NOTE *Surgery*

Inhibitory effect for proliferation of oral bacteria in dogs by tooth brushing and application of toothpaste

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ABSTRACT. To investigate inhibitory effect for oral bacterial proliferation, we divided 12 dogs into 3 groups; scaling alone (C; control group), brushing (B) and application of toothpaste (P). Before scaling (Pre) and at 0 to 8 weeks after scaling (0–8 w), we collected oral bacteria from the dental surface every week and counted them using a bacterial counter. The results demonstrated a significant reduction in the number of oral bacteria for group B relative to Pre and group C, as well as for group P relative to group C at 5–7 w. Consequently, brushing may inhibit an increase in the number of oral bacteria, and toothpaste may be effective at a certain level, although not more than that of brushing.
KEY WORDS: dog, lactoperoxidase system, oral bacteria, tooth brushing, toothpaste

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The common procedure by a veterinarian to prevent periodontal diseases in dogs is scaling [1]. However, without some following oral care, the deposition of calculi immediately starts, and the oral cavity environment deteriorates again. Consequently, long-term preventive efficacy of periodontal diseases by only scaling can't be expected [5]. Therefore, oral care by owners after scaling is important to prevent the deposition of plaque and calculi as much as possible [5]. Tooth brushing is considered to be the most effective method to prevent periodontal diseases for dogs [1, 7]. However, in the present circumstances, the tooth brushing by owners has not yet prevailed. Therefore, alternative oral cares which are easier and less stressful for dogs than tooth brushing are needed.

As one of the means, there is the toothpaste which has the effect to inhibit the activity of oral bacteria by supporting the one of the innate defense mechanisms in saliva; lactoperoxidase system [9, 10].

In this study, as the oral care after scaling, the efficacy of daily tooth brushing and application of toothpaste were verified by measuring the number of oral bacteria and the calculus index.

This study used 12 beagles (9 males and 3 females, aged 4 to 6 years old and weighing 9.6 kg to 12.8 kg) that seemed to be clinically healthy without severe periodontitis. The dogs were equally randomized into 3 groups (4 dogs in each group); control group with scaling only (C), brushing group with tooth brushing once daily after scaling (B) and toothpaste group with application of toothpaste once daily after scaling (P). The dogs were housed together within their respective groups during the study period, fed with the same dry food (TC-2, Oriental Yeast Co., Ltd., Tokyo, Japan) once daily and freely supplied with water. This study was approved by the Animal Care and Use Committee, Faculty of Applied Biological Sciences, Gifu University.

At the start of the experiment, a combination of medetomidine (30 μg/kg, Domitor; Nippon Zenyaku Kogyo Co., Ltd., Fukushima, Japan), midazolam (0.15 mg/kg, Dormicum; Astellas Pharma Inc., Tokyo, Japan) and butorphanol (0.1 mg/kg, Vetorphale; Meiji Seika Pharma Co., Ltd., Tokyo, Japan) was intramuscularly administered to all dogs for sedation. Supragingival calculi were removed using an ultrasonic scaler (Osada ENAC 10WA; Osada Medical Co., Ltd., Tokyo, Japan), and removal of subgingival calculi and root planing were performed using a curette scaler. After completion of these procedures, atipamezole hydrochloride (0.15 mg/kg, Atipame injection, Kyoritsu Seiyaku Co., Tokyo, Japan) was intramuscularly administered to reverse the sedative effect of medetomidine.

For group B, brushing was performed with a dental brush for dogs (Ci Shuwawa; Ci Medical, Hakusan, Japan) within 30 min after eating once daily, by the same operator using the Bass technique. The Bass technique involves wiggling while applying the tip of a toothbrush on an interface between the teeth and the gingivae at an angle of 45 degree. Each dog had its own tooth brush that was utilized every day during the study period.

For group P, the toothpaste (C.E.T. toothpaste chicken flavor; Virbac Japan Co., Ltd., Osaka, Japan) was applied within 30 min after eating once daily. Two grams of toothpaste was applied on the bimaxillary tooth surface with finger of operator without spreading out by fingertip massage.

In all dogs, samples for determination of oral bacteria were collected, and the number of bacteria was measured before

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scaling (Pre), immediately after scaling (Week 0) and every week from Week 1 to 8. After 24 hr from the meal of the day before, the buccal surfaces of the left and right maxillary forth premolars were rubbed back and forth 3 times with the sterile cotton swab in a parallel position to the surface using a sample collection device under the constant pressure of 20 ± 5 g (Fig. 1). The samples were set in the bacterial counter device, and the number of bacteria was measured (Bacterial Counter, DU-AA01 NP-H, Panasonic Healthcare Co., Ltd., Tokyo, Japan).

In all dogs, the calculus index evaluation was performed on the canine and maxillary forth premolar of left and right sides on Pre, Week 0 and Week 8. As shown below, the calculus index was evaluated with four stages, and the measured value was determined by the average value of the 4 teeth.

0: no calculus present

1: supragingival calculus covering not more than one third of the exposed tooth surface

2: supragingival calculus covering more than one third but not more than two thirds of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth.

3: supragingival calculus covering more than two thirds of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth.

Statistical analysis was conducted using the Mann-Whitney *U* test. $P<0.05$ was considered statistically significant.

Figure 2 shows the number of oral bacteria at Pre and in each week from Week 0 to 8 in groups C, B and P. In group C, the number of bacteria increased to the same level as Pre at Week 1 and kept the same level until Week 8. In group B, the number of bacteria did not increase drastically, and it kept rather low level and had been significantly lower than Pre. In group P, the number of bacteria increased to nearly the level of Pre at Week 1, however, it showed a tendency to decrease gradually thereafter.

On the comparison among the three groups, the numbers of oral bacteria were not significantly different at Pre and Week 0 and lower than the detection limit (1×10^5 colony-forming units (CFU)/m*l*) at Week 0 in all the groups. In group B from Week 1 to 8, the number of oral bacteria was significantly lower than that of group C. In the meantime, the number of bacteria in group P from Week 5 to 7 was significantly lower than that of group C, however, it was significantly higher than that of group B on Week 1, 2, 5 and 7.

Figure 3 shows the test result of calculus index in Pre, Week 0 and 8 in the three groups. There was no significant difference of calculus index between the values of Pre and Week 0 in each group. However, on Week 8, there were observed significant differences in each intergroup; between groups C and B, C and P, and B and P.

Periodontal disease is caused by plaque. If we can prevent plaque accumulation, this disease will not occur [1, 4]. The plaque forms a biofilm consisting of a large number of bacteria, which attaches to the dental surface [4]. Furthermore, the calculus results from deposition of the salivary components on the dental surface, and to its coarse surface, the plaque is easy to attach [4]. Therefore, the number of oral bacteria may be an indicator of the development of periodontal disease, as well as deposition of the calculus may be that of plaque attachment. Brushing after scaling is the best way to remove the plaque biofilm [1]. Evaluating the number of oral bacteria and the calculus may provide an indicator of dental care to prevent periodontal disease.

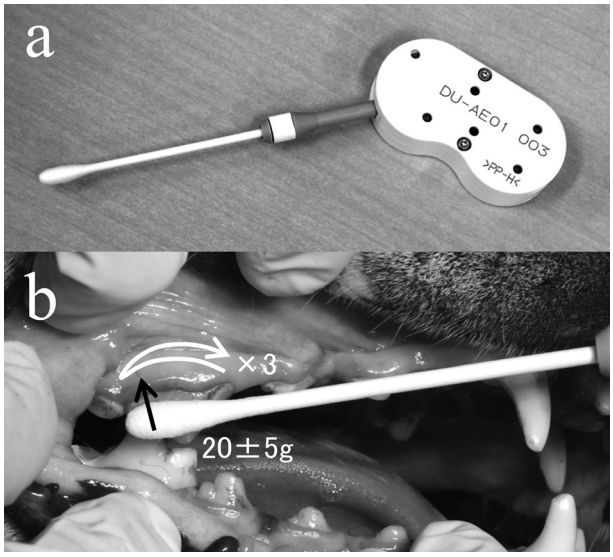

Fig. 1. Sampling from tooth surfaces using a sample collection device under constant pressure. A weight of 20 ± 5 g was loaded on the buccal surface of the left and right maxillary fourth premolars using a sample collection device under constant pressure with a sterile cotton swab (a), and the buccal surface was rubbed back and forth 3 times using the cotton swab in a parallel position to the surface to collect samples (b).

This study used a bacterial counter with DEPIM (dielectrophoretic impedance measurement) to quantitatively evaluate the number of oral bacteria. This system captures bacteria in proximity to an electrode in liquids by electrophoresis and determines the density of bacteria (CFU/m*l*) in the sample by measuring the change of impedance. DEPIM has a high correlation with conventional culture methods and is a measurement method that can detect the number of bacteria comprehensively [2, 3, 6]. DEPIM, the method which is able to measure the number of bacteria regardless of the strains, is considered to be suitable for determining the number of oral bacteria which are various in strains and consist of unidentified bacteria or difficult-to-culture bacteria [8, 11]. Certainly, we cannot directly measure the number of causative bacteria for periodontal disease using Bacterial Counter. Additionally, the method used to collect bacteria in this study can only collect them on the dental surface with potential occurrence of errors. However, in the presence of these errors, this method can comprehensively measure viable bacteria collected. Thus, we considered it possible to know an increase or decrease in the numbers of the causative bacteria for periodontal disease based on the measuring values, which will enable comparison among individual groups.

In this study, the number of oral bacteria in group C

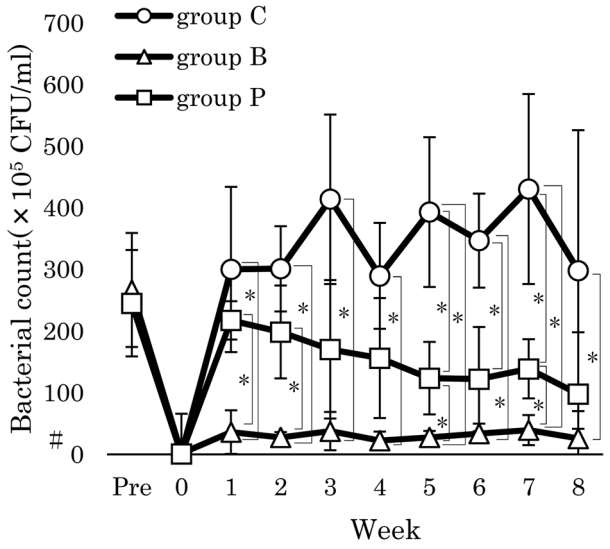

Fig. 2. Changes in the number of oral bacteria in each group. In group C, the number of bacteria increased to the same level as Pre at Week 1 and kept the same level until Week 8. In group B, the number of bacteria did not increase drastically, and it kept rather low level and had been significantly lower than Pre. In group P, the number of bacteria increased to almost the same level of Pre at Week 1 and then decreased gradually. *There was a significant difference at the same time (P<0.05). #Lower than the detection limit (1 × 10^5).

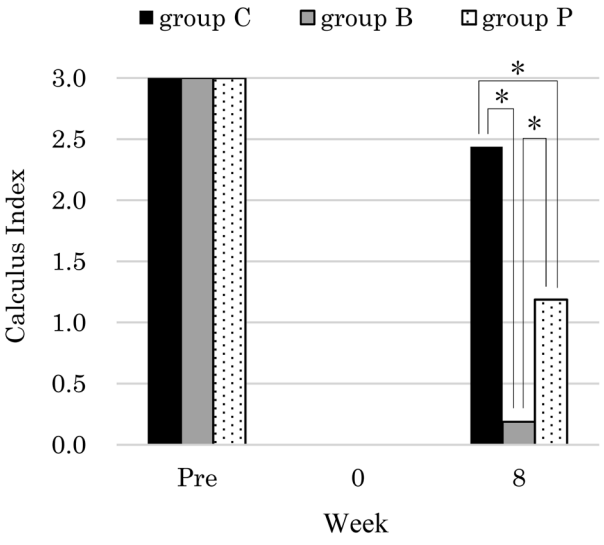

Fig. 3. Calculus index in Pre, Week 0 and 8 in the three groups. There was no significant difference of calculus index between the values of Pre and Week 0 in each group. On Week 8, there were observed significant differences in each intergroup. *There was a significant difference at the same time (P<0.05).

decreased lower than the detection limit at Week 0, but increased to almost the same level as the value of Pre at Week 1 (Fig. 2). This indicates that scaling can decrease the number of oral bacteria temporally, but is extremely low in its efficacy to inhibit the increase of oral bacteria long term [5]. Meanwhile, since brushing can mechanically remove the oral bacteria and plaques on the tooth surfaces, it is considered to be the most effective as an inhibitory method of proliferation of oral bacteria [1, 4, 5]. Reportedly, it takes more than 48 hr for the plaques to become calculi in dogs' mouths. It is possible to prevent the deposition of calculi by removing plaques on the tooth surface by brushing once daily [4]. The number of oral bacteria in group B did not increase drastically, rather kept a low level and had been significantly lower than Pre (Fig. 2). Furthermore, the calculus index was significantly lower than the other 2 groups (Fig. 3). It is confirmed that brushing has a 70% sterilization rate in humans [7]. Our study also indicated the high sterilization rate of brushing in dogs. In the evaluation of the calculus index in group B, almost no calculus deposition was observed. Those results indicated that calculus deposition could be prevented by removing plaques through once daily brushing.

Tooth brushing is considered the most effective method for prevention of periodontitis, but many dogs have difficulty accepting this procedure. Therefore, we focused on the antibacterial activity in saliva. One of the antibacterial activities in saliva is lactoperoxidase system [10]. Hydrogen peroxide and thiocyanate ions in saliva react by catalysis of lactoperoxidase, producing hypothiocyanate ions and water. The hypothiocyanate ions have the antibacterial activity [9, 10]. However, it is generally agreed that the amount of the hypothiocyanate ions produced through the endogenous reaction are insufficient for the antibacterial effect [9, 10]. The toothpaste used in this study contains hypothiocyanate ions and lactoperoxidase, and reinforces the lactoperoxidase system to allow sufficient functionalization of its antibacterial activity [9, 10]. As mentioned above, we could observe the effects of the dual enzyme system on bacteria, but there have been no reports of a study on toothpaste using this system. Therefore, this study investigated the effects of toothpaste application alone. In this study, the number of oral bacteria in group P increased to nearly the level of Pre at Week 1, however, it showed a tendency to decrease gradually (Fig. 2). This result indicated that it required time for hypothiocyanate ions to increase to the level of antibacterial activity, or in some cases, it requires sufficient time until the effect is adequately reflected regarding the decrease in the number of oral bacteria, as hypothiocyanate ions exhibit the bacteriostatic effect by inactivating the metabolizing enzymes.

Comparing the results in this study, the number of oral bacteria in group B was significantly lower than that of group C, and the calculus index on Week 8 of group B was significantly lower than the both groups; C and P (Fig. 3). Those results indicated that once daily brushing is extremely effective to decrease the number of oral bacteria and to prevent the deposition of calculi. In the meantime, group P did not have enough effect for decreasing the number of oral bacteria and preventing the deposition of calculi, compared with group B. However, compared with group C, the number of bacteria was significantly lower from Week 5 to 7, and the

calculus index was also significantly lower at Week 8. Those results indicated that the continuous use of this toothpaste has a certain effect to prevent periodontal diseases.

As for dental care by owners, daily tooth brushing is ideal, but the application of toothpaste does require less time and labor than brushing. In this study, we should have established a control group that used toothpaste containing no active ingredient. However, dogs move their mouths for a few sec when using toothpaste, and presumably, such movement may involve minimal salivation or mechanical factor, compared with their behavior of eating foods. Thus, we determined that the chemical effect of the toothpaste by dual enzyme system was demonstrated. The application of toothpaste is considered to be worth, suggesting as an easy dental care for owners who do not care for their dogs' teeth.

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