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ERYTHROCYTES AND THEIR VALUE IN PATHOGENESIS OF PARODONTIUM INFLAMMATORY DISEASES

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Abstract

The value of experimental animals (white rats) erythrocytes in pathogenesis of parodontium inflammatory disease was searched. The dynamics of Hb changes, erythrocytes number and hematocrit were surveyed. Periodontitis was moulded by special diet. When periodontitis developed oxidative stress and enhanced generation of reactive oxygen species were observed as well as changes in the number of erythrocytes, hemoglobin and erythrocyte indices. The latter were caused by membrane destructive processes in erythrocytes, decrease in their absolute number due to hemolysis, and changes in hematocrit due to blood redistribution.

Key words: erythrocytes, hemoglobin, hematocrit, periodontitis.

Erythrocytes are blood corpuscles that are unique because they constantly contact with oxygen, transporting it to all tissues, but do not use oxygen for themselves. Erythrocytes, possessing exclusively anaerobic metabolism, do not contain the main oxygen-consuming systems: mitochondria and endoplasmic net [2, 6]. The formation of energy in them occurs through substrate phosphorylation of ADP in glycolysis reactions, they are not capable of protein synthesis and do not have DNA. On the other hand, erythrocytes are cells that constantly contain oxygen in hemoglobin and are as resistant as possible to the damaging

effects of its active forms [5]. Permanent interaction with oxygen causes auto-oxidation of hemoglobin of red blood cells with the formation of superoxide radicals, as well as other reactive oxygen species, mainly hydrogen peroxide and hydroxide radicals [1, 3, 4, 7, 9]. To protect against them in erythrocytes, there is a powerful system of anti-peroxide and anti-radical protection: SOD, catalase, glutathione peroxidase, glutathione reductase, and others.

The objective: to appraise the significance of erythrocytes in the pathogenesis of periodontitis.

Materials and methods. Erythrocytes were the object of the study. Experimental periodontitis in rats was caused by the use of a light consistency diet with high carbohydrate content from O. I. Evdokimov in O. I. Sukmansky and O. A. Makarenko [8] modification. The diet's formulation: wheat flour - 34%, dry non-fat cow milk -30%, starch - 20%, sugar - 15%, cooking salt - 1%. In order to accelerate the simulation, in addition to the diet of rats, reoxidized sunflower oil (1 ml per a rat) was added, which was obtained by heating it in the presence of 2% copper sulfate for 6-10 hours until the peroxide value exceeds 30 units.

Blood was taken from the tail vein of rats in 10-12 hours after the last meal. As an anticoagulant, 7.2 mg of K2EDTA was used (in terms of rats' body mass according to Rybolovlev Yu.P.) [10]. The working suspension of erythrocytes was obtained by means of a three-fold wash with a solution of low ionic strength Liss (manufacturer "Hematologist Ltd.") with a centrifugation regime at 2700 rpm for 8 minutes. The prepared suspension of erythrocytes was diluted in a ratio of 1: 200. Blood parameters of hemograms were determined on the automatic hematologic analyzer MINDRAV BC-3000. The study of hemogram parameters included the determination of the number of erythrocytes (NE), hemoglobin (Hb), hematocrit number (Ht), expressing the content of erythrocytes in the total blood volume (normally the number of hematocrit is 0.36 - 0.48 g / l). The average hemoglobin content in each erythrocyte was determined by the color index, which was calculated by dividing the number of hemoglobin in units of Sali on the doubling of the first digits of the number of red blood cells (with their number exceeding one million) [11]. Erythrocyte indices corresponded to the average volume of erythrocytes (VEav). Morphometry of peripheral blood red blood cells was investigated for forming-up histograms of erythrocytes distribution by their content and Hb, erythrocytes geometric parameters and their statistical characteristics [12].

All experiments were carried out in compliance with the International Principles of European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (Strasbourg, 1986). All manipulations that caused pain were

performed with ethanal-sodium anesthesia (40 mg / kg body weight intraperitoneally) [11]. To exclude the influence of seasonal and daily oscillations (Zolotukhin S. E., et al., 1991) on the indicators studied, the main researches were conducted in the autumn-winter period, in the morning.

The statistical processing of the data obtained was carried out using the Statistica for Windows 8.0 software package. Student's t-criterion and correlation analysis were used. The results were considered valid at $p < 0.05$.

Results of the research and their discussion. It has been established that during periodontitis in the body of rats there is an increase in the formation of active forms of oxygen in all terms of the study.

The number of TBA-active products (TBA-AP) increased by 1.8; 4.2; 3,3 and 2,1 times in 1, 3, 6 hours and in a day. We believe that the source of oxygen active forms generation is blood copcuscles, and the oxidation substrate is membrane blood cells, as well as plasmatic lipoproteins. Investigation of the qualitative and quantitative composition of blood cells in our experiment allowed to find significant shifts of all indicators. Thus, the number of peripheral blood red blood cells in the first 30 minutes of the experiment increased by 20.9% relative to control, and then decreased and remained 8% -10% less than in the control for up to 150 minutes. Subsequently, in 3 hours after the beginning of the experiment, there was a sharp decrease of NE (2.2 times), which slightly increased in a day. However, in general, NE concentration in this period remained significantly lower than in control (by 20%). Dynamics of hemoglobin concentration changes were similar (Table 1).

*Thus, Hb level increased by 8% in 30 min after the beginning of the experiment, and then decreased by 10% -15% for the next 120 minutes. By 180 minutes of the experiment NE’s reduction reached 30% relative to control. After 6 hours and in a day, Hb concentration remained 25% and 30% lower than control, respectively.

The maximum decrease in NE and Hb in the blood timing-wise coincided with the peak increase in TBA-active products, which most likely indicates active hemolysis of erythrocytes in this period. The results obtained are in agreement with the data of the literature that in the various influences on erythrocytes, in particular, hydrogen peroxide, there is oxidation and denaturation of hemoglobin (the formation of so-called Heinz bodies), which is accompanied by the release of heme / gemin - ferriprotoporphine IX [13]. In this case, exogenous hemin is able to be easily embedded in the membrane, destabilizing it and causing hemolysis [8]. Changes in NE and Hb may also be due to fluctuations of the hematocrit number indicating redistribution of blood and hemodynamic impairment. The rate of

hematocrit in experimental animals increased sharply in the first 30 minutes of the experiment (by 26% relative to control), indicating blood clotting; in 60 min Ht returned to normal. However, after 3 hours, Ht declined sharply, amounting to only 57% of the control level, and practically did not recover to the ascending level in a day, remaining at 24% compared with control. The average volume of erythrocytes did not change significantly during the day after the experiment beginning. At the same time, 90 and 180 minutes after, VEav was slightly reduced and only in a day recovered to the level of control.

Table 1

Dynamics of hemoglobin changes, number of red blood cells and hematocrit in periodontitis

($X \pm S_X$)

Period of observation	Indicators		
	NE, 10^{12}	Hb, g/l	Ht, l/l
control	6.75 ± 0.12	116.0±3.6	36.5 ± 1.8
30 min	8.23 ± 0.25 *	125.0 ± 2.9 *	46.0 ± 1.2 *
60 min	6.1 ± 0.18 *	104.0 ± 2.2 *	33.6 ± 1.7
90 min	6.3 ± 0.15*	98.0 ± 4.1*	33.0 ± 2.0
120 min	6.5 ± 0.11 *	100.0 ± 3.7 *	35.1 ± 2.2
150 min	6.4 ± 0.12 *	99.0 ± 3.8 *	35.0 ± 2.2 *
180 min	4.1 ± 0.46 *	81.0 ± 4.0 *	23.0 ± 3.0 *
6 hs	4,0 ± 0,51 *	88,0 ± 3,6 *	21,0 ± 2,1 *
24 hs	4.8 ± 0.44 *		28.0 ± 1.1 *

Notes:

1. n – in every 30 min. = 3 rats;
2. n - total number - 27 animals;
3. * p - <0,05 in relation to control.

The average content of hemoglobin in the erythrocyte (HbEav) varied during the experiment wavelike: 30 minutes after the beginning of the experiment this indicator declined by 12% relative to control and was similar to that 150 minutes after the beginning of the experiment (by 10%). A day later, the average content of hemoglobin in the erythrocyte was almost restored to the ascending level. The average concentration of hemoglobin in erythrocytes was similar in dynamics, while the changes in this index were reliable in terms of control in all terms of observation. The smallest value of CHbEav is marked after 30 minutes after the introduction of sodium nitrite, the most - in 6 hours.

The results of erythrocytic indices study showed (Table 2) that in periodontitis, the average volume of erythrocyte does not significantly change, while the average content of hemoglobin in erythrocyte - HbEav - and the average concentration of hemoglobin in

erythrocytes - CHbEav - decrease in 30 minutes, and then increase (in 3 and 6 hours). At the same time, in 24 hours, HbEav reaches the level of control, and CHbEav remains low.

Table 2

Dynamics of erythrocytic indices changes in periodontitis (X ± SX)

Period of observation	Indicators		
	NEav	HbEav	CHbEav
Control	54.1 ± 1.2	17.2 ± 0.1	317.0 ± 2.7
30 min	55.9 ± 1.6	15.2 ± 0.3 *	271.0 ± 6.8 *
60 min	55.1 ± 1.9	17.1 ± 0.4	309.0 ± 10.1
90 min	52.4 ± 1.5	15.5 ± 0.4 *	297.0 ± 7.9 *
120 min	53.9 ± 1.5	15.4 ± 0.1*	285.0 ± 11.0 *
150 min	55.9 ± 1.2	15.0 ± 0.4 *	268.0 ± 7.9 *
180 min	56.0 ± 1.3	19.8 ± 0.6 *	352.0 ± 6.6 *
6 hours	52.5 ± 1.2	22.0 ± 0.2 *	419.0 ± 6.0 *
24 hours	58.0 ± 1.3 *	17.0 ± 0.1 *	292.0 ± 6.3 *

Notes:

1. n – in every 30 min. = 3 rats;
2. n - total number - 27 animals;
3. * p - <0,05 in relation to control.

Perhaps slight changes (sizes' reduction) in the volume of red blood cells, which we observed during periodontitis during first 6 hours is due to partial dehydration and compression of cells due to the discovery of calcium-dependent potassium channels (Gardos effect), which occurs under the action of oxidants - products of peroxidation

Our assumption is based on literature data, which shows that the action of oxidizing agents (phenazine methsulphate, tertbutyl hydroperoxide) on red blood cells leads to the activation of calcium-dependent potassium channels. The authors

believe that the activation of Gardos' channels is the general property of the cellular response in oxidative influences [12]. When the amount of red blood cells is reduced, an increase in HbEav and CHbEav takes plaace, while the concentration of hemoglobin in blood decreases. Later on, the decrease in cell volume changes by its increase, owing to plasmolysis due to deep membrane-destructive changes. On erythrocytes' hemolysis indicates a sharp decrease in their number and hemoglobin concentration starting from 180 minutes of the experiment. Thus, we believe that this dynamics of erythrocytic indices is due to membrane-destructive processes in erythrocytes, changes in their absolute number as a result of hemolysis, and changes in hematocrit due to redistribution of blood.

Conclusions:

1. Changes in the number of erythrocytes, hemoglobin and erythrocytic indices in periodontitis take place due to membrane-destructive processes in erythrocytes, a decrease in their absolute number due to hemolysis, and changes in hematocrit due to redistribution of blood.

2. Changes in erythrocytes are oxygen-dependent mechanisms in the pathogenesis of periodontitis.

References:

1. Vladimirov Yu. A. Free Radicals in Biological Systems / Yu. A. Vladimirov // Sorovsky Educational Journal. - 2000. - T.6, No. 12. - P. 13-19.

2. Korkushko O. V. Changes in the oxygen transport function of blood in arterial hypoxemia in elderly and senile people / O. V. Korkushko, A. V. Pisaruk, E. O. Asanov [and others] // Bukovinsky Medical Bulletin. - 2011. - T. 15, No. 3 (59). - P. 200-204.

3. Romanova V. E. Influence of chronic ischemia on the energy exchange of the brain of rats with varying sensitivity to oxygen deficiency / V. E. Romanova, H. N. Chernobayev, V. V. Dudchenko [and others] // Hypoxia in medicine. - 1996. - No. 3. - P. 58.

4. Chesnokova N. P. Molecular-cellular mechanisms of induction of free radical oxidation under conditions of pathology / N. P. Chesnokova, E.V. Ponukalina, M.N. Bizenko // Modern problems of science and education. - 2006. - No. 6. - P. 21-26.

5. Camillo D.G. Oxygen and life span: chronic hypoxia as a model for studying HIF-1α, VEGF and NOS during aging / D. G. Camillo, G. Bianchi, M. Cacchio [et al.] // Respiratory Physiology and Neurobiology. - 2005 - Vol. 147, No. 1. - P. 31-33.

6. Dorthe M. Is there a molecular connection between hypoxia and aging? / M. Dorthe, N. Katschinski // Experimental Gerontology. - 2006 - Vol. 41. - P. 482.

7. Skulachev VP The phenomena of programmed death. Mitochondria, cells and organs: the role of active forms of oxygen / V.P. Skulachev // Sorovsky educational journal. - 2001. - T. 7, No. 6. - P. 4-10.

8. Sukmansky O. I., Makarenko O. A. Experimental model of generalized periodontitis // Herald of Dentistry. - 2006. - № 2. - P. 2 - 3.

9. Kozlyanin N. P. Physiological antioxidant system of the gums and bones of the alveolar process in norm and at pathology / N. P. Kozlyanin: Synopsis. Cand. Biol. Sciences Thesis. - Kiev, 1990. - 16 p.

10. Rybolovlev Yu. P. Recalculation of the Constant of Biological Activity / Yu P. P. Rybolovlev, D. P. Sidlyarov, N. I. Afonin // Sb. "Toxicological aspects of safety of the finished dosage forms". - M.: Medical Book, 1981. - P. 9 - 21.

11. Sernov L. N. Elements of Experimental Pharmacology / L. N. Serov, V. V. Gatsura // - M., Medicine, 2000. - 352 p.

12. Kovach I.V. Biochemical indices of blood serum of rats at experimental gingivitis and caries that caused toxic factors / I.V. Kovach // Bulletin of Dentistry. - 2005. - No. 2. - P. 11-14.

13. Nikol'skaya I. S. Reaction of the blood system to hypoxic hypoxia and circulation of hematopoietic stem cells / I. S. Nikolsky // Environment and Health. - 2012. - No. 2. - P. 79-80.