Comparative analysis of some mouthrinses on the production of volatile sulfur-containing compounds

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Rösing CK, Jonski G, Rølla G. Comparative analysis of some mouthrinses on the production of volatile sulfur-containing compounds. Acta Odontol Scand 2002;60:10–12. Oslo. ISSN 0001-6357.

Oral malodor is mainly caused by the presence of volatile sulfur-containing compounds (VSC) produced by proteolytic periodontopathic bacteria in the oral cavity. Different solutions have been used as mouthrinses, trying to reduce malodor, and a large number is on the market. The aim of this study was to compare the effect of three commercially available mouthrinses with a simple inexpensive solution of zinc (zinc acetate 0.1%) on the production of VSC in vivo. Two of the solutions contained triclosan, one of them with fluoride and the other with sodium bicarbonate, and the third one contained herbal components. Seven healthy subjects rinsed with cysteine to induce production of VSC at baseline. After halitosis induction and VSC measurements, the subjects rinsed with the test solution, and mouth air VSC analyses were then performed by means of gas chromatography subsequent to repeated cysteine rinses after 30, 60, and 120 min. The data were calculated as percentage reduction of VSC from baseline. The percentage reduction of VSC decreased over time for all experimental groups. Zinc acetate had clearly the highest percentage reduction, starting from 95.68% at 30 min and with 69.27% after 2 h. The three other mouthrinses produced a VSC reduction of 23.92%–49.86% after 30 min, decreasing to 13.06%–37.09% after 2 h. One-way ANOVA ($P = 0.05$) was applied, and comparisons showed no differences between the commercially available solutions, but zinc acetate was significantly better than these. It may be concluded that some commercial mouthrinses are markedly less effective than a simple and cheap solution of zinc acetate. □ *Halitosis; mouthrinses; volatile sulfur compounds; zinc acetate*

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Halitosis is known to be related mainly to oral conditions. The production of volatile sulfur-containing compounds (VSC) in the mouth, in particular hydrogen sulfide and methyl mercaptane, is the main source of oral malodor. These compounds are the end products of microbial degradation of sulfur containing amino acids from the diet, desquamated epithelial cells, serum, and saliva. Periodontopathogenic bacteria in periodontal niches and in the crypts on the dorsum of the tongue play an important role in the development of halitosis (1).

VSC are of importance not only because of the unpleasant odor but also because they may have a pathogenic potential for periodontal disease by penetrating the cell membranes and disturbing the metabolism of the cells (2).

The importance of halitosis has led to the formulation of different commercial products that are claimed to have anti-halitosis effect. The mechanisms of action of these solutions are in general due to their antimicrobial or oxidizing properties or to their capacity to specifically inhibit the formation of VSC, even in the presence of oral bacteria (3).

Products for inhibition of halitosis are also herbal, triclosan-containing solutions and products containing zinc, cupric, or stannous ions. The effects of metal ion salts have been extensively studied both against plaque and halitosis in vivo and in vitro (4–9). These studies show that zinc salts appear to be the most promising, with a favorable cost–benefit relationship, because staining of the teeth is rare. The assumed mechanism of action of zinc solutions against the production of VSC is probably due to its affinity to sulfur and its capacity to oxidize thiol groups in the sulfur-containing precursors of VSC into non-volatile substances (4). When H_2S is dissolved in water (or saliva), it is dissociated in two steps:

$$H_2S \longrightarrow H^+ + HS^-$$
$$HS^- \longrightarrow H^{++} + S^{--}$$

Both anions will form insoluble sulfides with zinc and thus inhibit formation of VSC. Cysteine, which is an important substrate for VSC formation as such, is also oxidized by Zn.

Wåler (4, 5) has studied the anti-VSC production capacity of zinc ions in rinsing solutions and with chewing gum, reporting that they were able to inhibit the VSC.

The aim of the present study was to compare the effect of three commercially available mouthrinses, claimed to have effect on halitosis, with a simple non-commercial 0.1% aqueous solution of zinc acetate on the production of VSC in vivo.

The hypothesis that was tested was that the simple inexpensive aqueous solution of zinc acetate was more effective than other commercial products, based on different mechanisms, on the production of VSC.

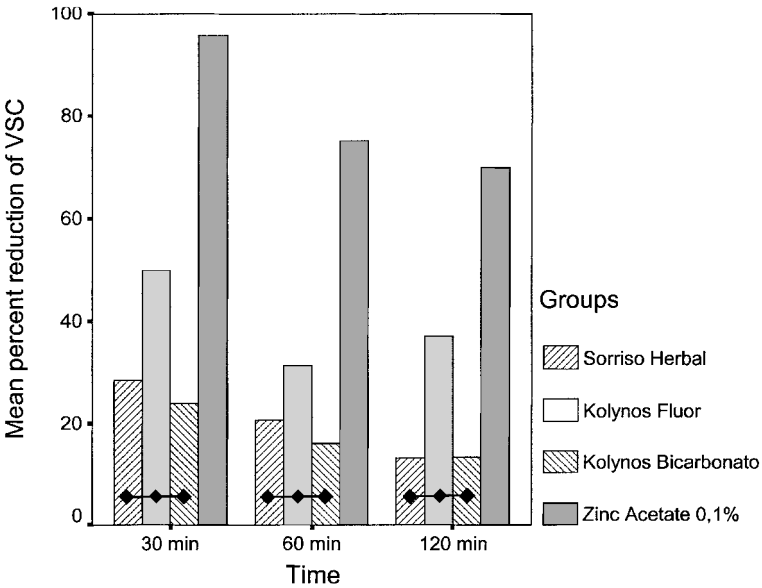

Fig. 1. Mean percentage reduction of volatile sulfur compounds (VSC) for the tested mouthrinses (Sorriso Herbal, Kolynos Fluor, Kolynos Bicarbonate, and zinc acetate, 0.1%) for 120 min ($n = 7$ for each solution, crossover design). The horizontal bars indicate means that are not statistically different in the time period.

Materials and methods

Test panel

Seven healthy subjects (aged 29–46 years) volunteered for the study. On the test days the volunteers were asked to refrain from their habitual oral hygiene measures. In the morning, after breakfast, the test panel had appointments at the Clinical Dental Laboratory, Faculty of Dentistry, University of Oslo, to participate in the experiment.

Collection of samples and solutions tested

The mouth air samples were collected by aspiration of air by means of a 10-ml syringe. The air was injected directly into a GC-14B gas chromatograph (Shimadzu, Japan), equipped with a flame photometric detector, a 366-cm × 0.32-cm Teflon column packed with 5% polyphenyl ether–0.05% phosphoric acid on 40/60 mesh Chomosorb T, and an auto-injection system with a 3-ml sample loop. The column conditions were as follows: column temperature, 70°C; nitrogen gas flow rate, 32 ml/min; hydrogen gas flow rate, 125 ml/min; and air flow rate, 43 ml/min (6).

The subjects rinsed first with 5 ml of a 6-mM solution of L-cysteine (Sigma Chemicals, St. Louis, Mo., USA) (10). After this rinse the mouth was kept closed for 90 sec, after which mouth air was aspirated by means of a 10-ml syringe connected to the apparatus, injected into it, and analyzed for the presence of VSC directly in the gas chromatograph. After this baseline measurement the volunteers rinsed for 1 min with 10 ml of one of the following solutions: Sorriso Herbal (a herbal-containing mouthrinse manufactured by Kolynos do Brasil), Kolynos Fluor (a fluoride-and triclosan-containing mouthrinse manufactured by Kolynos do Brasil), Kolynos Bicarbonato (a triclosan-and sodium bicarbonate-containing mouthrinse manufactured by Kolynos do Brasil), and a non-commercial aqueous solution of zinc acetate, 0.1% (Sigma Chemicals), which served as a positive control.

The cysteine rinses and the following mouth-air analyses were repeated after 30, 60, and 120 min. The experiment was run under a crossover design. The test persons were randomly assigned to each of the different mouthrinses, and at least 5 days elapsed between each experiment as a washout period. Rinsing with each of the test solutions was done only once. VSC levels after the time intervals were compared with baseline results for each volunteer, for each one of the four test solutions.

Analysis of the data

The percentage reduction in relation to baseline was calculated for each subject. Mean values of these reduction figures were calculated for each experimental solution. One-way ANOVA and post-hoc LSD were applied to the data. The chosen alpha level was 0.05.

Results

The results of the present investigation are shown in Fig. 1. Zinc acetate, 0.1%, showed a markedly higher effect in reducing VSC than the commercially available solutions. The herbal, the triclosan + fluoride, and the triclosan +

sodium bicarbonate had a low level of reduction and did not differ statistically.

The reduction figures for the three commercially available products show that already 30 min after rinsing the effect was reduced by at least 50%. When the same analysis was performed after 60 and 120 min, the reduction had dropped even more, to 10%–40%.

On the other hand, the percentage reduction by zinc acetate, 0.1%, was of 95.68% after 30 min and 75.01% and 69.27% after 60 and 120 min, respectively. The zinc solution was significantly better than the commercial products tested.

Discussion

The methods used in the present investigation include the use of gas chromatography and a halitosis-inducing method (10). Gas chromatography is the preferred method for measuring VSC in the mouth. The halimeter, which is frequently used in clinical studies, has the disadvantage that it is unstable and only measures total VSC and not the individual products. The halimeter is also known to be sensitive to tobacco smoke and to chlorine-containing compounds. Gas chromatography enables differentiation between hydrogen sulfide and methyl mercaptane and gives reproducible results. Many studies have been performed with this technique, and it is considered to be the standard VSC measurement (1, 10, 11).

The halitosis induction method used in the present study involved rinsing with cysteine. It is known that this has an immediate effect in enhancing the production of oral VSC (10, 12). This procedure is convenient and reproducible and enables experimental designs with smaller test panels that other procedures previously used (10).

The experiment showed a much better effect from the solution of zinc acetate than from the different commercial products. The hypothesis was then confirmed. The efficacy shown in the present study supports the hypothesis. Several other studies have shown that zinc salts are effective against VSC production (4–7). No negative control was included, as it is generally accepted that a cysteine rinse consistently produces an increase in VSC formation (10). This was shown also in the present study by the minimal influence of the herbal and the bicarbonate products.

The herbal solution and the triclosan with fluoride or with sodium bicarbonate showed only a slight reduction of the VSC in the test panel, an effect that is scarcely of clinical importance. Although these three solutions are claimed by the manufacturers to be effective against malodor, their effect must be considered questionable. Triclosan has previously been studied as an anti-VSC agent. Niles et al. (8) and Sharma et al. (9) tested triclosan-containing dentrifices both by means of gas chomatography and organoleptically and showed positive results, contrary to ours. However, there are indications that the nature of the solvents or detergents used in combination with triclosan is essential (13)

Zinc is inexpensive and does not cause dental stain, as many antibacterial agents do. It can serve as a gold standard with which new commercial products should be compared before they are marketed.

The clear-cut results of the present investigation lead to the conclusion that not all mouthrinses claiming to act on oral malodor are more effective than a simple and cheap metal salt solution such as zinc acetate 0.1%.

Acknowledgement.—Cassiano Kuchenbecker Rösing is on leave of absence from Departments of Periodontology, Federal University of Rio Grande do Sul, Porto Alegre, and Lutheran University of Brazil, Canoas, Brazil.

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Received for publication 5 March 2001
Accepted 20 June 2001