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# Clinical and microbiological effects of a subantimicrobial dose of oral doxycycline on periodontitis in dogs

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ABSTRACT

Doxycycline is regarded as an effective treatment for periodontal inflammation. In humans, it has been shown that the long-term administration of a subantimicrobial dose of doxycycline (SDD) does not induce antimicrobial effects on the subgingival microflora and furthermore does not affect antimicrobial susceptibility. The present study was designed to evaluate the influence of oral administration of SDD on normal periodontal microflora and antimicrobial susceptibility in dogs. Experimental periodontitis was induced in 12 experimental dogs using a silk and wire-twisted ligature for 60 days. After the periodontitis induction period, the ligature was removed, and dental cleaning (subgingival and supragingival ultrasonic scaling) was performed. The dogs were randomly assigned to one of two groups: an SDD group with six dogs receiving 2 mg/kg PO once daily and a control group with six dogs receiving a placebo. At weeks 0, 4 and 8, clinical periodontal parameters were evaluated. After the clinical assessments, subgingival plaque was sampled and then cultured in an anaerobic system for one week, and the total anaerobes, *Porphyromonas* spp., *Bacteroides* spp. and *Pasteurella* spp. counts were investigated. Using the agar dilution method, the minimum bactericidal concentration of doxycycline was evaluated and the resistance for doxycycline was monitored during this experimental phase.

The clinical periodontal status of the SDD group was significantly improved compared to the control group ($P < 0.05$). Bacterial counts were not significantly different between the two experimental groups ($P > 0.05$), and antibacterial resistance was not established in the SDD group during the experimental periods ($P < 0.05$). These results suggest that the once daily oral regimen of 2 mg/kg of doxycycline could serve as a SDD in dogs.



## Introduction

Doxycycline (DOX) is a member of the tetracycline group of antibiotics that is effective in the treatment of periodontal disease (Golub et al., 1991). It has an antimicrobial and an anti-inflammatory effect due to the inhibition of matrix metalloproteinases (MMPs) (Golub et al., 1991, 1995; Choi et al., 2004; Emingil et al., 2004a; Lee et al., 2004). Long-term administration of antibiotics can lead to antimicrobial resistance, but the DOX-mediated inhibition of MMPs occurs below the antimicrobial dose (Lee et al., 2004). Therefore, the approach involving a subantimicrobial dose of DOX (SDD) has been applied to the treatment of inflammatory lesions, including periodontitis and arthritis that occur due to the host response in humans (Nordström et al., 1998).

Previous studies have demonstrated that the administration of SDD after subgingival root planing can resolve chronic periodontal inflammation (Golub et al., 1995; Choi et al., 2004; Emingil et al., 2004a, and b). Furthermore, in human studies the long-term administration of SDD has been shown not to induce antimicrobial effects on the subgingival microflora and therefore does not affect antimicrobial susceptibility (Thomas et al., 2000; Walker et al., 2000).

In contrast, the effect of SDD in dogs has not been determined for routine therapeutic use, including periodontal treatment. In a previous study, SDD in dogs was evaluated using high-performance liquid chromatography, and its efficacy was revealed in middle-aged dogs with periodontitis (Kim et al., 2013). However, the SDD efficacy study used uncontrolled periodontitis-predisposed animals and did not consider the environmental variables, including calculus and periodontal inflammation which could affect prognosis (Kim et al., 2013). Furthermore, alteration of the subgingival microflora and antibiotic resistance after the administration of SDD was not previously evaluated. Periodontitis could worsen due to overgrowth of more resistant and pathogenic microorganisms if the

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**Table 1**
Scoring for periodontal parameters.

| Parameters | Score | |
|---|---|---|
| PI | 0 | No plaque |
| | 1 | A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface. |
| | 2 | Moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin which can be seen with the naked eye. |
| | 3 | Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin. |
| GI | 0 | Absence of inflammation |
| | 1 | Mild inflammation – slight change in color and little change in texture |
| | 2 | Moderate inflammation – moderate glazing, redness, edema and hypertrophy. Bleeding on pressure. |
| | 3 | Severe inflammation – marked redness and hypertrophy. Tendency to spontaneous bleeding. ulceration |
| PPD | The distance between the gingival margin and the bottom of the probeable pocket | |
| CAL | The distance between the cementoenamel junction and the bottom of the probeable pocket | |
| BoP | 0 | Absence of bleeding within 10 s following probing |
| | 1 | Presence of bleeding within 10 s following probing |

PI, plaque index; GI, gingival index; PPD, periodontal pocket depth; CAL, clinical attachment loss; BoP, bleeding on pressure.

accumulation of SDD suppresses the normal susceptible microflora (Edlund et al., 1996).

In human medicine, it has been shown that SDD treatment as an adjunct to dental cleaning was effective and did not affect the subgingival microflora (Caton and Ryan, 2011). The present study was performed to evaluate the effect of SDD as an adjunctive therapy to dental cleaning, and to assess any alteration of subgingival microflora and its antimicrobial resistance after a 2-month administration of SDD.

## Materials and methods

The protocol for this study was approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-100609-4; date of approval 9 June 2010).

Twelve standard Beagles, approximately 1.5 years old (eight females and four males) without periodontitis, were used. Exclusion criteria included the administration of any systemic medications during the 1-month period prior to the study. All experimental procedures, including sampling and clinical periodontal evaluations, were performed under general anesthesia using a combination of medetomidine (0.01 mg/kg; Domitor, Orion Pharma), tramadol (2 mg/kg; Toranzin, Samsung Pharm) and a commercial combination of zolazepam and tiletamine (2.5 mg/kg; Zoletil 50, Virbac Laboratories), administered via intramuscular injection.

### *Experimentally induced periodontitis*

For the preparation of healthy gingiva, all teeth were scaled and polished using a piezoelectric ultrasonic scaler (ART-SP2, Bonart), and tooth brushing was performed once a day without anesthesia for the following 2 weeks. During this prophylactic period, the Beagles were fed a hard pellet diet to reduce plaque accumulation (Martuscelli et al., 2000). After the preparation period, experimental periodontitis was induced on the left maxillary second premolar (PM2), third premolar (PM3) and fourth premolar (PM4), as well as the left mandibular PM3, PM4 and first molar (M1) using a twisted-wire (Spooled ligature wire; ClassOne Orthodontics) with 2-0 silk (Silk 2-0, Ailee) ligature (Kim et al., 2012). For the promotion of plaque formation, soft-moistened food was given for the following 60 days. The ligatures were checked daily and lost ligatures were repaired immediately under general anesthesia.

### *Evaluation of clinical effects of SDD on periodontitis*

Sixty days after the periodontitis induction, the ligatures were removed and the clinical periodontal parameters were recorded prior to the initiation of treatment to evaluate the baseline (week 0) periodontal status. The clinical periodontal parameters included the plaque index (PI), gingival index (GI), periodontal pocket depth (PPD), clinical attachment loss (CAL) and bleeding on probing (BoP) (Table 1; Löe and Silness, 1963; Silness and Löe, 1964; Wennström et al., 2001). The measurements were performed at the mesiobuccal, buccal and distobuccal gingival margins of each tooth using individual sterile dental probes (XP23-W Williams Explorer-Probe, Osung). Subgingival plaque samples were taken using different sterilized dental curettes (GR3-4 Hu-friedy type Gracey curette, Osung) from the left maxillary PM4 and left mandibular M1 in each dog.

Clinical periodontal parameters were assessed prior to the subgingival sample collection because the sampling of plaque by using a dental curette could affect periodontal parameters such as the PI and BoP. Collected samples were weighed and stored below 4 °C in coded micro-centrifuge tubes until they were cultured. After the clinical examination and sampling, all dogs received subgingival and supragingival ultrasonic scalings at week 0.

The dogs were randomly divided into two groups. The dogs in the SDD group ($n = 6$, 36 teeth) received 2 mg/kg/day of DOX in a gelatin capsule. The dogs in the control group (placebo; $n = 6$, 36 teeth) received the empty gelatin capsule only. All medications were administered orally once daily for 8 weeks, 30 min after the morning meal. The clinical conditions of each dog were checked daily. On weeks 4 and 8, their clinical periodontal status was re-evaluated, and subgingival plaque samples were collected again in the same manner. All measurements and sample collections were performed in a blinded manner, by a single experienced clinician who was not informed of the experimental group to which each animal had been assigned.

### *Periodontal plaque culture*

The sampled plaque and subgingival debris were diluted 100-fold into anaerobic-sterilized and lactated Ringer's solutions. The diluted solutions were gently sonicated to scatter the plaque and were further diluted $10^4$-fold. After vortexing, 100 μL aliquots of $10^{-6}$ solutions were dispensed onto commercial Trypticase-soy blood agar (TSBA) plates containing 5% sheep blood (TSBA plates, Hankang Media) and spread with sterile glass rods. The plates were incubated in a completely anaerobic system (AnaeroPack-Anaero; Rectangular jar, Mitsubishi Gas Chemical Co) at 37 °C for 1 week. After incubation, each sample was assessed by counting visible colonies, which were separately counted according to the morphology of each colony with or without haemolysis. Total anaerobic counts were also evaluated. The sampled colonies were sent to a laboratory (Macrogen[1]), and the colonies were identified by 16s rRNA sequencing using universal primer. Finally, the counts of the three most predominant species were evaluated.

The minimal bactericidal concentration (MBC) of DOX was also determined on week 0 and was determined for the plaque suspension by the modified agar dilution technique in a range of concentrations between 1 and 16 μg/mL using a TSBA plate (Walker et al., 1979). The plates were also incubated in a completely anaerobic environment at 37 °C for 1 week. The lowest DOX concentration that yielded no growth of a visible colony was considered as the MBC. To evaluate the alteration of antimicrobial resistance, the plaque dilutions sampled at week 8 were cultured again on the DOX diluted TSBA plate, which contained the titrated MBC of DOX at week 0.

### *Statistical analyses*

Clinical periodontal parameters of each group were expressed as means ± standard deviation (SD). Statistical analyses were performed using a commercial software program (PASW 18.0, SPSS). To evaluate the clinical effect of SDD and the subgingival bacterial counts over time, the data from weeks 0, 4 and 8 were assessed using a repeated measures analysis of variance (repeated measures ANOVA). Tukey's method was used as a post hoc test. Linear mixed model was performed to identify whether there was association between groups and time flow. Model selection was based on the Akaike information criteria (AIC). To compare periodontal status and bacterial counts between the groups, the data from each week were also compared using a Student's *t* test. *P* values of less than 0.05 were considered statistically significant.

[1] See: http://macrogenlab.com/ (accessed 26 September 2015).