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Original Article

Endogenous Intoxication in Development of Experimental Periodontitis of Bacterial-Immune Genesis

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Abstract

Introduction: Excessive lipoperoxidation is accompanied by accumulation of peroxidation products and depletion of antioxidant reserves, which cause hyperenzymemia and the accumulation of toxic substances. The level of endotoxicosis is determined by the content of hydrophilic and hydrophobic products in the blood.

Aim: To define the pathogenetic role of endogenous intoxication in the dynamics of development of experimental periodontitis of bacterial-immune genesis.

Materials and methods: The experiment was conducted on rats. The animals were divided into three groups: group 1 – control group/intact; group 2 – model periodontitis at 7 days; group 3 – model periodontitis at 30 days. The experimental bacterial-immune periodontitis was induced by injection into the tissue of the periodontal complex of the microorganisms' mixture diluted with egg's albumin. For the study, we selected the blood serum in which the content of middle molecular weight molecules and erythrocyte intoxication index were determined. The results were statistically analyzed by means of non-parametric indices methods.

Results: The development of generalized periodontitis is characterized by the occurrence of oxidative stress, which leads to violation of the metabolism in the mucous membrane of the oral cavity resulting in the accumulation of toxic products and the development of endogenous intoxication. The results show that the content of middle molecular weight molecules (aromatic amino and chain amino acids) determined on day 7 of the experiment was 1.11 times higher than that of the control group ($p<0.01$) and by 1.16 times ($p<0.01$), respectively. Comparing the levels of the above hydrophilic components of endogenous intoxication at 30 days of experimental periodontitis, we found a probable increase in these indicators compared with those at 7 days of the experiment. Studying the level of erythrocyte intoxication index, we found that at 7 days of experimental periodontitis this index was 1.28 times higher ($p<0.01$) than that of the intact group and continued to increase at 30 days.

Conclusions: The dynamics of experimental periodontitis of bacterial-immune genesis show that the highest rates of endogenous intoxication are found in the late stages of the dynamics of the inflammatory process in the periodontium, namely at 30 days of the experiment, which may indicate chronic inflammation.

Keywords

endogenous intoxication, erythrocyte intoxication index, lipid peroxidation, middle molecular weight molecules, periodontitis

INTRODUCTION

Generalized periodontitis is a progressive inflammation of the periodontal complex. Ninety-five percent of the population has gingival inflammation. This disease is the second most common after caries because, according to statistical studies, it occurs in one form or another, especially in people over 50 years old.[1] This pathological process includes inflammation of the periodontal ligament, gingiva, and alveolar bone.[2,3] Chronic periodontitis is much more likely to lead to tooth loss than caries is, so finding out the mechanisms of occurrence, development, methods of prevention, and treatment of this inflammatory disease has been and is a topical issue in modern science.[4,5]

One of the key links in the pathogenesis of any inflammatory disease, including those in the maxillofacial area, is endogenous intoxication, an important pathophysiological mechanism of which is the activation of lipid peroxidation.[6,7] Excessive lipoperoxidation is accompanied by accumulation of peroxidation products and depletion of antioxidant reserves, which causes hyperenzymemia and the accumulation of toxic substances.[8,9] Endogenous intoxication is characterized by increased formation and accumulation of toxic metabolites, which in turn lead to the destruction of plasma and cytoplasmic membranes.[10] The level of endotoxicosis is determined by the content of hydrophilic and hydrophobic products in the blood. Middle molecular weight molecules belong to the hydrophilic components of endogenous intoxication. They are formed due to increased proteolysis and have an alternative effect on the body due to high functional activity.[11,12] Thus, the periodontium itself becomes a focus of formation of toxic metabolites, in excess concentrations of which not only have a toxic effect on periodontal tissues, impairing cell metabolism, but also provoke significant disorders of metabolic processes in the body.[13]

That is why the study of the level of middle molecular weight molecules (MMWM) in the serum and erythrocyte index of intoxication in simulated bacterial-immune periodontitis is a reliable criterion.

AIM

To define the pathogenetic role of endogenous intoxication in the dynamics of development of the experimental periodontitis of bacterial-immune genesis.

MATERIALS AND METHODS

The experiment was conducted on 26 white outbred rats weighing 180-200 g, which were fed the usual diet of vivarium. According to the Commission on Bioethics of I. Horbachevsky Ternopil National Medical University (protocol No. 56 of 08.01.2020), violations of moral and ethical norms during the research work were not detected. The study was performed according to the general rules and regulations of the European Convention for the Protection of Vertebrate Animals that are used for experimental and other scientific purposes (Strasbourg, 1986), and the General Ethical Animal Experimentation (Kyiv, 2001). The animals were divided into three groups: group 1 – control/intact (10 rats); group 2 – animals in which the model periodontitis was at 7 days of experiment (8 rats); group 3 – animals in which the model periodontitis was at 30 days (8 rats). The experimental bacterial-immune periodontitis in the experimental animals was caused by injection into the tissue of the periodontal complex of the microorganisms mixture (Staphylococcus and Streptococcus) diluted with egg's albumin.[14] To enhance the immune response, an injection of complete Freund's adjuvant was simultaneously injected into the rats paws. At the time of performance of the studies with animals of group 3, on day 14, the injection of the pathogenic and adjuvant was repeated. The experimental animals were exsanguinated under thiopental anesthesia at 7 days and at 30 days. Then the blood serum was selected for further research.

To determine the content of middle molecular weight molecules, the acid-soluble fraction was isolated from the serum. It was obtained by adding 1.8 ml of 10% solution of trichloroacetic acid to 0.2 ml of serum. Subsequent centrifugation was performed at 3000 rpm for 30 minutes. The isolated 0.5 ml fraction was diluted 1:10 with distilled water and the optical density was determined at 254 nm (chain amino acids) and 280 nm (aromatic amino acids) against distilled water on a spectrophotometer. The results were expressed in conventional units, numerically equal to the extinction.[15]

The method of determining the erythrocyte intoxication index (EII) was based on information about erythrocytes as a universal adsorbent, which allows to estimate the level of EII by changing the sorption capacity of erythrocytes polar, almost impermeable to their methylene blue membrane. In a test tube containing 1 ml of 3.8% sodium citrate solution, 4 ml of blood was taken, mixed and the erythrocytes were separated by centrifugation for 10 min at 3000 rpm. Plasma was removed. 1 ml of erythrocyte mass was transferred to a test tube containing 3 ml of a solution of methylene blue (0.025%) prepared in saline. The samples were stirred and incubated for 10-12 min at room temperature, then centrifuged again for 10 min at 3000 rpm. The supernatant was transferred to a cuvette and the optical density relative to saline was determined at a wavelength of 630 nm on a spectrophotometer. The amount of absorbed dye (as a percentage) was calculated from the difference between the optical density of the initial dye solution and the dye solution after incubation with erythrocytes.[16]

The results were statistically analyzed by means of non-parametric indices methods using the STATISTICA 10.0 software (Statsoft, USA). The reliability of the differences in values between independent quantitative values was determined with a normal distribution according to the Mann-Whitney U criterion.[17]

RESULTS

The results show that the content of middle molecular weight molecules, which was determined at a wavelength of 254 nm in serum (chain amino acids), increased on the 7th day of the experiment by 1.11 times ($p<0.01$), compared with the intact group **(Table 1)**. When comparing the level of the above hydrophilic components of endogenous intoxication at 30 days of experimental periodontitis **(Fig. 1)**, we found a probable increase by 1.30 times in these indicators compared with these at 7 days of the experiment ($p<0.01$). It should be noted that this figure was also significantly higher relative to the control group of animals (by 1.44 times; $p<0.01$).

At the early stage of development of experimental periodontitis, i.e. at 7 days, there was also an increase by 1.16 times of the middle molecular weight molecules in serum, which was determined at a wavelength of 280 nm (aromatic amino acids) ($p<0.01$).

Later, at 30 days of experimental periodontitis development, the concentration of aromatic amino acids in the composition of medium molecules significantly increased compared with that at 7 days of the experiment, this type of middle molecular weight molecules increased significantly (by 1.60 times; $p<0.05$). When comparing its level relative to the control group of animals, it should be noted that it was higher (by 1.85 times; $p<0.01$) **(Fig. 2)**.

As a result of the study of the total toxic effect on the erythrocyte membranes of the blood of experimental animals in experimental bacterial-immune periodontitis – the level of erythrocyte intoxication index (EII), significant changes were also found **(Table 1)**. In particular, it was found that at 7 days of the formation of experimental periodontitis in rats, this index was 1.28 times higher ($p<0.01$) than that of the intact group.

The results at 30 days of the experiment showed that the level of EII in blood continued to increase (by 1.67 times; $p<0.01$) compared with the groups of animals with experimental periodontitis at 7 days of the experiment **(Fig. 3)**. However, it was significantly higher (by 2.14 times; $p<0.01$) compared to the control group of white rats.

Table 1. The content of middle molecular weight molecules in the serum and erythrocyte index of intoxication of experimental animals in different periods of development of experimental periodontitis (M ± m)

Conditions and indicators of the experiment	Control. Intact group	White rats with experimental periodontitis	
Experiment duration (days)	-	7	30
Number of animals	10	8	8
$MMWM_{254}$, condit. units	353.80±4.53	392.25±5.10 $p_1<0.01$	509.25±5.26 $p_1<0.01$; $p_2<0.01$
$MMWM_{280}$, condit. units	144.30±4.67	167.00±5.84 $p_1<0.01$	267.00±7.96 $p_1<0.01$; $p_2<0.01$
Erythrocyte intoxication index, %	31.06±1.88	39.85±0.96 $p_1<0.01$	66.46±1.73 $p_1<0.01$; $p_2<0.01$

p_1 - significance of differences relative to control/intact animals; p_2 - significance of differences in animals with experimental periodontitis at 7 days of the study.

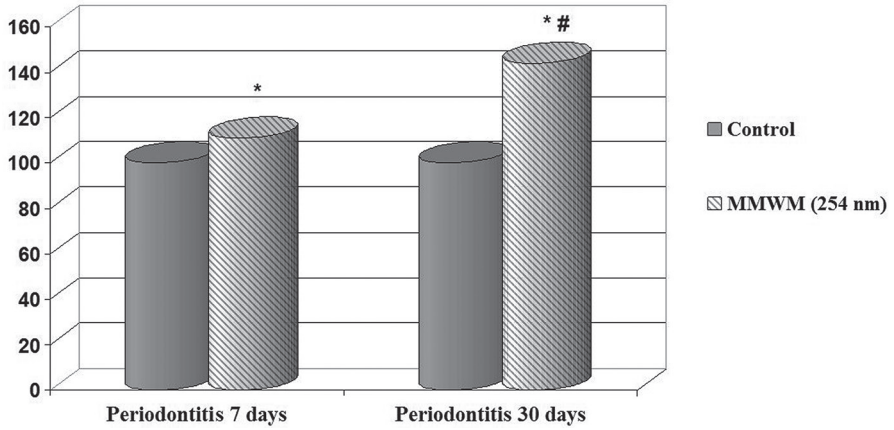

Figure 1. Dynamics of the content of $MMWM_{254}$ in the blood of white rats with experimental periodontitis (in % of control). *: significant differences in relation to the control/intact animals ($p<0.01$); #: significant differences in relation to the animals with periodontitis at 7 days of the study ($p<0.01$).

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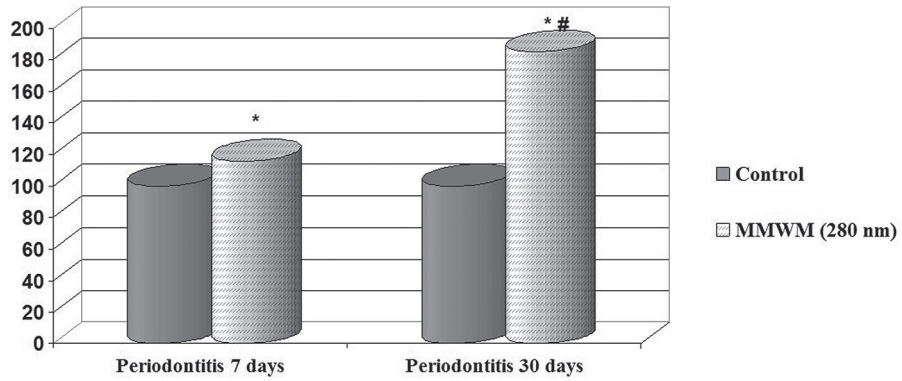

Figure 2. Dynamics of the content of $MMWM_{280}$ in the blood of white rats with experimental periodontitis (in % of control). *: significant differences in relation to the control/intact animals ($p<0.01$); #: significant differences in relation to the animals with periodontitis at 7 days of the study ($p<0.01$).

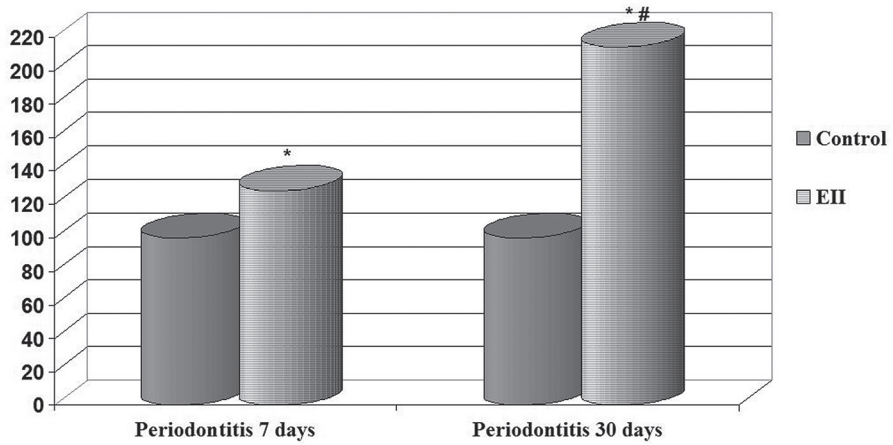

Figure 3. Dynamics of erythrocyte intoxication index in the blood of white rats with experimental periodontitis (in % of control). *: significant differences in relation to the control/intact animals ($p<0.01$); #: significant differences in relation to the animals with periodontitis at 7 days of the study ($p<0.01$).

DISCUSSION

The development of experimental periodontitis, like any other inflammatory process[18], is accompanied by an increase in the intensity of lipid peroxidation processes[19], resulting in an increase in endogenous intoxication[20]. Increased permeability of erythrocyte membranes is a reflection of the disorders of the functions and structure of plasma membranes of all cells of the body. The erythrocyte index of intoxication characterizes the inhibition of sorption activity (including methylene blue), which is a consequence of the rearrangement of lipid components of cell membranes and a decrease in the functional capacity of erythrocytes due to exposure to toxic substances.[21] These indicators are the proof of increased destructive processes, inhibition of the detoxifying properties of the body and the accumulation of intermediate toxic products, mainly lipoperoxidation, in the serum, which are formed during the development and course of bacterial-immune experimental periodontitis.[23]

Endogenous intoxication not only accompanies most inflammatory diseases, but is also an important factor in their pathogenesis and, in many cases, determines the possible adverse effects, because an important feature of MMWMs is their high biological activity. They have neurotoxic activity, inhibit protein synthesis, promote hemolysis of erythrocytes, inhibit erythropoiesis and enzyme activity, and cause a state of secondary immunosuppression.[22] MMWMs are also able to block cell receptors by binding to the active sites of the albumin molecule competing with regulatory peptides, thus disrupting the process of humoral regulation.[24] Therefore, the formation of endogenous intoxication syndrome is an important part of the pathogenesis of periodontitis.

CONCLUSIONS

In the modeling of bacterial-immune periodontitis in laboratory rats, the development of endogenous intoxication was established. It was detected by increasing the level of markers of this process – middle molecular weight molecules and the level of erythrocyte intoxication index. The highest rates of endogenous intoxication were found in the

later stages of the dynamics of the inflammatory process in the periodontium, namely at 30 days of the experiment, which may indicate chronic inflammation. An increase in the level of EII and in the number of MMWM indicate an increase in the permeability of erythrocyte membranes and significant activation of catabolic processes in experimental periodontitis, which leads to metabolic disorders and accumulation in tissues of products that can cause endogenous intoxication.

Author contributions

A.D.: writing of the article, contributed in the gathering and listing data, the data analysis and interpretation, the idea and the planning of the study, critical review of the article, final approval of the manuscript. The author has full access to all data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The author made a significant contribution to the manuscript about its conception, writing and final approval, read and approved the submission of the manuscript. The manuscript has not been published and is not being considered for publication elsewhere, in whole or in part in any language.

Conflict of Interest

The author declares no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Эндогенная интоксикация в развитии экспериментального пародонтита бактериально-иммунного генеза

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Резюме

Введение: Избыточная липопероксидация сопровождается накоплением продуктов перекисного окисления и истощением антиоксидантных резервов, что вызывает гиперферментемию и накопление токсических веществ. Уровень эндотоксикоза определяют по содержанию в крови гидрофильных и гидрофобных продуктов.

Цель: Определить патогенетическую роль эндогенной интоксикации в динамике развития экспериментального пародонтита бактериально-иммунного генеза.

Материалы и методы: Эксперимент проведён на крысах. Животные были разделены на три группы: 1 группа – контрольная группа/интактные; 2-я группа – модель пародонтита на 7-е сутки; 3-я группа – модель пародонтита на 30-е сутки. Экспериментальный бактериально-иммунный пародонтит вызывали введением в ткань пародонтального комплекса смеси микроорганизмов, разведённой яичным альбумином. Для исследования отбирали сыворотку крови, в которой определяли содержание молекул средней молекулярной массы и индекс интоксикации эритроцитов. Результаты были подвергнуты статистической обработке с помощью методов непараметрических показателей.

Результаты: Развитие генерализованного пародонтита характеризуется возникновением оксидативного стресса, что приводит к нарушению обмена веществ в слизистой оболочке полости рта, что приводит к накоплению токсических продуктов и развитию эндогенной интоксикации. Результаты показывают, что содержание молекул средней молекулярной массы (ароматических аминокислот и цепочечных аминокислот), определённое на 7-е сутки эксперимента, было выше, чем в контрольной группе, в 1.11 раза (p<0.01) и в 1.16 раза (p<0.01) соответственно. Сравнивая уровни вышеперечисленных гидрофильных компонентов эндогенной интоксикации на 30-е сутки экспериментального пародонтита, мы обнаружили вероятное увеличение этих показателей по сравнению с таковыми на 7-е сутки эксперимента. Изучая уровень индекса эритроцитарной интоксикации, мы установили, что на 7-е сутки экспериментального пародонтита этот показатель был в 1.28 раза выше (p<0.01), чем у интактной группы, и продолжал увеличиваться на 30-е сутки.

Заключение: Динамика экспериментального пародонтита бактериально-иммунного генеза показывает, что наиболее высокие показатели эндогенной интоксикации обнаруживаются на поздних стадиях динамики воспалительного процесса в пародонте, а именно на 30-е сутки эксперимента, что может свидетельствовать о хроническом воспалении.

Ключевые слова

эндогенная интоксикация, эритроцитарные индексы интоксикации, перекисное окисление липидов, среднемолекулярные молекулы, пародонтит