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ORIGINAL ARTICLE

Effect of water containing organic acids on aspiration pneumonia-causative bacteria in the biofilm on the tooth surface

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Abstract *Background/purpose:* The tooth surface is a source of oral microbes in dentulous individuals, it is difficult for elderly people requiring nursing care to perform mechanical tooth cleaning by themselves. The objective of this study was to investigate the antimicrobial effect of water containing organic acids (WOA) made by some organic acids as food additives on chemical cleaning for elderly people on aspiration pneumonia-causative bacteria in the biofilm on the tooth surface.
Materials and methods: Ninety-six specimens made from bovine incisors were divided into four groups and incubated with one of four aspiration pneumonia-causative bacteria. Each group was further divided into six subgroups according to treatment as follows: control group (DW), chlorhexidine gluconate solution group (CHX), WOA group (WOA), ultrasonic treatment in distilled water group (DW-U), ultrasonic treatment in chlorhexidine gluconate solution group (CHX-U) or ultrasonic treatment in WOA group (WOA-U). After treatment, the levels of viable microbes in the biofilm were evaluated by quantitative adenosine triphosphate analysis and compared among the six groups.
Results: For every evaluated microbe, there were significant differences between DW and WOA, and DW and WOA-U. However, there was no significant difference among the WOA, DW-U, CHX-U and WOA-U groups. These results suggested that the antimicrobial effect of WOA on microbes attached to the tooth surface was similar to that of ultrasonic cleaning.
Conclusion: WOA has an antimicrobial effect on microbes in the biofilm on the tooth surface.

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Introduction

Aspiration pneumonia is caused by silent aspiration of oral microbes present in the mouth and pharynx.[1–3] The control of oral microbial flora by oral health care is an effective preventive measure.[4] Ryu et al.[5] found that the level of adherence of tongue coating and denture plaque was related to the total number of salivary anaerobic bacteria in edentulous subjects. Yasui et al.[6] reported that periodontal pathogens were detected at a high rate on the dorsum of the tongue, denture base and artificial teeth. The tooth surface is also a source of salivary microbes in dentulous individuals.[4,7] These reports confirm that the tooth surface, tongue and dentures are foci for oral microbes, and that controlling the number of microbes in these areas is important for effectively reducing the aspiration of oral microbes. Currently available antimicrobial mouthwashes such as chlorhexidine gluconate and povidone-iodine pose a risk of causing anaphylactic shock depending on the concentration and application method.[8,9] Because of these shortcomings, the development of an antimicrobial mouthwash that is suitable for elderly people requiring nursing care is required.

In this study, we focused on water containing organic acids (WOA). The main components of WOA are organic acids such as citric acid and lactic acid, commonly used as food additives for humans.[10]

WOA has been shown to exhibit an antimicrobial effect against planktonic and resin-attached aspiration pneumonia-causative microbes as well as against total anaerobic bacteria attached to dentures in use.[10] This report suggested that its antimicrobial effect may extend to oral microbes attached to tooth surfaces. However, the drug sensitivity of oral microbes attached to tooth surfaces differs from that of planktonic microbes, and adherent form of oral microbes attached to tooth surface differ from to dentures.[11,12] So it is necessary to investigate the effect of WOA on microbes forming a biofilm on the tooth surface.

Streptococcus sanguinis (*S. sanguinis*) initially adheres to the pellicle as a dental plaque bacterium.[13] *Porphyromonas gingivalis* (*P. gingivalis*), *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pneumoniae* (*S. pneumoniae*) are bacteria that cause aspiration pneumonia.[14–16] The objective of this study was to investigate the antimicrobial effect of WOA on aspiration pneumonia-causative bacteria in the biofilm on the tooth surface.

Materials and methods

Preparation of WOA and specimens

WOA was prepared using the BIOioNURSE (Separator System Kogyo, Nara, Japan). Water was ultrapurified using a reverse osmosis membrane via an ion-exchange resin. A total of 10,000 ppm of citric acid, 2,000 ppm of lactic acid, and trace amounts of oxalic acid and tartaric acid were added to 1 L of ultrapure water. The concentration of total organic acids was adjusted to 3% by volume by adjusting the quantity of acids. The water was colorless and transparent, with a pH of 2.19 and an oxidation-reduction potential of 250 mV.[10]

Ninety-six bovine incisors were used to prepare specimens for this study. The incisors were polished and washed by ultrasonic cleaning. In the preliminary experiment, we confirmed that there was no significant difference on number of bacterial adhesion between specimens after washing by ATP assay, so we considered that the specimens were enough clean. The incisors were cut into 5-mm lengths from the incisal edge perpendicular to their major axis using a diamond disc under running water. The cut surfaces of the specimens were polished using silicon carbide abrasive paper up to #1000 under running water to standardize the surface roughness. The surface roughness of the enamel of the specimens was analyzed before treatment within a single group using a scanning electron microscope (SEM) with a three-dimensional shape analysis function (3DSEM: Era-8900Fe, Elionix, Tokyo, Japan). The mean surface roughness (Ra) was determined using SEM images that were taken at a magnification of $\times$2000. Each specimen was measured at three randomly selected points. It was confirmed that there were no significant differences in the ATP levels of bacteria attached to the cut surface among the specimens under the initial polishing conditions as a preliminary experiment (data not shown). The specimens were divided randomly into four groups for incubation with the following microbes: 1) *S. sanguinis*, 2) *P. gingivalis*, 3) *S. aureus*, and 4) *S. pneumoniae*. Each group was divided randomly into six subgroups ($n = 4$) that were immersed in three solutions with or without ultrasonic treatment. Each specimen was weighed using an electronic scale (LA230S Sartorius, Tokyo, Japan) to infer the surface area of the specimens.

Whole saliva without stimulation was collected from four healthy adult volunteers (2 men, 2 women; mean age 29 $\pm$ 1 years) as described previously.[17,18] The Ethics Committee of Tokyo Dental College approved collection of saliva (#554). The tooth specimens were treated with the saliva for 10 min at room temperature to form a pellicle on the surface.

Antimicrobial effect of WOA on microbes in the biofilm on the tooth surface

S. sanguinis ATCC 10556, *P. gingivalis* ATCC 33277 and *S. aureus* 209P, obtained from the Department of Microbiology, Tokyo Dental College, and *S. pneumoniae* GTC 261 obtained from the Department of Microbiology, Gifu University Graduate School of Medicine, were used in this study. *S. sanguinis* and *S. pneumoniae* were maintained on Todd Hewitt agar plates (Becton Dickinson, Franklin Lakes, NJ, USA), *S. aureus* was maintained on trypticase soy agar plates (Becton Dickinson), and *P. gingivalis* was maintained on trypticase soy agar plates (Becton Dickinson) supplemented with hemin (5 μg/mL), menadione (0.5 μg/mL), and 10% defibrinated horse blood. To obtain cells in the late log phase, *S. sanguinis* was precultured in brain heart infusion broth at 37 °C for 24 hours under anaerobic conditions (10% CO_2, 10% H_2 and 80% N_2). *P. gingivalis* was first cultured in trypticase soy broth with 1 mL of hemin and menadione per liter at 37 °C for 6 days under anaerobic conditions. *S. aureus* and *S. pneumoniae* were first cultured in trypticase soy broth at 37 °C for 12 hours for