Inflammatory diseases of periodontal tissues mostly cause damage to the hard and soft tissues, which surround and support the tooth. Structural complexity of the components of periodontal tissues, including gingival bacteria and the products of their metabolism, as well as a variety of response factors of the macroorganism hinder the disclosure of etiopathogenetic mechanism of inflammatory diseases of the periodontal tissues [1].

In recent years some factors that have an influence on the development of gingivitis and periodontitis have been revealed [2]. On summing up investigations of recent years, R.C.Williams [3] has presented an up-to-data model of inflammatory diseases of periodontal tissues placing particular emphasis on the immune inflammatory response of the macroorganism to the invading microbes of dental plague.

Neutrophil leukocytes (NL) perform the protective role of periodontal tissues as they have a variety of substances necessary for destruction of pathogenic microorganisms. When the number of NL decreases or their functional efficiency gets disturbed, diseases of periodontal tissues set in [4]. Neutrophil leukocytes perform the protective role of macroorganism through different consecutive stages. Its final stage is phagocytosis of microorganisms, destroying them in the phagolysosome [5]. Along with phagocytosis, microbes, virus-infected cells, and cancer cells can be destroyed by NL, releasing their lysosomal granular content into the extracellular environment [6]. It has been established that NLs on leaving healthy periodontal tissues spontaneously release certain amount of active forms of oxygen [7].

Active forms of oxygen produced in the respiratory burst of NL (superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxy-radical (·OH), and singlet oxygen (O$_2^*$)) [8, 9] make up an effective bactericidal NL system. Intensity of the respiratory burst depends on the nature of NL stimulator and concentration [10]. Active forms of oxygen, being potent oxidants, not only cause destruction of bacteria [11], but they can also damage the tissues of the macroorganism [12].

In recent years, most of scientists [13, 14], who investigate various diseases and oxidative metabolism of leukocytes, focus attention on superoxide anion generated by NL. Then, by Haber-Weiss reaction:

$$\text{H}_2\text{O}_2 + \text{O}_2^- \rightarrow \text{OH}^- + \cdot \text{OH}$$

or as a result of Fenton reaction:

$$\text{H}_2\text{O}_2 + \text{Fe}^{3+} \rightarrow \text{Fe}^{2+} + \text{OH}^- + \cdot \text{OH}$$

hydroxyl-radical or some other similar toxic forms of oxygen can be produced [15].

Superoxide anion determination is mostly done by the method of lucigenin-dependent chemiluminescence (CL) [11, 13]. The aim of the present study was to investigate the generation of superoxide anion in NL of peripheral venous blood of patients with periodontitis and of healthy subject by the method of lucigenin-dependent chemiluminescence. Stimulating leukocytes with opsonized zymosan, nonopsonized E.coli, and E.coli 055:BB endotoxin (LPS).

**MATERIALS AND METHODS**

In performing investigations of superoxide anion generation, neutrophil leukocytes (NL) were obtained from peripheral venous blood of 16 periodontitis patients and 10 healthy subjects. Superoxide anions production was measured by a lucigenin-enhanced chemiluminescence assay.
The difference in mean age and sex of patients and of those in the control group was not significant (p=0.05).

Superoxide anion generation was investigated by lucigenin-dependent chemiluminescence (CL) method as proposed by L.G.Korkina et al. [12]. Chemiluminescence measurements were performed at the Department of Biochemistry of Kaunas University of Medicine using a liquid scintillation counter “Delta-300”. Lucigenin, zymosan, lyopolysacharides (LPS), as well as Hanks balanced salts solution (pH=7.3) were obtained from Sigma Chemical Co. (USA), and E.coli sample ATCC 25922 was grown at the Microbiology Laboratory of Kaunas Medicine University Clinics. Specimens of 25922 was grown at the Microbiology Laboratory of Kaunas from Sigma Chemical Co. (USA), and Hanks balanced salts solution (pH=7.3) were obtained using a liquid scintillation counter “Delta-300”. Chemistry of Kaunas University of Medicine using a liquid scintillation counter “Delta-300”.

Preparation of leukocyte specimens

For assessment of superoxide anions, leukocytes were obtained from peripheral venous blood of patients with periodontal diseases and from that of healthy control subjects. Ten milliliters of venous blood were taken in the morning before meals. Blood clotting was controlled with heparin (20 units/ml). Plastic test tubes containing blood were positioned at an angle of 45 degrees and were kept for 1 hour at 37°C. Then the supernatant layer of plasma rich in leukocytes was aspirated and diluted with Hanks balanced salt solution (HBSS) up to 5 ml. Then, this cell suspension in portions of 1 ml was taken into cuvettes used for chemiluminescence investigation, putting aside 1 ml of the suspension for counting up the number of leukocytes and the percentage of their composition.

Investigation of NL superoxide anion generation

Cuvettes containing leukocyte suspension were placed in a thermostat with water, gradually adding 0.1 ml of lucigenin (20 units/ml). Plastic test tubes containing blood were positioned at an angle of 45 degrees and were kept for 1 hour at 37°C. Then the supernatant layer of plasma rich in leukocytes was aspirated and diluted with Hanks balanced salt solution (HBSS) up to 5 ml. Then, this cell suspension in portions of 1 ml was taken into cuvettes used for chemiluminescence investigation, putting aside 1 ml of the suspension for counting up the number of leukocytes and the percentage of their composition.

Investigation of NL superoxide anion generation

In the control group was not significant (p≤0.001). It is necessary to note that the above-mentioned difference was particularly distinct due to the effect of E.coli LPS. If lucigenin-dependent CL of NL 1X10⁹ of peripheral venous blood of patients suffering from periodontitis reaches its maximum 407±893 imp/min, the analogous CL of healthy control subjects is only 381±67 imp/min. It is also evident that stimulation of NL of blood of patients with periodontitis and of subjects with intact periodontium by means of opsonized zymosan, non-opsonized E.coli, or LPS, in the main intensifies lucigenin-dependent CL. Acted by opsonized zymosan, 1X10⁹ NL of peripheral venous blood taken from patients with periodontitis, reaches 387±65 cpm, and it has been found to be statistically reliably (p≤0.001) higher than that of analogous cases in the control group (137±14 cpm).

Nonopsonized E.coli intensified much more lucigenin-dependent CL of NL of peripheral venous blood taken from periodontal patients and from control healthy subjects. However, in the group of patients with periodontitis, it was in the main (p≤0.001) higher (2422±337 cpm) than in the analogous control group (65±103 cpm).

It is of interest to note that the above-mentioned difference was particularly distinct due to the effect of E.coli LPS. If lucigenin-dependent CL of NL 1X10⁹ of peripheral venous blood of patients suffering from periodontitis reaches its maximum 407±893 imp/min, the analogous CL of healthy control subjects is only 381±67 imp/min. It is necessary to note that the NL reaction of peripheral venous blood of subjects in the control group to E.coli LPS was much weaker than the reaction to non-opsonized E.coli (p≤0.001).
Lucigenin-dependent CL changes in NL of peripheral venous blood of both groups of patients under study due to opsonized zymosan, nonopsonized E.coli, and E.coli LPS are presented in Fig 1. It shows that due to the effect of opsonized zymosan and E.coli LPS, the above-mentioned CL reaches its maximum after 15 minutes, while under the effect of non-opsonized E.coli, it takes about 30 min to reach its maximum after stimulation.

DISCUSSION

Periodontal disease is an inflammatory disorder caused by bacteria of dental plaque [17]. Recent investigations have shown that some dental plaque bacteria can penetrate deep into the tissues [18], their toxins penetrating biological membranes [19]. NL cells have been found to be the very first to migrate into tissues as a response of the macroorganism to the invaded microorganisms [20]. As NL cells migrate to the infected site, their activity undergoes some changes manifested in biological effects, including elevated production of active forms of oxygen. An increase in the activity of NL is an important non-specific immunological factor of the organism surveillance, hindering the penetration of microbes. At the same time this factor can cause damage to the tissues of the organism [21]. This is particularly characteristic of the action of NL superoxide anion released during the respiratory burst into the surrounding [22].

When drawing a comparison between the effect of lucigenin-dependent CL, which is induced by opsonized zymosan, non-opsonized E.coli, and LPS (see Table 1 and Fig.1) of NL of peripheral venous blood taken from patients with periodontitis, it is known that [23] lucigenin-dependent CL of NL indicates only to the fact that superoxide anion is produced by NL cells and on undergoing a series of intermediate reactions, superoxide anion can attain various toxic forms of oxygen.

It is supposed [5] that active forms of oxygen produced in vivo can inactivate antiproteinases present in biological fluids, thus increasing the activity of proteases. In recent years, lots of research works [24] have been published, placing emphasis on the importance of proteases in causing inflammatory diseases of the periodontium. Besides, active oxygen forms can activate NL-produced metal proteinases. This makes it possible to presume that such active forms of oxygen produced by NL are particularly important factors causing tissue damage [25].

It is expected that performing investigations in this research area will make it possible to better understand the causative factors of diseases of the periodontal tissues, and to work out effective methods of prevention and treatment of such diseases.

Table 1. Lucigenin-dependent chemiluminescence of peripheral venous blood NL of investigation groups patients.

<table>
<thead>
<tr>
<th>Investigation groups</th>
<th>n</th>
<th>Non-stimulated</th>
<th>After stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 × 10^7 NL chemiluminescence (cpm)</td>
<td>opsonized zymosan</td>
</tr>
<tr>
<td>Periodontitis patients</td>
<td>16</td>
<td>387±65</td>
<td>1361±169</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>137±14</td>
<td>492±56</td>
</tr>
<tr>
<td>p</td>
<td>≤0,001</td>
<td>≤0,001</td>
<td>≤0,001</td>
</tr>
</tbody>
</table>

REFERENCES


Received: 22 08 2003 Accepted for publishing: 27 09 2003