პერფუზიის დაწყებამდე ვენური სტაზი, ჰეპატო-ცელულური ნეკროზი ან ld-MaS არ დაფიქსირდა. sd-MaS-ს დაფიქსირდა უჯრედების 3%-ზე ნაკლებში. მონონუკლეური ანთებითი უჯრედების პორტული ინფილტრატები გამოვლინდა მხოლოდ რამდენიმე უბანში. მსუბუქად გამოხატული შერეული ld-MaS და sd-MaS აღინიშნა უჯრედების 5% და 10%-ზე ნაკლებ რაოდენობაში, შესაბამისად, 4 და 8-საათიანი in vivo პერფუზიის შემდეგ. ანალოგიურად, ზომიერი ვენური ჰიპერემია აღინიშნა 6-დან 1 შემთხვევაში 4-საათიანი პერფუზიის შემდეგ, ხოლო 6-დან 2 შემთხვევაში 8-საათიანი პერფუზიის შემდეგ. ნეკროზული ჰეპატოციტების და მონონუკლეური უჯრედებით ინფილტრირებული პორტული ტრიადების

რაოდენობა არ აღემატებოდა 10% და 15%, შესაბამ-ისად. მიუხედავად ზემოაღნიშნულისა, რაიმე განსხ-ვავება ბილიარული ტრაქტის დაზიანების ხარისხში – ქოლესტაზი ან სადინარების გაფართოება – რაც შეესაბამებოდა ექსპერიმენტის პირობებს არ დადგ-ინდა.

დონორის ღვიძლის ჰისტოლოგიური შეფასების საერთაშორისო კრიტერიუმების გათვალისწინებით, "გულით მკვდარ" დონორებში ღვიძლის 8-საათიანი in vivo პერფუზია, ჩვენ მიერ შემუშავებული მო-პულსაცი სისხლმიმოქცევის აპარატის გამოყენებით, უზრუნველყოფს ღვიძლის დამაკმაყოფილებელ კონსერვაციას, რაც წარმოადგენს მისი შემდგომი წარმატებული ტრანსპლანტაციის წინაპირობას.

CONSEQUENCES OF MICROSEQUENCES OF MICROCIRCULATORY DISTRURBANCES OF MICROCIRCULATORY DISTURBANCES OF ORAL MUCOSA IN MODELING OF RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by synovial hyperplasia and the destruction of cartilage and bone [14] which could be described as chronic destructive arthritis characterized by proliferating pannus, in which synoviocyte proliferation, increased expression of extracellular matrix (ECM) proteins, immune cell infiltration, and neovascularization are characteristic findings [16].

Many methods of prevention of RA are created with point of view that cells of immune system are involved in pathogenesis with their central place [12] and injuring of microcirculatory bed (MCB). The diagnosis and management of the patient with RA must be performed with understanding of oral cavity involvement and periodontitis development as possible hypothesis about a link between periodontitis and rheumatoid arthritis [5] could be important key in relief of such patients. Till now many aspects of connection between periodontitis and rheumatoid arthritis are unexplored and there is a necessity for experimental investigation to clarify the pathogenesis of this disease, for understanding management of primary prevention and pathogenetically based treatment of patients with RA including their clinical manifestations in the oral cavity [5,10] and one of possible way could be correction o microcirculatory disturbance which needs verification for rheumatoid arthritis.

The aim of this study was to determine importance of microcirculatory disturbance of oral mucosa in modeling of rheumatoid arthritis.

Material and methods. We performed experimental investigation for a morphological study of the state of the tissues of the oral mucosa in RA in order to eliminate the influence of somatic pathology and the influence of social factors. An experimental study was conducted on 30 animals (laboratory white male mice of eight weeks of age). RA was simulated according to the method of Ikuo Kato in the modification proposed previously [6,7] using bovine serum albumin and picrylchloride. Mice were immunized by regular administration of an emulsion of 4 mg / ml of 1:1 bovine serum albumin and picrylchloride. Animals were injected with 0.1 ml of the emulsion intrascineously in the back and tail. Starting from day

21, the degree of arthritis induced by visual assessment of the paws and the surrounding area was evaluated every 5 days. Ankle edema was measured using digital calipers. Each paw was assessed on a scale from 0 to 4 as follows: 0 without visible changes; 1 - erythema and slight swelling; 2 - erythema and edema, extending to the ankles and one or two toes; 3 - erythema and edema, extending to the metatarsal joints and more than two toes; and 4 - ankylosing deformity with joint swelling. The scores for each paw were added to obtain a cumulative score from 0 to 16. Last stage (ankylosing deformity with joint swelling) was not obtained in any animal. Thus, animals with a formed RA were counted when there were balls from 4 to 12 (in 2 animals, the sum of points was less than 4 and they were excluded from conducting morphological studies). Further, the animals (28 mice) were divided into 3 groups (I, II, III) according to severity by principle described below (in the results of the study) depending on the sum of the points. Another 10 intact animals served as a control group. The animals were taken out of the experiment by the method of cervical dislocation in accordance with the Helsinki Declaration [8], European Convention for the protection of vertebrate animals (18.03.1986), European Economic Society Council Directive on the Protection of Vertebrate Animals (24.11.1986) after approval from the Regional Ethical Review Board at State Establishment "Dnipropetrovsk Medical Academy" protocol №1 (15.01.2016).

The specimens of soft tissues of the oral cavity were stained with hematoxylin and eosin [2], according to van Gieson, according to Rego after the routine proceeding. The microscopic study was performed on a microscope "Olympus BX-41" with subsequent processing by the program "Olympus DP-soft version 3.2". Morphometric studies were performed in the gingival zone which was chosen for morphological interpretation. Volumes of specific vascular density in microcirculatory bed (MCB), specific density of connective tissue in lamina propria and specific area of tissue with ischemia were estimated for each group (%). Black area of tissue was detected as area of ischemia is slides staining according to Rego.

Statistical analysis of the study results was performed on a personal computer using Microsoft Excel and Statistica-10 database software. The criteria of non-parametric statistics were used in order to assess the significance of differences in sample populations. Statistical comparison was performed using Mann-Whitney test for statistical analysis. Spearman's rank correlation coefficient (r) was counted for measure of the strength of relationship between paired data [15]. The accepted level of significance was p<0.05.

Results and their discussion. As a result of the performed experiment, we obtained 28 animals with visual assessment of the severity of induced arthritis of the paws and the adjacent area, which amount of balls was from 4 to 12. As mentioned above, the animals visually revealed the following changes: erythema and slight swelling; erythema and edema extending to the ankles and one or two toes; erythema and edema extending to the metatarsal joints and more than two fingers, while ankylosing deformity with swelling of the joint we were not able to achieve either the water case. Changes were uniform in the limbs in all selected animals, i.e. there were no animals that had 0 points on one limb and 2 or more points on the other limb. Changes in all four paws differed by no more than 1 point. There is principle of the final distribution of animals for the studied groups in Table 1.

Examination of the oral cavity of group of animals with simulated rheumatoid arthritis has revealed that the mucous membrane is pale, compacted, and no focal changes in the visible tissues. Modeling of rheumatoid arthritis did not change the microscopic organization of the oral mucosa and the oral mucosa is covered with a multi-layered squamous non-squamous epithelium with the exception of the gum surface. Already in the study of drugs stained with hematoxylin and eosin, there is a non-uniform thickness of the squamous epithelium, where on one side there are areas of its thinning to one or two rows of cells, on the other side of the zone of uneven thickening.

Signs of inflammatory changes, which are characterized by the presence of both focal and diffuse clusters of inflammatory cells among which lymphocytes predominate have been identified in groups with RA modeling. The presence of superficial damage of epithelial cells up to the appearance of single erosive defects is noted. Most epithelial cells of the superficial layer are characterized by pronounced vacuolization of the cytoplasm, which is commonly regarded as a manifestation of hydropic dystrophy. Basal epithelial cells are high, narrow, which, apparently, is a consequence of their proliferative activity. At the same time in all layers of the epithelium there is a moderately pronounced edema.

Morphofunctional state of the MCB has been changed in all groups with RA modeling in comparison with the control group with pronounced disorder observed in the vessels of the periodontium. The vasculature has been characterized by uneven blood filling with background of desolate vessels that have fallen lumens and presence of sharply expanded blood-filled capillaries. Mucoid and fibrinoid swelling have been observed. Endotheliocytes are flattened more often, with signs of desquamation. Simultaneously signs of sclerotic processes have been noted in perivascular space in lamina propria. Vascular density of MCD is decreased with depending of RA severity from 17.28±1.91% to 5.76±1.01% (table 2) according to morphometric studies in comparison with the intact group (p<0.05). Spearman's rank coefficient indicates a strong correlation relationship between specific densities of MCB vessels and RA severity (r=0.74).

Important data have been revealed in lamina propria of the mucosa with muscular lamina involvement in slides stained according to Rego: the intensely stained black or dark gray foci have been detected in different tissue structures that are an indicator of ischemia development. Spearman's rank coefficient indicates a strong correlation relationship between ischemic area and RA severity (r=0.72). It could be connected with changes in MCB and Spearman's rank coefficient confirms it (r=0.82). Development of sclerotic changes in the lamina propria of the mucosa could be consequences of described changes with increased area of connective tissue from 21.37±2.82% to 34.97±2.26 % in severe cases. Spearman's rank coefficient proves connection of described processes (r=0.71).

As result of our work we can corroborate that the pathogenesis of RA includes changes in MCB and connective tissue that is important for oral mucosa as it had been described in previously published woks [4, 13]. Disorganization of connective tissue, disturbance of MCB development, consequences of ischemic injuries are important for development of periodontitis. We have shown that vascular dysfunction may link development of hypoxia with activation of connective tissue that leads to sclerotic changes in oral mucosa. Sclerotic process is final stage of oral mucosa transformation as hypoxic injuring of tissue is realized in inflammation with appearance signs of periodontitis as changes in epithelium and lamina propria as it was described before [1,3,19].

Our results are combined with previously published woks about development of periodontal pathology in rheumatoid arthritis. Pathogenesis of periodontal inflammation might involve inhibition of cell death, through the apoptotic factors, due to the DNA damage by the product of catalysis [9,11] with highest levels activity found at sites of chronic inflammation.

Table 1. The distribution of animals in the studied groups

Groups	Options for the distribution of the sum of points on the limbs	Sum of points		
		4-6	7-9	10-12
I	1111 1112 1122	2 3 4		
II	1222 2222 2223		4 4 3	
III	2233 2333 3333			4 3 1
Total	28 animals:	9	11	8

Table 2. Changes of the oral mucosa in different severity of rheumatoid arthritis

Groups / Index	Intact	I	II	III	RA (all)
Specific vascular density MCB (%)	17,28±1,91	12,75±1,06	9,02±0,88*	5,76±1,01*	9,18±1,21*
Specific density of connective tissue (%)	21,37±2,82	25,86±1,18	29,98±1,92*	34,97±2,26*	30,27±1,48*
Specific area of tissue with ischemia (%)	5, 42±0,71	15,19±1,14*	20,70±1,34*	24,14±1,68*	20,01±1,24*

* p < 0.05 significant difference between groups with RA modeling and intact animals.

We suggest and support [18] that a synthesis of current and emerging therapeutic interventions might provide the basis for an improved strategy aimed at preventing MCB injuries that is logical and attractive, but requires further investigation in oral cavity relation. Continue of performed investigation could be connected with enhancement of microcirculatory function in RA and reducing hypoxic influence [11,17]. Inflammatory process in the oral cavity could be characterized by morphological picture with inflammatory, degenerative, microcirculatory changes which are accompanied by disturbance of nitric oxide synthase with significant disturbance of it activity [18] and changes in antioxidant balance [9] that also could be used in creation of preventive measures directed for correction of oral status patients with rheumatoid arthritis.

Conclusion.

1. Disturbance of oral mucosae microvasculature is formed in rheumatoid arthritis with strong correlation relationship between specific densities of microcirculatory bed vessels and rheumatoid arthritis severity (r=0.74).

2. Development of ischemic area indicates strong correlation relationship between ischemic area and rheumatoid arthritis severity also (r=0.72) and it could be connected with changes in microvasculature (r=0.82). Development of sclerotic changes in the lamina propria of the mucosa could is characterized by increased area of connective tissue from 21.37±2.82% to 34.97±2.26 %.

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SUMMARY

CONSEQUENCES OF MICROSEQUENCES OF MICROCIRCULATORY DISTRURBANCES OF MICROCIRCULATORY DISTURBANCES OF ORAL MUCOSA IN MODELING OF RHEUMATOID ARTHRITIS

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Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by synovial hyperplasia and the

destruction of cartilage and bone with unclear morphogenesis of pathological changes in oral cavity. Simultaneously microcirculatory disturbance is important link of pathogenesis in many pathological conditions in oral cavity with inflammatory consequences. The aim of this study was to determine importance of microcirculatory disturbance of oral mucosa in modeling of rheumatoid arthritis.

Experimental investigation has been performed with modeling RA on laboratory white male mice according to described before method. Investigated groups were formed according to severity manifestation as ankle changes using digital calipers measuring. The specimens of soft tissues of the oral cavity were stained with hematoxylin and eosin, according to van Gieson, according to Rego after the routine proceeding. Morphometric studies were performed with estimation of volumes of specific vascular density in microcirculatory bed, density of connective tissue in lamina propria and area of tissue with ischemia.

It was detected that disturbance of oral mucosae microvasculature is formed in rheumatoid arthritis with strong correlation relationship between specific densities of microcirculatory bed vessels and rheumatoid arthritis severity (r=0.74). Development of ischemic area indicates strong correlation relationship between ischemic area and rheumatoid arthritis severity also (r=0.72) and it could be connected with changes in microvasculature (r=0.82). Development of sclerotic changes in the lamina propria of the mucosa could is characterized by increased area of connective tissue from 21.37±2.82% to 34.97±2.26 %.

Keywords: rheumatoid arthritis, oral mucosa, periodontitis, histology, experiment.

РЕЗЮМЕ

ПОСЛЕДСТВИЯ НАРУШЕНИЯ МИКРОЦИРКУЛЯЦИИ СЛИЗИСТОЙ ОБОЛОЧКИ ПОЛОСТИ РТА ПРИ МОДЕЛИРОВАНИИ РЕВМАТОИДНОГО АРТРИТА

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Ревматоидный артрит (РА) представляет собой хроническое воспалительное аутоиммунное заболевание, характеризующееся синовиальной гиперплазией и разрушением хряща и кости с неясным морфогенезом патологических изменений в полости рта. Одновременно с этим нарушение микроциркуляции является важным звеном патогенеза при многих патологических состояниях полости рта с воспалительными последствиями. Целью данного исследования было определение значения нарушения микроциркуляции слизистой оболочки полости рта при моделировании ревматоидного артрита.

Экспериментальное исследование было выполнено путем моделирования РА на лабораторных белых мышах-самцах согласно описанной ранее методике. Исследуемые группы были сформированы в соответствии с выраженностью тяжести в виде изменений лодыжки с использованием цифровых измерителей. Микропрепараты мягких тканей ротовой полости после рутинной проводки окрашивали гематоксилином и эозином, по Рего, по ван Гизону. Морфометрические исследования проводили с оценкой объемов удельной плотности сосудов микроциркуляторного русла, плотности соединительной ткани в собственной пластинке слизистой и площади ткани с ишемией.

Обнаружено, что при ревматоидном артрите формируется нарушение циркуляции слизистой ротовой полости с сильной корреляционной зависимостью между удельной плотностью сосудов микроциркуляторного русла и тяжестью ревматоидного артрита (r=0,74). Развитие ишемии также указывает на сильную корреляционную связь между площадью ишемии и тяжестью ревматоидного артрита (r=0,72), и это может быть связано с изменениями в микроциркуляторном русле (r=0,82). Развитие склеротических изменений в собственной пластинке слизистой оболочки характеризуется увеличением площади соединительной ткани с 21,37±2,82% до 34,97±2,26%.

რეზიუმე

პირის ღრუს ლორწოვანი გარსის მიკროცირკულაციის დარღვევის შედეგები რევმატოიდული ართრიტის მოდელირების დროს

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რევმატოიდული ართრიტი ქრონიკული ანთებითი აუტოიმუნური დაავადებაა,რომელსაც ახასიათებს სინოვიური ჰიპერპლაზია, ძვლისა და ხრტილის დაშლა და პირის ღრუში უცნობი მორფოგენეზის პათოლოგიური ცვლილებების განვითარება. ამასთანავე, მიკროცირკულაციის დარღვევა წარმოადგენს პათოგენეზის მნიშვნელოვან რგოლს პირის ღრუს ბევრი დაავადების პათოგენეზში ანთებითი შედეგებით.

წინამდებარე კვლევის მიზანს წარმოადგენდა პირის ღრუს ლორწოვანი გარსის მიკროცირკულაციის დარღვევის მნიშვნელობის გარკვევა რევმატოიდული ართრიტის მოდელირების დროს.

ექსპერიმენტული კვლევა ჩატარდა რევმატოიდული ართრიტის მოდელირების გზით ლაბორატორიულ თეთრ მამრ ვირთაგვებზე ადრე აღწერილი მეთოდიკით. საკვლევი ჯგუფები შეიქმნა კოჭის მიდამოში განვითარებული და ციფრული გამზომი ხელსაწყოებით შეფასებული ცვლილებების სიმძიმის მიხედვით. პირის ღრუს რბილი ქსოვილების მიკროპრეპარატები იღებებოდა ჰემატოქსილინ-ეოზინით, რეგოს მიხედვით, ვან-გიზონის მიხედვით. მორფომეტრიული კვლევები ჩატარდა მიკროცირკულაციური კალაპოტის სისხლძარღვების ხვედრითი სიმჭიდროვის,ლორწოვანის გარსის საკუთარ ფირფიტაში შემაერთებელი ქსოვილის სიმჭიდროვის და იშემიური ქსოვილის ფართობის შეფასებით.

დადგენილია, რომ რევმატოიდული ართრიტის დროს პირის ღრუს ლორწოვან გარსში ვითარდება ცირკულაციის დარღვევა ძლიერი კორელაციური კავშირით მიკროცირკულაციური სისხლძარღვების ხვედრით სიმჭიდროვესა და რევმატოიდული ართრიტის სიმძიმეს შორის (r=0,74). იშემიის განვითარება ასევე მიუთითებს ძლიერი კორელაციური კავშირის შესახებ იშემიის ფართობსა და რევმატოიდული ართრიტის სიმძიმეს შორის (r=0,72) და ეს შესაძლოა დაკავშირებული იყოს ცვლილებებთან მიკროცირკულაციურ კალაპოტში (r=0,82). სკლეროზული ცვლილებები ლორწოვანი გარსის საკუთარ ფირფიტაში ხასიათდება შემაერთებელი ქსოვილის ფართობის ზრდით 21,37±2,82%-დან 34,97±2,26%-მდე.