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Improvement of periodontal status by green tea catechin using a local delivery system: A clinical pilot study

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The purpose of this study was to determine the usefulness of green tea catechin for the improvement of periodontal disease. The minimum inhibitory concentration (MIC) and bactericidal activity of green tea catechin against black-pigmented, Gram-negative anaerobic rods (BPR) were measured. Hydroxypropylcellulose strips containing green tea catechin as a slow release local delivery system were applied in pockets in patients once a week for 8 weeks. The clinical, enzymatic and microbiological effects of the catechin were determined. Green tea catechin showed a bactericidal effect against *Porphyromonas gingivalis* and *Prevotella* spp. *in vitro* with an MIC of 1.0 mg/ml. In the *in vivo* experiment, the pocket depth (PD) and the proportion of BPR were markedly decreased in the catechin group with mechanical treatment at week 8 compared with the baseline with significant difference. In contrast, PD and BPR were similar to the baseline and the value at the end of the experimental period in the placebo sites of scaled groups. The peptidase activities in the gingival fluid were maintained at lower levels during the experimental period in the test sites, while it reached 70% of that at baseline in the placebo sites. No morbidity was observed in the placebo and catechin groups without mechanical treatment. Green tea catechin showed a bactericidal effect against BPR and the combined use of mechanical treatment and the application of green tea catechin using a slow release local delivery system was effective in improving periodontal status.

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The development of gingivitis is accompanied by substantial increases in the number of Gram-negative anaerobic rods (1–5). Among them, *Porphyromonas gingivalis* and *Prevotella* spp. have been strongly implicated in periodontitis. Reduction of the periodontopathic microflora by scaling and supragingival plaque control can lead to an improvement of the periodontal status (6–8). Complete removal of plaque and calculus is more difficult in deep than in shallow pockets. Hence, the failure of periodontal treatment may be the result of plaque and calculus remaining after therapy (9, 10). Therefore, the use of drugs to treat plaque-associated periodontal diseases is required. Dental applications of local delivery and controlled drug release have been proposed. A number of reviews (11–13) addressed the local application of antimicrobial agents to the subgingival area for the treatment of periodontitis.

Green tea catechin has been reported to be useful for prevention of periodontal disease (14, 15). We have investigated the inhibitory effect of green tea catechin on collagenase activity (14) and our results have suggested that green tea catechin may be useful for prevention of periodontal disease.

In the present study, we studied the effect of green tea catechin against black-pigmented, Gram-negative anaerobic rods (BPR) and the usefulness of green tea catechin applied using a slow-release local delivery system with commercial hydroxypropylcellulose (HPC) as a carrier for improvement of periodontitis.

Material and methods

Green tea catechin and chemicals

The green tea catechin used in this study was well-purified Sunphenon® (Taiyo Kagaku, Yokkaichi, Mie, Japan) prepared from Japanese green tea (16). HPC was purchased from Wako Chemical (Tokyo, Japan).

Assay of minimum inhibitory concentration (MIC)

Prevotella intermedia ATCC25611, *Prevotella nigrescens* ATCC33563 and ATCC25261 and *P. gingivalis* ATCC-33277 and 381 were used in this study. The MIC to each test bacterial strain in green tea catechin was measured by the liquid culture medium dilution method using GAM (Nissui, Tokyo, Japan) supplemented with hemin (1 μg/mL) and menadione (0.5 μg/mL). Exponential-phase cells (approximately 1×10^6) were added to GAM broth containing 250, 500, 1000 or 2000 μg of green tea catechin per mL and incubated for 48 h in an anaerobic glove box (Forma Scientific anaerobic system model 1024). Antibacterial activity was determined by calculation of colony forming units (CFU)/mL. The minimum green tea catechin concentrations at which no increase was observed in initial inoculated bacterial number on brucella agar (Difco Laboratory, Detroit, MI, USA) with 5% rabbit blood containing hemin, menadione and 0.1% kanamycin sulfate (BBK) plates were defined as the MIC.

Bactericidal effect of green tea catechin

The procedure of bactericidal test has been described previously (17). Briefly, growing bacterial cells were collected, washed three times with reduced transport fluid (RTF) (18) and suspended to an OD_{540} of 1.0. Samples (10 μL) of the cell suspension were added to 2 mL of RTF containing 8 times the MIC of green tea catechin. The mixtures was incubated for 30, 60, 90 and 120 min at 37 °C anaerobically, and then samples of the mixture were immediately diluted and spread on BBK plates. The number of CFU was counted after 5 days of incubation at 37 °C under anaerobic conditions.

Preparation of HPC strips

HPC powder with or without 5% green tea catechin was dissolved with ethanol and lyophilized. The dry sheet was cut into strips 2 mm in width, 4 mm in length and 0.3 mm in thickness.

Dissolution test of green tea catechin from HPC strips

In vitro dissolution tests were performed according to the procedure of Noguchi *et al.* (19). Briefly, 300 mL of distilled water containing the test strip 2.5 cm in width, 5.0 cm in length and 0.3 mm in thickness was rotated at 37 °C, 100 r.p.m. The green tea catechin released into the solution was determined by monitoring absorbance at 277 nm using a spectrophotometer at selected intervals. The cumulative amount of green tea catechin released was calculated at each time point.

Subjects

The subjects were six volunteers (three males and three females, 41–64 years of age) with advanced periodontitis, but with no systemic disorders. From each volunteer who had a pair of deep pockets bilaterally, two pockets were selected; one for administration of the test agent and the other for placebo. The pocket depths (PD) were approximately 5 mm. PD was measured to the nearest millimeter using a standard periodontal probe. All subjects gave their informed consent to participation in this study.

Experimental schedule

The subjects were divided randomly into the scaled and non-scaled groups, three subjects each. Tooth scaling and root planing were performed after determining basal peptidase activity, PD and microbial assay in the scaled group. In the other three subjects in the non-scaled group, tooth scaling and root planing were not performed during the experimental period. The test or placebo strips were inserted into the pocket of subjects in both the scaled and non-scaled groups. The measurement of peptidase activity and the insertion of strips were performed every week until week 7. At the end of the experimental period, week 8, microbiological tests and the determination of PD and peptidase activity were performed.

Microbiological examination

Gingival crevicular fluid (GCF) was obtained using paper points placed in each site for 10 s, placing these quickly in 500 μL of RTF, and then immediately in an anaerobic glove box. Each sample was sonicated, diluted, and plated on BBK without kanamycin for total bacteria. The plates were incubated at 37 °C for 7 days. Growing colonies were counted and total bacterial numbers were determined. BBK medium was used for BPR.

Measurement of peptidase activity

Peptidase activity was measured using the SK-013 method (20), using commercial Periocheck periodontal diagnostic kits (Sunstar Inc., Takatsuki, Japan). The procedure has been described previously (21). Briefly, three paper points were placed in the pocket for 30 s, then transferred into a small vial containing substrate, chromogen and ascorbic oxidase solution, followed by agitation for 10 s. The mixture was then incubated at 37 °C for 15 min. The optical density was measured spectrophotometrically at 666 nm. Enzymatic activity was calculated from a standard curve and recorded as trypsin units (U/mL).

Statistical analysis

Data shown are from three separate experiments and were analyzed statistically by calculating means and standard deviations of the mean. Differences among experimental sites were evaluated by Student's *t*-test.

Results

Antibacterial activity of green tea catechin

Determination of MIC The MICs of green tea catechin against *Prevotella* spp. and *P. gingivalis* are shown in Table 1. The MICs for *P. gingivalis*, *P. intermedia* and *P. nigrescens* were 1.0 mg/mL.

Table 1. MIC of green tea catechin to *Porphyromonas* and *Prevotella* spp

Microorganism	MIC (mg/mL)			
	0.25	0.5	1	2
P. intermedia ATCC25611	+ *	+	–	–
P. nigrescens ATCC33563	+	+	–	–
P. nigrescens ATCC25261	+	+	–	–
P. gingivalis 381	+	+	–	–
P. gingivalis ATCC33277	+	+	–	–

* + : growth; –: no growth.

Bactericidal activity The antibacterial effects of green tea catechin on resting bacterial cells of *P. gingivalis* ATCC33277, *P. intermedia* ATCC25611 and *P. nigrescens* ATCC33563 were investigated. Resting bacterial cells from these three strains were killed linearly by green tea catechin within 120 min. The time course curve of *P. intermedia* ATCC25611 as a representative strain is shown in Fig. 1A. Figure 1B shows the bactericidal activity curve of *P. intermedia* after 90 min treatment with a different concentration of green tea catechin. The antibacterial effect was dependent on concentration. *P. gingivalis* and *P. nigrescens* showed similar results to *P. intermedia*: Green tea catechin showed bactericidal effects against all three bacteria.

Effects of green tea catechin on periodontal disease

In vitro green tea catechin release from HPC strips More than 80% of green tea catechin was released from HPC strips within 2 h. Complete release was obtained within 4 h (data not shown).

Examination of clinical parameters The average PD in the test sites of the scaled group was reduced from 4.7 mm at baseline to 3.3 mm at week 8. In contrast, PD showed no change at the end of the experiment in the placebo sites of scaled group and the placebo (data not shown) and test sites of non-scaled groups (Table 2).

Measurement of peptidase activity Changes of peptidase activity are shown in Fig. 2. Peptidase activity was 0.36 U/mL at baseline in the test sites of scaled group. The value decreased to

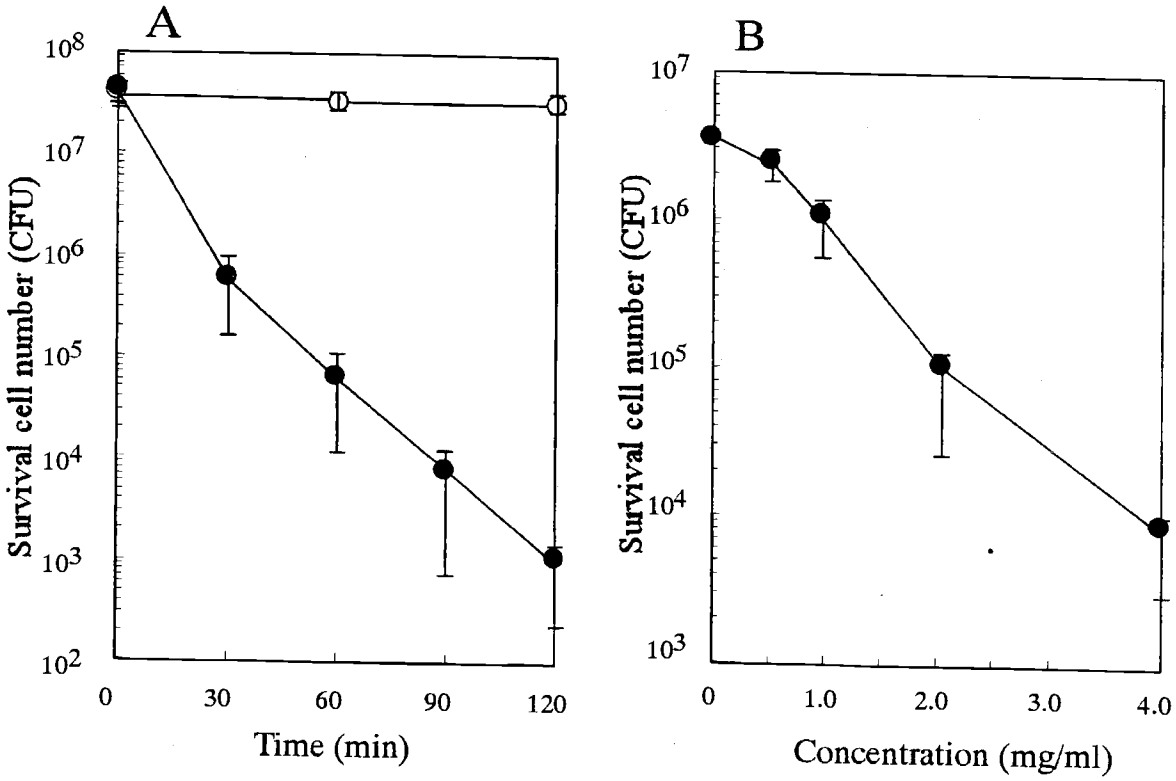

Fig. 1. Bactericidal effect of green tea catechin on *P. intermedia* ATCC25611 cells. A. Time course curve. ●: with 4 mg/mL catechin; ○: without catechin. B. Survival cell number after 90 min treatment with various concentration of green tea catechin. Bar indicates standard deviation.

Table 2. Changes of pocket depth by the application of HPC strips with or without green tea catechin in periodontitis

	Pocket Depth (mm ± SD)		
	Non-scaled group	Scaled group	
Period	Test site	Placebo site	Test site
Baseline	4.9 ± 0.6	5.0 ± 0.8	4.7 ± 0.8
Week 8	5.3 ± 0.9	4.9 ± 0.8	3.3 ± 0.5 *

*$P < 0.05$.

0.09 U/mL at week 1 after scaling and root planing and remained at a low level until the end of the experiment. In the placebo sites of scaled group, peptidase activities at baseline and at week 1 were similar to those in the test sites. However, the value increased slowly from week 4 and was markedly elevated at week 8. Finally, peptidase activity in the placebo sites of the scaled group reached 70% of that at baseline. In the non-scaled group, in both the placebo and the test sites, peptidase activity remained almost the same as baseline during the experimental term. At the end of the experiment, no decrease in activity was observed.

Microbiological examination Numbers of total cultivable bacteria and BPR are shown in Table 3. Changes in percentage of BPR in total cultivable bacteria are shown in parentheses in Table 3. The ratio of BPR was 10.6% at baseline, and was markedly decreased to 0.02% at the end of the experimental period in the test sites of the scaled group. However, in the placebo sites of the scaled group, the ratio of BPR was similar to the baseline and the value at the end of the experimental period. Moreover, the ratio of BPR increased from 8.7% to 11.3% at week 8 compared with baseline in the test sites of the non-scaled group.

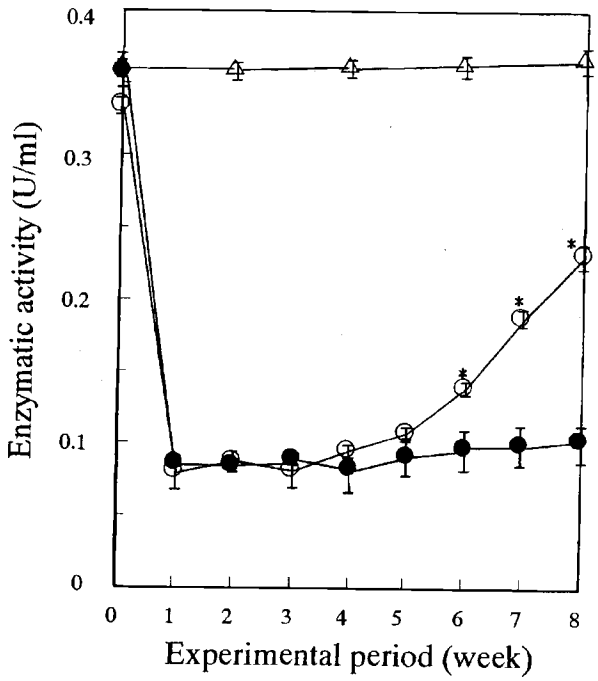

Fig. 2. Time course of changes in peptidase activity (U/mL) for test sites of scaled group (●), placebo sites of scaled group (○) and test sites of non-scaled group (△). Bar: standard deviation; *Significant difference at $P < 0.01$.

Discussion

There have been many reports (1–5) that periodontal disease is associated with complex microflora. Members of the BPR are known to be associated with various forms of destructive periodontal disease (1–4). BPR have been shown to have pathogenic potential and produce virulence factors that may play important roles in periodontal breakdown (1–4). Thus, antibiotics such as tetracycline or minocycline have been used experimentally to eliminate or reduce the pathogenic BPR associated with periodontal disease (6, 22, 23). In the course of our investigations of natural substances, which have the potential of effectively preventing periodontitis (14, 17) and dental caries (24, 25), we have focused on green tea, which is commonly drunk in the world especially in Asia. Sunphenon®, which is prepared from Japanese green tea and consists of catechin (16), inhibits collagenase activity (14), the growth of *Streptococcus mutans* (16) and the adherence *P. gingivalis* to oral epithelial cells at concentrations below 0.25 mg/mL (15). The antibacterial effects of green tea catechin on BPR showed bactericidal action in this study (Fig. 1).

The problems of administering drugs systemically, such as adverse reactions and appearance of antibiotic-resistant bacteria, have focused attention on methods of applying the drug directly to the target site (26). Antimicrobial controlled-release and local delivery systems using several carriers for the treatment of periodontitis have been reviewed (11–13). We have focused on HPC as a material for slow release of green tea catechin into the subgingival pocket. HPC strips containing tetracycline or chlorhexidine were inserted into the periodontal pocket for treatment of periodontitis (19). HPC is a water-, methanol- and ethanol-soluble white powder with properties of plasticity and no toxicity. Noguchi *et al.* (19) used HPC strips as a slow release and local delivery system and reported that tetracycline was released completely from HPC strips within 4 h *in vitro*, and that the drug

Table 3. Colony counts of total cultivable bacteria and BPR from periodontal pockets at baseline and week 8

	Number of bacteria, CFU/mL, × 10^6, mean ± SD					
	Non-scaled group		Scaled group			
	Test site		Placebo site		Test site	
Period	Total	BPR	Total	BPR	Total	BPR
Baseline	20.6 ± 8.3	1.8 ± 1.0 (8.7)*	15.3 ± 4.5	1.3 ± 0.4 (8.5)	25.5 ± 1.5	2.7 ± 1.6 (10.6)
Week 8	22.1 ± 10.5	2.5 ± 0.9 (11.3)	15.7 ± 7.0	1.1 ± 0.6 (7.0)	17.9 ± 11.1	0.003 ± 0.003 (0.02)**

*Parentheses: Percentage against total bacteria.
**BPR at week 8 in test site of scaled group was significantly less ($P < 0.01$) than BPR at baseline in test site of scaled group and at week 8 in test site of non-scaled and placebo site of scaled groups.

remained in the periodontal pocket for at least 24 h *in vivo*. We obtained similar *in vitro* results (data not shown). Catechin can be maintained at an effective concentration in the periodontal pocket, although we could not determine the concentration of catechin in the pocket because of the lack of a suitable assay method. The improvements of clinical parameters and the results of peptidase activity and bacterial experiments indicated that green tea catechin continued to be released from HPC strips into the subgingival pocket until the end of the experimental period. The ratio of BPR to total cultivable bacteria in the test sites of scaled group remained low during the experimental period. The result suggests that green tea catechin may be absorbed by the oral epithelial cells in the subgingival pocket and may inhibit the growth of BPR for 24 h. In the non-scaled group without tooth scaling and root planing, peptidase activity, PD and BPR number were not improved during the experimental period. These results suggest that the development of periodontitis may partly prevent by regular tooth scaling and root planing.

BPR produce tissue destructive enzymes such as collagenase and peptidase. These enzymes may play a role in destroying the gingival tissues, in osteoclast breakdown and in the progress of the development of periodontitis. Golub *et al.* (27) reported that tetracycline, by directly inhibiting collagenolytic enzymes activity, may be therapeutically useful in treating diseases such as periodontitis characterized by excessive collagen breakdown. We have reported previously (14) that green tea catechin inhibited collagenase activity in the GCF *in vitro* and green tea catechin treatment of collagen inhibited its hydrolysis by collagenase. Continuous application of green tea catechin on a daily basis may be a useful and practical method for the prevention of periodontal disease. It will be necessary to carry out long-term observations on the effects of the green tea catechin. The development of a new ointment base that can maintain long-term effectiveness in periodontal tissue by one-time drug administration is expected. The slow release and local delivery system described here using HPC strips containing green tea catechin is an effective method for improvement of periodontitis.

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