Original

Inhibitory Effects of Zinc Chloride and Citric Acid on Release of Volatile Sulfur Compounds

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Accepted for publication, May 17, 2013)

Abstract: Research into halitosis (commonly referred to as “bad breath”) has been increased recent years. Public awareness of halitosis has also increased, as a result of improved attention to hygiene, and the number of dental patients being treated for halitosis is increasing globally. Halitosis is known to be caused by volatile sulfur compounds (VSCs), formed by decomposition of sulfur-containing amino acids, and these can lead to infection of periodontal tissue. Previous studies have shown that zinc chloride and citric acid effectively suppress VSC release. In this study, we compared the VSC-inhibiting effects of zinc chloride alone, and of a zinc chloride/citric acid combination, in artificial saliva and in distilled water, each containing hydrogen sulfide. Our results showed that the inhibition of VSC release from distilled water achieved using citric acid/zinc chloride (18%) was less than that obtained using zinc chloride alone (27%). However, 100% inhibition was achieved in artificial saliva using the combined solutions, compared with 18% using zinc chloride alone. We believe that at the pH of the artificial saliva, some hydrogen sulfide is present as bisulfide ions, which readily react with zinc ions. Also, zinc ions form a chelate with citric acid, preventing formation of insoluble, unreactive zinc hydroxide. Our results suggest that a zinc chloride/citric acid combination would be an effective treatment for halitosis.

Key words: Halitosis, Citric acid, Zinc chloride, Volatile sulfur compounds

Introduction

Halitosis is the third-most-common cause of dental visits in North America, and in Japan there are many potential patients suffering from halitosis¹⁾. Halitosis has been classified by the World Health Organization (WHO) in the International Classification of Diseases (ICD-10). The rapid advances in halitosis research in recent years have shown that halitosis is caused by volatile sulfur compounds (VSCs). The main components of VSCs are hydrogen sulfide, methanethiol, and dimethyl sulfide²⁾. VSCs are generated by the decomposition of sulfur-containing amino acids in saliva, blood, and epithelial cell. The invasion of periodontal tissue by pathogens and endotoxins is caused by VSCs because they increase the permeability of oral mucosa and inhibit protein synthesis by human gingival fibroblasts³⁾.

Various studies have reported the inhibition of VSCs for treating halitosis. The inhibitory effect of zinc chloride on halitosis has been identified in many basic clinical studies⁴⁻⁶⁾. Recently, we

reported that bismuth oxychloride, which is widely used as a synthetic pearl essence in cosmetics, is an effective VSC inhibitor⁷⁾. In a previous in vitro study, we discovered that citric acid and zinc chloride effectively inhibited VSC formation in artificial saliva. The aim of this study was to examine the synergistic effect of citric acid and zinc chloride in artificial saliva. We compared the effect on VSCs of a combination of citric acid and zinc chloride in artificial saliva with that of zinc chloride alone

Materials and Methods

The reagents used were citric acid, zinc chloride, and hydrogen sulfide (Wako, Tokyo, Japan). A GV-100s gas detector chamber (Gastec, Tokyo, Japan) was used to collect gas samples. Artificial saliva was prepared according to the method reported in the literature⁸⁾.

Each test solution (5 ml) was placed in a test-tube, and the test-tube was covered with a stopper. 0.1 mL of hydrogen sulfide (2.0 mg/ml) in toluene solution was added to the test solution, and the mixture was stirred vigorously for 1 min. The sample was then transferred to a 200-ml flask and allowed to stand at ambient temperature, keeping the test-tube tightly closed. After 5 min, the

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Table 1. Inhibitory Effects of Zinc Chloride and Citric Acid on Hydrogen Sulfide.

Entry	Additives	Solvent	Release of VSC (ppm)	Inhibition of the release of VSC (%)
1		Distilled water	440	
2	ZnCl ₂ ^a	Distilled water	32	27
3	ZnCl ₂ ^a , citric acid ^b	Distilled water	36	18
4		Artificial saliva	44	0
5	ZnCl ₂ ^a	Artificial saliva	36	18
6	ZnCl ₂ ^a , citric acid ^b	Artificial saliva	0	100

a: 0.1 % of ZnCl₂ solution was prepared by addition of 0.1 g of ZnCl₂ into a 100 ml of test sample solution.

b: 0.2% of citric acid solution was prepared by addition of 0.2 g of citric acid into a 100 ml solution of test sample solution. VSC inhibitory rate was calculated based on the value of hydrogen sulfide.

gas inside the flask was collected in the gas chamber to detect gases and measure their concentrations⁷⁾. Similar tests were carried out using distilled water, instead of the test sample solution, as a negative control. The inhibitory effect on VSC release was determined using the following formula:

Inhibition of VSCs release

$$= \frac{[(\text{the initial concentration of VSCs in the test solution}) - (\text{concentration of VSCs gas released in the test solution})]}{(\text{the initial concentration of VSCs in the test solution})}$$

Results

The results are summarized in Table 1. We detected 44 ppm of hydrogen sulfide gas in the distilled water used as the negative control (entry 1). In the case of addition of 0.1w/v% zinc chloride solution to distilled water, inhibition of the release of VSCs was 27% (entry 2). The addition of a combination of 0.1w/v% zinc chloride and 0.2w/v% citric acid solutions to distilled water inhibited VSC release by 18% (entry 3). When a 0.1w/v% zinc chloride solution was added to artificial saliva at pH 6.8, the inhibition of the release of VSCs was estimated to be 18w/v% (entry 5). When 0.1w/v% zinc chloride and 0.2w/v% citric acid solutions were added to artificial saliva at pH 6.8, the inhibition of VSC release was 100% (entry 6).

Discussion

Currently, many treatments for halitosis are commercially available. In the United States, combinations of chlorhexidine with other substances, which kill VSC-producing bacteria in the oral cavity, are commercially available⁸⁻¹⁰⁾. However, an immediate effect is not produced by such formulations. Moreover, mouth rinses containing essential oils and ethanol are commercially available, but toxicity is a problem with formulations containing more than 25% ethanol¹⁰⁾.

Zinc chloride removes breath odor because the non-volatile zinc ions combine with VSCs⁴⁾. Furthermore, zinc ions bind to sulfur-containing amino acids, inhibiting the production of VSCs.

VSC inhibition strongly inhibits the proteolytic enzyme activities of bacteria. The inhibition of VSCs also suppresses degradation of the cellular components of saliva¹¹⁾. This *in vitro* study compared the rates of inhibition of VSCs using either zinc chloride alone, or a combination of zinc chloride and citric acid. We used a zinc chloride solution of concentration 0.1w/v% in this study. There have been no reports of side effects such as hypersensitivity for this zinc chloride concentration. The rate of inhibition of VSC release in distilled water by 0.1 w/v% zinc chloride was 27% (entry 2). Zinc chloride inhibited the release of VSCs, but zinc chloride precipitation was observed. This precipitation was caused by the formation of zinc hydroxide under neutral conditions¹²⁾. When 0.2w/v% citric acid was added to the 0.1w/v% zinc chloride distilled water solution, no precipitation was observed because the chelation effect of the citric acid suppressed the production of zinc hydroxide. However, the rate of inhibition of VSC release was still low. No effective increase in inhibition was observed using citric acid in distilled water. The rate of inhibition of the release of VSCs by 0.1w/v% zinc chloride in artificial saliva solution at pH 6.8 was estimated to be 18%, the same as that in distilled water. To our surprise, when a combination of 0.1w/v% zinc chloride and 0.2w/v% citric acid solutions was added to artificial saliva at pH 6.8, the rate of inhibition of VSC release increased to 100%. The artificial saliva was a buffered solution controlled at pH 6.8. In general, the major species in a hydrogen sulfide distilled water solution is hydrogen sulfide, as a result of the weak acidity of hydrogen sulfide. Bisulfide ions are present in the artificial buffer solution at pH 6.8, although hydrogen sulfide is still a major species. Bisulfide ions are more reactive than hydrogen sulfide, so zinc ions bind to the bisulfide ions. However, the zinc chloride is transformed into insoluble, unreactive zinc hydroxide under neutral conditions. The addition of citric acid to the zinc chloride solution prevents the production of zinc hydroxide by chelation of zinc and citric acid, thus stabilizing the zinc chloride. A combination of zinc chloride and citric acid in an artificial saliva solution reacts with hydrogen sulfide and significantly inhibits VSC release.

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