки задніх лап повного ад'юванта Фрейнда з наступним щоденним відтворенням імобілізаційного стресу з 14 по 28 добу експерименту. Розвиток ад'ювантного артриту супроводжувався погіршенням загального стану щурів та клінічними ознаками місцевої і генералізованої запальної реакції. При гістологічному дослідженні виявлено зміни в епітелії (розширення міжклітинних просторів, вакуолярну дистрофію і некроз епітеліоцитів), системну дезорганізацію сполучної тканини (деструкція, набряк, розшарування і розпушування колагенових волокон, гістіо-лімфоцитарна інфільтрація) та генералізовану вазопатію (набряк інерстицію, периваскулярні геморагії, порушення цілісності стінок судин, повнокрів'я та крововиливи). Виявлені зміни свідчили про можливий негативний вплив ревматоїдного артриту на стан тканин язика, поглиблення та обтяження патологічного процесу в слизовій оболонці порожнини рота при розвитку коморбідних станів.

Ключові слова: експериментальний ревматоїдний артрит, щури, ад'ювант Фрейнда, морфологічні зміни, слизова оболонка, м'язи язика.

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Recently, comorbid conditions have become especially important, including a combination of dental diseases with systemic disorders in the human body. Rheumatoid arthritis is the most common disease among systemic autoimmune connective tissue disease. It is a chronic inflammatory disease of the joints with a complex autoimmune pathogenesis, which leads to slow destruction of the joints, often in combination with extra-articular manifestations: lesions of the heart, lungs, kidneys, eyes, etc. [3, 8, 14]. However, some aspects of the association of rheumatoid arthritis with tongue disease are still poorly understood.

According to the authors [5, 10, 13], patients with rheumatoid arthritis are often diagnosed with multiple caries, generalized periodontitis, gingivitis, glossitis, diseases of the temporomandibular joint, changes in the oral mucosa and Sjogren's syndrome. Patients with rheumatoid arthritis complain of a burning sensation and pain in the tongue, with a long course of the disease there is a complete atrophy of the lingual papillae, the tongue becomes smooth, "polished". Vasculitis, submucosal hemorrhage with a

transparent film, through which petechiae are translucent, can occur in the thickness of the oral mucosa [9, 11]. Therefore, the issue of the influence of systemic autoimmune disease of the joints on the condition of the tissues of the mucous membrane of the tongue remains relevant, which will improve the effectiveness of treatment of patients.

In isolated studies [1, 12], microcirculatory disorders in the oral mucosa have been identified in the simulation of rheumatoid arthritis in experimental animals and their association with periodontal disease. At the same time, in order to deepen knowledge about the development of comorbid conditions (combination of lesions of the tongue tissues with rheumatoid arthritis), little attention is still paid to studying the nature of morphological changes in the epithelium, connective tissue and muscles of the tongue on an experimental model of autoimmune disease.

The purpose of the study was to investigate the general condition of experimental rats with simulating rheumatoid arthritis, changes in the epithelium, connective tissue and muscles of the tongue during the development of autoimmune disease.

Materials and methods. Experimental studies were performed in accordance with the international principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

To identify the nature of changes in the mucous membrane and muscles of the tongue in the development of rheumatoid arthritis, 25 mature white rats of herd breeding of both sexes (aged 3–3.5 months), with an average weight of 169.1±1 g (with a deviation from the average weight of not more than 10 %) were selected. 2 groups of animals were formed (equal numbers of males and females): Group I – 10 animals (control), Group II – 15 (main). The rats were kept in metal cages at a temperature of 22–26^0 C, air humidity of 65–75 %, the usual light regime "night–day", a standard mixed feed diet with free access to water. Group I (intact animals) were on a vivarium diet. Rheumatoid arthritis was simulated in group II rats according to the method of A. G. Kurygin [6]: a single intradermal injection of 0.07–0.08 ml of complete Freund's adjuvant into the hind leg pads – inactivated BCG culture (2 mg/ml) in an oily medium (1 part of lanolin and 30 parts of vaseline oil). This agent is one of the most adequate, able to reproduce the autoimmune process – the stage of pre-disease adjuvant arthritis and to extrapolate the obtained data to humans [4, 7]. According to the author's method, rats were subjected to daily immobilization stress by fixing the animals upside down on laboratory machines for 1 hour to reproduce directly a model of rheumatoid arthritis with pronounced manifestations of polyarthritis from the 14th to the 28th day. Animals were observed daily for 28 days after administration of Freund's adjuvant. Clinical assessment of the general condition of experimental animals under conditions of formation of adjuvant arthritis was carried out on the parameters of the appearance of animals, the condition of the joints, motor activity, behavior change, zoo-social relations, the ability to respond to external stimuli.

Hematological parameters, which were determined by the standard method in all groups of animals on the 28th day of the experimental study (counting white blood cells in the Goryaev chamber after lysis of red blood cells with 20 volumes of 5 % acetic acid solution; leukocyte formula was determined microscopically by blood smears stained by Giemsa), were used as criteria for the development of chronic autoimmune pathological conditions. After the blood test, all animals of the main and control groups were sacrificed with propofol anesthesia at a dose of 60 mg/kg by total bloodletting.

For histological examination of the tongue structure of experimental rats, the latter was cut off with eye scissors. Rats' tongue fragments were fixed in 10 % neutral formalin solution, dehydrated in a series of increasing concentrations of ethanol, enlightened in xylene and embedded in paraffin. Sections 5 μm thick were made, stained with hematoxylin-eosin and Van Gieson's picrofuchsin. It was examined in an OLIMPUS BX 41 light microscope (Japan) using x4, x10, x40 lenses and an x10 eyepiece. They were photographed with a digital camera.

Statistical processing of the study results was performed using a Microsoft Office Excel 2017 (Microsoft Corporation, USA) with the extension "Real Statistics" in the environment of Windows XP Professional (Microsoft Corporation, USA). The reliability of the obtained hematological parameters was assessed by Student's t-test. The difference was considered statistically significant at $p<0.05$.

Results of the study and their discussion. At the beginning of the experiment, a visual inspection of the rats revealed that the animals of both groups were active, the motor activity of the joints is not limited, and the general emotional state was not disturbed. Under the conditions of modeling rheumatoid

arthritis, the death of 5 out of 15 animals of the main group was registered (one for days 6, 7, 15, 16 and 17, respectively). In other periods of observation (until the end of the experiment), all animals survived. No fatalities were observed among the 10 intact rats.

1–3 days after administration of Freund's adjuvant, rats showed changes in the frequency and depth of respiration, increased alertness, some hyperactivity (excitability, aggression, and irritability), and the appearance of a pain reflex, exophthalmos and ptosis, changes in response to external stimuli. Specific clinical signs of a local inflammatory reaction were revealed: an increase in the volume of the limb, edema of the ventral part of the leg, redness of the skin of the damaged paw, the appearance of ulcers on it in 4 animals. Over the next three days, the clinical signs of the local inflammatory process intensified: swelling and redness of the skin increased, the fur skin in the area of the affected joints was lumpy, apparently moist. A significant decrease in the mobility of the affected limb and a gentle mode of pressing on this paw were revealed.

In the next 7–14 days of modeling adjuvant arthritis, the disease progressed and a generalized reaction developed. During this observation period, skin ulcers were detected in all rats on the ventral part of the foot, on the phalanges and on the knee joint of the limb into which Freund's adjuvant was administered. In addition, edema and hyperemia of the skin, joint pain on the intact limb, as well as an increase in the inflammatory process in the joint area of both limbs were observed. According to visual observations, there were decreased mobility, decreased overall activity and response to external stimuli. Food and water consumption decreased significantly.

It should be noted the negative dynamics and significant deterioration of the general condition of rats during the reproduction of immobilization stress from the 14th day of the experiment. The highest mortality was found in the first days of daily fixation of animals. Then, up to the 28th day, despite the daily hourly effect of immobilization stress, a slow improvement in the general condition of the experimental animals and symptoms of local inflammatory reaction of joints were observed. The appearance of cyanosis of the extremities and auricles indicated the development of a chronic inflammatory process. In order to confirm the development of experimental rheumatoid arthritis on the 28th day of the study, we performed a study of blood parameters in experimental animals in comparison with those intact rats, the results of which are shown in table 1.

Table 1

Indicators of the study of peripheral blood of experimental animals with simulated rheumatoid arthritis, (M±m)

Parameter	Main group, n=10	Control group, n=10
White blood cells, 10^9/L	23.7±0.79***	12.6±1.19
Lymphocytes, %	52.3±1.16***	68.8±1.31
Monocytes, %	3.7±0.39	2.8±0.67
Segmented, %	31.2±1.26***	23.6±1.08
Stab, %	6.8±0.67***	2.2±0.41
Eosinophils, %	5.05±0.47***	2.7±0.51
Plasma cells, %	0.93±0.25***	0
ESR, mm/h	11.2±1.69***	3.5±0.56

Note: the difference between the main and control groups: * ≤0.05; ** p≤0.01; *** p≤0.001.

Established leukocytosis, lymphocytopenia, eosinophilia, neutrophilia and accelerated erythrocyte sedimentation rate confirmed the development of the autoimmune process – rheumatoid arthritis in experimental rats.

To identify features of the structure of the mucous membrane of the tongue in rheumatoid arthritis, we studied the morphological structure of the squamous epithelium, vessels of the microcirculatory tract, muscle and nerve fibers of the tongue of intact rats. The study found that in intact rats, the tongue has a structure characteristic of this organ and consists of a muscular body covered with a mucous membrane (fig. 1).

The structure of epithelial layer, its lamina propria with numerous papillae (filamentous, fungiform, foliate, circumvallate), blood and lymphatic vessels, nerve fibers and endings, and muscular body did not differ morphologically in structure from the literature described in the literature.

After 28 days, there were pronounced changes in the structure of the mucous membrane and the structure of the muscular body of the tongue in the group of animals with experimental rheumatoid arthritis.

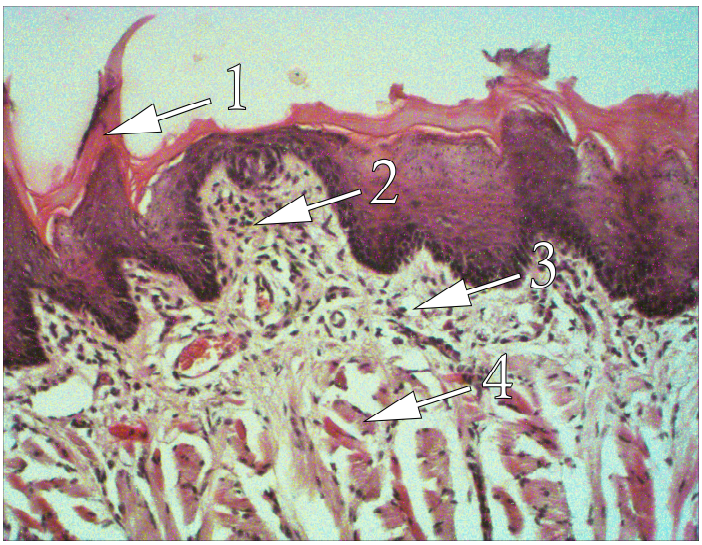

Fig. 1. Histological structure of the tongue of intact rats (Group I): 1. Filamentous papillae. 2. Fungiform papillae. 3. Lamina propria. 4. Bundles of muscle fibers. Hematoxylin-eosin staining. X10 Lens. X10 Eyepiece.

Pathological changes in the epithelium of the mucous membrane of the tongue were established: dilation of intercellular spaces, vacuolar dystrophy, atrophic and necrobiotic changes of epitheliocytes of the epithelial lining of the tongue. The thickened epithelial layer contains an increased number of lymphocytes; its stratum corneum is stratified. Collagen fibers in the basement membrane are loosened, and numerous lymphocytes and histiocytes are present between them.

Histio-lymphocytic infiltration is present in the papillary layer of the mucous membrane. There is hyperplasia and hypertrophy of fibroblasts, as well as hypertrophy of collagen fibers in the lamina propria of the mucous membrane. Collagen fibers are disorganized in some areas.

The vessels of the microcirculatory tract of the papillary and reticular layers of the mucous membrane of the tongue are full-blooded, and their lumens are dilated. Perivascular interstitial edema and histiolymphocytic infiltration were expressed in the papillary and reticular layers of the mucous membrane, whereas in intact rats such manifestations were absent. The lumens of the lymphatic vessels in the papillary and reticular layers of the mucosa were also dilated and filled with lymph. There is a hyperemia in capillaries of the muscular body.

The lumens of blood capillaries in the reticular and papillary layer of the mucous membrane are dilated and full-blooded. The walls of most blood capillaries are not solid. Endothelial cells in the walls of blood capillaries were heterogeneous in structure. Thus, along with normochromic cells similar to those in intact rats, cells with enlightened cytoplasm were detected, indicating edema of endothelial cytoplasm. In addition, there are areas of desquamation of endothelial cells. The basement membrane in the walls of the capillaries is loose. There are areas of hemorrhage per diapedesis around the blood capillaries (fig. 2).

The lumens of the venules are also dilated, sharply congested. The endothelial lining in the venule wall is also not solid. Areas of endothelial cell desquamation, stasis, marginal standing of leukocytes and their diapedesis through the walls were detected. In some places in the venules there are parietal thrombi. The permeability of the walls of the venules is impaired. This was indicated by severe edema, inertia, perivascular hemorrhage, as well as an increased number of tissue basophils and leukocytes in the perivascular spaces.

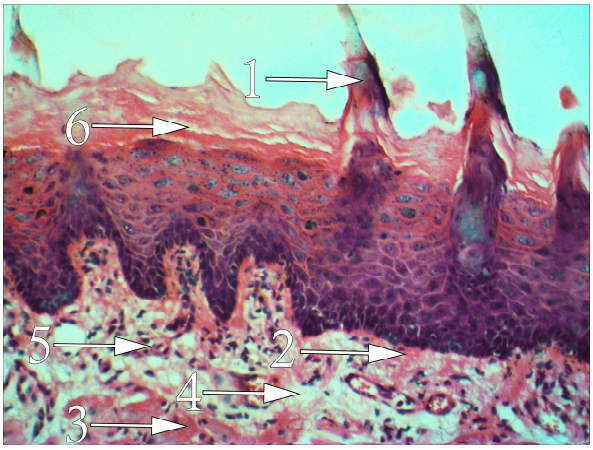

Fig. 2. Histological structure of the tongue of rats in the main group (Group II), 28th day. Perivascular edema of the interstitium and destruction of collagen fibers in the reticular layer of the mucous membrane, as well as thickened and stratified stratum corneum: 1. Filamentous papillae. 2. Lamina propria. 3. Bundles of muscle fibers. 4. Severe perivascular edema of the interstitium. 5. Edema and destruction of collagen fibers in the reticular layer of the mucous membrane. 6. Thickened and stratified stratum corneum. Hematoxylin-eosin staining. X10 Lens. X10 Eyepiece.

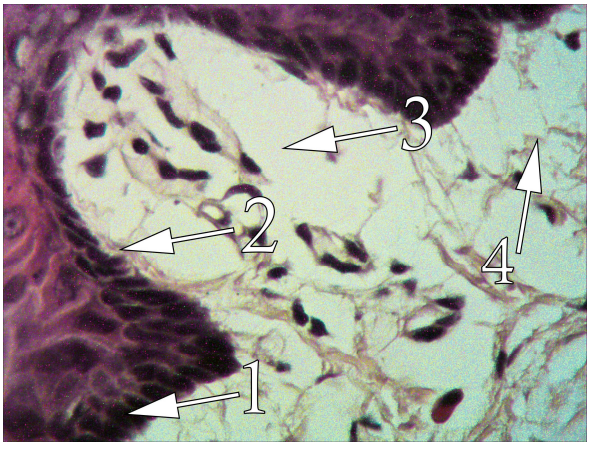

Fig. 3. Histological structure of the tongue of rats in the main group (Group II), 28th day. Severe perivascular edema of the interstitium, as well as edema and destruction of collagen fibers in the papillary layer of the mucous membrane: 1. Epithelial cells of the basement layer. 2. Edema of the basement membrane. 3. Severe perivascular edema of the interstitium. 4. Edema and destruction of collagen fibers in the reticular layer of the mucous membrane. Hematoxylin-eosin staining. X40 Lens. X10 Eyepiece.

The lumens of the arterioles are narrowed due to the location of parietal thrombi in them, which in some cases occupied most of it. Red blood cells were found in the lumen of the arterioles, which indicated a violation of blood flow (fig. 3).

The endothelial layer in the arterioles was not continuous. Focal areas of endothelial cell desquamation were detected. Hypertrophy and hyperplasia of smooth myocytes are expressed in the middle membrane of the arterial wall. In the outer shell there was fibroblast hyperplasia and hypertrophy of collagen fibers. Plasma infiltration of arteriole walls and severe perivascular leukocyte infiltration and significant interstitial edema were also detected. There is an edema of the connective tissue around the muscle and nerve fibers.

Some of the muscle fibers are similar in the structure to those in intact animals. However, some muscle fibers did not show cross-striping. There were also foci of necrotized and shortened muscle fibers. Macrophages and numerous eosinophils are located around such fibers. Interstitial edema and leukocyte infiltration are expressed in the connective tissue of endomysium and perimysium (fig. 4).

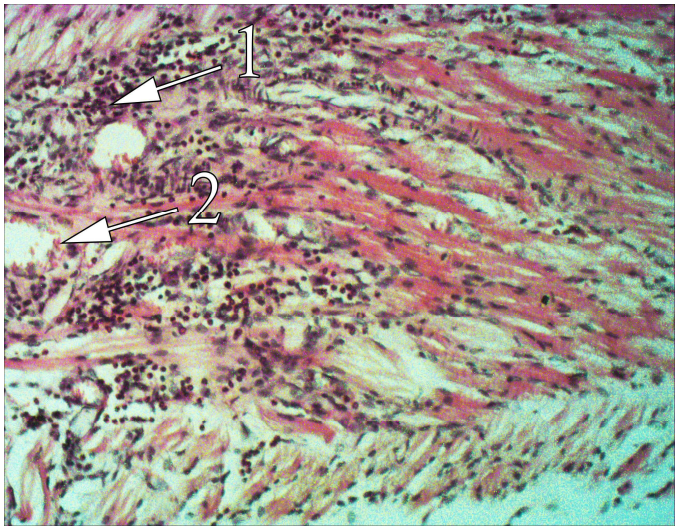

Fig. 4. Histological structure of the tongue of rats in the main group (Group II), 28th day. Pronounced leukocyte infiltration of endomysium and parietal thrombi in venules: 1. Pronounced leukocyte infiltration of the endomysium. 2. Parietal thrombi in venules. Hematoxylin-eosin staining. X10 Lens. X10 Eyepiece.

We obtained the results of deterioration of the general condition of rats (excitability, aggression, irritability, the appearance of pain reflex, exophthalmos and ptosis, changes in response to external stimuli) and the development of local and generalized inflammatory response (increase in the limb volume, edema of the ventral part of the foot, hyperemia of the first damaged skin and then intact limb, the appearance of ulcers, a gentle mode of pressing the paw), which confirmed the adequacy of reproducing the model of rheumatoid arthritis proposed by A. G. Kurygin [6]. Hematological parameters evidenced by the development of chronic autoimmune pathological process on the 28th day of the experiment: leukocytosis, lymphocytopenia, eosinophilia, neutrophilia and accelerated erythrocyte sedimentation rate in rats.

Obviously, detected changes in the epithelium of the mucous membrane of the tongue (dilation of intercellular spaces, vacuolar degeneration and necrosis of epithelial cells), systemic connective tissue disease (destruction, edema, stratification and loosening of collagen fibers, histio-lymphocytic infiltration) and generalized vascular pathology (violation of the integrity of the walls of vessels of the microcirculatory bed, expansion of their lumens, increased permeability for blood corpuscles, plethora and hemorrhage), can be considered a negative effect of adjuvant arthritis, as indicated by the authors [2, 4, 7]. In our opinion, the main changes in the structure of the studied organ are associated with dystrophic and necrobiotic disorders in the vessel walls of the microcirculatory tract. This was indicated by severe edema, inertia, perivascular hemorrhages, as well as an increased number of tissue basophils and white blood cells in the perivascular spaces. This confirmed the isolated data of experimental morphological studies [1, 12] on the importance of microcirculatory disorders in the pathogenesis of lesions of the oral mucosa in autoimmune disease and the need for their correction.

Conclusion

When reproducing an experimental model of rheumatoid arthritis, it was found that changes in blood vessels and connective tissue reflect generalized vascular pathology and systemic connective tissue disorder. Dystrophic and necrobiotic vascular disorders in the microcirculatory system cause increased permeability of vascular walls to plasma and blood corpuscles. Microangiopathies have a chronic inflammatory nature with pronounced sclerotic processes. The established changes in the connective tissue are due to hemodynamic disorders and increased vascular permeability. In the blood capillaries that supply blood to muscle fibers, there is a sludge of red blood cells, the walls of arterioles are thickened due to hyperplasia of smooth myocytes in the middle membrane, in the lumen of venules – marginal

position of white blood cells, their adhesion and diapedesis through the walls. In our opinion, changes in the structure of the mucous membrane and muscles of the tongue in experimental rheumatoid arthritis indicate a negative impact of autoimmune disease on the condition of the tissues of the tongue, which can contribute to the deepening and burdening of the pathological process in the development of comorbid conditions.

Prospects for further research are the study of clinical features of lesions of the oral mucosa in combination with rheumatoid arthritis, the development of methods for their treatment and prevention and testing in the experimental model of autoimmune disease.

References

1. Glazunov OA, Fesenko DV, Gargin VV, Nakonechnaya OA. Izmenenie kletochnoy reaktsii slizistoy rotovoy polosti pri modelirovanii revmatoidnogo artrita. East European Scientific Journal. 2019; 8(48): 23–28. [in Russian]
2. Hladkyh F, Stepaniuk N, Vernygorodskyi S. Makro-ta mikroskopichne doslidzhennya vplyvu 2-fenil-3-karbetoksy-4-dymetylaminometyl-5-oksybenzofuranu hidrohlorydu (vinboronu) na gastrotoksychnist ibuprofenu za umov eksperymentalnoho revmatoyidnoho artrytu u shhuriv. Path of Science. 2017; 3(10): 7001–7018. doi:10.22178/pos.27-8 [in Ukrainian]
3. Zhdan VM, Lebid VG, Haymenova GS, Ishheykina JuO. Faktory ryzyku ishemichnoyi khvoroby sertsya u khvoryh na revmatoyidnyj artryt. Aktualni problemy suchasnoyi medytsyny: Visnyk Ukrayinskoyi medychnoyi stomatologichnoyi akademii. 2020; 20(1): 95–99. doi: https://doi.org/10.31718/2077–1096.20.1.95 [in Ukrainian]
4. Kaladze NN, Zagorulko AK, Memetova JeJa. Morfogenez tkani timusa u eksperimentalnykh zhivotnyh s modelirovannym adyuvantnym artritom. Zdorovye rebyonka. 2011; 1:144–7. [in Russian]
5. Kulygina VM, Povshenjuk AV. Rezultaty klinichnoho obstezhennya patsiyentiv iz zakhvorjuvannyamy slyzovoyi obolonky porozhnyny rota, poyednanymy z revmatoyidnym artrytom. Buk Med Herald. 2015; 1(73): 88–92. doi: https://doi.org/10.24061/2413–0737.XIX.1.73.2015.20 [in Ukrainian]
6. Kurygin AG, izobretatel; Jaroslavskaya gos. med. Akademiya, patentoobladatel. Sposob modelirovaniya revmatoidnogo artrita. Patent Rossiyskoy federatsii N. 2305328. 2007 Avg 27. [in Russian]
7. Mohort MA, Seredynska NM, Kyrychok LM, Prytula, TP, Myslyvets SO, Pavliuk GV, et.al. Dynamika integralnykh pokaznykiv zhyttjediyalnosti bilykh shhuriv z eksperymentalnym revmatoyidnym artrytom za umov mono-ta kompleksnoho zastosuvannya nesteroyidnykh protyzapalnykh likarskykh zasobiv i antagonista kaltsiyu. Farmakolohiya ta likarska toksykolohiya. 2012; 4: 32–39. [in Ukrainian]
8. Muravyov JuV. Vnesustavnye projavleniya revmatoidnogo artrita. Nauchno-prakticheskaya revmatologiya. 2018; 56(3): 356–362. doi: https://doi.org/10.14412/1995–4484–2018–356–362 [in Russian]
9. Truhan DI, Tarasova LV, Truhan LJu. Izmeneniya organov i tkaney polosti rta pri zabolevaniyakh serdechno-sosudistoy sistemy i soedinitelnoy tkani i ikh lekarstvennoy terapii. Consilium Medicum. 2014; 16 (1): 79–82. [in Russian]
10. Abrao AL, Santana CM, Bezerra AC, Batista de Amorim RF, Branco da Silva M, Henrique da Mota LM, et al. What rheumatologists should know about orofacial manifestations of autoimmune rheumatic diseases. Rev Bras Reumatol. 2016; 56(5): 441–450. doi: https://doi.org/10.1016/j.rbr.2015.08.011
11. Crincoli V, Anelli MG, Quercia E, Piancino MG, Comite MD. Temporomandibular disorders and oral features in early rheumatoid arthritis patients: an observational study. Int J Med Sci. 2019; 16(2): 253–263. doi: 10.7150/ijms.28361
12. Fesenko D, Glazunov O, Nakonechna O, Nazaryan R, Gargin V. Consequences of microsequences of microcirculatory distrurbances of oral mucosa in modeling of rheumatoid arthritis. Georgian Med News. 2019; 10 (295): 133–140.
13. Gualtierotti R, Marzano AV, Spadari F, Cugno M. Main oral manifestations in immune–mediated and inflammatory rheumatic diseases. J Clin Med. 2019; 8(1): 21. doi: https://doi.org/10.3390/jcm8010021
14. Shaw M, Collins BF, Ho LA, Raghu G. Rheumatoid arthritis–associated lung disease. Eur Respir Rev. 2015; 24: 1–16. doi: 10.1183/09059180.00008014

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