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Tissue reaction to orthodontic tooth movement in acute and chronic corticosteroid treatment*

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Structured Abstract

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Objectives – To study tissue reaction to orthodontic loading during the course of short- and long-term corticosteroid administration.

Design – 'Split-mouth' design to perform orthodontic tooth movement in 64 six-month-old male rats divided into groups: no drug administration (n = 19), acute (n = 22) and chronic (n = 23) 8 mg/kg/day corticosteroid treatment. Performed in the Department of Orthodontics at Aarhus University.

Experimental Variable – The upper left first molar was moved for 21 days. Bone markers were administered at 7 and 2 days before sacrifice. Histological sections were cut at the coronal level.

Outcome Measure – Tooth movement rate, alveolar socket area, the relative extension of alveolar wall with erosion, and the mineralizing surfaces were measured and compared in the three groups.

Results – Tooth movement rate increased in the chronic group. The mechanical load induced an enlargement of the alveolar wall that was less pronounced in both medicated groups. In the acute group the drug suppressed bone resorption and formation without mechanical stimulus. Force application resulted in significant increase in the relative extension of resorption and formation in both drug groups; it was particularly pronounced in the chronic group.

Conclusion – Because acute corticosteroid ingestion reduces bone turnover, in these patients orthodontic treatment might best be postponed until a time the patient is free of the drug. Chronic steroid ingestion leads to an increased biological reaction to mechanical perturbation indicating that the orthodontic force level should be reduced and controlled more frequently in patients on chronic steroid treatment.

Key words: alveolar bone turnover; corticosteroid; histomorphometry; orthodontics; rats

Introduction

The state of tissues that surround the teeth is influenced by local factors related to teeth and occlusion, as well as, the general metabolism of the total skeleton. The reaction to orthodontic forces has been described (1–3) and it has been shown that orthodontic tooth movement may be influenced by general and local administration of pharmaceutical agents (4–9). As the prevalence of allergies and diseases that need corticosteroid treatment is on the increase, it can be anticipated that an important number of orthodontic patients can present variations from normal bone turnover because of this steroid (10). In most of the published animal experiments that studied glucocorticoid administration and orthodontic tooth movement, the glucocorticosteroid dose has been high. These high doses made the animals osteoporotic. Daily injections (15 mg/kg) of glucocorticosteroid drug caused a marked state of osteoporosis in a short time period in the rabbit (11–13) and even higher doses (25 mg/kg) have been used in cats (14). The dosages used in the above-mentioned studies, however, are not compatible with the concentrations recommended for use in humans, either for short or long durations. Yamane et al. (15) used a dosage of 10 mg/kg for only 7 days. Ong et al. (16) used a therapeutic dosage of 1 mg/kg in young rats short-term, thus avoiding the risk of secondary hyperparathyroidism.

A proper evaluation of the effect of combined orthodontic treatment and metabolic condition requires measures of clinical outcome i.e. the rate of orthodontic tooth movement, and histological and histomorphometric data. The latter allows for the analysis of the bone remodeling patterns through the quantification of both the resorptive and formative components of the remodeling cycle, combined with the description of structural changes. While the influence of the corticosteroids on bone metabolism has been well described (17), the effect of combined corticosteroid treatment at therapeutic dosages and orthodontic forces on bone behavior remains elusive. This is because studies that investigated the role of corticosteroid treatment have not simultaneously analyzed the resorptive and the formative components of bone remodeling and the structural changes of the alveolar bone. These reports give only a partial answer to the question (11, 16).

On this background the aims of this study were defined to be the following:

1. To study the effect of short-term and long-term therapeutic dose corticosteroid administration on orthodontic tooth movement rate.
2. To study the effect of such dosage and application of corticosteroid on alveolar bone structure.
3. To evaluate the modification of bone remodeling pattern subsequent to orthodontic loading in combination with the above-mentioned dosages of the drug.

Materials and methods

Sixty-four 6-month-old outbred male Wistar rats with a body weight of 350–500 g were obtained from Møllegaards Breeding and Research Centre (Ejby, Denmark). They were housed paired in cages in a room with a 12:12-h artificial light cycle, at room temperature and humidity according to the National Research Council's guide for the care and use of laboratory animals. Rats were divided at random into three groups: a chronic group (n = 23) that received pharmacological treatment for 7 weeks (weeks 1–7), and orthodontic treatment for 3 weeks (weeks 5–7), an acute group (n = 22) that received pharmacological treatment and orthodontic treatment simultaneously for 3 weeks (weeks 5–7), and a control group (n = 19) without any pharmacological treatment but that received orthodontic treatment for 3 weeks (weeks 5–7). All animals were killed at the end of week 7; see Fig. 1.

The experimental groups received 8 mg/kg/day of methylprednisolone ('Solu-medrol'; Pharmacia and Uphjolm Company, Kalamazoo, MI, USA) subcutaneously every 24 h for the prescribed number of days (18). Body weight was checked weekly. All rats received a standard diet (Altromin, Brogaarden, Gentofte, Denmark) and tap water *ad libitum*. Orthodontic tooth movement was generated by the insertion of a 25 g Sentalloy® (GAC, Ctr. Iship, NY, USA) closed coil orthodontic spring between the upper left first molar and upper incisors (Fig. 2). The appliance was inserted under general anesthesia, induced by a subcutaneous injection of 0.1 ml/100 g body weight of Immobilon® (Pherrovet, Malmø, Sweden) and reversed by the same amount of Revivlon® (Pherrovet). The spring was left in

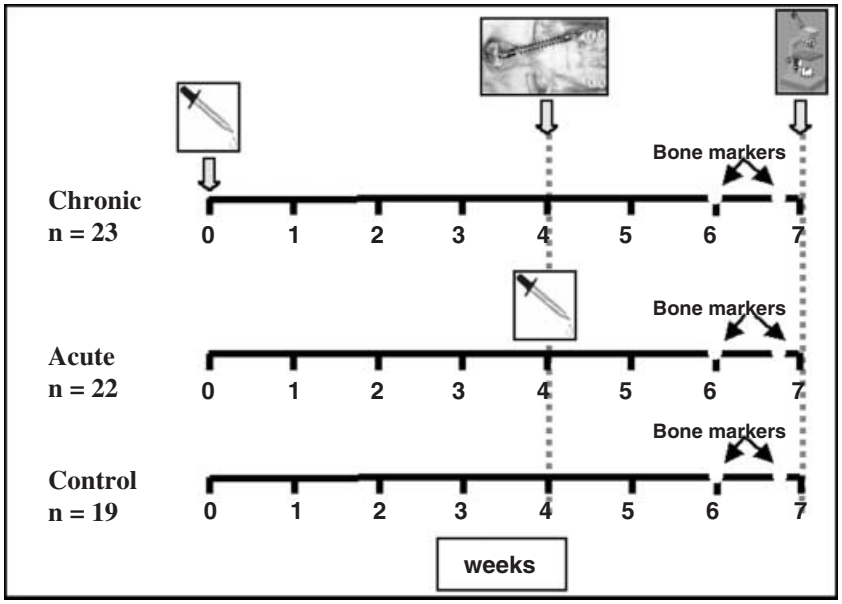

Fig. 1. Flow diagram illustration the treatment of the rats in the three groups.

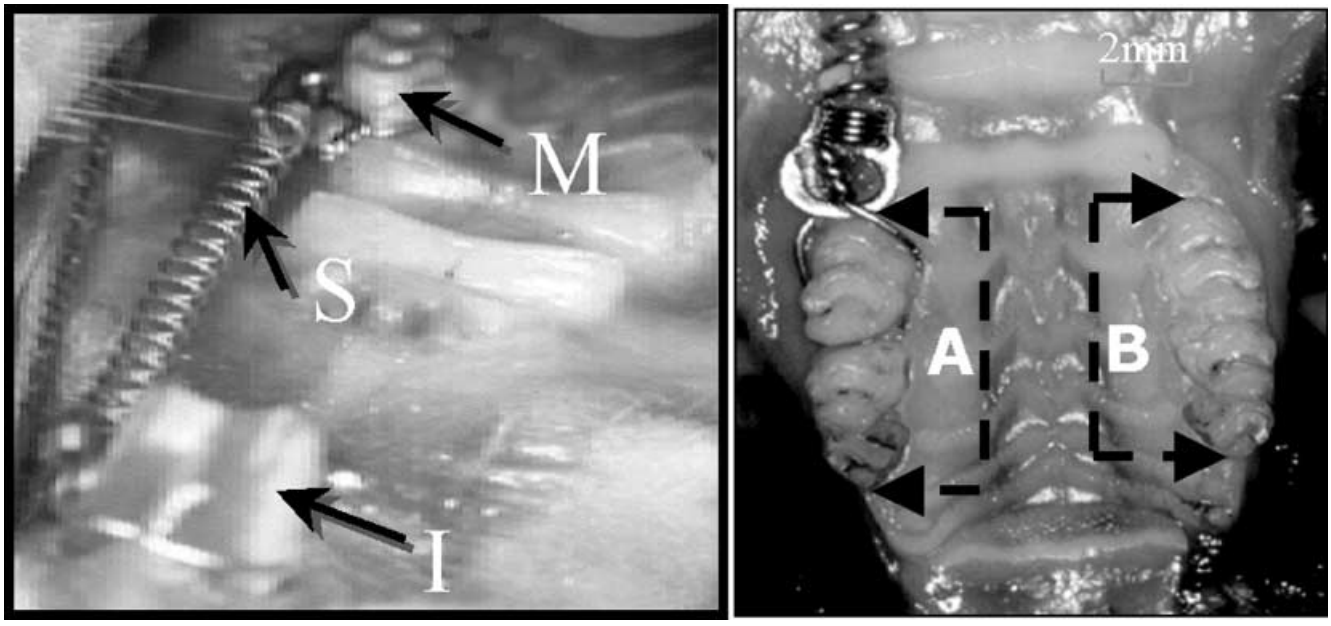

Fig. 2. Appliance used to move the molars mesially. (a) Detailed picture of the coil spring (S) extending from the incisors (I) to the upper left first molar (M). (b) Indication of the measurement procedure. The distance between the mesial side of the first molar and the distal side of the third molar was measured, and that of the treated side (A) subtracted from the one of the untreated side (B).

place for 21 days, in order to generate a mesial movement of the first molar (Fig. 2). With the purpose of evaluating the bone turn-over 15 mg/kg of tetracycline and 20 mg/kg of calceine were administered intraperitoneally respectively 7 and 2 days before killing (19).

To limit the influence of inter-animal variation in response to metabolic stimuli, a split-mouth design was used and the untreated contralateral side served as control side. All animals were sacrificed with an overdose of CO_2 and their maxillae excised. The distance between the mesial surface of the first and the distal surface of the third molar was measured bilaterally with an electronic caliper (Digimatic-Mitutoyo, Telford, UK). Tooth movement was estimated by subtracting the mean of the repeated measured values from the untreated and treated sides as described by Hong et al. (20) (Fig. 2). The error of the method based on double measurements has already been described (21).

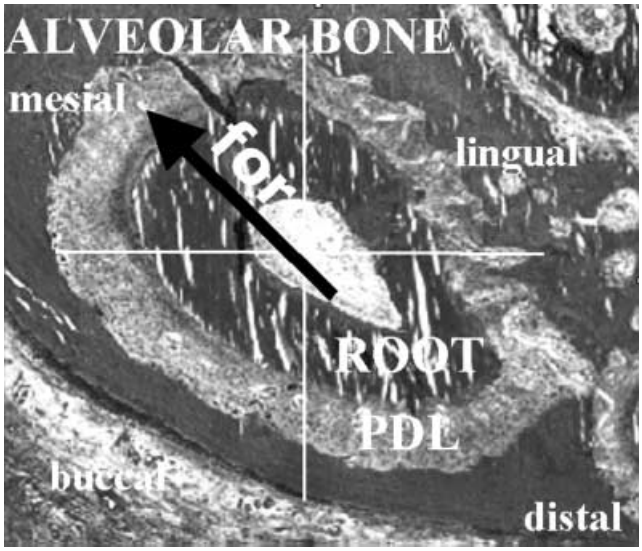

Fig. 3. Micrograph of the horizontal section of the molar root showing the areas where the measurements were performed.

The excised undecalcified maxillae were then embedded in methylmethacrylate and paraocclusal sections were cut at the coronal level (Fig. 3). Seven-micrometer thick sections were stained with modified Goldner trichrome, while the adjacent was cut at 10 μm and left unstained for fluorescent microscopy. Tissues surrounding the mesial root were investigated on the treated and contralateral untreated side and the following histomorphometric parameters were determined:

1. Alveolar socket area (AS): the cross-section of the four quadrants of the alveolar socket surrounding the root expressed in mm^2. A custom-made radial grid with 32 equally distanced lines in ramification from the central point was placed in the right eyepiece of the microscope, with the central point corresponding with the center of the root. An image analysis system was applied in order to measure the distance from the center of the grid and the intersection of with the outline of the alveolar wall. When no bone was present, the border of the alveolar socket was considered as corresponding to the next anatomical structure; i.e. the periosteal surface of the maxillary bone or the neighboring roots. The alveolar socket was divided into quadrants: M = mesial, B = buccal, D = distal, L = lingual by a cross centered in the middle of the root and oriented according to the midpalatal suture at an inclination of approximately 15° (Fig. 2). The measurements were performed by a custom-made software image analysis system at a 40× magnification.
2. Relative extension of alveolar wall covered by erosion surfaces (ES/BS) (22) was determined by means of a Zeus II integrating reticule (Carl Zeiss GmbH, Jena, Germany) with equidistant parallel lines. The percentage of intersections hitting the resorption lacunae, defined as scalloped surfaces with or without osteoclasts, was measured on the mesial (M) and the distal (D) site of the alveolar socket. A total of 150 intersections with bone on each site were recorded at a 200× magnification.
3. Relative extension of mineralizing surfaces on the alveolar bone surrounding the root (MS/BS) (22). The percentage of lines intersecting bone labeled with both tetracycline and calceine was measured at 200× magnification under fluorescent light using the same system described above for the ES/BS.

Statistical analysis

The obtained parameters were described statistically and evaluated by means of a repeated measurement three-way analysis of variance. The quadrants and the sides (treated and untreated) were the repeated measurements within the groups. The pharmacological treatment (0 = no treatment, 1 = acute, 2 = chronic) was the between subject factor. An *a posteriori* test for pairwise comparison (Student–Newman–Keuls test) was performed to evaluate whether a statistically significant difference existed between the three groups. All data were analyzed by using the statistical software SPSS for Windows (SPSS Inc., Chicago, IL, USA) and the level of significance was chosen to be 5%.

Results

The pharmacological treatment resulted in a moderate weight loss, which was more pronounced in the chronic than in the acute group. This weight loss was accentuated by the appliance insertion. The rate of tooth movement was significantly faster in the chronic group than in the control ($p < 0.02$) and the acute group ($p < 0.05$). The difference between the control and acute group was not significant (Table 1).

Table 1. **Tooth movement in pharmacologically treated and untreated rats in mm**

Treatment	n	Mean	SD
Control	19	0.18	0.122
Chronic	23	0.28*	0.152
Acute	22	0.21†	0.115

*Significantly different from the control group.
†Sig. to the chronic.

The surface area of the alveolar bone socket was significantly increased by orthodontic treatment in all three groups. Medicated rats differed from the controls on both the treated and the untreated sides: total surface area of the alveolar bone was significantly reduced in the medicated rats. But orthodontic treatment increased the surface area of the alveolar bone significantly in all three groups. This increase with mechanical perturbation, however, was greater in the chronic group compared with the acute (Table 2).

The amount of erosion on the mesial and distal aspect of the bone surface was significantly reduced in the acute group, while the chronic group showed an increase (Table 3). Both sides (orthodontically treated or not) and sites (mesial and distal) had a significant impact on the extension of the resorbing surface: the mesial sides in all three groups on the treated quadrants showed a significantly larger percentage of resorption compared with the untreated sides. In addition, an interaction between the treatment-related changes and the corticosteroid therapy was found. Interestingly, in the control rats orthodontically treated teeth exhibited an increase in resorption on the mesial and a reduction on the distal side. In the acute group the resorption on the treated side had reached the level of the controls on the mesial side but was larger on the distal side. In the chronic group the difference between the mesial and the distal sides was even more pronounced (Table 3).

The relative extension of the mineralizing surface around the untreated teeth was significantly reduced only in the acute group on both the mesial and the distal sides. When adding the orthodontic forces a significant increase was observed in both groups but significantly more in the acute group (Table 4).

Table 2. **Area of the alveolus at the coronal level in mm^2**

	Drug regime					
	Controls		Acute		Chronic	
Alveolar socket area (AS)	Mean	SD	Mean	SD	Mean	SD
Sites						
Treated side						
Mesial	1.36	0.12	1.17	0.24	1.25	0.31
Buccal	1.20	0.31	0.91	0.37	1.12	0.31
Distal	1.17	0.17	0.82	0.23	0.79	0.21
Lingual	0.61	0.10	0.72	0.28	0.78	0.26
Total	1.09*	0.18	0.91*†	0.28	0.98*†‡	0.27
Untreated side						
Mesial	1.38	0.38	1.27	0.92	1.14	0.27
Buccal	0.88	0.24	0.55	0.22	0.63	0.24
Distal	0.89	0.12	0.69	0.23	0.73	0.20
Lingual	0.50	0.06	0.61	0.35	0.63	0.27
Total	0.91	0.20	0.78†	0.43	0.79†	0.24

*Significantly different from the untreated side.
†Significantly different from the control group.
‡Significantly different from the acute group.

Table 3. **Relative extension of erosion (ES/BS) of the alveolar wall in the three groups (%)**

	Control				Acute				Chronic			
	Mesial		Distal		Mesial		Distal		Mesial		Distal	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Untreated	4.7	1.5	13.7	4.9	3.4†	3.3	7.7	3.2	6.6	4.6	8.8	3.5
Treated	18.6*	7.2	10.6	7.2	19.0*	4.0	18.8*	3.2	25.8*‡	2.6	12.2	1.6

*Significantly different from the untreated side.
†Significantly different from the untreated side in the control and chronic group.
‡Significantly different from the distal side.

Table 4. **Relative extension of mineralizing surface (MS/BS) of the alveolar wall in the three groups (%)**

	Control				Acute				Chronic			
	Mesial		Distal		Mesial		Distal		Mesial		Distal	
	Mean	SD	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Untreated	19.5	9.9	8.6	7.44	5.9†	3.4	3.6†	2.4	18.1	1.9	10.4	3.0
Treated	18.7	9.56	22.9	10.4	20.7*	4.0	28.4*	3.7	30.7*	3.9	34.5*	3.9

*Significantly different from the untreated side.
†Significantly different from the untreated side in the control and chronic group.

Discussion

We studied the changes in the response of alveolar bone upon acute and chronic systemic glucocorticoid administration in a rat model with and without orthodontic forces. The rat model is the standard method for such studies (23–25). Because we had used super elastic springs, that are capable of delivering constant force without decay, any concerns of inconsistent force system could be ignored (26). As the inter-animal variability was still high the split mouth design seemed justified (27). Also, the 7-week experimental period (drug administration and orthodontic treatment) was chosen in order to interfere with bone metabolism for the duration of, at least, one remodeling cycle (sigma), that at 6 months it is considered to be about 21 days (28).

Previously published studies (11, 13) have been carried out on rabbits. Although a bigger size animal is an advantage, having to deal with continuously erupting molars may flaw the results. The molars studied in this experiment were fully erupted at the time of the experiment. It could be argued that the change in function, occlusion, and the eruptive component of the appliance might influence the results. This effect, however, would be identical in the drug-treated and control animals. Thus, the background noise from the continuously erupting incisor would not affect the comparative results.

The tooth movement measurements used in this study as described by Hong et al. (20) is likely to underestimate tooth movement because of the transseptal fiber pull. The upper incisors were not used as reference landmarks as the anchorage loss or continual eruption would bias the readings. Implants were not used either for the concern of regional effect in the bone around the implants.

Data from high-dosage glucocorticoid administration in the osteoporotic animal have been reported (11). Also reported are results from the studies of Ong et al. (16). These workers used a therapeutic dosage of 1 mg/kg, but our data cannot be compared with theirs because the age and the type of animal was not the same, and the frequency of administration differed. The present experiment made use of a therapeutic glucocorticoid dose in order to simulate clinical situations (18). The alveolar bone effects of physiological

doses of glucocorticosteroid administration as specified in this study with and without orthodontic movement have not been previously investigated. Yasumura (29) examined the response of alveolar bone in rats after 2 weeks of glucocorticosteroid administration; a period below even one sigma period. The present study with a total duration of 7 weeks was covering several sigma periods and verified that the drug had an influence on the tissue reaction to orthodontic forces.

The rate of tooth movement increased in both drug groups although significantly only in the chronic group. The parameters measured had varied between the acute and chronic groups indicating that a different clinical reaction can be anticipated in patients undergoing corticosteroid treatment. The explanation is probably different in the two groups. The trend towards increased rate of tooth movement in the acute group could possibly be explained as a reflection of the transition state from the short-term effect to the long-term effect of the drug (30). The lack of a significant increase in the rate of tooth movement could be explained by a modification of the mechanical properties of the periodontal ligament in the early phase of orthodontic tooth movement in short-term drug administration, as shown by Ong et al. (16). In the chronic group a likely explanation of the increased rate of tooth movement could be that the experiment has lasted long enough for the increased turnover rate to be established as a result of an increased sensitivity to PTH. Indeed, the effects of drug therapy are known to change after a period when a secondary hyperparathyroidism may develop. The time scale of this event in the rat has not been clarified (28, 31–33). The increased tooth movement rate observed in the chronic group is in line with the results of Ashcraft et al. in a corticosteroid-induced osteoporotic rat model (11) and of other studies with high metabolic rate conditions (5, 21, 34–37).

Tooth movement had, as would be expected, a considerable impact on the magnitude of the alveolar bone response. This influence was also modulated by the administration of corticosteroids, probably because of the differences in the stress/strain distribution in a stiffer periodontal ligament.

The decrease in resorption seen in the untreated sites in the acute group corroborates the observations of Ong et al. (16), who reported a drug induced inhibition of the clastic activity in the PDL. In the acute group the observation of a decreased percentage of resorption in association with a decreased percentage of bone formation suggests that in the acute phase of the drug therapy bone remodeling seems to slow down.

An important interaction was noted between mechanical perturbation and the drug, leading to an increase in the extension of mineralizing surfaces exceeding what was seen in the control animals. In the chronic group, the degree of resorption was doubled on the mesial aspects of the treated side. This corresponded to the increased rate of tooth movement. It should be remembered that the original distal drift is reversed in the experimental situation. A possible explanation for this finding may be the long duration of treatment in the chronic group (more than 2 sigma), which may have led to a secondary hyperparathyroidism. This condition would then be reflected in an increased activation frequency and an alteration of the BMU period (38), resulting in a transient moderate osteoporotic state.

The evaluation of formation was influenced by the fact that single and double labels were pooled. In the case of extremely intense labeling it may be difficult to separate, with any precision, the single- from the double-labeled sites (39). The high percentage of depository surfaces reflecting a rapid bone turnover has been described earlier in tooth movement (1, 40). One could inadvertently examine the transient-state basic remodeling units. If this is committed, it could explain the large variation seen (30, 41). On the mesial aspect we might have generated a localized rapid acceleration phenomenon (RAP) where bone surface was subjected to a high local stress by the orthodontic appliance. This could lead to decreased resorption in some sites because of ischemia and increased in others reflecting a local repair process.

Histomorphometric analyses confirmed that the glucocorticoid drug therapy elicits a noticeable change in the bone turnover rate. In the short duration drug therapy orthodontic tooth movement was not affected, but at a tissue level the remodeling process seemed delayed, as less remodeling in the absence of mechanical loading was observed. In the chronic group, however, the tooth movement rate did increase, possibly as a result of the induction of a secondary hyperparathyroidism.

Clinically, the present study suggests that it is possible to treat patients undergoing corticosteroid therapy, with minimum of adverse effects. Patients who are

within the short-term phase of the drug, as for example, in hay fever therapy or other perennial or short-lived allergy cases, may be recommended to postpone orthodontic treatment until the chronic phase is over and the patient is off the medication. If the patient is already undergoing orthodontics, however, then appliance adjustments should be minimal, and the appointments scheduled with longer intervals, as bone turnover will be delayed. Tooth movement could most likely take longer in the acute phase of glucocorticoid therapy. In long-term drug therapy, our data seem to indicate that the rate of tooth movement will be increased. The orthodontic appliance can, therefore, be controlled as usual or more frequently.

It is important to note that the glucocorticosteroid therapy is not only dose dependent but also time dependent. In all cases of chronic glucocorticosteroid drug therapy there is an initial increase in bone loss that slows down after a period of about 12 months in humans (38). Thus, the clinical implications of this study should be limited to below the 12-month period. With this knowledge in hand, it is now necessary to concentrate on corticosteroid-induced induced root resorption.

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