Effect of Corticosteroids on Orthodontic Tooth Movement in a Rabbit Model

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Introduction: *While there are a growing number of studies on the effects of medications on orthodontic tooth movement (OTM), only few studies have investigated the role of corticosteroids, despite their widespread use. The aim of the current study was to evaluate the effects of triamcinolone acetonide injection on OTM in a rabbit model.* ***Study design:*** *Sixteen one-month old rabbits were randomly divided into two groups: Eight rabbits had triamcinolone acetonide (1mg/kg/day) administered IM daily for 21 days (test group) while the remaining eight rabbits received no drug (control group). The rabbits in both groups had a tube bonded to the upper central incisors and a stainless steel helical spring was inserted in tube slot to apply 50 cN distal force. After 3 weeks, the rabbits were sacrificed and the distance between mesial corners of incisors was measured. The incisors are associated tissue was processed for histology and the apical and cervical area of the roots evaluated. An observer who was blind to the study groups evaluated the specimens.* ***Results:*** *All appliance-treated incisors in test and control groups showed evidence of tooth movement. The distance between the incisors was significantly greater in the triamcinolone acetonide treated group compared to the control group ($P<0.001$). Histological examination revealed an increased number of resorption lacunae and decreased number of cuboidal osteoblastic cells around the apical and cervical area of the Incisor roots in the test compared to the control group ($P<0.01$).* ***Conclusion:*** *Treatment with triamcinolone acetonide is associated with increased tooth movement in rabbits via increased resorptive activity in the alveolar bone.*
Keywords: *triamcinolone acetonide, orthodontic tooth movement, resorptive activity.*

INTRODUCTION

Since its discovery, corticosteroids in 1949, have been used in many therapeutic regimens for the treatment of a wide variety of conditions such as rheumatoid arthritis, dermatitis, allergies, asthma, in hormonal contraceptives and as immunosuppressive medications after organ transplantation.[1,2] Corticosteroids are also used as anti-inflammatories in dentistry. For example, endodontists use the triamcinolone acetonide derivative Ledermix (Lederle Laboratories, Cyanamid GmbH, Munich), to control pulpal inflammation.[3] The anti-inflammatory effect of corticosteroids is via blockage of phospholipase A2 and the suppression of the synthesis of COX-1 and COX-2, leading to the inhibition of the synthesis of prostaglandins and leukotrienes.[4]

Chronic corticosteroid treatment is also known to induce bone loss and osteoporosis.[5] The mechanism of action of corticosteroids on bone is less clear. It has been postulated that they interfere with the resorption and deposition cycle in normal bone remodeling, resulting in a reduced bone formation and increased bone resorption.[5] Studies have shown that corticosteroids have various effects on cells of osteoblastic lineage, including inhibition of osteoprogenitor differentiation. They also induce apoptosis in osteoblasts and osteocytes, which will decrease bone formation.[6]

When orthodontic forces are applied to teeth, bone resorption must occur on the pressure side to permit tooth movement. Various studies have investigated the effects of medications, vitamins and dietary supplements on orthodontic tooth movement (OTM).[7]

As corticosteroids are known to effect bone turnover it is important to understand whether they influence OTM. Two animal studies have reported an increased rate of tooth movement following the administration of corticosteroids,[8,9] however two other studies showed no increased rate of tooth movement.[10,11] In light of this contradictory data, more experimental data is needed to further elucidate the effects of corticosteroids on tooth movement. As the epidemiologic surveys show that the prevalence of diseases

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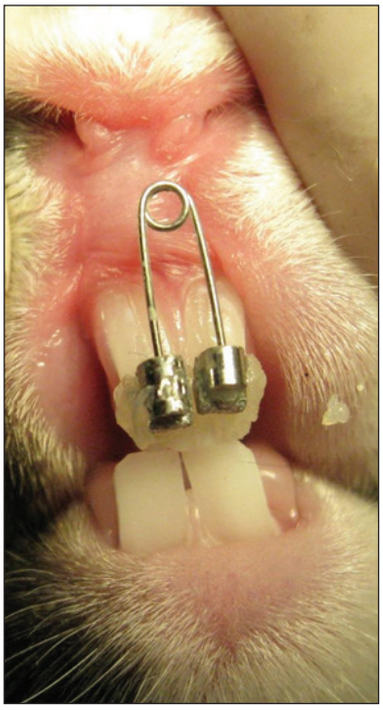

Figure 1. Design of appliance. Modified tubes bonded to the middle of the clinical crown of the central incisors by resin composite.

that are treated with corticosteroid treatment especially in children are increasing, it is predicted that a growing number of orthodontic patients will be taking corticosteroids during OTM.[12] Consequently, the current study was designed to investigate the role of triamcinolone acetonide, a long acting corticosteroid on OTM. Triamcinolone acetonide is an injectable solution, in which the active ingredient triamcinolone is present in the form of crystals. The half-life of the drug is 2-5 hours and due to slow absorption of these crystals at the site of injection, its effect is amplified.[13] To the best of our knowledge, this is the first study to investigate the role of triamcinolone acetonide on orthodontic tooth movement.

MATERIALS AND METHOD

The protocol for this study was approved by the local animal ethics committee. Sixteen one-month old New Zealand White rabbits, with a mean weight of 1kg were used. The New Zealand white rabbit model was selected as it has been previously used to study the effect of medications on OTM. They also share similar reactions with humans to diseases and medications.[8] Immature rabbits were selected to limit hormonal effects. The rabbits were fed a standard pellet diet with tap water at libitum for one month prior to the commencement of the study. Rabbits were randomly divided into a test and a control group (n=8 for each group); the rabbits in the test group received daily intramuscular injections of triamcinolone acetonide (1mg/kg/d; TriamHEXAL® 40, Hexal, Germany) for 21 days. The doasge was based on Baofeng *et al* study (1 mg/kg/day of methylprednisolone), which has similar potency to triamcinolone, in a rabbit model.[14] The short duration of drug administration was used to decrease risk of iatrogenic hyperadrenocorticism and hyperparathyroidism.[14,15] The protocol of drug administration was described in previous studies by Ashcraft, Kalia and Ong *et al*.[8-10] The rabbits in the control group did not receive any medication.

Table 1. Summary of tooth movement and histomorphomentry results

	Test (n=8)	Control (n=8)	p-value
Fibroblasts	70.3±8.6	68.4±11.7	0.720
Osteoblasts	69.1±11.3	93.6±17.6	<0.005
Resorptive lacunae	4.1±1.2	1.8±0.7	<0.001
Movement (mm)	8.1±0.3	4.4±0.3	<0.001

All results are presented as Mean ± SD. Tooth movement in millimeters (mm) and number of each cell type determined in five high power fields (HPF) in the control and test groups.

Orthodontic Appliance Treatment

Rabbits were anesthetized with an intramuscular injection of ketamine hydrochloride (Ketalar™; 100 mg/kg body weight). For the application of orthodontic force, modified tubes were bonded to the middle of the clinical crown of the maxillary central incisors by resin composite. 50 cN of reciprocal force, measured by a dynamometer, was applied to the teeth with a spring, bent from 0.014-inch (0.35 mm) stainless steel wire using Akin method[16] (Figure 1). All the springs had similar diameter and height. The wires were inserted in tube slot and activated only once from one arm. Flowable composite was used to secure the spring in the tube. The force level was measured with a dynamometer gauge, and the springs were not reactivated during the experimental period.

After 21 days, a caliper standardized to 0.1mm by an examiner blind to the treatment regimen measured the distance between mesial areas of incisors. The percentage of agreement within ±0.1 mm between repeated measuring of tooth distance was 90%. The mean values for each group were calculated and the difference between the baseline and post-treatment measurements was recorded. All measurements were done by the same examiner.

Histological Preparation and Analysis

After the 21-day experimental period, rabbits in both groups were euthanased with ketamine and perfused with 10% formalin by injection into the aorta. Maxillary incisor segments were dissected out, fixed in 10% buffered formalin and decalcified using Decalcifer-II® (Surgipath, Richmond, IL). The specimens were embedded in paraffin and serially sectioned at 4 to 5 micrometer intervals in the horizontal direction by Microtom (MicromHM 325,GMI Inc., Minnesota) to allow visualization of the both the pressure and tension sides of the incisor in the same section. To ensure that this procedure was the same for each examined tooth, the five sections were directly located next to the apical and cervical area of the roots. The sections were stained with hemotoxylin and eosin (H&E). Cells were considered osteoblasts based on their cuboidal appearance and apposition to bone surfaces.[17,18] Cells were considered fibroblasts if they were elongated and spindle shaped. Fibroblasts could be seen at a distance from bone surfaces. Resorption lacunae were identified based on their scalloped appearance on or within bone.[17,18]

Images were subsequently captured with a digital camera coupled to the microscope. An observer who was blind to the study groups evaluated the specimens. Cell and lacunae counts for each section were performed twice, and the mean calculated, and intra-operator error determined following repeated blind measurements.

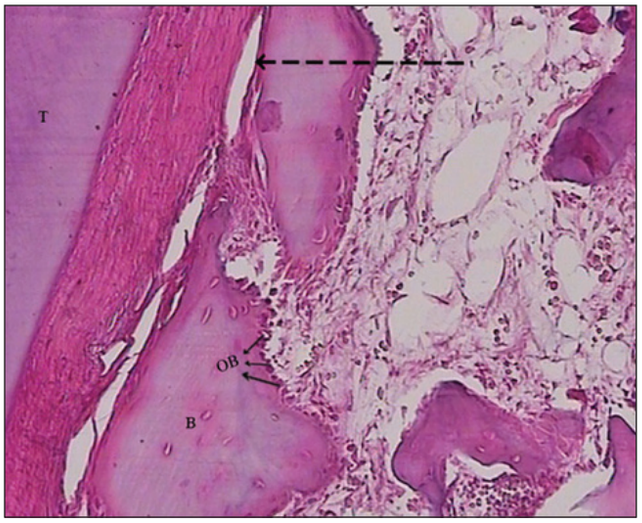

Figure 2. Tension side of tooth from triamcinolone treated rabbit. The tooth (T) and alveolar bone (B) can be clearly seen. The bone shows a layer of cuboidal cells (presumed to be osteoblasts (OB)). Direction of force is shown by dotted arrow. (H&E original magnification 100X)

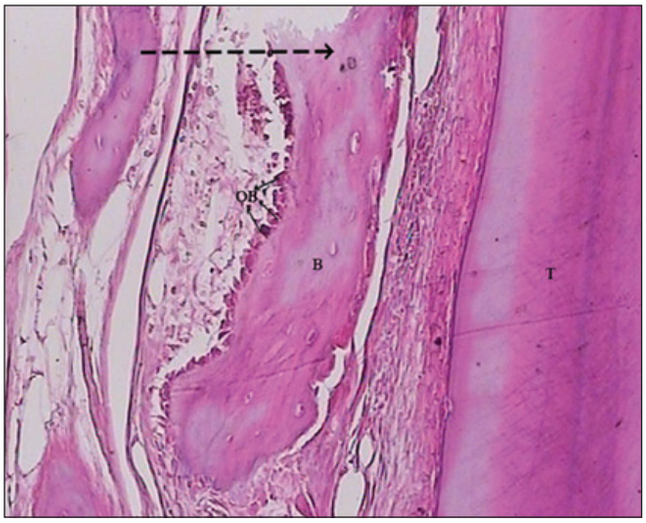

Figure 4. Tension side of tooth from the control rabbit. The tooth (T), alveolar bone (B) can be clearly seen. The bone shows a layer of cuboidal cells (presumed to be osteoblasts (OB). Direction of force is shown by dotted arrow. (H&E original magnification 100X)

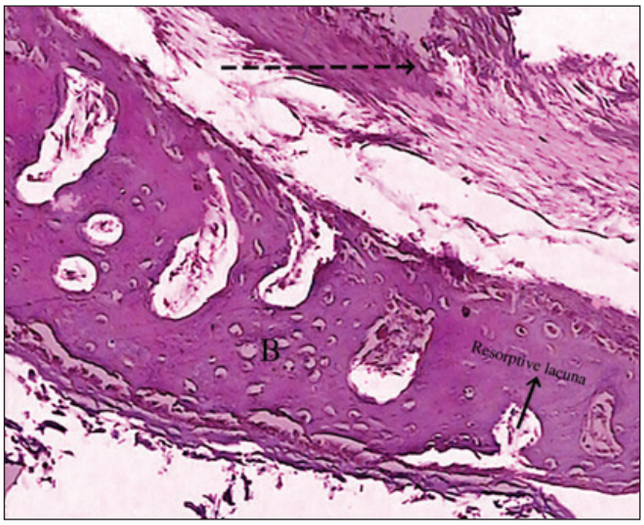

Figure 3. Pressure side of tooth from triamcinolone treated rabbit. The alveolar bone (B) adjacent to the periodontal ligament (PDL) shows a large number of resorption pits (shown with arrows), indicative of the presence of active osteoclasts. Direction of force is shown by dotted arrow.(H&E original magnification 100X)

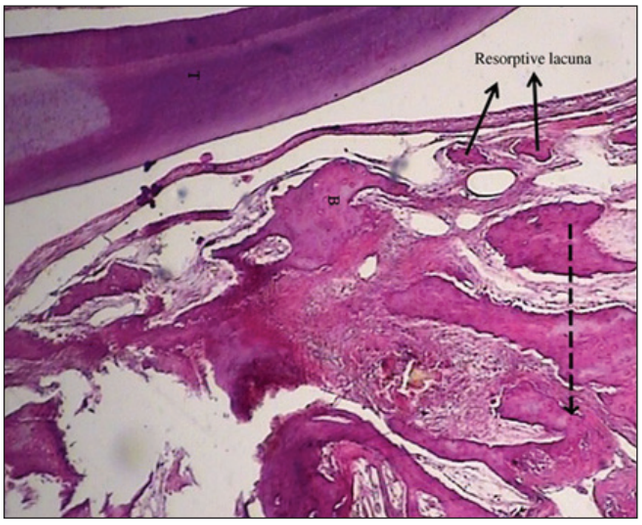

Figure 5. Pressure side of tooth from the control rabbit. The alveolar bone (B) resorption pits (shown with arrows), indicative of the presence of active osteoclasts. The tooth (T) can be clearly seen. Direction of force is shown by dotted arrow.(H&E original magnification 100X)

All statistical analysis was done via SPSS 11.0 software (SPSS Inc., Chicago, IL). All data were tested for normality and a t-test was used to compare data that were normally distributed; otherwise the Mann-Whitney test was used to test for statistical significance ($P \leq .05$).

RESULTS

The tooth movement and histomorphometric results are summarized in Table 1. There was no loss of the bonded appliance. Loss of weight in the control group was similar with the test group. A gap was observed between the incisors in all the rabbits and no sign of inflammation was observed in the gingiva. No complications were observed in any of the animals.

The amount of tooth movement was significantly greater in triamcinolone acetonide administered group (8.1 ± 0.3 mm) compared to the control group (4.4 ± 0.3 mm) ($P < .001$).

Examination of the histological sections showed a large number of active osteoblasts aligned along the bone surfaces on the tension side of the control and treated samples. On the pressure side fewer osteoblasts were visible; however resorption lacunae and even large cavities were visible in the boney trabeculae (Figures 2-5).

Whereas, there were no significant differences in the number of fibroblasts in the treated and control samples (70.3 ± 8.6 and 68.4 ± 11.7 respectively, P=0.72), there were significantly fewer osteoblasts in the treated samples compared to control (93.6 ± 17.6 and 69.1 ± 11.3 respectively, P= 0.005). Further, the number of resorptive lacuna

Table 2. Summary of studies invesutgating the effect of corticosteroids on OTM.

	Corticosteroid type	Dosage (mg/kg/d)	Induction period	Duration of treatment	Model	Result
Ashcraft et al (1992)	Cortisone acetate	15	4 days	14 days	Rabbit	Significant increase in OTM
Yamane et al (1997)	Hydrocortisone	10	7 days	20 (hours)	Rat	Hydrocortisone inhibited tooth movement
Ong et al (2000)	Prednisolone	1	12 days	12 days	Rat	No Significant effect on OTM
Kalia (2004)	Methylprednisolone	8	49 days	21 days	Rat	Significant increase in OTM
			0 day			No Significant effect

in the triamcinolone acetonide treated group (4.1 ± 1.2) was significantly higher than the control group (1.8 ± 0.7) (P≤.001). Repeated cell counts by the same examiner differed by no more than 5%.

DISCUSSION

According to the National Institute of Allergy and Infectious Diseases (NIAID), asthma and allergic diseases such as food allergy, hay fever and eczema are common for all age groups in the United States and elsewhere in the world. In the US, asthma affects more than 17 million adults and more than 7 million children while hay fever and other respiratory allergies affect nearly 10 percent of children under 18 years old.[19] Based on this projection, these conditions are among the most common diseases that can be encountered in an orthodontic practice which are treated with various forms of corticosteroids.

Although corticosteroids are widely used, there has been limited information on their effects on OTM. In the current study we evaluated the effects of a systemic corticosteroid, triamcinolone acetonide, on tooth movement in New Zealand white rabbits. Triamcinolone is widely used in the treatment of joint disease and arthritis. The effect of this medication on OTM has not been investigated in prior studies.

In the current study we determined that the administration of triamcinolone acetonide was associated with increased tooth movement. Four previous studies investigated the effect of corticosteroids on OTM (Table 2). Our results are similar to those of Ashcraft *et al* [8] who reported an increase in OTM in rabbits treated with cortisone acetate for 4 days before and then for 14 days during the application of an orthodontic force of approximately 100 cN. Similarly Kalia *et al* [9] reported that when methylprednisolone was given for an induction period of 7 weeks and then orthodontic force for 3 weeks, there was an increase in OTM. Ashcraft *et al* [8] suggested that the high doses they used (15 mg/kg/d) may have resulted in osteoporosis, which could indirectly result in the increased rate of tooth movement. However increased OTM in our study is unlikely to be due to steroid-induced osteoporosis because of two major differences between the two studies: first, Ashcarft had an induction period of 7 weeks and secondly they used a significantly higher dose of cortisone acetate. Although triamcinolone acetate is more potent, 1 mg/kg/d of triamcinolone is estimated to be equivalent to 2.4 mg/kg/d of cortisone acetate, which is still significantly lower than the 15mg/kg/d of cortisone.[8] Further, a recent study characterizing corticosteroid induced osteoporosis in rabbits indicated that significant changes in bone mineral density were only seen after 10 weeks of treatment with methylprednisolone (1mg/kg/d)[14] which as noted before has a similar potency to triamcinolone.

Ong *et al* [10] reported that the corticosteroid prednisolone had no effect on OTM. They administered prednisolone at (1mg/ kg/d) to rats for an induction period of 12 days, followed by an experimental period of 12 days. In their study, the first molar was moved mesially with a force of 30 cN. The difference in results between their study and ours may be due to the shorter duration of OTM used by Ong (12 days vs 21 days), that prednisolone is less potent than triamcinolone, that lower force was used compared to our study (30cN vs 50cN), and/or due to differences between rats and rabbits.

They noted that while OTM up-regulated growth hormone receptor (GHR) and IGF-1 receptor (IGF-1R) immunoreactivity, prednisolone reduced this up-regulation. The authors stated that suppression of GHR and IGF-I immunoreactivity in steroid-treated animals suggested that the mechanism whereby bone resorption and deposition, necessary for orthodontic tooth movement, may be inhibited by prednisolone.[10,20]

Yamane *et al* [11] compared the rate of tooth movement in response to an orthodontic force by using a time-lapse videotape recorder following 7 days treatment with hydrocortisone injections (10 mg/kg/d), or saline. They reported that the hydrocortisone actually inhibited tooth movement over the first 20 hours. The authors attributed this finding to the reported increase in the mechanical strength of the periodontal ligament of rats under corticosteroids. However the very short duration of OTM makes it difficult to assess what the effects of hydrocortisone treatment would have been over longer OTM treatment durations.

To determine if triamcinolone acetonide was causing effects on bone turnover, the number of osteoblasts were determined to assess bone formation and the number of resorption lacunae to assess bone resorption. Osteoblasts were distinguished from fibroblasts based on well-established morphological features as described in the materials and methods.[17,18] Further, confirmation of the identity of various cells can be achieved histochemically using alkaline phosphatase staining to confirm the identity of osteoblasts and tartrate resistant phosphatase (TRAP) staining for osteoclasts.

Histologically, it was noted as expected that resorption occurred predominantly on the pressure side of the teeth while bone formation was occurring on the tension side. Results indicated that triamcinolone acetonide was associated with increased number of resorption lacunae and decreased number of presumed osteoblasts. This appears to correspond to the increased amount of tooth movement. Our results are in agreement with our general knowledge of the functions of corticosteroids on bone metabolism such as the induction of apoptosis in osteoblasts and prolonging the lifespan of osteoclasts, thereby decreasing bone formation, and increasing bone resorption.[4]

It is important to note that OTM is affected by various factors, such as the magnitude and duration of the orthodontic force applied, shape of the roots, and the mechanical characteristics of the periodontal ligament.[21] The differences in the results of the investigations focusing on the effect of steroids on OTM might reflect the combined effects of the dosages, the induction periods, and the relative anti-inflammatory activity of the corticosteroids tested (Table 2). It seems as if corticosteroids stimulate OTM, but this depends on the relative potency of the corticosteroid used and the administration protocol. Future studies with multiple groups controlling for dosage, duration and the relative potency of steroid are needed to further our understanding of the potential effects of corticosteroids on OTM.

From a clinical point of view, the main issue with corticosteroids is that they are used for a wide range of medical reasons and their use for orthodontic reasons would not be appropriate. The systemic use of other drugs has not been met with widespread acclaim because of the detrimental effects elsewhere in the body rather than the desired local reaction.[7] Therefore, it is expected that the systemic administration of triamcinolone would have similar disadvantages.

The present study suggests that patients who are being treated with steroids for allergy cases such as hay fever[22] need to have their orthodontic appliances controlled more frequently, since our data suggest that the rate of tooth movement will be increased by the administration of corticosteroids. The orthodontist should also note that there is a risk of limited bone formation on the tension side of teeth, resulting in a loose tooth.

For future studies, it is suggested that researchers use a design with further control groups: a group with corticosteroid injection without orthodontic tooth movement, and a further control group with a sham injection of sterile phosphate buffered saline. Also, it should be pointed out that rate of the experimental tooth movement is higher in the initial phase than in the latter phase. Additional data, on the number of osteoclasts and the area of resorption lacunae would also be informative.

CONCLUSION

Triamcinolone acetonide was associated with accelerated tooth movement in rabbits *via* the suppression of osteoblastic activity and increased resorptive activity in alveolar bone.

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