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N. Yu. Emelyanova, V. Yu. Galchinskaya, T. N. Bondar
GI «L.T. Malaya Therapy National Institute of the NAMS of Ukraine», Kharkov

FEATURES OF THE METABOLISM OF NITRIC OXIDE IN PERIODONTAL DISEASES IN PATIENTS WITH COPD IN COMBINATION WITH CHD

e-mail: natadenta@gmail.com

The paper considered the role of nitric oxide in the pathology of periodontal tissues in patients with chronic obstructive pulmonary disease (COPD) in combination with coronary heart disease (CHD). It was found that in the blood plasma there was a decrease of total level of stable metabolites in patients with COPD as compared to the control group, as well as a significant increase of expression of inducible NO synthase in the materials the gingival mucosa.

Key words: chronic obstructive pulmonary disease, periodontitis, stable metabolites of nitric oxide, NO-synthase

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In terms of prevalence, chronic obstructive pulmonary disease (COPD), according to current publications, occupies one of the leading positions in the pathology structure of the bronchopulmonary system and is manifested by a persistent progressive restriction of airway patency associated with an increased chronic inflammatory response to the action of harmful particles or gases [15]. According to the conducted studies, the most common concomitant COPD pathology is coronary heart disease (CHD), which combination, according to different authors, is 55% [7]. At present time, the study of the role of nitric oxide (NO) in the pathogenesis of a number of somatic diseases (in particular COPD and CHD) is of great interest, which is due to participation of this important biological mediator in a variety of physiological and pathophysiological processes in the body. In addition, NO is involved in the etiopathogenesis of most periodontal tissue diseases [18]. It is known that the development of oxidative stress in patients with COPD is combined with increased NO production as a result of activation of the NO-synthase system and increased content of its stable metabolites. The half-life of NO is from 2 to 30 s, so its direct analysis becomes extremely difficult. To assess its concentration, the determination of the total content of stable metabolites of nitrates and nitrites (NO2, NO3) is used [13]. Generally, NO is produced by a group of isoenzymes or NO-synthases (NOS), each of which has a certain biological significance [5]. A few seconds after the stimulation of calcium-dependent constitutive endothelial (eNOS) and/or neuronal (nNOS) NOS, a small amount of NO is produced, which causes relaxation of smooth muscles and blood vessels, inhibition of aggregation and adhesion of blood cells [15]. Inducible NOS (iNOS) produces a large amount of NO for a long time. The main function of this NO is to participate in the immune processes (antitumor protection, antipathogenic reactions). Induction of iNOS is often initiated by inflammatory cytokines or endotoxins) [3]. Excess of NO as a result of iNOS expression can promote the accumulation of active forms of oxygen, mediate constrictive effects through activation of vascular permeability and cause inflammatory edema [2]. A number of studies have shown the essential role of NO and enzymes responsible for its formation in the pathogenesis of periodontal tissue diseases. The study of the dynamics of these changes in patients with combined pathology is of considerable scientific and clinical interest; however, despite the intensity of the conducted studies their results are often heterogeneous and contradictory [8].

The purpouse of the paper was to study the total concentration of stable metabolites of NO in the blood plasma and to determine the expression of NOS in gingival biopsy material in patients with COPD in combination with CHD.

Material and methods: Study included 156 patients: group 1 consisted of 85 patients (COPD in combination with CHD), group 2 involved 30 patients with COPD and group 3 involved 41 patients with CHD. The control group was represented by 20 healthy volunteers, matched by sex and age. The diagnosis of COPD was established according to the order of the Ministry of Health of Ukraine No. 555 of 27 Jun 2013 and the provisions of GOLD (Global Initiative for Chronic Obstructive Lung Disease) 2011-2015 [6]. The diagnosis of CHD was verified according to the recommendations of the European Society of Cardiology on the basis of a clinic, stress tests, HM, ECG and coronary angiography. All patients were examined by a dentist to determine the condition of periodontal tissues with the registration of periodontal parameters. The content of stable metabolites NO - (NO2 + NO3) in blood plasma was determined by the spectrophotometric method according to the Griss reaction after the reduction of nitrate to nitrite by zinc dust [9]. Deproteinization of plasma samples was carried out with 55 mM ZnSO4 and 75 mM NaOH in a volume ratio of 2: 5. Measurement of the optical density of the colored complex was carried out on a semiautomatic biochemical

analyzer CHEM-7. The expression of eNOS and iNOS was determined in biopsy materials of the gingival epithelium by immunohistochemical method using monoclonal antibodies from ThermoScientific. To differentiate tissue structures, the sections were additionally stained with Mayer's hematoxylin. The visualization was carried out using the Ultra Vision LP detection system (ThermoScientific). The prevalence of expression of the studies enzymes was assessed by the relative area of immunopositive structures (%) using Bio Vision computer morphometric program. The statistical processing was carried out using "SPSS 13" programm. Nonparametric statistical methods were used to analyze the data. The results were expressed as median (Me), 25th and 75th percentile (25-75%). To compare two independent samples, the Mann-Whitney U test and the χ 2 criterion were used. Wilcoxon test was used for dependent samples.

Results and discussion: The examination of the periodontal status of patients gave the following results: more than 90% of patients with declared somatic diseases had pathology of periodontal tissues with various clinical manifestations (Fig. 1).

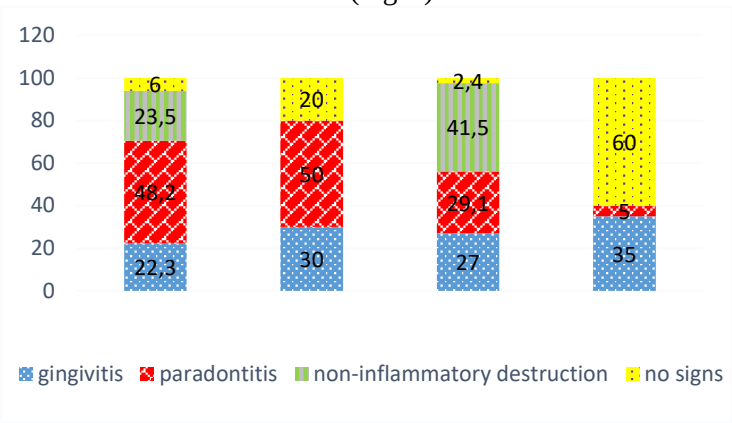

Fig.1. Structure of lesions of periodontal tissues in patients of different groups.

It should be noted that all patients with somatic pathology and 35% of patients of the control group had inflammatory diseases of periodontal tissues, gingivitis of various morphological forms without significant differences between the groups. The highest frequency of periodontitis was observed in patients diagnosed with COPD (groups 1 and 2), in contrast to the group of patients with diagnosed CHD, where the noninflammatory destruction of periodontium of different severity came to the fore (more than 40%) of periodontological pathology.

It is remarkable that all patients with COPD had an erased pattern of gingival lesion, which is probably due to the anti-inflammatory effect of inhaled glucocorticosteroids, which are taken by these patients. Determination of the total content of stable metabolites in blood plasma revealed significant decrease of this parameter in all groups of patients with somatic pathology in comparison with practically healthy persons (Table 1). The lowest values of total metabolites were observed in the group of patients with comorbid pathology. It should be noted that some studies showed conflicting results. So, according to some authors, the progression of COPD is accompanied by an increase of the content of metabolites of nitric oxide in the blood and condensation of exhaled air [11]. However, the results obtained by us are more consistent with the results of other authors who noted significantly more pronounced disturbances of endothelial dysfunction and parameters characterizing stiffness of the vascular stack in patients with chronic heart failure in combination with COPD as compared to patients without COPD [10]. In this study, the plasma component of the endothelial function was studied by the concentration of NO metabolites and it was found that it was significantly lower in patients with comorbid pathology than in patients with isolated CHD. Similar results were obtained by other authors who showed a significant decrease of the content of stable metabolites of nitric oxide in the blood plasma in COPD [4]. Due to technical difficulties and the failure of some patients to undergo gingival epithelial biopsy, the expression level of eNOS and iNOS was determined in 50 patients of the group 1, 16 patients of group 2, and 22 patients of group 3 and in 7 practically healthy individuals. All the results of the studied parameters are presented in Table 2.

Table 1

The content of stable metabolites of nitric oxide in the blood plasma of the patients of study groups (Me, q25-q75)

Patient group	NO2/NO3, µmol/l	Significance level of differences
Control (n=	20.77 (17.09; 26.58)	
1 group (n=	10.67 (6.02;17.29)	p=0.001
2 group(n=	12.58 (5.11; 20.36)	p=0.001
3 group (n=	14.34 (9.99; 21.16)	p=0.004. p1=0.026

Note: p - the level of significance of differences vs. control, p1 - the level of significance of differences vs. group 1.

Inflammatory, dystrophic, discirculatory changes were detected in the mucous membrane of gingiva in patients of groups 1 and 2 with the history of COPD, accompanied by increase of NOS expression. Expression of iNOS was increased by more than 3 times; increase of eNOS expression was less pronounced and was predominantly noted in the extravascular space. First of all, this is due to the activity of the inflammatory processes in the gingiva that induce increased iNOS expression and activity. In addition, this

category of patients has pronounced impairment of the hygienic state of the oral cavity, which contributes to the accumulation of endotoxins and can also lead to revealed increase of iNOS expression.

Table 2

Expression of eNOS and iNOS in preparations of the gingival biopsy material of the patients of study groups (Me, q25-q75)

Patient group	eNOS, %	Significance level of differences	iNOS, %	Significance level of differences
Control (n=7)	6.058 (5.951; 6.431)		4.944 (3.898; 5.604)	
Group 1 (n=50)	7.547 (6.593; 8.316)	p=0.001	14.944 (6.952; 19.023)	p=0.001
Group 2 (n=16)	7.733 (6.446; 8.555)	p=0.005	18.204 (5.614; 19.432)	p=0.004
Group 3(n=22)	6.753 (6.245; 7.889)	p=0.001 p1=0.037	5.821 (4.911; 10.874)	p=0.001 p1=0.039

Note: p - the level of significance of differences vs. control, p1 - the level of significance of differences vs. group 1.

Increased activity of iNOS and eNOS in the soft tissues of the oral cavity of experimental animals was noted by a number of authors in the modeling of atopic pathology against pronounced inflammatory and destructive processes [14]. Our findings are to some extent consistent with other studies that have shown that an increase of the level of NO in the oral cavity is associated with periodontal disease and is associated with increased iNOS expression in gingival biopsy specimens and also in gingival fibroblasts of cell cultures [1, 12] It is shown that through activation of iNOS increases the production of NO by macrophages and polymorphonuclear leukocytes, which contributes to the damage of periodontal tissue and leads to the progression of periodontitis [18]. To determine the possible relationship between the studied parameters, a correlation analysis was performed which results are presented in Table 3. In patients with comorbid pathology, an inverse correlation was found between the expression of iNOS and eNOS in the epithelium of the oral cavity with NO2 / NO3 content in the blood plasma ($r = -0.605$, $p = 0.001$) and ($r = -0.359$; $p = 0.047$). In patients with isolated COPD, a direct correlation was established between the expression levels of iNOS and eNOS in the epithelium of the oral cavity ($r = 0.700$, $p = 0.003$). In group 3, a group of patients with isolated IHD, a decrease in the total amount of nitrogen oxide metabolites was associated with an increase of eNOS expression in the epithelium of the oral cavity ($r = -0.827$; $p = 0.003$).

Undoubtedly, changes in the expression of NO synthases are primarily caused by local disturbances of the structural and functional state of the oral cavity in patients, both with isolated and associated pathologies. At the same time, the correlation links of isoenzyme expression and the content of stable NO metabolites in plasma suggest that the compensatory mechanisms can be involved in the pathological process in the oral cavity. It is likely that with increasing duration and severity of COPD, respiratory insufficiency progresses and gas exchange disorder occurs leading to tissue hypoxia. Under the influence of hypoxia, microcirculation changes occur, the development of pro-inflammatory cytokines is initiated, which in turn helps maintain a chronic inflammatory process in the periodontium. Thus, in patients with COPD, when the systemic content of stable metabolites of nitric oxide associated with endothelial dysfunction decreases, an increase of NOS expression in the gingival mucosa is noted, which contributes to the damage of periodontal tissue and leads to the progression of periodontitis in this category of patients.

Table 3

Identified correlation relationships between parameters in the groups

Group	Parameter	Value	NO2/NO3	iNOS,%	eNOS, %
1	NO2/NO3	Coefficient of correlations Significance, p	1 -	-0.605** 0.000	-0.359* 0.047
1	iNOS, %	Coefficient of correlations significance, p	-0.605** 0.000	1 -	0.093 0.519
1	eNOS, %	Coefficient of correlations significance, p	-0.359* 0.047	0.093 0.519	1 -
2	NO2/NO3	Coefficient of correlations significance, p	1 -	0.252 0.430	0.245 0.443
2	iNOS, %	Coefficient of correlations significance, p	0.252 0.430	1 -	0.700** 0.003
2	eNOS, %	Coefficient of correlations Significance, p	0.245 0.443	0.700** 0.003	1 -
3	NO2/NO3	Coefficient of correlations significance, p	1 -	0.159 0.640	-0.827** 0.002
3	iNOS, %	Coefficient of correlations significance, p	0.159 0.640	1 -	0.228 0.307
3	eNOS, %	Coefficient of correlations significance, p	-0.827** 0.002	0.228 0.307	1 -

This allows us to assume that the balance between physiological, regulatory and/or cytotoxic properties in the oral cavity is primarily due to the local expression of NO-producing enzymes, as well as the oxidative status of cells where NO effects are synthesized and realizes.

Conclusion

1. More than 90% of patients with COPD in combination with CHD have pathology of periodontal tissue with various clinical manifestations. The highest incidence of periodontitis was observed in patients diagnosed with COPD (groups 1 and 2), in contrast to the group of patients with diagnosed CHD, where non-inflammatory destruction of periodontium of different severity came to the fore (more than 40%) of periodontological

pathology. In patients with COPD and CHD, a statistically more significant decrease in the level of stable metabolites of NO in the blood relative to practically healthy individuals was found, the most pronounced in patients with comorbid pathology.

2. In patients with a combination of COPD and CHD, the expression of iNOS in the gingival mucosa increased more than by 3 times, the increase of eNOS expression was less pronounced and was predominantly noted in the extravascular space. An increase of local expression of NO-producing enzymes can contribute to damage of periodontal tissue and the progression of periodontitis in such patients.

Prospectsof the further *and deeper study of the role of NO in the onset and progression of diseases such as COPD and periodontitis will complement the standard treatment regimens taking into account pathogenetic effects.*

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Реферати

ОСОБЛИВОСТІ МЕТАБОЛІЗМУ ОКСИДУ АЗОТУ ПРИ ЗАХВОРЮВАННЯХ ПАРОДОНТУ У ПАЦІЄНТІВ З ХОЗЛ В ПОЄДНАННІ З ІХС

Ємельянова Н.Ю., Гальчинська В.Ю., Бондар Т.М.

У статті розглядається роль оксиду азоту при патології тканин пародонта у пацієнтів з хронічними обструктивними захворюваннями легень (ХОЗЛ) в поєднанні з ішемічною хворобою серця (ІХС). Виявлено, що в плазмі крові відбувається зниження сумарного рівня стабільних метаболітів у пацієнтів з ХОЗЛ відносно групи контролю, а також спостерігається істотне підвищення експресії індуцібельной NO-синтази в біоптатах слизової оболонки ясен.

Ключові слова: хронічна обструктивна хвороба легень, пародонтит, стабільні метаболіти оксиду азоту, NO-синтази.

ОСОБЕННОСТИ МЕТАБОЛИЗМА ОКСИДА АЗОТА ПРИ ЗАБОЛЕВАНИЯХ ПАРОДОНТА У ПАЦИЕНТОВ С ХОБЛ В СОЧЕТАНИИ С ИБС

Емельянова Н. Ю., Гальчинская В. Ю., Бондарь Т. Н.

В статье рассматривается роль оксида азота при патологии тканей пародонта у пациентов с хронической обструктивной болезнью легких (ХОБЛ) в сочетании с ишемической болезнью сердца (ИБС). Выявлено, что в плазме крови происходит снижение суммарного уровня стабильных метаболитов у пациентов с ХОБЛ относительно группы контроля, а также наблюдается существенное повышение экспрессии индуцибельной NO-синтазы в биоптатах слизистой оболочки десны.

Ключевые слова: хроническая обструктивная болезнь легких, пародонтит, стабильные метаболиты оксида азота, NO-синтазы.

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