EFFECT OF FLUORIDE ON HUMAN NEUTROPHIL OXIDATIVE BURST

Ingrida Pacauskiene,a Nomeda Baseviciene,b Pajauta Paipalieneb

Kaunas, Lithuania

Summary: Luminol-dependent chemiluminescence (LMDCL) and lucigenin-dependent chemiluminescence (LGDCL) of venous blood neutrophils in 53 periodontally healthy adult control subjects and 52 adult patients with chronic periodontitis were measured after incubation of the neutrophils with 5, 10, 50 µM, 7.5 mM, and 15 mM NaF, followed by activation with nonopsonized E. coli and opsonized zymosan. The chemiluminescence level (CL) of the neutrophils was found to depend on the concentration of NaF in the incubation medium and the state of the periodontal tissues. Although 5 and 10 µM F– had no influence on the CL, LMDCL and LGDCL of the activated neutrophils reproducibly increased at 50 µM F–. At 7.5 and 15 mM F–, LMDCL and LGDCL of the activated neutrophils decreased. The level of CL of activated neutrophils is consistently higher and most significant at a concentration of 50 µM F– for periodontitis patients than for healthy subjects, except for LMDCL of activated NLs by E. coli.

Keywords: Chemiluminescence (CL); Fluoride ion; Human neutrophils; Lucigenin-dependent chemiluminescence; Luminol-dependent chemiluminescence; Periodontitis; Reactive oxygen species.

INTRODUCTION

After more than five decades of intense dental usage, reports of toxic effects from fluoride, even at low levels of intake, are appearing with increasing frequency.1–3 Minute doses of fluoride below those causing dental fluorosis are reported to have adverse systemic effects on laboratory animals.4 Fluoride has also been found to alter mitotic activity of lymphocytes5 and to influence the function of neutrophilic leukocytes (NLs).6 Comprising a major part of crevicular granulocytes, NLs are very important for protecting periodontal tissues from pathogenic microorganisms.7–9 Moreover, increased generation of reactive oxygen species during interactions between NLs and microorganisms may have considerable influence on the destruction of periodontal tissues.10,11

Exposure of NLs to F– has been shown to affect the release of superoxide.12,13 At present, however, no data appear to be available indicating to what extent topically applied F– affects the oxidative response of human neutrophils to bacterial stimuli in healthy subjects and in patients with chronic periodontitis.

The aim of the present study was to explore and compare the effect of sodium fluoride at concentrations which may occur in the oral cavity on the oxidative activity of human venous blood NLs in a group of patients with chronic periodontitis and a healthy control group without periodontitis.

aFor Correspondence: Ingrida Pacauskiene, MD, PhD, a,bClinic of Dental and Oral Diseases, Kaunas Medical University, Eiveniu 2, Kaunas, Lithuania. E-mail: ingridapacauskiene@takas.lt
MATERIALS AND METHODS

The study included two groups of subjects 18 to 50 years of age: patients with chronic periodontitis (n = 52) and a group of periodontally healthy persons (n = 53). The periodontal index (PI) in the healthy subjects was equal to 0, whereas in patients with chronic periodontitis it was 5.78±0.7, i.e., significantly expressed periodontal tissue lesions. The ages and sex ratio of the patients with chronic periodontitis did not differ significantly from those of the healthy control subjects (p>0.05).

None of the subjects had any systemic disorders during the preceding three months, none had used drugs, and none had harmful intake habits (i.e., cigarette smoking or/alcohol abuse). All used the tap water from the city water supply containing 0.16 to 0.18 ppm F⁻ and did not use any additional fluoride preparations except fluoridated toothpaste.

For assessment of the degree of periodontal lesions the Russell periodontal index was used. Differential leukocyte count was performed with the haematology analyzer “Advia 120”.

The suspension of NLs was obtained by the method of spontaneous sedimentation from 10 mL of venous blood of the above-mentioned subjects taken early in the morning after overnight fasting.

Luminol- and lucigenin-dependent chemiluminescence (LMDCL and LGDCL) was measured by the method of Korkina et al. The measurements were performed with a scintillation β meter. Test tubes with suspensions of leucocytes were kept in water thermostats at a temperature of 37 ºC. To these suspensions an aqueous luminol or lucigenin solution of 50 µM final concentration was added, and chemiluminescence (CL) of nonstimulated NLs was then measured. For evaluation of the fluoride effect, a solution of NaF was poured into the test tubes so that final concentrations of F⁻ in the leucocyte suspension were equal to 5, 10, and 50 µM, 7.5 mM, and 15 mM. Corresponding amounts of Hank’s solution were added to test tubes with control leucocyte suspensions. CL was recorded after 3, 15, 30, 45, and 60 min of incubation with the NaF solutions.

For recording CL of stimulated NLs after 3 min of incubation with NaF, two different assays were carried out simultaneously by the addition of two different activators to the same leucocyte suspension: 0.1 mL of E. coli strain ATCC 25922 suspension grown for 24 hr (1x10⁹ cells/mL) and 0.05 mL of opsonized zymosan solution (2.5 mg/mL). Zymosan was opsonized by the method of Mayo. CL of NLs was recorded and counted according to the method of Korkina et al. after 5, 15, 30, and 45 min of stimulation by the above-mentioned bacterial treatment.

For statistical evaluation of the data, the SPSS statistical program package was used to calculate the arithmetic mean of the series of values, the standard deviations (SD), and the level of significance. A p value >0.05 was considered insignificant. For determining the normal distribution, the Kolmogorov-Smirnov test was used.
RESULTS

The venous blood of the healthy subjects and patients with chronic periodontitis did not differ appreciably in erythrocyte count and haemoglobin level (p>0.05), whereas the leukocyte count was significantly higher (p<0.05) in the blood of patients with chronic periodontitis (7.0±0.5x10^9) than in the blood of healthy subjects (4.8±0.3x10^9).

Very low concentrations of F⁻ (5 and 10 μM) had no appreciable influence on CL values of NLs activated by opsonized zymosan and nonopsonized E. coli. However, after incubation of NLs with 50 μM F⁻ and activation with opsonized zymosan and nonopsonized E. coli, LMDCL and LGDCL increased significantly (p<0.001) compared with controls not treated with F⁻ (Figures 1 and 2). LMDCL and LGDCL of NLs taken from patients with chronic periodontitis significantly exceeded corresponding values of the healthy subjects (p<0.001), except of LMDCL of activated NLs by E. coli.

After incubation of the NLs with higher concentrations of NaF (7.5 and 15 mM F⁻), LMDCL and LGDCL in both subject groups decreased with opsonized zymosan and nonopsonized E. coli and differed significantly from controls not treated with fluoride (Figures 1 and 2).

![Figure 1. LMDCL and LGDCL of neutrophils after 30-minute activation by E.coli and their dependence on F⁻ concentration in the incubation medium](image-url)
After incubation of NLs taken from periodontitis patients with 7.5 and 15 mM of F\(^-\), LMDCL and LGDCL significantly exceeded analogous values of the healthy subjects (p<0.001) in the presence of opsonized zymosan. However, after activation of NLs by nonopsonized *E. coli* incubated with NaF at the above-mentioned F\(^-\) concentrations, both the periodontitis and control groups did not differ in LMDCL (p>0.05).

After stimulation for 45 min of NLs by nonopsonized *E. coli* incubated with NaF, LMDCL reached its maximum in both groups of subjects. In these specimens of NLs, LGDCL reached its maximum after a 30-min stimulation.

**DISCUSSION**

It is known that LMDCL of NLs shows a summed-up total extracellularly and intracellularly produced amount of reactive oxygen species, whereas LGDCL of NLs indicates superoxide is released by these cells.\(^{19}\) In evaluating LMDCL and LGDCL of NLs, more data are supplied by the ratio between the LGDCL and LMDCL values.\(^{16}\) The higher the LGDCL of NLs, the greater is the probability that the latter will have tissue-damaging effects. Our results reveal that this ratio increased after incubation with NaF, and in the group of patients with chronic periodontitis it was 1.1–1.4 times greater than in the group of healthy subjects.
According to the data of our study, the effect of NaF on the release of reactive oxygen species was dependent on the F– concentration. The exact mechanisms of fluoride action, however, are still not well investigated. It is considered that F– directly activates G proteins of NLs and induces intracellular production of superoxide.\textsuperscript{20} It is also thought that F– influences changes of [Ca\textsuperscript{2+}] necessary for production of superoxide.\textsuperscript{2}

Our results showing that 50 µM F– has an activating action on CL of NLs indirectly support the findings by Gutierrez et al.\textsuperscript{21} They concluded that low concentrations of NaF activate neutrophil phagocytosis. Similar data were obtained by Shapira et al.,\textsuperscript{13} who found that very low concentrations of amines and stannous fluoride activate neutrophil superoxide generation. Although NLs have a protective function, data from contemporary investigations show that superoxide anions exert a considerable influence on the destruction of periodontal tissues.\textsuperscript{10,11,13,22,23}

Based on the results of various reported investigations, it is reasonable to assume that topical application of fluoride agents may activate NLs for performing a protective periodontal function, but it may also have a negative effect on the pathogenesis of periodontitis.

In summary, CL of neutrophils activated by nonopsonized \textit{Escherichia coli} and opsonized zymosan depends on the concentration of added sodium fluoride and on the state of the periodontal tissues. LMDCL and LGDCL of the activated neutrophils reproducibly increase at a concentration of 50 µM F– and decrease at concentrations of 7.5 and 15 mM F–. The level of CL of activated neutrophils is consistently higher and most significant at concentration of 50 µM F– for periodontitis patients than for healthy subjects, except for LMDCL of activated NLs by \textit{E. coli}.

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