Effect of a Mouthwash Containing Cetylpyridinium and Zinc Chloride on Oral Malodor

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The aim of this study is to clinically evaluate the effect of a mouthwash containing cetylpyridinium and zinc chloride on reduction of morning oral malodor in healthy subjects measured by organoleptic measurement and a portable sulfide monitor.

A total of 8 healthy non-smoking male volunteers were enrolled in this study of crossover design consisting of two experimental phases. The subjects were instructed to rinse the mouth with 10 mL of the experimental mouthwash containing cetylpyridinium and zinc chloride for 30 seconds at the first phase. At the second phase after a one-week washout period, each subject rinsed with distilled water as a control.

All experiments were conducted at around 8:30 a.m. and oral malodor was measured using organoleptic measurement and a portable sulfide monitor just before rinsing with the experimental mouthwash or control (baseline), 1 hr, 2 hrs, and 3 hrs after rinsing.

The mouthwash containing cetylpyridinium and zinc chloride reduced morning oral malodor up to 3 hrs after rinsing. Organoleptic score and concentrations of volatile sulfur compounds after use of the experimental mouthwash significantly decreased with time, and the decreases were significantly different between the mouthwash and control. In conclusion, the mouthwash containing cetylpyridinium and zinc chloride is significantly effective on reduction of morning oral malodor in healthy subjects by 3 hrs.

Key words: Cetylpyridinium; Mouthwash; Oral malodor; Volatile sulfur compounds; Zinc chloride

Ⅰ. INTRODUCTION

Oral malodor is the offensive odor of breath originating from the oral cavity itself or its neighboring structures. Oral malodor is a very common problem, complained by up to 50% of the adult population, and is known to be the result of oral causes in about 80 to 90% of cases.[1-4] It is well documented that volatile sulfur compounds (VSCs) such as hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH), and dimethyl sulfide ($(CH_3)_2S$) are the major malodorous components of oral malodor,[5] while the role of other gases seem insignificant.[6] These VSCs are produced by the action of oral bacteria in breaking down the organic substances such as epithelial cells, salivary and serum proteins, and food debris.[7,8]

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Based on such information, the treatment of oral malodor is directed in two ways, the mechanical and the chemical methods. The mechanical methods including tooth brushing, dental flossing, and tongue cleaning reduce oral malodor by cleaning mechanically the bacteria and the organic substances involved in production of VSCs. Because those are the traditional methods for oral health, if practiced properly, side effects are rare. The chemical method is use of chemical agents in the form of mouthwash or spray, etc. containing antibacterial or anti-malodor chemicals. The mechanical and chemical methods can be used in an independent or complementary way.[1,9]

Because many people complaining of oral malodor are already using mechanical methods in a daily basis, they usually want chemical methods for additional use. So there are increasing demands of chemical agents that are effective on oral malodor and safe for long-term use.[10-13] There have been several chemical agents that are introduced to be effective for reducing oral malodor.

Cetylpyridinium and zinc chloride have been shown to react chemically with VSCs and also have antimicrobial activity while no evident side effects have been reported.[14,15] The aim of this study was to clinically evaluate the effect of a mouthwash containing cetylpyridinium and zinc chloride on reduction of morning oral malodor in healthy subjects measured by organoleptic measurement and a portable sulfide monitor.

II. MATERIALS AND METHODS

1. Subjects

A total of 8 healthy non-smoking male volunteers (38.4 ± 12.7) were enrolled in this crossover study. Subjects who had any systemic disease, evident periodontal problem, undergoing antibiotic or antimicrobial therapy were excluded. The subjects received written and verbal explanation about the study and signed on a written consent form to participate in. We excluded female subjects since the menstrual cycle is known to affect oral malodor status.[16,17] International Review Board at Seoul National University Dental Hospital approved this clinical study (Approval No. CRI11031).

2. Study design

This study was conducted in terms of crossover design. The subjects were instructed to rinse the mouth with 10 mL of the experimental mouthwash for 30 seconds at the first phase. After a one-week washout period, the subjects rinsed the mouth with 10 mL of distilled water as a control at the second phase. The experimental mouthwash was a commercially available product (Halicare-G, H-Bio co., Seoul, Korea), which containing 0.025% cetylpyridinium chloride, 0.22% zinc chloride, glycerin, mint oil, and citric acid.

3. Oral malodor measurement

Subjects were instructed to abstain from strong-smelling foods such as garlic, onion, scented cosmetics, and alcohol at least for 24 hrs before the measurements. The breakfast and oral hygiene practice in the very morning of the experiment day.[18] Oral malodor was measured at around 8:30 a.m. just before rinsing with the experimental mouthwash or distilled water (baseline), 1 hr, 2 hr, and 3 hrs after rinsing. Oral malodor was measured by organoleptic method as described previously[19,20] and a portable sulfide monitor (Halimeter®, Model RH-17K, Interscan, Chatsworth, CA. USA).

1) Organoleptic measurement

The level of oral malodor was assessed organoleptically as described previously.[19,20] The subjects were instructed to close their mouths for 3 minutes in an upright sitting position prior to each sample collection. For this process, we used the “negative pressure method,” which entraps mouth air rapidly and minimizes the loss of highly volatile components. To accomplish this, we used a gastight

syringe with a 6-cm-long and 3-mm- diameter polytetrafluoroethylene (PTFE) tube instead of a syringe needle. The plunger was pulled to the 10-mL position with the valve closed to create a vacuum state inside the barrel. The PTFE tube was located in the intraoral area, 4 cm from the mandibular anterior teeth and 1 cm above the dorsal tongue surface. The mouth air was collected into the syringe barrel by opening and closing the valve. During sample collection, the subjects were instructed to hold their breath to avoid lung air interruption.

A disposable paper cup was used to perform the organoleptic test. A small hole was made at the base of the cup to insert a disposable 6.5-mm plastic straw. A thin wax film was used to seal the connection. For a more precise organoleptic test, one examiner placed the cup over his nose, and another examiner expelled the sample through the plastic tube into the cup. Two trained judges evaluated the collected air. When the two judges gave different scores, a mean score was used.

The organoleptic rating scale previously described by De Boever and Loesche was applied.[21)]

0 = no appreciable odor
1 = barely noticeable odor that is of low intensity and within acceptable limits
2 = slight to moderate odor that is clearly noticeable and slightly unpleasant
3 = moderate to high odor that is clearly noticeable, unpleasant, and of moderate intensity
4 = offensive odor of strong intensity

The inter-examiner reliability, using Cohen's kappa test, was 0.4.

2) Portable sulfide monitor

The intraoral concentration of VSC was measured in terms of a portable sulfide monitor (Halimeter®, Model RH-17K, Interscan, Chatsworth, CA. USA) as the instruction of manufacturer. A disposable 6.5-mm plastic straw was attached to the air inlet of the monitor. Each patient was asked to close their mouth for 3 minutes before measurements and then instructed to open their mouth slightly so the straw could be inserted 4 cm into the oral cavity. The subjects breathed through his/her nose during the measurement. The peak levels measured were determined in parts per billion (ppb) sulfide equivalents by direct readings from the analog scale of the monitor. Three consecutive measurements were done and the result was calculated as a mean of the 3 measurements.

4. Statistical analysis

The differences between the two mouthwashes were compared with a Student's t-test. The differences between before and after rinsing at each examination point were analyzed with paired t-tests. To detect significant differences of malodor changes, repeated-measures ANOVA was applied. The inter-examiner reliability was analyzed with Cohen's kappa test. For all analyses, a 5% significance level was used.

Ⅲ. RESULTS

1. Organoleptic measurement

Changes of means and standard deviations of organoleptic scores are presented in Table 1. At baseline, the mean score in experimental mouthwash group was 1.6, and the mean score in distilled water

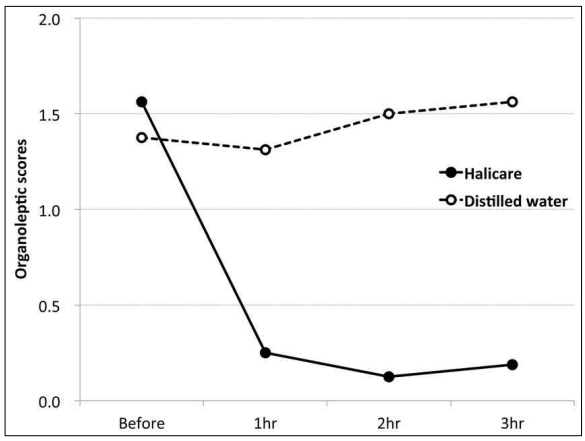

Fig. 1. Changes of the organoleptic scores.

Table 1. Mean changes of the organoleptic scores

Organoleptic score (Mean ± S.D.)	Before	1hr	2 hrs	3 hrs
Halicare	1.6 ± 0.7	0.3 ± 0.4**	0.1 ± 0.2***	0.2 ± 0.4***
Distilled water	1.4 ± 0.8	1.3 ± 0.6	1.5 ± 0.5	1.6 ± 0.9

S.D., standard deviation.
**: p<0.01, ***: p<0.001, significant difference in comparison with the baseline (before) value by paired t-test.

group was 1.4. There was no statistically significant difference between the two baselines. In experimental mouthwash group, the mean of organoleptic scores fell to 0.3, 0.1, and 0.2 at 1 hr, 2 hrs, and 3 hrs post-rinsing, respectively. Statistically significant decreases in oral malodor compared with baseline score were evident by 3 hrs. In distilled water group, on the other hand, the score dropped to 1.3 at 1hr post-rinsing but it increased thereafter to 1.5 and 1.6 at 2 hrs and 3 hrs post-rising, respectively (Fig. 1). The scores in experimental mouthwash group were significantly lower than those in distilled water group at 3 hrs post-rinsing (p<0.01). In the repeated-measures ANOVA analysis results, the concentrations in experimental group significantly decreased with time, and the decreases were significantly different between the groups (Table 3).

2. Portable sulfide monitor measurement

Changes of means and standard deviations of VSC concentrations are presented in Table 2. At baseline, the mean concentration in experimental mouthwash group was 239.3 ppb, and the mean concentration in distilled water group was 209.5 ppb. There was no statistically significant difference between the two baselines. In experimental mouthwash group, the mean concentration fell to 55.3, 59.0, and 66.3 ppb at 1 hr, 2 hrs, and 3 hrs post-rinsing, respectively. Statistically significant decreases in oral malodor compared with baseline concentration were evident by 3 hrs. In distilled

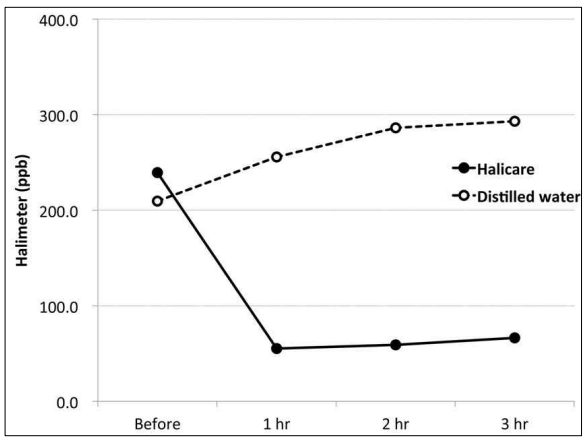

Fig. 2. Changes of the VSC concentrations.

Table 2. Mean changes of the VSC concentrations

Halimeter (ppb) (Mean ± S.D.)	Before	1 hr	2 hr	3 hr
Halicare	239.3 ± 99.6	55.3 ± 14.3**	59.0 ± 13.2**	66.3 ± 18.6**
Distilled water	209.5 ± 105.0	255.8 ± 138.8*	286.3 ± 138.9*	293.1 ± 138.0*

S.D., standard deviation.
*:p<0.05, **:p<0.01, significant difference in comparison with the baseline (before) value by paired t-test.

Table 3. Time and group effect on the organoleptic scores and VSC concentrations

P values	Time	Group	Time by Group
Organoleptic score	0.000	0.004	0.000
Halimeter	0.002	0.004	0.000

Differences were analyzed by repeated-measures ANOVA.

water group, on the other hand, the concentration increased thereafter to 255.8, 286.3, and 293.1 ppb at 1hr, 2 hrs, and 3 hrs post-rising, respectively (Fig. 2). The concentrations in experimental mouthwash group were significantly lower than those in distilled water group at 1hr, 2 hrs, and 3 hrs post-rising ($p<0.05$, $p<0.001$, $p<0.01$, respectively). In the repeated-measures ANOVA analysis results, the concentrations in experimental group significantly decreased with time, and the decreases were significantly different between the groups (Table 3).

Ⅳ. DISCUSSION

In this explorative trial, we compared two mouthwashes; one with cetylpyridinium and zinc chloride, and the other with only distilled water, to assess the effectiveness of a formulation of anti-malodor agent with cetylpyridinium and zinc chloride compared with a negative control. The relative effectiveness in comparison with other formulation of anti-malodor agents was not analyzed in this study. The results of this study show that a mouthwash with cetylpyridinium and zinc chloride significantly lowers the organoleptic scores and concentrations of VSCs and that such anti-VSC effects lasted up to 3 hrs after rinsing. Previous studies have showed that the quaternary ammonium compound, cetylpyridinium chloride inhibits bacterial growth and the accumulation of plaque,[15,22] and reduces VSC level.[14,23-26] The anti-malodor effects of cetylpyridinium chloride seemed less than other potent anti-malodor solutions such as chlorhexidine, zinc chloride and chlorine dioxide. However, compared to other substances, cetylpyridinium chloride showed no side effect even after long-term use.[15] Interestingly, reports showed that cetylpyridinium chloride and zinc mouthrinse had a significant synergistic effect on the reduction of VSC concentration.[27]

On the other hand, zinc chloride has been well known as a potent anti-malodor agent.[14,26,28-30] But it has a puckery taste at higher concentrations showing a better halitosis reducing effect. Concentration of 0.1% level was found acceptable, but showed only minor effects. The unpleasant-metallic taste may be overcome by mixing with other ingredients. In this study, the experimental solution contained 0.25 % zinc chloride but its metallic taste was not obvious due to the mint oil flavor added in the product.

The zinc ion is assumed that it oxidizes thiol groups in the sulfur-containing precursors of VSCs and converts them into non-volatile substances due to their affinity to sulfur[31,32]. Cupric and stannous ions have more anti-VSC effects than zinc ion, but they are known to cause discoloration of teeth. The antibacterial properties of zinc ions is known as bacteriostatic and may be less related to the anti-VSC effects.[33,34]

Cetylpyridinium chloride and zinc chloride have a different mode of action for reduction of oral malodor and this may provide a synergistic co- action. The most common sources of protein for malodor production include salivary mucins and epithelial cell components, both of which contain numerous glycoproteins.[35,36] Glycoprotein proteolysis depends on the initial removal of the carbohydrate side chains.[37]

Sterer et al. suggested that oral malodor production involves two stages:[38]

1) Deglycosylation of glycoproteins by Gram-positive bacteria in outer layers of biofilm.
2) Proteolysis and amino acid utilization of the protein core to yield VSCs by Gram-negative bacteria in the deeper layers of the biofilm.

This suggestions explain how physiologic malodor could develop and avoiding food could worsen physiologic malodor, especially in the morning.[36] Because deglycosylation of glycoproteins decreases in a situation that there are abundant glucoses in the oral cavity, cetylpyridinium chloride might play a role in the first stage by inhibiting bacterial growth and the accumulation of biofilm. Zinc chloride might take part in the second stage by inhibiting amino acid utilization through oxidizing thiol groups of amino acids.

In this study, we evaluated morning breath, which is known as the worst status of physiologic malodor. While pathologic malodor can be easily controlled by various methods such as tooth brushing, tongue scraping, and periodontal therapy, the control of physiologic malodor is more sophisticated and requires more than routine oral hygiene.[39] Cetylpyridinium chloride and zinc chloride reduced physiologic morning oral malodor by 3 hrs. It might have been the result of their synergistic co-action. Moreover, cetylpyridinium chloride and zinc chloride, both have minimal side effects after long-term use. This experimental solution is very promising as a chemical agent to manage oral malodor in the morning on a daily usage protocol, as well as pathologic oral malodor. The limitation of this study design is using only negative control in terms of distilled water without positive control and not including pathologic malodor group and female group. Henceforth, further research to evaluate relative effectiveness in comparison with other anti-malodor agents in more various subjects is necessary.

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국문초록

세틸피리디늄(Cetylpyridinium) 및 염화아연(Zinc chloride)을 함유한 구강 양치액의 구취제거 효과에 대한 연구

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전통적으로 항구취 제제로 널리 쓰여 온 세틸피리디늄 및 염화아연이 함유된 양치액은 장기간의 사용에도 부작용이 없이 구취를 효과적으로 감소시키는 것으로 알려져 있다. 본 연구에서는 세틸피리디늄 및 염화아연을 주성분으로 한 구강 양치액의 아침 구취에 대한 효과를 관찰하고자 했다. 총 8명의 건강한 남자를 대상으로 crossover study를 진행했으며 1주일의 washout 기간을 두었다. 대상자들에게는 30초간 실험 용액 10 mL를 양치하게 했으며 1주일 후에 같은 방법으로 증류수를 양치하게 했다. 구취의 측정은 오전 8:30 분에 이루어졌으며 양치 전, 양치 후 1시간, 2시간, 3시간에 평가를 하였다. 구취의 평가는 관능구취검사와 이동성 황화합물 측정기(Halimeter)를 이용하였다. 세틸피리디늄과 염화아연을 함유한 구강 양치액은 양치 후 3시간까지 아침 구취를 감소시켰다. 관능구취검사 수치와 휘발성 황화합물의 농도는 실험군에서 시간에 따라 유의한 감소를 보였고 그 감소 양상은 두 군 간에 유의한 차이를 보였다. 결론적으로, 세틸피리디늄과 염화아연을 함유한 구강 양치액은 건강한 대상자의 아침 구취를 3시간까지 유의하게 감소시킬 수 있어 구취제거를 위한 양치액으로 그 효용성이 매우 크다.

주제어: 구강 양치액; 구취; 세틸피리디늄; 염화아연; 휘발성 황화합물