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# Effect of Japanese Green Tea Extract on Canine Periodontal Diseases

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Asaccharolytic pigmented *Porphyromonas* strains were isolated from the plaque of dogs with gingivitis and periodontitis. Various species of *Porphyromonas*, including *P. endodontalis*, *P. gingivalis*, *P. circumdentaria* and unclassified species, were detectable. Canine *Porphyromonas* were sensitive to Japanese green tea extract (JGTE). We examined the effects of dietary JGTE on periodontal diseases. A special diet was prepared on the basis of the minimum inhibitory concentration (MIC: 0.8 mg/ml) of JGTE to several canine *Porphyromonas* species. Growth of all *Porphyromonas* isolates was inhibited at this concentration. After 2 mths, the percentage of canine *Porphyromonas* significantly decreased in the plaque microbiota. Concurrently with the observed decrease in percentage of these bacteria, gingival inflammation was inhibited. However, no change in the calculus index was recorded during the observation period. Levels of oral malodour varied among the dogs and diet with JGTE was effective in the inhibition of oral malodour. We concluded that JGTE was effective in the inhibition of canine periodontal diseases.

KEY WORDS: green tea extract; canine *Porphyromonas*; periodontal disease.

## INTRODUCTION

It has been reported that periodontal diseases are frequently found in dogs.[7,16] Asaccharolytic pigmented *Porphyromonas* species are predominant isolates from the plaque of dogs with gingivitis and periodontitis.[6,8,16] Recently, the asaccharolytic pigmented *Porphyromonas* have been divided into the following species; *P. asaccharolytica*, *P. endodontalis*, *P. gingivalis*, *P. salivosa*, *P. circumdentaria*, and *P. canoris*.[12]

The current view of periodontal disease is that different states of gingival health are correlated with different subgingival bacterial floras.[9,17,18] Slots and Listgarten[18] reported that *P. gingivalis* levels were elevated in active sites in humans and that monitoring these bacteria in advanced periodontal lesions might greatly augment the assessment of treatment efficacy and the risk of further pocket breakdown. Thus, prevention and treatment of the disease have targeted specific bacteria such as *P. gingivalis*.

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Japanese green tea contains polyphenolic compounds (PC) such as tannins.[5,15] These compounds exist naturally in plants, and they possess potentially valuable properties.[3,10,13,15,19] Canine periodontal pathogens such as *P. asaccharolytica* (reclassified as *P. circumdentaria*) may be sensitive to the JGTE, as are *P. gingivalis* human isolates.[5] It is possible that the JGTE could be useful as an anti-periodontitis agent via microbiota control in dogs.

In this study, we tried to inhibit periodontal diseases through diet containing JGTE. This report describes the effects of JGTE which include inhibition of both gingival inflammation and oral malodour. In addition, the percentages of *Porphyromonas* in the subgingival microbiotas were significantly decreased.

## MATERIALS AND METHODS

### *Subjects*

Twelve adult dogs (four German Shepherd, three Labrador Retriever, three Golden Retriever, one Pekinese, one Yorkshire Terrier) were studied.

Their mean age was 3·1 ± 2·6-yrs-old (1·0- to 8·6-yrs-old). Ten dogs were affected with periodontal diseases (seven dogs had mild/moderate inflammation of the gingiva and other three dogs had severe inflammation of the gingiva with natural bleeding). Two dogs had healthy gingivae.

*Polyphenolic compounds from Japanese green tea and preparation of diet*

PC were extracted from the leaf of *Camellia sinensis* with 95 per cent hot ethanol. After filtration and charcoal treatment, samples were analysed for composition by high-performance liquid chromatography and gas chromatography, as previously described.[5] Various oral bacteria including the genus *Porphyromonas* were used for determination of minimum inhibitory concentration (MIC).[5] On the basis of the MIC results, a special diet with JGTE (0·8 mg/g) was prepared by Funabashi Farm Co. (Chiba, Japan).

*Identification and examination of genus* Porphyromonas

Subgingival plaque was taken from the maxillary premolars of the dogs with a scaler. Each specimen was immediately placed in sterile oxygen-free tubes containing 10 ml of GAM broth (Nissui Co., Tokyo, Japan). GAM broth containing each specimen was placed in an anaerobic glove box (Hirasawa Co., Tokyo, Japan) with 80 per cent $N_2$–10 per cent $H_2$–10 per cent $CO_2$, as previously described.[6] The diluted suspension of each sample was plated on Brain heart infusion agar (Difco) with 7 per cent horse blood for total counts, and Brucella HK agar (Kyokuto Co., Tokyo, Japan) with 7 per cent horse blood for counts of genus *Porphyromonas* as black-pigmented bacteria. Identification of representative colonies was carried out using API 20A and API-ZYM systems (API system S. A., Montalieu, France). The count of the *Porphyromonas* was calculated from the number of colonies multiplied by the dilution factor. The total viable count and the percentage of *Porphyromonas* in the microbiota was calculated.

*Inhibitory effect of Japanese green tea extracts on growth of* Porphyromonas

The growth inhibitory effect of PC was examined at the concentration of 0·8 mg/ml as previously described.[5]

*Parameters of periodontal disease*

The degree of periodontal disease was estimated according to the criteria of the Gingival Index (GI) system by Löe and Silness[11] with some modifications described previously.[7] Gingival inflammation was examined on the buccal aspect of all teeth.

Formation of dental calculus was rated by means of the calculus index system which expresses the degree of oral hygiene in an index, described previously.[7] This method was a modified index system of the Oral Hygiene Index (OHI) by Green and Vermillion.[2]

Measurement of oral malodour was determined using a volatile sulphur compound sensor and methyl mercaptan standard (Tokuyama Soda Co., Tokyo, Japan) to assess oral malodour directly from three points in the oral cavity (e.g., on the dorsum of the tongue and two sites (right and left) near the specific periodontal pockets of maxillary premolar). Individual data were expressed as the mean. Production of volatile sulphur compounds from isolated *Porphyromonas* was also examined by the sensor.

*Experimental protocol*

All dogs were fed the commercial control diet (ES, Funabashi Farm Co., Chiba, Japan) for 1 mth, prior to being put on the experimental diet containing JGTE (0.8 mg/g) for 2 mths. Dogs were given the feeds twice daily during the test period.

The percentage of the genus *Porphyromonas* in the subgingival flora, Gingival Index, Calculus Index, and oral malodour were examined before and after feed of the diet with JGTE.

*Statistical analysis*

Wilcoxon (matched-pairs) signed-ranks test was used for comparison between groups of control diet and diet with JGTE. Sample variance of oral malodour was too large in control diet group to analyse the data, because the machine can be calibrated for oral malodour at the concentration ranged from 0·00 to 9·99 ppm (maximum level/minimum level÷1000). Therefore, statistical analysis for oral malodour level was done after logarithmic conversion.

## RESULTS

*Detection of genus* Porphyromonas *during the control diet period*

Various strains of the *Porphyromonas* (*P. endodontalis*, *P. gingivalis*, *P. circumdentaria* and

Table 1. Selected characteristics of isolates of the genus *Porphyromonas* which may enable differentiation of species: comparison of canine isolates with known canine, feline and human members of the genus

| Strain | Selected characteristics* 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | No of isolates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *P. asaccharolytica* ATCC 25260 | − | − | − | − | − | − | − | + | | | |
| *P. endodontalis* ATCC 35406 | − | − | − | − | − | − | − | − | | | |
| *P. gingivalis* ATCC 33277 | − | − | + | − | − | − | + | − | | | |
| *P. gingivalis* VPB 3492 | + | − | + | − | − | − | + | − | | | |
| *P. salivosa* NCTC 11632 | + | + | + | + | + | − | + | − | | | |
| *P. circumdentaria* NCTC 124969 | + | − | − | − | − | − | − | − | | | |
| *P. canoris* NCTC 12835 | + | − | − | + | − | + | + | − | | | |
| Isolates as *P. endodontalis* | − | − | − | − | − | − | − | − | + | + | 27 |
| Isolates as *P. gingivalis* | − | − | + | − | − | − | + | − | + | + | 3 |
| Isolates as *P. circumdentaria* | + | − | − | − | − | − | − | − | + | + | 8 |
| Unclassified isolates group A | − | + | + | − | − | − | − | − | + | + | 3 |
| Unclassified isolates group B | − | − | + | + | − | − | + | − | + | + | 3 |
| Unclassified isolates group C | − | − | − | − | − | − | + | − | + | + | 3 |

*Selected characteristics 1–8: *Int. J. Syst. Bacteriol.* 44: 204–208, 1994 by D. N. Love *et al.*
1: catalase activity, 2: lipase activity, 3: Trypsin-like activity, 4: Chymotrypsin activity, 5: α-galactosidase activity, 6: β-galactosidase activity, 7: N-acetyl-β-glucosaminidase activity, 8: α-fucosidase activity, 9: production of volatile sulphur compounds, 10: sensitive to JGTE (0·8 mg/ml).

Table 2. Effects of Japanese green tea extracts on various parameters in dogs with obvious periodontal diseases

| Parameters | Means ± SE in dogs fed: Control diet | Diet with JGTE | *P** |
|---|---|---|---|
| Percentage *Porphyromonas* | 38·5 ± 6·1 | 15·6 ± 5·2 | <0·01 |
| Gingival index | 0·51 ± 0·12 | 0·13 ± 0·02 | <0·01 |
| Calculus index | 0·87 ± 0·18 | 0·82 ± 0·18 | NS |
| Oral malodour (ppm) | 3·05 ± 1·25 | 0·42 ± 0·06 | <0·01† |

*Wilcoxon (matched-pairs) signed-ranks test was done. NS: not significant.
†Statistic analysis was done after logarithmic conversion.

unclassified species) were detectable during the control diet period (Table 1). Almost all of the black-pigmented bacteria belonged to the genus *Porphyromonas*, especially those from the plaque samples of dogs with gingival inflammation (GI>0·2). They were indole-positive and asaccharolytic in the API 20A system. Forty-seven *Porphyromonas* isolates were obtained from subgingival plaque of dogs with gingivitis/periodontitis. They were divided into six groups by the selected characteristics in API-ZYM system (Table 1). *P. canoris* could not be isolated from subgingival plaque. All isolates produced volatile sulphur compounds. All of them were sensitive to JGTE at the concentration of 0·8 mg/ml (Table 1). This value has been previously reported to be the MIC of several strains of *Porphyromonas*.[5]

*Effects of diet with JGTE on periodontal disease*

Diet with JGTE (0·8 mg/g) was prepared on the basis of previously reported MIC results. Before feeding the diet with JGTE, dogs were fed a control diet. After 2 mths, the percentage of canine *Porphyromonas* significantly decreased (Table 2), as did the degree of gingival inflammation (Table 2). However, the calculus index was constant for the observation period (Table 2).

*Effect of diet with Japanese green tea extracts on oral malodour formation*

The level of oral malodour varied among the dogs fed the control feed and it seemed that the variation of oral malodour was associated with the presence of the genus *Porphyromonas* (data not shown). Diet with JGTE was effective in the inhibition of oral malodour (Table 2). All isolates (strains of *Porphyromonas*) produced volatile sulphur compounds (Table 1). Gas obtained from 24 h culture (GAM liquid medium) included the volatile sulphur compounds in high level (>10 ppm: higher than the values for which the machine is calibrated).

## DISCUSSION

The present study investigated the effects of diet containing 0·8 mg/g JGTE on canine periodontal disease. The diet program was controlled and the diet showed potentially valuable anti-periodontopathic activities in dogs. The effect can associate inhibition of bacterial growth, anti-inflammation and inhibition of oral malodour. In the first step, growth inhibition of periodontopathic bacteria such as genus *Porphyromonas* may occur. Reductions in gingival inflammation and oral malodour may result from changes in the composition of the oral microbiota.

The JGTE were found to effectively inhibit the growth of canine oral bacteria *in vitro*.[5] The results suggest that the inhibitory effect on *Porphyromonas* species may be caused by the direct inhibition of its growth. Furthermore, the green tea extracts were not only effective against *Porphyromonas* species but also to other canine oral bacteria.[5] The inhibitory effect of plaque deposition could be induced by the diet with JGTE.

A number of oriental medicinal plants such as Japanese green tea, Chinese green tea, oolong tea, and black tea, are rich in tannins. Various tannins have been isolated from these plants, and some of their effects on coexisting substances, including antibacterial effects, are well known. Ooshima *et al.*[14] examined the inhibitory effect of oolong tea extract containing polymerised polyphenols on plaque deposition in human volunteers. They suggest that the inhibitory effect on human plaque deposition may be caused by the inhibition of glucan synthesis and not by the antimicrobial effect of oolong tea extract. There are many differences between the present study and that reported by Ooshima *et al.*,[14] including: (1) the use of dogs or human volunteers, each of which has a different oral microbiota; (2) the use of different varieties of tea; (3) differences in dietary regime; (4) length of test period; (5) disease measured (if periodontal disease or dental caries). However, polyphenolic compounds in various tea preparations were found to alter the oral microbiota in both studies and may be useful in the prevention of oral diseases.

PC in green tea extracts contained five major compounds: ( − )-epigallocatechin gallate (EGCg), ( − )-epigallocatechin (EGC), ( − )-epicatechin gallate (ECG), ( − )-epicatechin (EC), D-(+)-catechin.[5] It has been shown that several PC such as EGC, EGCg possesses strong bactericidal as well as antibacterial activities. A common characteristic of these components is the presence of a galloradical (pyrogallol).[15]

Love *et al.* placed several groups of the organisms in the genus *Porphyromonas* from the canine oral cavity. One group, which comprised 12 of the 259 phenotypically characterised isolates, was recently described as a new species (*P. canoris*).[12] Recently, further new species, *P. cangingivalis* and *P. cansulki*, were reported by these authors.[1] At about the same time, two new species, *P. gingivicanis* and *P. crevioricanis*, were proposed for black-pigmented, asaccharolytic, anaerobic, gram-negative, rod-shaped organisms isolated from the gingival crevicular fluids of beagles.[4] Our unclassified isolates appeared to be different from these species on the basis of selected characteristics using the API-ZYM system. The genus *Porphyromonas* is clearly a heterogeneous one and encompasses a diverse range of isolates from distinct areas, distinct canine species, or distinct disease types.

Oral malodour originates in the oral cavity as the results of microbial metabolism.[20] Many of the isolates obtained in this study produced strong malodour when grown in the laboratory. Oral malodour was clearly inhibited by the diet with JGTE. The reduction of oral malodour may be related to inhibition of bacterial metabolism. Diet is made up of a complex mixture and various commercial diets have been used for dogs. Canine periodontal diseases are often considered to be diet-related disorders and a soft diet favours rapid accumulation of dental plaque, which may result in periodontitis. Application of green tea extracts to diet may be a useful method for the prevention of canine periodontal diseases.

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