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Modulatory Effect of Ammonium Ions on Human Neutrophil Oxidative Burst in Response to Bacterial Stimuli

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SUMMARY

Neutrophils (polymorphonuclear leucocytes) are the principal cells of the host defense system. Consequently, if periodontal pathogen – derived substances in the gingival crevice significantly inhibit their function, they could shift the bacterial – host balance in favour of the bacteria. The aim of investigation was to determine if the ammonium ions (periodontal pathogens produce substantial amounts) affects the main neutrofils oxidative function – reactive oxygen species generation measured by luminol and lucigene– dependent chemiluminescence. Our results show that ammonium ions both in the group of non-periodontally diseased persons and in periodontitis patients have significant inhibitive effect on luminol- and lucigene-dependent chemiluminescence, which depends on NH_4Cl concentration (5 mM, 10 mM, 25 mM, 50 mM), i.e. inhibitive effect increases with increasing concentration of NH_4Cl . It shows that the presence of ammonia in the gingival crevice may increase the risk of development of periodontal disease.

Key words: chemiluminescence, lucigene, luminol, neutrophilic leucocytes, ammonium ions

INTRODUCTION

Inflammatory diseases of periodontal tissue and dental caries are the mostly prevalent dental diseases and the main causes of teeth-loss [1,2]. There were many studies performed that reported direct or reverse correlation between these lesions [3,4]. Direct correlation was observed only in those cases when overhanging edges of filling or prosthesis were found [5]. However, in cases of expressed lesion of periodontal tissue, much lower incidence of dental caries comparing to control group (healthy periodontal tissue) was found in some studies, i.e. reverse correlation was established [6]. Identification of factors responsible for this reverse correlation could help to understand etiology and pathogenesis of both dental caries and diseases of periodontal tissue.

Over the last years attention has grown towards the fact that alkalinization of the oral cavity could be one of the preventive measures [7]. Research proves that one of the major factors for alkalinization of dental plaque pH is ammonia (ions of ammonium), which is a product of dental plaque bacteria, produced in dissociation of urea and deaminization of amino acids [8]. Urea and bacteria, which dissociate it, are important components of dental sulcus and dental plaque [9,10]. Urea metabolism and formation of ammonium ions, while having alkalizing effect on plaque pH and neutralizing dental plaque acids, are useful for hard dental tissue. Latest research indicates, that ureolytic bacteria (disseminating urea into ammonia) can be very useful in prevention of dental caries [11]. There is also research showing that adults who were more resistant to dental caries had a bigger ability to produce ammonium ions in plaque [12].

Researchers report that amount of urea in the dental sulcus of patients with healthy periodontal tissue is almost equal to the amount of urea in saliva. However, when periodontal pathology is present, the amount of urea significantly decreases because of its higher dissociation into ammonia [13]. It is known, that increased pH of gingival fluid is related to more severe lesions of periodontal tissue [14]. However, the data on action of ammonium ions on periodontal tissue and their role in the development of periodontal diseases is not comprehensive.

It is known that neutrophilic leucocytes performs protective function for periodontal tissue. These cells, while forming a major part of gingival sulcus leucocytes, are primary defensive chain, protecting periodontal tissue from invasion of pathogenic microorganisms [15]. Decreased function of these cells can initiate development of pathology of periodontal tissue. Therefore, it is important to investigate effect of ammonium ions on one of the most important neutrophilic leucocytes defensive mechanisms – oxidative-bactericide system.

MATERIAL AND METHODS

Characteristics of the sample

Two groups of persons were formed for the study: adult periodontitis patients and non-periodontally diseased (with healthy periodontal tissue). Adult periodontitis patients were selected from the pool of patients who consulted dentist for treatment of periodontal tissue inflammation at the Consultation polyclinics of Stomatology at Kaunas University of Medicine. These patients were examined clinically and radiologically. Only those patients who showed pronounced symptoms of periodontitis (PI>5) were included. Study patients did not have any systematic diseases and did not take any medications during last three months.

The group of periodontitis patients consisted of 30 persons, aged 26-34 years. There were 14 female (46.7%)

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and 16 male (53.3%) patients included in the study.

The control group without periodontitis was formed from 30 persons selected from the staff of Kaunas University of Medicine, who did not have any systematic diseases. Those persons were aged 26-34 years; there were 20 female (66.7%) and 10 male (33.3%) persons included.

Clinical study

Participants of the study completed special questionnaire, which consisted of several parts: documental, data of anamnesis, data of objective examination, as well as clinical and laboratory data.

While assessing the grade of periodontal tissue lesion, Russell periodontitis index (PI) was used [16]. Based on this index, healthy periodontal tissue was graded as 0-0.2, gingivitis – 0.3-2.0 and periodontitis – 2.1-8.

The morphologic blood composition was examined: number of erythrocytes, amount of hemoglobin, formula of leucocytes were examined using blood analyzer "Coulter STKS", and erythrocyte sedimentation was examined using micrometer of Panchenkov.

Examination of chemiluminescence of venous blood neutrophilic leucocytes

Lucigene- and luminol-dependent chemiluminescence was measured using method of Korkina LG et al. [17]. Measurement was performed using scintillate beta register (Delta 300, Model 6891) of the Department of Biological and bioorganic chemistry at Kaunas University of Medicine. Chemiluminescence was measured 5, 15, 30, 45 and 60 minutes after incubation with NH_4Cl solution of 5mM, 10mM, 25mM and 50mM concentration and activation with non-opsonized E. coli.

Table 1. Characteristics of study groups.

Study groups	Median age (years)	PI index (according to Russell)
Non-periodontally diseased persons N=30	29.4±2.2	0.1±0.6
Periodontitis patients N=30	31.9±3.2	5.83±0.28

RESULTS AND DISCUSSION

Both study groups did not significantly differ by age ($p>0.05$). PI of healthy persons was 0.1±0.6 and of periodontitis patients 5.83±0.28 (Table 1).

Amount of hemoglobin and amount of erythrocytes in venous blood of non-periodontally diseased persons and periodontitis patients did not differ statistically significantly ($p>0.05$); however, venous blood of periodontitis patients contained significantly higher amount of leucocytes comparing with non-periodontally diseased persons, $p<0.05$ (Table 2).

While NL interacts with antigens during oxidative gale, light emission occurs [chemiluminescence (CL)], which depends on formed active forms of oxygen. It is known, that luminol-dependent NL chemiluminescence indicates total extra- and intracellular amount of produced active oxygen forms; while lucigene-dependent NL chemiluminescence indicates superoxide anion produced by these cells, which

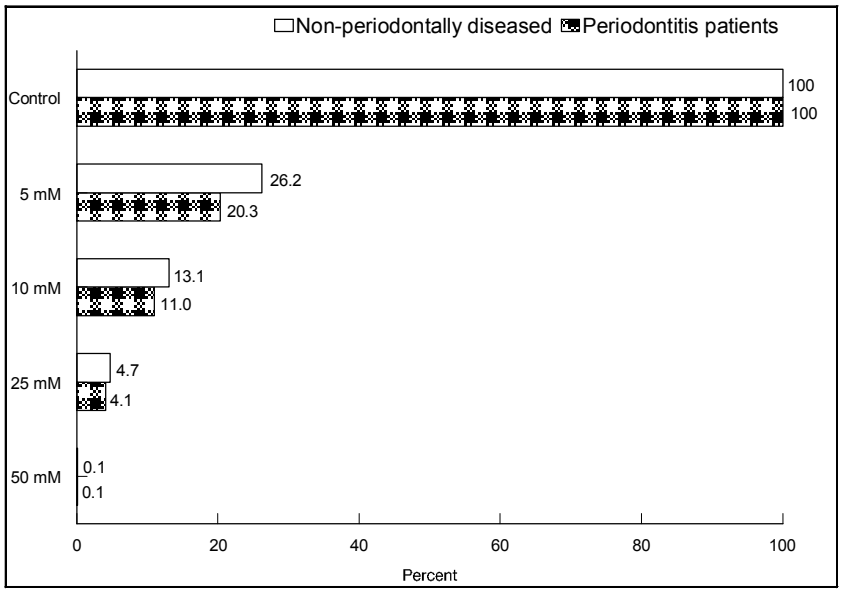

Figure 1. Dependence of inhibition effect of luminol-dependent NL chemiluminescence on concentration of NH_4Cl in non-periodontally diseased and periodontitis patients.

Table 2. Venous blood laboratory test data of examined persons.

Study group	Amount of hemoglobin (g/l)	Amount of erythrocytes x10^{12}/l	Amount of leucocytes x10^9/l
Non-periodontally diseased patients n=30	137.6±5.4	4.5±0.2	4.7±0.3*
Periodontitis patients n=30	136.7±9.2	4.6±0.4	7.0±0.5*
			*p<0,05

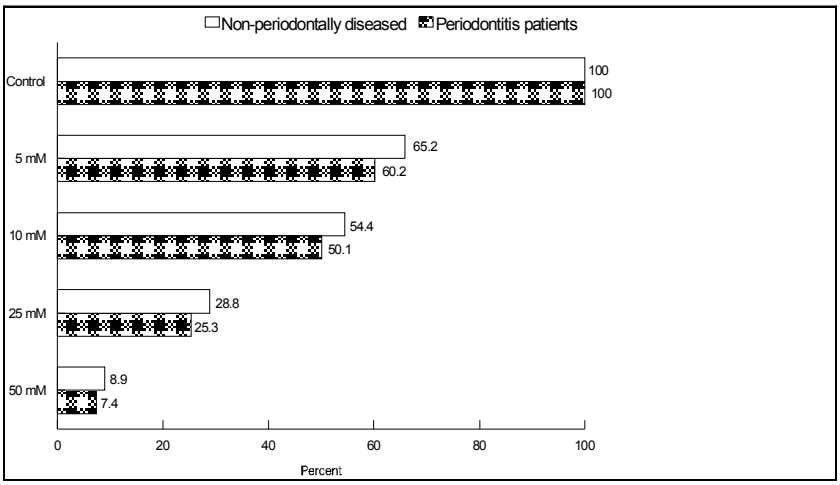

Figure 2. Dependence of inhibition effect of lucigene-dependent NL chemiluminescence on concentration of NH_4Cl in non-periodontally diseased and periodontitis patients.

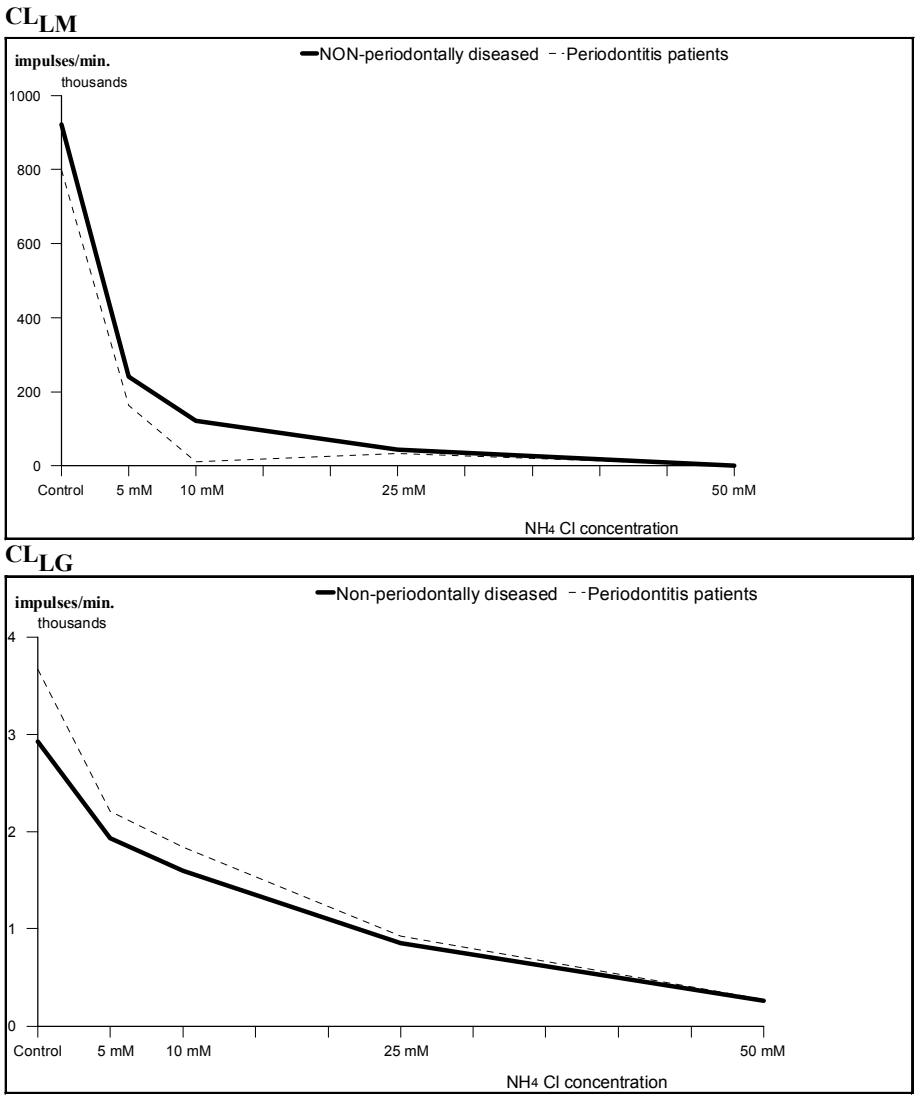

Figure 3. Venous blood NL luminol- (LM) and lucigene- (LG) dependent CL due to the effect of non-opsonized *E. coli* ($1x10^9$ cells/m) affected by NH_4Cl (5mM, 10 mM, 25 mM, 50 mM) in non-periodontally diseased and periodontitis patients.

due to Haber Weiss reaction transmutes into another extremely active form of oxygen – hydroxyl radical [18].

Our results show that ammonium ions both in the group of non-periodontally diseased persons and in periodontitis patients have significant inhibitive effect on luminol- and lucigene-dependent chemiluminescence, which depends on NH_4Cl concentration, i.e. inhibitive effect increases with increasing concentration of NH_4Cl (Fig. 1,2). Other authors report similar results as well. However, in their investigations synthetic activator was used and only luminol-dependent chemiluminescence was measured. As we know, investigations of the effect of NH_4Cl on NL oxidative function, while activating indicated cells with activators of biological origin, have higher clinical weight, since these cells are always influenced by microorganisms present in oral cavity. We also investigated lucigene-dependent chemiluminescence, indicating superoxide anion excreted by NL, which transmutes into extremely active form of oxygen – hydroxyl radical, supposedly having a big impact on lesion of periodontal tissue [19,20]. Our results indicate, that lucigene-dependent CL in both groups is inhibited by ammonium ions two times lower than luminol-dependent CL (Fig. 3). We can state that ammonium ions more significantly inhibit the release of active oxygen forms responsible for organism protection from bacteria than the release of active oxygen forms harmful to organism.

Inhibited production of active oxygen forms, using NH_4Cl concentration of 5, 10, 25 and 50 mM, can have negative effect on defensive function of NL in fighting the pathogens.

Hence, examination of effect of ammonium ions on neutrophilic leucocytes oxidative function could elucidate its role in development of periodontal diseases. The more we understand about pathological factors, the more opportunities we have to prevent the disease or select the treatment strategy.

The data of our research on the effect of ammonium ions on oxidative function of neutrophilic leucocytes and breathing speed of isolated mitochondria proves that ammonium ions can be one of risk factors for lesion of periodontal tissue. Patients, who have alkaline pH of saliva and gingival sulcus as well as low dental caries index, have increased risk for diseases of periodontal tissue because of harmful effect of ammonium ions on this tissue. The attention should be paid towards this, while selecting preventive measures for the oral hygiene. These patients should not use alkalinizing preventive measures.

CONCLUSIONS

1. Ammonium ions (dental plaque bacteria product) inhibit neutrophilic leucocytes – cells protecting periodontal tissue from invasion of pathogenic microorganisms, oxidative function:

NH_4Cl significantly inhibits the release of free oxygen radicals in non-periodontally diseased and periodontitis patients;

2. Ammonium ions more significantly inhibit the release of active oxygen forms responsible for organism protection from bacteria than the release of active oxygen forms harmful to organism: lucigene dependent chemiluminescence in both groups is inhibited by NH_4Cl two times lower than luminol-dependent chemiliuminescence.

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