

Effects of a Peroxidase-System-Containing Toothpaste on Whole Saliva in Vivo

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I. INTRODUCTION

Within the oral cavity, there are a number of mechanisms that protect the body from a variety of harmful agents. The nonspecific host defense includes physical, chemical, and humoral mechanisms. Within the latter category, much attention has been focused upon the lysozyme, lactoferrin, and salivary peroxidase systems¹⁾. The peroxidase systems in human saliva consist of peroxidase enzymes, hydrogen peroxide(H₂O₂), and thiocyanate ion(SCN⁻¹). All components of the peroxidase systems are normal constituents found in human saliva. The major sources of human whole saliva peroxidases are the salivary glands²⁾ and thiocyanate ion is also present in parotid³⁾, submandibular⁴⁾, and whole saliva⁵⁾,

which is derived not only endogenously during detoxification reaction between thiosulfate and cyanide but also exogenously after ingestion of the anion, its esters, or other precursor compounds such as nitriles and isothianates. Hydrogen peroxide(H₂O₂) is produced by several oral bacteria in which especially *S. sanguis*, *S. mitis*, and *S. mutans* excrete large amounts of hydrogen peroxide(H₂O₂) in the present of glucose⁶⁾.

The peroxidase enzymes catalyze the oxidation of the thiocyanate ions(SCN⁻¹) by hydrogen peroxide(H₂O₂) to generate the oxidized forms, hypothiocyanous acid(HOSCN) or the hypothiocyanite anion(OSCN⁻¹)⁷⁻⁹⁾. Peroxidase-system-generated HOSCN/OSCN⁻¹ are found to be antibacterial, especially at low pH^{10,11)}.

The oxidation of sulfhydryl(SH) groups of enzymes and proteins has been considered to be the key to the antimicrobial action of salivary peroxidase systems. This oxidation reaction inactivates bacterial enzymes, including hexokinase, glyceraldehyde-3P-dehydrogenase, with subsequent inhibition of the whole glycolytic pathway^{6,7)}. The peroxidase systems also block glucose uptake, inhibit amino acid transport, damage the inner membrane leading to leakage of amino acids

and K⁺ ions from bacterial cell, and disrupt electrical gradients⁷⁾.

Previous in vitro studies support that salivary peroxidase systems are antimicrobial against many oral bacteria¹²⁾, fungi¹³⁾, and virus¹⁴⁾. Among the susceptible bacteria are such clinically important species as *lactobacilli*, *streptococci* and *actinomyces*⁹⁾.

The limiting component for the production of HOSCN/OSCN⁻ is hydrogen peroxide (H₂O₂), of which normal level is too low to activate the biological inhibiting system in saliva¹⁵⁾. Therefore, previous attempts to enhance the activity of oral peroxidase systems have been based on the addition of H₂O₂-generating enzymes to toothpastes or mouthrinses^{16,17)}. However, studies using toothpastes other than Biotene[®] (Laclede Professional Products, Gardena, CA, USA), a peroxidase-system-containing toothpaste, have not reported any increase in salivary HOSCN/OSCN⁻ levels. The clinical effects are still controversial¹⁾.

Biotene[®] was the unique toothpaste which contained the complete components of the peroxidase system. Since development, it was effective in inhibiting the growth of a number of microorganisms in vitro tests. Although very little in vivo study existed, two preliminary studies reported that peroxidase system administered by a toothpaste improved gingival health in irradiated head and neck cancer patients¹⁸⁾, reduced periodontal probing depth, decreased pocket bleeding in radiation - induced xerostomic patients when compared with a common fluoridated toothpaste¹⁹⁾.

However, recent studies in healthy subjects reported that the use of Biotene[®] twice a day did not affect the levels of *mutans streptococci*, *lactobacilli*, total streptococci, or total microbial flora in whole saliva and dental plaque^{20,21)}. The fact that saliva samples in these studies were not

collected within maximum generation time of HOSCN/OSCN⁻ after toothbrushing indicated that the bacteria could have recovered from the inhibitory effects of HOSCN/OSCN⁻.

The objectives of the first study on healthy subjects were to assess the levels of HOSCN/OSCN⁻ generated in human stimulated whole saliva after the use of a peroxidase-system-containing toothpaste (Biotene[®]) and then to determine the maximum generation time of HOSCN/OSCN⁻. The effects of a 2-week daily use of this toothpaste were evaluate on the values of salivary flow rate, pH, viscosity, and the levels of *S. mutans* and *lactobacilli*, the principle oral cariogenic bacteria.

The second study on xerostomic patients was designed to determine the maximum generation time of HOSCN/OSCN⁻ after the use of toothpaste containing or devoid of peroxidase system and to compare the effects of use of the each toothpaste on the levels of *S. mutans* and *lactobacilli* before with those at maximum generation time of HOSCN/OSCN⁻ after toothbrushing. The comparison between experimental group and control group was also conducted for the purpose of this study.

II. MATERIALS AND METHODS

1. Subjects and saliva collection

the study on healthy subjects

Two different types of studies on healthy subjects were performed. Seven healthy dental students (age; 23-25 years) participated in one cross-over study, separated by a 1-week washout period, that assessed the levels of HOSCN/OSCN⁻ and determined the maximum generation time after toothbrushing with toothpaste containing or devoid of peroxidase system. Saliva samples were collected between 9:00 a.m.

and 10:00 a.m. Gumbase-stimulated whole saliva was collected before toothbrushing, at 5, 15, 30, and 60 min. after toothbrushing with peroxidase-system-containing toothpaste (Biotene®) or fluoridated toothpaste devoid of peroxidase system. These samples were centrifuged at 2500 rpm for 15 min. for assay of hypothiocyanite.

Sixteen healthy dental students (age: 22-29 years) were included in the other study that evaluated antimicrobial effects of Biotene® after a 2-week daily use. After baseline measurements, half the participants were randomly given peroxidase-system-containing toothpaste (Biotene®) while the other half were given fluoridated toothpaste devoid of peroxidase system. All the subjects were asked to use the same amount (1 inch) of the experimental toothpaste twice a day for two minutes and advised to refrain from using any other oral hygiene or antimicrobial products. Gumbase-stimulated whole saliva was collected at maximum generation time of HOSCN/ OSCN⁻ (30min.) after toothbrushing, before and after a 2-week daily use of allocated toothpaste. Saliva samples were collected between 9:00 a.m. and 10:00 a.m. and immediately submitted to microbial assay.

The subjects who were excluded from this study, included those who were currently in medication, maintained some type of infectious disease and smokers. The participants of this study were not allowed to eat, drink, or smoke prior to saliva collection.

the study on xerostomic patients

Eight patients who had pronounced xerostomia as measured by the drooling test, attended a 1-week crossover trial with Biotene and a separate control toothpaste. The salivary secretion rates of stimulated whole saliva were equal to or less than 3.5 ml while the mean values were 2.8 ml for 5 minutes.

Gumbase-stimulated whole saliva was collected by the same method as the first study with the exception of collecting time. Stimulated whole saliva was collected before toothbrushing, at 15, 30, and 60 min. after toothbrushing with Biotene® or fluoridated toothpaste devoid of peroxidase system and centrifuged at 2500 rpm for a 15 min. for assay of hypothiocyanite. Saliva samples collected before toothbrushing and at maximum generation time of HOSCN/ OSCN⁻ (30min.) after toothbrushing with Biotene® or control toothpaste were used for microbial assay.

2. Chemical assay of HOSCN/OSCN⁻

The levels HOSCN/OSCN⁻ in stimulated whole saliva were quantified by the method described by Aune and Thomas²²⁾ and modified by Pruitt et al²³⁾. The concentration of HOSCN/OSCN⁻ was spectrophotometrically analyzed by the reaction with the colored anionic monomer of 5,5-dithiobis-(2-nitro-benzoic acid)(Nbs)₂ and calculated from the difference of absorbance at 412 nm following the oxidation of 5-thio-2- nitrobenzoic acid(Nbs) to 5,5-dithiobis-(2-nitro- benzoic acid)(Nbs)₂ by OSCN⁻ ions assuming a molar absorption coefficient of 13600 M⁻¹ cm⁻¹ 24).

3. Determination of salivary flow rate of stimulated whole saliva

After collection of gumbase-stimulated whole saliva, flow rate was determined by calculating the volume (milliliters) of collected saliva per minute.

4. Salivary pH determination

Salivary pH was assessed electrometrically with microprocessor-based pH/ion meter DP-880 (Dong-Woo Medical Co., Seoul, Korea). Before each measurement, the electrode was standardi-

zed with pH 4.0 and 7.0 buffer solution to ensure the accuracy of the results. The same electrode was used in each recording throughout the experiment. For increased accuracy, the pH of collected saliva samples were measured within a few minutes after collection.

5. Salivary viscosity determination

Viscosity measurements were performed at 37.0 ± 0.2 °C with model LVT Wells-Brookfield cone-and-plate digital viscometer(Brookfield Engineering Laboratories, Stoughton, MA, U.S.A.). A 0.8-degree cone(model No. CP-40) was utilized in this experiment. A 0.5-1.0 ml of the collected sample was used in each test and the viscosity values were recorded in centipoise(cps) at shear rate 450.0 sec^{-1} .

6. Microbial assay

After serial tenfold dilutions, the bacteria samples were plated as follows: *S. mutans* and *lactobacilli* were cultivated on Mitis Salivarius Bacitracin(MSB) and Rogosa SL agar plates respectively. *S. mutans* were incubated for 2 days in an anaerobic jar(DiFco Co. Ltd) at 37 °C and *lactobacilli* were incubated for 3 days at 37 °C. After the appropriate incubation period, the number of colony forming units(CFU/ml) was determined.

7. Statistical analysis

T-test and analysis of variance(ANOVA) with multiple comparison were used to compare the mean values and standard deviations. Oneway ANOVA with multiple comparison was used to compare differences in the generation of hypothiocyanite ion between different collecting times.

III. RESULTS

the study on healthy subjects

The levels of HOSCN/OSCN⁻ were significantly increased in stimulated whole saliva from the group that used peroxidase-system-containing toothpaste at 30 min. after toothbrushing with mean level of $25 \mu M$. There were no significant differences before toothbrushing, at 5, 15, and 60 min. after toothbrushing between the two groups(Table 1, Fig. 1). The concentration of HOSCN/OSCN⁻ in the group that used peroxidase-system-containing toothpaste gradually increased up to 30 min. but were found to be irregular in the control group.

The values of salivary flow rate, pH and viscosity at shear rate 450.0 sec^{-1} in stimulated whole saliva were not significantly different between before and after a 2-week daily use of the experimental toothpastes(Table 2).

After a 2-week daily use of experimental toothpastes, the levels of *S. mutans* and *lactobacilli* were relatively lower in the group that used toothpaste containing peroxidase system but statistically no significant differences were observed (Table 3, Fig 2, 3).

the study on xerostomic patients

The levels of HOSCN/OSCN⁻ in stimulated whole saliva of xerostomic patients were significantly increased after using both experimental toothpastes. Results indicated that they were significantly higher in the group that used toothpaste containing peroxidase system (Biotene[®]) at 30 min. with a mean level of $61.7 \mu M$ and at 60 min. with a mean level of $59.8 \mu M$ after toothbrushing (Table 4, Fig. 4).

The levels of *S. mutans* and *lactobacilli* at 30 min. after the use of toothpaste containing peroxidase system($p < 0.01$) or toothpaste devoid of peroxidase system($p < 0.05$) were significantly

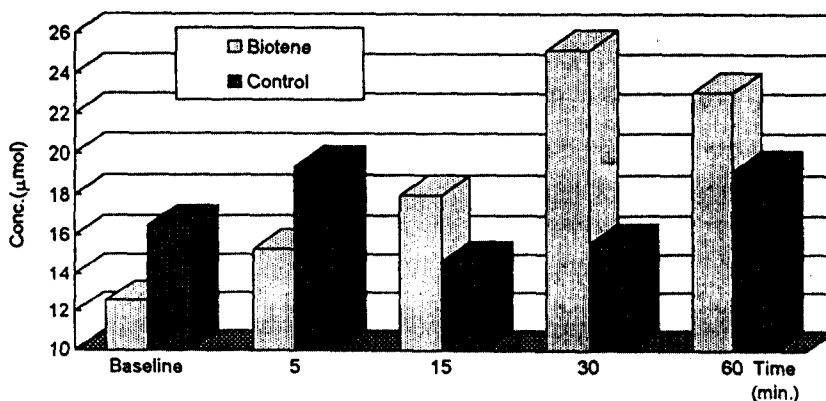


Fig 1. Comparison of effects of brushing with experimental toothpastes on the generation of hypothiocyanite in stimulated whole saliva of healthy subjects

Table 1. Effects of experimental toothpastes on the generation of hypothiocyanite in stimulated whole saliva of healthy subjects

Time Conc. (µ mol)	Baseline mean ± S.D.	T ₅ mean ± S.D.	T ₁₅ mean ± S.D.	T ₃₀ mean ± S.D.	T ₆₀ mean ± S.D.	difference in each group (ANOVA)
Biotene group (N = 7)	12.6 ± 10.2	15.3 ± 7.7	18.0 ± 8.7	25.2 ± 6.8	23.1 ± 5.0	(T ₀ , T ₃₀)* (T ₀ , T ₆₀)* (T ₅ , T ₃₀)** (T ₅ , T ₆₀)* (T ₁₅ , T ₃₀)*
Control group (N = 7)	16.5 ± 11.3	19.4 ± 9.6	14.7 ± 9.8	15.6 ± 8.6	19.2 ± 11.7	(T ₅ , T ₆₀)** (T ₁₅ , T ₆₀)*
difference between groups (t-test)	N.S.	N.S.	N.S.	*	N.S.	

* : Statistically significant (p < 0.05)

** : Statistically significant (p < 0.01)

N.S. : Not significant

T_a : Time after toothbrushing(a min.)

SD : Standard deviation

lower than those of before toothbrushing. The group that used toothpaste containing peroxidase system measured a significantly lower level of *S. mutans* (p < 0.05) than the control group. The levels of *lactobacilli* were slightly lower in the

group that used peroxidase-system-containing toothpaste(Biotene®) but no statistically significant difference was observed (p = 0.065) (Table 5, Fig 5, 6).

Table 2. The values of salivary flow rate , pH, and viscosity at shear rate 450.0 sec⁻¹ before and after a 2-week daily use of the experimental toothpastes in stimulated whole saliva of healthy subjects

		Biotene group (N = 8)	Control group (N = 8)	difference between groups (t-test)
Flow rate(ml/min.)	Baseline(mean ± S.D.)	9.0 ± 4.3	6.4 ± 1.9	N.S.
	2 weeks(mean ± S.D.)	8.3 ± 4.2	6.2 ± 1.3	N.S.
difference in each group (t-test)		N.S.	N.S.	
Salivary pH	Baseline(mean ± S.D.)	7.1 ± 0.3	7.1 ± 0.2	N.S.
	2 weeks(mean ± S.D.)	7.0 ± 0.3	7.0 ± 0.2	N.S.
difference in each group (t-test)		N.S.	N.S.	
viscosity(cps)	Baseline(mean ± S.D.)	0.30 ± 0.12	0.30 ± 0.28	N.S.
	2 weeks(mean ± S.D.)	0.29 ± 0.13	0.29 ± 0.10	N.S.
difference in each group (t-test)		N.S.	N.S.	

N.S. : Not significant S.D. : Standard deviation

Table 3. Effects of a 2-week daily use of the peroxidase containing toothpaste on the numbers of bacteria in stimulated whole saliva of healthy subjects.

Colony Forming Units(CFU/ml)		Biotene group (N = 8)	Control group (N = 8)	difference between groups (t-test)
<i>S. mutans</i> (× 10 ⁷)	Baseline (mean ± S.D.)	5.9 ± 7.7	4.9 ± 4.8	N.S.
	2 weeks, (mean ± S.D.)	2.8 ± 2.6	2.7 ± 2.7	N.S.
<i>Lactobacilli</i> (× 10 ³)	Baseline (mean ± S.D.)	1.5 ± 3.4	1.5 ± 2.3	N.S.
	2 weeks (mean ± S.D.)	2.3 ± 3.2	3.4 ± 3.1	N.S.

N.S. : Not significant S.D. : Standard deviation

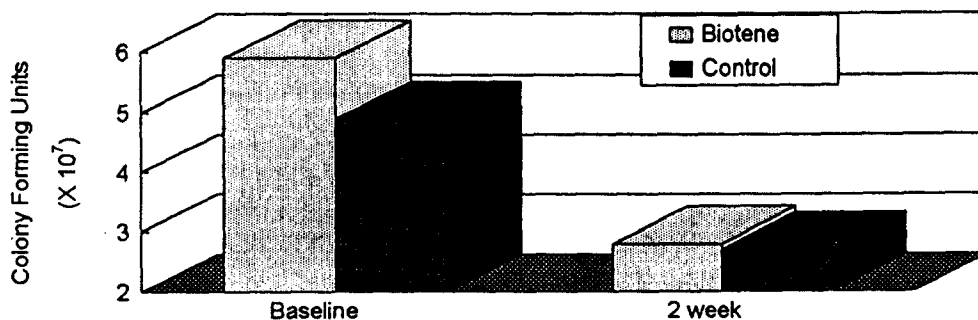


Fig. 2. Comparison of effects of a 2-week daily use of experimental toothpastes on the numbers of *S. mutans* in stimulated whole saliva of healthy subjects.

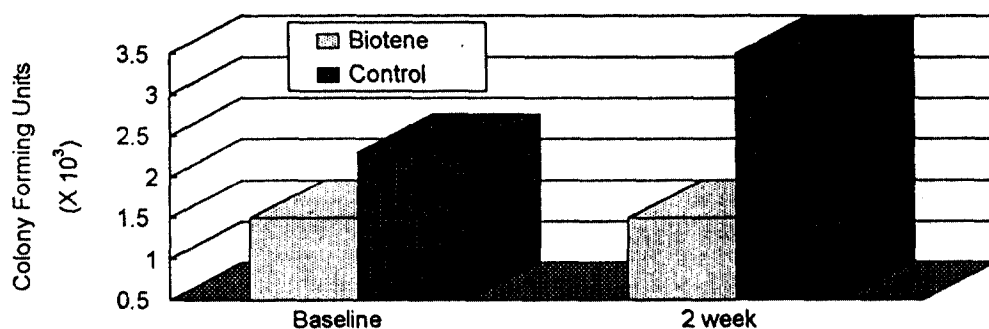


Fig. 3. Comparison of effects of a 2-week daily use of experimental toothpastes on the numbers of Lactobacilli in stimulated whole saliva of healthy subjects.

Table 4. Effects of experimental toothpastes on the generation of hypothiocyanite in stimulated whole saliva of xerostomic patients

Conc. (μ mol)	Time	Baseline mean ± S.D.	T ₁₅ mean ± S.D.	T ₃₀ mean ± S.D.	T ₆₀ mean ± S.D.	difference in each group (ANOVA)
Biotene group (N = 8)		14.4 ± 13.0	37.8 ± 16.1	61.7 ± 6.8	59.8 ± 10.8	(T ₀ ,T ₁₅)* (T ₀ ,T ₃₀)** (T ₀ ,T ₆₀)** (T ₁₅ ,T ₃₀)** (T ₁₅ ,T ₆₀)**
Control group (N = 8)		10.9 ± 11.0	16.1 ± 9.9	25.4 ± 10.8	22.7 ± 14.0	(T ₀ ,T ₁₅)* (T ₀ ,T ₃₀)** (T ₀ ,T ₆₀)** (T ₁₅ ,T ₃₀)**
difference between groups(t-test)		N.S.	N.S.	**	*	

* : Statistically significant (p < 0.05)

** : Statistically significant (p < 0.01)

N.S. : Not significant

SD : Standard deviation

T_a : Time after toothbrushing (a min.)

IV. DISCUSSION

The role of oral microorganisms as a major factor in the aetiology of dental diseases is now clearly recognized. However, not all patients have either the ability or motivation to control the microbial factors to prevent associated diseases. Thus, there has been an increasing interest in

both natural and artificial methods that can improve the efficiency of the usual oral hygiene methods and additional inhibition of bacteria left behind after routine oral hygiene care, which is worthy of investigation. A number of oral antimicrobial chemicals have been employed in inhibiting oral bacteria and preventing oral disease but the long-term use of artificial

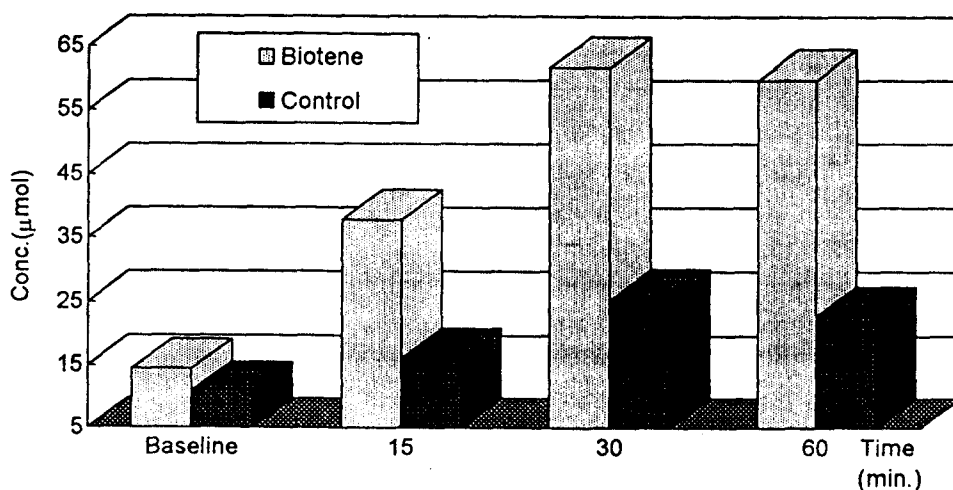


Fig. 4. Comparison of effects of brushing with experimental toothpastes on the generation of hypothiocyanite in stimulated whole saliva of xerostomic patients

Table 5. Effects of use of experimental toothpastes on the numbers of bacteria in stimulated whole saliva of xerostomic patients.

Colony Forming Units(CFU/ml)		Biotene group (N = 8)	Control group (N = 8)	difference between groups (t-test)
<i>S. mutans</i> ($\times 10^6$)	Baseline (mean \pm S.D.)	7.78 \pm 4.17	10.81 \pm 10.00	N.S.
	30 min. after toothbrushing (mean \pm S.D.)	1.21 \pm 0.81	6.28 \pm 5.22	*
difference in each group (t-test)		**	*	
<i>Lactobacilli</i> ($\times 10^4$)	Baseline (mean \pm S.D.)	15.85 \pm 9.57	12.98 \pm 8.31	N.S.
	30 min. after toothbrushing (mean \pm S.D.)	3.48 \pm 3.12	7.23 \pm 5.22	N.S.
difference in each group (t-test)		**	*	

* : Statistically significant ($p < 0.05$)

** : Statistically significant ($p < 0.01$)

N.S. : Not significant

SD : Standard deviation

antimicrobial agents may give rise to serious side effects. Therefore, it has been expected that the remedy containing antimicrobial substances proper to saliva could be safely utilized to prevent

and treat oral diseases.

The peroxidase antimicrobial system is a naturally occurring system which has been proven to be both bacteriostatic and bactericidal

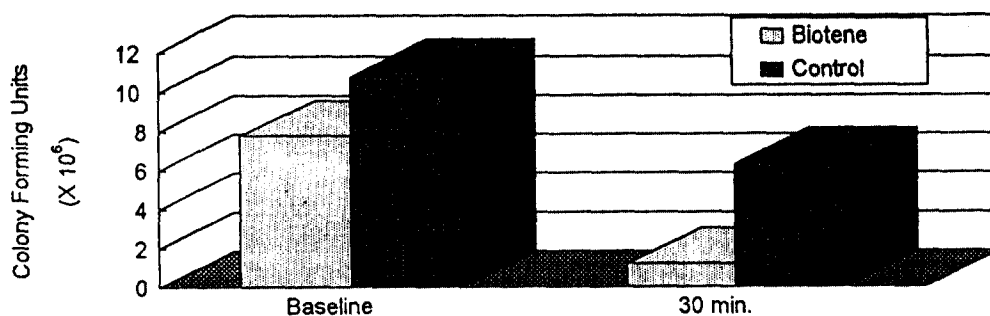


Fig. 5. Comparison of effects of experimental toothpastes on the numbers of *S. mutans* in stimulated whole saliva of xerostomic patients.

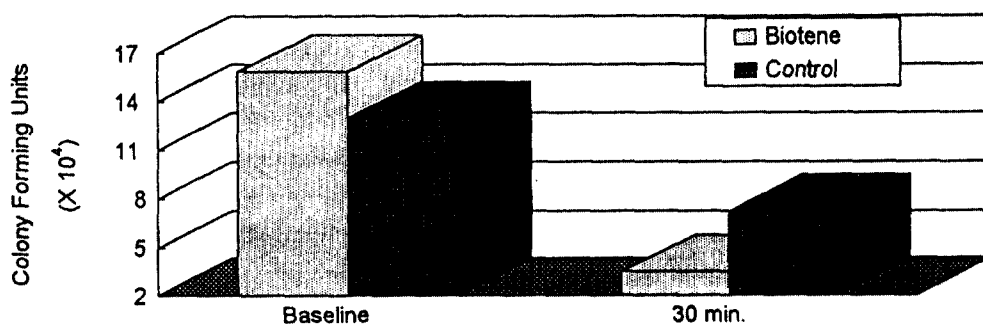


Fig. 6. Comparison of effects of experimental toothpastes on the numbers of *Lactobacilli* in stimulated whole saliva of xerostomic patients.

to a variety of gram-positive and gram-negative microorganisms by producing HOSC⁻/OSCN⁻ as an antibacterial agent¹⁴. The inverse relationship between oral microorganisms and salivary HOSC⁻/OSCN⁻ level suggested the possible clinical effectiveness of enhancement of HOSC⁻/OSCN⁻ generation in human mouth. A number of clinical and laboratory trials have been undertaken to prove it¹⁸.

The results confirmed that the levels of HOSC⁻/OSCN⁻ in stimulated whole saliva of healthy subjects significantly increased by using peroxidase-system-containing toothpaste (Biotene[®]) and also showed a different generation pattern in comparison with the previous study¹⁸ with unstimulated whole saliva where the decom-

position of HOSC⁻/OSCN⁻ after initial notable generation was completed within 20 min. of brushing. Because saliva contains a number of oral microorganisms and chewing promotes their exfoliation from teeth and oral mucosa, we analyzed stimulated whole saliva as an indirect indicator for evaluating the number of oral microorganisms during the whole experiment. The rapid increase of antibacterial component up to 30 min. after toothbrushing supported the use of peroxidase-system-containing toothpaste to be effective in the control of oral microorganisms.

However, several studies in contrast to expectations, have indicated that using peroxidase-system-containing toothpaste (Biotene[®]) twice daily did not affect the levels of microorganisms

in whole saliva or dental plaque^{20,21}). Though statistically significant differences were not observed, results of the experiment on microorganisms showed that peroxidase containing toothpaste might be clinically effective in reducing oral microorganisms even in healthy subjects. Besides, if that the concentration of HOSCN/OSCN⁻ in stimulated whole saliva was lower than that of unstimulated whole saliva²⁰, actual reduction of oral microorganism might be more extensive at rest in oral cavity. It can be hypothesized that such a difference between the results and those of previous studies was mainly due to the collection time of saliva samples. In the study, saliva was collected in maximum generation time of hypothiocyanite and bacterial recovery might not have been allowed to some extent.

The flow rate of stimulated whole saliva was measured immediately after toothbrushing for objective evaluation of the function of salivary gland because the saliva is one of major determinants of oral environment through mechanical cleansing and protective function²⁵). Salivary pH and viscosity at shear rate 450.0^{-1} were also estimated. A 2-week daily use of the experimental toothpastes had no effect on salivary flow rate, pH, and viscosity of stimulated whole saliva collected immediately after toothbrushing, which may show that these factors, especially flow rate, were principally affected more by mechanical stimulation of toothbrushing than chemical effects of peroxidase-system-containing toothpaste. It is also considered that measuring stimulated whole saliva is not an effective method of salivary flow rate.

Xerostomia(dry mouth) is a clinical condition which is characterized by a desiccation of the intraoral tissues. The most common reason is reduced saliva production(sialopenia). Therefore, an objective assessment of salivary function, by measuring salivary flow rates, was indicated

before the diagnosis of salivary hypofunction in the second study. Normal physiological activities of the mouth, including talking, eating, chewing, and swallowing are often intolerable in the patient with xerostomia. Besides, they are more susceptible to oral infectious diseases because many patients with xerostomia have incomplete buffer capacity and can not maintain covering salivary coating(pellicle). Xerostomic patients also have a reduced quantity and quality of peroxidase systems, which may be main cause that we can expect more beneficial effects of peroxidase-system-containing toothpaste in xerostomic patients.

In the second study on xerostomic patients, the levels of HOSCN/OSCN⁻ in stimulated whole saliva also reached the maximum generation time at 30 min. after toothbrushing. The levels were higher and remained longer than those of healthy subjects.

Comparing microorganisms at 30 min. after toothbrushing between the two groups, levels of *S. mutans* and *lactobacilli* were lower in the group using toothpaste containing peroxidase system and moreover *S. mutans* were significantly lower($p < 0.05$). A possible explanation to the results may be due to obvious increase of HOSCN/OSCN⁻, lower salivary pH in xerostomic patients, and incomplete mechanical cleansing by toothbrushing.

The above results suggested that the use of peroxidase-system-containing toothpaste can be effective in enhancing the oral defense mechanisms even in healthy individuals and play an important role in restoring saliva's own defense systems of patients with hyposalivation or xerostomia.

V. CONCLUSIONS

This study was performed to assess the effects

of the use of a toothpaste containing peroxidase system on stimulated whole saliva of healthy subjects and xerostomic patients

The first study was performed to assess the levels of HOSCN/OSCN⁻ generated in human stimulated whole saliva of healthy subjects after using a toothpaste containing the peroxidase system to determine the maximum generation time. The effects of a 2-week daily use of this toothpaste on the values of salivary flow rate, pH and viscosity, and the levels of *S. mutans* and *lactobacilli* were also examined in healthy patients. In the second study on xerostomic patients, maximum generation time of HOSCN/OSCN⁻ was also determined and then the levels of *S. mutans* and *lactobacilli* were evaluated before and after toothbrushing. The author came to the following conclusions.

1. The levels of HOSCN/OSCN⁻ were significantly increased in stimulated whole saliva of healthy subjects in the group that used peroxidase-system-containing toothpaste at 30 min. after toothbrushing ($p < 0.05$) than those in control group.
2. The flow rate, pH, and viscosity in stimulated whole saliva of healthy subjects were not significantly affected after a 2-week daily use of experimental toothpastes.
3. The levels of *S. mutans* and *lactobacilli* in healthy subjects after a 2-week daily use of experimental toothpastes were relatively lower in the group using peroxidase-system-containing toothpaste but no significant differences were observed ($p = 0.206$ for *lactobacilli*, $p = 0.944$ for *S. mutans*).
4. The levels of HOSCN/OSCN⁻ in the stimulated whole saliva of xerostomic patients were significantly higher in the group using toothpaste containing peroxidase system at 30 min. ($p < 0.01$) and 60 min. ($p < 0.05$) after

toothbrushing.

5. The levels of *S. mutans* and *lactobacilli* in xerostomic patients at 30 min. after use of toothpaste containing peroxidase system ($p < 0.01$) or toothpaste devoid of peroxidase system ($p < 0.05$) were significantly lower than those before toothbrushing.
6. The levels of *S. mutans* at 30 min. after toothbrushing in stimulated whole saliva of xerostomic patients were significantly lower in the group that used toothpaste containing peroxidase system than those in control group ($p < 0.05$).
7. The levels of *lactobacilli* at 30 min. after toothbrushing in stimulated whole saliva of xerostomic patients were relatively lower in the group that used peroxidase-system-containing toothpaste but no statistically significant differences were observed.

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PEROXIDASE SYSTEM을 함유한 세치제가 생체에서 전타액에 미치는 영향에 대한 연구

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Peroxidase system은 구강내 고유의 항균체계로서 항세균물질인 HOSCN/OSCN⁻을 생산하여 다양한 그래ם 양성 그리고 그래ם 음성 세균에 대해 살균 및 정균작용을 나타낸다.

본 연구는 peroxidase system을 함유한 세치제가 건강한 대상자와 구강건조증 환자의 자극성 전타액에 미치는 영향에 대해 각각 연구하였다.

첫 번째 연구에서는 peroxidase system을 함유한 치약을 사용한 후 건강한 대상자의 자극성 전타액내 생성되는 HOSCN/OSCN⁻의 양을 측정하여 최대 생성 시간을 결정하였다. 그 후 건강한 대상자에게 임의로 실험치약과 대조치약을 할당하여 2주간 사용하도록 한 후 HOSCN/OSCN⁻ 최대 생성 시간에 타액을 채취하여 치약 사용전과 2주후의 타액분비량, pH, 점도도 그리고 *S. mutans*와 *lactobacilli*에 대한 평균효과를 비교하였다.

구강건조증 환자를 대상으로 한 두 번째 연구에서도 peroxidase system을 함유한 세치제를 사용한 후 생성되는 HOSCN/OSCN⁻의 최대생성시간을 측정하였으며, peroxidase system을 함유한 세치제가 *S. mutans*와 *lactobacilli*에 미치는 영향을 양치전과 양치후 HOSCN/OSCN⁻의 최대생성시간에 타액을 채취하여 비교하였다. 이와같은 실험을 통해 다음과 같은 결론을 얻었다.

1. 건강한 대상자의 자극성 전타액내의 HOSCN/OSCN⁻의 농도는 양치후 30분후 대조군에 비해 peroxidase systems을 함유한 세치제를 사용한 군에서 유의성있게 높게 나타났다($p < 0.05$).
2. 건강한 대상자의 자극성 전타액의 타액분비량, pH, 타액점도도는 peroxidase system을 함유한 세치제를 2주간 사용한 후에도 유의한 변화를 나타내지 않았다.
3. 2주간 실험치약들을 사용한후 건강한 대상자의 자극성 전타액내 *S. mutans*와 *lactobacilli*의 양은 peroxidase system을 함유한 세치제를 사용한 군에서 상대적으로 낮게 나타났으나 통계학적 유의성은 없었다.($p = 0.206$: *lactobacilli*, $p = 0.944$: *S. mutans*).
4. 구강건조증 환자의 자극성 전타액내의 HOSCN/OSCN⁻의 농도는 양치후 30분($p < 0.01$)과 60분($p < 0.05$)에서 대조군에 비해 peroxidase system을 함유한 세치제를 사용한 군에서 유의성있게 높았다.
5. 구강건조증 환자의 양치후 30분에 채취한 자극성 전타액내의 *S. mutans*와 *lactobacilli*의 양은 peroxidase system을 함유하거나($p < 0.01$) 함유하지 않은($p < 0.05$) 치약을 사용한 군 모두에서 양치전에 비해 유의성있게 감소하였다.
6. 양치후 30분에 채취한 구강건조증 환자의 자극성 전타액내 *S. mutans* 양은 peroxidase system을 함유한 세치제를 사용한 군에서 대조군에 비해 유의성있게 낮았다($p < 0.05$).
7. 양치후 30분에 채취한 구강건조증 환자의 자극성 전타액내 *lactobacilli* 양은 peroxidase system을 함유한 세치제를 사용한 군에서 대조군에 비해 상대적으로 낮게 나타났으나($p = 0.067$) 통계학적 유의성은 없었다.

주요어 : 타액, peroxidase system, 치약, hypothiocyanite, 미생물