Anti-demineralizing Potential of Bottled Sugar-free Green Tea Beverages *in vitro*

Yoshiharu Mukai¹, Kazuko Kamijo², Yukio Hirata² and Toshio Teranaka¹

¹Division of Restorative Dentistry, Department of Oral Medicine, Kanagawa Dental College
²Division of Sociological Approach in Dentistry, Department of Dental Sociology, Kanagawa Dental College

Abstract: The purpose of this study was to evaluate the influence of a bottled green tea beverage on dentin demineralization with a demineralization gel system *in vitro*. Samples were cut from bovine root dentin. Each sample was immersed in 8% methylcellulose gel onto which a layer of green tea beverage was placed. For comparison, sugar-free coffee beverage, deionized water, and 0.8-ppm F solutions were used. After two weeks, the treatment solutions were replaced with demineralization solution. The mineral profiles and mineral loss values of the lesions were obtained by transversal microradiography (TMR) after one-week demineralization. The green tea, coffee, and fluoride solution treatments induced a significantly thicker surface layer when compared with the deionized water treatment. In particular, the mineral volume % of the demineralized dentin specimens treated with green tea was approximately seven times higher than that of the de-ionized water treatment. The green tea treatment showed significantly lower mineral loss than the other three treatments. TMR measurements clearly showed that the sugar-free bottled green tea beverage inhibited dentin lesion progression, presumably due to the effect of sub-ppm fluoride levels.

Key words: green tea, dentin, demineralization, microradiography, fluoride

Introduction

Improved dental care offered to the adult population because of better knowledge of the prevention and treatment of periodontal disease is expected to lead to the retention of a high proportion of teeth which could previously have been lost at an early age. Also, in older individuals root caries on exposed root surfaces has become an increasingly important problem¹. Numerous clinical and laboratory studies conducted in recent decades have demonstrated the caries-preventive effects of fluoride. However, there have been few reports on the effect of fluoride in green tea for dentin caries prevention. The production of sugar-free green tea beverages is increasing, and is estimated at over 2.6 million kiloliters in 2005 in Japan². Generally, the anti-cariogenic potential of green tea is widely recognized. The habit among Japanese people of drinking sugar-free green tea, because of the caries-inhibiting components contained in the tea, will significantly contribute to preserving and improving the oral health of the population. Onishi *et al.*³,⁴ reported epidemiological evidence of the caries-preventive effect of drinking tea. The effects of green tea extract on
caries inhibition in hamsters and on acid resistance of human tooth enamel have been investigated using caries scores and dissolved Ca measurement\(^5\). These studies revealed the effects of green tea on caries inhibition indirectly; however, observations of lesion progression affected by green tea using transversal microradiography (TMR), which is a standardized technique for evaluating demineralization and remineralization of teeth, have not been reported. The purpose of the present study was to clearly show the influence of green tea beverages on dentin demineralization using TMR measurements in vitro.

Materials and Methods

1. Measurement of fluoride concentrations
We measured the fluoride concentration in a bottled green tea beverage (Iemon, Suntory, Japan) and compared it to a control beverage, a canned sugar-free coffee beverage (UCC-Black, UCC, Japan). We mixed 0.5 ml of TISABIII buffer solution (940911, Thermo, Japan) with 5 ml of each sample. Fluoride in each sample was measured using an ion meter (9609 BN Ionplus, Thermo, Japan) with a fluoride ion electrode.

2. Preparation of specimens and demineralization procedures
Ten extracted bovine lower incisors were used. A diamond-coated wire-sectioning machine (Well type 3242, Walter Ebner, Germany) was used to separate roots from crowns, and each root was cut in half longitudinally. Experimental surfaces (approx. \(5 \times 3 \) mm\(^2\)) were prepared by cutting 1 mm from the buccal and lingual root surfaces with the same machine, and \(1 \times 3 \) mm\(^2\) windows were created with acid-resistant varnish. Experimental groups were 1) bottled green tea beverage, 2) sugar-free coffee beverage, 3) 0.8-ppm F solution as sodium fluoride (NaF), and 4) de-ionized water (Table 1). For the 0.8-ppm F solution, tenfold solution (8-ppm F) was prepared by using sodium fluoride powder (Wako, Japan), and it was diluted to 0.8-ppm F with de-ionized water. For each of the four experimental groups, we placed five randomly selected tooth sections at the bottom of a plastic container and immersed them in 120 ml of 8% methylcellulose gel (Methocel MC, Fluka, Switzerland) at 37°C for 24 hrs. We then poured 200 ml of the appropriate treatment solution (green tea, coffee, 0.8-ppm F solution, or de-ionized water) onto each gel layer.

After two weeks, demineralization was performed referring to the demineralization method of Ingram et al.\(^6\). The treatment solutions were replaced with demineralization solution (1.5 mmol/L calcium chloride (CaCl\(_2\)), 0.9 mmol/L potassium dihydrogen phosphate (KH\(_2\)PO\(_4\)), 0.1 mol/L acetic acid). The pH was adjusted to 5.0 with 1 mol/L potassium hydroxide (KOH). The demineralization process was allowed to continue for one week at 37°C (Fig. 1).

3. Microradiography
Three 300-\(\mu\)m-thick sections were cut perpendicularly to the experimental surface from each specimen using the same sectioning machine for specimen preparation. The sections were placed on a Perspex holder in a droplet of water and covered with a thin polyester sheet to avoid dentin shrinkage\(^7\). The sections were then radiographed on a high-resolution glass film plate (High-Resolution Glass Plate, Konica, Japan) with a nickel-filtered Cu-K\(\alpha\) source operating at 25 kV and 15 mA for 20 minutes (PW 3830, Spectris, UK) with a 13-step aluminum wedge ranging from 0 to 300-\(\mu\)m thickness. Radiographic images of the sections and aluminum step wedge were analyzed using a microscope/video-camera/microcomputer setup and software\(^8\) (TMR2000, Inspektor Research System, The Netherlands). Mineral loss values (\(\Delta Z\)) of the lesions were measured and the values of thin sections were averaged for each sample. On

<table>
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<th>Table 1</th>
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<td>mineral loss values ((\Delta Z): vol% (\times \mu)m)</td>
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<tr>
<td>Green tea (lemon)</td>
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<td>Coffee (UCC-Black)</td>
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<td>0.8-ppm F (NaF)</td>
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<td>De-ionized water</td>
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There was a statistical difference between values marked with different letters (\(p < 0.05\)). n = 5
TMR microradiograms, the volume percentage of mineral is plotted versus the distance from the outer surface. \( \Delta Z \) is the integrated difference between the microradiogram of the sample with mineral loss and that of the sound sample.\(^9\)–\(^{12}\) (Fig. 2).

4. Statistical analysis

Comparisons of fluoride concentrations between two groups were tested by the unpaired t test, and differences of \( \Delta Z \) among groups were tested by one-way ANOVA followed by Duncan’s multiple range test, using SPSS version 10.1 for significance at the level of \( p < 0.05 \).

Results

1. Amount of fluoride of the solutions

Data are the mean ± SD of 3 samples. The concentration of fluoride in the green tea beverage was 0.845 ± 0.051 ppm, whereas that in the sugar-free coffee beverage was 0.015 ± 0.001 ppm. A significant difference was detected between two groups at \( p < 0.05 \).

2. Mineral profiles and mineral loss by lesions

Figures 3 and 4 show representative TMR images and average lesion profiles of each treatment. Green tea, coffee, and fluoride solution treatments induced significantly higher radiodense surface layers compared with the deionized water treatment. In particular, the green tea treatment induced a thicker surface layer than the other treatments. The green tea treatment showed a significantly lower \( \Delta Z \) (3,485.2 \( \text{vol}\% \times \mu \text{m} \)) than the other three treatments (3848.6 – 4,836.8 \( \text{vol}\% \times \mu \text{m} \)) at \( p < 0.05 \) (Table 1).

Discussion

This study quantitatively showed that green tea beverage has the potential to inhibit dentin demineralization \textit{in vitro}. While it is possible to determine the acid resistance of human tooth
enamel by polarized light-microscopy and measuring the Vickers microhardness\textsuperscript{13}, these techniques do not precisely quantify the amount of mineral changes in these tissues. In the present study, we calculated the mineral loss value from TMR images. This evaluation technique, in which precise levels of mineral loss are shown as $\Delta Z$, is widely recognized as a standard method in demineralization and remineralization studies of both enamel and dentin\textsuperscript{7–12,14–19}.

Significant differences in $\Delta Z$ between the green tea treatment and others were detected by analyzing TMR images. As ten Cate\textsuperscript{20} has shown, sub-ppm levels of fluoride in saliva are effective in shifting the balance from demineralization to remineralization. Therefore, this level of fluoride in a green tea beverage will contribute to reduce the $\Delta Z$ against acid attack. The fluoride concen-
treatment of the bottled green tea beverage was almost 0.8 ppm. This value is similar to those reported by Friedman21 and Udijanto22. As mentioned above, the caries-preventive effects of fluoride are widely recognized, but to clarify whether the acid resistance was induced by fluoride in the green tea beverage, we made an experimental group with a solution of NaF whose fluoride concentration was 0.8 ppm. In the methylcellulose gel system for demineralization, each treatment solution was put on the gel and replaced with demineralization solution after two weeks. This ‘soaking’ period allowed the fluoride of the treatment solutions to penetrate into the gel. Tooth specimens from the green tea treatment showed a thicker surface layer than those from the NaF treatment. Moreover, the mean ΔZ of specimens in the green tea beverage treatment was significantly lower than that of specimens in the NaF treatment. This result suggests that fluoride in green tea mainly contributes to produce the anti-demineralizing effect, however some other components may also have roles. This is consistent with the previously reported finding that, in addition to fluoride incorporation, other components in tea may affect the dissolution rate of hydroxyapatite21. Yu13 reported that, besides fluoride, other effective components for caries inhibition were mainly composed of relatively large molecules such as tannin and catechin. Hayakawa23 reported that the combination of aluminum and fluoride ions in green tea may increase the acid resistance of hydroxyapatite and those very minor components of a green tea infusion and/or their interactions with other components play important roles in increasing the acid resistance of hydroxyapatite. The present results do not confirm which components influence the inhibition of dentin demineralization, but they suggest that frequent consumption of green tea beverages may contribute to the acid resistance of dentin, presumably due to the combined effects of sub-ppm fluoride levels. Ingestion of green tea beverages may help prevent root caries.

Conclusions

1. The fluoride concentration of sugar-free bottled green tea beverage (0.8 ppm) was significantly higher than that of coffee beverages.

2. The bottled green tea treatment showed a significantly lower ΔZ than the other three treatments (DW, coffee, and 0.8-ppm F solution).

3. TMR measurements clearly showed that the green tea beverage inhibited the progression of dentin lesions.

References


