Short Communication

The effect of some metal ions on volatile sulfur-containing compounds originating from the oral cavity

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Halitosis originates mainly from the oral cavity, and the volatile sulfur-containing compounds (VSC) are the major contributors of the unpleasant odor. Anaerobic G^- bacteria use sulfur-containing amino acids in their production of VSC. Zinc has been shown to inhibit production of odiferous VSC, and the mechanism proposed has been that zinc, with its affinity for sulfur, oxidizes thiol groups and thereby inhibits the precursors of VSC. The aim of the study was to investigate whether, and to what extent, other metal ions with affinity for sulfur exert the same effect and whether a correlation exists between the sulfur affinity and VSC-inhibiting activity of these metals. VSC levels were measured on the 'morning breath' of 10 test subjects, using a portable sulfide monitor. The mouthrinses tested were aqueous solutions of zinc chloride, zinc citrate, stannous fluoride, cuprous gluconate, ferrous gluconate, and silver acetate, and they contained equimolar amounts of metals (1.47 mmol/l). The results showed that the ranking of Zn^{++} and Sn^{++} differed in the clinical test compared with sulfur affinity, and likewise with Ag^+ and Fe^{++}. It may therefore be concluded that there is no positive correlation between the inhibiting effect of metal ions on VSC and their affinity for sulfur. □ *Bad breath; halitosis; sulfur volatiles*

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It has been shown that the volatile sulfur-containing compounds (VSC) hydrogen sulfide, methyl mercaptan, and dimethyl sulfide are the major components constituting bad breath originating from the oral cavity (1, 2). Conditions that favor the retention of anaerobic G^- bacteria are thought to contribute to the odor production (3, 4). Such retentive areas may be the dorsum of the tongue and the interproximal areas of the teeth (5, 6). Another predisposing factor is periodontal pockets (7). Sulfur-containing amino acids and peptides serve as substrate for the G^- anaerobic bacteria in their production of VSC. Components resulting from cellular breakdown or caseins from dairy products may contribute. VSC have also been shown to penetrate the tissues and interfere with the cell function and metabolism and be involved in the initiation and progression of periodontal disease (8–10).

It is well established that aqueous solutions of zinc salts have a major effect on VSC, reducing or eliminating the unpleasant odor (11–14). It is assumed that the mechanism of effect is that zinc ions, due to their affinity for sulfur, oxidize thiol groups in the sulfur-containing precursors of VSC, or the odiferous substances themselves, to non-volatile substances (8). Several other metal ions have high affinity for sulfur; some even have considerably higher affinity for sulfur than for zinc (15). A selection of non-toxic metal ions with a wide variety of sulfur affinity was chosen for study. The aim of the present study was to examine to what extent these metal ions are able to inhibit VSC formation in the oral cavity. The hypothesis to be tested was that there is a positive correlation between the sulfur affinity of the metal ions and their effect as inhibitors of VSC production.

Materials and methods

The VSC measurements were performed with a Halimeter RH17E from Interscan, USA. This instrument enables measurement of air collected in the oral cavity, not including expiration air. A vacuum pump transfers the mouth air sample via a straw directly to the sensors of the instrument. These electrochemical sensors are designed to record the total amount of VSC in parts per billion (ppb) in the air sample.

The test panel was first examined for base-line values of VSC (morning breath), in accordance with the manufacturer's instructions. To collect mouth air, the mouth was kept closed for 90 sec before sampling. The straw was inserted and positioned above the posterior part of the dorsum of the tongue, touching neither the oral mucosa nor the tongue. No breathing was allowed during each sampling; the mouth was kept open at approximately 1.5 cm, and the peak value was recorded. Measurements were made in triplicate. When the variation was less than 10%, the mean value was calculated and accepted.

After the base-line values were established, the test panel rinsed for 1 min with 10 ml of each of the

different test solutions, and subsequent measurements were carried out as described above. The mouthrinses consisted of aqueous solutions of zinc chloride, zinc citrate, stannous fluoride, cuprous gluconate, ferrous gluconate, and silver acetate. All solutions were prepared with 1.47 mmol/l of metal ions. This concentration is equivalent to a 0.02% zinc chloride solution, which has been used in previous studies and was chosen to facilitate comparisons. All test subjects used all test solutions. The respective solutions were used on separate days, and the study was carried out in accordance with a double-blind design. A water rinse was used as a negative control.

Ten test subjects (age, 9–45 years) volunteered for the study, and informed consent was obtained from the participants. The subjects displayed slight to medium unpleasant 'morning breath' by organoleptic evaluation. This was recorded at a mean level of 230 ppb by the Halimeter. The test subjects had no pathologic periodontal pockets. To perform measurements of VSC levels on morning breath, the participants were instructed to brush their teeth with water only in the evening before testing. Furthermore, the participants were not allowed to eat, drink, smoke, or perform any kind of oral hygiene on the morning of testing.

The sulfur affinity of the metal ions was obtained from chemical tables (15), which gave the following ranking: $Cu^{++} > Sn^{++} > Zn^{++} > Fe^{++} > Ag^{+}$.

The stannous fluoride and silver acetate were purchased from Sigma Chemicals (St. Louis, Mo., USA), ferrous and cuprous gluconate from Aldrich (Steinheim, Germany), and zinc chloride from Merck (Darmstadt, Germany), and zinc citrate was a gift from Ello, Kristiansund, Norway. All chemicals were analytical grade.

Statistical analysis was carried out using a one-tailed paired Student's t test.

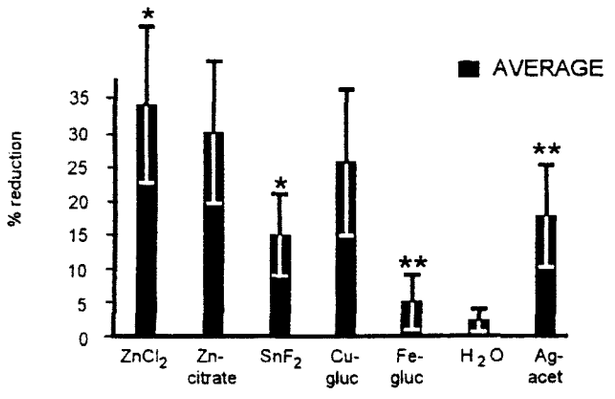

Fig. 1. Histogram showing the reduction (means and standard deviations in percentage) of volatile sulfur compounds (VSC) after mouthrinses with 1.47 mmol/l aqueous solutions of the different metal salts. The significant differences are marked with asterisks: *P = 0.02, **P = 0.01.

Results

The results in Fig. 1, given as mean percentage reduction of VSC levels, showed that the numerical values gave the following ranking of the VSC-inhibiting effect: $Zn^{++} > Cu^{++} > Ag^{+} > Sn^{++} > Fe^{++}$. The relevant comparisons in the present context are as follows: Zn ions are statistically more effective than Sn ions (P = 0.02), and Ag ions are statistically more effective than Fe ions (P = 0.01). It was furthermore shown that Cu ions were not statistically different from Zn ions. Zinc chloride was most effective, giving a reduction of 35%, whereas zinc citrate gave a comparable reduction of 31%. Cu ions were second best and gave a VSC reduction of 26%. Ag ions gave a reduction of 18%, and Sn ions 15%. Fe ions had the lowest effect of 5%, not significantly different from the water control.

Discussion

The sulfide monitor used in the present study is based on electrochemical sensors recording VSC levels of mouth air in ppb. Halitosis may in some cases also involve compounds other than VSC, but the conclusions in the present study relate to orally produced VSC only. This was found acceptable, as VSC are acknowledged as the dominant component of halitosis (1, 2). The Halimeter has been extensively used in related studies and is well suited to show relative VSC levels, as in the present study (16–20).

The results (Fig. 1) showed that the tested metal ions, with the exception of ferrous ions, had significant inhibiting effect on VSC production in the oral cavity compared with the water control. Zinc citrate and zinc chloride differed numerically, although not significantly. The difference between the two zinc solutions, containing the same amount of zinc, may be due to the well-known complex formation in the organic zinc citrate solution, making fewer ions available for reactions. The ranking of the metal ions in accordance with their inhibiting effect, based on numerical values, was shown to be $Zn^{++} > Cu^{++} > Ag^{+} > Sn^{++} > Fe^{++}$ in our clinical test, whereas a ranking by sulfur affinity gives $Cu^{++} > Sn^{++} > Zn^{++} > Fe^{++} > Ag^{+}$ (15). That Zn ions were more effective than Sn ions, and Ag ions were more effective than Fe ions, was statistically significant in the clinical test, as opposed to their ranking by sulfur affinity, as given above. Interestingly, Cu ions were not statistically different from Zn ions in the clinical test in spite of having a markedly higher sulfur affinity ($K_{sp} = 10^{-40}$ versus $K_{sp} = 10^{-24}$, respectively) (15). It thus seems safe to conclude that the VSC inhibiting effect is not positively correlated to sulfur affinity. Properties other than sulfur affinity are probably important for the clinical effect of metal ions against VSC production.

The standard deviation was rather large due to the considerable individual variations. Such variations are

to be expected in clinical measurements of an unstable medium like mouth air. However, the data obtained in the present study were sufficiently clear to give statistical significance and thus allow the conclusion stated above. The age range of the test panel was large, since some children were included because of high VSC base-line concentrations.

It is well known that zinc ions have high affinity for carboxyl and phosphate groups (21), which presumably are exposed on the surface of oral tissues, on bacteria, and in salivary macromolecules. This binding probably involves displacement of counterions (that is, calcium) and may give rise to a long-term retention of zinc in the mouth. It may be speculated that a gradual subsequent release of the adsorbed zinc by competing calcium ions from newly secreted saliva may provide a mechanism resulting in a slow release of zinc. These ions may thus be available for oxidation of thiol groups present in VSC or their precursors. The balance between the strength of the binding and the affinity for sulfur could be a factor relevant to the clinical effect of metal ions.

Metal ions in the mouth may rapidly become oxidized and thus lose their affinity for specific sites, such as sulfur. Of the metals tested, zinc is the least reactive in this respect and is the ion with the lowest standard reduction potential. It appears conceivable that this may be a factor that makes zinc most effective in VSC inhibition. Besides, zinc participates in a wide range of regulatory mechanisms and may well be involved in the enzymatically regulated process by which the sulfur-containing amino acids are metabolized to yield methyl mercaptan, hydrogen sulfide, and dimethyl sulfide. Furthermore, that zinc is the smallest of the ions tested could also be significant by affecting steric relationships. The ionic radius of Zn^{++} is 0.74A, compared with an Sn^{++} radius of 1.12A.

It has previously been shown that metal ions have an antiplaque effect when used in aqueous solutions as mouthrinses (22). Stannous fluoride solutions in particular have been extensively investigated (23–25). Both stannous and cuprous ions have a higher plaque-inhibiting capacity than zinc ions. The plaque-inhibiting capacity has been shown to be due to oxidization of thiol groups in glycolytic enzymes (26), thereby causing a reduced metabolic activity in plaque bacteria. This effect is thus positively correlated to sulfur affinity, contrary to the inhibition of VSC production as discussed above. The mechanisms involved in the inhibition of VSC production and the inhibition of plaque formation thus seem to be based on two different biologic activities exerted by the same ion.

Conclusion

The present study showed that there is no positive correlation between the affinity of metal ions for sulfur and their inhibiting effect on VSC production in the oral cavity.

The unique property of zinc as a VSC inhibitor is thus dependent on mechanisms other than sulfur affinity, even though this probably is an essential factor.

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