Effect of Green Tea Beverages on Efficacy of Povidone-Iodine

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Povidone-iodine (PVP-I) gargling solution is used to prevent bacterial infection, but because of its unpleasant smell and metallic taste, people often drink a flavoring agent, such as green tea, immediately after gargling. Green tea beverages, however, have antioxidant activity and may decrease the antibacterial activity of PVP-I. In this study, we investigated the interaction between green tea beverages and PVP-I. Addition of O-i ocha® or O-i ocha koiaji® caused color changes of PVP-I from amber to yellow-brown or to orange-yellow, respectively. Ascorbic acid (0.03 mg/ml) caused no color change in PVP-I. The antioxidant activity was highest in O-i ocha koiaji®, followed by Hajime®, Nama cha®, O-i ocha®, and Flavancha® in that order. Sou ken bi cha® and ascorbic acid (0.03 mg/ml) showed poor antioxidant activity. All six green tea beverages that we examined inhibited the antibacterial activity of PVP-I towards *Streptococcus salivarius*, *Streptococcus mutans* (*S. mutans*) and *Streptococcus sanguis* (*S. sanguis*). O-i ocha koiaji® showed the most potent inhibitory activity. The antioxidant activity was significantly correlated with the minimum inhibitory dilution ratio of green tea beverages for *S. sanguis* ($r = 0.763$, $p < 0.05$) and *S. mutans* ($r = 0.854$, $p < 0.01$). These findings indicate that green tea beverages reduce the antibacterial activity of PVP-I in proportion to their antioxidant activity. In conclusion, beverages with antioxidant activity, such as green tea beverages, should not be consumed immediately after PVP-I gargling.

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INTRODUCTION

Consumption of green tea beverages has greatly increased following the introduction of polyethylene terephthalate (PET) bottles.[1] Green tea catechins have significant antioxidant, anticarcinogenic, anti-inflammatory, thermogenic, probiotic, and antimicrobial propertiesin, and green tea beverages are regarded as health-promoting.[2–7] On the other hand, povidone-iodine (Isodine®, PVP-I), a complex of iodine with 1-polyvinyl-2-pyrrolidone, has antibacterial activity owing to the oxidative activity of free iodine released from the complex.[8,9] As PVP-I has a broad antibacterial spectrum and strong bactericidal activity, it is often used to treat injuries to skin/mucosa, or infected skin.[10,11] It is also used as a gargling solution (Isodine® gargle) to prevent bacterial infection.[12] However, it can induce nausea and/or vomiting, especially in young children and patients undergoing treatment with anticancer drugs, because of its unpleasant smell and metallic taste.[9] Therefore, people often drink flavoring agents, such as green tea beverages, immediately after PVP-I gargling.

Sodium thiosulfate, a potent antioxidant agent, causes a significant color change and decreased antibacterial activity of PVP-I.[13–16] Catechins have an antioxidant activity, and so might also have an inhibitory effect on the antibacterial activity of PVP-I. The purpose of this study is to investigate the influence of green tea beverages on the antibacterial activity of PVP-I.

MATERIALS AND METHODS

Materials —— PVP-I (Isodine® gargle) was obtained from Meiji Seika Kaisha, Ltd. (Tokyo, Japan). Ascorbic acid and metaphosphoric acid were purchased from Sigma Aldrich Japan Co., Ltd. (Tokyo, Japan). 2-Morpholinoethanesulfonic acid (MES) was from MP Biomedicals, LLC (Ohio, U.S.A.). 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Trolox was from Wako Pure Chemicals Co., Ltd. (Osaka, Japan). Brain Heart Infusion Broth (BHI) was purchased from Nissui Pharmaceutical Co., Ltd. (To-

kyo, Japan).

All the bottled green tea beverages studied were obtained commercially: O-i ocha® and O-i ocha koiaji® from Ito En Co., Ltd. (Tokyo, Japan), Nama cha® from Kirin Beverage Co., Ltd. (Tokyo, Japan), Hajime® and Sou ken bi cha® from Coca•Cola National Beverage Co., Ltd. (Tokyo, Japan) and Flavancha® from Suntory Foods Co., Ltd. (Tokyo, Japan). *Streptococcus salivarius* ATCC9222 (*S. salivarius*), *Streptococcus mutans* OMZ175 (*S. mutans*) and *Streptococcus sanguis* ATCC10556 (*S. sanguis*) were gifts from the Laboratory of Bacteriology, Graduate School of Biomedical Sciences, Hiroshima University.

Effect of Green Tea Beverages on the Color of PVP-I —— According to the instructions of Isodine® gargle, 4 ml of PVP-I was diluted with 60 ml of distilled water to make PVP-I test solution.[17] The color change of PVP-I upon addition of 2 ml of green tea beverage or ascorbic acid (0.03 mg/ml) to 50 ml of PVP-I test solution was measured.

Measurement of Ascorbic Acid Concentration in Green Tea Beverages —— Ascorbic acid concentration in green tea beverages was determined by high-performance liquid chromatography (HPLC) (LC-10ADvp, Shimadzu, Kyoto, Japan). Briefly, 3 ml of green tea was mixed with 27 ml of 5% metaphosphoric acid filtered through a 0.45 μm filter. The filtrate was diluted 10 times with distilled water before injection into the HPLC system. The HPLC system consisted of a reverse-phase column [Shodex Asahipak, Tokyo, Japan, NH2P-50 4E (4.6 × 250 mm)] and an ultraviolet absorbance detector set at 254 nm. The mobile phase consisted of a mixture of 20 mM NaH_2PO_4 + 30 mM H_3PO_4 and acetonitrile (20 : 80 v/v), the flow rate was 1.0 ml/min, and the column temperature was 35°C.

Measurement of Antioxidant Activity —— Ascorbic acid was dissolved in 5% metaphosphoric acid buffer. Trolox was dissolved in ethanol. Green tea beverages were diluted appropriately with water.

Ascorbic acid (0.003 mg/ml) or 10-fold-diluted green tea beverage (30, 60, 90, 120 and 150 μl) was mixed with 200 mM MES buffer (pH 6.0, 500 μl) and 500 μl of 400 μM DPPH in ethanol (final concentration: 200 μM). The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance at 520 nm due to DPPH was measured with a SpectraMax® Plus^{384} spectrophotometer (Molecular Devices, Tokyo, Japan). We used Trolox as a control standard because it is a stable antioxidant that is widely used as an index of antioxidant activity.[18] The remaining antioxidant activity of green tea beverages was evaluated at 24, 48 and 72 hr after opening the bottles.

Effect of Green Tea Beverages on Antibacterial Activity of PVP-I —— The BHI plus PVP-I medium was prepared by diluting 4 ml of PVP-I to 30 ml with BHI.[17] The antibacterial activity was evaluated by adding 100 μl of serial dilutions of each green tea beverage or ascorbic acid (0.03 mg/ml) to 100 μl of the BHI plus PVP-I medium containing 2 × 10^5 cells/ml of bacteria on 96-well plates. The mixture was incubated at 37°C for 24 hr, and the antibacterial activity of PVP-I was evaluated in terms of the presence or the absence of colonies to identify the minimum inhibitory dilution ratio.

Statistical Analysis —— Data are presented as mean ± standard deviation (S.D.). The statistical significance of differences was evaluated by using analysis of variance (ANOVA) followed by a post hoc test. A value of $p < 0.05$ was considered significant.

RESULTS

Color Changes of PVP-I after Adding Green Tea Beverages

Color changes of PVP-I from amber to yellow-brown with O-i ocha®, to orange-yellow with O-i ocha koiaji®, and to blackish brown with Sou ken bi cha® were observed. Ascorbic acid (0.03 mg/ml) caused no significant change in the color of PVP-I (Fig. 1).

Concentration of Ascorbic Acid in Green Tea Beverages

The concentrations of ascorbic acid in the green tea beverages were measured by HPLC, and the values obtained (means ± S.D., $n = 5$) were 0.023 ± 0.003 mg/ml (Hajime®), 0.028 ± 0.001 mg/ml (Nama cha®), 0.025 ± 0.0002 mg/ml (O-i ocha®), 0.039 ± 0.004 mg/ml (O-i ocha koiaji®) and 0.038 ± 0.006 mg/ml (Flavancha®). The average was approximately 0.030 mg/ml. However, the concentration of ascorbic acid in Sou ken bi cha® was only 0.005 ± 0.001 mg/ml, much lower than the others ($p < 0.01$).

Antioxidant Activity in Green Tea Beverages

As shown in Fig. 2, the antioxidant activity of all green tea beverages increased almost linearly with increasing dose, up to 60 μl. The highest antioxidant activity was seen in O-i ocha koiaji®, followed by

Fig. 1. Effect of Some Green Tea Beverages on the Color of PVP-I

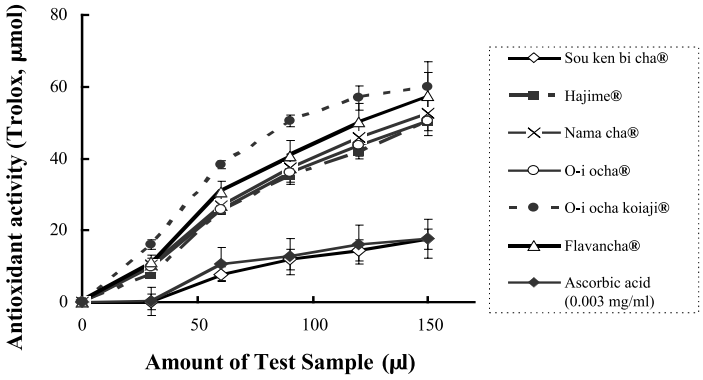

Fig. 2. Antioxidant Activities of Various Green Tea Beverages
Each point represents the mean ± S.D. of 5 experiments.

Hajime®, Nama cha®, O-i ocha®, and Flavancha®, respectively. Sou ken bi cha® and ascorbic acid (0.03 mg/ml) showed poor antioxidant activity.

None of the green tea beverages showed any significant change of its antioxidant activity for up to 72 hr after opening the bottle (data not shown).

Effect of Green Tea Beverages on Antibacterial Activity of PVP-I

PVP-I showed antibacterial activity towards *S. salivarius*, *S. mutans* and *S. sanguis*, and the green tea beverages inhibited this activity. Except for Sou ken bi cha®, all green tea beverages showed an inhibitory effect at 8- to 16-fold dilution and a few, including O-i ocha koiaji®, were effective even at 32-fold dilution (Fig. 3).

Correlation between Antioxidant Activity and Minimum Inhibitory Dilution Ratio of Green Tea Beverages

As shown in Fig. 4, there were significant correlations between the minimum inhibitory dilution ratio and the antioxidant activity of green tea beverages in the cases of *S. sanguis* ($r = 0.763$, $p < 0.05$) and *S. mutans* ($r = 0.854$, $p < 0.01$), though in the case of *S. salivarius* the correlation was not statistically significant ($r = 0.606$, $p < 0.10$).

DISCUSSION

It is considered that the antibacterial activity of PVP-I is due to the slow release of iodine, which shows a potent oxidative activity.[18,19] Therefore, chemicals with antioxidant activity are expected to reduce the efficacy of PVP-I. Indeed, it is well known that the color of PVP-I changes gradually from brown to yellow and then to colorless as increasing amounts of sodium thiosulfate are added, and sodium thiosulfate also decreases the antibacterial activity of PVP-I.[14,15] Three green tea beverages examined caused color changes of PVP-I. Because of the presence of starch in Sou ken bi cha®, it is considered that the iodine-starch reaction is responsible for the color change to blackish brown. O-i ocha koiaji® and O-i ocha® caused color changes to yellow-brown and orange-yellow, respectively. They are produced by the same company, and O-i ocha koiaji®

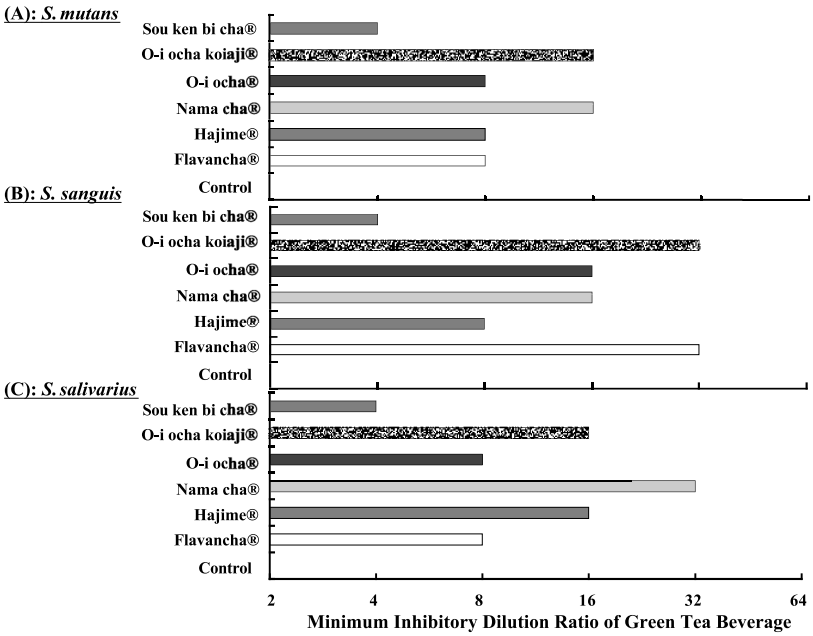

Fig. 3. Effect of Various Green Tea Beverages on Antibacterial Activity of PVP-I Towards *S. mutans* (A), *S. sanguis* (B) and *S. salivarius* (C)

Each column indicates the minimum inhibitory dilution ratio.

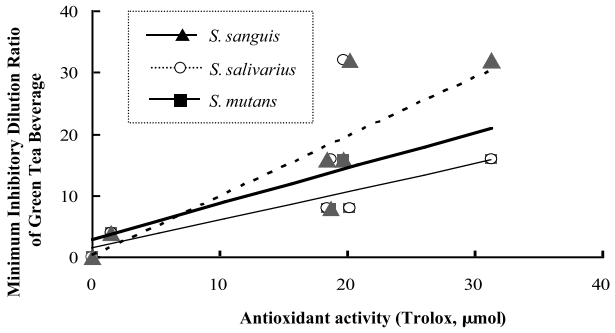

Fig. 4. Correlations between Antioxidant Activity and Minimum Inhibitory Dilution Ratio of Various Green Tea Beverages

S. sanguis (correlation coefficient $r = 0.845$, $p < 0.01$), *S. mutans* ($r = 0.867$, $p < 0.01$), *S. salivarius* ($r = 0.606$, $p < 0.10$).

is said to have a higher content of catechins than O-i ocha®. Ascorbic acid (0.03 mg/ml) caused no significant change in the color of PVP-I. Further, the ascorbic acid contents of all the green tea beverages except Sou ken bi cha® were similar, and close to 0.03 mg/ml. These results suggest that the difference in content of antioxidant catechins may be at least partly responsible for the observed difference between O-i ocha koiaji® and O-i ocha®. Therefore, it seemed likely that green tea beverages would inhibit the antibacterial activity of PVP-I.

In fact, all six different green tea beverages inhibited the antibacterial activity of PVP-I towards *S. salivarius*, *S. mutans* and *S. sanguis*, and O-i ocha koiaji® showed the most potent inhibition. The antioxidant activity was correlated with the minimum inhibitory dilution ratio of green tea beverages for *S. sanguis* ($r = 0.763$, $p < 0.05$) and *S. mutans* ($r = 0.854$, $p < 0.01$). These findings indicate that green tea beverages reduce the antibacterial activity of PVP-I in proportion to their antioxidant activity.

In conclusion, beverages with antioxidant activity, such as green tea beverages, should not be taken concomitantly with, or shortly after, PVP-I gargling solution.

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