Effect of Short-term Steroid Use (Prednisolone) on Bone Healing around Implants: An Experimental Study on Dogs

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Abstract

Introduction: Prednisolone is a glucocorticoid used for treatment of immune-mediated inflammatory disorders such as rheumatoid arthritis and lupus erythematosus. There is no consensus regarding the effect of short-term steroid use on implant osseointegration. This study aimed to evaluate the short-term effect of prednisolone on the osseointegration process in dogs. Materials and Methods: The 2nd, 3rd, and 4th mandibular premolar teeth of 8 mature male mixed-breed dogs were bilaterally extracted under general anesthesia. After 3 months of healing, the dogs were allocated into study (receiving 4 mg/day prednisolone for 4 weeks followed by 2 mg/day for another 4 weeks) and control groups (4 dogs per each group). Six implants (bone level) were inserted in the mandible of each dog. In 4 dogs (2 in each group), the reverse torque and the bone-implant contact (BIC) were evaluated at 1 week post-operatively and in the remaining dogs at 4 weeks. Data were analyzed using two-way ANOVA with 95% confidence interval. Results: The reverse torque of all implants at 1 and 4 weeks postoperatively was at the highest value of implant ratchet. Microscopic evaluation revealed that the BIC was significantly greater in controls in comparison to the prednisolone group (P-value<0.05). In addition, the BIC of both groups significantly increased at 4 weeks compared to 1 week (P-value<0.05). The newly formed bone around implants at 1 and 4 weeks postoperatively was woven and lamellar, respectively. Conclusion: Prednisolone has the potential to disrupt the osseointegration process.

Key Words: Dental implants, Steroids, Bone, Osseointegration

Introduction

Branemark introduced the concept of osseointegration for the first time in 1969 [1]. Osseointegration refers to a direct structural and functional bone-to-metal interface without interposition of non-bone tissue. The bone could become so fused with the titanium oxide layer of the dental implant surface that the two could not be separated without fracture. Based on the literature, osseointegration is defined as a cicatricial event leading to bone formation at the surface of the inserted implants. The outcome of osseointegration is the fixation of implant to the alveolar bone via the newly formed bone [2].

While the osseointegration process involves bone formation, it is dependent on the turnover and remodeling of alveolar bone. As a result, various factors have the potential to affect the osseointegration process such as implant characteristics, surface properties, primary stability, loading condition, and intake of systemic medications during the osseointegration process [3-5].

Immunosuppressive drugs may interfere with the osseointegration process. In addition, it has been reported that long-term intake of glucocorticoids has adverse effects on osseointegration and success rate of dental implants [6]. Increasing the humane serum levels of glucocorticoids in vitro to supraphysiological doses decreases the ability of osteoblasts to differentiate. Fluprednisolone, paramethasone, prednisone, prednisolone and methylprednisolone have comparable therapeutic indices. Contrariwise, the therapeutic indices of dexamethasone, betamethasone and triamcinolone are lower than those of prednisolone; they are less looked-for for routine use and should be set aside for specially selected cases [7]. Corticosteroid treatment is commonly used in rheumatologic and inflammatory diseases and to diminish postoperative pain and protracted soft tissue swelling after elective surgery. Consequently, glucocorticoids might lead to a serious delay of bone healing [6].

Evidence on the effect of short-term glucocorticoid therapy on osseointegration is limited; Carvas et al. [8] observed that administration of methylprednisolone led to significant reduction of BIC in rabbits after 12 weeks. As glucocorticoids have anti-inflammatory properties in short-term administration, there exists a possibility that it would interfere with the osseointegration process [9]. Hence, the aim of the current study was to evaluate the effect of prednisolone on osseointegration process in dogs. The null hypothesis of the study was that there would be no significant differences in osseointegration between the study and control groups.

Materials and Methods

This experimental study was approved in the Ethics Committee of Isfahan University and was conducted in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals. The purpose of the current study was to evaluate the short-term effect of prednisolone on osseointegration process in dogs.

Study sample

To evaluate the study hypothesis, 8 mature male mixed-breed dogs aged 16-20 months and weighing between 11 and 13 Kgs were selected. Canines were excluded from the study if domesticated, had rabies, uncontrollable behavior, or were aggressive.

Surgical procedure

Surgery was performed in three stages. In the first stage, the teeth (2nd, 3rd, and 4th mandibular premolars) were extracted.

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In the second stage, implants were placed and in the third stage, implants were removed by a trephine drill.

First stage

To induce general anesthesia, 1% acepromazine (0.2 cc/kg), 10% ketamine (10 mg/kg) and atropine (0.04 mg/kg) were administered. Anesthesia was maintained with halothane. Following general anesthesia, a full thickness flap was elevated at the mandibular-premolar region (from the 1st to the 4th premolar). Next, the 2nd, 3rd, and 4th premolar teeth of each quadrant were sectioned buccolingually and extracted using a periotome. Then, the flap was sutured with 4-0 nylon (Mersilk, Ethicon Co., Livingston, UK). Sutures were removed after 1 week.

Group allocation and second stage surgery

After 3 months of healing (following the first stage surgery), the dogs were allocated into study (4 dogs) and control (4 dogs) groups. In the study group, dogs received oral prednisolone (4 mg/day) for 4 weeks, which was continued with the dosage of 2 mg/kg for another 4 weeks. Dogs in the control group received oral placebo. A blood sample was taken every 2 weeks to ensure significant reduction in leukocyte population during corticosteroid therapy. At the end of the 4th week of placebo and prednisolone administration, 6 implants were inserted in the mandible of each dog in the two groups. The second stage of surgery was performed under general anesthesia. The night prior to surgery, all dogs received 20000 IU penicillin and streptomycin (1 g/10 kg) (corresponding to 4 days of antibiotic therapy). After 4 days, antibiotics were administrated again to maintain the coverage until the 8th day. At this stage, a crestal incision was made at the mandibular premolar region and three identical bone level implants with 3.4 mm diameter and 10mm length (Dental implant, DENTIS implant company, Seongseoseo-ro, Dalseo-gu, Daegu, Korea) were placed bilaterally at the 2nd, 3rd, and 4th mandibular premolar sites. Flaps were sutured with non-absorbable suture and the implants were submerged. In the study group, dogs received anti-acid treatment to prevent gastrointestinal side effects of corticosteroids. In addition, antibiotics were prescribed to prevent infection.

Third stage surgery and BIC evaluation

Implants placed in 4 dogs (2 dogs per each group) were evaluated one week after the second stage surgery while the remaining were evaluated at 4 weeks postoperatively. Following anesthesia, a blinded operator measured the reverse torque of all implants with implant ratchet (Dental implant kit, DENTIS implant company, Seongseoseo-ro, Dalseo-gu, Daegu, Korea). Reverse torque ranged from 0 to 65 N.Cm for biomechanical measurement of osseointegration. All implants were removed by a trephine drill (size: 10 mm) and stored in 10% formalin solution. Specimens were mounted in resin blocks and sectioned (Accutom 50, Struers, Copenhagen, Denmark) mesiodistally twice to a thickness of 50 µm. Sections were fixed on a microscope slide and stained by hematoxylin and eosin. Stained sections were observed under a light microscope at ×40 magnifications to measure the BIC (*Figure 1*). Samples were re-examined with Photoshop software version 7.0 (San Jose, CA, USA).

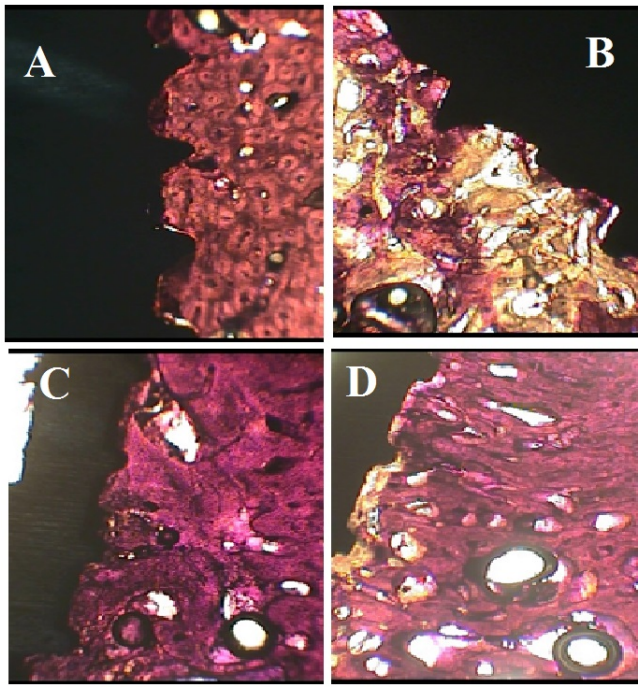

Figure 1*. Bone-implant contact at ×40 magnification. Notes: The BIC significantly increased at 4 weeks (A, C) in comparison to 1 week (B, D). Furthermore, the BIC of the study group (A, B) was significantly smaller than that of the control group (C, D).*

Statistical analysis

Appropriate descriptive statistics (mean, standard deviation, minimum, and maximum) were computed. The data were analyzed using SPSS version 11.5 (Microsoft, Chicago, IL, USA) and two-way ANOVA with 95% confidence interval. P value<0.05 was considered statistically significant.

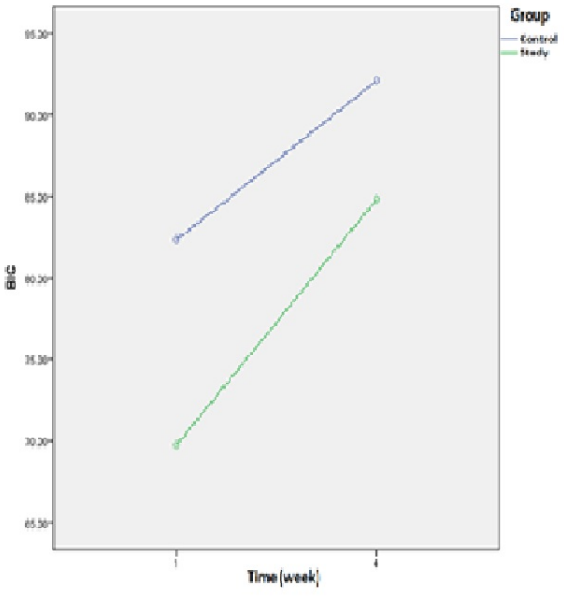

Figure 2. *The changes in BIC over time in the study and control groups.*

Results

Eight mature dogs with a mean age of 17.12 ± 1.29 months and a mean weight of 11.91 ± 0.83 kg received a total of 48 implants. The dogs were randomly divided into two groups: 4 dogs received prednisolone and 4 dogs received placebo. Based on the Kolmogorov-Smirnov test, the data were normally distributed (P-value>0.05).

The reverse torque of all implants was the same and at the maximum value in both groups. The mean BIC of the implants is presented in *Table 1* and *Figure 2*. According to two-way ANOVA (*Table 2*), no significant interaction was observed between time and implant group (P-value>0.05). In addition, the BIC significantly increased at 4 weeks in comparison to 1 week (P-value<0.05). Furthermore, the BIC of the study group was significantly smaller than that of the control group (P-value<0.05).

Table 1. The BIC of implants according to the treatment group and time intervals.

Time	Group	Number of dogs	Number of implants	Minimum	Maximum	Mean	Standard deviation
1 week	Study	2	12	53	80	69.75	6.38
	Control	2	12	80	86	82.33	2.19
4 weeks	Study	2	12	78	91	84.58	4.54
	Control	2	12	86	100	92.08	3.59

According to histological evaluation, the newly formed bone was woven and lamellar in all samples at 1 and 4 weeks, respectively.

Table 2. Results of two-way ANOVA.

Factor	Df	Mean Square	F	P-value
Intercept	1	324229.69	16383.69	< 0.001
Group	1	1210.02	61.14	< 0.001
Time	1	1813.02	91.61	< 0.001
Group * Time	1	77.52	3.92	0.054

Discussion

Recent studies suggest that early bone healing phase is the key to successful osseointegration. This phase is characterized by an inflammatory reaction with increased secretion of prostaglandins by osteoblasts. Glucocorticoids have been suspected to negatively affect fracture healing [6]. However, the effect of their short-term use on osseointegration has not been well studied [6]. The aim of the current study was to evaluate the effect of short-term administration of prednisolone on the osseointegration process of implants in dogs. The null hypothesis was that there would be no significant differences between prednisolone and control groups regarding osseointegration. The null hypothesis was refuted as the BIC of the control group was significantly greater than that of the prednisolone group.

According to the results of the current study, all implants in both groups had maximum reverse torque. The reverse torque test was introduced by Roberts et al. [10] in 1984 and was later developed by Johansson and Albrektsson [11,12]. It is among the most reliable techniques to assess the implant-alveolar bone integrity and is very accurate in estimating the clinical BIC. However, the reverse torque test is an aggressive method, which is highly destructive and should solely be used in animal models [13,14].

The results of the current study revealed that short-term administration of prednisolone had a negative effect on the osseointegration process. The BIC of the study group at both 1 and 4 weeks was significantly smaller than that of the control group. In accordance with the present findings, Carvas et al. [8] found deleterious changes in BIC of implants inserted in rabbit tibias after 18 weeks of methylprednisolone administration. Although the findings of Carvas et al. [8] and the current study are comparable, there are several differences in the study design and interpretations. They inserted implants in the tibia of rabbits, which has less similarity to the human jaw [15] while we used a canine model. Moreover, they investigated the long-term effect of corticosteroids while we investigated the short-term effect of prednisolone.

Based on the literature, 18-week administration of corticosteroids is sufficient to induce osteoporosis in an animal model [16]; however, osteoporosis is a long-term side effect of corticosteroids. A short-term effect of corticosteroids is their anti-inflammatory activity, which is beneficial in immune-mediated systemic disorders including lupus erythematosus, rheumatoid arthritis, polyarteritis nodosa, asthma, and allergy [17]. In the mentioned disorders, the first treatment step is to administer high doses of glucocorticoids to acutely suppress this process. Following the resolution of signs of the disorder, the dosage of the glucocorticoid is

gradually decreased [18]. In the current study, to simulate the treatment protocol of the aforementioned disorders in human, the adjusted dosage of prednisolone for a canine model was used (4 mg/day prednisolone for 4 weeks followed by 2 mg/day for another 4 weeks) [19]. The anti-inflammatory properties of prednisolone are due to lipocortin-1 (annexin-1) synthesis. Lipocortin-1 suppresses phospholipase A2 and eicosanoid production. It also inhibits various leukocyte inflammatory events including epithelial adhesion, chemo taxis, migration, and phagocytosis. Hence, glucocorticoids not only suppress the immune response, they also inhibit synthesis of prostaglandins and leukotriene (two main inflammatory markers). Glucocorticoids inhibit the synthesis of prostaglandins at the level of phospholipase A2 and cyclooxygenase (COX-1 and COX-2) [9]. Chikazu et al. [20] demonstrated that the activity of COX-2 was essential for the osseointegration process. In addition, the first stage of osseointegration involves an inflammatory phase [21,22]. In the current study, the implants were inserted after 4 weeks of prednisolone administration. Similarly, Carvas et al. [8] inserted implants following 6 weeks of methylprednisolone administration. The BIC percentages in the prednisolone group at both 2 and 4 weeks in our study could be explained by the fact that prednisolone inhibits the inflammatory phase of osseointegration. At 1 week postoperatively, type of the newly formed bone around all implants was woven; while at 4 weeks, histological evaluation revealed lamellar bone around all implants. The change in bone types was in line with the increase in BIC.

One of the advantages of the current study design was that it enabled assessment of the short-term effects of prednisolone on the osseointegration process without the interference of an underlying inflammatory disorder for which corticosteroids may be administrated (i.e. lupus erythematosus). However, it should be noted that this study was conducted on an animal model and thus, generalization of the results to humans must be done with caution.

In conclusion, within the limitations of the current study, the results showed that short-term administration of prednisolone attenuated the osseointegration process, which could be regarded as a side effect in treatment of patients with systemic disorders including lupus erythematosus, asthma, and allergy in need of dental implants.

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