Effects of oral hygiene products containing lactoperoxidase, lysozyme, and lactoferrin on the composition of whole saliva and on subjective oral symptoms in patients with xerostomia

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This study evaluates the effects of two oral hygiene products containing nonimmunoglobulin antimicrobial agents on whole saliva and on subjective oral symptoms in patients with xerostomia. Twenty patients used a lactoperoxidase-system-containing toothpaste (Biotene®) combined with the use of a mouthrinse (Biotene®), comprising also lysozyme and lactoferrin, for 4 weeks. Saliva samples were collected at base line, after 4 weeks' use of the products, and at the end of a 4-week washout period. Samples were analyzed for selected biochemical and microbiologic factors. The effects on subjective oral symptoms were also recorded. A 4-week daily use of toothpaste and mouthrinse relieved the symptoms of oral dryness in 16 patients. The levels of salivary hypothiocyanite, lysozyme, lactoferrin, or myeloperoxidase activity did not change, but there was a significant decrease in salivary pH ($P < 0.05$), total peroxidase activity ($P < 0.05$), and total protein content ($P = 0.01$). In patients with the lowest salivary flow rates ($n = 5$) a significant ($P \leq 0.04$) increase was detected in salivary hypothiocyanite concentrations. No major changes occurred in salivary microflora. The products relieved subjective oral symptoms in most xerostomic patients, but this was not necessarily related to the presence of antimicrobial agents. □ *Antimicrobial agents; mouthrinse; saliva; toothpaste; xerostomia*

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The significance of human saliva not only for oral health but also for the general health is obvious (1–3). The basic role of saliva is to protect the oral environment and the upper gastrointestinal tract against various insults (4, 5). The antibacterial, antiviral, and antifungal factors of saliva are either nonimmunoglobulin, innate agents (such as lysozyme, lactoferrin, peroxidase systems, agglutinins) or immune, acquired agents (IgA, IgG, IgM) (6).

The significance of saliva is often underestimated until a dysfunction of the salivary glands develops (7–11). Dryness of mouth is a common clinical complaint (11, 12). In an adult population in Sweden 14–39% reported symptoms of xerostomia, the highest prevalences being in women and the elderly (13). Subjective feelings of dry mouth have also been found in patients with normal salivary flow rates (8), but complaint of oral dryness is most often a symptom of salivary gland hypofunction.

Treatment of the symptoms of dry mouth has been mainly palliative. Stimulation of remaining salivary function or various saliva substitutes have been recommended (14–16). Oral hygiene products containing natural oral antimicrobial agents in combinations are among the recent approaches to relieve dry mouth symptoms and simultaneously enhance saliva-mediated protection against infections (17). One important defense mechanism is the peroxidase system, which is antimicrobial against several oral microorganisms including mutans streptococci, lactobacilli, fungi, and some viruses (18). Peroxidase enzymes catalyze the oxidation of thiocyanate ions (SCN^-) to hypothiocyanite ion ($OSCN^-$) and hypothiocyanous acid (HOSCN) (19), which are antimicrobial (20, 21). The limiting component for the production of HOSCN/$OSCN^-$ in whole saliva is hydrogen peroxide (H_2O_2) (22). The salivary peroxidase system can be enhanced in vivo by adding small amounts of H_2O_2-generating enzymes to toothpastes or mouthrinses (18, 22–24).

Biotene® toothpaste comprises all the components of the peroxidase system: peroxidase enzyme (lactoperoxidase), SCN^- ions, and H_2O_2-generating enzymes, combined with other prophylactic agents, such as sodium monofluorophosphate and xylitol. Healthy persons with normal salivary secretion rate do not notably benefit by using a lactoperoxidase-system-containing toothpaste such as Biotene (24, 25), but in xerostomic patients Biotene improves gingival health compared with a control dentifrice (26). In addition, combining the use of Biotene toothpaste with a lactoperoxidase-containing gel (Oralbalance®) in patients with hyposalivation significantly decreases gingival inflammation (27).

Biotene products have been developed to relieve

Table 1. Characteristics of the subjects on the basis of age, sex, clinical diagnosis, number of daily medications, and subjective oral symptoms

Subject	Age (years)	Sex	Diagnosis	Medications	Subjective oral symptoms
1	62	F	Sjögren's syndrome, rheumatoid arthritis	5	Difficulty talking, itching mouth, metal taste
2	62	F	Sjögren's syndrome, rheumatoid arthritis	None	Difficulty talking, burning mouth, glossitis
3	51	F	Sjögren's syndrome, diabetes mellitus, lactose intolerance	1	Difficulty talking, itching mouth, glossitis, metal taste
4	53	M	Sjögren's syndrome, rheumatoid arthritis, tumour in parotid gland	1	Difficulty talking, minor aphthae
5	69	F	Extrapyramidal syndrome, blepharospasm	None	Difficulty talking and eating, burning mouth, glossitis, mucosal ulcers
6	52	F	Sjögren's syndrome, rheumatoid arthritis, diabetes mellitus	5	Difficulty eating and swallowing, glossitis, bad taste
7	61	F	Sjögren's syndrome, rheumatoid arthritis, hypothyroidism	3	Difficulty talking, vesicles on buccal mucosa
8	54	F	SLE*	3	Difficulty talking and eating, sore throat, glossitis, bad taste
9	60	F	Sjögren's syndrome, SLE Malignant lymphoma '94	2	Difficulty talking, burning mouth, glossitis
10	54	F	Sjögren's syndrome, SLE, hypothyroidism	4	Difficulty talking, burning mouth, angular cheilitis, glossitis
11	56	F	Sjögren's syndrome, mitral prolapse	1	Difficulty talking, itching mouth, glossitis
12	58	M	Xerostomia, osteoarthritis	1	Difficulty talking, mucosal ulcers, bad taste
13	52	F	Depression	5	Difficulty talking, mucosal ulcers, recurrent candidosis, bad taste
14	56	F	SLE, thyroid cancer '78, gout	9	Difficulty talking and eating, burning mouth, glossitis, recurrent candidosis
15	41	F	Sjögren's syndrome	1	Itching mouth
16	59	F	Sjögren's syndrome, larynx spasm	1	Difficulty talking, burning mouth, bad taste
17	61	M	Wegener granulomatosis	5	Difficulty talking, mucosal ulcers, bad taste
18	69	F		1	Burning mouth, ulcers
19	47	F	Sjögren's syndrome, rheumatoid arthritis	1	Difficulty talking, angular cheilitis, metal taste
20	26	F	Sjögren's syndrome	1	Difficulty eating, recurrent candidosis, bad taste

* SLE = systemic lupus erythematosus.

symptoms of oral dryness and to restore the salivary antimicrobial capacity of patients with hyposalivation or xerostomia. Therefore, in this study our aim was first to analyze the effects of Biotene toothpaste, combined with the use of Biotene mouthrinse (a nonalcoholic rinse, which contains peroxidase system, lysozyme, and lactoferrin), on levels of selected salivary antimicrobial factors and microbial flora in patients with dry mouth. The second aim was to evaluate the possible effects of these commercially available products on subjective symptoms and complaints of dry mouth among the study group.

Materials and methods

Subjects and study design

Twenty patients (17 women and 3 men; mean age, 55 years; range, 24–69 years) with chronic dry mouth symptoms (Table 1) and paraffin-stimulated salivary flow rate < 1.0 ml/min (28) participated in this clinical study. Thirteen subjects had been diagnosed as having Sjögren's syndrome at Turku University Hospital, Turku, Finland, and seven subjects were patients from the University Dental Clinic, Turku. All subjects had a natural dentition, but one had a removable denture in addition to his natural teeth. Detailed description of the individuals on the basis of age, sex, clinical diagnosis, and number of different daily medications is presented in Table 1. All subjects were informed about the purpose of this study.

At the first visit all subjects were interviewed about their subjective health condition, and the effect of xerostomia on the patients' everyday life was scored on a four-grade scale. A standardized form was used, and the following questions were asked: 'Does the feeling of dry mouth cause you very strong discomfort (1), quite a lot of discomfort (2), minor (3), or no (4) harm?' Symptoms of the lips, oral mucosa, tongue, and pharynx, sensations of burning or itching mouth, and problems with speech, eating, or swallowing and disturbances of taste were recorded. A comprehensive medical and dental history, including medications and dietary and dental habits, was obtained from each individual. At base-line appointment a complete oral examination was performed after sampling of saliva.

All participants were advised to brush with the experimental toothpaste (Biotene) for 1 min and then rinse the mouth with the test mouthrinse (Biotene) for 30 sec at least twice daily, in the morning and in the

evening during the 4-week test period. They were instructed to use the same amount of toothpaste as they did usually. This amount was later calculated to be 0.54 ± 0.39 g. The amount of mouthrinse used by each patient was calculated to be 5.62 ± 1.74 ml/rinsing. The subjects were asked to refrain from using other dental or antimicrobial products and to maintain their normal dietary and smoking habits during the experiment.

After 4 weeks' use of the test products the participants were asked to complete a questionnaire concerning their opinions and subjective feelings about the two experimental products. Saliva samples were collected, and the subjects were asked to return to their normal oral hygiene methods. During the following 4-week washout period the subjects were allowed to use any toothpaste and mouthrinse except the two test products. After this 4-week period the third saliva sample was collected. Since similar toothpaste or mouthrinse without antimicrobial proteins, sodium lauryl sulfate, xylitol, and/or alcohol was not available, no control group could be formed. However, because Biotene products are known to generate antimicrobial agents in vivo (24), the specific aim was to evaluate longitudinally the efficacy and acceptability of these products by using the base-line status and washout periods as references.

Oral examinations

A complete oral examination of each subject was made. Any changes in the oral mucosa were recorded. Dental caries was registered with explorer and mirror under standard conditions with optimal light. Caries data were expressed as decayed, missing, and filled teeth (DMFT) and surfaces (DMFS). A surface was recorded as carious if it had a lesion with a detectably softened floor, undermined enamel, or softened wall. A value of five surfaces was ascribed for teeth missing due to caries. The third molars were excluded from the analysis. The values for DMFT and DMFS indices (mean ± *s*) were 24.1 ± 3.7 and 89.4 ± 28.2, respectively.

The periodontal status was evaluated by probing the pocket depth at four (mesial, buccal, distal, and oral) sites on all teeth, using a calibrated periodontal probe with a point diameter of 0.45 mm. Bleeding on probing (BOP) was registered at the same sites as follows: 0 = no bleeding within 10 sec after probing; 1 = bleeding within 10 sec after probing. BOP was 62.0 ± 19.4% of the studied tooth sites.

Test products

The two experimental products were gifts from Tam-Drug Co., Vantaa, Finland, manufactured by Biopole Co., Bruxelles, Belgium. The test dentifrice (Biotene) contained sodium monofluorophosphate (0.15% F^-), xylitol (1%), and a nonionic surfactant, Arasolve 200®, which is a polyoxyethylene 20 isohexadecyl ether. Biotene contained in addition lactoperoxidase (from bovine milk; 454 ABTS enzyme units/mg, equivalent to 0.02%), $KSCN^-$, and a dextrose–glucose oxidase system for H_2O_2 generation. The pH of the toothpaste was 6.5.

The test mouthrinse (Biotene) contained lactoperoxidase, lysozyme, lactoferrin, xylitol, hydrogenated starch, propylene glycol, hydroxyethyl cellulose, aloe vera, poloxamer 407, sodium benzoate, benzoic acid, zinc gluconate, aromatic compound (832318-A), color (Cl 42090), and water and had a pH of 5.2.

Collection and treatment of saliva samples

Paraffin-stimulated whole saliva was collected from each subject at base line, after 4 weeks' use of the test products, and at the end of the 4-week washout period. The subjects were asked not to eat or smoke for 1 h before sampling, which was always done between 0800 h and 1100 h. Saliva was collected for 5 min, but if the secretion rate was poor, the collection continued further for a maximum of 20 min. The secretion rate was determined in milliliters per minute, the salivary buffer capacity was assessed with the Dentobuff Kit (Orion Diagnostica, Espoo, Finland), and the pH with a pHM62 Standard pH Meter (Radiometer, Copenhagen, Denmark). Immediately after sampling, 100 µl of fresh and uncentrifuged saliva was used for the hypothiocyanite ($HOSCN/OSCN^-$) assay. For microbiologic analysis a 100-µl aliquot was transferred to a tube containing 1 ml of tryptic soy broth (TSB) (Oxoid, Basingstoke, UK), supplemented with 10% glycerol. Aliquots of fresh uncentrifuged saliva were also withdrawn for lactoferrin and lysozyme analysis. The samples were stored at −20 °C until analyzed.

The rest of the collected saliva was centrifuged at 18,000 *g* for 10 min at +4 °C. Portions of centrifuged saliva needed for the analysis of total salivary proteins, thiocyanate (SCN^-), total salivary peroxidase, and myeloperoxidase activity were stored frozen at −20 °C before analysis.

Chemical assays

Hypothiocyanite ($HOSCN/OSCN^-$) concentration was analyzed by reaction with the colored anionic monomer of 5,5-dithiobis-(2-nitro-benzoic acid) $(Nbs)_2$ as described by Pruitt et al. (29). The thiocyanate (SCN^-) ions were quantified with the ferric nitrate method described by Betts & Dainton (30).

Lysozyme activity was estimated with *Micrococcus lysodeikticus* diffusion plates (Lysozyme Kit, Kallestad Laboratories, Chaska, Minn., USA) using lyophilized human urine lysozyme as a standard. The lactoferrin levels were quantitated with a noncompetitive avidin–biotin enzyme immunoassay (31). Human colostral lactoferrin (Sigma Chemical Co., St. Louis, Mo., USA), further purified by affinity chromatography,

Table 2. Nonimmunologic defense factors in paraffin-stimulated whole saliva after 4 weeks' use of the test products compared with base line and 4-week washout period

	Base line, mean ± *s*	4 weeks, mean ± *s*	Washout, mean ± *s*	Significance†
Flow rate (ml/min)	0.3 ± 0.2	0.3 ± 0.3	0.3 ± 0.2	NS
pH	7.0 ± 0.4*	6.8 ± 0.5	6.8 ± 0.5	$P < 0.05$
Total protein (mg/ml)	3.0 ± 1.0*	2.8 ± 1.4	3.1 ± 1.4	$P = 0.01$
Lysozyme (μg/ml)	18.8 ± 13.0	14.9 ± 11.9	13.4 ± 5.7	NS
Lactoferrin (μg/ml)	36.8 ± 40.4	50.9 ± 74.1	34.9 ± 53.2	NS
Total salivary peroxidase (mU)	2.1 ± 0.7*	1.5 ± 0.9	1.6 ± 0.6	$P < 0.05$
Myeloperoxidase (mU)	0.9 ± 0.4	0.9 ± 0.5	0.7 ± 0.3	NS
Thiocyanate (mM)	2.2 ± 1.5	2.0 ± 1.2	3.1 ± 1.4*	$P < 0.001$
Hypothiocyanite (μM)	52.9 ± 38.9	62.4 ± 32.5	44.2 ± 49.4	NS

† Student's paired *t* test.
* Statistically significantly different from the values after the 4-week test period (middle).

was used as a standard. The absorbances in lactoferrin assay were read with an automatic spectrophotometer (Titertek Multiscan, Eflab Oy, Helsinki, Finland).

Salivary peroxidase activity was measured at +22 °C by following the rate of oxidation of 5-thio-2-nitrobenzoic acid (Nbs) to $(Nbs)_2$ by $OSCN^-$ ions generated during the oxidation of SCN^- by salivary peroxidase (32). SCN^- was replaced by Cl^- in the assay mixture to determine the myeloperoxidase activity in human saliva (33). Protein content was measured by the method of Lowry et al. (34), using bovine serum albumin as a standard.

Microbiologic assays

For microbiologic analysis the TSB tubes were thawed and vortexed thoroughly for 1 min. After serial 10-fold dilutions the bacteria were plated as follows: total streptococci and mutans streptococci on mitis salivarius (Difco Laboratories, Detroit, Mich., USA) and mitis salivarius agar supplemented with bacitracin, respectively. The mitis salivarius agar plates were supplemented with 20% sucrose (BDH Chemicals Ltd., Poole, UK) and incubated aerobically for 2 days in 37 °C. The mitis salivarius bacitracin agar plates were supplemented with both sucrose and 0.5 μg/ml bacitracin (Sigma Chemical Co.) and incubated for 3 days in air containing 7% CO_2 at 37 °C. Lactobacilli were cultivated on Rogosa SL agar plates (Difco Laboratories) and incubated anaerobically for 3 days at 37 °C. The total anaerobic flora was determined by plating samples on blood agar plates containing 5% bovine blood (Orion Diagnostica) and incubated anaerobically for 2 days at 37 °C. The growth of *Candida* species was determined by plating serially diluted samples on Sabouraud dextrose agar plates (Difco Laboratories). The plates were incubated aerobically for 2 days at 37 °C. After appropriate incubation times the number of colony-forming units (CFU/ml saliva) was determined.

Statistics

Student's paired *t* test was used for statistical analysis. *P* values of ≤ 0.05 were considered statistically significant.

Results

At base-line examination 35% ($n = 7$) of the 20 individuals had healthy oral soft tissues, 25% ($n = 5$) had angular cheilitis, and two patients beefy red tongue. Of the subjects 80% ($n = 16$) had dry, sticky oral mucosa, 15% ($n = 3$) had ulcerations, and 15% ($n = 3$) white, lichen-like lesions on the buccal mucosa. The periodontal examination showed probing pocket depths of 4–6 mm on at least one tooth in 65% ($n = 13$) of the patients and 6 mm or more in 2 of the 20 patients.

At the initial visit 17 of the 20 subjects reported that the feeling of dry mouth disturbed their everyday life quite a bit, 6 of them having very strong discomfort of xerostomia. Oral dryness caused minor ($n = 2$) or no ($n = 1$) harm to only a few individuals.

A 4-week daily use of a lactoperoxidase-system-containing toothpaste and mouthrinse gave good relief of dry mouth symptoms for 5 subjects, and 11 subjects felt the effect slightly relieving. Two participants reported a smarting feeling in the mouth after using either of the regimens, and two did not feel any change. Sixteen subjects were satisfied with the taste of the dentifrice, two said it was too strong, and two felt it too mild. The mouthrinse was considered to be too strong by seven individuals; the rest of the test group liked the taste. All but one patient wanted to continue daily use of the test toothpaste, and three quarters the test mouthrinse, after this trial.

The values for the studied nonimmunologic antimicrobial factors in paraffin-stimulated whole saliva are presented in Table 2. A 4-week daily use of the test products had no effect on salivary flow rate. The salivary buffer capacity was good in 35%, intermediate

Table 3. Microbiologic variables (log CFU/ml) in paraffin-stimulated whole saliva after 4 weeks' use of the test products compared with the base line and 4-week washout period

	Base line, mean ± *s*	4 weeks, mean ± *s*	Washout, mean ± *s*	Significance†
Mutans streptococci	6.6 ± 1.0	6.8 ± 0.9	6.8 ± 1.0	NS
Lactobacilli	6.2 ± 0.9	5.9 ± 1.1	6.2 ± 1.1*	$P < 0.05$
Total streptococci	8.1 ± 0.4	7.8 ± 0.5	7.7 ± 0.5	NS
Total anaerobes	8.7 ± 0.6	8.7 ± 0.4	7.7 ± 0.5*	$P < 0.001$
Candida species	3.6 ± 2.1	3.2 ± 2.0	4.3 ± 0.8*	$P < 0.01$

† Student's paired *t* test.
* Statistically significantly different from the values after the 4-week test period (middle).

in 60%, and low in 5% of the subjects at base line, and no major changes were observed during the study.

The levels of salivary lysozyme, lactoferrin, or myeloperoxidase activity did not change significantly during the test period. No significant increase was noticed in the peroxidase-system-generated HOSCN/$OSCN^-$ in saliva, but salivary pH, total peroxidase activity, and total protein content decreased slightly during the 4 weeks' use of the products. The concentration of SCN^- did not increase during the experimental period but showed a significant ($P \leq 0.0008$) increase during the washout period (Table 2).

Because considerable differences existed in salivary flow rate within the study group, the analysis was also done separately for those having the lowest ($n = 5$) or highest ($n = 5$) base-line secretion rates (0.19 ± 0.14 and 0.59 ± 0.04 ml/min, respectively). In these analyses, a remarkable increase in subjects with low flow rate was found in salivary HOSCN/$OSCN^-$ ($P \leq 0.04$) and, in two of five subjects, also in lysozyme and lactoferrin (Fig. 1) but not in pH or other biochemical or microbiologic variables (data not shown). Thus, of those components that were incorporated in Biotene products only HOSCN/$OSCN^-$ increased significantly in whole saliva samples during the experimental period.

At base line 80% of the subjects harbored *Candida* species, and no significant changes were observed during the experiment (Table 3). Overall, the use of Biotene did not affect the microbial levels in saliva. During the washout period the number of total anaerobes decreased, and the number of *Candida* species increased significantly.

Discussion

Xerostomia often provokes unpleasant oral symptoms such as burning mouth, difficulty with speech, chewing, and swallowing, and impairment of taste (9, 35). Patients with strongly reduced secretion of saliva have an increased risk of developing rampant caries, periodontal diseases, and mucosal inflammations (5, 36), probably also in the upper gastrointestinal tract (2). These consequences may partly be due to the decreased output of saliva-mediated host defense factors (37, 38).

Since systemic stimulation of salivary flow is difficult, most studies have focused on symptomatic therapies with locally applied agents (14, 16, 17). Our present results show that products containing innate antimicrobial agents, lactoperoxidase system, lactoferrin, and lysozyme, relieved the oral symptoms in 16 of the 20 xerostomic patients. Toothpaste was preferred over the mouthrinse, but for most patients the combination was also well accepted. Similar results of a decreased frequency of subjective complaints has been reported with a combination of a Biotene toothpaste and Oralbalance gel, the latter also containing the lactoperoxidase system components (27). The acceptability of these products is probably related to the lack of alcohol in the mouthrinse and to the lack of foaming agents in the toothpaste. The commonest detergent used in dentifrices is sodium lauryl sulfate (SLS) which, by denaturating the oral mucin layer, may induce mucosal lesions in subjects prone to recurrent aphthous ulcers (39). Thus, the lack of mucosa-irritating alcohol and SLS can be considered positive in products meant for patients with dry and painful oral mucosal surfaces. Banoczy et al. (27) recently reported enhanced keratinization and decreased number of inflammatory cells in both gingival and buccal smears of xerostomic patients who had used a Biotene/Oralbalance combination for 1 month. Similarly, van Steenberghe et al. (26) reported reduced gingival inflammation in xerostomic patients after the use of Biotene toothpaste when compared with a placebo dentrifrice. Our results together with the previous ones suggest that the daily use of these products gives relief to many oral complaints in most patients with xerostomia. Because we could not form a control group with similar products lacking antimicrobial proteins, SLS, xylitol, and/or alcohol, the possibility of a placebo effect cannot, however, be totally excluded. Thus, this study does not prove that the relief in oral symptoms is necessarily connected with the presence of the antimicrobial agents in these products.

It is also difficult to claim that the presence of antimicrobial proteins in these products could affect the oral microflora in a positive manner or perhaps even prevent oral diseases. Although our previous study (24) showed that the HOSCN/$OSCN^-$ levels in saliva of

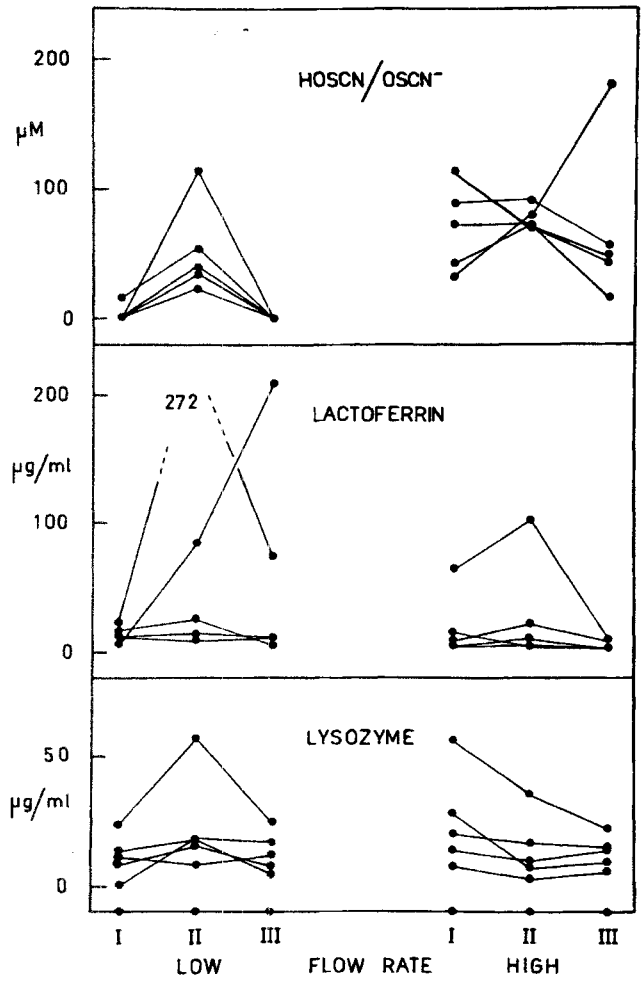

Fig. 1. Concentration of hypothiocyanite ($HOSCN/OSCN^-$), lactoferrin, and lysozyme in whole saliva at base line (I) and after 4-week test (II) and washout (III) periods. Values from five subjects with lowest (left) and highest (right) salivary flow rates are presented. The only statistically significant differences were found in $HOSCN/OSCN^-$ levels of low secretors between I and II ($P \leq 0.04$) and II and III ($P < 0.03$); Student's paired t test).

subjects with normal secretion rate increased significantly during and after brushing with Biotene toothpaste, in a longitudinal analysis no changes in salivary levels of total streptococci, mutans streptococci, lactobacilli, or total anaerobes could be detected. Banoczy et al. (27) made similar observations of mutans streptococci, lactobacilli, and yeasts in xerostomic patients, whereas Mulligan et al. (40) found a significant decrease in salivary total anaerobes after the use of Biotene mouthrinse. It should be emphasized that the abundant literature on the antimicrobial effects of $HOSCN/OSCN^-$ indicates that these agents are mainly bacteriostatic and only in unusual cases bactericidal (41). Therefore, as observed also in the present study, is is not surprising that consistent and clinically significant changes in salivary microflora have not been observed in connection with the use of these products. The reason for a slight increase in salivary yeasts during the washout period is not known. So far, the metabolic effects have been studied only in subjects with normal salivary flow rate, but Biotene toothpaste did not reduce the accumulation rate, acidogenicity, or sucrose-induced acid production by dental plaque (25). Although salivary SCN^- levels could be affected by smoking habits (18), SCN^- ions are not the limiting factor for oral $HOSCN/OSCN^-$ generation (22). Therefore, it is unlikely that smoking has any role in the results of this study.

The above findings with oral bacteria do not, however, exclude the possibility that introduction of $HOSCN/OSCN^-$, lactoferrin, or lysozyme into the mouth of xerostomic patients could have other important protective functions for the host. Very low concentrations of $HOSCN/OSCN^-$ have recently been shown to kill orally transmitted viruses, such as herpes simplex type 1, respiratory syncytial virus, and echovirus type 11 (42, 43) and also human immunodeficiency virus (44). Furthermore, the lactoperoxidase system protects mucosal cells from the toxicity of hydrogen peroxide (45, 46) by converting H_2O_2 generated by oral bacteria and leukocytes to nontoxic $HOSCN/OSCN^-$. Especially in a dry mouth the presence of $HOSCN/OSCN^-$ in sufficient amounts could be beneficial, instead of excessive endogenous accumulation of hydrogen peroxide (47).

Our results clearly demonstrate for the first time that among patients with dry mouth the short-term increase of salivary $HOSCN/OSCN^-$ is possible, and the levels can be raised to those detectable in patients with more normal salivary secretion rate (37). Therefore, obviously greater benefit of the incorporation of nonimmunoglobulin agents in oral hygiene products is gained by patients who have severe xerostomia (stimulated flow ≤ 0.2 ml/min) than in those who have subjective complaints of dry mouth but the salivary flow rate is notably higher. In spite of the concept of adding physiologic, host-derived antimicrobial agents into the mouth of patients who have dry mouth, the long-term efficacy of these products in preventing oral diseases remains to be shown.

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