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MARKERS OF INFLAMMATION AND ANTIOXIDANT PROTECTION IN THE ORAL FLUID OF CHILDREN WITH DIFFERENT BODY MASS INDEX BEFORE AND AFTER THE USE OF A MUCOSE–ADHESIVE GEL BASED ON LIPOPOLYSACCHARIDE

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In medical practice, bacterial endotoxins are used primarily to stimulate immunity and nonspecific resistance of the organism. Lipopolysaccharide has a pro-inflammatory effect, stimulating the production of pro – inflammatory cytokines by leukocytes (TNF – α, IL – 1, IL – 6, etc.). However, in small doses, endotoxin stimulates the body's defense systems, transferring them from a state of rest to the stage of active action. Such properties of small doses of lipopolysaccharide served as the basis for its clinical use. Oral applications of the proposed lipopolysaccharide-based gel normalize oral microbiocenosis (decrease of urease activity and degree of dysbiosis), and significantly increase the level of inflammation markers in all groups of children by body mass index, contributing to the transition of inflammatory processes to the active phase; significantly reduce the level of pathological processes in the parodontium of children due to the activation of physiological inflammation.

Key words: children, inflammation, body mass index, oral fluid, lipopolysaccharide, dental diseases.

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МАРКЕРИ ЗАПАЛЕННЯ ТА АНТИОКСИДАНТНОГО ЗАХИСТУ В РОТОВІЙ РІДИНІ ДІТЕЙ З РІЗНИМ ІНДЕКСОМ МАСИ ТІЛА ДО ТА ПІСЛЯ ЗАСТОСУВАННЯ МУКОЗО-АДГЕЗИВНОГО ГЕЛЮ НА ОСНОВІ ЛІПОПОЛІСАХАРИДУ

У медичній практиці бактеріальні ендотоксини застосовуються насамперед для стимуляції імунітету та неспецифічної резистентності організму. Ліпополісахарид має протизапальну дію, стимулюючи вироблення прозапальних цитокінів лейкоцитами (ФНО-б, ІЛ-1, ІЛ-6 та ін.). Однак у малих дозах ендотоксин стимулює захисні системи організму, переводячи їх із стану спокою в стадію активної дії. Такі властивості малих доз ліпополісахариду послужили основою для його клінічного застосування. Пероральне застосування запропонованого гелю на основі ліпополісахаридів нормалізує мікробіоценоз у порожнині рота (зниження активності уреази та ступінь дисбіозу) та суттєво підвищує рівень маркерів запалення у всіх групах дітей за індексом маси тіла, сприяючи переходу запальних процесів у активну фазу; значно знижують рівень патологічних процесів у пародонті у дітей за рахунок активізації фізіологічного запалення.

Ключові слова: діти, запалення, індекс маси тіла, ротова рідина, ліпополісахарид, стоматологічні захворювання.

The work is a fragment of the research project: "Correction of pathogenetic mechanisms of metabolic disorders in oral tissues in patients depending on environmental and nutritional factors that affect carbohydrate and lipid metabolism", state registration No. 0118U0006966.

As it is known, one of the most important inducers of inflammation is intestinal endotoxin – lipopolysaccharide (ET–LPS) [14]. Already in minimal doses (for children 0.5–2.5 mcg/day or 0.002–5.0 g per 100 g of dosage form), it causes the development of an inflammatory reaction, which is manifested by an increase in antimicrobial potential due to reactive oxygen species [9].

LPS were studied from the point of view of ET involvement in the pathogenesis of certain diseases (cardiovascular, gynecological, diseases of the upper respiratory tract and lungs, neurological diseases, diabetes mellitus, etc.), including dental diseases, as well as the development of dysbiosis. In dental practice, LPS are used in cases of chronic infectious inflammatory diseases of the maxillofacial region and in chronic recurrent stomatitis. Pyrogenic effect is noted with the introduction of LPS [15]. The efficacy of pyrotherapy in many delayed, chronic infectious diseases is associated with immunostimulation. In addition, the effectiveness of pyrotherapy in some diseases is associated with an increase in vascular permeability and tissue barriers not only for immune cells and for factors of humoral immunity, but also for antibiotics and other drugs. [3]. Dysbiotic and inflammatory reactions in the oral cavity are protective in relation to the parodont tissues.

Every year, the percentage of children and adolescents with metabolic syndrome and, in particular, who are overweight, is growing, which is caused by an unfavorable environmental situation, excessive stress loads, the spread of bad habits and unhealthy lifestyles [8], as well as a deterioration in the quality and structure of nutrition of children and adolescents, as well as a sedentary lifestyle. At the same time, there are only a few reports devoted to the study of changes in the microbiocenosis of the oral cavity in children with alimentary – constitutional obesity [4].

In medical practice, ET – LPS is used primarily to stimulate immunity and nonspecific resistance of the body. The most famous drugs are pyrogenal, prodigiosan, banquetom, pyrexal, salmozan, zymosan, etc. Currently, LPS-based preparations are increasingly used in dentistry due to the emergence of new purified bacterial lysates. The main indication for the appointment of bacterial polysaccharides is the chronic course of infectious diseases [13].

Unreasonably, little attention has been paid to the problem of prevention and treatment of periodontal diseases with the use of LPS preparations in adolescents. Thus, further study of various aspects of the targeted action of preparations based on LPS in the complex therapy of chronic inflammatory diseases of the maxillofacial region and periodontal diseases, in particular, is a promising area of modern dentistry.

The purpose of the work was to determine the therapeutic and prophylactic effect of a mucosa-adhesive gel containing lipopolysaccharide on the level of inflammatory response in the tissues of the oral cavity in children with different body mass index.

Materials and methods. The study involved 54 children of 12 years of age: 31 students of No.6 secondary school of Chernomorsk and 23 students of Odesa gymnasium No. 1. After assessing their physical development, all children were divided into three groups depending on BMI: normotrophy (BMI=20–25), hypertrophy (BMI>25), hypotrophy (BMI<20). The research was carried out in groups of children separately from Odesa and the city of Chernomorsk, assuming that children live in cities of the same climatic zone, but study in different educational institutions. We also took into account the different social level of families (living conditions, the nature of food, parental employment, the ability of families to engage children in different sections, etc.).

The preparation gel "Pyrogenal" was used (Order No. 431 dated 21.07.2011; GR certificate No. 257/II–300200000 dated 21.07.2011. Manufacturer: Institute of Epidemiology and Microbiology (Russian Federation)), used in the composition of mucose – adhesive gel (TU U 20.4–13903778–032: 2012) with a concentration of LPS 2 mcg/ml.

Children during 2 weeks, before bedtime, after brushing their teeth used the "Pyrogenal" gel in a dose of 0.5 ml per one application on the gums. The study was carried out in children of all BMI groups before the application of the gel and 2 weeks after the end of the application.

Biochemical analysis was performed in the liquid portion of the mixed oral fluid of children. The collection of saliva was carried out in the morning, before meals, in centrifuge tubes for 10 minutes [7].

The determination of the activity of urease in the oral fluid was carried out by a method based on the ability of urease to split urea to ammonia, which with Nessler's reagent gives a yellow color. The intensity of the color of the sample is directly proportional to the activity of urease, which was expressed in μcatal/l of ammonia, formed in 1 second in 1 liter of oral fluid [7].

Determination of the level of lysozyme in the oral fluid of children was carried out by the bacteriolytic method, based on the ability of lysozyme to lyse the walls of bacteria. The degree of clarification is proportional to the activity of lysozyme, which was expressed in u/l of oral fluid [5].

The degree of dysbiosis was calculated from the ratio of the relative activities of urease and lysozyme according to Levitsky [6]. Normally, in healthy individuals, this indicator equals 1. In the presence of dysbiotic phenomena>1.

In saliva, the level of inflammation markers was determined: the content of malondialdehyde (MDA) and elastase activity [1]. MDA content – by reaction with 2 – thiobarbituric acid. Normally, the concentration of MDA in the oral fluid is 0.3±0.07 mmol/l.

The degree of inflammation in the oral cavity was assessed by the level of hydrolysis of the synthetic substrate N–t–BOC–L–alanine–p–nitrophenyl ester (BOC) ("Sigma", USA) by the Visser method. Elastase activity is expressed in microcatals per 1 liter of oral fluid. Normally, elastase activity in the oral fluid is 8.0±1.0 μkat/l.

The results were processed by variational and statistical methods of analysis on an IBM PC in SPSS SigmaStat 3.0 and StatSoft Statistica 6.0 software using Student's t-test [2].

Results of the study and their discussion. In the system of antimicrobial protection of the oral cavity, the most important role is played by lysozyme – an enzyme that destroys bacteria and viruses, and at the same time activates immunoglobulins and phagocytic leukocytes. It was found that the activity of lysozyme in the oral fluid correlates with the level of nonspecific and specific antimicrobial factors. At the same time, a decrease in the activity of this enzyme naturally causes an increased reproduction in the oral cavity of conditionally – pathogenic and pathogenic microflora.

Urease activity reflects the degree of contamination of conditionally – pathogenic microbiota, which synthesizes this enzyme into the oral cavity. By the level of urease activity, one can indirectly estimate the level of microbial contamination.

The results of biochemical studies clearly demonstrate the results of the effect of "Pyrogenal" gel applications on the periodontium of 12-year-old children with different BMI. Table 1 shows changes in the activity of urease and lysozyme in the oral fluid of children with normo-, hypo- and hypertrophy in Odessa and Chornomorsk, separately.

Table 1

Effect of LPS gel applications on urease and lysozyme activity in the oral fluid of children with different BMI

No.	Groups	Urease activity, мкат / l		Lysozyme activity, u / l	
		before	after	before	after
	Odessa				
1	BMI=20–25	0.071±0.009	0.130±0.012 p_1<0.001	78±10	96±27 p_1>0.05
2	BMI>25	0.214±0.010 p<0.001	0.133±0.061 p_1>0.05	147±18 p<0.005	101±18 p_1<0.05
3	BMI<20	0.177±0.014 p<0.01	0.147±0.015 p_1>0.05	149±20 p<0.005	105±18 p_1>0.05
	Chernomorsk				
1	BMI=20–25	0.046±0.008	0.045±0.014 p_1>0.05	83±13	71±14 p_1>0.05
2	BMI>25	0.130±0.011 p<0.001	0.054±0.012 p_1<0.001	76±12 p>0.05	75±10 p_1>0.05
3	BMI<20	0.120±0.010 p<0.01	0.103±0.019 p_1>0.05	53±11 p<0.05	62±9 p_1>0.05

Note: significance of differences calculated: p – compared to gr. 1; p_1 – in comparison with the indicator before applications.

However, in the group of children with BMI=20–25 (Odesa), the urease activity after the application of the mucose – adhesive gel increased by 1.4 times, while in the same group of children from Chernomorsk there were no changes. Urease activity in the group of children with BMI>25 decreased by 1.6 times (Odesa) and 2.4 times (Chernomorsk).

A month after the application of the gel to the gums of the examined children, it was found that the activity of lysozyme in the oral fluid in 12-years-old children with different BMI indices remained practically unchanged. At the same time, in the oral fluid of Chernomorsk children with BMI=20–25 and BMI>25, the activity of lysozyme is significantly reduced, which indicates an insufficient level of antimicrobial protection in the oral cavity of these children.

The results of biochemical studies of the oral fluid in 12-year-old children with different BMI indicate a significant decrease in urease activity from by 1.2 to by 2.4 times in children with BMI deviations from the norm in both settlements, both in the direction of hypo – and hypertrophy. This fact indicates a decrease in the level of contamination with bacterial microbiota in the oral cavity in children of the observation groups.

The state of "antimicrobial protection and opportunistic microbiota" system in the oral cavity is clearly reflected in the indicator of the degree of dysbiosis. Table 2 shows the results of determining the degree of oral dysbiosis by the method of A.P. Levitsky. At the initial stage of the study, the degree of dysbiosis only in the oral cavity of Odesa children with BMI> 25 exceeded by 1.6 times the normal index (p<0.05). After application of the LPS gel, the degree of oral dysbiosis in overweight children was normal (table 2).

Table 2

Influence of "Pyrogenal" gel applications on the degree of oral dysbiosis in children with different BMI (M±m)

No.	Groups	Degree of oral dysbiosis			
		Odesa		Chernomorsk	
		before	after	Before	after
1	BMI=20–25	1.00±0.12	1.06±0.09 p_1>0.05	1.09±0.08	1.12±0.19 p_1>0.05
2	BMI>25	1.61±0.22 p<0.05	0.97±0.18 p>0.05 p_1<0.05	3.08±0.35 p<0.001	1.15±0.10 p>0.05 p_1>0.001
3	BMI<20	1.30±0.18 p>0.05	1.04±0.11 p>0.05 p_1>0.05	4.02±0.59 p<0.001	2.63±0.32 p<0.001 p_1<0.05

Note: significance of differences calculated: p – compared to gr. 1; p_1 – in comparison with the indicator before applications.

Before treatment in the oral cavity of children of Chernomorsk city with deviations in body weight towards both hypotrophy and hypertrophy, the degree of dysbiosis significantly exceeded that indicator in

children with normal body weight. After a course of applications with LPS gel in the oral cavity of overweight children, the studied parameter returned to normal (p>0.001) and decreased by 2.7 times. In children with BMI<20, despite a decrease by 1.5 times, it remained quite high (p<0.001) (table 2). After application of the LPS gel, the degree of oral dysbiosis in overweight children was normal. On the background of deviations in body weight both towards hypo – and towards hypertrophy in the oral cavity, microbiocenosis disorders (urease activity) occur, which are more pronounced in children of Chernomorsk, and a decrease in protective reactions (decrease of elastase activity and MDA level) is observed Odesa children.

Table 3 shows the results of determining of inflammation markers in the oral fluid.

The application of the gel with LPS to the gingival mucosa contributed to an even greater increase in the activity of elastase in the oral fluid, as evidenced by the results obtained 1 month after the start of the application. The data obtained show the growth of this marker by 1.5–6.7 times, which indicates the activation of the inflammatory process in the parodontium, and in children of all observation groups with different BMI indicators, both in Odesa and Chernomorsk.

Table 3

Influence of "Pyrogenal" gel applications on the level of inflammation markers in the oral fluid of children with different BMI

No.	Groups	Elastase activity, мкat/l		MDA content, mmol/l	
		before	after	Before	after
Odesa					
1	BMI=20–25	0.197±0.020	0.368±0.075 p_1<0.05	0.202±0.018	0.287±0.054 p_1<0.05
2	BMI>25	0.076±0.009 p<0.01	0.322±0.040 p_1<0.001	0.167±0.020 p>0.05	0.299±0.080 p_1<0.05
3	BMI<20	0.072±0.010 p<0.001	0.483±0.119 p_1<0.001	0.130±0.018 p<0.05	0.284±0.05 p_1<0.05
Chernomorsk					
1	BMI=20–25	0.150±0.018	0.365±0.062 p_1<0.05	0.149±0.013	0.166±0.17 p_1>0.05
2	BMI>25	0.176±0.020 p>0.05	0.232±0.043 p_1<0.05	0.134±0.015 p>0.05	0.207±0.080 p_1<0.05
3	BMI<20	0.176±0.018 p>0.05	0.287±0.020 p_1<0.05	0.152±0.012 p>0.05	0.215±0.05 p_1<0.05

Note: significance of differences calculated: p – compared to gr. 1; p_1 – in comparison with the indicator before applications.

Noteworthy is the low activity of elastase in the oral fluid of children in Odessa before the use of a gel based on lipopolysaccharide. The largest amount of elastase is determined in neutrophils. Since this enzyme is of neutrophilic origin, it can be assumed that there is a delayed immunodeficiency in children with deviations in the body weight of Odessa, both in the direction of hypotrophy and in the direction of hypertrophy. Neutrophil elastase reduces the inflammatory response to invasion by microorganisms.

An interesting fact is the maximum increase in the level of elastase in Odessa schoolchildren with BMI<20 (by 6.7 times), while in 12-year-old children of Chernomorsk the maximum increase in the level of this indicator was recorded in the group with BMI=20–25 (by 2.4 times). Elastase is characterized by a powerful destructive effect on tissue proteins. The activity of this enzyme is significantly increased in the oral fluid during inflammatory processes in the oral cavity.

The same table presents the results of a study of another marker of inflammation, the MDA level, which characterizes the intensity of lipid peroxidation (LPO) in the oral cavity of children in the observation groups. Analysis of the oral fluid before treatment revealed a slight decrease in the content of this indicator in all groups of children with BMI abnormalities. Normally, the content of MDA in the oral fluid is 0.17±0.02 mmol/l.

After applying the gel with LPS, an increase in the MDA level was noted, the maximum increase was recorded in Odessa children with hypotrophy (by 2.2 times). In schoolchildren in Chernomorsk, the maximum increase in the MDA level was found in children with hypertrophy – by 1.54 times.

Most of the LPS have been studied in terms of the participation of endotoxin in the pathogenesis of certain diseases (cardiovascular, gynecological, diseases of the upper respiratory tract and lungs, neurological diseases, diabetes mellitus, etc.), including dental diseases, as well as the development of dysbiosis [11]. However, unreasonably little attention has been paid to the problem of prevention and treatment of periodontal diseases in adolescents with the use of LPS preparations. Previously, we assumed that an insufficient level of inflammatory response might be the cause of the formation of chronic parodontitis [10].

The drug **"**Pyrogenal**"** used by us is a highly active nonspecific immunomodulator of a wide spectrum of action; causes activation of the reticuloendothelial, hypothalamo – pituitary and fibrinolytic systems. Pyrogenal increases the general and specific resistance of the body, affects the thermoregulatory centers of the hypothalamus. Pyrogenal activates macrophages, enhances phagocytosis, stimulates the production of interleukin – 1, which causes the proliferation of a number of body cells (fibroblasts, endothelial cells, hematopoietic cells, etc.); interleukin – 2, which is needed to support the growth of lymphocytes (primarily T-cells), induction of endogenous interferon. An increase in the functional activity of phagocytes leads to an increase in the antimicrobial resistance of the organism, acceleration of the formation of antibodies. As a result of the activation of cells of the macrophage series and the secretion of cytokines by them, the functional activity of both the cellular and humoral immune response increases [12, 13].

The fact of shifts in the microbiota of the oral cavity in children with hypo- and hypertrophy requires the appointment of more correct differentiated schemes of prevention and therapy for not only renewing the micro – landscape of the oral cavity, but also to reduce the inflammatory process of the mucous tissues in it. This situation requires more frequent and detailed dental examinations in children with deviations in BMI, which in this case it is advisable to carry out timely and effective pharmacotherapy, especially preventive measures in this contingent of children in order to prevent major dental diseases.

Thus, further study of various aspects of the targeted action of preparations based on LPS in the complex therapy of chronic inflammatory diseases of the maxillofacial region and parodontal diseases, in particular, is a promising area of modern dentistry.

Conclusions

The course of application of the gel with LPS normalizes oral microbiocenosis (decreased urease activity and the degree of dysbiosis), with the exception of children with BMI<20, and increases the level of inflammatory markers (elastase and MDA), contributing to the transition of inflammatory processes to the active phase. Oral applications of the **"**Pyrogenal**"** gel significantly reduce the level of pathological processes in the parodontium of children due to the activation of physiological inflammation.

Prospects for further research are in the development of an effective method for the prevention of major dental diseases in schoolchildren, depending on the body mass index, with the use of an appropriate treatment and prophylactic complex.

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