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Effect of a protease-containing tablet with rough surface on the number of bacteria on the tongue

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Although it is thought that protease-containing tablets with rough surfaces may be effective for cleaning the tongue surface, no studies have been reported. We investigated how the surface roughness and enzyme content of the tablets decreased the number of bacteria on the tongue. Four types of tablets were used : tablets comprised of several sugars with different solubilities with and without protease (actinidine), and tablets of one type of sugar, also with and without protease. The subjects were 20 healthy young adults and 20 elderly persons requiring support/nursing care. All gave informed consent. They used two tablets of each of the four types, and their efficacy at removing the tongue coating was evaluated employing a crossover study design.

The number of bacteria on the tongue was significantly decreased in the enzyme-containing tablets compared to the non-enzyme-containing group. When the surface property was considered, no significant difference was noted between the tablets prepared with several types of sugar and those prepared with a single type in either of the groups. It was suggested that the presence of protease is more effective at removing the tongue coating than was the rough surface formed by several sugars. (J Osaka Dent Univ 2015 ; 49 : 165–170)

Key words : Protease-containing tablet ; Bacteria ; Tongue

INTRODUCTION

Volatile sulfides cause physiological bad breath,[1] and volatile sulfides produced by bacterial putrefaction of the tongue coating account for 60% of those volatile sulfides. These sulfides are known as the main cause of physiological bad breath.[2] The prevention of aspiration pneumonia by oral cavity cleaning, including removal of the tongue coating, has been reported,[3] and coating removal is essential for not only young people conscious of bad breath, but also for weak, elderly people who are unable to practice proper oral hygiene.

Generally, the tongue coating is removed by mechanical cleaning using tooth and tongue brushes. Although these methods are effective,[4] it has been noted that they may cause trauma to the tongue mucosa and induce the vomiting reflex.[5, 6] For these reasons we examined chemical tongue coating-removal methods.

Many studies[7–10] have reported that protease is effective at removing the tongue coating, which is comprised of oral mucosa-derived desquamated epithelial cells and bacteria.[11–13] Yoshimatsu *et al.* reported that rough tablets containing a kiwi fruit-derived cysteine protease, actinidine, were effective at removing the tongue coating and preventing bad breath.[7] This tablet is comprised of three types of sugar with different solubilities. Because the surface becomes rough as the tablet is dissolved in the oral cavity, mechanical cleaning of the tongue coating is expected. However, no study has reported on how the surface properties of the tablet, and the presence or absence of an enzyme in the tablet, affect tongue cleansing.

In the elderly receiving support and nursing care, it has been reported that atrophy and reduction of movement of the lingual muscles occur in addition to a vertical drop in the laryngeal and hyoid bone posi-

tions as a result of aging and sarcopenia, leading to difficulty in pushing the dorsum of the tongue against the palate.[14, 15] Therefore, the elderly may not be able to roll tablets around with their tongue and obtain a mechanical cleansing effect through the roughness of the tablet. We investigated how the enzyme and surface properties of the tablets cleansed the dorsum of the tongue in elderly persons requiring support/nursing care, in whom the mechanical cleaning effect of tablets is likely to decrease. This group was compared with healthy young adults.

MATERIALS AND METHODS

Subjects

Since this study was conducted to investigate the effect of tablets on removing the tongue coating when dissolved in the mouth, we selected as the subjects 20 healthy young adults with tongue coating, 9 males and 11 females with a mean age of 30 ± 2 years (the young adult group) and 20 elderly persons requiring support/nursing care, 8 males and 12 females with a mean age of 71 ± 3 years (the elderly group). The elderly subjects, who required support/nursing care, were selected from two facilities to avoid the influence of their environment. Ten subjects were selected who had been admitted to an elderly care facility, and 10 subjects who visited a long-term care sanatorium-type geriatric health service facility. All subjects were performing oral care by themselves.

Subjects with sufficient consciousness and cognitive function levels to avoid erroneous swallowing of tablets were selected. To investigate the tongue-cleaning effect in daily living activities, the subjects continued usual tongue and oral cleaning habits until the morning of the experiment. The study was adequately explained to all subjects, and consent to participate in the study was obtained.

Study design

Crossover studies of the four types of tablet described above were performed in all subjects. The 40 subjects were randomly divided into four groups, each consisting of 10 subjects. Each group used one of the four tablet types described earlier. The number of oral bacteria was measured immediately before and after use of the tablet. After a washout period of one-week or longer, the same test was performed using a different type of tablet.

Tablets

The tablets were composed of the ingredients shown in Table 1. They had a diameter of 13 mm and a weight of 1.0 g.[7] The four types of tablets were prepared based on the presence or absence of protease (actinidine) and number of compounded sugars. One group contained the protease compounded with three sugars (enzyme +, rough +), one contained the protease compounded with one sugar (enzyme +, rough −), one contained no protease compounded with 3 sugars (enzyme −, rough +), and one contained protease compounded with one sugar (enzyme −, rough −). The subjects mechanically dissolved the tablets by rolling them on the dorsum of their tongue without chewing. Two tablets of the same

Table 1 Composition of the four types of tablets used

Component	Tablet type			
	enzyme +, rough +	enzyme +, rough −	enzyme −, rough +	enzyme −, rough −
Sorbitol	70%	80%	72%	82%
Erythritol	5%	−	5%	−
Reduced palatinose	5%	−	5%	−
Particulate silica	1%	1%	1%	1%
Other ingredients	17%	17%	17%	17%
Total	100%	100%	100%	100%

Enzyme : 50 U/g when converted into acid protease

type were used for each experiment, and the second tablet was used after the first tablet had dissolved. The time until the complete dissolution of the second tablet was measured. The safety of the tablets has been confirmed.[7]

Evaluation of the number of bacteria on the tongue

The number of bacteria on the tongue was measured using a simple oral bacteria measurement device, the Bacterial Counter (DU-AA 01 NP-h, Panasonic Healthcare, Tokyo, Japan). Samples were collected by scraping about 1 cm of the tongue from the intersection point of a line connecting the bilateral first molars and the median line of the tongue with a 20-gram force forward and backward five times using a sterile swab attached to the Bacterial Counter. To reduce technical errors, all bacterial samples were collected by only two investigators. The sample swabs were immersed in 5 mL of sterile distilled water attached to the device and applied to the Counter, which counts bacteria in sterile distilled water by electrophoresis.[16]

Statistical analysis

The numbers of bacteria on the tongue before and after use of the tablets and the rate of change were subjected to paired two-way layout analysis of variance using Fisher's least-significant difference method in all four of the tablet groups in the healthy young adult and elderly groups.

Ethical considerations

This study was performed after approval by the directors of the nursing facilities and Ethics Committees of Osaka Dental University (Approval number 110797). The objective of the study was sufficiently explained to the subjects orally and using documents. The subjects were informed that their participation was voluntarily, that non-enrollment would not affect their treatment in the facility, and that the study would be immediately discontinued if they decided to drop out, even after they had consented to cooperate. Consideration for the protection of personal information was explained, and the survey was performed after obtaining their consent.

Results

Before use of the tablets (baseline), as shown in Table 2, no significant difference was noted in the number of bacteria on the tongue among the four tablet groups either within the young adult or the elderly groups, consisting of 40 subjects in total. The order of administration of the four types of tablet was randomly assigned. A significant difference was noted between

Table 2 Effect of tablets on the number of bacteria on the tongue before tablet use in the clinical trials by analysis of variance

Factor	F value	P value	Significance
Number of bacteria on the tongue among the four tablet-based groups	0.53	0.662	−
Young adult and elderly group	13.09	0.0004	*
Reciprocal action	0.175	0.914	−

*$p < 0.01$

Table 3 Number of bacteria on the tongue before and after using each type of tablet analyzed by two-way layout analysis of variance using Fisher's least-significant difference method

Group	Enzyme	Rough	Before using tablet	After using tablet	Significance
Young adults (n = 20)	+	+	1.42 ± 0.22	0.29 ± 0.09	*
	+	−	1.54 ± 0.25	0.36 ± 0.08	*
	−	+	1.08 ± 0.20	0.42 ± 0.08	*
	−	−	1.32 ± 0.32	0.55 ± 0.10	*
Elderly persons requiring support/nursing care (n = 20)	+	+	2.67 ± 0.68	0.62 ± 0.25	*
	+	−	2.36 ± 0.37	0.61 ± 0.14	*
	−	+	2.18 ± 0.43	0.88 ± 0.20	*
	−	−	2.09 ± 0.38	0.77 ± 0.18	*

Mean number of bacteria ± SE ($\times 10^7$), *$p < 0.01$

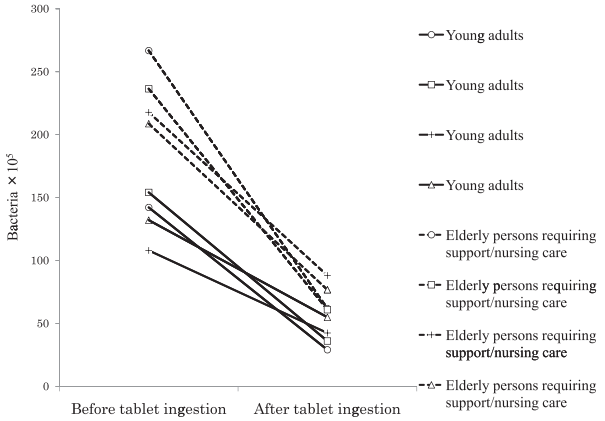

Fig. 1 Number of bacteria on the tongue before and after tablet use.

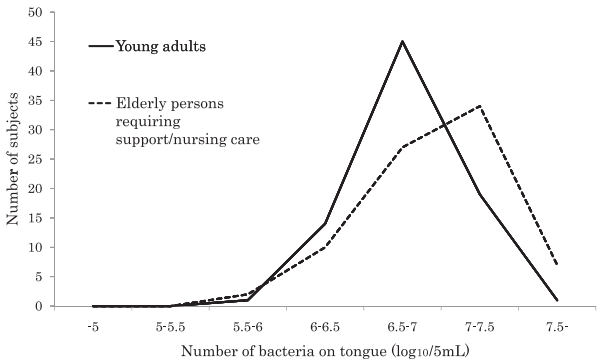

Fig. 2 Histogram of the number of bacteria on tongue before tablet use.

the young adult and elderly groups ($p < 0.01$).

Numbers of bacteria on the tongue before and after use of the tablets

As shown in Table 3, a significant decrease in bacteria was noted after using the tablet in each group ($p < 0.01$). The number of bacteria decreased in both the elderly and young adult groups, as shown in Fig. 1. The distribution of the number of bacteria on the tongue before the use of the tablets is shown in Fig. 2. The distribution of bacteria in the elderly was greater (shifted to the right) than in the young adult group.

The number of bacteria on the tongue after the use of tablets

Figure 3 shows the rate of decrease in the number of bacteria on the tongue after tablet use. The rates were

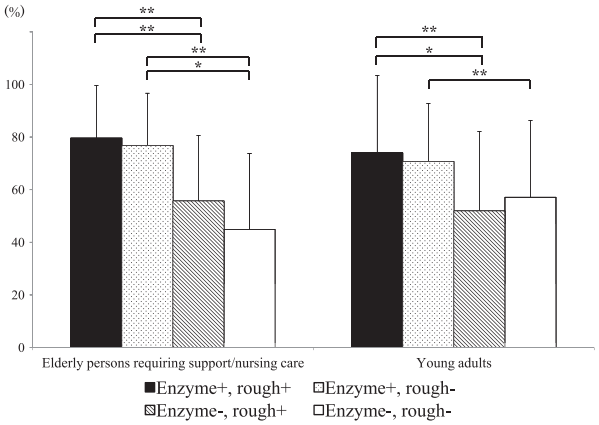

Fig. 3 Rate of decrease in the number of bacteria on the tongue after tablet use (**$p < 0.01$, *$p < 0.05$).

80%, 77%, 56% and 45% in the (enzyme +, rough +), (enzyme +, rough −), (enzyme −, rough +) and (enzyme −, rough −) groups, respectively, in the young adult group, and 74%, 70%, 52% and 57%, respectively, in the elderly group. This data shows a significant differences between the (enzyme +, the rough +) and (enzyme −, rough +), between the (enzyme +, rough +) and (enzyme −, rough −), between the (enzyme +, rough −) and (enzyme −, rough +), and between the (enzyme +, rough −) and (enzyme −, rough −) in the young adult group, and between the (enzyme +, rough +) and (enzyme −, rough +), between the (enzyme +, rough +) and (enzyme −, rough −), and between the (enzyme +, rough −) and (enzyme −, rough +) in the elderly group. No significant difference in the decrease in the number of bacteria was noted after use of each type of tablet between the young adult and elderly groups.

DISCUSSION

Methods

The tongue coating supports bacterial growth, similar to dental plaque, and is considered an oral biofilm.[6] Bacteria that grow on the dorsum of the tongue produce volatile sulfides through putrefaction, causing bad breath. Moreover, a high concentration of volatile sulfides may destroy periodontal tissue.[17] The tongue coating has been noted as a hotbed for bacteria causing aspiration pneumonia,[18] indicating the importance of its removal in elderly requiring nursing care.

The tongue coating is comprised of desquamated epithelial cells and proteins, mainly components of Streptococcus, Staphylococcus, and white blood cells.[13] It has been clarified that the coating can be removed by chemical cleaning with protease and mechanical cleaning with the rough surface of protease-containing tablets.[7–10] However, it has not been investigated which is better. By compounding several sugars with different solubilities in the tablets, the surface roughness can be increased when the tablet is dissolved in saliva.

The level of roughness is lower when only one type of sugar is compounded, and the surface area is smaller.[19] Thus, we investigated the effect of roughness by changing the number of sugars compounded. Sugars are dissolved by saliva and by being manipulated with the tongue. This effect increases when the tablet is rolled around by the tongue. To investigate the influence of the tongue on the mechanical cleansing by the tablets, two groups, elderly requiring nursing care and young adults, were involved in the study.

Removal of the tongue coating has been evaluated by measuring the number of bacteria on the tongue and by visual evaluation of adhering tongue-coating bacteria using culture techniques.[7,20] Because visual evaluation lacks objectivity, we used a simple oral bacteria measurement device, the Bacterial Counter. Because it is capable of scraping the tongue with a pressure of 20 g using an attached apparatus, operator differences are minimized.

A strong correlation between bacterial counts measured by the counter and the culture method has been reported.[16] In addition, the device is portable, and the number of bacteria can be immediately measured by a simple procedure. In this study, the elderly subjects were utilizing nursing care facilities, and the bacteria were counted at the facilities. For these reasons, we chose the Bacterial Counter as the measurement device.

RESULTS

As shown in Table 2, the number of bacteria on the tongue was significantly different between the young adult and elderly groups before the use of tablets. More tongue coating was observed in the elderly than in the young adults prior to the study, the same as reported by Konda *et al.*[21] The distribution of the number of bacteria on the tongue before use of the tablets is shown in Fig. 2. Similar to the findings reported by Tashiro *et al.*[16] the distribution peak moved to the right in the elderly, indicating a higher level of bacteria. However, compared to the study reported by Tashiro *et al.,* the number of bacteria on the tongue in the elderly group was low. This may have been caused by the inclusion of subjects with only a mild decline in physical function and little need of nursing care when visiting the nursing care facility.

The number of bacteria on the tongue significantly decreased after use of each type of tablet, clarifying that cleansing of the tongue coating can be attained by only the mechanical stimulation of rolling the tablets on the tongue for 15 minutes. The (enzyme +, rough +) and (enzyme +, rough −) groups correspond to the protease-containing tablet with rough surface and the placebo groups, respectively, in the study reported by Yoshimatsu *et al.* The significant differences between the groups and changes that we observed were consistent with those in their study.[7] However, the rate of the tablet-induced decrease in the number of bacteria was significantly greater in the enzyme than in the non-enzyme group, suggesting that the presence of the enzyme is more effective than the rough surface properties in decreasing the bacterial count. Although the bacterial count before tablet use was significantly different between the groups, the rate of decrease after tablet use was not significantly different, suggesting that the tablet shape was similarly effective for the elderly group.

The biochemical method of cleansing the tongue using protease-containing tablets with a rough surface is preferable because it is simpler than mechanical tongue-cleaning methods using tools and less likely to cause adverse mechanical stimulation. In addition, its simplicity and chemical effect help promote mechanical cleansing when used by patients with reduced physical function, such as frail elderly.

We evaluated the effects of the presence of an enzyme and the surface properties of tablets on cleansing of the dorsum of the tongue in young adults and in

elderly requiring nursing care. We found that the presence of enzymes is more effective than rough surface properties in removing the tongue coating, and that a combination of the two maximizes the effect.

A summary of this paper was presented at the Chugoku, Shikoku and Kansai branches of the Japanese Society of Prosthetics on September 6, 2014, in Kurashiki, Japan.

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