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EXPERIMENTAL APPROVAL OF THE APPLICATION OF BIOFLAVONOID ANGIOPROTECTOR FOR COMPENSATION OF THE INFLUENCE OF HORMONAL CONTRACEPTIVES ON PARODONT

Abstract. *In the pathogenesis of periodontal diseases of women, an imbalance of sex hormones is important. It is considered as one of the provocative factors of periodontal disease. According to the literature, oral contraceptives may cause changes in periodontium similar to changes in puberty and pregnancy. For the correct choice of the method of treatment of these lesions, it was necessary to conduct an experimental investigation on animals. Aim. To determine the characteristics of the periodontal disease of women taking oral hormonal contraceptives for the purpose of planning pregnancy, determine the possible periodontoprotective effect of bioflavonoid angioprotector when using a hormonal contraceptive. Material and methods. A survey was conducted on 110 women, the main group was 80 women taking oral contraceptives, control 30 women who did not take them. To quantify the state of periodontal tissues, the Schiller-Pisarev test, the Green-Vermillion Hygiene Index, the Gingival Index (GI) by H. Loe, J. Silness, the Bleeding Index by H.R. Muhlemann, the index PMA by S. Parma, the index of the dental plaque of interdental gaps (API) by Lange. As a contraceptive used the drug "Yarina", containing ethinyl estradiol and drospirenone. As a bioflavonoid angioprotector, the drug Normoven was used, containing diosmin and hesperidin. Studies were performed on white female rats that received daily per os contraceptives at a dose of 2 µg / kg (ethinyl estradiol) and drospirenone at a dose of 200 µg / kg. The number of rats simultaneously received an angioprotector at a dose of 80 mg / kg (calculated on bioflavonoid). The duration of the experiment is 30 days. Blood cell count, biochemical parameters of blood serum and gums (urease, lysozyme, elastase, catalase, MDA), as well as periodontal bone tissue (phosphatase, calcium, protein) were determined. The degree of atrophy of the alveolar process was determined morphometrically. Results. The investigation showed that inflammatory and degenerative-inflammatory diseases of the periodontal tissue were detected in 78 (97.5%) women in the primary and in 28 (93.3%) women in the control group. They found an unsatisfactory state of oral hygiene in both groups. In 70 (89.7%) women in the primary group and 23 (82.1%) controls, chronic catarrhal gingivitis was detected; Generalized periodontitis was detected in 8 (11.3%) women in the primary group and in 5 (18.9%) women in the control group. Established with the introduction of a contraceptive development of inflammation in the gum, a decrease in the mineralizing activity of the bone (AP / CF) and an increase in the degree of atrophy of the alveolar process. Simultaneous introduction of an angioprotector stimulates mineralizing activity and reduces the degree of atrophy of the alveolar process bone. Conclusions Analysis of the data shows a significant level of inflammatory and dystrophic-inflammatory periodontal disease in the examined women. Attention is drawn to a somewhat lesser extent of their prevalence in women taking contraceptives. Bioflavonoid angioprotector "Normoven" has a periodontoprotective effect when using a hormonal contraceptive.*

Key words: *periodontal disease, oral contraceptives, bioflavonoid, angioprotector.*

Introduction. To date, periodontal disease is the most important problem in modern dentistry due to their significant distribution and treatment difficulties [3, 5, 9, 19, 21, 29].

According to the literature, oral contraceptives may cause changes in periodontia to be similar to changes in puberty and pregnancy [8, 25, 28]. This is manifested by an increase in propensity to gingivitis, an increase in the depth of pockets when sensing. Prolonged use of oral contraceptives can accelerate the progression of periodontal disease [24, 27]. Some authors believe that low doses of estrogen and progesterone, widely used in modern contraceptives, have little effect on periodontal tissue [27]. Other authors state an increase in the depth of periodontal pockets, a violation of the integrity of epithelial attachment, and an increase in the level of gingival index (GI) six months after taking oral contraceptives [23].

In our previous experimental work [2], it was found that in rats receiving hormonal contraceptive "Yarina" (containing ethinylestradiol and drospirenone) for 30 days, development of gingivitis and systemic inflammation was observed against the background of a decrease in the neutrophil and eosinophil content in the blood and an increase in gums of the antioxidant-prooxidant API index.

Objective. Taking into account certain contradictions in literary data, it was interesting to determine the effect of oral contraceptives on the state of women's periodontal tissues and to experimentally substantiate the methods of treating these lesions of periodontal disease.

Materials and methods. To achieve the goal, a clinical-radiological examination of 110 women was conducted. The main group was 80 (72.73%) women who took oral contraceptives for the purpose of planning pregnancy. The control group comprised 30 (27.27%) women who applied for the purpose of rehabilitation at the dental center of the O.O. NMU. Bohomoltsy and university students. Basically, they were patients of the reproductive age from 19 to 35 years old who received at least 1 year of low-dose oral contraceptives containing 0.03 mg ethinylestradiol and 3 mg drospirenone.

The presence of inflammation in the periodontal tissues was determined using the Schiller-Pisarev test [26]. The assessment of the hygienic state of the oral cavity was estimated using the OHI-S OHIM-S Oral Hygiene Index for Green Vermillion [6, 7].

To determine the intensity of inflammation in the periodontal tissues, the Papillary-Marginal-Alveolar Index (PMA) for C.Rarma [20], the Gingival Index (GI) for H. Loe, J. Silness [16], the Bleeding Estimation (PBI) for HR Muhlemann [18], index of dental plaque of interdental gaps (API) for Lange [10]. The condition of bone tissue was determined using orthopantomogram. Diagnosis of periodontal defeat was established by the classification of MF Danilevsky [4].

As a hormonal contraceptive, the combined oral contraceptive "Yarina" was manufactured by the company "Bayer Pharm Pharma AG" (FRG). One tablet contains 30 micrograms ethinylestradiol and 3 mg drospirenone (an analogue of progesterone). The daily estrogen dose was 0.3 µg per rat or 2 µg / kg, and the daily dose of drospirenone was 200 µg / kg.

As a bioflavonoid angioprotector, the drug "Normenen" was used by PJSC "Kiev Vitamin Plant" (Ukraine). Each rat received 14 mg of bioflavonoids (80 mg / kg) per day.

Experiments were carried out on 21 white rat of the Vistar line (females, 5 months, mean live weight 170 ± 6 g), which were divided into 3 groups of the group: 1 – intact rats, 2 received contraceptive, 3 received contraception and "Normoven". Both drugs were injected per os. The duration of the experiment is 30 days. Euthanasia of animals was performed under thiopental anesthesia (20 mg / kg) by total bleeding from the heart.

In the blood, the hemoglobin, erythrocytes, leukocytes and leukocyte formula were determined [1]. The blood serum determined the protein content [17], the activity of urease [11], lysozyme [11], elastase [12], catalase [12], and the content of malodialdehyde (MDA) [12]. The antioxidant-prooxidant index of the API [127] was calculated for the ratio of the catalase and the content of MDA.

The homogenate of the gums was determined by the activity of urease, lysozyme elastase, catalase, MDA content and API index. According to the ratio of relative activity of urease and lysozyme, the degree of dysbiosis according to AP Levytsky [13] was calculated.

The homogenate of the alveolar appendix of the lower jaw was determined by the activity of alkaline (LF) and acidic (KF) phosphatase [14],

calcium content [13, 14] and protein. The ratio of LF and KF calculated the mineralization index (MI) [15], and the ratio of calcium and protein content - the degree of mineralization [13].

The experiments were subjected to standard processing [22].

Results and their discussion. The analysis of the results showed that 78 (97.5%) women in the primary and 23 (76.67%) women in the control group had inflammatory and degenerative-inflammatory diseases of the periodontal tissue. Among inflammatory diseases, chronic catarrhal gingivitis was more often diagnosed with mild to moderate severity. It was detected in 70 (89.7%) women in the main group and 17 (56.67%) in the control group. Their main complaints were an unpleasant sensation in the gums, gummy edema. In the main group, they were noted in 37 (52%) women. Complaints of bleeding gums were noted in 24 (34.3%) women. In the control group, gastric edema was noted in 16 (69%) women, bleeding gums - in 7 (43.8%) women.

Dystrophic-inflammatory diseases of periodontal tissues were detected in 10 (12.5%) women of the main group, among them 7 (8.75%) women were diagnosed with generalized periodontitis of the initial stage of chronic course, and in 3 (3.75%) women generalized periodontitis And the degree of chronic flow. In the control group, dystrophic-inflammatory diseases were

detected in 2 (6.67%) women, in particular, generalized periodontitis of the initial degree of chronic course was detected. The oral hygiene estimates for women in the main group fluctuated within the 1.2-3.0 OHI-S index, an average of 2.1, and the ARI index ranged from 14% to 100% - an average of 37.7%. In the control group, the values of these indices were respectively 2.4 (index (OHI-S) and 31.3% - API index.

This is largely attested to the poor state of oral hygiene in both groups. The PMA index in the main group ranged from 34.8%, while in the control group it was 29.2%. The gut bleeding of the gums, which was determined by the RVI index, was for women of the main group within the range of 0.95 points and, respectively, 0.78 points for women in the control group. The gingival index (GI) for women in the main group was 1.8 points, while in the control group it was 2.2 points.

Thus, a comparative analysis of the state of periodontal tissues in women in the examined groups indicates an unsatisfactory state of their marginal gums due to unsatisfactory state of hygiene.

Attention is drawn to a slightly higher level of prevalence in women taking contraceptives. They also noted a higher level of lesions of periodontal tissues with dystrophic-inflammatory diseases (generalized periodontitis).

The results of the cytological analysis of blood are presented in Table 1, from which it is evident

Table 1

Effect of angioprotector "Normoven" on cytological parameters of blood of rats, who received a hormonal contraceptive

Indexes	Intact	Contraceptive	Contraceptive + Normoven
Hemoglobin, g / l	164±2	156±8 p>0,05	155±4 p<0,05; p ₁ >0,8
Erythrocytes, x 10 ¹² /l	5,65±0,35	5,33±0,12 p>0,1	5,47±0,21 p>0,3; p ₁ >0,3
Leukocytes, x 10 ⁹ /l	5,18±0,50	5,35±0,44 p>0,5	3,33±0,09 p<0,05; p ₁ <0,05
Neutrophils (N), %	30,7±4,1	25,0±2,0 p>0,05	16,0±3,7 p<0,05; p ₁ <0,05
Lymphocytes (L), %	65,0±5,0	70,0±0,8 p>0,05	80,0±4,7 p<0,05; p ₁ <0,05
Monocytes, %	0,75±0,75	2,0±0,5 p>0,05	0,5±0,5 p>0,05; p ₁ <0,05
Eozinophils, %	3,5±1,7	3,0±1,7 p>0,5	3,5±1,0 p=1; p ₁ >0,5
Lymphocytic index (L/N)	2,12±0,18	2,80±0,23 p<0,05	5,00±0,49 p<0,01; p ₁ <0,05

Notes: p – compared to gr. "Intact"; p₁ – in comparison with gr. "Contraceptive".

that the level of leukocytes, monocytes, eosinophils and the proportion of neutrophils is significantly lower in rats receiving normal, but the proportion of lymphocytes and lymphocytic index (the ratio of lymphocytes and neutrophils) is increasing.

Table 2 presents the results of the determination of a number of biochemical parameters of blood serum in rats. It is seen that in rats, which received normal, significantly increases the activity of urease, but the activity of catalase decreases.

Table 3 presents the results of biochemical studies in the gums of rats. From these data it is clear that normalization increases the activity of urease, but significantly reduces the activity of lysozyme.

Table 4 shows the state of bone marrow periodontal disease. It is seen that the administration of normotensive increases the activity of LF, somewhat reduces the activity of CF, resulting in a significant increase in mineralization index (MI).

Table 2

Effect of angioprotector "Normenen" on biochemical parameters of blood serum of rats, who received a hormonal contraceptive

Indexes	Intact	Contraceptive	Contraceptive + Normoven
Protein, g/l	63,0±1,0	65,0±0,9 p>0,05	62,5±2,1 p>0,5; p ₁ >0,05
Urease, ukat/L	0,62±0,17	0,77±0,11 p>0,3	1,49±0,39 p<0,05; p ₁ <0,05
Lysozyme, unit / l	80±2	77±5 p>0,3	66±4 p<0,05; p ₁ >0,05
MDA, mmol / l	0,96±0,09	1,11±0,07 p>0,05	0,98±0,09 p>0,5; p ₁ <0,05
Elastase, ukat/l	115±5	142±5 p<0,05	148±14 p<0,05; p ₁ >0,3
Catalase, ukat/l	0,56±0,02	0,52±0,02 p>0,05	0,36±0,01 p<0,01; p ₁ <0,01
API	5,83±0,20	4,68±0,19 p<0,05	4,65±0,15 p<0,05; p ₁ >0,7

Notes: p – compared to gr. "Intact"; p₁ – in comparison with gr. "Contraceptive".

Table 3

Effect of angioprotector "Normoven" on the biochemical parameters of gums in rats, who received a hormonal contraceptive

Indexes	Intact	Contraceptive	Contraceptive + Normoven
Urease, ukat/kg	0,48±0,04	0,33±0,03 p<0,05	0,44±0,03 p>0,3; p ₁ <0,05
Lysozyme, unit / kg	246±22	231±27 p>0,3	155±21 p<0,05; p ₁ <0,05
MDA, ukat/ kg	16,7±1,4	13,9±0,7 p<0,05	15,3±1,6 p>0,3; p ₁ >0,3
Elastase, ukat/kg	44,6±2,6	56,9±5,2 p<0,05	63,3±3,8 p<0,05; p ₁ >0,05
Catalase, ukat/ kg	8,1±0,3	9,0±0,4 p>0,05	8,7±0,5 p>0,05; p ₁ >0,3
API	4,85±0,25	6,47±0,34 p<0,05	5,69±0,35 p>0,05; p ₁ >0,05

Notes: p – compared to gr. "Intact"; p₁ – in comparison with gr. "Contraceptive".

Table 4

Effect of angioprotector "Normoven" on biochemical parameters of bone tissue of the alveolar appendix of the mandible of rats, who received a hormonal contraceptive

Indexes	Intact	Contraceptive	Contraceptive + Normoven
LF, ukat/ kg	224,6±5,7	208,0±5,4 p<0,05	234,0±6,1 p>0,1; p ₁ <0,05
KΦ, ukat/ kg	3,2±0,3	3,3±0,3 p>0,5	2,9±0,3 p>0,3; p ₁ >0,3
Calcium, moles /kg	2,27±0,17	2,17±0,14 p>0,3	2,07±0,28 p>0,3; p ₁ >0,3
Protein, g / kg	14,57±0,94	13,86±1,15 p>0,3	15,44±1,08 p>0,3; p ₁ >0,3
MI	70,2±2,6	63,0±3,0 p>0,05	80,7±4,1 p<0,05; p ₁ <0,05
CM, g/g	6,23±0,45	6,26±0,48 p>0,5	5,36±0,25 p<0,05; p ₁ >0,05

Notes: p – compared to gr. "Intact"; p₁ – in comparison with gr. "Contraceptive".

The obtained data testify to a certain periodontoprotective effect of normoven.

Conclusions. 1. Women who took oral contraceptives revealed a higher incidence of periodontal disease, requiring appropriate correction of their treatment.

2. Experimental studies have shown that the use of a bioflavonoid angioprotector "Normoven" stimulates the mineralizing activity of periodontal bone tissue, reduces the atrophy of the alveolar appendix.

3. These changes occur on the background of lowering the level of lymphocytes in the blood and increasing the degree of dysbiosis in the gums.

References:

1. Bazarnova MA. *Rukovodstvo po klinicheskoy laboratornoy diagnostike [Manual of Clinical Laboratory Diagnostics].* Ch. 1. Kiyev: Vyscha shkola;1981. 55 p.

2. Borysenko AV, Levytskyi AP, Vatankha TV. *Vlyaniye hormonalnoho kontratseptyva na sostoye parodonta krysa. Topical Issues of Science and Education.* 2017;4:21-5.

3. Chyzhevskiy IV. *Klinichne ta hiiienichne obhruntuvannia profilaktyky kariiesu zubiv u ditei u promyslovo rozvynutomu rehioni [avtoref].* Kyiv: 2010. 38 p.

4. Danilevskiy N.F. *Sistematika bolezney parodonta. Vlsnik stomatologiyi.* 1994;(1):17–21.

5. Danilevskiy NF, Sidelnikova LF, Tkachenko AG. *Rasprostranennost osnovnykh*

stomatologicheskikh zabolevaniy i sostoyanie gigenyi polosti rta u naseleniya razlichnykh regionov Ukrainyi. Sovremennaya stomatologiya. 2006;(2):14-6.

6. Green JC, Vermillion JR. *The oral hygiene index: A method for classifying oral hygiene status. J Am Dent Assoc.*1960;61:172-5.

7. Green JC, Vermillion JR. *The simplified oral hygiene index. J Am Dent Assoc.* 1964;68:7-10.

8. Kalkwarf KL. *Effect of oral contraceptives therapy on gingival inflammation in humans. Journal of Periodontology.* 1978;32:560-3.

9. Kosenko KM. *Epidemiolohiia osnovnykh stomatolohichnykh zakhvoriuvan u naselennia Ukrainyi i shliakhy yikh profilaktyky. [avtoref].* Kyiv: 1994. – 45 p.

10. Lange DE. *Die Anwendung von Indices zur Diagnose der Parodontopathien. Dtsch Zahnarzl Z.* 1978;33:108-11.

11. Levitsky AP, Makarenko OA. *Selivanskaya I. A. [i dr.]. Fermentativnyy metod opredeleniya disbioza polosti rta dlya skringa pro- i prebiotikov: metodicheskie rekomendatsii [Enzymatic methods for determination of oral dysbiosis for screening pro- and prebiotics: method guidelines].* Kiev: GFC; 2007. 22 p.

12. Levitsky AP, Denga OV, Makarenko OA. *Biokhimicheskie markery vospaleniya tkaney rotovoy polosti: metodicheskie rekomendatsii [Biochemical markers of inflammation of oral*

- cavity tissue: method guidelines]. Odessa: KP OGT; 2010.16 p.
13. Levitsky AP, Denga OV, Selivanskaya IA. The method of estimation of the degree of dysbiosis (dysbacteriosis) of organs and tissues. Patent of Ukraine 43140. IPC (2009) G01N 33/48. Application number u 200815092. Date of filling: 26.12.2008. Publ.: 10.08.2009. Bul. № 15.
 14. Levitsky AP, Makarenko OA, Denga OV. Eksperimentalnye metody issledovaniya stimulyatorov osteogeneza: metodicheskie rekomendatsii [The experimental methods of the study of osteogenesis stimulators]. Kiev:GFK; 2005. 50 p.
 15. Levitsky AP, Makarenko OA, Khodakov IV. The enzymatic method of the estimation of the state of osseous tissue. Odeskiy medychnyy zhurnal. 2006;3:17-21.
 16. Löe H, Silness J. Periodontal disease in pregnancy. Acta Odontol Scand. 1963;21:533-6.
 17. Lowry ON, Rosebrought NJ, Porr AL. Protein measurement with Folin phenol reagent. J Biol Chem. 1951;193:265-75.
 18. Mühlemann HR, Son S. Gingival sulcus bleeding — a leading symptom in initial gingivitis. Helv Odontol Acta. 1971;15:107-10.
 19. Ostapko OI. Naukove obgruntuvannia shliakhiv ta metodiv profilaktyky osnovnykh stomatolohichnykh zakhvoriuvan u ditei v rehionakh z riznym rivnem zabrudnennia dovkillia. [avtoref]. Kyiv: 2011. 38 p.
 20. Parma C. Parodontopathin. Leipzig: 1960. 203 p.
 21. Pavlenko OV, Antonenko MYu, Sidelnykov PV. Planuvannia likuvalno-profilaktychnoi dopomohy khvorym na heneralizovanyi parodontyt na osnovi otsinky ryzyku urazhennia parodontu. Sovremennaia stomatolohyia. 2009;1:56-61.
 22. Rebrova OYu. Statisticheskiy analiz meditsynskikh dannyykh. Primeneniye paketa prikladnykh program «Statistika» [Statistical analysis of medical data. Application of the software package "Statistics"]. Moskva: Media Sfera; 2002.
 23. Seck-Diallo A, Cissé ML, Benoist HM, Diouf A, Ahnoux-Kouadio A, Diallo T, Sembene M, Moreau JC, Diallo PD. Periodontal status in a sample of Senegalese women using hormonal contraception. Odontostomatol Trop. 2008;31(121):36-42.
 24. Soory M. Hormonal factors in periodontal disease. Dent Update. 2000;27(8):380-3.
 25. Sperber GH. Oral contraceptive hypertrophic gingivitis. J Dent Assoc S Afr. 1969;24:37-40.
 26. Svrakov D, Atanasova E. Parodontopatii (etiologiya, klinika i lechenie). Sofiya: Gosudarstvennoe izdatelstvo «Meditsina i fizkultura»; 1962. 212 p.
 27. Taichman LS, Eklund SA. Oral contraceptives and periodontal diseases: Rethinking the Association based upon analysis of national health and nutrition examination survey data. J Periodontol. 2005;76:1374-85.
 28. Tilakaratne A, Soory M, Ranasinghe A. Effects of hormonal contraceptives on the periodontium, in a population of rural Sri-Lankan women. Journal Clinical Periodontology. 2000;27:753-7.
 29. Tkachenko AH. Osoblyvosti klinichnoho perebihu, likuvannia ta profilaktyky heneralizovanoho parodontytu u osib molodoho viku 18-25 rokov [avtoref]. Kyiv: 2006. 20 p.