Original Article

Effect of ovarian activity on orthodontic tooth movement and gingival crevicular fluid levels of interleukin-1β and prostaglandin E_2 in cats

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ABSTRACT

Objective: To evaluate whether there is any correlation between ovarian activity and two potent bone-resorbing mediators (prostaglandin E_2 [PGE_2], interleukin-1β [IL-1β]) secreted from the gingival crevicular fluid (GCF) during orthodontic tooth movement.

Materials and Methods: Eighteen female cats were included in this study. Animals were randomly divided into three groups (estrous, anestrous, and ovariectomized groups), each having six queens. Estrous was induced by administration of 150 IU equine chorionic gonadotropin (eCG) to queens of the estrous group. A closed-coil spring, applied with 80 g of tipping force to the canine, was attached between the maxillary canine and mini-implant. GCF was collected on days 0, 6, and 12 from each cat to examine PGE_2 and IL-1β during orthodontic tooth movement in cats. The PGE_2 and IL-1β levels were determined with enzyme-linked immunosorbent assay.

Results: There was no significant difference ($P > .05$) between anestrous and the ovariectomized groups in terms of tooth movement on days 6 and 12 of distalization. In contrast, tooth movement in the estrous group was lower ($P < .05$) than in the remaining two groups (anestrous and ovariectomized). The mean PGE_2 and IL-1β levels of the canine teeth of the estrous groups were significantly lower than the remaining two groups on days 6 and 12 ($P < .05$) of coil spring applications.

Conclusions: These results indicate that ovarian activity can affect orthodontic tooth movement and GCF levels of IL-1β and PGE_2 in cats. (*Angle Orthod.* 2013;83:70–75.)

KEY WORDS: IL-1β; PGE_2; Tooth movement; Estrous; Cat

INTRODUCTION

In recent years, many researchers have attempted to analyze the histological changes in periodontal tissues after tooth movement. Those studies showed extensive cellular activities in the mechanically stressed periodontal ligament (PDL) involving fibroblasts, osteoblasts, osteocytes, endothelial cells, and endosteal cells.[1] Tooth movement is performed through the formation and resorption of bone in response to compression and tension.[2] Pressure in the PDL induced by orthodontic forces induces vascular changes that cause cellular activation and release of proinflammatory molecules such as cytokines, prostaglandins, and proteinases.[3]

Prostaglandin E_2 (PGE_2) has been well described as a potent stimulator of bone resorption,[4] and its production is modulated in part by interleukin-I (IL-1). There are two forms of IL-1, alpha (α) and beta (β), that are most involved in bone metabolism.[5] There have been numerous studies in vitro models correlating the role of IL-1β and PGE_2 with orthodontic tooth movement.[6–12] A human fibroblast cell has been shown to respond to mechanical stress with increased production of PGE_2.[6] Many studies have shown an increase in proinflammatory cytokines in the PDL. However, the direct effect of mechanical loading on IL-1β from gingival fibroblasts is not yet clear.[13–15]

In addition to all this, sex hormones such as estrogen, progesterone, and androgen play an essential role in bone metabolism. It is known that estrogen

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deficiency is a major factor in the development of postmenopausal osteoporosis.[16] It is reported that osteoporosis and estrogen deficiency are risk factors for alveolar bone density loss in postmenopausal women.[17] Animal and human studies have shown that the levels of sex steroids, including progesterone and estradiol, fluctuate in accordance with estrous or menstrual cycle.[18,19] Responses to the orthodontic force may also vary in accordance with the phase of the menstrual cycle. Osteoclast activity can be inhibited by estrogen in a direct or indirect manner, thereby modulating bone resorption.[20]

The aim of this study was to determine whether there is any variation in levels of PGE_2 and IL-1β in the gingival crevicular fluid (GCF) during orthodontic tooth movement in accordance with phase of the estrous cycle.

MATERIALS AND METHODS

Eighteen female cats (2–4 years old) were included in this study after the approval of the local Ethics Committee. Animals were randomly divided into three groups (estrous, anestrous, and ovariectomized groups), each having six queens. Estrous was induced by administration of 150 IU equine chorionic gonadotropin (eCG; Folligon, Intervet, The Netherlands) to queens of the estrous group.[21] Blood estradiol was measured by radioimmunoassay (RIA) using an estradiol RIA kit (Biosource Europe SA, Nivelles, Belgium). Experimental protocols for teeth movement were performed on the anestrous group animals during nonbreeding season (blood estradiol levels were at basal value). Reproductive tracts (ovaries and uterine tissue) of queens selected for the ovariectomized group were removed by surgical operation. All orthodontic applications were carried out under general anesthesia induced by ketamine hydrochloride (20 mg/kg, intramuscularly [IM]) and xylazine (2 mg/kg, IM) administration.

Experimental Tooth Movement

Screw-shaped titanium mini-implants (1.6 × 6 mm; Dewimed, Tuttlingen, Germany) were applied on the mouth of cats under general anesthesia. Mini-implants were implanted on the zygomatic bone of each cat. A closed-coil spring (G&H Wire Company force medium 200 g) was stretched between the maxillary canine and mini-implant. The spring was fixed by steel ligatures on the teeth to provide retentive undercuts (Figure 1). When stretched, the coil spring applied 80 g tipping force to the canine; the activation continued for 12 days. At the end of the activation period, the miniscrew and coil springs were removed. For measurement of the amount of experimental tooth movement, a silicone impression of the maxillary dentition was taken on days 0, 6, and 12 for production of a plaster model. In each maxilla, the contralateral canine remained untreated. The distance between the mesial side of the second premolar tooth and the distal side of the canine tooth was measured by an electronic caliper (150 mm HS/R3/1A; Knuth Machine Tools, KG, Wasbek, Germany) with an accuracy of ±0.01 mm. Tooth movement was measured twice by the same researcher. Mean values were calculated for each animal.

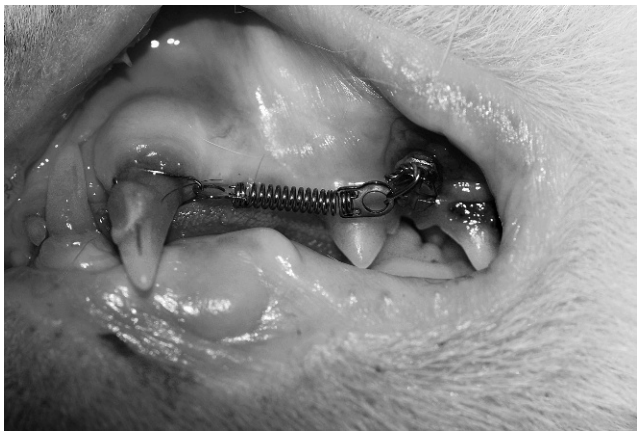

Figure 1. A closed-coil spring fixed between maxillary canine and mini-implant. Results are the mean ± SEM of six independent experiments ($P < .05$).

GCF Collection

Each crevicular site was isolated with cotton rolls. Supragingival plaque was removed with cotton pellets before the GCF collection, and a gentle air stream was directed toward the tooth surface for 5 seconds for drying the area.[22] GCF was collected from the distal side of the tooth with standardized sterile strip papers (Periopaper, Harco, Tustin, Calif) inserted 1 mm into the gingival crevice and left in situ for 30 seconds. Paper strips were placed into plastic vials after the collection of GCF. The mean volume of GCF was calculated for each sample as previously described.[23] The volume of GCF in the periopaper was measured with a Periotron 8000 (Harco). The paper strips were stored in individual vials at −70°C until further processing.

Cytokine Measurement

To determine the IL-1β levels, a mercantile IL-1β enzyme-linked immunosorbent assay (ELISA) kit (BioSource International, Camarillo, Calif) was used; 50 μL of each sample and standards was applied to each well precoated with anti-human IL-1β antibodies, in duplicate; 50 μL of biotinylated antibody containing

0.01% thimerosal was then added, and the plate was sealed and incubated at room temperature (20°C–25°C) for 3 hours. After washing three times with wash buffer, streptavidin-horseradish peroxidase (S-HRP) solution, diluted 1:400 with S-HRP dilution buffer, was applied, and the plate was incubated for 30 minutes. After washing three times, 100 μL of premixed tetramethylbenzidine substrate was added and allowed to react for 30 minutes in the dark; 100 μL of stop solution (0.18 mol/L sulfuric acid) was added to terminate the reaction, and the optical density was measured at 450 nm.

To determine PGE_2 levels, a mercantile PGE_2 ELISA kit (BioSource International) was used; 50 μL of each sample and standards was applied to the assigned wells in duplicate; 50 μL of antibody and conjugate were added, and the plate was incubated at room temperature (20°C–25°C) for 1 hour on an orbital shaker (Hoefer Pharmacia, San Francisco, Calif). After washing each well four times with wash buffer, 150 μL of enzyme substrate was added; 30 minutes later, 100 μL of stop buffer (0.1 mol/L sulfuric acid) was added, and optical density was measured at 450 nm.

Statistical Analysis

One-way analysis of variance (ANOVA) and multiple-comparison analysis were performed to compare the amount of canine movement between groups. Means and standard deviations of the IL-1β and the PGE_2 concentrations were obtained for the each experimental site. ANOVA determined whether there were any significant overall differences among the groups (estrous, anestrous, and ovariectomized) at each time. In addition, to identify the significance in each pair of groups (estrous, anestrous, and ovariectomized), multiple-comparison analysis was also performed for the time when the significant difference was noticed. The data thus collected were assessed using SPSS 16.0 statistical software (SPSS Inc, Chicago, Ill).

RESULTS

The coil springs and mini-implants were well tolerated by the cats through the experimental period. Application did not cause irritation of the soft tissues. No weight loss was observed. Means and standard errors for distal movement of maxillary canines on days 6 and 12 are shown in Figure 2. There were no statistical differences ($P > .05$) between anestrous and ovariectomized groups on days 6 and 12 of distalization. In contrast, there was a significant difference ($P < .05$) between the estrous and remaining two groups (anestrous and ovariectomized cats) in terms of tooth movement.

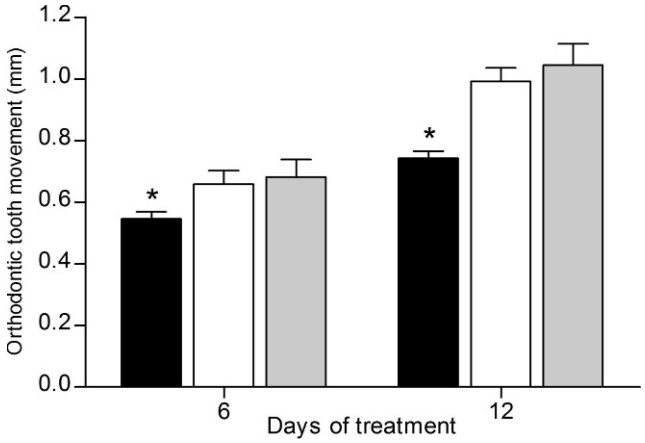

Figure 2. Orthodontic tooth movement in estrous (■), anestrous (□), and ovariectomized (■) animals. Results are the mean ± SEM of six independent experiments ($P < .05$).

The mean PGE_2 and IL-1β levels of the canine teeth of the estrous groups were significantly lower than in the remaining two groups on days 6 and 12 ($P < .05$) of coil spring application. The statistical analysis of the PGE_2 and IL-1β in response to the application of orthodontic force is shown in Figures 3 and 4.

The mean PGE_2 values of the canine teeth of estrous groups were significantly ($P < .05$) lower than the remaining two groups on days 6 (29.3 ± 4.4 pg vs 51.4 ± 2.8 and 55.7 ± 3.7 pg for estrous, anestrous, and ovariectomized animals, respectively) and 12 (40.1 ± 3.3 pg vs 52.5 ± 4.4 and 55.7 ± 7.1 pg for estrous, anestrous, and ovariectomized animals, respectively); however, there were no statistical differences ($P > .05$) between the anestrous and the ovariectomized groups on days 6 and 12 of distalization. For IL-1β, significant differences ($P < .05$) between estrous and the remaining two groups were noted on days 6 (7.3 ± 0.81 pg vs 16.6 ± 1.46 and

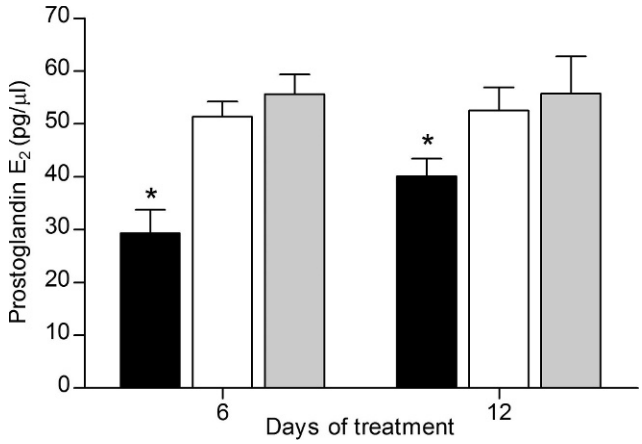

Figure 3. Prostoglandin E_2 levels in estrous (■), anestrous (□), and ovariectomized (■) animals. Results are the mean ± SEM of six independent experiments ($P < .05$).

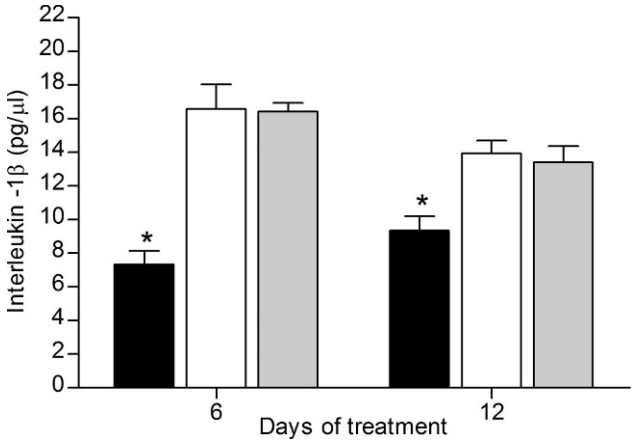

Figure 4. IL-1β levels in estrous (■), anestrous (□), and ovariectomized (■) animals. Results are the mean ± SEM of six independent experiments ($P < .05$).

16.4 ± 0.52 pg for estrous, anestrous, and ovariectomized animals, respectively) and 12 (9.3 ± 0.87 pg vs 13.9 ± 0.78 and 13.4 ± 0.98 pg for estrous, anestrous, and ovariectomized animals, respectively), but there were no statistical differences ($P > .05$) between the anestrous and the ovariectomized groups on days 6 and 12 of distalization.

DISCUSSION

Previous studies have shown that when an orthodontic force is applied to periodontal tissues, inflammatory cytokines and prostaglandins are expressed.[12] Davidovitch et al.[3] demonstrated immunohistochemically that IL-1β and PGE_2 could be detected in the periodontal tissues of cat canine teeth following the application of a tipping force. Their study provided the first experimental evidence to support the suggestion that cytokines regulate some remodeling processes during orthodontic therapy. So we focused on cytokines but used a different diagnosis method.

Yeh and Rodan[7] reported an increase in PGE_2 synthesis when tensile forces were applied to bone cells cultured on collagen ribbons.[7] Harell et al.[8] reported that prostaglandins are important mediators of mechanical stress. Yamasaki et al.[9] studied the effect of prostaglandin (PGE_2) injection into the gingiva around orthodontically treated canines. They reported that the rate of tooth movement on the PGE_2-injected side was twice as fast as that on the side injected with the medium only.

Clinical and animal studies reported by various researchers have identified the role of prostaglandins (PGE_1 and PGE_2) in stimulating bone resorption.[10] IL-1 is a known potent cytokine that facilitates the fusion, activation, and survival of osteoclasts,[11] thus contributing to the initiation of bone resorption. It is reported that the amount of IL-1β in human gingival fibroblasts increases during orthodontic movement.[6,12]

In the present study, we used GCF. Analysis of GCF is a useful and advantageous method, especially for human in vivo studies.[24] It is noninvasive, and repetitive sampling from the same site is achievable regardless of the number. The analysis of specific constituents in the GCF may provide quantitative biochemical indicators that can be used to evaluate the local cellular metabolism, reflecting the bone-remodeling process and periodontal health status during orthodontic treatment.[25]

Our results show that GCF IL-1β and PGE_2 activity was significantly different in estrous groups. Tooth movement was the greatest in the cats receiving orthodontic force principally during anestrous and in the ovariectomized groups, whereas it was the least in those that received such force principally during estrous. It is well known that the serum estradiol level varied according to the phase of the estrous cycle, with a peak during estrous and a nadir during anestrous. It has been demonstrated that tooth movement was closely related to the activity of osteoclasts that can be inhibited by estrogen.[26,27] It has been reported that low estrogen levels stimulate the osteoblastic production of bone resorption–related factors, such as IL-1 and -6, macrophage colony-stimulating factor, and tumor necrosis factor-alpha.[28] These factors may induce bone loss by affecting the differentiation and activity of osteoclasts. It has also been demonstrated that estrogen inhibits tooth movement by increasing the bone mineral content and bone mass and by reducing the bone resorption rate.[29] Several studies[2,30–32] have shown that estrogen deficiency accelerated tooth movement.

Yamashiro and Yamamoto[30] evaluated the effect of ovariectomy and reported significant increases in the histomorphometric parameters of bone resorption and formation in the alveolar bone in ovariectomized rats compared with the sham-operated group of rats. In a study on ovariectomized rat models, it was demonstrated that estrogen deficiency affects trabecular bone volume and the number of trabeculae in the alveolar bone.[2] In another study, five 10-week-old female Wistar rats undergoing ovariectomy were investigated as the experimental group, and the other five without ovariectomy served as the control group; tooth movement in the ovariectomized group was found to be more rapid than in the nonovariectomized group.[32]

In accordance with previous studies, the extent of tooth movement is associated with the ovarian activity in present studies. Therefore, consistent with previous studies,[33–35] negative correlations were observed between levels of estradiol (high in the estrous term in

cats) and markers of bone resorption (PGE_2 and IL-1β) in this study.

In the present study, we examined ovarian activity-dependent variations in the principal female sex hormone (estrogen), cytokines of bone turnover in the GCF, and orthodontic tooth movement and the possible relationships between them in cats, as there is no information available for this species. Consequently, this is the first study to determine the relationship between cytokines in GCF, ovarian activity, and orthodontic tooth movement in cats.

CONCLUSIONS

- PGE_2 and IL-1β expressions were markedly increased by the orthodontic forces in ovariectomized and anestrous groups, which were reflected in a greater rate of tooth movement in these two groups. These increased expressions of cytokines might be related to the estradiol level, which was almost totally lacking in the ovariectomized and anestrous groups.

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