Preventive Effect of Green Tea Polyphenols against Dental Caries in Conventional Rats

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The effects of green tea polyphenols, inhibitors of various biological activities of cariogenic bacteria in vitro, on caries development were examined using conventional rats. A total of 96 male rats were divided into eight groups and the rats in the test groups were given tea polyphenols ranging from 0.1% to 0.5% in their cariogenic diet or drinking water for 40 days. Total fissure caries lesions was significantly reduced by the addition of tea polyphenols to the diet or in the drinking water. Diet containing 0.1% tea polyphenols demonstrated about 40% reduction of total fissure caries lesions. No toxic effect of tea polyphenols on rats were observed under these experimental conditions.

It is likely that dental caries of both humans and animals are causally associated with the indigenous cariogenic streptococci in their mouths.1) The bacterial virulence factors responsible for their cariogenicity are recognized to use sucrose from food as a substrate for both growth and adherent insoluble glucan synthesis.2) Following to this concept, many investigators had attempted to remove the virulence factors of cariogenic streptococci by examining the effects of substituting sugars with sucrose such as xylitol,3) coupling sugar,4) and palatinose.5) Dextranase has also been used to reduce the formation of glucan from sucrose by the bacteria.6)

We have investigated the relationship between Japanese green tea, which is usually drunk after every meal as a custom in Japan, and dental caries. It was observed that tea polyphenols, the major components of green tea, are inhibitors of growth, glucan synthesis, and cellular adherence of cariogenic streptococci in vitro.7,8) Previously, some investigators examined the relationship between the contents of fluorides in tea and its preventive effects against dental caries in vitro,9) or in field tests.10) Most investigators found that fluorides are effective in preventing dental caries. However, since green tea extract was more effective than the fluorides tested, and the amount of fluorides in tea is insufficient to cause this effect, there is a possibility of the presence of an unknown substance responsible for the preventive activity against dental caries in the tea extract.11)

In our previous papers we described how (-)-epigallocatechin, (+)-gallocatechin, (−)-epicatechin gallate, and (−)-epigallocatechin gallate, the major components of tea polyphenols, inhibited various metabolic activities of the cariogenic streptococci in vitro. These activities include growth, glucan synthesis, and cellular adherence to glass surfaces.7,8) This study reports the effects of tea polyphenols on fissure caries induced by indigenous cariogenic bacteria, which are natural inhabitants of the mouths of conventional rats.

Materials and Methods

This study was carried out on 96 conventional NRC-Wistar male rats (Nihon Rat Ltd., Saitama, Japan). All animals were weaned 20 days after birth and randomly distributed into eight groups. After weaning, the rats were fed on a cariogenic diet (modified formula diet 200012) consisting of 30% sucrose, 26% corn starch, 28% skim milk powder, 6% whole wheat flour, 3% alfalfa powder, 4% brewer's yeast, 1% liver powder, and 2% sodium chloride. Tea polyphenols used in this study were “Sunphenon” (Taiyo Kagaku Co., Ltd., Yokkaichi, Japan), prepared from a hot-water extract of green tea.7) It contains mainly of (+)-catechin (2.9%), (−)-epicatechin (6.8%), (+)-gallocatechin (12.9%), (-)-epigallocatechin (16.5%), (−)-epicatechin gallate (6.6%), (-)-gallocatechin gallate (8.5%), and (−)-epigallocatechin gallate (21.3%). Other components were caffeine (9.9%), sugars (5.1%), amino acids and peptides (2.7%), ash (0.3%), and moisture. The amounts of Sunphenon blended in the powdered diet or dissolved in the drinking water were as follows: group 1 was the control group, groups 2, 3, and 4 had 0.1, 0.2, and 0.5% added to the drinking water, groups 5, 6, and 7 had 0.1, 0.2, and 0.5% added to the diet, and group 8 had 0.1% in the drinking water and 0.2% in the diet.

Fifteen or 40 days later, bacteria on their teeth (6 rats/group) were cultured as follows. A lower molar segment was swabbed for ten seconds with a sterile cotton swab, and this was suspended in 0.9 ml sterile phosphate-buffered saline water (pH 7.0). One-tenth ml of the appropriately diluted samples were plated on Mitis Salivarius medium (Difco, Detroit) containing 15% sucrose (MS) and MS with added bacitracin (0.2 units/ml) (MSB) medium.13) The plate were incubated at 37°C for 72 hr, and the characteristic colonies of cariogenic streptococci were counted on MSB medium and total streptococci colonies were counted on MS medium. The rats were kept in stainless steel cages with wire bottoms (4 rats/cage, 22±2°C and 55±10% RH). Diet and drinking water were available ad libitum, and the amounts consumed were measured weekly (diet) or daily (drinking water). On 40th day, the rats were killed and their jaws were removed. One half of the lower and upper jaws were soaked in neutral buffered formaldehyde solution (pH 7.0), and stained with 0.5% fuchsin solution for 4 hr. The fixed jaws were bisected with a steel saw. Caries was divided into three grades (A, enamel lesions; T, lesions reaching the dentinocemental junction, and B, advanced dentin lesions).14,15)

At the end of the experiment, blood biochemistry was analyzed; total

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**Table**: Experimental Design

<table>
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<th>30</th>
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<tr>
<td>Caries analysis</td>
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Fig. 1. Experimental Design.
protein, albumin/globulin, glucose, glutamate-oxaloacetate transaminase (GOT), and glutamate-pyruvate transaminase (GPT) were analyzed with the A/GB-Test Wako, Glucose B-Test Wako, and GPI UV-Test Wako (Wako Pure Chemical Industries, Ltd., Osaka).

The difference between the control group and the test groups were statistically evaluated using Student's t-test or the chi-square test.

**Results**

All the rats appeared healthy throughout the experiment. Mean (± SD) weight gains of rats in groups 1–8 were 173.72 ± 18.83 g, 184.18 ± 27.59 g, 177.63 ± 13.60 g, 157.06 ± 22.20 g, 165.80 ± 18.13 g, 178.51 ± 22.64 g, 162.23 ± 21.76 g, and 160.39 ± 26.30 g, respectively. Mean weight gains did not differ significantly between the control (group 1) and the tests (groups 2–8). The average daily intake of tea polyphenols were 15.8–106.0 mg/rat (Table I).

Furthermore, blood biochemical tests (glucose, albumin/globulin, total protein contents, GOT, and GPT) showed no significant difference between the control group and the test groups. Tea polyphenols preparations were not toxic to rats.

The number of total fissure caries lesions, the sum of enamel lesions (A), lesions reaching the dentinoenamel junction (T) and advanced dentin lesions (B), was significantly reduced by the addition of tea polyphenols to the diet or drinking water (Table II and Fig. 2). Furthermore, serious caries (T+B lesions) was reduced more than total caries (A+T+B lesions) in all the test groups. The group receiving 0.1% tea polyphenols in the diet demonstrated 37.7% and 46.2% reductions in A+T+B and T+B lesions, respectively. A visual comparison of teeth of rats fed on cariogenic diets with and without (control) tea polyphenols is shown in Fig. 3.

Because of the large variations in counts of bacteria in each group, there were no significant differences in the percentages of cariogenic streptococci to total Gram positive streptococci between the control group and each test group (Fig. 4). However, there was a strong trend indicating that the percentages became lower in the test groups at the final day (40th day) than the 15th day, especially groups 5 and 6.

**Discussion**

In recent years, much attention has been focused on various medicinal actions of green tea. These actions were analyzed pharmacologically and biochemically. Preventive effect of green tea against dental caries is one of these actions, and it has become a subject of considerable interest. It was previously found that 0.1% tea polyphenols inhibit the various biological activities of cariogenic streptococci in vitro. However, higher concentrations of tea polyphenols (at 0.1–0.5%) were used in this study since the mouth is an open environmental system. Higher concentrations were also necessary to overcome the dilution effect of saliva and foods in the mouth.

The rat dental caries were induced by the indigenous cariogenic bacteria when a sucrose diet was given. As shown in Fig. 2, the caries-inducing activity was markedly reduced by the addition of tea polyphenols in the diet or in the drinking water. Moreover, tea polyphenols in the diet were more effective than when in the drinking water. Such result may be caused by the longer stay of diet in the mouth in comparison with the drinking water. It is likely that the constant cariogenic challenge presented by the diet impacted into the sulci was overcome by tea polyphenols being present together.

Tea polyphenols are thought to affect the microbial flora.
of the mouth. In this experiment, although not statistically significant, the percentages of cariogenic streptococci in total Gram positive streptococci in plaque decreased substantially in all groups receiving tea polyphenols in the diet or in the drinking water on the final day in comparison with that on the 15th day. At the end of the experiment the percentages for the tests (groups 2—8) were all lower than that for the control group. While it is impossible to draw any conclusions about the effect of tea polyphenols on microbial colonization of the mouth, it does appear that tea polyphenols from the diet or drinking water can affect the plaque flora, especially the activity of cariogenic streptococci.

In this experiment, tea polyphenols did not cause any side effects detectable by visual inspection of rats throughout the experiment. Weight gains of rats fed on diet or drinking water containing tea polyphenols up to 0.5% were comparable to those in the control. However, in a preliminary test, 1% tea polyphenols in diet had no effect on body weight gains, while 1% tea polyphenols in drinking water reduced body weight gains. The bitterness of 1% tea polyphenols in drinking water reduced drinking, which might have affected feeding.

In summary, frequent daily intake of 0.1% tea polyphenols, when provided in a cariogenic diet containing sucrose or in the drinking water, significantly reduced fissure caries. This could be partially explained by a possible effect of tea polyphenols on weakening the growth and activities of cariogenic streptococci in the plaque. Green tea, which has been consumed as a drink in Japan for about 1000 years, is one of the safest drinks. The amount of tea polyphenols used in this study was observed to be not toxic. The result reported here strongly suggest that the use of tea polyphenols in foods might be effective in preventing dental caries.

References