Pilot Study to Assess the Potential of New Moisturizing Agents for Oral Dryness

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Abstract

Background/Purpose: Products to relieve symptoms of xerostomia have mainly been targeted at moisturizing the oral cavity. However, comprehensive treatment should aim at the following three goals: 1) promoting saliva secretion; 2) maintaining a moisturizing effect; and 3) cleaning the oral cavity. We examined the effects of a new mouthwash in terms of these three effects.

Materials and Methods: Effects of the mouthwash or water on saliva secretion were compared using the Saxon test in 8 adults with below-average saliva secretion. In terms of moisturizing effects, the cell survival rate was calculated after cells were processed with or without phosphate-buffered saline, followed by drying. Cleaning effects were examined using glass slides coated with vegetable oil, lard and flour residues to simulate accumulated food residues, placed into test tubes with 20ml of mouthwash or water, mechanically agitated for 30s, and then dried. Slide weight after washing was divided by that before washing, and ratios were compared between water, the new mouthwash and a commercial mouthwash.

Results: Saliva secretion was significantly higher with the new mouthwash (5.62 ± 2.07 g/2min) than with water (4.88 ± 1.97 g/2min; p=0.0051). Viable cell counts after drying were significantly better with the new mouthwash (93.5 ± 7.5%) than without (67.6 ± 8.9%; p<0.001). Cleaning rate was significantly higher with the new mouthwash (68.9 ± 2.58%) than with water (21.1 ± 3.41%; p<0.001), and was not significantly different from that with the commercial mouthwash (66.3 ± 5.97%).

Conclusion: The components of this new mouthwash appear to promote saliva secretion, and have moisturizing and cleaning effects, suggesting potential as an effective mouthwash.

Keywords:
xerostomia, mouthwashes, saliva secretion, moisturizing effect, cleaning effect

Introduction

Saliva is known to play important roles in maintaining oral health through antibacterial activity, natural purification and mucosal protective effects. Nevertheless, complaints about oral dryness and xerostomia have become increasingly common in recent years, and these symptoms are frequently seen in the elderly (1–3). In addition, some studies have indicated that oral dryness is caused by decreases in saliva production, adversely affecting oral health and function (4–7). Some methods used for xerostomia include administration of artificial saliva and the use of pharmacological agents to improve saliva secretion (8, 9). However, results in those studies have indicated that either patients with xerostomia experienced little benefit, or the duration of administration was insufficient to remedy oral dryness.

In recent years, several varieties of oral moisturizing gel to relieve oral dryness have gradually come into use (10, 11). These moisturizing gels are easier to retain in the oral cavity than artificial saliva, and such gels can easily be used...
by a caregiver or by elderly individuals themselves. These oral moisturizing products have therefore been made available to nursing-care facilities and for at-home care. Some studies have shown that oral moisturizing gels for xerostomia offer evanescent advantages for xerostomia caused by preoperative radiotherapy or Sjögren’s syndrome (12–14).

Moisturizing gels have various effects, supplying moisture within the oral cavity, wherein the moisturizing components retained in the oral cavity are used to relieve the symptoms of xerostomia. This type of product used to relieve symptoms of xerostomia was developed to moisturize the inside of the oral cavity. However, simply moisturizing the oral cavity is insufficient for xerostomia care. A comprehensive approach should aim to achieve the following three goals:

1) increase salivation to supply moisturizing effects (promoting saliva secretion);
2) maintain moisture in the oral cavity (moisturizing effect); and
3) clean the oral cavity (cleaning effect).

However, at present no moisturizing gels simultaneously promote saliva secretion, or offer moisturizing and cleaning effects.

We therefore aimed to develop a new mouthwash with these effects. The present study was undertaken to clarify the various effects of the components and their characteristics.

**Materials and Methods**

*New mouthwash*

The new mouthwash tested in this study was based on MONDAHMIN®, a non-alcohol (Earth Chemical, Tokyo, Japan) with the addition of polyoxyethylene cetyl ether (CETETH-25), ethylene diamine tetra-acetic acid disodium (EDTA-2Na salt) and cetylpyridinium chloride (CPC). MONDAHMIN® is well known to show high detergency and strong sterilizing properties (15), and is a popular, commercially available sterile cleaning agent in Japan. The new mouthwash also includes seaweed extract to promote saliva secretion and betaine for moisturizing effects. In addition, these materials were extracted from natural seaweed, but information on the density of these additives is confidential. However, the combination ratio of each ingredient is CPC: EDTA-2Na:CETETH-25 = 1:2:20.

**Judging effects in promoting saliva secretion**

In a preliminary experiment, we selected 8 adults (5 males, 3 females; mean age, 45.7 ± 12 years) with less saliva secretion under stimulated conditions than the average adult. Subjects ate a meal 1 h prior to commencement of the experiment, and then fasted. In addition, after entering the laboratory, after 15 min of rest, subjects left 20 ml of test solution or water (distilled water) in their mouth, gargled for 30 s, spat out the contents, then rested for another 30 min. Saliva secretion under stimulation (Saxon test) was then measured. The same experiment was also conducted on another day, with subjects blinded to the solution used (0.1% seaweed extract water solution or water).

For the Saxon test, a piece of gauze (30 cm × 30 cm, sterile; Hakujuji, Tokyo, Japan) was left in the mouth, and the subject was requested to perform chewing movements at a frequency of once per second for 2 min. The weight of the gauze was then measured using an electronic balance (BL-320S®; Shimadzu, Kyoto, Japan), and that value was subtracted from the weight of the gauze prior to placement in the mouth to calculate saliva secretion in grams per 2 min (16).

The present work was conducted in accordance with the Declaration of Helsinki. This study was approved by the ethics committee at Nihon University School of Dentistry at Matsudo (EC 14-040).

**Judging moisturizing effects**

Moisturizing effects were evaluated using cultured cells according to the methods described by Mori et al. (17) and Morio et al. (18). First, the Ca9–22 human gingival squamous carcinoma cell line (Japanese Collection of Research Bioreresources Cell Bank) was cultured with Eagle’s minimum essential medium (Wako Pure Chemical Industries, Osaka, Japan), 10% fetal bovine serum (Gibco, Waltham, MA) and 0.5% antibiotics (penicillin-streptomycin solution; Wako Pure Chemical Industries) at 37 °C and under 5% CO2. Cells were cultured in a 96-well plate for 2 days until confluence.

After removing the medium, cells were washed using phosphate-buffered saline (PBS), and incubated for 15 min in 100 μl of test solution contain moisturizing ingredients or PBS alone as a control. These solutions were aspirated, and cells were left to dry for 10 min at room temperature (temperature, 30°C; relative humidity, 45%). Next, 100 μl of the above medium was added and 10 μl of Cell Counting Kit-
8 (Dojindo Laboratories, Kumamoto, Japan) was added to the well after the drying process, which was then left to stand for 3 h at 37°C and under 5% CO₂. A microplate reader (Multiskan GO; Thermo Fisher Scientific, Waltham, MA) was used to measure the cell survival rates by absorbance at 450 nm. The survival rate was calculated relative to an assumed survival rate of 100% with PBS, using the following formula: cell survival rate = absorbance of the treated group/ absorbance of the PBS group.

**Judging cleaning effects**

Simulated food stains produced from carbohydrates and fats were used to evaluate the wash effect of the new mouthwash. Glass slides (18 mm × 42 mm) were coated with food materials including 30% vegetable oils, 20% lard, 50% flour to simulate build-up in the oral cavity, then dried for use as test boards. Test boards were placed into 50 ml plastic test tubes with 20 ml of each type of liquid (new mouthwash, water, commercially available mouthwash (MONDAHMIN® non-alcohol), and agitated mechanically (BW10L, Yamato Scientific, Tokyo, Japan) for 30 s. The test board was then removed, gently washed in pure water, and weighed after drying for 1 h. The weight of the test board after shaking and drying was divided by the weight of the test board before placement in the test tube to calculate the cleaning rate, reflecting the cleaning effect.

**Statistical analyses**

Results are expressed as means ± standard deviation. Paired t-test was performed to test for saliva secretion with the test agent and purified water. One-way analysis of variance (ANOVA) and the Bonferroni method was performed to test for cell numbers and cell survival rates with the test agent and purified water. For cleaning rate, one-way ANOVA and the Tukey method were performed to compare results between water, commercially available cleaning agent, and test agent.

These analyses were performed using SPSS Statistics version 20 software (IBM Japan, Tokyo, Japan). Values of p < 0.05 were considered statistically significant.

**Results**

**Promotion of saliva secretion**

Saliva secretion was significantly higher with the new mouthwash (5.62 ± 2.07 g/2 min) than in the control group with purified water (4.88 ± 1.97 g/2 min; p = 0.0051) (Table 1).

**Moisturizing effects**

In terms of cell survival rate, viable cell counts after drying were significantly better with the new mouthwash containing moisturizing ingredients (sodium hyaluronate, betaine) (93.5 ± 7.5%) than without (67.6 ± 8.9%; p < 0.001) (Table 2).
Cleaning effects

Cleaning rate with the new mouthwash was 68.9 ± 2.58%, significantly higher than the 21.1 ± 3.41% with water (p < 0.001). This effect was comparable to that of the commercial mouthwash (66.3 ± 5.97%) (Table 3).

Discussion

In order to achieve comprehensive treatment of xerostomia, this study developed the following three concepts for a new mouthwash: 1) promote saliva secretion to increase the wetting effect (saliva secretion effect); 2) maintain the moisturizing effect in the oral cavity (moisturizing effect); and 3) remove food residue from the oral cavity (cleaning effect). The experimental results showed that the new mouthwash benefits saliva secretion, moisturizing and cleaning, combining to offer a new type of mouthwash.

Promotion of saliva secretion

Due to the reduced amount of saliva and reduced saliva self-purification function, causing the emergence of various diseases, promotion of saliva secretion can be considered the ideal countermeasure to xerostomia. To improve saliva flow, drugs used to promote saliva secretion have contained cevimeline hydrochloride hydrate (19) and/or pilocarpine hydrochloride hydrate (20). Cevimeline hydrochloride works by directly stimulating muscarinic M3 receptors in the acinar cells of the salivary glands, thus stimulating the secretion of serous saliva (21). However, while the efficacy of cevimeline has been demonstrated, side effects such as nausea and vomiting have been reported (22). This drug treatment is thus not necessarily effective for everyone.

We noticed that the “umami” taste component could promote saliva secretion in a form that can be simply and easily applied to anyone. We all know that taste and saliva secretion are associated. Reports have indicated that of the five basic tastes (sweet, bitter, sour, salty, and umami), umami is the best in promoting saliva secretion effects (23). Glutaminic acid, inosinic acid, and guanylic acid are taste ingredients that provide the umami taste. Sasano et al. also reported that palpitation, sweating, nausea, diarrhea and dizziness have all been observed in elderly patients taking parasympathomimetic drugs. To circumvent this problem, glutamate, which produces umami taste, was demonstrated to increase salivary secretion and thereby improve hypoguesia by enhancing the gustatory-salivary reflex (23).

We found that makonbu, which has been used since ancient Japanese times to refine broth, contains very high levels of umami components. The components of various seaweed extracts can be used as food material, and in particular, the high salivation promotion effect of makonbu-extract is well known in the Japanese food known as washoku. Our results showed that, when compared with water, the new mouthwash containing seaweed extract had marked effects in promoting saliva secretion.

Moisturizing effects

Glycerin and cellulose derivatives are often used to moisturize the inside of the oral cavity. These derivatives can be applied as liquids with sticky characteristics to increase the retention time in the oral cavity (24). Apart from such moisturizing effects, the aim should be to include components that protect and reduce irritation of the oral mucosa. We noticed that betaine (trimethylglycine), a natural substance derived from beet, has very good water retention characteristics. At the same time, this substance can protect the oral mucosa from irritation (25). However, betaine lacks the quality of stickiness, and is thus poorly remained in the oral cavity. To allow betaine to remain in the oral cavity, addition of sodium hyaluronate to the test solution improves retention and the moisturizing effect (26). In this study, we used a human oral squamous cell carcinoma cell line (Ca9–22), in accordance with the method.

| Table 3. Cleaning rate treated test mouthwash |
|---------------------------------------------|
| Water                                      |
| mean | SD  | commercially available cleaning agent | mean | SD  | Test agent | mean | SD  | F(2,6) | P            |
| 21.1  | 3.41| 66.25  | 5.97 | 68.89 | 2.58 | 121.18 | <0.001 |
| Multiple comparison                        |
| water<test agent, commercially available cleaning agent |
of Mori et al. Ca9-22 reflects intraoral mucosa, as it was derived from human epithelial cells. We found that viable cell counts after drying were significantly better with the new mouthwash containing moisturizing ingredients (sodium hyaluronate, betaine) than without.

Our preparation was thus formulated using betaine and sodium hyaluronate with the expectation of improved moisturizing and protective effects.

Cleaning effects

Due to the reduced saliva secretion in xerostomia, fluidity in the oral cavity is reduced, causing the inside of the oral cavity to become prone to the accumulation of food residue, peeling of the epithelium, and pro-inflammatory depositions (27). In the present study, the cleaning effects of the new mouthwash were evaluated using a simulation made of vegetable oils, lard, flour and other food sources. The resulting residue was not easily removed using water, but the new mouthwash showed much better cleaning effects, suggesting increased efficacy for cleaning the oral cavity. The new mouthwash was based on MONDAHMIN®, which is currently on the market in Japan, with the addition of polyoxyethylene cetyl ether (CELUETH-25), ethylene diamine tetra-acetic acid disodium (EDTA-2Na salt) and cetlypyridinium chloride. The active effect of CELUETH-25 is to remove oils from the oral cavity (28), while the EDTA-2Na salt acts to enhance the cleaning effect. Moreover, cetlypyridinium chloride has both a sterilization effect and a decay prevention effect (29). In combination, this provides disinfection and antiseptics (30). The new mouthwash also included seaweed extract to promote saliva secretion and betaine for moisturizing effects. Table 3 shows that the detergency of the new mouthwash was comparable to that of the currently marketed mouthwash. None of these ingredients have problems in terms of safety or adverse effects. From the above results, our cleaning test results appear mainly due to the CELUETH-25, with EDTA-2Na providing supporting effects.

Overall effects

The results indicate that this new mouthwash provides very good saliva secretion, moisturizing and cleaning effects. Unlike the low-level, traditional oral function moisturizers focused on improving “washing” and “moisturizing”, this mouthwash facilitates recovery of the original oral function through the promotion of saliva secretion. This represents a major characteristic of the new mouthwash. If symptoms can be alleviated in the elderly and individuals prone to xerostomia, this mouthwash will provide a great benefit in maintaining or improving the oral environment.

One of the limitations of this study is that we were unable to use various controls. In the future, actual use of the new mouthwash on patients with xerostomia, young individuals and the elderly will be investigated in order to validate the present findings.

Conclusion

The components of this new mouthwash promote saliva secretion, and have moisturizing and cleaning effects, suggesting its promise as an effective mouthwash.

Acknowledgments

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Conflicts of interest

The authors have no conflicts of interest to declare.

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