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Antimicrobial Effect of Silver Ionised Water Prepared with the Sintering Coating Method

—Effect on Acid Production in Plaque

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Abstract

Purpose: To investigate the effect of silver ionised water on acid production in plaque. **Methods:** After injecting 0.5 mL of silver ionised water (concentration: 5 ppm) produced with the sintering coating method in the sensor part of the pH metre, plaque collected from the oral cavity by one platinum loop was mixed in. Immediately after that, a 5% sucrose solution (1 g/20 mL) was added and the pH was continuously measured for 30 minutes at 1-minute intervals (A). Similarly, silver ionised water was mixed with 5% sucrose solution in the same way as in (A) at 3 (B), 5 (C), 10 (D) and 15 minutes (E) after plaque contamination. The pH was measured at 1-minute intervals. The pH of the purified water containing no silver ionised water mixed with plaque and sugar solution at the same time was measured and used as a control. Each experiment was conducted three times, and the pH measured every minute was compared as a percentage of the pH at the beginning of the measurement (100%). **Results:** Analysis of variance of the repeated measurements to determine the effect of silver ionised water on the decrease in pH revealed a main effect of silver ionised water and an interaction between time and group [$F_{(1.302,20.826)} = 39.145$, $p < 0.01$]. Multiple comparisons using Dunnett's method showed a significant decline in the rate of decrease in pH from B to E as compared with that in the control ($p < 0.01$). **Conclusion:** Silver ionised water was found to inhibit the acid production in plaque.

Keywords

Silver Ionised Water, Antimicrobial Effect, Plaque, Stephan Curve, pH

1. Introduction

Silver is a metal that has been known for a long time. It is rarely produced natu-

rally as natural silver and is found in trace amounts in sulphide minerals such as copper, lead and zinc. Silver has long been known to have strong antibacterial [1] [2] [3] and antifungal properties [4]. In the medical field, silver ions have been used as an antimicrobial agent in the treatment of burns [5] and as a disinfectant for dialysis [6].

In the dental field, the antibacterial effect of orthodontic brackets coated with silver ions [7], the antibacterial effect of a resin base containing antibacterial particles with silver ions [8] and the antibacterial property of silica glass containing silver [9] have been reported. In addition, as an effect on plaque, the rate of plaque adhesion was reported to be suppressed after mouth washing with silver-ion water [10].

Microorganisms in plaque on the surface of teeth are known to decompose sugar to produce acid, which demineralises the teeth. Since the lowering of pH by acid was reported by Stephan [11], the mechanical removal of plaque has been recognised as the first choice for caries prevention. In addition to tooth brushing, mouthwash can be used as a sanitising method, but no studies have been conducted to clarify the effect of silver ionised water on plaque on teeth. In this study, we investigated the effect of silver ionised water on the acid production of plaque for application in clinical dentistry.

2. Methods

2.1. Preparation of Silver-Ion Water

Silver-ion water was prepared by adding silver salt compound powder to pure water and then dissolving it. This solution was applied to the metal surface and sintered with nitrogen to form a layer containing sintered silver nitride on the metal surface. The layer was immersed in distilled water, and the silver-ion water eluted from it was used for the experiment [12].

2.2. Experimental Method

The silver-ion water concentration was measured with an atomic absorption spectrophotometer (Hitachi, Ltd., Model 180-30, Japan), and 5 ppm was used. After injecting 0.5 mL of the silver ionised water in the sensor of a pH metre (LAQUA twin, Horiba, Japan) with a micropipette, the water was left for 5 minutes and when the pH of the silver ionised water stabilised owing to the influence of carbon dioxide in the air, 1 platinum loop (about 2 mg) of plaque was mixed in. Immediately after that, 50 μL of 5% sucrose solution (1 g/20mL) was added and the pH was continuously measured for 40 min at 1-min intervals (A). Similarly, sugar solution of the same concentration was added to the silver ionised water for 3 (B), 5 (C), 10 (D), and 15 minutes (E) after plaque contamination and the pH was measured for 30 minutes at 1-minute intervals. On the other hand, as a control, the plaque and sugar solution were mixed simultaneously in 0.5 mL of purified water (Ken-ei Pharmaceutical Co., Ltd., Japan) and the pH was measured. Plaque was collected from the oral cavity of experimenters

who had not brushed their teeth for a day. The experiment was started at 14:00 in the laboratory.

3. Statistical Analysis

The data obtained was expressed as a percentage of the value of pH at the beginning of the measurement as 100%, and the average value of three times was obtained. Repeated-measures analysis of variance was performed using the control and silver-ion water immersion time as factors to confirm the main effect and interaction of the rate of decrease in pH and time. Multiple comparison tests using the Dunnett's method were conducted as subsequent tests. The SPSS Statistics 26 statistical software was used for statistical processing, and the significance probability was set at <5%.

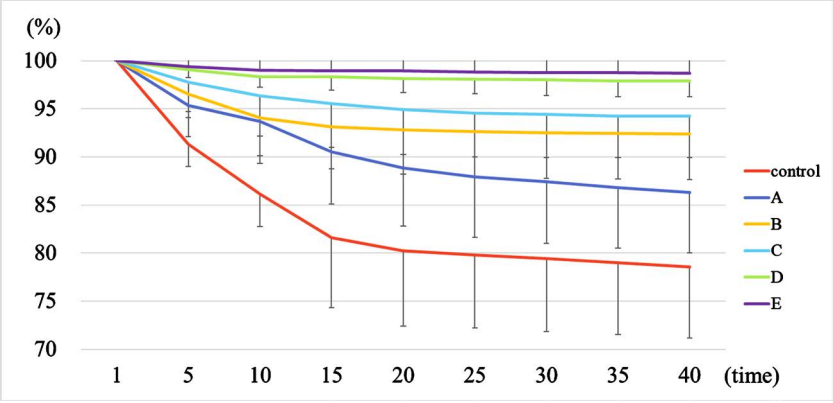
4. Results

The results are shown in the graph in Figure 1. The analysis of variance for repeated measurements showed a main effect of silver ionised water on the decrease in pH and an interaction between time and group [$F_{(1.302,20.826)} = 39.145$, $p < 0.01$]. The decrease in pH may be due to the mutual influence of two factors, immersion time in silver ionised water and time after sucrose incorporation. Multiple comparisons using Dunnett's method showed a significant decrease in the rate of decrease in pH from B to E as compared with the control ($p < 0.01$).

5. Discussion

5.1 Silver Ionised Water

Two types of manufacturing methods have been used for silver ionised water, the conventional electrolysis method [13] in which silver ions are generated by passing an electric current through pure silver electrodes in water and the



Sugar solution was added to the silver ionised water for immediately (A), 3 (B), 5 (C), 10 (D), and 15 minutes (E) after plaque contamination and the pH was measured for 40 minutes at 1-minute intervals. The data obtained was expressed as a percentage of the value of pH at the beginning of the measurement as 100%. Multiple comparisons using Dunnett's method showed a significant decrease in the rate of decrease in pH from B to E compared to the control ($p < 0.01$).

Figure 1. Successive changes in the rate of pH descent.

pharmaceutical method (zeolite) [14] in which a compound containing silver-ion components is added to water. Owing to the characteristics of each manufacturing method, the common practice is to purify silver-ion water in the case of the electrolysis method and silver-ion concentrated solution in the case of the pharmaceutical method. The silver-ion water used in this study was produced using the sintered coating method [12] that is, the dissolved solution of silver salt compounds was applied to the metal surface and then sintered to form a layered silver nitride sintered body, which was then immersed in pure water to elute the silver-ion water. The method was used in this experiment because it is cheaper and safer than the conventional methods and has the feature of being able to produce a large amount of silver-ion water at once, which is thought to be advantageous when considering the commercialisation of oral care products in the future.

According to the 'Outline of the Review of Water Quality Standards' by the Ministry of Health, Labour and Welfare of Japan in 2003 [15], the safety of silver ions is not regulated by the water quality standards and the guideline values for monitoring items, and is set at 0.01 mg/L by the chemical standards, which are other standards. According to the toxicity assessment, the lifetime oral intake of silver as a NOAEL (no observed adverse effect level) for humans is approximately 10 g. The contribution of drinking water to this NOAEL is usually considered negligible, and no health guideline values need to be established. The scientific opinion [16] on the safety evaluation of silver zeolite A (silver-zinc, sodium, ammonium and aluminosilicate) with 2% - 5% silver content as a food product concluded that silver-ion concentrations not exceeding 0.05 mg Ag/kg food causes no safety concerns for consumers. As for the absorption of silver ions in the body, silver ions have been shown to combine with chloride ions to form insoluble salts and when silver ions come into contact with hydrochloric acid in stomach acid, they immediately become insoluble AgCl, which is not absorbed by the human body, and are discharged directly from the body.

As this experiment was conducted outside the oral cavity, we used relatively high silver-ion water concentrations within the safety standards. However, on the basis of the results of this experiment, we are considering conducting oral experiments with lower concentrations in the future.

5.2. Analysis of the Results

The decrease in pH by the control was due to the bacteria in the plaque consuming the sugar solution, showing the so-called typical Stefan curve [11] and after 40 minutes, the pH was 20% lower than at the start. The pH change in (A) was similar to that of control at first, but after 5 nin, the rate of descent clearly decreased; after 40 min, the rate of descent was 10% lower than at the start. In (B) and (C), the rate of descent was suppressed compared to the control and (A). In (D) and (E), almost no decrease in pH was observed.

In summary, the present experiments showed that silver-ion water affected

the plaque from a relatively early stage and almost stopped the acid production of plaque after 10 minutes of immersion (D).

5.3. Antimicrobial Mechanism of Silver Ions

There are several reports on the disinfection principle of silver ions. The most popular theory is that silver ions with a (+) charge adhere to the cell walls of bacteria with a (−) charge, impairing their ability to synthesize proteins and inhibiting cell division [15].

As the cell walls of gram-negative bacteria are thinner than those of gram-positive bacteria, the effect of silver ions is better for gram-negative bacteria. The indigenous bacteria in the oral cavity are mainly gram-positive bacillus such as *Actinomyces* and *Corynebacterium* and gram-positive coccus such as Streptococcus and *Streptococcus pneumoniae* [16], it may take some time for the silver ions to take effect.

5.4. Effect on Acid Production in Plaque

Morishita *et al.* [10] investigated the inhibitory activity of Silver Zeolite (SZ) mouthwash on plaque formation. As a result, they reported that plaque formation was significantly ($p < 0.05$) reduced by mouth washing with 3% (*w*/*w*) concentration of SZ powder twice a day in subjects who had interrupted oral cleaning with toothbrush for 5 days. It is unclear how this report is affected by the factors such as the different contents of the foods and drinks taken by the subjects during the experiment and the difference in saliva secretion rate of the subjects were not standardised.

On the other hand, the results of a test conducted by the Japan Food Analysis Center, which investigated the effect of silver ionised water on the viable counts of oral bacteria, *Prophyromonas gingivalis* and *Streptococcus mutans*, [17] showed that the viable counts of both groups of bacteria decreased after 5 minutes of exposure as compared with the control group. From these reports, we can conclude that silver ionised water has a positive effect on the bacterial count.

These reports suggest that silver ionised water has a bactericidal activity against oral bacteria and inhibits the growth of plaque. In this experiment, acid-producing ability, which is evidence of bacterial activity, was measured directly with a pH metre and the effect of silver ionised water on oral bacteria was clarified. Pre-sleep mouthwash with silver ionised water is expected to contribute to the prevention of aspiration pneumonia in the elderly.

6. Conclusion

In this study, we found that 5-ppm silver ionised water suppressed plaque acid production and decreased the pH level.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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