

Orthodontic Tooth Movement in the Prednisolone-Treated Rat

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Abstract: Adverse effects of corticosteroids on bone metabolism raise concerns as to whether steroid treatment may influence orthodontic movement. This study examined the effect of prednisolone on orthodontic movement using an established rat model. The corticosteroid treated group (N = 6) was administered prednisolone (1 mg/kg) daily, for a 12-day induction period; the control group (N = 6) received equivalent volumes of saline. On day 12, an orthodontic appliance was placed which exerted 30 g of mesial force to the maxillary first molar. Animals were sacrificed on day 24 and tooth movement was measured. Sagittal sections of the molars were stained with haematoxylin and eosin, and for tartrate-resistant acid phosphatase (TRAP) activity. While there were no significant differences in the magnitude of tooth movement between the 2 groups, steroid-treated rats displayed significantly less root resorption on the compression side and fewer TRAP-positive cells within the PDL space on the same side. This suggests steroid treatment suppressed clastic activity. (*Angle Orthod* 2000;70:118–125.)

Key Words: Prednisolone; Orthodontic tooth movement; Rat; TRAP; Root resorption

INTRODUCTION

Corticosteroids (CS) are a group of related compounds derived from cholesterol that have widespread effects on a diverse range of organs.¹ Synthetic steroids are used as anti-inflammatory and immunosuppressive agents in the treatment of a wide range of chronic medical conditions. Prednisolone is a moderately potent synthetic steroid which is a useful prototype to study the effects of steroids on both humans and animals.^{1,2} Numerous systemic side effects associated with the long-term use of prednisolone therapy have been documented, including disturbances of mineralized tissue metabolism and wound healing, an increased risk of infection, and suppression of somatic growth, chondrogenesis, and osteogenesis.¹

Prolonged administration of synthetic CS in moderate to large doses, is associated with bone loss and osteoporosis.³ Corticosteroids interfere with the coupling of resorption and deposition cycle in normal bone, which results in reduced bone formation and increased bone resorption. Even low

dosages of steroids have been shown to directly affect cells of osteoblastic lineage,⁴ with impaired differentiation of osteoprogenitor cells into osteoblasts, and decreased collagen formation by mature osteoblasts. Corticosteroids also cause a reduction in bone mass indirectly by inhibiting gonadotrophin and sex steroid production thereby inducing a state of hypogonadism.³ Recently, bone resorption has been shown to remain unchanged or to decrease slightly in healthy young male volunteers, after administration of low doses of prednisolone.⁴

Active osteoclasts, osteoclast-like cells, and their mononuclear precursors exhibit a high content of a specific cytochemical marker, tartrate-resistant acid phosphatase (TRAP),⁵ which is thought to participate in or signal active bone resorption.⁶ While there is some evidence that expression of TRAP is hormonally regulated,⁵ the expression of steroids on the expression of TRAP has not been determined.

Despite the well-documented effects on skeletal tissues, little is known about the consequences of CS on orthodontic treatment. Orthodontic tooth movement relies on the efficient remodeling of bone and involves the coordinated processes of resorption and deposition. Typically, force application to a tooth causes the coronal portion of the root to move in the direction of the line of force, compressing the periodontal ligament (PDL) and alveolar bone in those areas and stretching the PDL on the opposite side of the root. The compression results in bone resorption and the tension stimulates bone formation. Exogenous administration of steroids may threaten this balanced physiologic response to orthodontic forces and affect treatment in a number of

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ways. For example, exogenous steroids may disrupt the rate of orthodontic tooth movement, or influence the stability of the posttreatment result. Both these effects were seen in an animal study where rabbits were treated with high doses of cortisone acetate (15 mg/kg), which produced significantly more rapid orthodontic tooth movement and subsequent relapse compared with the controls.⁷ This study was of limited application because the doses of steroid were so high that osteoporosis resulted. The effects of low doses are of greater clinical interest and relevance. The aim of the present study was to examine the effects of a low-dose prednisolone treatment on orthodontic tooth movement using a well-established rat model.

MATERIALS AND METHODS

Animals

Twelve 9-week-old male Wistar rats, obtained from the Central Animal Breeding House, University of Queensland, were used in this study. The average weight of the rats was 270.5 g. The Institutional Ethics Committee granted ethical clearance, and the study followed the guidelines prescribed for animal experimentation by the National Health and Medical Research Council of Australia. Animals were acclimatized for 5 days in plastic cages (two to a cage) with a standard 12-hour light-dark cycle, and fed a diet of finely ground laboratory food ad libitum. The soft food diet minimized any discomfort to the animal following orthodontic appliance insertion and minimized the risk of appliance displacement. Body weights of all rats were measured daily. Of the 12 animals used in this study, 6 served as controls and 6 were treated with prednisolone. The steroid-treated group was fed a daily dose of 1 mg/kg oral prednisolone (Fisons Pty Ltd, Sydney, Australia) in saline, using a stomach tube, for a 12-day induction period and for the next 11 days while the orthodontic appliance was in situ. The control group was fed an equivalent volume of saline (Astra Pharmaceuticals Pty Ltd, Sydney, Australia) using a stomach tube over the same time period. The dose of 1 mg/kg is within recommended therapeutic levels for rats,⁸ and is associated with anti-inflammatory and some immunosuppressive effects.⁹ This dosage has been used in our laboratory on immature and mature Lewis rats and does not result in the failure to gain weight observed with a higher dose of 5 mg/kg.¹⁰

Orthodontic appliance treatment

Following acclimatization, an orthodontic appliance was inserted on the maxillary left first molar, and a mesially directed force of 30 g was applied. The force level was verified using a Dynamometer (Correx, Dentarum, Newtown, Pa., USA) measuring gauge. Force level was measured on insertion while the animal was sedated. The orthodontic appliance (Figure 1) consisted of a stretched

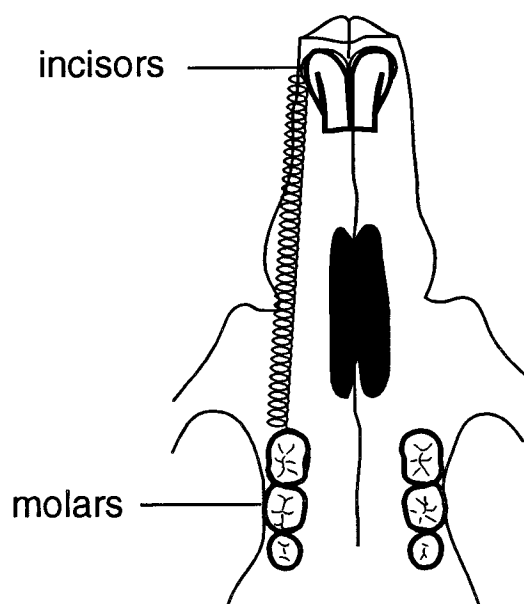


FIGURE 1. Orthodontic appliance.

closed coil spring (0.008" × 0.032" Elgiloy spring, Rocky Mountain Dental Products Co, Denver, Co., USA) ligated between the maxillary left first molar and 2 maxillary central incisors. Incisors were notched on the mesial and distal surfaces to ensure maximum retention of the spring. The molar on the right side was used as a nonappliance control. During appliance insertion, rats were sedated by subcutaneous injection of fentanyl citrate/fluanisone and midazolam (Sublimaze & Hypnovel) 0.15 to 0.2 mL per 100 g body weight. One rat from the control group died following complications associated with the use of these sedatives. This orthodontic appliance is based on a modified technique described by Brudvik and Rygh.^{11, 12}

The appliance was activated immediately upon insertion and was not reactivated during the experimental period. At 12 days postappliance insertion, animals were sacrificed using carbon dioxide asphyxiation. The magnitude of tooth movement was determined by measuring the relative separation between the first and second maxillary molars, using vernier calipers with sharpened tips (accurate to 0.1 mm) to measure the distance between the mesial occlusal pits of these molar teeth. Measurements were recorded intra-orally prior to appliance insertion, both on the appliance (left) side and nonappliance (right) side, and again immediately after sacrifice. All measurements at each time, were repeated 5 times for each side of the maxilla for each animal. The same operator performed all measurements. The group mean values were calculated and the difference between the pretreatment and posttreatment measurements was recorded as the magnitude of tooth movement.

Histological preparation

Following animal sacrifice, maxillae were immediately removed, fixed in Bouin's solution (1.2% picric acid, 10%

formaldehyde and 5% glacial acetic acid) for 24 hours, and demineralised in 0.25 M ethylenediaminetetraacetic acid (EDTA) (10%, pH 7.2) at 4°C for 14 days. Paraffin sagittal sections were prepared (parallel to the long axis of the first molar, at a thickness of 5 µm) and mounted on 3-aminopropyltriethoxysilane (APES) coated glass slides. Sections closest to the midline of the crown, which contained roots, radicular tissue, and pulp were examined. Two serial sections from each animal were stained with haematoxylin and eosin (H & E), and 2 serial sections for TRAP activity.

TRAP histochemistry was used to identify clastic cells and their precursors. TRAP activity was demonstrated using naphthol ASTR-phosphate (in N,N-dimethyl formamide) as a substrate and pararosaniline-HCL as a coupling agent in L(+)- tartaric acid, at 37°C for 30 minutes. Sections were counterstained with 0.2% methyl green. TRAP activity produced a red-colored reaction product. Specificity was verified by the use of negative controls in which the substrate naphthol AS-BI phosphate was excluded during incubation.

Histological analysis

The 22 hemi-maxillae harvested from the 6 steroid-treated (ST) and 5 nonsteroid-treated (NST) control animals were divided into the following 4 subgroups for histological examination: (1) no-steroid, no-appliance controls (NNA) (2) no-steroid, appliance controls (NAP), (3) steroid, no-appliance (SNA), and (4) steroid, appliance groups (SAP). Histomorphometry was performed on the regions surrounding the distal root (coronal one-third) of the maxillary first molars using light microscopy. Areas for measurement were the mesial and distal aspects of the distal root, corresponding to compression and tension sides, respectively, in the appliance groups. Sections were examined at ×40 and ×100 magnification using a calibrated graticule to determine the width of the PDL and the lengths of root resorption and the hyalinized zone.

Cell counts were performed on sections stained for TRAP using a precalibrated 10 × 10-graticule eyepiece micrometer (Olympus, Tokyo, Japan), at ×400 magnification. Total TRAP positive cell numbers were counted from 10 adjacent small squares (area 24.5 µm²) oriented vertically along the surface of the alveolar bone and cementum, and in the PDL as shown in Figure 2. Both mono- and multinucleated TRAP-positive cells in the PDL, and multinucleated TRAP-positive cells along the alveolar bone and root surfaces were counted on both compression and tension sides.

Quantitative data

Weight measurements were performed 3 times daily on each animal, and the mean of the 3 measurements was used to estimate the percent body weight gain or loss for each animal. Individual percent of weight gain or loss were averaged to derive the group mean.

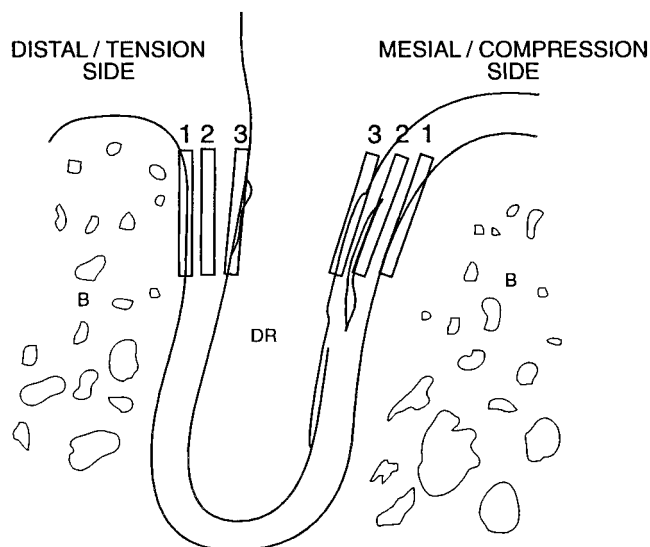


FIGURE 2. Boxes representing cell count sites on the distal root (DR): (1) along the alveolar bone (B) margin; (2) in the central region of the periodontal ligament; and (3) along the distal root surface.

The magnitude of tooth movement was calculated using the mean distance (5 repeated measurements) between the mesial occlusal pits of the first and second molars, intra-orally at the time of appliance insertion and intra-orally at the time of animal sacrifice. The mean magnitudes of tooth movement for each subgroup (SNA, NNA, SAP and NAP) were calculated, and differences compared using analysis of variance (ANOVA), with $P < .05$.

The histomorphometric measurements involving root length, PDL width, root resorption length, and hyalinized zone length, were performed on 2 serial sections from each animal within the 4 subgroups (SNA, NNA, SAP, NAP). Each section was measured twice, yielding 4 measurements from which the average value for each animal was derived. These were then combined to yield the group mean value. Differences between subgroups were compared using ANOVA.

Cell counts for each section were performed twice, the mean calculated, and intra-operator error determined following repeated blind measurements. Cell counts were performed on 2 sections per specimen and differences in counts were determined using ANOVA with a level of significance at $P < .05$.

RESULTS

Animal health and measurement error

All animals appeared healthy during the study. Food and water consumption appeared to be unaffected by the orthodontic appliance. Except for a temporary episode of weight loss in all animals for 1 to 2 days following appliance insertion, there was an overall gain in weight throughout the

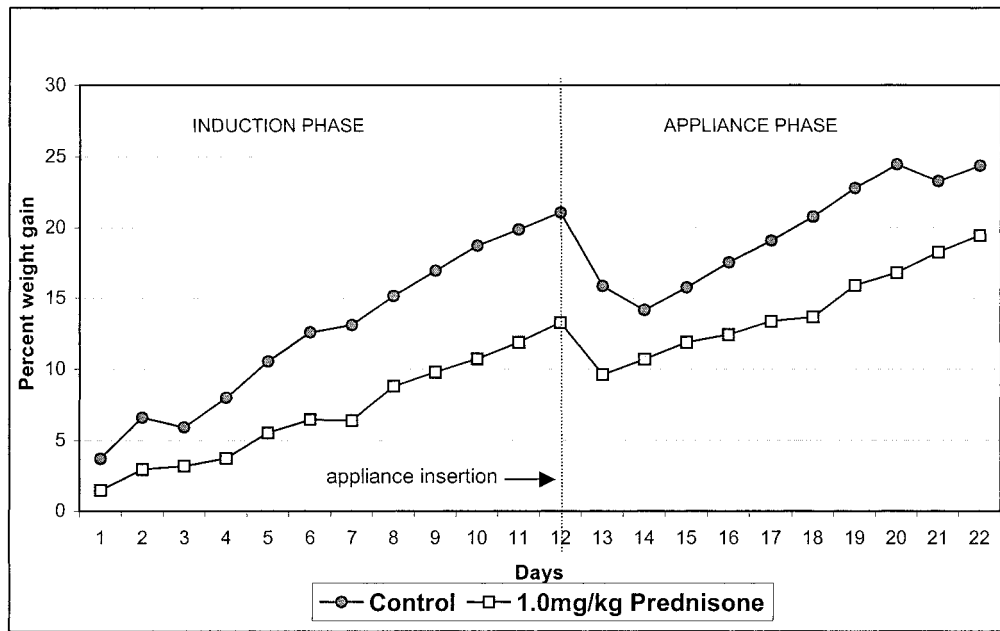


FIGURE 3. Percent weight gain (of original weight) for control and steroid treated rats during the experimental period.

induction and experimental periods (Figure 3). Steroid-treated animal weight gain was similar to NST controls (Table 1). There was good to high agreement for measurements undertaken by the one investigator (Dr Ong). Agreement for intra-examiner measurements and counts was not less than 97%.

Tooth movement

All appliance-treated molars in the SAP and NAP animals showed evidence of tooth movement, with the development of spacing between the first and second molars. No space was observed between the second and third molars, indicating lack of mesial movement of the second molar during the experiment. However, mesial movement of the first molar may induce changes in the transeptal fibers between the first and second molars and result in some mesial movement of the second and third molars. Sections of the second and third molar roots did not demonstrate the histological features, characteristic of orthodontic tooth movement, observed for the first molar. No tooth movement was evident on the nonappliance side. There was no significant difference in the group mean magnitude of tooth movement between the SAP and NAP groups (Table 1).

Width of PDL space

The width of the PDL space on the compression side was significantly wider in both the appliance groups compared with the nonappliance groups ($P < .05$). Width of the PDL space on the tension side in the appliance group was significantly wider compared with the nonappliance group ($P < .001$, Table 1).

Length of hyalinized zone

The length of the distal root available for histological measurements was similar for all groups (mean value of $1415 \mu\text{m} \pm 430 \mu\text{m}$). Hyalinized zones in the PDL space on the mesial surface (compression zones) were observed only in appliance groups (Table 1). The extent and location varied considerably from animal to animal. Generally, the hyalinized area was located between the coronal and middle thirds of the root. No difference in length of the hyalinized zone was observed between the SAP and NAP groups ($P > .05$). Hyalinized lengths ranged from $0 \mu\text{m}$ to $377 \mu\text{m}$ in the NAP group, and from $0 \mu\text{m}$ to $503 \mu\text{m}$ in the SAP group. Four of the 6 animals in the SAP group and 2 of the 5 animals in the NAP group had $0 \mu\text{m}$ hyalinized lengths.

Root resorption was apparent on the compression surface of the distal root for both appliance groups. Most resorption took place apical and coronal to the hyalinized zone, rather than directly adjacent to it. No resorption took place at the apex of the roots. Compression-side root resorption lengths for the NAP group were greater compared with those for the SAP group ($P < .001$, Table 1). Occasional sporadic pitting resorption affecting the distal aspect of the root surface was observed in the NNA and SAP groups. This resorption appeared to be of a limited nature, distinct from that observed on the compression surfaces.

TRAP activity

TRAP-positive cells were detected in all groups studied. The intensity of TRAP activity was greatest in areas associated with compression-induced resorption and repair (Fig-

TABLE 1. Measurements Recorded for Control and Steroid-Treated Animals

Group ^a	Parameter ^a	Mean (SD) ^a	Significance ^a
Control	Percent weight gain	24.5 (6.4)	} ns
Steroid		23.4 (4.0)	
NAP	Magnitude of tooth movement (mm)	0.3 (0.04)	} ns
SAP		0.3 (0.06)	
NNA	Width PDL (μ m), compression	124.3 (9.1)	} ns } $P < .05$
SNA		128.1 (7.2)	
NAP		174.6 (24.2)	
SAP		173.5 (29.4)	
NNA	Width PDL (μ m), tension	146.7 (4.9)	} ns } $P < .001$
SNA		145.5 (5.3)	
NAP		308.7 (18.7)	
SAP		293.4 (8.8)	
NAP	Length hyalinized zone (μ m)	155.1 (167.4)	} ns
SAP		118.8 (206.0)	
NAP	Root resorption (μ m) compression	687.4 (54.5)	} $P < .001$
SAP		513.4 (73.9)	

^a SD indicates standard deviation; NAP, no-steroid, appliance controls; SAP, steroid, appliance group; NNA, no-steroid, no-appliance controls; SNA, steroid, no-appliance group; PDL, periodontal ligament; and ns, nonsignificant. NNA and NAP (N = 5); SNA and SAP (N = 6).

ure 4). On the compression side, TRAP activity was observed in the coronal and mid thirds of the root, and along the adjacent margins of the alveolar bone as well as within the marrow spaces. Staining was particularly intense along the coronal third of the root and adjacent bone surface.

TRAP-positive cells were present at the apical and coronal peripheries, but not within the areas of hyalinization. TRAP-positive multinucleated cells were located along both root and alveolar bone surfaces, as well as occasionally within the PDL (Table 2). Mononuclear TRAP-positive cells were present in the coronal third of the PDL and in areas adjacent to root resorption and hyalinized zones.

There were significantly fewer TRAP positive cells counted in the mesial (compression) side PDL in the SAP group compared with the NAP control group, ($P < .001$, Table 2). Significantly more TRAP-positive cells were present on the alveolar bone surface and along the root surface, on the mesial surface, in the NAP group compared with the SAP group. In all subgroups, the occasional TRAP-positive cell was detected along the alveolar bone margins on the distal aspect, in the coronal and middle thirds. However, numbers were very low and too few cells were available for statistical analysis. Sections from controls were generally negative for TRAP activity and only a few sections contained the occasional TRAP-positive cell.

DISCUSSION

In this study, an orthodontic appliance which applied a mesial force of 30 g to a rat molar produced orthodontic tooth movement and characteristic histological reactions.¹¹⁻¹⁵ This reaction included widening of the PDL on the tension side, narrowing of the PDL on the compression side, areas

of hyalinization on the compression side, frontal alveolar bone resorption on the compression side, and root resorption affecting the compression side root surface. The use of 1 mg/kg prednisolone did not appear to affect the magnitude of tooth movement. In addition, the overall morphology of tooth movement, twelve days after appliance activation, was largely unaffected by steroid treatment, except for a reduction in the length of root resorption along the compression side. Histochemically, fewer TRAP positive cells were present in the compression side of the PDL in the ST group compared with controls.

The steroid treatment protocol did not impair the overall health or weight gain of the animals. All animals showed a temporary loss of weight at the time of appliance insertion, consistent with the findings of Brudvik and Rygh.¹⁰ The short duration of corticosteroid administration in the present study makes the possibility of iatrogenic hypercortisolemia and hyperparathyroidism remote.

There was no difference in the magnitude of tooth movement between the ST and NST groups, and the values recorded for tooth movement were similar to those obtained by King and Keeling.¹⁶ However, controversy exists as to the effects of corticosteroids on tooth movement. As noted previously, Ashcraft et al⁷ induced orthodontic molar tooth movement for 14 days in corticosteroid-induced osteoporotic rabbits, and showed a greater rate of tooth movement in ST rabbits. In contrast, Yamane et al¹⁷ reported that tooth movement in rats was inhibited by 10 mg/kg per day of hydrocortisone, while Davidovitch et al¹⁸ showed slower tooth movement in cats treated with cortisone acetate (12.5 to 25 mg/day). These differences may be explained by variations within animal species studied, forces used to move

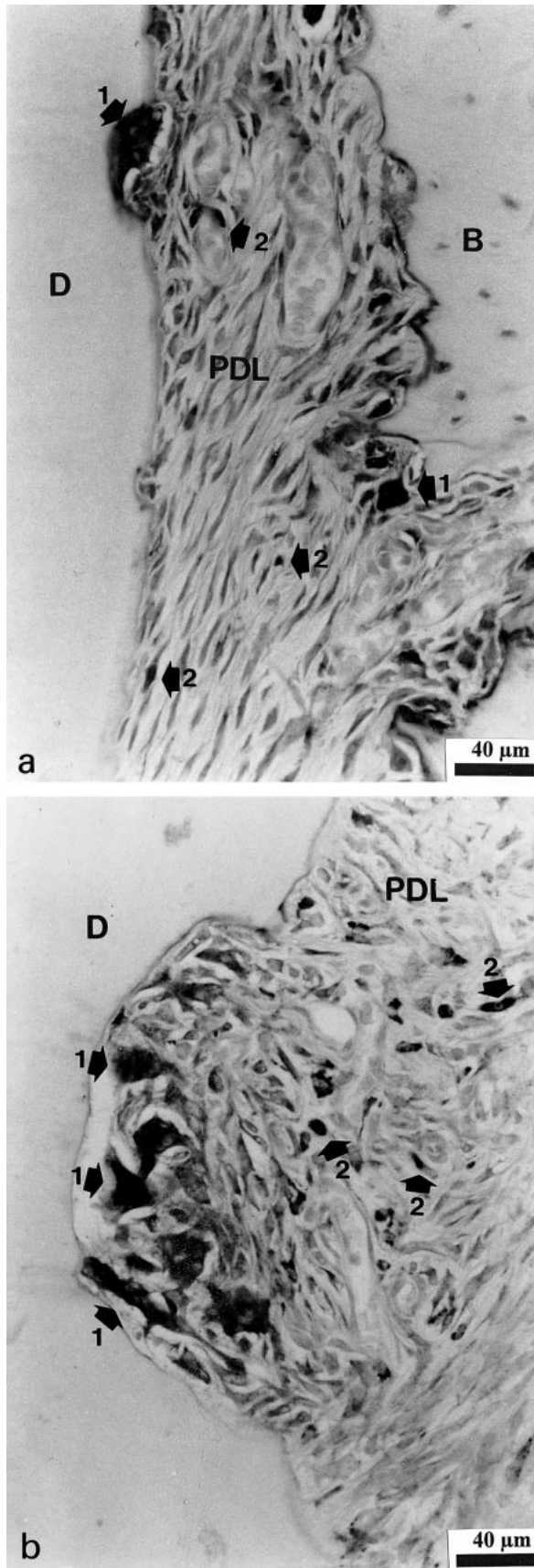


TABLE 2. Number of TRAP-Positive Cells Counted on the Alveolar Bone, PDL and Root Surface in the Zone of Compression

Group ^a	Location ^a	Number of		Significance
		Measurements	Mean (SD) ^a	
NAP	Alveolar bone surface	20	10.15 (2.68)	} <i>P</i> < .05
SAP		24	8.73 (0.97)	
NAP	PDL	20	17.25 (8.04)	} <i>P</i> < .001
SAP		24	10.42 (3.71)	
NAP	Root surface	20	4.37 (2.22)	} <i>P</i> < .01
SAP		24	5.79 (1.02)	

^aSD indicates standard deviation; NAP, no-steroid, appliance controls; SAP, steroid, appliance group; and PDL, periodontal ligament. Too few TRAP-positive cells were present on the tension side for statistical analysis. NNA and NAP (N = 5); SNA and SAP (N = 6).

teeth, duration of the experiment, dosage and time interval of administration, and potency of the steroid used. The present study used a standardized technique for inducing orthodontic tooth movement in rats as described previously by Brudvik and Rygh.¹¹ This technique mimics orthodontic tooth movement in humans.

There was a significant increase in the mesial (compression) PDL width in both appliance groups compared with the nonappliance controls (*P* < .05). Frontal alveolar bone resorption along the compression side is a normal component of remodeling associated with orthodontic tooth movement.¹³ In addition, root resorption caused by compression contributed to the observed increase in width of the PDL in the appliance groups. The mean values of the mesial PDL width in this study were comparable to those recorded by Brudvik and Rygh¹³ for the same time period. They recorded the widest PDL widths between days 10 and 14. In the present study, the presence of steroids did not have any effect on the width of PDL measured when compared with the NST groups. Only 1 time interval was examined in the present study, hence, significant effects of prednisolone on cell activity at earlier time intervals cannot be ruled out.

Previous studies have reported that hyalinization of the PDL precedes root resorption during orthodontic movement, and that resorption is often seen in areas adjacent to the hyalinized zones, especially in the early stages.^{11,13,14} Brudvik and Rygh,^{13,14} and Hellsing and Hammarstrom¹⁹ showed that the reorganization of necrotic tissues evokes substances that activate root resorption and removal of the hyalinized zone before repair can occur. They claimed that continued root resorption was associated with the persis-

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FIGURE 4. Histological sections of the first molar root on compression side from a steroid-treated animal showing TRAP activity in multinuclear (1) and mononuclear (2) cells in: (a) the region cervical to the hyalinized zone, and (b) a site of root resorption. Bone (B), Dentine (D) and periodontal ligament (PDL). Bar represents 40 µm.

tence and the removal of the necrotic tissue. This premise is supported by the findings in the present study, where root resorption on the compression side root surface could be observed in the absence of hyalinization. Root resorption has been reported to occur in rats after 7 days of active appliance wear.¹⁹

The results of the present study demonstrate a reduction in the extent of root resorption along compression sites in ST animals. This may represent either a suppression of clastic-inflammatory activity or conversely, facilitated early repair. As the mean hyalinized zone lengths in ST and NST animals were not significantly different, improved repair of the root is unlikely to explain the result obtained. The suppression of clastic-inflammatory activity may be reflected in the lower TRAP cell counts in steroid-treated animals. Orthodontic tooth movement will induce an inflammatory response in the periodontal ligament and result in bone resorption on the compression side and bone deposition on the tension side. During normal physiological bone metabolism resorption and deposition occur in the absence of inflammation. As prednisolone is an anti-inflammatory agent, it may lower the TRAP activity associated with inflammation during tooth movement, without suppressing the rate of bone resorption necessary for tooth movement.

Active osteoclasts, osteoclast-like cells, and their mononuclear precursors exhibit a high levels of TRAP activity.^{5,11} Although strong TRAP activity has been assumed to be a major cytochemical marker of osteoclasts, TRAP activity has been demonstrated in both osteoblasts and osteocytes.^{11,20} In the present study, strong TRAP activity was consistently associated with multinucleated cells and resorptive areas along the compression side PDL, alveolar bone, and root surface in both control and steroid-treated animals. There was a significant reduction in TRAP activity in the SAP group compared with the NAP control group. This apparent reduction in clastic activity may provide a possible explanation for the reduction in root resorption length measured along the compression side root surface. Whether this represents a reduction in actual clastic cell numbers or merely a reduction in cytoplasmic TRAP activity remains to be determined. King and Keeling¹⁶ and King et al²¹ noted biphasic bone remodeling during orthodontic tooth movement, with bone resorption and formation occurring in alternate waves. Steroids may disrupt this pattern of bone remodeling during tooth movement.

CONCLUSION

The present study was conducted using 1 mg/kg of prednisolone administered for a period of 23 days to mature male Wistar rats (n = 6). An orthodontic appliance was used to exert a force of 30 g on the maxillary first left molar. Prednisolone treatment did not affect the magnitude of orthodontic tooth movement compared with the NST controls. However, the length of root resorption along the

mesial compression side and TRAP activity in the compression side PDL were reduced, suggesting a suppression of clastic activity had taken place.

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