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Growth Hormone Receptor and IGF-I Receptor Immunoreactivity During Orthodontic Tooth Movement in the Prednisolone-Treated Rat

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ABSTRACT

Bone remodeling during tooth movement is regulated by local and systemic factors. Two regulators of bone metabolism are growth hormone (GH) and insulin-like growth factor-I (IGF-I). Their effects are mediated via binding to GH receptor (GHR) and IGF-I receptor (IGF-IR) in target tissues. Corticosteroids may affect the activity of these growth factors. This study examined the effect of prednisolone on GHR and IGF-IR expression in dental tissues following orthodontic tooth movement. The corticosteroid-treated group (N = 6) was administered prednisolone (1 mg/kg) daily and the control group (N = 6) received equivalent volumes of saline. An orthodontic force (30 g) was applied to the maxillary first molar. Animals were sacrificed 12 days postappliance insertion. Sagittal sections of the first molar were stained for GHR and IGF-IR immunoreactivity. GHR and IGF-IR cell counts were elevated following appliance-treatment. Orthodontic tooth movement appeared to up-regulate GHR and IGF-IR immunoreactivity, but this up-regulation was reduced following prednisolone treatment. The suppression of GHR and IGF-I immunoreactivity in steroid-treated animals infers the mechanism whereby bone resorption and deposition, necessary for orthodontic tooth movement, may be inhibited by prednisolone. However, at 12 days postappliance insertion, no difference in orthodontic tooth movement was observed following low-dose prednisolone treatment.

KEY WORDS: Bone, Corticosteroid, Resorption.

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Orthodontic tooth movement evokes a cascade of cellular responses within the enveloping alveolar bone and periodontal ligament (PDL). Bone remodeling and the metabolism associated with this tooth movement are regulated by a large number of local and systemic growth factors. The widespread use of therapeutic corticosteroids today raises concerns with regard to their effects on the production and activity of these growth factors during orthodontic tooth movement. The side effects associated with the long-term use of prednisolone therapy include disturbances in mineralized tissue metabolism and wound healing, and suppression of somatic growth, chondrogenesis and osteogenesis.¹⁻³

Two major regulators involved in bone metabolism are growth hormone (GH) and its mediator, insulin-like growth factor-I (IGF-I). GH is an important regulator of postnatal skeletal growth and development and promotes stem-cell differentiation and proliferation. It acts both indirectly and directly to influence bone and chondrocyte cell function and differentiation.^{4,5} The indirect actions of GH are mediated by

locally and possibly hepatic produced IGF-I.⁶ GH has been shown to actively participate in bone remodeling by stimulating osteoblast formation⁷ and osteoclastic bone resorption.⁸

IGF-I has an active role in bone remodeling, and its production is GH dependent. IGF-I is potent in assays for the promotion of growth, development, and functioning of most tissues.⁹ It influences osteoblastic function in all stages of development and increases the replication of cells of the osteoblastic lineage.¹⁰ Osteoclasts actively engaged in bone resorption have been found to express IGF-I and IGF-I receptor (IGF-IR) mRNA.¹¹

Corticosteroids are suggested to disrupt the GH/IGF-I axis by suppressing GH levels and increasing total IGF-I levels¹² and may deplete the bone marrow stroma of osteogenic precursors.¹³ High concentrations of steroids cause a decrease in protein, RNA, and DNA synthesis in bone cells.^{14–17} In addition, corticosteroids alter the expression of a number of genes in osteoblastic cells, including those for growth factors and receptors.¹⁰ GH and IGF-I have specific receptors to which they bind and interact.^{18,19} GH receptor (GHR) expression in the periodontium is initially reduced and subsequently enhanced during orthodontic tooth movement²⁰ and during tooth eruption alveolar bone osteoblasts and osteoclasts demonstrate GHR immunoreactivity.^{21,22} Steroids have also been shown to down-regulate GHR, resulting in a reduction of bone formation in vivo.^{10,23} Orthodontic tooth movement requires a functioning bone metabolism that permits efficient bone resorption and deposition following the application of a force. Therefore, the aim of this study was to examine the effect of low-dose prednisolone treatment on the expression of GHR and IGF-IR around the distal root of the maxillary first molar during orthodontic tooth movement using a well-established rat model.

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Animals and prednisolone treatment

Twelve 9-week-old male Wistar rats (average weight 270.5 g) obtained from the Central Animal Breeding House, University of Queensland, Brisbane, Australia, were used in this study. Animals were examined in two groups: experimental (n=6) and control (n=6). 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 per cage) with a standard 12-hour light/dark cycle. They were fed a diet of finely ground laboratory food (Rat and Mouse Cubes, Norco, Brisbane, Australia) ad libitum. Body weights of all rats were measured daily. The experimental group was administered daily doses of 1 mg/kg oral prednisolone in saline (Panafcortelone 1®, Fisons Pty Ltd, Sydney, Australia), using a stomach tube, for a 12-day induction period and during the experimental period. The control group was administered an equivalent volume of saline (Sodium Chloride 0.9%, Astra Pharmaceuticals Pty Ltd, Australia). The dose of 1 mg/kg prednisolone has been used in our laboratory previously.²⁴ It is within recommended therapeutic levels for rats and is associated with anti-inflammatory and some immunosuppressive effects.²⁵

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 orthodontic appliance consisted of a stretched closed coil spring (0.008 inch × 0.032 inch Elgiloy spring, Rocky Mountain Dental Products Co, USA) ligated between the maxillary left first molar and 2 maxillary central incisors as previously described.²⁴ The molar on the right side was used as the nonappliance control. The magnitude of tooth movement was determined by measuring the relative separation between the first and second maxillary molar using vernier calipers with sharpened tips inserted into occlusal pits.²⁴ The distance between the mesial occlusal pits on the first and second molars was measured intraorally before appliance insertion and immediately after sacrifice. Measurements were performed by the same operator (CKLO) and were repeated five times for each side of the maxilla.

Rats were sedated during appliance insertion using subcutaneous injections of fentanyl citrate/fluanisone and midazolam (Sublimaze & Hypnovel) 0.15–0.2 mL per 100 g body weight. One animal in the nonsteroid control group died from complications associated with the use of these sedatives.

Histological preparation


At 12 days postappliance insertion, rats were euthanised using carbon dioxide asphyxiation. Maxillae were immediately removed and dissected into halves, fixed in Bouin's solution (1.2% picric acid, 10% formaldehyde, and 5% glacial acetic acid) for 24 hours and demineralized 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 to a thickness of 5 µm) and mounted on 3-aminopropyltriethoxysilane 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 for GHR and two serial sections for IGF-IR expression.

Immunohistochemistry

Deparaffinized sections were incubated with the appropriate antibody and processed for determining the distribution of GHR and IGF-1R. Staining was carried out using the specific 3-layered streptavidin peroxidase technique to detect bound antibody, as described previously in detail for rat tissues for GHR²⁶⁻²⁸ and IGF-IR²⁹ using well-characterized antibodies.

Briefly, sections were deparaffinized, hydrated, and incubated in 0.03% hydrogen peroxide in phosphate buffered saline (pH 7.3) for 10 minutes at 25°C to block endogenous peroxidase. Nonspecific protein binding was eliminated with rabbit serum for 1 hour at 25°C. They were then incubated with antibody to GHR or IGF-IR overnight at 4°C. Binding was identified by the addition of biotin conjugated anti-immunoglobulin followed by streptavidin horseradish peroxidase. A substrate containing tris HCL, 3,3-diaminobenzidine and peroxide was used to develop the slide sections. Slides were counterstained with Mayer's haematoxylin, dehydrated, and mounted. All incubations were made in humidified chambers. Controls included a negative control (omitting the primary antibody) and a positive control (rat liver tissue for GHR and rat digestive tract for IGF-IR).

Histological analysis

The 22 hemimaxillae harvested from the six steroid-treated (ST) and five nonsteroid-treated (NST) control animals were divided into the following 4 groups for histological examination: (1) no-steroid, no-appliance controls (NNA) (2) no-steroid, appliance-treated controls (NAP), (3) steroid-treated, no-appliance (SNA) animals, and (4) steroid- and appliance-treated (SAP) animals. Histomorphometry was performed on the regions surrounding the distal root (coronal one-third) of the first molar using a light microscope. Cell counts were limited to the mesial and distal sides of the distal root, corresponding to compression and tension sides, respectively, in the appliance groups (Figure 1 ). Cell counts were made with a precalibrated 10x10-graticule-eyepiece micrometer (Olympus, 24 OC-M, 10/10 SQ, Tokyo, Japan) mounted on a light microscope under 400x magnification. Total cell numbers were counted from 10 adjacent small squares (area 24.5 m²) orientated vertically along the surface of the alveolar bone and cementum, and the PDL.

GHR and IGF-IR immunoreactive cells stained brown. The main immunoreactive cell types counted along the alveolar bone surface and root surface were mononuclear blast-like and multinucleated clast-like cells. Within the PDL, fibroblast-like, mononuclear, and multinucleated clast-like cells were identified as being positively stained. The GHR and IGF-IR positive cell counts were performed on two serial sections from each animal within the four groups (NNA, NAP, SNA, SAP). Each section was measured twice, yielding four measurements from which the average value for each animal was derived. The combined values for each animal within a particular group were then averaged to yield the group mean value. The differences in the mean values for each of the four groups were compared using one-way analysis of variance (ANOVA) with significance at $P < .05$.

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Animal health and measurement error

Food and water consumption appeared to be unaffected by the presence of an appliance. There was an overall gain in weight throughout the induction and experimental periods (controls 24.5 ± 6.4 g and steroid-treated 23.4 ± 4.0 g) that was not significantly different between the groups.²⁴ There was good to high agreement for measurements undertaken by the one investigator (CKLO). Agreement for intra-examiner cell counts was not less than 97%.


Tooth movement

All appliance-treated molars showed evidence of tooth movement, as previously described.²⁹ No significant difference in the magnitude of tooth movement was present in controls compared with steroid-treated animals. No tooth movement was evident on the nonappliance side.

Specificity of immunoreactivity

Incubation of the sections without the primary antibody for GHR and IGF-IR produced no staining. Positive staining was clearly evident in liver tissue for GHR and gastric epithelium for IGF-IR.

Growth Hormone Receptor expression

Cells in the PDL and along the adjacent mineralized tissue surfaces demonstrated immunoreactivity for GHR (Figure 2 ). In the appliance groups, multinucleated clast-like cells were located in resorption lacunae on the root surface and along the adjacent alveolar bone undergoing remodeling on the compression-side and within the PDL. There was a variable expression of GHR immunoreactivity among the multinucleated cells, with some, but not all, clast-like cells staining positive. The greatest number of GHR-positive cells was found within the PDL. Most positively stained cells in this region were mononuclear and fibroblast-like. Cells staining positive along the alveolar bone were osteoblast-like cells. Osteocytes stained negative in all specimens. Along the root surfaces, cells staining positive appeared to be cementoblasts.

Generally, orthodontic tooth movement enhanced GHR immunoreactivity and steroid treatment reduced GHR immunoreactivity (Figure 3). In all the areas investigated except for the root surface on the mesial aspect, there were significantly fewer GHR-positive cells associated with the SAP group compared with the NAP group ($P < .05$). The SNA group expressed the least number of GHR positive cells in all areas.

Nonappliance groups expressed fewer GHR-positive cells compared with appliance groups in all areas examined except for the mesial root surface where there were no statistical differences between the NNA and NAP groups and between the SNA and SAP groups.

IGF-I- Receptor expression

Cell types expressing IGF-IR were similar to those that expressed GHR (Figure 2). The greatest numbers of positive cells were located in the PDL. These cells appeared to be fibroblast-like and mononuclear cells. Osteoblast-like cells along the alveolar bone surface and cementoblasts were positively stained. Multinucleated osteoclast-like cells were variable in expression of IGF-IR and positive cells were located on the resorbing alveolar bone, in resorption defects on the root surface, and in the PDL.

The pattern of IGF-IR immunoreactivity and staining intensity was similar to that noted for GHR (Figure 4). Co-localization of GHR and IGF-IR was variable and random. The positive cell counts were generally fewer for IGF-IR compared with GHR, and the significant differences between the groups for IGF-IR were less than for GHR.

As for GHR, steroid-treatment reduced the overall number of IGF-IR positive cells in tissue sections. The SAP group had significantly fewer IGF-IR positive cell numbers in all cell-count areas compared with the NAP group ($P < .05$) except on the tension side alveolar bone and root areas. The SNA group showed consistently fewer positive cells in all areas investigated compared with NNA and NAP groups ($P < .05$).

Appliance groups showed a variable increase in positive IGF-IR cell counts compared with nonappliance groups. Along the mesial alveolar bone surface, the NAP group had more IGF-IR positive cell numbers compared with the NNA group ($P = .041$). The SAP group expressed greater IGF-IR positive cell numbers compared with the SNA group along the mesial root surface ($P = .028$), mesial PDL ($P = .028$), and distal alveolar bone surface ($P = .024$). There were no significant differences in IGF-IR positive cell numbers between the NNA and NAP groups and between the SNA and SAP groups along the distal root surface and distal PDL.

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In this study, administration of 1 mg/kg prednisolone had a suppressive effect on cell immunoreactivity for GHR and IGF-IR in most dental and paradental tissues. Tooth movement induced by an orthodontic appliance upregulated the immunoreactivity for GHR and IGF-IR to varying degrees but suppression by prednisolone was still evident. No difference in the magnitude of tooth movement was observed in the prednisolone-treated group.

The rat model adapts well to orthodontic forces and maximum deformation of molar alveolar tissues is achieved at force levels between 20–40 g, beyond which there is no further deformation and no further increase in tooth movement.³⁰ The force level of 30 g selected for the present study produces effective orthodontic tooth movement without any adverse effects.²⁴ The duration of appliance wear was based on previous studies which showed that 10–14 days are required for one complete bone remodeling cycle to take place.^{30–32} Bone resorption activity peaks on the compression side 7 days after appliance activation and a peak rate of osteoclastic activity occurs after 7 to 8 days of appliance therapy.³⁰ This is followed by a second wave of osteoclastic activity towards the end of appliance decay at days 12–14. Bone turnover at pressure and tension sites remains elevated for up to 16 days following activation although the orthodontic forces have substantially decayed.³¹

The present study noted a pattern of overall reduction in cellular GHR immunoreactivity associated with the steroid-treated animals during orthodontic tooth movement. Similarly, methylprednisolone (400 µg/day) administered orally, has been shown to reduce hepatic GH binding and markedly suppress plasma GH binding protein and GHR expression.¹¹ Glucocorticoids are thought to decrease tissue sensitivity to GH by decreasing the number of available GHRs.³³ This is supported by the observation of reduced GHR immunoreactivity in steroid-treated animals noted in the present study. However, glucocorticoids do not always appear to down-regulate GHR and dexamethasone has been shown to increase GHR expression in cultured rat pancreatic islets.³⁴ In contrast, our in vivo data indicated that the GHR was down-regulated by continuous glucocorticoid treatment in a variety of tooth- and bone-forming cells during orthodontic tooth movement. At 12–14 days after the application of an orthodontic force, osteoclast activity peaks³¹ and GHR immunoreactivity in periradicular tissues is also elevated.²⁰ Suppression of GHR and IGF-IR immunoreactivity in cells associated with orthodontic tooth movement may indicate that cell function is altered by prednisolone treatment, which may arise from a lack of available precursors.^{28,35}

Endocrine factors which regulate the expression of GHR include GH, insulin, IGF-IR, thyroxine and sex steroids.¹⁹ GHR mRNA has been shown to be present in most tissues in the rat and correlates with ligand binding.³⁶ The control of GHR gene expression is complex, involving at least seven alternative promoters and is dependent on tissue specific, developmental, hormonal, and nutritional factors.¹⁹ Previous studies reported the expression of GHR in cells showing morphological signs of differentiation and proliferation.^{37,38} The

expression of GHR was linked to the functional state of the various cell populations during odontogenesis, in particular, the onset of cytodifferentiation. [4.5](#)

Similarly, cellular IGF-IR expression was reduced in steroid-treated animals. These findings differ from those of Bennett et al. [39](#) who conducted experiments on cultured rat bone cells from foetal calvaria in which IGF-IR numbers were increased by exposure to dexamethasone. However, the latter effect of increased receptor numbers was only observed in conditions of overall increased cell numbers. Receptor expression may vary with stage of development in cells from foetal and mature bone, and in vivo compared with in vitro studies. Indeed, in other studies cortisol has been shown to down-regulate IGF-IR mRNA levels and IGF-IR expression. [40.41](#)

IGF-I in plasma and other body fluids can be bound to IGF binding proteins (IGFBP). The role of IGFBPs in bone cell function is unclear at present. These carrier proteins are believed to determine the bioavailability of IGF-I and to modulate its biologic actions. [9.42](#) Unfortunately, the exact mechanism by which these binding proteins regulate or modulate the interaction of IGF with their receptors is still unclear. They are believed to function in several ways: by binding to IGF-I and decreasing the bioavailability of IGF-I; by enhancing the binding of IGF-I to its receptor, thereby potentiating its activity in skeletal tissues; by controlling the transport of the IGF to specific cell types; by direct IGF-I independent actions on target cells; and by increasing the half-life of IGF-I in serum. [4.6.43](#) Dexamethasone has been shown to suppress IGFBP in rat osteoblast-like cells [44](#) and high doses of cortisol have been shown to inhibit the production of IGFBP in vitro. [43](#) Since IGF-I is believed to up-regulate its own receptor, [45](#) and prednisolone decreases IGF-I production, this may contribute to the loss of IGF-IR observed in the current study.

At 12 days postappliance activation, no difference was detected in orthodontic tooth movement between control and prednisolone-treated animals. However, in the rabbit where a higher dosage of corticosteroid induced osteoporosis, rapid orthodontic tooth movement and increased relapse were observed. [46](#) The ability of corticosteroid to affect bone metabolism is related to dosage, duration, and the specific medication. Orthodontic movement in patients with corticosteroid-induced osteoporosis may require special consideration. Where osteoporosis is related to increase bone turnover and the rate of resorption exceeds deposition, orthodontic tooth movement may be accelerated but relapse will occur. However, where osteoporosis is related to a depression in resorption and deposition rates, orthodontic tooth movement may be slowed. While prednisolone-treatment reduced the number of tartrate-resistant acid phosphatase-positive cells in the rat PDL during tooth movement and the incidence of root resorption, no inhibition of tooth movement was observed. [24](#) The effect of a low-dose prednisolone medication on orthodontic tooth movement may be negligible or, at the very worst, may slow tooth movement and subsequently require a prolonged retention period. It is possible that other growth factors and cytokines compensate for the disturbances in GH and IGF-I activity induced by prednisolone and allow orthodontic tooth movement to proceed normally.

CONCLUSION [Return to TOC](#)

This study reports that a low-dose systemic prednisolone reduced GHR and IGF-IR immunoreactivity in dental and paradental tissues in the rat. Although orthodontic tooth movement upregulated GHR and IGF-IR immunoreactivity to varying degrees, this effect was relatively reduced in animals treated with prednisolone. Tooth movement was not inhibited in rats treated with a low-dose systemic prednisolone.

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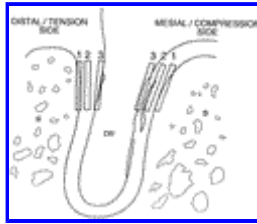
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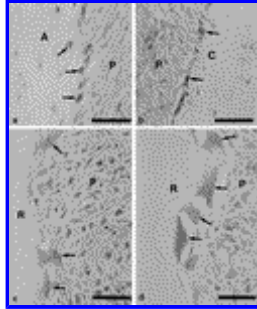
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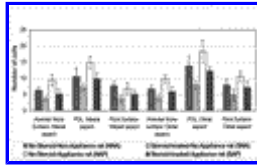
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FIGURE 1. Boxes representing cell count sites on the distal root (DR): (1) along the alveolar bone (B) margin; (2) in the central region of the periodontal ligament; and (3) along the distal root surface



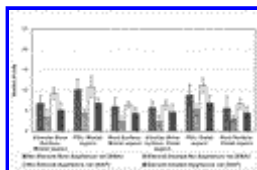
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FIGURE 2. Histological sections from the mesial surface of the distal root of the first molar showing GHR immunoreactivity in the NAP group: (a) osteoblasts (arrowed), (b) cementoblasts (arrowed), (c) osteoclasts (arrowed) located in resorption lacunae on the root (R) surface; and IGF-1R immunoreactivity in the NAP group: (d) osteoclasts (arrowed) located in resorption lacunae on the root (R) surface. Alveolar bone (A), cementum (C), periodontal ligament (P) and distal root (R). Bar represents 50 μ m



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FIGURE 3. Mean GHR cell counts along the alveolar bone and root surfaces and PDL. (Mean values and standard deviations). SAP compared with NAP, $P < .05$, except for the root surface on the mesial aspect



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FIGURE 4. Mean IGF-1R cell counts along the alveolar bone and root surfaces, and PDL. (Mean values and standard deviations). SAP compared with NAP, $P < .05$, except for the bone and root surface on the distal aspect. SNA had significantly fewer positive-cells compared with NNA and NAP, $P < .05$

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