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Disorders of the antioxidant defense system of the organism in periodontitis and the use of various types of removable prosthetic constructions

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In 20-70% of patients, inflammatory reactions of the mucous membrane and the periodontal complex are observed, associated with the use of removable dentures made of basic plastics. The aim of our research was to investigate the disruption of the antioxidant defense system in rats with experimental bacterial-immune periodontitis under the conditions of using acrylic and nylon bases of removable dentures. Experimental periodontitis was induced in experimental animals by injecting a suspension containing a mixture of microorganisms (Staphylococcus aureus and Streptococcus hemolyticus) based on egg protein into the periodontal tissues. The state of the antioxidant system was assessed by the activity of enzymes such as superoxide dismutase, catalase, ceruloplasmin, glutathione peroxidase, glutathione reductase and reduced glutathione on the 30th day of the experiment both without and with different types of removable denture bases fixed in the oral cavity of experimental animals. In animals with experimental periodontitis and acrylic dentures, increased catalase, superoxide dismutase, ceruloplasmin activity, and decreased glutathione levels were found compared to rats with nylon dentures. The results show differences in the effects of different types of denture bases on the antioxidant defenses of the organism.

Key words: periodontium; periodontitis; antioxidants; oxidative stress; lipid peroxidation; removable prosthetics; prosthesis base; acrylic prosthesis; nylon prosthesis.

INTRODUCTION

In prosthetic dentistry, the issue of biological compatibility of dentures and their effect on oral tissues is relevant [1]. In this regard, of great interest is both the direct impact of orthopedic structures made of various materials on the homeostasis of the oral cavity and periodontal tissue, and the impact of changes in homeostasis on adaptation to dentures [2]. In response to the influence of the prosthesis, chronic pathological processes of periodontal tissues and the mucous membrane of the prosthetic bed, which acts as an entrance gate for both microorganisms and components of the orthopedic structure, may occur [3].

In 20-70% of patients, inflammatory reactions of the mucous membrane are observed, associated with the use of removable dentures made of basic plastics [4]. The causes of its occurrence are mainly due to local factors caused directly by the removable denture and the base material (mechanical, thermal, allergic and toxic irritation) [5]. The periodontal response to removable dentures also depends on individual reactivity [6]. Acrylic plastics are subject to biological destruction in the oral cavity, as a result of which the formed products affect the factors of specific and nonspecific resistance, which are manifested by the suppression of the state of local immunity [7].

Removable dentures should be considered a strong combined irritant [8]. The denture and the base material negatively affect various elements of the oral cavity homeostasis, which is normally strictly balanced. The impact of dentures can be aggravated by violations of their manufacturing technology, especially in pathologies of the protective system of the oral cavity [9].

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As indicated by numerous studies a significant role in the development of stomatitis is played by increased lipid peroxidation and a decrease in antioxidant defense factors [10]. When prosthetics with removable dentures oxidative modification of oral fluid proteins is observed and, as a result, a reduction in the antioxidant activity of saliva and an increase in lipid peroxidation processes [11]. In patients, lipids peroxidation levels in the blood and oral fluid increase, and the activity of antioxidant defense enzymes changes [12].

The aim of our research was to investigate the disruption of the antioxidant defense system in rats with experimental bacterial-immune periodontitis under the conditions of using acrylic and nylon bases of removable dentures.

METHODS

The experiments were conducted on clinically healthy male white rats weighing 150-200 g, maintained under vivarium conditions under hygiene standards and good laboratory practice (GLP).

The experimental animals were randomly selected and divided into four groups: group I – intact animals, control (n = 10); group II – animals with periodontitis on the 30th day of the study (n = 8); group III – animals with periodontitis on the 30th day of the study with acrylic bases (n = 8); group IV – animals with experimental periodontitis on the 30th day of the study with nylon bases (n = 8).

Dentures were manufactured using standard methods: acrylic bases were made by thermal polymerization of the polymethacrylate material "Villacryl H Plus" ("Zhermack", Poland) [13], and nylon bases were made by compression molding of the thermoplastic material "Vertex ThermoSens" ("Vertex", Netherlands) [14]. The orthopaedic structures were designed so as not to cover the occlusal surfaces of the teeth and were fixed on both central incisors of the lower jaw and tightly fitted to the alveolar ridge. Taking into account the physiology of the dentofacial system of experimental animals, daily monitoring and, if necessary, correction of the quality of attachment of the denture bases was carried out.

Experimental bacterial-immune periodontitis in experimental animals was induced by injection of a mixture of microorganisms (Staphylococcus aureus та Streptococcus hemolyticus), suspended in egg white, into periodontal tissue [15]. The components of the cell wall of gram-positive bacteria are lipoteichoic acids, peptidoglycan, and lipoproteins, which are triggers for the development of inflammation through toll-like receptors 2. These molecules are keys in pathogen recognition and triggering of the innate immune system. To enhance the immune response complete Freund's adjuvant rats were simultaneously administered. This procedure was repeated on the 14th day of the experiment to confirm the effectiveness of induction and chronicity of periodontitis of bacterial-immune origin [16]. On the 30th day, the experimental animals were euthanized by exsanguination under anesthesia using sodium thiopental and blood serum was collected to determine the activity of superoxide dismutase, catalase, and the content of the antioxidant defense glutathione.

Determination of superoxide dismutase activity (SOD) was carried out according to the method based on the ability of the enzyme to inhibit the reduction of nitrotetrazolium blue. The determination of catalase activity was based on the ability of hydrogen peroxide to form a stable colored complex with ammonium molybdate, the intensity of which is inversely proportional to the activity of catalase in the substrate under study. The intensity of the color was measured on a SF-46 spectrophotometer at 410 nm. The level of ceruloplasmin in blood serum was determined according to the method based on the study of the optical density of the oxidation products of n-phenylenediamine in the presence of ceruloplasmin. Its amount is proportional to the intensity of the color. The optical density was determined on a SF-46 spectrophotometer at 530 nm and expressed in milligrams per liter (mg/l). The principle of the method for determining the concentration of reduced

glutathione was the interaction of 5,5-dithiobis (2-nitrobenzoic) acid (Ellman's reagent) with the SH-groups of the substrate under study. This formed a thionitrophenyl anion, the amount of which was directly proportional to the content of SH-groups. Glutathione reductase activity was calculated in the control and experimental samples by the difference between the amounts of NADPH2 consumed in the enzymatic reaction of reduction of oxidized glutathione in the centrifuge spectrophotometrically (SF-46, 340 nm). Glutathione peroxidase activity was calculated from the difference between the amount of reduced glutathione in the control (without H_2O_2) and experimental samples and expressed in mmol/min×l [17].

All procedures were carried out in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and the "General Ethical Principles of Animal Experiments" (Kyiv, 2001). The study was approved by the Bioethics Commission of the I. Horbachevsky Ternopil National Medical University of the Ministry of Health of Ukraine (protocol No. 78 dated August 18, 2024).

The results were analyzed using nonparametric statistical methods in the STATISTICA 10.0 software (StatSoft, USA). To perform statistical processing of the obtained results, analysis of variation series was used - calculation of the arithmetic mean and its standard error (M and m). The reliability of differences between independent quantitative variables with normal distribution was assessed using the Mann-Whitney U-test using nonparametric characteristics. The critical value of the level of statistical significance (P) for all types of analysis was taken <5% ($P < 0.05$) [18].

RESULTS AND DISCUSSION

On the 30th day of the development of experimental periodontitis, a decrease in SOD activity in the blood serum was recorded by 2.55 times ($P < 0.001$) compared with the indicators of the control group of animals. It is worth noting that these values were also lower than in rats with periodontitis on the 30th day, which had prosthetics with removable structures. In particular, SOD activity decreased by 2.02 times ($P < 0.001$) when using an acrylic base and by 1.49 times ($P < 0.001$) when using a nylon base (Table 1).

When comparing the activity of the studied enzyme on the 30th day of the development of experimental periodontitis in animals with removable bases made of polymethacrylate and thermoplastic nylon plastics relative to the intact group, its decrease was found to be 1.27 times ($P < 0.01$) and 1.71 times ($P < 0.001$), respectively. Comparative analysis showed a statistically significant difference between the levels of superoxide dismutase in groups with different types of prosthetics. In rats with acrylic bases, the activity of this antioxidant enzyme was higher by 1.35 times ($P < 0.01$) compared to nylon structures.

The study of catalase activity, one of the key enzymes of antioxidant defense, in blood serum demonstrated the opposite nature of changes compared to SOD activity, although the degree of severity of these changes was somewhat lower. In particular, on the 30th day of the development of experimental bacterial-immune periodontitis, was recorded an increase in catalase activity in blood serum by 1.54 times ($P < 0.001$) compared to the indicators of the control group.

During the same period of development of the inflammatory process in the tissues of the periodontal complex, but under the condition of prosthetics with acrylic bases, an increase in catalase activity was observed in the blood serum by 1.07 times ($P < 0.05$) compared to the group of animals on the 30th day without prosthetics. However, compared to the control group, this indicator remained at a rather high level, exceeding it by 1.65 times ($P < 0.001$). This indicates more intensive use of the pool of this enzyme with the preservation of reserve potentials for antioxidant protection.

Using a thermoplastic nylon base in experimental bacterial-immune periodontitis caused an increase in catalase levels by 1.44 times ($P < 0.001$) compared to the values of intact rats. However, this indicator was lower compared to the results of animals with an inflammatory process in periodontal tissues on the 30th day of the experiment without prosthetics, although the difference was statistically insignificant ($P > 0.05$). Compared to the group with bases made of polymethacrylate material, the concentration of this antioxidant decreased by 1.15 times ($P < 0.01$).

In animals with experimental bacterial-immune periodontitis, on the 30th day of the study, the SOD/Catalase ratio significantly decreased – 3.96 times ($P < 0.001$) compared to intact animals. In rats of the third experimental group, which underwent prosthetics with acrylic bases, this indicator on the 30th day was 1.92 times ($P < 0.001$) higher compared to animals of the same period without prosthetics. However, despite this upward trend, the value remained lower than in the control group – 2.06 times ($P < 0.001$).

Analyzing the indicator of this ratio, it should be noted that when using nylon orthopedic structures, it also decreased, that is, it was lower compared to the control data (by 2.48 times; $P < 0.001$) and increased by 1.60 times ($P < 0.001$) compared to its level in animals without prosthetics. The results obtained during the experiment indicate a significant overstrain of one component of the antioxidant system with a simultaneous weakening of the other. This causes a violation of coordination in the work of antioxidant enzymes and, as a result, a decrease in the effectiveness of anti-radical protection of tissues [19]. It is worth noting that in animals with nylon prostheses against the background of periodontitis, the SOD/Catalase ratio was lower compared to the indicators of rats that were installed with acrylic bases. However, the differences found were not statistically significant ($P > 0.05$).

Ceruloplasmin is also an important antioxidant, as it plays a key role in neutralizing free radicals through its enzymatic activity, in particular in the processes of iron oxidation. It

Table 1. SOD, catalase and ceruloplasmin levels in the blood serum of experimental animals with experimental bacterial-immune periodontitis and with fixation of prosthetic bases (M ± m)

Conditions and indicators of the study	Control (intact) group (n = 10)	Animals with experimental bacterial-immune periodontitis		
		No prosthetics (n = 8)	Acrylic base (n = 8)	Nylon base (n = 8)
SOD (conditional units/ml)	0.166 ± 0.005	0.065 ± 0.003 $P_1<0.001$	0.131 ± 0.005 $P_1<0.01$; $P_2<0.001$	0.097 ± 0.002 $P_1<0.001$; $P_2<0.001$; $P_3<0.01$
Catalase (μcat/l)	0.168 ± 0.005	0.258 ± 0.005 $P_1<0.001$	0.277 ± 0.010 $P_1<0.001$; $P_2<0.05$	0.241 ± 0.008 $P_1<0.001$; $P_2>0.05$; $P_3<0.01$
SOD / Catalase	0.99 ± 0.03	0.25 ± 0.02 $P_1<0.001$	0,48 ± 0.03 $P_1<0.001$; $P_2<0.001$	0.40 ± 0.02 $P_1<0.001$; $P_2<0.001$; $P_3>0.05$
Ceruloplasmin (mg/l)	4.68 ± 0.06	8.15 ± 0.11 $P_1<0.001$	7.24 ± 0.06 $P_1<0.001$; $P_2<0.001$	6.00 ± 0.05 $P_1<0.001$; $P_2<0.001$; $P_3<0.001$

Note: here and in Table 2 P_1 – statistical significance relative to control; P_2 – statistical significance relative to experimental periodontitis animals with no crowns; P_3 – statistical significance relative to experimental periodontitis animals with stamped crowns.

binds ionized iron, preventing its participation in Fenton reactions, which cause the formation of highly reactive hydroxyl radicals. Unlike catalase and superoxide dismutase, which eliminate hydrogen peroxide and superoxide anions, ceruloplasmin is also involved in the regulation of copper metabolism and has anti-inflammatory properties, which makes it an important element of antioxidant defense. The synergy of its functions with SOD and catalase provides comprehensive protection of cells from oxidative stress and prevents tissue damage [20].

In animals of the second experimental group, on the 30th day of the development of the inflammatory process in periodontal tissues, an increase in the level of ceruloplasmin in blood plasma by 1.74 times ($P < 0.001$) was recorded compared to the control group.

In rats of the third experimental group on the 30th day with polymethylmethacrylate constructs, the ceruloplasmin levels remained elevated, exceeding the value of the control group by 1.55 times ($P < 0.001$). When comparing the concentration of this antioxidant in the serum of both above-mentioned experimental groups, it was found that its level was 1.13 times higher ($P < 0.001$) in animals studied on the 30th day without prosthetics.

When using nylon base plastics, a decrease in the content of this antioxidant in the blood was observed compared to the 30th day of the inflammatory process without prosthetics and with acrylic prostheses (1.36 times; $P < 0.001$ and 1.21 times; $P < 0.001$, respectively), and at the same time its level was higher than in control animals (1.28 times; $P < 0.001$).

It was found that on the 30th day of the development of experimental bacterial-immune periodontitis, the content of reduced glutathione in the blood was significantly lower than the indicators of intact animals (by 3.41 times; $P < 0.001$).

In the group of animals with acrylic prosthetic structures, its level in the blood serum decreased by 1.23 times ($P < 0.001$) compared to the data of rats that did not have prostheses on the 30th day of inflammation in the periodontal tissues (Table 2). In addition, this indicator remained at a lower level compared to the intact group, where it was reduced by 4.19 times ($P < 0.001$).

In the next experimental group, on the 30th day of the development of the inflammatory process in the periodontal complex and under the condition of using nylon prostheses, the content of reduced glutathione in the blood serum increased (by 1.13 times; $P < 0.01$) relative to the groups of animals with experimental bacterial-immune periodontitis without prosthetics. Compared to the group of animals with acrylic bases, it was also higher (by 2.06 times;

Table 2. Indicators of the glutathione link of the antioxidant defense system in the blood serum of experimental animals with experimental bacterial-immune periodontitis and under the condition of fixation of prosthetic bases (M ± m)

Conditions and indicators of the study	Control (intact) group (n = 10)	Animals with experimental bacterial-immune periodontitis		
		No prosthetics (n = 8)	Acrylic base (n = 8)	Nylon base (n = 8)
Reduced glutathione (mmol/l)	12.98 ± 0.31	3.81 ± 0.09 $P_1<0.001$	3.10 ± 0.06 $P_1<0.001$; $P_2<0.001$	6.39 ± 0.44 $P_1<0.001$; $P_2<0.001$; $P_3<0.001$
Glutathione reductase (mmol/min×l)	0.874 ± 0.006	0.352 ± 0.010 $P_1<0.001$	0.317 ± 0.004 $P_1<0.001$; $P_2<0.05$	0.682 ± 0.007 $P_1<0.001$; $P_2<0.001$; $P_3<0.001$
Glutathione peroxidase (mmol/min×l)	0.668 ± 0.005	0.163 ± 0.004 $P_1<0.001$	0.257 ± 0.010 $P_1<0.001$; $P_2<0.001$	0.427 ± 0.009 $P_1<0.001$; $P_2<0.001$; $P_3<0.001$

$P < 0.001$). However, compared to the control group, the level of reduced glutathione in the blood serum remained at a rather low level (it was lower by 2.03 times; $P < 0.001$).

As for the changes in the activity of the enzymatic link of the glutathione system, it turned out that the activity of glutathione peroxidase in animals with inflammation in the periodontal complex was statistically significantly lower on the 30th day of the experiment, both without prosthetics and with prosthetic constructions, compared to the control values.

Thus, in the group of animals on the 30th day of development of simulated periodontitis without bases the activity of glutathione peroxidase was lower by 4.10 times ($P < 0.001$), compared to the control group. In rats with inflammation during the same period, but with acrylic bases, a statistically significant increase in indicators was observed (by 1.58 times; $P < 0.001$) compared to the previous study group. However, in them, the activity of this enzyme was lower than the control values (by 2.60 times; $P < 0.001$).

During the development of inflammation with the use of nylon prostheses, the activity of glutathione peroxidase significantly increased compared to the results on the 30th day of experimental bacterial-immune periodontitis without prosthetic structures (by 2.62 times; $P < 0.001$) and with acrylic prostheses for the same period of the study (by 1.66 times; $P < 0.001$), but remained lower by 1.56 times ($P < 0.001$) compared to the intact group. On the 30th day of the experiment, the activity of glutathione reductase was reduced by 2.48 times ($P < 0.01$) compared to the control. It is worth noting that at the same period of the inflammatory process under the conditions of acrylic prosthetics, a decrease in the indicator by 2.22 times ($P < 0.05$) was observed compared to the group without prosthetic bases. In addition, the level of glutathione reductase activity remained lower than control values, where it was reduced by 1.76 times ($P < 0.001$).

The use of nylon prosthetic bases demonstrated the opposite trend: glutathione reductase activity significantly increased compared to the data obtained on the 30th day after the start of the experiment without prosthetics (1.94 times; $P < 0.001$), and was also higher compared to the group using acrylic structures (2.15 times; $P < 0.001$). However, when comparing glutathione reductase activity in animals with nylon prostheses with the intact group, this indicator was statistically significantly lower (1.28 times; $P < 0.001$).

The increase in the level of lipid peroxidation products in the blood under conditions of experimental bacterial-immune periodontitis indicates damage to cell membranes and increased oxidative stress, which can affect the antioxidant defense systems of the body [21]. The analysis of biochemical parameters reflecting the state of the enzymatic component of the antioxidant system (superoxide dismutase, catalase) and indicators of non-enzymatic antioxidant defense (ceruloplasmin and glutathione system) demonstrated that during the development of the inflammatory process in periodontal tissues, the activity of antioxidant enzymes changes in different directions. This depends on the presence and type of prosthetic structures in the oral cavity of experimental animals, as well as the influence of pathogenic factors.

With the increase in catalase activity, the entire antioxidant defense system of the body is strengthened and launched, aimed at the effective neutralization of lipid peroxidation products. These products are formed in excessive quantities during the development of the inflammatory process in periodontal tissues. The main role of catalase in such a situation is to prevent the penetration of toxic metabolites into the bloodstream, which can cause oxidative stress if they accumulate. Oxidative stress, in turn, is a key factor in damage to cellular structures (alteration) and can cause a prolonged inflammatory process, complicated by systemic manifestations and potential pathological changes in the body [22].

In addition, changes in the glutathione system, particularly the level of reduced glutathione and the activity of the enzymes glutathione reductase and glutathione peroxidase deserve attention. Glutathione antioxidant protection in maintaining the balance of redox processes in periodontal cells has an important role [23]. In this modeled pathology a decrease in glutathione levels can lead to the accumulation of free radicals, which, in turn, contributes to the development of inflammatory processes in gingival tissues. Oxidative stress associated with glutathione deficiency can activate inflammatory molecules, such as cytokines and metalloproteinases, which negatively affect the structure and function of the periodontium [24]. Thus, the glutathione system is important in regulating inflammatory reactions and maintaining periodontal health.

The decrease in the activity of the antioxidant system when using denture bases may be associated with the toxic or inhibitory effect of the materials on metabolic processes in the cells of the oral cavity. Nylon and acrylic structures secrete low-molecular substances or monomers that have a toxic effect on cells [25]. The result is the inhibition of the activity of the enzymes of the antioxidant system. Contact of basic plastics with the oral mucosa also causes microtraumas that change the redox balance. It should also be noted that prostheses can alter the composition of the microflora, which in turn affects the intensity of oxidative stress.

Therefore, the results obtained regarding the changes in the activity of the antioxidant defense system in all the studied groups of experimental periodontitis and under prosthetic conditions indicate the important role of this system in the mechanisms of development of this pathology.

CONCLUSIONS

1.The inflammatory process in the tissues of the periodontal complex, caused by the combined influence of bacterial and immune factors, is characterized by dynamic changes in the functioning of the antioxidant defense system, which is manifested by increased activity of both enzymatic and non-enzymatic components and depends on the presence and type of prosthetic structures.

2. Various base materials affect the intensity and nature of changes in the antioxidant potential of blood plasma: acrylic bases demonstrated a moderate effect on the indicators, whereas nylon designs caused more significant fluctuations in enzyme activity. This confirms the important role of the antioxidant system, particularly the glutathione link, in the mechanisms of development and regulation of inflammatory processes in periodontal tissues, and the body's response to prosthetics.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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ПОРУШЕННЯ СИСТЕМИ АНТИОКСИДАНТНОГО ЗАХИСТУ ОРГАНІЗМУ ПРИ ПАРОДОНТИТІ ТА ВИКОРИСТАННІ РІЗНИХ ТИПІВ ЗНІМНИХ ПРОТЕЗНИХ КОНСТРУКЦІЙ

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У 20–70% пацієнтів спостерігаються запальні реакції слизової оболонки та пародонтального комплексу, пов'язані з експлуатацією знімних протезів, виготовлених з базисних пластмас. Метою нашої роботи було дослідити порушення системи антиоксидантного захисту у щурів при експериментальному бактеріально-імунному пародонтиті за умов використання акрилових та нейлонових базисів знімних зубних протезів. У тварин експериментальний пародонтит моделювали ін'єкційним введенням у тканини пародонта суспензії, що містила суміш мікроорганізмів (Staphylococcus aureus і Streptococcus hemolyticus) на основі яєчного білка. Стан антиоксидантної системи оцінювали за активністю таких ферментів, як супероксиддисмутаза, каталаза, церулоплазмін, глутатіонпероксидаза, глутатіонредуктаза та відновлений глутатіон на 30-й день експери-

менту як без, так і з зафіксованими в ротовій порожнині тварин різних типів базисів знімних зубних протезів. У тварин із пародонтитом та акриловими конструкціями було виявлено підвищення активності каталази, супероксиддисмутази та церулоплазміну, а також зниження концентрації глутатіонової ланки порівняно з показниками у щурів з нейлоновим протезуванням. Результати показують відмінності у впливі різних типів базисів протезів на стан антиоксидантного захисту організму.

Ключові слова: пародонт; пародонти; антиоксиданти; оксидативний стрес; перекисне окиснення ліпідів; знімне протезування; базис протезу; акриловий протез; нейлоновий протез.

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