J. Dent. 1994; **22**: 279-282

Action of sodium fluoride on phagocytosis by systemic polymorphonuclear leucocytes

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ABSTRACT

Alterations in phagocytosis appear to be important in the onset and development of periodontitis. We investigated new substances that may be of use in the treatment of periodontitis. In a preliminary study, we tested the effect of sodium fluoride on phagocytosis by circulating polymorphonuclear leucocytes (PMNs) in 10 replicate assays using blood from six healthy subjects. Sodium fluoride was tested at concentrations of 1.0 μm to 4.0 μm against *Streptococcus oralis*, *Streptococcus mutans*, *Streptococcus sobrinus* and *Streptococcus sanguis*. The phagocytic index against all microorganisms increased significantly at all concentrations of sodium fluoride assayed; this effect was dose dependent. Sodium fluoride appeared to stimulate phagocytosis via two mechanisms: an apparent increase in bacterial susceptibility to phagocytosis, and direct stimulation of phagocytosis by PMNs.

KEY WORDS: Oral streptococci, Phagocytosis, Polymorphonuclear, Sodium fluoride

J. Dent. 1994; **22**: 279-282 (Received 4 December 1992; reviewed 21 April 1993; accepted 28 January 1994)

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INTRODUCTION

The role of polymorphonuclear leucocytes (PMNs) in the origin of periodontitis is well known. Most informative studies have investigated the chemotactic disorders, at both the systemic and local levels, in the periodontium. These chemotactic alterations in PMNs are intrinsic in nature, and are linked to the depletion of certain receptors[1-3]. Other leucocyte functions have also been examined in many studies of periodontitis with deficient chemotaxis. Although some authors have reported adhesiveness and phagocytosis to be normal[4,5], most have found them to be diminished[5-10]. Several studies that have centred on different substances used to correct PMN deficiencies—especially chemotactic anomalies—have yielded disappointing results[11-13]. We describe the *in vitro* effects of sodium fluoride, a widely used compound in dentistry, on phagocytosis by PMNs, as a possible alternative approach to stimulating leucocytes.

MATERIALS AND METHODS

All experiments were carried out ten times at 15-day intervals, to avoid testing patients in whom extractions were done within short intervals, as a test of the replicability of our approach.

Subjects

All assays were performed with venous blood from six healthy 22-year-old males with no immunological or periodontal abnormalities. The subjects had taken no medication during the 60 days preceding the study, and at no time throughout the study showed clinical signs of infection. Any immunological, clinical or haematological alterations detected during the experiment led us to discard the results from previous samples, and start a new cycle of blood sampling until ten consecutive normal samples were obtained.

In each experiment, serum levels of fluoride were determined according to the method of Villa[14]. Other analytical parameters determined were the complement components C3, C4 and total complement (C50), total leucocyte count and differential blood counts.

Sodium fluoride

Sodium fluoride (S1504, Sigma Chemical Co., St Louis,

0300-5712/94/050279-04

MO, USA) was dissolved in isotonic saline solution and tested at the following concentrations: 1.0, 2.0 and 4.0 μm.

Microorganisms

Four species of *Streptococcus* were used to test the phagocytic index: *S. oralis* NCTC 11427, *S. mutans* NCTC 10832, *S. sobrinus* ATCC 33487 and *S. sanguis* NCTC 7863. All microorganisms were suspended in Hank's solution (041-04175M, HBSS, Gibco, Paisley, UK) to give a dilution of 1×10^8 bacteria ml^{-1}.

Preparation of polymorphonuclear leucocytes

PMNs were obtained from 40 ml of venous blood and isolated with the technique described by Gutiérrez *et al.*[15], based on dextran sedimentation (17-0340-01, Pharmacia Fine Chemicals, Uppsala, Sweden) in 6% phosphate buffered saline (BR-14a, Oxoid Ltd, UK), followed by sodium diatrizoate Ficoll density gradient centrifugation (17-0840-03, Pharmacia LKB Biotechnology, Upsala, Sweden). Erythrocytes were removed from the cellular sediment by hypotonic lysis, and the final concentration of PMNS was adjusted to 1×10^6 cells ml^{-1} with the help of a Neubauer chamber. The PMNs were then redissolved in different concentrations of HBSS with and without 25% autologous serum, to reproduce the situation existing *in vivo*, in which serum factors are present.

Phagocytosis studies[16, 17]

Phagocytosis was studied by placing PMNs and HBSS with serum in contact with different concentrations of sodium fluoride and different microorganisms in suspension (Effect A). To determine whether the results of these experiments were due to the action of fluoride on bacteria (Effect B) or on phagocytes (Effect C), we studied the two effects separately, and compared the results to the findings in control experiments without fluoride (Effect D).

Effect A

To 0.45 ml of PMNS suspension in HBSS with serum and 0.1 ml of one of the concentrations of sodium fluoride was added 0.45 ml of bacterial suspension. The mixture was incubated for 60 min at 36 ± 1 °C in an atmosphere with 5% CO_2, then washed three times, cytocentrifuged at 900 ***g*** and stained with acridine orange. The phagocytic index (PI) was calculated as the mean number of bacterial cells phagocytosed per 100 leucocytes counted by microscopy.

Effect B

0.1 ml of one of the sodium fluoride solutions was added to 0.9 ml of bacterial suspension, and the mixture was incubated for 30 min at 36 ± 1 °C in an atmosphere with 5% CO_2. The bacterial cells were washed twice and resuspended in 0.9 ml isotonic saline solution. To 0.45 ml aliquots of this suspension was added 0.45 ml of PMN stock suspension in Hank's solution with serum. This mixture was incubated for 60 min at 36 ± 1 °C, then washed three times and cytocentrifuged at 900 ***g*** and stained with acridine orange. The PI was calculated as described above.

Effect C

0.1 ml of different concentrations of sodium fluoride were added to 0.9 ml PMN suspension. The mixture was incubated for 30 min at 36 ± 1 °C in an atmosphere with 5% CO_2, and washed as described above, after which the PMNs were resuspended in 0.9 ml Hank's solution with serum. To 0.45 ml of the stock PMN suspension was added 0.45 ml of bacterial suspension, and this mixture was incubated at 36 ± 1 °C in an atmosphere of 5% CO_2. The cells were washed three times, cytocentrifuged and stained, and PI was calculated as described above. In these assays, serum was not present initially, in order to investigate the direct effect of sodium fluoride on PMNs.

Effect D

The procedure was the same as that described above for Effect A, except that no sodium fluoride was added.

Observations were made by one experimenter who was aware of the objective of the study, and another experimenter who was blind. The findings reported by both observers were the same.

Statistical analysis

The values for the different variables were compared with Mann-Whitney's U test.

RESULTS

All results are given as the mean of 10 determinations and mean of six subjects.

Previous analyses

Serum levels of fluoride never surpassed 0.27 μm. All other analyses (C3, C4, total complement, total leucocyte count and differential blood count) yielded results within the normal range for the subject's age and sex.

Effect of sodium fluoride on phagocytosis by PMNs

The phagocytic index in PMNs not treated with sodium fluoride ranged from 1.5 ± 0.01 for *S. oralis* and *S. sanguis* to 1.5 ± 0.08 for *S. mutans*. The PI against all microorganisms increased significantly ($P < 0.01$) in the

Table I. Effect of sodium fluoride on phagocytosis by systemic polymorphonuclear leucocytes

Effect	*[NaF]**	*Phagocytic indices with different microorganisms (means of 10 replications)* S. oralis	S. mutans	S. sobrinus	S. sanguis
Effect A*	1 μm	2.1 ± 0.06	2.3 ± 0.05	2.8 ± 0.08	1.9 ± 0.06
	2 μm	2.4 ± 0.09	2.09 ± 0.07	4.6 ± 0.08	2.2 ± 0.07
	4 μm	4.6 ± 0.05	4.76 ± 0.08	4.6 ± 0.08	3.9 ± 0.09
Effect B†	1 μm	1.7 ± 0.01	1.84 ± 0.08	2.7 ± 0.05	1.7 ± 0.08
	2 μm	1.8 ± 0.06	1.7 ± 0.08	3.0 ± 0.05	1.9 ± 0.09
	4 μm	4.9 ± 0.08	4.63 ± 0.06	5.2 ± 0.05	4.8 ± 0.07
Effect C‡	1 μm	1.7 ± 0.08	1.9 ± 0.06	3.4 ± 0.07	1.7 ± 0.07
	2 μm	1.7 ± 0.01	1.7 ± 0.05	4.6 ± 0.07	1.9 ± 0.01
	4 μm	4.0 ± 0.06	4.5 ± 0.1	4.9 ± 0.07	3.8 ± 0.05
Effect D§	—	1.5 ± 0.01	1.5 ± 0.08	1.5 ± 0.06	1.5 ± 0.01

*Phagocytosis by PMNs after treatment with NaF and incubation with bacteria.
†Effect of NaF on bacteria.
‡Effect of NaF on PMNs.
§Untreated control bacteria and PMNs.
[NaF], Sodium fluoride.

presence of all concentrations of sodium fluoride. As shown in *Table I*, the largest increases were obtained with 4.0 μm NaF. There were no significant differences between the effect of sodium fluoride for different species of streptococci or between different concentrations of NaF. However, the increase in PI was significant ($P < 0.01$) when control values were compared with the findings after treatment of the bacteria (Effect B) or the PMNs (Effect C) with sodium fluoride.

We believe the concentration of sodium used did not affect the results.

DISCUSSION

Although fluoride is widely used in dental treatment to prevent dental caries, little is known of its action on cells of the imune system. Gabler and Leong[18] discovered that fluoride suppressed non-oxidative metabolism in PMNs, which explained the inhibition of phagocytosis and antimicrobial activity in PMNs. However, these researchers used such high concentrations of fluoride that on the basis of their results, 1000 times the concentration normally found in human plasma in geographical areas that use fluoridation would be necessary to achieve a 50% reduction in phagocytosis by PMNs. Similarly, other authors[19,20] showed that sodium fluoride inhibits locomotion and chemotaxis and adhesiveness in circulating PMNs. In contrast, our *in vitro* findings show that physiological amounts of fluoride, which would be readily achievable in plasma in populations residing in optimally fluoridated areas, significantly enhance the phagocytic capacity of PMNs (entry of the bacteria). Although this effect may not be so intense *in vivo*, our findings suggest that concentrations of 4.0 μm may well serve to treat cases of periodontitis in which the physiological basis is deficient chemotaxis, as the cells that migrate to the inflammatory focus may be better able to control the microbial virulence factors because of their improved phagocytic capacity. Phagocytic capacity can also be improved by treating the inflammatory focus with local irrigation several times a day at the concentrations used in this study.

The results of our experiments with different concentrations of sodium fluoride have several implications. At concentrations of 1.0, 2.0 and 4.0 μm, this compound increases phagocytosis of *S. oralis*, *S. mutans*, *S. sobrinus* and *S. sanguis* by PMNS, as shown by the significant increases in PI. The mechanism responsible for stimulating phagocytosis may operate synergistically on two fronts: by making bacterial cells more susceptible to phagocytosis, and by directly stimulating phagocytosis by PMNs. The action of sodium fluoride was dose dependent, i.e. the higher the concentration of NaF, the greater the increase in PI. Finally, sodium fluoride was equally effective in stimulating phagocytosis against all species of streptococci assayed, with no significant differences between any of the species. This suggests that the action of sodium fluoride is not species dependent.

Acknowledgements

We thank Ms Karen Shashok for translating the original manuscript into English.

This study was supported in part by the Education and Sciences Council of the Andalusian Regional Government through Project no. 082.

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