Instant and freshness effect of mouth rinses on type 1 (oral) halitosis

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ABSTRACT

Hygiene deficiency causes type 1 (oral) halitosis. There are short and long-term studies on the anti-halitosis effect of mouth rinses but less knowledge on their instant effects. The aim of this study was to compare instant and freshness effects of 8 mouth rinses on type 1 halitosis.

Ninety self-reported halitosis patients (19-58 y.o., median 31) were randomly divided into 9 groups. Cysteine (20 mM) challenge test was applied to obtain maximum halitosis level in the mouth of each patient. Single use of 8 different mouth rinses (R1-R8) and tap water (R0) were tested on each group (n=10). Afterward, patients were requested to score oral freshness effect of the mouth rinse on a 5-point scale (0, bad; 5, fresh). Minimum halitosis level was obtained by rinsing with 20 mMol $ZnCL_2$. In each step, oral gas (organic, NH_3, SO_2, H_2S, H_2) concentrations were quantified by using a portable multi-gas detector (MX6, IndSci, US). The ANOVA or Kruskal Wallis tests were used to compare the quantitative measurements.

R3 (Halitosil Zn) mouth rinse was found to be have the highest instant anti-halitosis effect while the R2 (Colgate plax) had the lowest. The sensation of freshness was highest in R7 (Oxyfresh power mouth rinse lemon-mint) and lowest in R8 (Signal expert protection). The freshness effect was not associated with the anti-halitosis effect ($r= 0.185$, $p=0.608$).

Mouth rinses containing $ZnCl_2$ without alcohol are instantly effective on halitosis. Mouth rinses containing ethyl and other alcohols (including glycol, sorbitol, menthol, eucalyptol, thymol, xylitol and eugenol) were found to be less effective on halitosis.

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***Keywords**: halitosis; mouthwashes; breath tests; hydrogen sulfide; ammonia.*

Efecto anti-halitosis instantáneo y efecto de frescura de los enjuagues bucales sobre la halitosis (oral) tipo I

RESUMEN

La deficiencia de higiene causa halitosis tipo 1 (oral). Se han reportado efectos anti-halitosis a corto o largo plazo de los enjuagatorios bucales, pero se desconocen sus efectos instantáneos. El objetivo de este estudio fue comparar el efecto instantáneo y de frescura de 8 enjuagues bucales en la halitosis tipo 1.

Noventa pacientes (19-58 años, mediana 31) que reportaron sufrir halitosis se dividieron aleatoriamente en 9 grupos. Se aplicó la prueba de provocación con cisteína (20 mM) para obtener el máximo nivel de halitosis en la boca de cada paciente. El uso individual de 8 enjuagues bucales diferentes (R1-R8) y agua del grifo (R0) se probó en cada grupo (n = 10). Posteriormente, se pidió a los pacientes que puntuaran el efecto de la frescura oral del enjuague bucal en una escala de 5 puntos (0, malo; 5, fresco). El nivel mínimo de halitosis se obtuvo con 20 mMol de $ZnCL_2$ enjuague. En cada paso, se cuantificaron las concentraciones de gases orales (orgánicos, NH_3, SO_2, H_2S, H_2) mediante el uso de un detector portátil de múltiples gases (MX6, IndSci, EE. UU.)Se encontró que el enjuague bucal R3 (Halitosil Zn) tiene un mayor efecto anti-halitosis instantáneo, mientras que el R2 (Colgate plax) fue el más bajo. El sentido de frescura fue mayor en el enjuague bucal R7 (enjuague bucal Oxyfresh power lemon-mint) mientras que fue bajo en R8 (protección experta de Signal). El efecto de frescura no se asoció con el efecto anti-halitosis ($r = 0.185$, $p=0.608$).

Los enjuagues bucales que contienen ZnCl2 sin alcohol son instantáneamente efectivos en la halitosis. Se encontró que los enjuagues bucales que contenían etil y otros alcoholes (incluidos glicol, sorbitol, mentol, eucaliptol, timol, xilitol y eugenol) son menos efectivos para el control de la halitosis.

***Palabras clave:** halitosis; enjuagues bucales; pruebas de aliento; sulfuro de hidrógeno; amoníaco.*

INTRODUCTION

Halitosis is a chronic endogenous malodor, etiologically classified as follows: physiological (type 0), oral (type 1), airway (type 2), gastroesophageal (type 3), bloodborne (type 4) and subjective (type 5).[1]

The dorso-posterior area of the tongue is the most important halitogenic site due to the presence of densely populated biofilm containing anaerobes [2] . The causes of type 1 halitosis are usually poor oral hygiene, plaque stagnation areas, gingivitis and tongue coating.[1-3]

Type 1 halitosis is mostly composed of volatile sulfur compounds (VSCs) but also contains other volatile aromatic compounds including amines (indole, skatole, pyridine, picoline, urea, ammonia, methylated amines, putrescine, cadaverine), short/medium-chain fatty acids or organic acids (propionic, butyric, acetic, valeric acids), alcohols (methanol, ethanol, propanol), volatile aliphatic compounds (cyclopropane, cyclobutane, pentane), aldehydes and ketones (acetaldehyde, acetone, benzophenone, acetophenone).[3] Presence of hydrogen sulfide (H_2S) in the mouth has been accepted as a representative criterium of halitosis. [4] VSCs have been used to compare anti-halitosis effect of mouth rinses tested.[5,6] Other oral gases were assumed to be absent from the mouth cavity of halitosis patients.[7]

Traditional dental or periodontal treatments supported by mouth rinses provide only temporary relief in most patients.[8] Commercially available products, such as mints, toothpaste, mouth rinses, sprays and chewing gums, attempt to mask oral malodor with pleasant flavors and fragrances. [9] According to the literature, good results were reported with chlorhexidine (CHX), while triclosan seems less effective. Essential oils and cetylpyridinium chloride (CPC) are only effective up to 2 -3 hours, and antimicrobial ingredients are only temporarily effective in reducing microorganisms.[9]

In a systematic review of the anti-halitosis effect of mouth rinses; most of the products were found to have beneficial effects in reducing oral malodor. Adequate evidence was provided by both short-term (less than 3 weeks) and long-term studies on the effect of CHX-, CPC- and zinc-containing mouth rinses on oral malodor.[10] The medium-term (less than 2 weeks) efficacy of mouth rinses was also investigated, and CHX, CPC, zinc salts and $ZnCl_2$ were found to be the most effective against oral halitosis.[11]

Patients with halitosis need to use a fast-acting mouth rinse before social approach. Anti-halitosis mouth rinses are usually expected to provide instant effect. There is currently little available knowledge on which are the most fast-acting, instantly effective mouth rinses.

The aim of this study was to quantify instant the anti-halitosis and freshness effects of 8 mouth rinses by comparing different gases using a multi-gas detector.

MATERIALS AND METHODS

Study design and population

This was a single-center, randomized, double-blind, parallel-group clinical trial with a single use of mouth rinses. The study enrolled 90 patients, of whom 52 were female (19-58 y.o., median 31) who self-reported oral malodor. More than 0.7 ppm H_2S in their mouth was confirmed by halitometry. Patients with possible extraoral causes of oral malodor such as upper respiratory infections, as well as those with taste and smell disorders, sinonasal disorders (nasal polyps, chronic rhinosinusitis, allergic rhinitis, septum deviation), any history of asthma, malignancy, head trauma, neurologic and psychiatric disorders (schizophrenia, obsessive-compulsive disorder, social anxiety disorder), metabolic and endocrine disorders (diabetes mellitus, hypogonadism, liver or kidney disease) were excluded. Pregnant or lactating women, patients taking antimicrobials, and smoking or drinking individuals were also excluded.

All subjects who took part in the experiment signed written informed consent, after having received an explanation of the protocol approved by the Ethics Committee of Cumhuriyet University (2016-05-02). This study was conducted in full accordance with the World Medical Association Declaration of Helsinki.

Patients were asked to avoid odorous foods (such as onion, garlic) in their diet for 48 h before their appointment, and to refrain from alcohol intake and smoking 12 h prior to the halitosis examination. Gas measurements were taken blindly between 09:00 and 11:30 a.m.

Baseline gas measurement

Patients were randomly divided into 9 groups. Each participant's baseline volatile organic compound (VOC), NH_3, SO_2, H_2S and H_2 levels in oral air were measured using a portable multi-gas detector (MX6, IndSci, US) following a previously described procedure.[12] These initial values of the 5 gases were used as individualized control data.

Cysteine challenge test

The cysteine challenge test[13] was carried out as follows: 5 ml of 20 mM (2.43 g/L) aqueous *L*-Cysteine solution (#1.02839.0100, Merck) was placed in the mouth and held in contact with the dorsal part of the tongue for 30 s to generate H_2S in the mouth to challenge oral halitosis.

After 3 min, emerging H_2S in the oral cavity was measured and recorded as maximum halitosis level for each halitosis patient.

After this stage, each group (n=10) gargled for 30s with 10 ml of one of the mouth rinses listed in Table 1.

Oral gas concentrations were read blindly and recorded. Immediately after rinsing, participants were requested to score the oral freshness effect (FE) of the mouth rinse by answering the question "How fresh does your mouth feel?" on a 5-point scale from 0 (bad taste) to 5 (extremely fresh). Answers were recorded.

Finally, to remove the odor of cysteine from the mouth, subjects gargled with 10 ml of 20 mMol $ZnCL_2$ solution (Tekkim, TK800000.01000, Tr) for 30 s, after which oral gases were re-read. This value represented the minimum halitosis level for each halitosis patient.

Statistical analysis

MedCalc v.18.5 software (MedCalc, Belgium) was used for all statistical analyses, and the data were reported as mean ± standard deviation (SD). The *Kolmogorov-Smirnov* test was used to show the normal distribution of quantitative measurements, and the ANOVA or *Kruskal Wallis* tests were used for the comparison of the quantitative measurements between the groups of more than two. A post-hoc test (using *Scheffe* or *Conover*) was performed for pairwise comparison of subgroups when the ANOVA test was positive (p less than the selected significance level). Pearson correlation coefficient was used to analyze the degree of association between two variables. A log transformation was used for the variables that were not normally distributed. The probability of making a Type I error (alpha, significance) is 0.05 in all tests.

Table 1: Mouth rinses used in this study and their content (in alphabetical order).

Number	Mouth rinse	Ingredients
R0	Tap water	Water
R1	Cb12	Water, glycerin, hydrogenated starch hydrolysate, alcohol, zinc acetate dihydrate. Chlorhexidine, diacetate, sodium fluoride, peg-40, hydrogenated castor oil, potassium acesulfame, citric acid, aroma.
R2	Colgate Plax	Water, glycerin, alcohol, propylene glycol, sorbitol, polysorbate 20, sodium benzoate, aroma, menthol, cetylpyridinium chloride, Sodium fluoride (0.025%), experimental sodium saccharin, cl4290.
R3	Halitosil Zn	Zinc chloride, sodium chloride, boric acid, deionized water, glycerin.
R4	Listerine total care zero	Water, sorbitol, propylene glycol, poloxamer 407, sodium lauryl sulfate, aroma, eucalyptol, zinc chloride, benzoic acid, sodium benzoate, methyl salicylate, thymol, sodium saccharin, sodium fluoride, menthol, sucralose, cl16035, cl42090, sodium fluoride (220ppm).
R5	Oderol	Chlorhexidine digluconate (0.025%), zinc lactate, mentha piperita, sucralose, deionized water.
R6	OralB proexpert	Water, glycerine, aroma, cetylpyridinium chloride, poloxamer 407, methylparaben, sodium saccharin, cinnamal, propylparaben, eugenol, cl4290.
R7	Oxyfresh power mouthrinse lemon-mint	Water xylitol, peg-40 hydrogenated castor oil, sodium chloride, Zinc acetate, Natural flavor, Citrus citrus medica limonum (lemon) fruit oil, Citrus grandis (grapefruit), Peel oil, Citrus aurantifolia (Lime) oil, Mentha viridis (spearmint) leaf oil, aloe barbadensis leaf juice sucralose, sodium citrate, citric acid, sodium hydroxide.
R8	Signal expert protection	Water, sorbitol, Peg-40, hydrogenated castor oil, potassium citrate, glycine, sodium benzoate, aroma (flavor), citric acid, zinc sulfate, sodium saccharin, sodium fluoride, propylene glycol, citrus medica limonum juice, Aloe barbadensis leaf extract, limonene, cl 42051, cl 47005.

RESULTS

Initial concentrations of the five gases (VOC, NH_3, SO_2, H_2S, H_2) for each mouth rinse group (n=10) are provided in the left column of Table 2. No significant differences were noted within the baseline readings of the patients. The decrease in baseline concentrations of 5 gases after 1) cysteine rinse, 2) mouth rinse tested and 3) $ZnCl_2$ solution are also shown in Table 2. Table 3 shows the average values of each gas, calculated from the data provided in Table 2.

After the cysteine rinse, H_2S gas increased in the mouth while all other gases decreased. Then,

Table 2: Initial concentrations of gases and their average decrease according to the each step of the study.

Mouth rinse	Gases	Initial concentration (ppm±SD)	Decrease (ppm±SD)		
			Cysteine	Mouth rinse	$ZnCl_2$
R0	VOC	1.27 ± 1,22	0.42 ± 0.40	0.28 ± 0.42	0.28 ± 0.46
	NH_3	5.70 ± 2.66	2.10 ± 1.37	2.8 ± 1.47	0.7 ± 0.48
	SO_2	0-00 ± 0.00	0.00 ± 0.00	0.00± 0.00	0.00 ± 0.00
	H_2S	1.28 ± 0.56	-10.17 ± 4.12	3.4 ± 2.36	7.22 ± 3.29
	H_2	11.20 ± 11.77	6.90 ± 6.22	3.3 ± 5.37	0.60 ± 1.42
R1	VOC	1.43 ± 0.44	0.24 ± 0.26	-36.53 ± 7.55	36.15 ± 7.79
	NH_3	3.7 ± 2.26	1.60 ± 1.57	-8.04 ± 4.1	8.54 ± 3.71
	SO_2	0.00 ± 0.00	0.00 ± 0.00	-0.06 ± 0.13	0.06 ± 0.13
	H_2S	1.29 ± 0.58	-10.02 ± 5.27	0.96 ± 2.16	9.54 ± 5.79
	H^2	16.8 ± 10.12	5.50 ± 5.16	-215.5 ± 94.34	215.4 ± 93.94
R2	VOC	1.52 ± 1.23	0.20 ± 0.35	-53.91 ± 19.08	53.22 ± 19.94
	NH_3	1.80 ± 0.91	0.20 ± 0.78	-1.40 ± 0.96	1.90 ± 0.99
	SO_2	0.00 ± 0.00	0.00 ± 0.00	-0.07 ± 0.11	0.07 ± 0.11
	H_2S	1.54 ± 0.87	-15.11 ± 10.25	1.03 ± 2.05	14.07 ± 11.03
	H_2	12.2 ± 10.62	2.00 ± 2.70	-218.2 ± 95.68	219.1 ± 93.72
R3	VOC	1.75 ± 0.96	0.38 ± 0.40	0.83 ± 0.56	0.4 ± 0.37
	NH_3	3.90 ± 2.21	1.63 ± 1.43	1.54 ± 1.43	0.45 ± 0.68
	SO_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	H_2S	1.42 ± 0.96	-17.10 ± 14.67	17.54 ± 15.25	0.32 ± 0.27
	H_2	15.00 ± 6.76	5.00 ± 3.31	6.63 ± 5.44	2.72 ± 2.61
R4	VOC	1.82 ± 1.11	0.40 ± 0.39	-25.03 ± 17.70	25.18 ± 18.03
	NH_3	3.20 ± 2.60	1.15 ± 1.28	-0.53 ± 0.77	0.38 ± 3.66
	SO_2	0.00 ± 0.00	0.00 ± 0.00	-0.046± 0.11	0.04 ± 0.11
	H_2S	1.52 ± 0.94	-13.88 ± 9.30	3.43 ± 5.20	10.9 ± 9.35
	H_2	6.76 ± 4.95	0.61 ± 3.68	2.84 ± 2.91	1.3 ± 1.31
R5	VOC	1.87 ± 0.61	0.40 ± 0.28	0.24 ± 0.59	0.79± 0.54
	NH_3	4.5 ± 3.54	0.78 ± 0.57	-2.35 ± 1.7	5.0± 3.8
	SO_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	H_2S	1.91± 1.36	-18.37 ± 16.42	10.93 ± 9.79	8.69± 8.15
	H_2	12.71± 10.26	5.07± 3.58	3.71± 5.36	3.42± 4.23
R6	VOC	1.76± 1.00	0.38± 0.56	-2.18± 1.45	2.43± 1.25
	NH_3	2.60±1.95	0.80± 0.63	-1.50± 0.97	2.60± 1.82
	SO_2	0.00± 0.00	0.00 ± 0.00	-0.21± 0.22	0.21± 0.22
	H_2S	1.62± 1.07	-14.94± 12.97	4.42± 4.45	10.98± 9.61
	H_2	12.40± 7.02	6.00± 4.49	-50.9±18.09	52.5±16.56
R7	VOC	2.43 ±1.01	0.40 ± 0.21	-3.75 ± 3.09	4.14± 3.14
	NH_3	2.60 ±1.80	0.72 ± 0.90	-2.90 ± 2.11	3.45± 2.06
	SO_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.000± 0.00
	H_2S	1.62± 1.03	-13.66± 9.15	11.87± 7.39	2.01± 3.26
	H_2	17.54± 9.16	4.45± 3.72	7.09± 4.90	2.27± 4.49
R8	VOC	2.16± 1.24	0.61± 0.45	-27.9± 16.19	27.69± 16.11
	NH_3	3.10± 3.48	1.71± 2.43	-6.07± 4.42	5.21± 5.38
	SO_2	0.00 ± 0.00	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00
	H_2S	1.32± 0.97	-10.97± 7.61	0.25± 3.53	11.18± 8.75
	H_2	17.42± 29.93	9.21 ± 17.44	3.85 ± 7.72	0 ± 3.13

Negative value means increase

depending on the chemical content of mouth rinse tested, gas concentrations changed. In the final step, after the $ZnCl_2$ solution, all gases decreased sharply, as shown in Table 3.

The average ratios of the inhibitory effect of each mouth rinse on all gases were calculated from Table 2 and sorted as follows (from highest to lowest): 57.88 % (R3), 42.12 % (R0), 11.13 % (R5), -41.39 % (R7), -201.98 % (R6), -344.09 % (R4), -357.93 % (R8), -1070.25 % (R1), -1245.17 % (R2). Negative values represent increase in gas concentrations in the mouth immediately after mouth rinse. R3 was the most effective and R2 the least effective mouth rinse on oral gases, while the others were found to have an intermediate effect.

The freshness effects of the mouth rinses were sorted as follows: R7> R5> R3> R4> R1> R0> R6>R8 (Fig 1). Freshness effect was not correlated with anti-halitosis effect (p=0.608, r= 0.185).

DISCUSSION

This study investigated instant anti-halitosis effects of 8 mouth rinses by comparing different gases using a multi-gas detector in patients with type 1 halitosis. Mouth rinse R3 was the most effective and R2 the least effective against oral gases.

Self-assessment is indeed the primary diagnostic criterium for halitosis because it is the reason why patients request halitosis examination. Self-assessment and/or feedback from other people in the subject's social environment have a prominent place among all diagnostic tests.[14-16] The presence of halitosis in participants was confirmed by halitometry. No self-assessed participant had less than 0.7 ppm H_2S in the oral cavity.

Sulfide detectors are generally used to measure halitosis. The Halimeter (Interscan, Chatsworth, CA) can only detect H_2S gas. Oral Chroma (Abimedical Corporation, Osaka, Jp) is sensitive to only three sulfurous gases. Nevertheless, because there are more than 700[17] or 3481[18] gases in the human breath, most of the gases would have been overlooked if such a sulfide detector had been used in this experiment.

The MX6 multi-gas detector has been used for halitosis examination in previous clinical studies.[7,12] According to the MX6 user manual[19], the hydrogen and photoionization sensors are sufficiently sensitive to more than 72 volatile compounds and 116 organic gases, including major contributors to type 1 halitosis (VSCs, alcohols, aldehydes, ketones, ammonia). A strength of this study was that it measured a wide range of gases, enabling us to compare not only 3 sulfurous gases but also non-sulfurous gases which can be present in the mouth of halitosis patients. On the other hand, there were some limitations, such as the facts that MX6 cannot

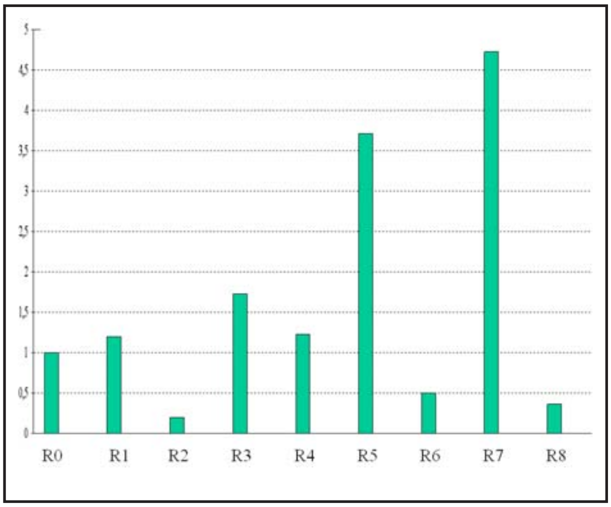

Fig. 1: Freshness effect of the mouth rinses scored by contributors (0 bad taste, 5 extremely fresh). R0-R8 are the mouth rinses listed in the Table 1.

Table 3: Average values of each gas at baseline, after cysteine, mouth rinse and $ZnCl_2$, respectively.

	Initial (ppm±SD)	Gas concentrations (ppm±SD) after:		
		Cystein	Mouth rinse	$ZnCl_2$
VOC	1.80 ±1.03	1.41 ± 0.87	17.60 ± 21.10 *	1.13 ±1.06*
NH_3	3.48 ± 2.70	2.29 ±1.90	4.44 ± 4.00	1.20 ± 2.10
SO_2	0.00 ±0.00	0.00 ± 0.00	0.03 ± 0.11	0.00 ± 0.00
H_2S	1.51 ± 0.96	15.4 ± 11.7	9.32 ± 8.6 *	0.98 ± 0.57
H_2	13.53 ±13.80	8.58 ± 8.30	52.3 ± 96.3*	3.89 ± 5.92

* statistically significant

distinguish which particular organic gas is present in a gas sample, and that some unpredictable cross-reactions can occur between gas sensors.

However, the effectiveness of oral healthcare products depends on active ingredients and their concentrations.[9] In the current study, the dose-dependent effects of ingredients could not be investigated because, due to business considerations, mouth rinse manufacturers are unwilling to disclose information regarding the ratio of active ingredients.

Some clinical parameters, such as oral hygiene, periodontal health, carious lesions, etc., were not considered because the aim of the study was not to detect causes of halitosis. Patients with periodontitis were allowed to enroll in the study because they are the most representative halitosis patients. The study does not cover the long-term effect of mouth rinses, which may differ from their instant effect.

Minimum halitosis level

Zinc at concentrations of 1% seems to be a safe, effective metal for inhibiting halitosis.[9] The zinc ion inhibition on VSC production has been largely attributed to its affinity for sulfur, although a restricted antimicrobial effect of zinc on plaque bacteria has been reported.[20]

$ZnCl_2$ is the most effective zinc salt against oral malodor due to its water-soluble structure. Zinc citrate has been found to be less effective because it is difficult to ionize, while other zinc salts show intermediate levels of effectiveness on oral halitosis.[21]

$ZnCl_2$ has already been used to achieve a minimum halitosis level in the mouth.[7,21,22] Unexpectedly, $ZnCl_2$ not only reduced H_2S but also reduced other gases. After $ZnCl_2$ rinsing, the VOC, NH_3, SO_2, H_2S and H_2 decreased 93.65%, 72.45%, 0%, 89.27%, 93.06%, respectively (data calculated from Table 2).

Maximum halitosis level (cysteine challenge test)

To challenge halitosis, using cysteine rinse in healthy individuals is often used and widely accepted way of designing such studies. [4,5,21,23,24] can be useful to distinguish type 1 halitosis from other types of halitosis.

Degradation of cysteine by oral bacteria is the biochemical process in the formation of artificial oral malodor. The magnitude of the H_2S (peak) response provides an idea of the tendency of the oral ecology to produce halitosis.[21] The H_2S peak value depends on the proteolytic capacity of the microbiota of an individual's mouth, independently of age and sex and momentary alterations of type 1 halitosis level. [7,12,21,22]

Usually, 8.23 mM L-cysteine rinse for 1 min, or 10 mM,[23] 6mM (pH 7.2)[5] or 6 mM (pH 7.1) for 20 s,[24] or 0.06 mM (pH 7.2) for 30 s have been used in the cysteine challenge test.

In this study, oral H_2S increased sharply after cysteine (20 mM) rinse, and oral bacteria produced H_2S by using cysteine, rather than carbohydrates, as an energy source. Other oral gases (H_2 or VOC) decreased, since carbohydrates were not used by bacteria during cysteine challenge test (Table 3).

Previous black and white approach to diagnosis of oral halitosis by one-time measurement with a halitometer may be an oversimplification.[1] It is assumed that halitosis fluctuates every 2 minutes throughout the day.[25] Single instant gas readings, or comparison of the reading to a predefined mathematical cut-off value, can lead to misdiagnosing halitosis. It might be more accurate to consider a scale between maximum and minimum levels for each individual. Oral gas readings can be compared with reference to these two points.[1,12] For this reason, this study determined maximum and minimum halitosis levels by using cysteine and $ZnCl_2$ solution, respectively. This procedure, which was used to diagnose halitosis in this study, can be called "scalar approach".

Effect of water on halitosis

Water rinse has been found not to reduce halitosis.[13] However, some evidence on this matter is conflicting, since rinsing with 15 ml of water for 30 s has been found to reduce oral H_2S by 30% - 50%.[26] In the present study, oral gas concentrations of VOC, NH_3, SO_2, H_2S and H_2 decreased 32.94, 77.70, 0.00, 29.69 and 23.25%, respectively, by rinsing with tap water (R0) (data calculated from Table 2). The anti-halitosis effect of water rinse probably simply consisted of a dilution effect on oral gases, which returned to initial values immediately after the water rinse. NH_3 was the most affected gas to be suppressed by water rinse (data not shown).

Mouth rinses

Good short-term results on halitosis were reported with CHX. However, CHX-containing mouth rinses are not suitable for long periods of use because they can destabilize oral microbiota or cause tooth staining, calculus formation, transient taste disturbance and harmful effects on the oral mucosa.[27-29] Rinsing with 0.2% alcohol-free CHX for 1 week caused irritation of oral mucosa and burning sensation, and disturbed taste perception compared to the placebo rinse.[29] In this mouth rinse series, R1, R2, R5 and R6 cannot be used for more than one week due to the possible adverse effect of their antiseptic content.

The present study found that CB12 mouth rinse reduced oral H_2S but increased other gases in the oral cavity. This was confirmed by an additional test; unfavorably halitosis treatment , this mouth rinse was found to spontaneously release very high concentration of VOC (52.96±8.20 ppm, n=10), NH_3 (3.6±1.1 ppm, n=10) and H2 (2057.2±154.7 ppm) in the headspace of its bottle at room temperature (data not shown).

Triclosan, and metal ions such as stannous and zinc, appear to be effective in controlling oral malodor. The effects of Triclosan (34.5 mmol/L) and Zinc Citrate (39.8 mmol/L), alone or in combination, have been tested in vitro for oral bacteria grown. Inhibitory effects were observed on oral bacteria except for *S. gordonii* and *S. oralis*.[30] However, the ability to kill bacteria may not truly reflect the success of the anti-halitosis effect. Antimicrobial mouth rinses merely suppress bacterial activity in the mouth for a limited time.[9]

Essential oils and CPC were found to be more effective than triclosan up to 2 or 3 h. Metal ions and oxidizing agents, such as hydrogen peroxide and chlorine dioxide, are active in neutralizing VSCs. Thymol, Eucalyptol, Menthol, CPC, ClO_2 + Zn-acetate containing mouthwashes reduce oral malodor within 4 h of a single usage. CPC was found to be the most effective ingredient.[31] In this study, R2 contained CPC, but was found to be the least effective, while another CPC containing R6 was found to have an intermediate level of effect on oral gases.

Silwood CJ et al,[6] tested six products on 6 volunteers according to 7 treatment regimens. Twenty ml of 5 of products were examined. VSC concentrations were recorded at 30 minutes intervals after administration. All products reduced VSC within 20 minutes of treatment. VSC concentrations returned to their baseline values within 5 h. The most effective oral health care products were found to be chlorite anion and chlorine dioxide. In accordance with the literature, the present study found that R3 and R7, which contain chlorite anion (Sodium chlorite), were both reasonably effective on oral gases.

Mouth rinses containing a combination of sodium chlorite and $ZnCl_2$ have been found to be more effective than those containing $ZnCl_2$ alone.[20] In the present study, R3 contained this combination that was found to be the most effective rinse.

Alcohol(s)

The term “alcohol” traditionally refers to ethanol (ethyl alcohol), although glycol, sorbitol, menthol, eucalyptol, thymol, xylitol and eugenol are also alcohols which may present in mouth rinses. All alcohols are mild skin irritants.[32] It has been reported that a high content of alcohol, an acidic pH and other ingredients constitute potential irritants individually or synergistically with the other components in the mouth rinse.[33]

The widespread use of alcohol-containing mouth rinses has also resulted in a number of reports of mouth rinse-associated adverse effects, including potential association with oral cancer.[34,35] There are controversial reports showing no causal relationship between the use of alcohol-containing mouth rinses and the development of oral cancer.[36] However, a hydroalcoholic vehicle containing 21.6 to 26.9 percent of alcohol in a mouth rinse meets the requirements of the FDA policy regarding fixed combinations of over-the-counter active ingredients with antigingivitis/antiplaque action.

Alcohol is not required in anti-halitosis mouth rinses since it exacerbates halitosis by drying the oral mucosa.[2,37-40]. Most manufacturers do not need to disclose alcohol content of rinses as ingredient but Listerine, Plax, Scope, Signal, ACT, Viadent and CB12 contain 26.9%, 7.5%, 18.9%, 14.5%, 6%, 10% and 1.7% of alcohol, respectively.[39] In the current study, R1 and R2 were clearly labeled as containing alcohol. In agreement with the literature, R1 and R2 were found to be the least effective on oral gases, possibly due to their alcohol content. There is not enough documentation to distinguish which alcohol contributes to oral malodor. Nevertheless, alcohols should not be preferred in the content of an anti-halitosis mouth rinse.

In 1978, Bernstein ML reported two cases of oral mucosa white lesions associated with Listerine mouth rinse, and subsequently reported the experimental induction of hyperkeratotic white lesions in hamster cheek pouches with the same mouth rinse.[33] The present study found that Listerine Total (R4) was not an effective mouth rinse on type 1 halitosis.

Instead of ethanol, R4, R6, R7 and R8 contained other alcohols such as sorbitol, glycol, thymol, eucalyptol, menthol and xylitol. These were found to have intermediate effectiveness on oral gases. All alcohol-containing mouth rinses (R1, R2, R4, R6, R8) released high concentrations of H_2 and VOC to the headspace of their bottle (data not shown). The reason for some gases increasing in the oral cavity immediately after rinsing may be the alcohol content of the mouth rinse tested.

On the other hand, alcohol-free mouth rinses (R3, R5) were found to be the most effective against oral gases. H_2 gas in the breath can be recognized by the MX6 and is also known as an indicator for inflammation. [12] It may be a good idea to monitor oral inflammation by detecting oral H_2 gas in the mouth cavity. However, SO_2 did not significantly alter within the study. Even though SO_2 is sulfurous, it might not be a halitosis gas. Further studies on oral halitosis gases are needed in the future.

Freshness effect

Even although the freshness effect of mouth rinses has been found to be independent of anti-halitosis action, it provides a feeling of psychological relief for patients. This is why it is important to assess the instant success of anti-halitosis mouth rinses. The three freshest mouth rinses (R7, R5, R3) are alcohol free, except R7, which contains xylitol, and all contained zinc salts.

CONCLUSIONS

1. Mouth rinses containing Zn salts, especially $ZnCl_2$, were instantly effective on halitosis.
2. Mouth rinses containing ethyl and other alcohols (glycol, sorbitol, menthol, eucalyptol, thymol, xylitol and eugenol) were less effective on halitosis than the alcohol-free mouth rinses.
3. Freshness effect was not associated with anti-halitosis effect.

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