Stomatologija, Baltic Dental and Maxillofacial Journal, 5:129-32, 2003

The Influence of Sodium Fluoride on Chemiluminescence of Neutrophilic Leukocytes

Ingrida Pacauskiene, Nomeda Baseviciene, Pajauta Paipaliene, Jonas Zekonis

SUMMARY

In spite of wide use of fluoridated dental products in dentistry, until now there are no available data on the influence of fluoride (F^-) on oxidative activity of neutrophil leucocytes, which play the major role in protecting periodontal tissues and oral mucosa from pathogens. The aim of the present study was to investigate and compare the effect of sodium fluoride on the oxidative activity of venous blood neutrophils of the periodontally healthy control subjects (n=53) and patients with chronic periodontitis (n=52).

Luminol-dependent chemiluminescence was measured after incubation of the neutrophils with NaF (5, 10 and 10 μM, 7.5 and 15 mM F^-) and activation with nonopsonized *E.coli* and opsonized zymosan.

The level of chemiluminescence of the neutrophils depended on concentration of NaF in the incubation medium and the state of periodontal tissues. Low concentration of fluoride (5 μM and 10 μM F^-) had no influence on the chemiluminescence. Luminol-dependent chemiluminescence of the non activated and activated neutrophils reliably increased at a concentration of 50 μM F^-. At concentrations of 7.5 and 15 mM F^-, chemiluminescence of non activated neutrophils in both the studied groups reliably increased, whereas chemiluminescence of the activated neutrophils decreased. At concentrations of 7.5 and 15 mM F^-, the level of chemiluminescence of non activated neutrophils was reliably higher in patients with periodontitis than that of healthy subjects.

According to the data of this study, NaF effecting the production of reactive oxygen species by neutrophilic leucocytes may influence the protection periodontal tissues from pathogenic microorganisms.

Key words: neutrophilic leucocytes, luminescence, fluoride, reactive oxygen species, periodontitis.

INTRODUCTION

Fluoride preparations are very widely used in preventing dental disorders. After a more than five-decade intense usage even in very small doses, reports on toxic effects of fluoride have been increasingly presented [13, 24, 17], as there appeared studies demonstrating that minute doses of fluoride, not causing fluorosis, may exert negative effect on animal organism [1]. Recently, it was revealed that fluoride may have a genotoxic effect [13], can alter mitotic activity of lymphocytes [14], and influence function of neutrophilic leucocytes [10].

Neutrophilic leucocytes (NL), comprising major part of crevicular granulocytes, are very important in protecting the periodontal tissues from pathogenic microorganisms [18, 15, 25]. Recent studies have shown that increased generation of active forms of oxygen during interaction between NL and microorganisms may have a great influence upon destruction of the periodontal tissues [12, 20]. However, it has not been yet established whether preventively used sodium fluoride (NaF) preparations could influence the species of reactive oxygen released by NL and play a role in the pathogenesis of chronic periodontitis.

***Ingrida Pacauskiene**, D.D.S., PhD.*
***Nomeda Baseviciene**, D.D.S., PhD.*
***Pajauta Paipaliene**, Assoc. Prof., D.D.S., PhD.*
***Jonas Zekonis**, Prof., D.D.S., PhD., Hab. Dr.*
Kaunas Medical University, Faculty of Odontology
Address correspondence to Dr.Ingrida Pacauskiene, Clinic of Dental and Oral Pathology, Eiveniu 2, Kaunas, Lithuania.
E-mail: ingridapacauskiene@takas.lt

MATERIAL AND METHODS

For performance of the study, two groups of subjects were formed: a group of patients with chronic periodontitis (n=52) and a group of healthy persons (n=53). The periodontal index (PI) in the healthy subjects was equal to 0, whereas in patients with chronic periodontitis, 5.78±0.7, i.e. the patients had significantly expressed periodontal tissue lesions. The age of the studied subjects ranged from 18 to 50 years. The patients with chronic periodontitis did not differ significantly from the healthy persons concerning the age and sex ($p>0.05$).

The studied subjects had no systemic disorders during the preceding 3 months, used no drugs, had no harmful habits (cigarette smoking or/and alcohol abuse). All the studied persons have been using as potable water the running water from the city water supply with content of fluoride in the range from 0.16 to 0.18 ppm and did not use any additional fluoride preparations except the tooth paste containing fluoride.

For assessment of the degree of periodontal lesions the Rusell periodontal index was used. Also, differential leucocyte count was performed.

Neutrophilic leucocytes were obtained by the method of spontaneous sedimentation [21] from 10 ml of venous blood of the above–mentioned donors, taken early in the morning after overnight fasting.

Luminol–dependent chemiluminiscence (CL) was measured by the method of L.G.Korkina et al [11]. The measurements were performed with a scintillation β-meter (Delta 300, Model 6891). For evaluation of the fluoride effect, solution of NaF was poured into the test tubes so that final concentrations of F^- in the medium were equal to 5 μM, 10 μM, 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 NaF solution.

Test tubes with suspension of leucocytes were kept in water thermostats at a temperature of 37ţ C, to which 0.1 ml of luminal solution (of 50μM final concentration) was added, and CL of nonstimulated NL was measured.

For measurement of CL of stimulated NL after 3 min of incubation with NaF, 0.1 ml of *E.coli suspension* (1×10^9 cells/ml) or 0.05 ml of opsonized by the method of K.Mayo zymosan (2.5 mg/ml) suspension were added to the tubes [16]. CL was recorded after 5, 15, 30 and 45 min of stimulation by the abovementioned cells.

For the statistical evaluation of the data, SPSS statistical program package was used, the arithmetic mean, the standard deviation (SD), the level of significance were calculated. The significance was accepted at the $p<0.005$.

RESULTS

The venous blood of the healthy subjects and patients with chronic periodontitis did not differ in the erythrocyte count and hemoglobine level ($p>0.05$), whereas leucocyte count was significantly ($p<0.05$) higher in the blood of the patients with chronic periodontitis ($7.0\pm0.5\cdot10^9$) than in the blood of healthy subjects ($4.8\pm0.3\cdot10^9$).

It was found that after 3-minute incubation of NL of healthy subjects and chronic periodontitis patients with 50 μM, 7.5 mM, and 15 mM FÆ, luminal-dependent background CL significantly ($p<0.05$) increased (Fig. 1) and was higher in periodontitis patients group ($p<0.001$). During longer incubation of NL with FÆ, CL of NL increased and reached its maximum after 30 min. Very low concentrations of FÆ (5 and 10μM) had no reliable influence upon CL values.

After 3-minute incubation of NL with 50 μM FÆ and activation with opsonized zymosan and nonopsonized *E.coli*, luminal-dependent CL significantly increased ($p<0.001$) compared with control specimens, not affected with fluoride (Fig.2, 4). Luminol-dependent CL of NL taken from patients with chronic periodontitis significantly exceeded analogous values of the healthy subjects ($p<0.001$).

After incubation of the NL with higher concentration NaF (7.5 and15 mM of FÆ), luminal-dependent LC of stimulated by opsonized zymosan and nonopsonized *E.coli* NL in both studied subject groups decreased, and significantly differed from controls not effected by fluoride (see Fig.2, 4). After incubation of NL with NaF and opsonized zymosan or nonopsonized *E.coli* at the abovementioned FÆconcentration, both the studied groups did not differ ($p>0.05$) in luminal-depended CL.

After 45-minute stimulation by nonopsonized *E.coli* and incubation of NL with NaF, luminal-dependent CL reached its maximum in both the studied groups (Fig.3).

DISCUSSION

During interaction between NL and antigens, in the phase of respiratory burst, light emission [chemiluminescence] occurs, which depends on the generated forms of reactive oxygen. It is known that luminal-dependent chemiluminescence of NL shows a summed up total extracellularly and intracellularly produced amount of active oxygen forms, which during occurring pathological states can damage body tissues [8, 20, 25].

It is considered that NL not stimulated by bacterial activators release by themselves a certain amount of the active forms of oxygen, whereas NL of patients with chronic periodontitis releases these substances even in greater amounts [6]. That is also supported by the data of this study. Besides, incubation of NL with NaF increases the release of these substances. Very low concentrations of NaF (5 and 10μM of FÆ) exerted no influence on the values of CL of leucocytes. CL of NL obtained from venous blood of the patients with chronic periodontitis and incubated with NaF was significantly higher than the analogous value of the healthy subjects ($p<0.05$).

The mechanism of the increased release of active forms of oxygen under influence of fluoride ions is yet not well known. It is considered that fluoride directly activates G proteins of NL and induces intracellular production of superoxide [2]. Other authors suppose that FÆ concentration

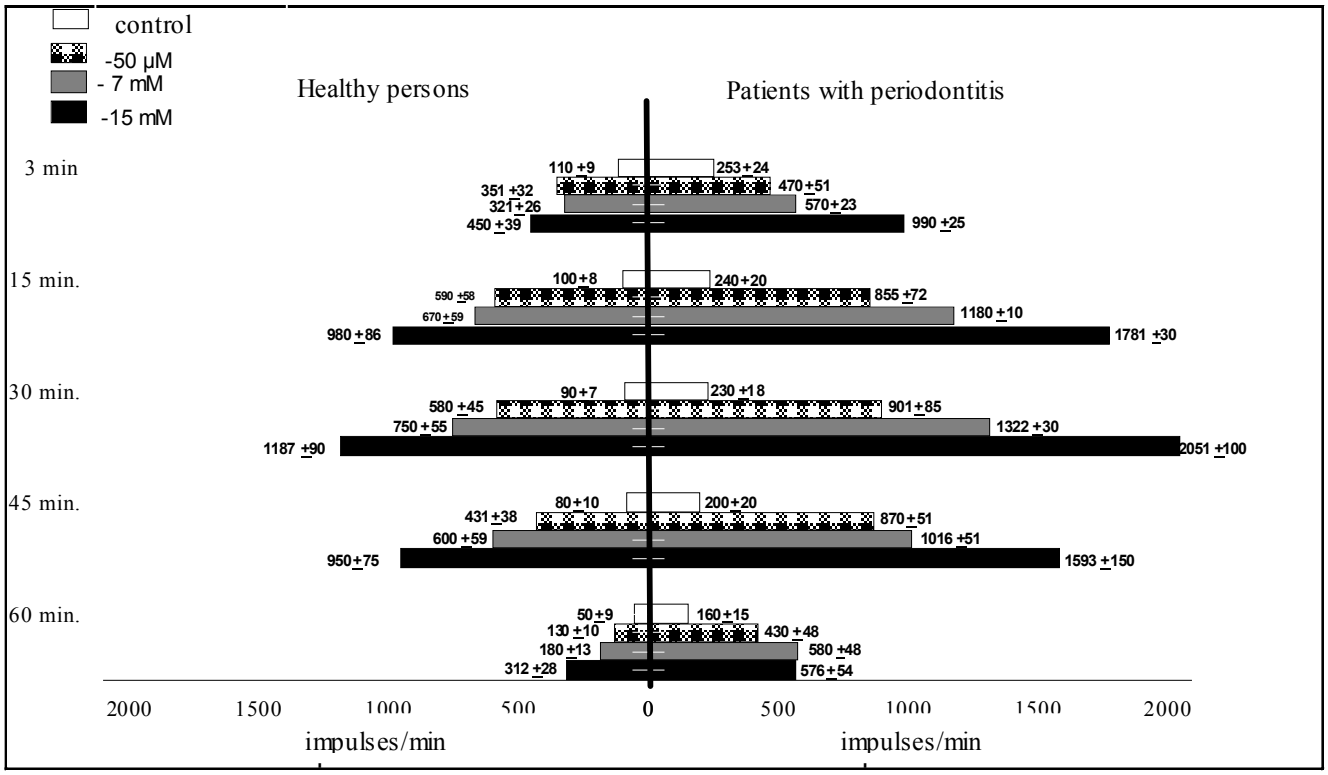

Figure 1. *Effect of NaF on luminal-dependent chemiluminescece of neutrophils*

higher than 5 mM exerts influence on changes of intracellular level of Ca^{2+} [10]. There is supposition that NaF at concentration of 10-20 mM may bind up the cellular surface Ca^{2+} necessary for production of superoxide [4]. The decreased concentrations of calcium ions on the outer surface of the cells may impact the release of calcium ions from the intracellular structures plasma membrane, mitochondria, and endoplasmic reticulum. This process is controlled by cAMP. It has been established that NaF induces an increase in the amount of cAMP proportionate to its concentration, and in this way augments concentration of the intracellular calcium, which stimulates the superoxide release [10]. Mechanisms of the action of fluoride on the release of superoxide may be different and depend on fluoride concentration. Higher than 10 mM NaF concentration may stimulate superoxide release by suppressing the activity of phosphoproteinphosphatase and indirect activation of NADPH system. The onset of the oxidative response is indicated by activation of NADPH oxidase, responsible for reduction of molecular oxygen into the reactive forms of oxygen.

The studies of action of NaF on oxidative function by stimulating the abovementioned cells with activators of biological origin are of even greater clinical importance, as quite always these cells encounter microorganisms in the oral cavity. During interaction between NL and antigens, the intensity of a burst of oxygen consumption and the values of chemiluminiscence depend on the origin and concentration of the activator of NL [9]. Performing the investigation, we activated NL with nonopsonized *E.coli* and opsonized zymosan. In both the studied groups of subjects luminal-dependent CL of the NL activated by opsonized zymosan was reliably higher ($p<0.001$) than analogous CL of the NL effected by nonopsonized *E.coli* (Fig. 2 and 4).

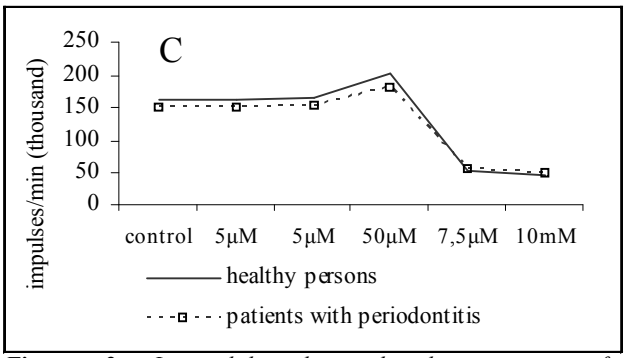

Figure 2. *Luminol-dependent chemiluminescence of neutrophils after 30-minute activation by E.coli and its dependence on F- concentration in the incubation medium.*

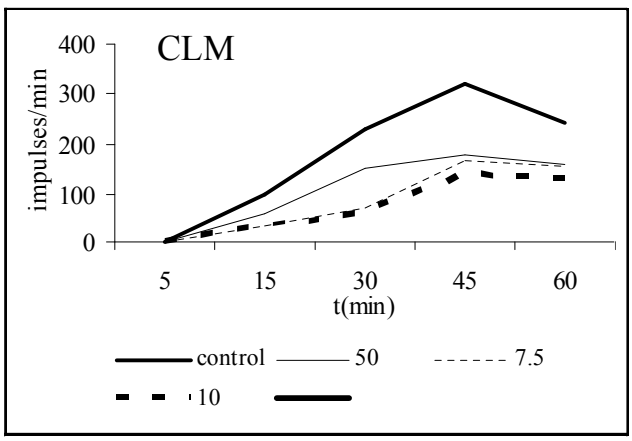

Figure 3. *Dynamic of the chemiluminescence of NL obtained from patients with periodontitis, activated with E.coli*

Very low fluoride concentrations (5 and 10 μM FÆ) had no reliable influence ($p>0.05$) upon CL values of the NL activated with opsonized zymosan or nonopsonized *E.coli*. It is interesting that concentrations of FÆ at the level of 50 μM or its high concentrations (7.5 and 15 mM) practically exerted different influence on the CL of NL taken from the venous blood of healthy subjects and patients with chronic periodontitis. In both the studied groups, incubation of NL with 50 μM FÆ reliably increased ($p<0.001$) CL of NL activated with opsonized zymosan or nonopsonized *E.coli*. However, high fluoride concentrations (7.5 and 15 mM of FÆ) suppressed CL of NL. Accurate fluoride action mechanisms are still not well investigated. The mechanisms of activating action of fluorine on release of superoxide may be different and dependent on concentrations of fluoride [10]. This assumption is also supported by the data of this study.

The data showing that 50 μM concentration of FÆ has an activating action on CL of NL indirectly support the data of the study conducted by J.Gutierrez and coauthors. They affirm that low concentrations of NaF activate neutrophil phagocytosis [7]. Similar data were obtained by L.Shapira and coauthors [22]: at very low concentrations amine- and stannous fluorides activate the neutrophil superoxide generation.

It is considered that preincubation of polymorphonuclear leucocytes with fluoride ions suppresses their response to possible activators [4, 10]. However, the indicated authors studied just effect of high fluoride concentrations on release of superoxide. These data are also supported by the data of our study, showing that higher concentrations of fluoride (7.5 and 15mM of FÆ) suppress CL of NL stimulated by the bacterial activators (Fig. 3). One of the theories enabling explanation of the suppressing effect of high doses of fluoride presupposes that NL, directly interacting with fluorine ions "exhaust", and when they are stimulated by bacterial activators they already are not able to increase production of the reactive forms of oxygen [23]. According to other authors, fluorine ions in great concentrations bind up extracellular Ca^{2+}. In the state of reduced inflow into the cell of the extracellular calcium, the release of superoxide is also diminished [10].

Although NL carriers out a protective function, the data of contemporary investigations have shown that their lysomal enzymes and active forms of oxygen may damage tissues of the body [12,19]. According to the opinion of other authors, the active forms of oxygen have even greater importance to these processes than proteolytic enzymes [5].

It was established that NL of patients with rapidly progressive periodontitis release greater amounts of superoxide [22]. However, in accordance with opinion of other authors, in certain pathological cases the function of NL may

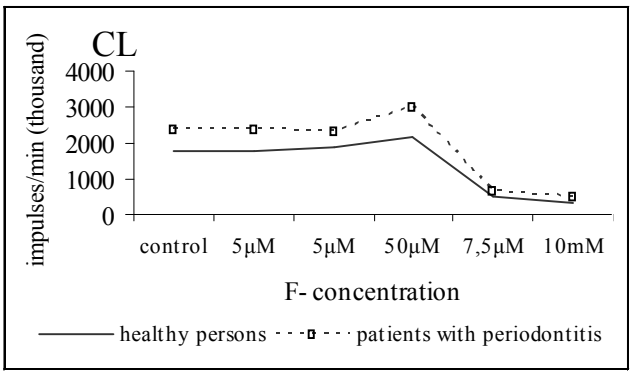

Figure 4. *Luminol-dependent chemiluminescence of neutrophils after 30-minute activation by opsonized zymosan and its dependence on F^- concentration in the incubation medium.*

become weaker [27]. According to the data of our study, the value of CL of nonactivated neutrophils of the patients with chronic periodontitis was higher than the analogous value of NL taken from the healthy subjects with normal periodontal tissues.

During topical application of fluoride agents, their concentration in the mouth may become equal to several dozens of μM; however, this concentration becomes much lower (0.026 mM) within some minutes and continues to be at this level for longer period of time [10]. There are reports in the literature that μM-level concentrations of fluoride may form in periodontal pockets [23]. Therefore, the effect of fluoride exerted upon NL in low-level concentrations may be of greater clinical importance than that in high concentrations. Basing on the results of accomplished investigations, it was possible to make a supposition that topical application of fluoride agents may activate the NL performing periodontal protective function and that may exert negative influence on the pathogenesis of periodontitis. Besides, the systematic usage of fluoride agents or getting them in greater quantities into the body from the environment and accumulating in the inside structures may lead to elevation of the concentration of fluorides in the blood plasma up to dozens of μM or even greater amounts [3]. Therefore, the above-mentioned levels of fluoride may exert general effect on the immune system of the human body.

CONCLUSIONS

Chemiluminescence of the nonactivated neutrophilic leucocytes, and the neutrophils activated by nonopsonized *Escherichia coli* and opsonized zymosan depends on the concentration of sodium fluoride added for incubation to the suspension of neutrophils obtained from venous blood of the studied persons and on the state of the periodontal tissues:

1. Low concentrations of fluoride (5μM and 10μM) have no influence on chemiluminescence of the neutrophilic leucocytes.
2. At 50μM FÆ concentration, luminol dependent chemiluminescence of the neutrophilic leucocytes significantly increases.
3. In the presence of high sodium fluoride concentration (7.5 and 15mM FÆ), chemiluminescence of the nonactivated neutrophils obtained from venous blood of the patients with chronic periodontitis is significantly higher than the chemiluminescence of the neutrophilic leucocytes taken from the venous blood of the healthy subjects.
4. Under the influence of 7.5 and 15mM FÆ concentration, in both the studied groups of persons, luminol-dependent chemiluminescence of the nonactivated neutrophilic leucocytes significantly increases whereas chemiluminescence of the activated neutrophils significantly diminishes.

REFERENCES

1. Boeckhhaebish EM, Oliveira RM. Systemic effects of fluoridated water on rats. *Fluoride* 1998; 31(2): 100-1.
2. Brom C, Brom J, KonigW. G protein activation and mediator release from human neutrophils and platelets after stimulation with sodium fluoride and receptor mediated stimuli. *Immunology* 1991; 73: 287-92.
3. Fajerskov O, Ekstrand J, Burt B. Fluoride in dentistry. 2nd ed. Copenhagen: Munksgaard; 1996. p. 40-180, 230-247, 275-343.
4. Gabler WL, Hunter N. Inhibition of human neutrophil phagocytosis and intracellular killing of yeast cells by fluoride. *Arch Oral Biol* 1987; 32: 363-6.
5. Gaboury JP, Anderson DC, Kubes P. Molecular mechanisms involved in superoxide-induced leukocyte-endothelial cell interactions in vivo. *Am J Physiol* 1994; 226(2): 637-42.
6. Guarnieri C, Zuccheli G, Bernardi F. Enhanced superoxide productionwith no change of antioxidant activity in gingival fluid of patients with chronic adult periodontitis. *Free Rad Res Comms* 1991; 15: 11-6.
7. Gutierrez J, Liebana J, Ruiz M, Castillo A. Action of sodium fluoride ob phagocytosis by systemic polymorphonuclear leucocytes. *J Dent* 1994; 22: 279-82.
8. Gyllenhammar H. Lucigenin chemiluminescence in the assessment of neutrophil superoxide production. *J Immunol Methods* 1987; 97: 209-13.
9. Hansen PR. Role of neutrophils in myocardial ischemia and perfusion. *Circulation* 1995; 91:1872-85.
10. Knoll-Köhler E, Büssem G. Short exposure of polymorphonuclear leucocytes to sodium fluoride suppresses the response to fMLP. *J Trace Elements Med Biol* 1999; 13: 68-75.
11. Korkina LG, Samochatova EV, Maschan AA. Release of active oxygen radicals by leucocytes of Fanconi anemia patients. *J Leukoc Biol* 1992; 52(3): 357-62.
12. Lamster IB, Oshrain RL, Celenti RS. Indications of acute inflammatory and hu,oral immune responses in gingival cervicular fluid. *J Periodontal Res* 1991; 74: 1468-74.
13. Li Y, Liang CK, Katz BP. Long-term fluoride exposure in drinking water and sister chromatid exchange frequency in human blood lymphocytes. *J Dent Res* 1995; 74: 1468-74.
14. Loftenius A, Andersson B, Butler JE. Fluoride augments the mitogenic and antigenic response of human blood lymphocytes in vitro. *Caries Res* 1999; 33: 148-55.
15. Matarasso S, Catiero C, Bizzarri L. The role of phagocytic cells in periodontal disease. *Minerva Stomatol* 1991; 40(4): 203- 10.
16. Mayo K, Held M, Wadstrom T. Helicobacter pylori – human polymorphonuclear leucocyte interaction in the presence of ammonia. *Eur J Gastroenterol Hepatol* 1997; 9: 457-61.
17. McDonagh MS, Whiting PF, Wilson PM. Systematic review of water fluoridation. *Br Med J* 2000; 321(7265): 855-9.
18. Myasaki KT. The neutrophil: mechanisms of controlling periodontal bacteria. *J Periodontol* 1991; 62(12): 761-74.
19. Moseley R, Waddington RJ, Embery G. Degradation of glycosaminoglycans by reactive oxygen species, derived from stimulated polimorphonuclear leucocytes. *Biochim Biophys Acta* 1997; 1362: 221-31.
20. Moseley R, Waddington RJ, Embery G. The modification of alveolar bone proteoglycans by reactive oxygen species in vitro. *Conn Tiss Res* 1998; 37: 13-28.
21. Rebecchi I, Novo N, Juliano Y. Oxidative metabolism and release of myeloperoxidase from polymorphonuclear leucocytes obtained from blood sedimentation in a Ficoll – Hypaque gradient. *Cell Biochem. Funct* 2000; 18:127-32
22. Shapira L, Gordon B, Warbington M. Priming effect of Porphyromonas gingivalis lipopolysaccharide on superoxide production by neutrophils from healthy and rapidly progressive periodontitis subjects. *J Periodontol* 1994; 65: 129-33.
23. Shapira L, Schatzker Y, Gedalia I. Effect of amine – and stannous fluoride on human neutrophil functions in vitro. *J Dent Res* 1997, 76(7): 1381-6.
24. Takahashi K. Fluoride-linked Dawn syndrome births and their estimated occurrence due to water fluoridation. *Fluoride* 1998; 31(2): 61-73.
25. Van Dyke TE, Vaikuntam J. Neutrophil function and dysfunction in periodontal disease. *Curr Opin Periodontol* 1994; 28: 19-27.
26. Waddington RJ, Moseley R, Embery G. Reactive oxygen species: a potential role in the pathogenesis of periodontal diseases. *Oral Dis* 2000; 6: 138-51.
27. Zafiropoulos GG, Flore-De-Jacoby L. PMN function in periodontal disease. *J Periodontol* 1992; 63: 1015.

Received: 22 10 2003
Accepted for publishing: 27 11 2003