Betaine-containing toothpaste relieves subjective symptoms of dry mouth

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Subjects with dry mouth often experience irritation of the oral mucosa when using sodium lauryl sulfate–containing products for oral hygiene. Betaine, or trimethylglycine, reduces skin-irritating effects of ingredients of cosmetics such as sodium lauryl sulfate. The aim of the present study was to compare the effects of a betaine-containing toothpaste with a regular toothpaste on the oral microbial flora, the condition of the oral mucosa, and subjective symptoms of dry mouth in subjects with chronic dry mouth symptoms. Thirteen subjects with chronic dry mouth symptoms and with a paraffin-stimulated salivary flow rate $\leq$ 1 mL/min participated in the double-blind crossover study. Ten subjects had a very low salivary flow rate ($\leq$0.6 mL/min). The subjects used both experimental toothpastes (with or without 4% betaine) twice a day for 2 weeks. Oral examinations and microbiologic sample collections were made at the base lines preceding the two experimental periods and at the end. Standardized questions on subjective symptoms of dry mouth were used when the subjects were interviewed at the end of the two experimental periods. No study-induced significant changes were observed in the microbiologic variables (plaque index, mutans streptococci, lactobacilli, *Candida* species) or in the appearance of the oral mucosa. The use of the betaine-containing toothpaste was, however, associated with a significant relief of several subjective symptoms of dry mouth. Betaine appears thus to be a promising ingredient of toothpastes in general and especially of toothpastes designed for patients with dry mouth. □ *Betaine; hyposalivation; Sjögren's syndrome; xerostomia*

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Xerostomia provokes unpleasant oral symptoms such as burning mouth, difficulty with speech, chewing, and swallowing, and impairment of taste (1, 2). Dry mouth also enhances susceptibility to oral candidosis (3, 4). Xerostomic patients usually show high levels of both mutans streptococci and lactobacilli, and they have an increased susceptibility to dental caries (4, 5). Dryness of the mouth is a common clinical complaint. In an adult population in Sweden 14%–39% reported symptoms of xerostomia, the highest prevalences being in women and the elderly (6). Subjective feelings of dry mouth have also been found in patients with normal salivary flow rates (7), but complaint of oral dryness is most often a symptom of salivary gland hypofunction.

Subjects with dry mouth often experience irritation of the oral mucosa when using products for oral hygiene. For example, alcohol in mouthrinses and sodium lauryl sulfate, a common detergent of toothpastes, irritate the dry mouth. Sodium lauryl sulfate may irritate the oral mucosa of persons with normal salivary flow rate as well; for example, recurrent aphthous ulcers were recently connected with the presence of sodium lauryl sulfate in toothpastes (8). Products have been specially designed for subjects with dry mouth—for example, saliva substitutes (for a review, see Ref. 9) and products containing natural oral antimicrobiologic agents (10, 11). Promising results in alleviating subjective sensations of dry mouth have been obtained with a lactoperoxidase-containing gel combined with a toothpaste that also contains components of the peroxidase system (12). Detergents with lower irritating properties have also been searched for (13). A novel approach is offered by betaine. Betaine, or trimethylglycine, was recently shown to reduce the skin-irritating effects of ingredients of cosmetics, such as sodium lauryl sulfate (14, 15). Betaine, which is common in nature, has unique skin-moisturizing properties (14). Thus it is a potential natural ingredient of toothpastes.

The aim of the present study was to compare the effects of a betaine-containing toothpaste with a regular toothpaste on the oral flora, the condition of the oral mucosa, and subjective symptoms of dry mouth in subjects with chronic dry mouth symptoms.

Materials and methods

Subjects and study design

Thirteen subjects (12 women and 1 man; mean age, 57 years; range, 28–76 years) with chronic dry mouth symptoms and paraffin-stimulated salivary flow rate $\leq$ 1.0 mL/min participated in this clinical study. Ten subjects had a very low salivary flow rate ($\leq$0.6 mL/min). Three of the subjects had full dentures. The dental history of the subjects has been reported earlier (11). A detailed description of the individuals on the basis of age, sex, clinical diagnosis, and the number of different daily medications is presented in Table 1. All subjects were informed about the purpose of this study. The study was

Table 1. Characteristics of the subjects: age, sex, stimulated saliva flow rate, diagnosis, and subjective oral symptoms

Subject	Age (years)	Sex	Saliva flow rate (mL/min)†	Diagnosis	Medications
1	76	F	0.3	Sjögren's syndrome	9
2	55	M	0.05	Sjögren's syndrome Rheumatoid arthritis Tumor in parotid gland	2
3	54	F	0.3	Depression	5
4	59	F	0.05	Sjögren's syndrome Mitral prolapse	–
5	28	F	0.4	Sjögren's syndrome	–
6	57	F	0	Sjögren's syndrome	1
7	43	F	0.5	Sjögren's syndrome	1
8	65	F	0.4	Sjögren's syndrome Rheumatoid arthritis	6
9	64	F	0.2	Sjögren's syndrome Rheumatoid arthritis	–
10	62	F	0.1	Sjögren's syndrome SLE*	–
11	48	F	1.0	Sjögren's syndrome	4
12	61	F	1.0	Sjögren's syndrome Larynx spasm	3
13	71	F	0.9	No diagnosed disease	1

* SLE = systemic lupus erythematosus.
† Mean of four measures.

approved by the Ethical Board of the Medical Faculty of the University of Turku.

The study was performed as a double-blind crossover study. The 13 subjects, who had participated in toothpaste studies previously, were invited to the study by mail. They also received by mail the instructions for the study 4 weeks before it was started. Seven of the subjects used the number-coded (1 or 2) toothpaste in the order 1–2 and six in the order 2–1. The two 2-week experimental periods were separated by a 3- to 4-week wash-out period. During the wash-out period and 1 month preceding the study the subjects were instructed not to use any oral hygiene products with strong antibacterial activity (such as chlorhexidine products). They were also instructed to follow their normal dietary habits and to use regular fluoride toothpaste of their own choice before the study and during the wash-out period.

The oral examination and the collection of the microbiologic samples were done at the two base-line points preceding the two experimental periods and at the end of them. At the first visit all subjects were interviewed about their subjective health condition. A comprehensive medical history, including medications, was obtained for each individual. Standardized questions (16) on subjective symptoms of dry mouth were used when the subjects were interviewed at the end of the two experimental periods. The six standardized questions monitored feelings of dry mouth during the nighttime and daytime, the need to drink during the night or at meals, and difficulties during eating or swallowing. In addition, we asked four questions to ascertain whether the subjects had itching of the tongue, the lips, or the oral mucosa in general or had problems with speaking. The subjects were asked to grade their subjective feelings of dry mouth on a three-graded scale (minor, medium, strong discomfort). They were also asked to compare their subjective dry mouth sensations during the use of the toothpaste with the sensations preceding the experimental period. Their opinions were recorded as decreases, no changes, or increases in the severities of the dry mouth sensations. The interview also included questions on the composition of the product. At the last visit the subjects were asked which toothpaste they preferred (1 or 2).

All participants were instructed to brush their teeth for approximately 3 min with the experimental toothpaste in the morning and in the evening. They were asked to apply approximately 500 mg toothpaste on their toothbrushes. The subjects who had dentures kept the dentures on when they brushed their teeth. The subjects were instructed to refrain from all oral hygiene procedures from the evening preceding the day of the appointment. They were also asked not to eat or drink for at least 1 h before the appointment.

Oral examinations

A complete oral examination was made of each subject. Any changes in the oral mucosa or the lips were recorded. The plaque index of the 10 dentulous subjects was measured from 6 teeth (Sillnes & Löe; dd 16, 21, 24, 36, 41, 44) in the 10 subjects who did not have dentures.

Test products

The composition of the two experimental toothpastes is shown in Table 2. The toothpastes were number-coded (1

Table 2. The main ingredients of the tested toothpastes (in percentage)

	Betaine toothpaste	Placebo toothpaste
Sorbitol (70%)	50.0	51.1
Water	19.5	22.4
Carboxymethylcellulose 7MF	1.0	1.0
Sodium fluoride	0.2	0.2
Sodium benzoate	0.1	0.1
Titanium dioxide	0.5	0.5
Saccharine	0.3	0.3
Tri-sodium phosphate	1.1	1.1
Betafin BP	4.0	–
Carbopol 980 NF	0.2	0.2
Colour 0.5%	0.1	0.1
Silicon dioxide Zeodent 113	20.0	20.0
Sodium lauryl sulfate	2.0	2.0
Colour 432	1.0	1.0
Total	100	100

or 2). The non-commercial toothpastes were manufactured for the study by Sophie-International (Helsinki, Finland). The betaine (trimethylglycine, $C_5H_{11}NO_2$) was from Cultor, Naantali, Finland.

Collection and treatment of salivary and microbiologic samples

Paraffin-stimulated whole saliva was collected for 5 min from each subject at the two base-line points and after the two experimental periods. The secretion rate was determined in milliliters per minute. To obtain microbiologic samples, the subjects rinsed their mouths with 5 mL of saline, and a 100-μL aliquot was transferred to a test tube containing 900 μL of tryptic soy broth (TSB) (Oxoid, Basingstoke, UK), supplemented with 10% glycerol. The microbiologic samples were stored at −20°C until analyzed within 2 weeks.

Microbiologic assays

For microbiologic assays the TSB tubes were thawed and vortexed thoroughly for 1 min. After serial dilutions the bacteria were cultured as follows: mutans streptococci on Mitis salivarius (Difco Laboratories, Detroit, Mich., USA) bacitracin agar plates (17) for 3 days in air containing 7% CO_2 at 37°C. Lactobacilli were cultured on Rogosa SL agar plates (Difco Laboratories) and incubated anaerobically for 3 days at 37°C. The total anaerobic flora was determined using blood agar plates containing 5% bovine blood and incubated anaerobically for 3 days at 37°C. *Candida* species were grown on Sabouraud dextrose agar plates (Difco Laboratories). The plates were incubated aerobically for 2 days at 37°C. The colony-forming units (CFU/mL) were determined.

Statistics

At the base lines the groups were compared using ANOVA. Student's paired t test was used to analyze the study-induced changes in the variables. The chi-square test was used to test the results of the interviews. P values of <0.05 were considered statistically significant.

Results

The crossover design had no effect on any of the results, and the results of the two groups were therefore combined. The mean saliva flow rates ($n = 4$) of each subject are shown in Table 1. The results were analyzed both for all subjects ($n = 13$) and for those subjects with a very low salivary flow rate ($\leq$0.6 mL/min, $n = 10$). No statistically significant changes in the saliva flow rates were observed during the study.

At base line 9 of the 13 subjects had medium to strong discomfort of dry mouth in the daytime and had to drink water often during the day. More than half of the subjects also had sensations of dry mouth during the nighttime. These subjects all had some problems with their oral mucosa, lips, and/or tongue, such as ulcerations. The four subjects who reported minor discomfort connected with the dry mouth had relatively healthy oral soft tissues, including the lips and the tongue.

All but one of the subjects harbored *Candida* species; high counts (CFU/mL $\geq 10^5$) were found in 38%. The subjects wearing full dentures carried no mutans streptococci, but the rest of the subjects showed high levels of mutans streptococci (CFU/mL $\geq 10^5$). All subjects harbored lactobacilli, and high counts (CFU/mL $\geq 10^5$) were detected in 85%.

No toothpaste-induced visible changes in the oral mucosa including the tongue and the lips were detected during the study in the two toothpaste groups. The 2-week use of the betaine-containing toothpaste, however, was associated with relieved subjective symptoms of dry mouth. No increases in the symptoms were observed, and for 8 of the 10 questions monitoring dry mouth at least 1 subject reported a decrease in the symptom. In the placebo toothpaste group increased symptoms of dry mouth were reported in association with 2 questions and a decrease (1 subject) only for 1 of the 10 questions. For the question 'Does your mouth feel dry during the daytime?' 60% of the very dry mouth subjects ($n = 10$) using the betaine toothpaste reported a decrease in the dry mouth sensations. In the placebo group no changes were observed. The result was statistically significant ($P = 0.003$). For the question on burning sensations of the tongue or oral mucosa one subject in the betaine group reported decreased symptoms, whereas four subjects in the placebo group had increased symptoms ($P = 0.06$). Three of the 10 subjects with very dry mouth in the betaine toothpaste group reported reduced oral dryness during the nighttime ($P = 0.06$) and a decreased need to drink water during daytime ($P = 0.06$). When all subjects with subjective dry mouth sensations were analyzed ($n = 13$), the results were similar; however, a statistically significant difference between the groups was detected only for the

Table 3. The effect of the use of the experimental toothpastes on microbiologic variables in subjects with stimulated saliva flow ≤ 0.6 mL/min ($n = 10$). B = base-line value, 2 wk = value after the experimental period. The values shown are means and standard deviations (*s*)

		Candida species		Mutans streptococci		Lactobacilli		Total anaerobes		Plaque index*	
		B	2 wk	B	2 wk	B	2 wk	B	2 wk	B	2 wk
Betaine toothpaste	Mean	3.7	3.9	4.8	4.7	5.2	5.1	7.2	7.3	1.1	1.1
	s	1.6	1.7	2.6	2.6	2.1	2.2	0.6	0.5	0.4	0.3
Placebo toothpaste	Mean	3.9	4.4	4.8	5.0	5.8	5.8	7.4	7.6	1.4	1.2
	s	0.8	0.9	2.6	2.7	1.2	1.1	0.5	0.3	0.4	0.2

* $n = 8$ (two of the subjects had full dentures).

question on relief of dry mouth sensations during the daytime ($P = 0.005$). Eight of the 10 with very dry mouth preferred the betaine toothpaste over the placebo toothpaste. The corresponding figure for all subjects was nine.

No study-induced significant changes were observed in the microbiologic variables in either the betaine or the placebo toothpaste group. The mean values of the microbiologic variables and plaque indexes of the dry mouth subjects are presented in Table 3.

Discussion

Betaine is a quaternary ammonium compound that does not possess any skin-irritating properties (14). It also reduces the skin-irritating properties of cosmetics in general and improves their skin compatibility and moisturizing properties (14, 15). Betaine is also shown to reduce the irritating properties of sodium lauryl sulfate in cosmetics (14). These properties make it a promising detergent-like ingredient of toothpastes as well. Sodium lauryl sulfate, the commonest detergent used in toothpaste, causes irritation of the oral mucosa especially in patients with dry mouth. Its use has also been connected with recurrent aphthous ulcers (8). Less irritating detergents have been actively searched for; for example, the dry mouth toothpaste (Biotene®) contains polyoxyethylene-20-isohexadecylether as the detergent. Recently, cocoamidopropyl-betaine, a zwitterionic detergent, was found to cause less mucosal irritation than sodium lauryl sulfate (13). A toothpaste for patients with dry mouth may thus contain a combination of betaine and sodium lauryl sulfate or some other detergent with a better compatibility with the oral mucosa. An optimal betaine-containing toothpaste for such patients may contain no detergents at all. The present results with a toothpaste containing 2% sodium lauryl sulfate, a concentration close to the highest concentrations generally used in toothpastes, and 4% betaine supports the above idea. Most of the dry mouth subjects in the present double-blind study preferred the betaine-containing toothpaste to the placebo toothpaste representing regular commercial fluoride toothpastes. The most important subjective symptoms of dry mouth, those experienced during the daytime, were also relieved during the use of the betaine-containing toothpaste. The improvement in the subjective symptoms of dry mouth were not reflected in the appearance of the oral mucosa or in the composition of the oral flora, but the 2-week study period may have been too short for such effects to appear.

According to the existing literature betaine does not appear to possess antibacterial activity against microorganisms. This seems to be true also of the oral bacteria *Streptococcus mutans*, *Actinomyces viscosus*, *Lactobacillus casei*, and *Candida albicans* (A. Le Bell et al., unpublished observations). On the other hand, some microbes are able to degrade betaine, and it is not accumulated in nature. Both aerobic and anaerobic microorganisms are known to degrade betaine (18, 19); no oral microbes have, however, been shown to degrade it. Our results for the dry mouth patients suggest that betaine, when used in the form of a toothpaste, has no effect on the levels of mutans streptococci, lactobacilli, or *Candida* species or on the amount of plaque. This finding is also supported by a study performed with 35 healthy adults, in which betaine was used as an ingredient of either lozenges or toothpaste. No changes in the oral flora or the wet weight of plaque were detected during the 2-week use of the betaine-containing test products (E. Söderling et al., unpublished observations).

In conclusion, betaine appears to be a promising ingredient of toothpastes in general—not only of toothpastes especially designed for patients with dry mouth but also for those who react to the mucosa-irritating effects of toothpaste components.

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