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Effect of a static magnetic field on orthodontic tooth movement in the rat

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SUMMARY Orthodontic tooth movement may be enhanced by the application of a magnetic field. Bone remodelling necessary for othodontic tooth movement involves clastic cells, which are tartrate-resistant acid phosphatase (TRAP) positive and which may also be regulated by growth hormone (GH) via its receptor (GHR). The aim of this study was to determine the effect of a static magnetic field (SMF) on orthodontic tooth movement in the rat. Thirty-two male Wistar rats, 9 weeks old, were fitted with an orthodontic appliance directing a mesial force of 30 g on the left maxillary first molar. The appliance incorporated a weight (NM) or a magnet (M). The animals were killed at 1, 3, 7, or 14 days post-appliance insertion, and the maxillae processed to paraffin. Sagittal sections of the first molar were stained with haematoxylin and eosin (H&E), for TRAP activity or immunohistochemically for GHR. The percentage body weight loss/gain, magnetic flux density, tooth movement, width of the periodontal ligament (PDL), length of root resorption lacunae, and hyalinized zone were measured. TRAP and GHR-positive cells along the alveolar bone, root surface, and in the PDL space were counted.

The incorporation of a SMF (100–170 Gauss) into an orthodontic appliance did not enhance tooth movement, nor greatly alter the histological appearance of the PDL during tooth movement. However significantly greater root resorption ($P = 0.016$), increased width of the PDL ($P = 0.017$) and greater TRAP activity ($P = 0.001$) were observed for group M at day 7 on the compression side. At day 14 no differences were observed between the appliance groups.

Introduction

Orthodontic tooth movement involves bone remodelling and requires a close interplay between bone formation by osteoblasts and bone resorption by osteoclasts. During tooth movement, mechanical stresses in the periodontal ligament (PDL) space induce cellular reactions, which may be enhanced by an electromagnetic field (EMF) of either static (SMF) or pulsed (PEMF) types (Stark and Sinclair, 1987; Blechman and Steger, 1995; Darendeliler *et al*., 1995). The desired dose-response for clinical application of EMF in orthodontics is produced by the simultaneous application of orthodontic force under the exposure of a light EMF of between 24–300 Gauss (Blechman and Steger, 1995).

While magnets have been used for many years in medicine and dentistry (Bondemark, 1994), controversy remains as to the actual biological effects and benefits of EMF application (Barker and Lunt, 1983; Clearly, 1993). EMFs enhance DNA, RNA and protein production in cell cultures (Rodan *et al*., 1978; Liboff *et al*., 1984; Farndale and Murray, 1985) and short-term EMF application is suggested to cause accelerated calcium uptake in cartilaginous embryonic chick limbs (Colacicco and Pilla, 1984). Such effects may contribute to the reported therapeutic benefits of EMF on fracture healing and bone metabolism during tooth movement (Stark and Sinclair, 1987; Bassett *et al*., 1974; Darendeliler *et al*., 1995). On the other hand, EMFs have been suggested to cause significant alteration

in cell metabolism, cytoskeleton structure, and morphology. These changes are reported to cause apoptosis of rat bone marrow osteoprogenitor and tendon fibroblast cells (Blumenthal *et al*., 1997), reduce growth and development of mouse bone marrow cells (Feinendiegen and Muhlensiepen, 1987), and interfere with hormone receptor interactions on the cell surface (Luben *et al*., 1982). Others reported that EMFs produce no detectable effect on the activity of fibroblasts, osteoblasts, and osseous tissues (Camilleri and McDonald, 1993; McDonald, 1993; Gonzalez-Riola *et al*., 1997).

Bone turnover is regulated by complex interactions involving systemic hormones and locally derived growth factors (Mohan and Baylink, 1991). Growth hormone (GH) influences normal bone metabolism, and is a major regulator of postnatal growth and development. GH is secreted by the anterior pituitary gland and acts directly on tissues via specific GH receptors (GHR; Leung *et al*., 1987; Matthews *et al*., 1989) or indirectly via the production of insulin-like growth factor I (IGF-I). GH stimulates differentiation of osteoblastic precursors (Loveridge *et al*., 1995), as well as odontoblasts and ameloblasts during odontogenesis (Zhang *et al*., 1992; Symons *et al*., 1994, 1996a; Young, 1995). GHR has also been immunolocalized in hepatic tissue, macrophages, osteoblasts, osteoclasts, and dental tissues (Kover *et al*., 1986; Zhang *et al*., 1992; Symons *et al*., 1994, 1996a,b; Loveridge *et al*., 1995; Young, 1995).

Recent investigations into the use of EMF during orthodontic tooth movement suggest that EMF affects the activities of cells in the periodontium by inducing faster bone resorption and deposition (Stark and Sinclair, 1987; Darendeliler *et al*., 1997). The cells associated with this hard tissue resorption and elimination of necrotic tissues are positive for tartrate-resistant acid phosphatase (TRAP) activity (Brudvik and Rygh, 1993a, 1994, 1995). To date, the cellular events that take place during the phases of orthodontic tooth movement under the influence of EMF have not been studied in detail. Therefore, the aim of this study was to investigate the effect of a SMF on dental and paradental tissues, and the distribution of TRAP activity and GHR expression in cells of the periodontium of the rat maxillary molar during orthodontic tooth movement.

Materials and methods

Animals

Thirty-two 9-week-old male Wistar rats (mean weight 356 g, range 310–420 g) were obtained from the Central Animal Breeding House, University of Queensland. Ethical clearance was granted by the Institutional Ethics Committee, and the experiment followed the National Health and Medical Research Council of Australia guidelines. Prior to appliance placement, the rats were allowed to acclimatize to the new environment for 5 days and were fed a diet of ground pellets with water *ad libitum*. Body weight was recorded daily during the acclimatization and experimental period. The percentage body weight gain or loss was calculated for each experimental day. Differences in weight gain between groups were determined using a Student's *t*-test

Induction of tooth movement

Following acclimatization, an orthodontic appliance was inserted on the left maxillary first molar and a mesially directed force of 30 g applied. The force level was verified using a dynamometer (Correx, Dentarum, USA) measuring gauge. The orthodontic appliance consisted of a stretched closed coil spring (0.008 × 0.032-inch Elgiloy spring, Rocky Mountain Dental Products Co., USA) ligated between the maxillary left first molar and two maxillary central incisors. To prevent slippage of the appliance, grooves were made along the lateral sides of the incisors and a figure-eight ligature tie used. This orthodontic appliance is based on a modified technique described by Brudvik and Rygh (1993a,b). In the non-magnet group (NM), a weight (stainless steel coil with the same dimensions and weight as the magnet) was incorporated into the appliance, and in the magnet group (M) a magnet (MagneForce ORMCO Pty Ltd., California, USA, with outer diameter = 3.5 mm, internal diameter = 0.7 mm, thickness

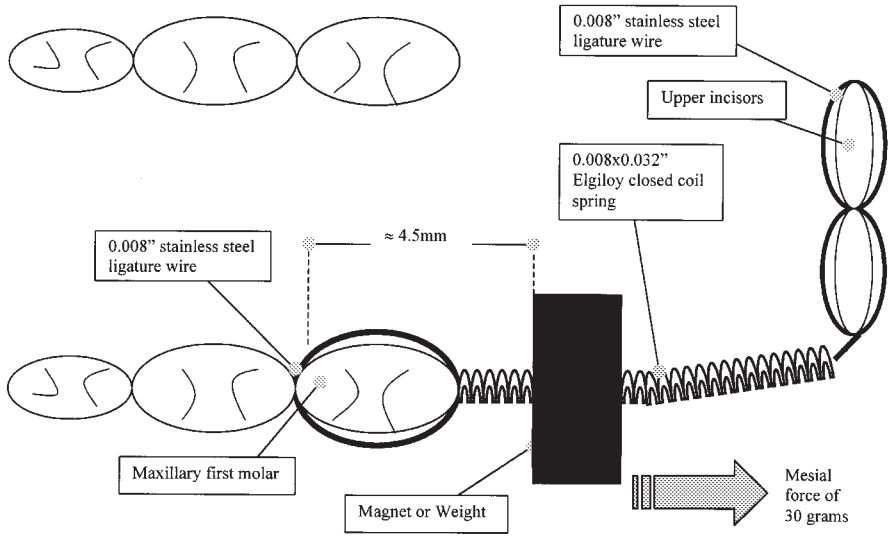

Figure 1 Orthodontic appliance used in the experiment.

= 2.0 mm) was placed 2 mm away from the mesial surface of the first molar (Figure 1). This ensured that a separation of approximately 4.5 mm was maintained between the pole surface of the magnet and the distal surface of the first molar. Magnetic flux density was measured prior to and at completion of the experiment using a hand-held digital Gauss/Tesla meter (FW Bell, Model 4048, Orlando, Florida, USA). Flux measurements were collected at every millimetre, from the pole surface of the magnet to a distance of 10 mm. Flux (Gauss) was plotted against distance (mm) to allow an estimation of the amount of magnetic flux density present on the experimental root structure and surrounding tissues. As the distal root was located directly beneath the distal surface of the first molar crown, the magnetic field strength (flux density) around this area, was determined to be between 100 and 170 Gauss. The molar on the right side was used as the non-appliance control.

Insertion of the orthodontic appliance was performed under general anaesthesia via a subcutaneous injection of fentanyl citrate/ fluanison midazolam (Janssen-Cilag Pty Ltd, Sydney, Australia/Alphapharm Pty Ltd, Sydney, Australia) at 0.15–0.2 ml/100 g body weight. The appliance was not reactivated during the experimental period. The residual forces in each appliance were measured at the end of each experimental period using a dynamometer (Correx). Four animals in each appliance group were killed at 1, 3, 7, or 14 days post-appliance insertion. For non-appliance controls (eight animals), the right maxilla from one animal in each appliance group, at each experimental period, was processed for histological measurements.

Measurements of tooth movement

The magnitude of tooth movement was determined by measuring the relative separation between the first and second molars, using vernier callipers (Dentarum) 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 and immediately following death, both on the appliance and the non-appliance (control) side. All measurements at each time, were repeated five times for each side of the maxilla for each animal. The same operator (BST) performed all measurements. The group mean values were calculated, and the difference between the pre- and post-treatment measurements was recorded as the magnitude of

tooth movement. A Student's *t*-test was used to compare the differences between groups.

Histological preparation

On death, the maxillae were immediately removed, fixed for 24 hours at 4°C in Bouin's solution (1.2 per cent picric acid, 10 per cent formaldehyde and 5 per cent glacial acetic acid), and washed twice in 0.5 M EDTA (pH 7.2) for 30 minutes. Prior to demineralization, the orthodontic appliance was removed and the maxillae demineralized for 10–12 days in 0.5 M EDTA (pH 7.2) at 25°C. Paraffin sagittal sections were prepared parallel to the long axis of the first molar, at a thickness of 5 μm as described by Symons and Seymour (2000). In order to achieve standardization of section location, maxillae were blocked in paraffin using a standardized orientation and mounted on the microtome in a standard manner. Serial sections from the buccal surface to the palatal surface were cut and sections from the central region of the distal root were used in the study. Each section contained the full length of the distal root, and the mesial and distal surface of the distal root and root canal were identified. The sections also contained a sagittal section of the dental crown showing pulp anatomy and the mesial root. Standard histological preparation for sections minimized discrepancies between specimens. Serial sections from each animal were stained with haematoxylin and eosin (H&E), for TRAP activity and GHR immunoreactivity. Staining of a second series of serial sections was performed. The sections were investigated using light microscopy at magnifications of ×100, ×200, and ×400.

Histological measurements

H&E stained sections were used for examination of tissue morphology and histological measurements. For standardization, the investigation was confined to the distal root. Areas for measurement in the appliance groups were the mesial and distal aspects of the distal root, corresponding to the compression and tension sides, respectively (Figure 2). The following were measured: the width of the PDL space on the compression and tension sides (the space between the root surface and most coronally located adjacent alveolar bone), the length of the main

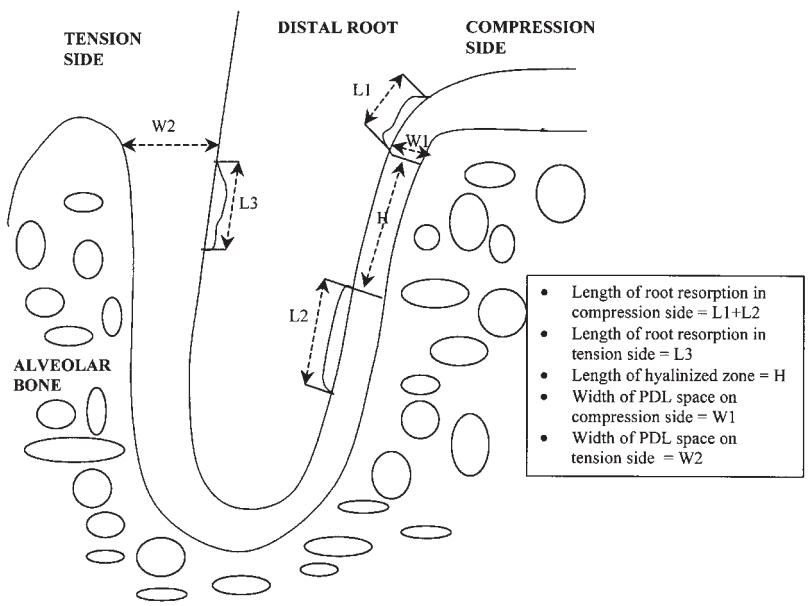

Figure 2 Investigated sites around the distal root of the maxillary first molar.

hyalinized zone on the compression side, and the length of root resorption lacunae on the compression and tension side in the apical coronal direction (expressed as a percentage of the total root length). Total root length on the tension side was measured as the vertical distance from the cemento-enamel junction to the root tip at the level of the apical foramina. On the compression side root length was measured from the root tip at the level of the apical foramina to the most coronal site on the root surface prior to the curvature of the furcation area. These measurements are similar to those undertaken by Brudvik and Rygh (1995). All measurements were carried out twice, the mean determined, and intra-operator error was assessed following repeated blind measurements. The measurements were made at ×100 magnification using a graticule eyepiece micrometer, accurate to 5 μ (Olympus, Tokyo, Japan), and differences between groups determined using analysis of variance (ANOVA), with a level of significance at $P < 0.05$.

TRAP activity. Demonstration of TRAP activity was performed using naphtol AS-BI phosphate (Sigma, Croydon, Victoria, Australia) as a substrate and paraosaniline-HCL (Sigma) as a coupling agent in L(+) tartaric acid (Sigma) at 37°C for 30 minutes. Slides were counterstained with 0.2 per cent methyl green solution (Sigma) and mounted. For the negative control slides, the substrate napthol AS-BI phosphate was omitted.

GHR immunohistochemistry. Sections were processed for detection of GHR according to the technique described by Symons *et al*. (1994, 1996b). Briefly, deparaffinized sections were hydrated and incubated in 0.03 per cent H_2O_2 in PBS for 10 minutes to block endogenous peroxidase. Antibody to GHR was applied. Binding was identified by the addition of biotin conjugated anti-immunoglobulin followed by streptavidin peroxidase. Slides were counterstained with haematoxylin. A negative control consisted of omission of the primary antibody and liver tissue was used as a positive control. Sections incubated with non cross-reacting rabbit GHR specific antibody or with GHR antibody pre-absorbed by recombinant rat GHR/binding protein do not produce a histochemical reaction (Symons *et al*., 1996b).

Cell counts. Cell counts were performed on sections stained for TRAP and GHR using a pre-calibrated 10 × 10 graticule eyepiece micrometer (Olympus, 24 OC-M, 10/10 SQ, Tokyo, Japan) at ×400 magnification. Positive cells along the alveolar bone, root surfaces, and in the PDL space on the compression and tension sides were counted from 10 adjacent small squares (area 24.5 μm^2), orientated vertically along the surface of the alveolar bone and cementum, and in the PDL, of the cervical half of the root. These cells were identified as osteoblast, cementoblast, and fibroblast in appearance, respectively. Mononuclear cells present in these regions were included in the count. Cell counts for each section were performed twice. The mean determined and intra-operator error was assessed following repeated blind measurements. Cell counts were performed on two sections per specimen and differences in counts were determined using analysis of variance (ANOVA), with a level of significance at $P < 0.05$.

Results

Animal weight gain and measurement error

All animals appeared healthy during the study. An initial reduction in body weight (mean of 10.7 g) followed appliance insertion, but the animals subsequently gained weight. No significant differences in the percentage of weight loss/gain between the appliance groups were observed. Food and water consumption appeared to be unaffected by the orthodontic appliance. There was good to high agreement for measurements undertaken by the one investigator (BST). Agreement for intra-examiner measurements was not less than 97 per cent.

Tooth movement and orthodontic appliance status

Orthodontic tooth movement was evidenced by a gradual increase in the width of the inter-dental

Table 1 Means of the parameters measured for controls (non-appliance side) and at 1, 3, 7, and 14 days following placement of an appliance without a magnet (NM) and incorporating a magnet (M).

Parameter	Site	Control (n = 16)	Appliance group (n = 8)	Day 1	Day 3	Day 7	Day 14
Tooth movement (mm)	n/a	nil	NM	0.18 ± 0.04	0.25 ± 0.02	0.24 ± 0.03	0.40 ± 0.04
			M	0.14 ± 0.04	0.26 ± 0.03	0.25 ± 0.02	0.41 ± 0.03
Force in appliance (g)	n/a	n/a	NM	22.5 ± 1.3	14.25 ± 1.3	10.5 ± 1.4	nil
			M	22.0 ± 1.3	17.5 ± 1.4	11.25 ± 1.3	nil
Width PDL (μ)	Compression	123 ± 5	NM	54 ± 8	48 ± 6	44 ± 9]*	110 ± 22
			M	55 ± 5	55 ± 5	95 ± 23]	142 ± 37
	Tension	157 ± 7	NM	243 ± 16	233 ± 10	248 ± 8	257 ± 13
			M	244 ± 5	240 ± 10	278 ± 72	257 ± 4
Percentage root length	Hyalinized zone	nil	NM	11 ± 2	22 ± 3	27 ± 2	10 ± 6
		M		19 ± 8	27 ± 5	24 ± 9	6 ± 5
	Root resorption	nil	NM	nil	nil	3 ± 2]*	35 ± 8
			M	nil	2 ± 2	20 ± 7]	37 ± 12

*$P < 0.05$.

space between first and second molars. No space was observed inter-dentally between the second and third molars indicating little or no mesial movement of the second molar. Mesial movement of the first molar may induce changes in the transeptal fibres between the first and second molars, and result in some mesial movement of the second molar. The magnitude of tooth movement for group NM was not statistically different from group M (Table 1). No orthodontic tooth movement was observed for the non-appliance side. The force remaining in the appliances decreased in a linear fashion to zero by day 14 (Table 1). No significant difference was noted for the magnetic flux density of the magnets used before or after the experiment, and the amount of magnetic flux density present in the region of the distal root was between 100–170 Gauss.

Width of the PDL space

In the appliance groups, the width of the PDL space on the compression side decreased from days 1 to 7 and increased by day 14. There were no significant differences noted in the width of the PDL space on the compression side at days 1, 3, and 14. However, at day 7, group M demonstrated a significantly greater width on the compression side (P = 0.017) compared with group NM, indicating an earlier return towards normality (Table 1). On the tension side, no significant differences were observed in the width of PDL space between the appliance groups. The histological appearance of sections from the non-appliance control side did not demonstrate tissue changes associated with orthodontic tooth movement.

Hyalinized zone and root resorption

A hyalinized zone was present within the PDL space on the compression side, the length of which increased from days 1 to 7 and then declined (Table 1). Although group M showed earlier formation and removal of the hyalinized zone, no significant differences were observed between the appliance groups. Root resorption was present on the compression side. The length of resorption lacunae increased with time and was only significantly greater (P = 0.016) in group M, compared with group NM, at day 7 (Table 1).

Specificity of immunohistochemical staining. Sections incubated without substrate for TRAP activity and omission of the primary antibody for GHR, were negative for TRAP activity or GHR immunoreactivity, respectively. Liver tissue showed positive immunoreactivity for GHR on hepatocytes.

Table 2 Mean number of TRAP active cells present at various sites on the compression and tension side for controls (non-appliance side), and at 1, 3, 7, and 14 days following placement of an appliance without a magnet (NM) and incorporating a magnet (M).

		Compression			Tension	
Day	Appliance (n = 8)	Alveolar bone bone	PDL	Root surface	Alveolar bone	PDL
1	NM	nil	0.25 ± 0.25	nil	nil	nil
	M	2.0 ± 1.23	nil	nil	1.5 ± 1.5	nil
3	NM	0.75 ± 0.75	0.75 ± 0.75	0.75 ± 0.75	1.0 ± 1.0 *	2.0 ± 1.16
	M	4.0 ± 1.41	4.0 ± 2.35	1.5 ± 0.87	4.25 ± 1.5	1.5 ± 1.5
7	NM	3.25 ± 1.6 **	15.13 ± 1.5	4.5 ± 1.19	3.25 ± 1.38	0.5 ± 0.5
	M	10.0 ± 0.40	16.13 ± 3.91	5.75 ± 2.69	4.0 ± 2.83	2.25 ± 2.25
14	NM	4.73 ± 2.36	5.5 ± 3.28	1.75 ± 1.03	nil	nil
	M	3.75 ± 3.12	8.5 ± 5.97	4.75 ± 2.75	nil	nil
Control (n = 16)	n/a	0.6 ± 0.2	nil	nil	0.25 ± 0.16	nil

*$P < 0.05$; **$P \leq 0.001$.

TRAP activity. Cell counts for TRAP activity are presented in Table 2. Figure 3 shows TRAP positive cells on the compression side at day 7 in a group M specimen. TRAP positive cells were observed from day 1, numbers peaked at day 7 and thereafter declined.

Along the alveolar bone surface on the compression side, higher cell counts for TRAP activity was recorded for group M, but only reached a significant level at day 7 (P = 0.001). The number of TRAP positive cells present in the PDL space and along the root surface on the compression side tended to be greater for group M, but this was not significant.

Root resorption on the compression side began at the periphery of the hyalinized zone, and coalesced to form a large area of resorption with subsequent removal of hyalinized tissue by the mono- and multi-nucleated TRAP positive cells. By day 14, extensive root resorption was present in both appliance groups, with 50 per cent of the animals in both groups having complete removal of the hyalinized zone. In animals with persistent hyalinized zones, TRAP activity remained intense. Repopulation of the resorbed lacunae by TRAP negative cells was observed in some day 14 specimens.

On the tension side, TRAP positive cells were observed on the alveolar bone surface and in the PDL space for the appliance groups at days 1–7,

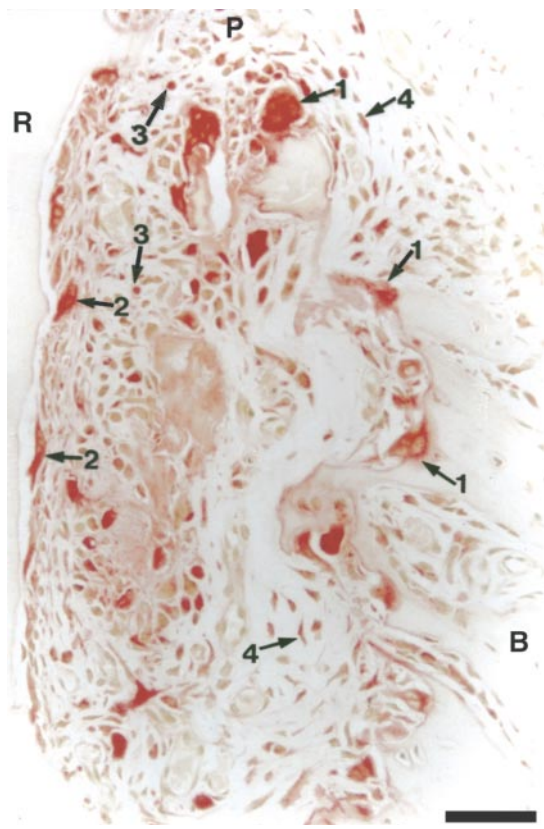

Figure 3 TRAP activity on the compresson side above the hyalinized zone from group M (day 7), showing staining in multinucleated clastic cells resorbing bone (1), clastic cells resorbing root (2), mononuclear cells (3), and fibroblasts-like cells (4). Root (R), periodontal ligament space (P), alveolar bone (B). Bar = 100 μm.

but was absent by day 14. The only difference observed between the appliance groups for the parameters measured on the tension side, was that group M demonstrated a significantly greater ($P = 0.046$) number of TRAP positive cells along the alveolar bone surface at day 3. No TRAP activity was observed along the root surface on the tension side for either group.

GHR immunoreactivity. Various cell types were positive for GHR (Figure 4). No significant differences were observed between the appliance groups for GHR cell counts. Generally, the number of GHR positive cells in the appliance groups showed an initial decline at day 3 and, thereafter, increased. By day 14, the number of GHR positive cells in the appliance groups returned to levels closer to those observed in the non-appliance control side (Table 3).

On the compression side, the number of GHR positive cells on the alveolar bone surface in the appliance groups decreased at days 1 and 3 ($P < 0.05$), and increased to a level similar to that of the non-appliance control side, by days 7 and 14. In the PDL space, there was a decline in the number of GHR positive cells at day 3 for the appliance groups, with group M recording significantly fewer ($P < 0.05$) than the control side. The number of GHR positive cells increased at days 7 and 14. By day 14, both appliance groups demonstrated a significantly greater ($P < 0.05$) number of GHR positive cells in the PDL space compared with the control side. The number of GHR positive cells on the root surface in the appliance groups, decreased significantly ($P < 0.05$) at day 3, but by days 7 and 14 increased to a level similar to that of the control side.

On the tension side, an initial decrease of GHR positive cells was observed along the alveolar bone surface at day 3, but was not statistically significant for both appliance groups compared with the control side. The number of GHR positive cells was noted to increase and was significantly greater ($P < 0.05$), compared with the control side, for group M at days 7 and 14, and group NM at day 14. The number of GHR positive cells present in the PDL space on the tension side was noted to decrease in the appliance groups at days 1 and 3, but was not significantly different from the non-appliance control side. Both appliance groups demonstrated a greater number of GHR positive at days 7 and 14 compared with the control side. Along the root surfaces, the number of GHR positive cells decreased by day 3, and gradually increased to a level similar to the control side at days 7 and 14.

Discussion

In this study, the incorporation of a magnet into an orthodontic appliance did not accelerate the rate of tooth movement of the rat maxillary first molar over a period of 14 days. Tooth movement induced by orthodontic appliances, with and without a magnet, resulted in the formation of a hyalinized zone, root resorption, compression of the PDL space, an increase in TRAP activity, and variation in GHR immunoreactivity. These histological features and distribution of TRAP activity are typical of those produced by orthodontic tooth movement (Kvam, 1969; Reitan, 1972; Rygh, 1973; Brudvik and Rygh, 1993a, 1994, 1995). The histological appearance of group M demonstrated an earlier formation and removal of the hyalinized tissue at days 1 and 7, respectively. There were few significant differences observed between groups NM and M for most of the parameters investigated. However, group M demonstrated a significantly greater width of PDL space ($P < 0.05$), length of root resorption ($P < 0.05$), and a greater number of TRAP positive cells ($P \leq 0.001$) lining the alveolar bone on the compression side at day 7. There was also an increased number of TRAP positive cell ($P < 0.05$) on the tension side at day 3, compared with group NM.

The incorporation of a magnet into the orthodontic appliance did not result in a significantly greater degree of tooth movement at any time interval studied. Both appliance groups demonstrated an initial tooth movement at day 1, reached a ‘plateau’ phase at days 3–7, and tooth movement continued to increase to day 14. Initial tooth movement is attributed to compression of the periodontium which is followed by formation of hyalinized tissue at subsequent experimental periods, indicated by cessation of tooth movement

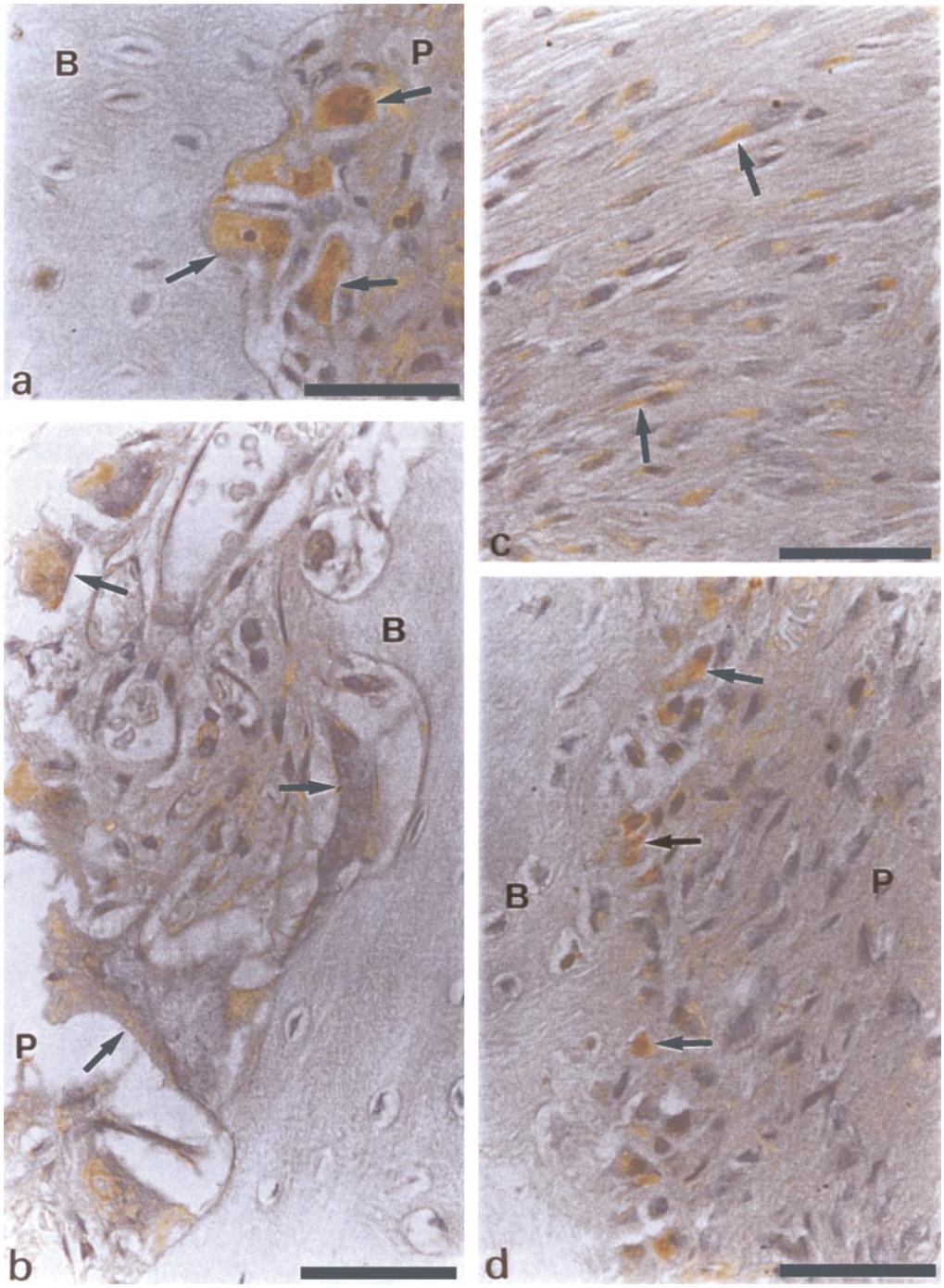

Figure 4 Section stained for GHR immunoreactivity from: (a) group NM, day 1, tension side showing GHR immunoreactive osteoclasts (arrow); (b) group M, day 3, compression side showing differential staining in multinucleated clastic cells (arrow); (c) group M, day 7, tension side showing positively stained periodontal fibroblasts (arrow); (d) group M, day 7, tension side showing positively stained osteoblasts (arrow). Root (R), periodontal ligament space (P), alveolar bone (B). Bar = 50 μm.

Table 3 Mean number of GHR immunoreactive cells present at various sites on the compression and tension side for controls (non-appliance side), and at 1, 3, 7, and 14 days following placement of an appliance without a magnet (NM) and incorporating a magnet (M).

		Compression			Tension		
Day	Appliance (n = 8)	Alveolar bone	Compression PDL	Root surface	Alveolar bone	Tension PDL	Root surface
1	NM	1.25 ± 0.75	4.5 ± 0.94	4.38 ± 0.55	6.5 ± 1.62	15.75 ± 2.05	10.5 ± 1.34
	M	3.0 ± 0.71	7.0 ± 2.5	4.38 ± 0.55	8.25 ± 2.09	13.5 ± 2.5	8.38 ± 1.75
3	NM	2.0 ± 0.41	5.0 ± 0.54	2.75 ± 0.85	5.88 ± 1.85	12.75 ± 2.56	7.5 ± 1.34
	M	2.25 ± 1.31	2.13 ± 2.13	0.75 ± 0.75	6.25 ± 1.25	8.88 ± 2.09	4.75 ± 1.6
7	NM	8.63 ± 3.02	15.13 ± 5.11	5.0 ± 2.04	8.75 ± 2.18	19.38 ± 3.04	9.5 ± 1.85
	M	6.88 ± 2.07	11.63 ± 4.49	3.88 ± 1.39	12.13 ± 3.31	17.63 ± 1.44	8.38 ± 2.77
14	NM	9.5 ± 1.06	17.5 ± 4.39	6.88 ± 2.54	11.63 ± 1.95	20.63 ± 2.73	11.63 ± 0.75
	M	11.5 ± 1.85	20.5 ± 3.35	7.88 ± 1.66	16.0 ± 0.91	18.75 ± 1.44	10.13 ± 0.97
Control (n = 16)	n/a	8.22 ± 0.7	9.53 ± 0.9	6.34 ± 0.8	7.72 ± 0.5	11.88 ± 1.0	7.88 ± 0.5

at the 'plateau' phase (Kvam, 1969; Reitan, 1972; Rygh, 1973; King and Fischlschweiger, 1982). During the 'plateau' phase, no resorption of the alveolar bone surface adjacent to the root (frontal resorption) occurs, while an undermining resorption of the alveolar bone takes place to allow for subsequent tooth movement (Brudvik and Rygh, 1993a, 1994). Recent reports suggest that the application of an EMF in conjunction with orthodontic forces can increase the rate of orthodontic tooth movement of incisors in guinea pigs, accompanied by the absence of the 'plateau' phase (Stark and Sinclair, 1987; Darendeliler *et al*., 1995). In the present study, the inclusion of a magnet into the orthodontic appliance did not appear to vary this phase. While the 'plateau' phase has been absent in other studies, the presence of this phase in the current investigation, may be due to the difference in the magnitude of the forces applied, the animal model, and tooth type investigated.

The advantage of the animal model used in this study was that forces were directed at moving a finitely erupting molar in a mesial direction that closely mimics events occurring during orthodontic tooth movement in humans. An orthodontic force of around 30 g has been shown to achieve maximum tooth movement in the rat without inducing significant cemental cratering (King and Fischlschweiger, 1982; Brudvig and Rygh, 1993a). The flux density in the region studied was 100–170 Gauss and is within the desired range suggested for orthodontic application in humans (Blechman and Steger, 1995). Previous studies on magnetic fields using the rat model have shown that 3 mT (Gonzalez-Riola *et al*, 1997) and up to 100 mT (Camilleri and McDonald, 1993) are well tolerated by tissues. However, extrapolation of results from the present study must be guarded as the application of orthodontic forces and magnetic fields are very high in relation to body weight of these small animals when compared with humans.

The width of the PDL space on the compression side was significantly greater ($P < 0.05$) in group M at day 7 when compared with group NM, while no significant differences in the length of the hyalinized zone were observed. It was also noted that one of the four specimens in group M had complete removal of the hyalinized tissue by day 7. Brudvik and Rygh (1995) observed that the width of the PDL on the compression side starts to increase towards the normal dimension by the seventh day post-appliance insertion. In this study, a similar observation was noted only for group M at day 7. Therefore, it is possible that the differences between the M and NM group at day 7 are attributed to the influence of a SMF on cellular activities in the PDL,

as suggested by Darendeliler *et al*. (1995). However, it should be noted that by day 14, both NM and M groups demonstrated a similar width of the previously compressed PDL space. Moreover, the suggestion that EMFs can produce a greater amount of bone formation and re-organization of the periodontium, which may reduce tooth mobility (Blechman and Steger, 1995; Darendeliler *et al*., 1995, 1997) could not be supported in this study. Both appliance groups did not show any differences in the histological features, which would indicate greater bone formation or early reversal from bone resorption to deposition at the tension side over the time intervals studied. However, the magnitude of the residual force remaining in each appliance was reduced to zero after 7 days. Therefore, at 14 days, the lack of significant differences in parameters between the appliance groups may be due to the absence of an orthodontic force.

Removal of hyalinized tissue was observed in conjunction with an increase in root resorption. Thus, a gradual increase in the percentage of root resorption on the compression side occurred concurrently with a reduction in the length of the hyalinized zone. In this study, significantly greater ($P < 0.05$) root resorption was noted for group M at day 7. This difference may be attributed to the effect of a SMF on the activity of cells associated with hard tissue resorption and removal of hyalinized tissues (Stark and Sinclair, 1987; Darendeliler *et al*., 1995). By day 14 both groups showed a similar amount of root resorption.

TRAP activity observed in this experiment gradually progressed to a maximum level by day 7, and was accompanied by an increase in the incidence of root resorption. On the compression side, group M demonstrated a greater number of TRAP positive cells. This was only significantly greater along the alveolar bone surface on the compression side at day 7. The elevation in TRAP activity may be attributed to the influence of a SMF on inducing faster recruitment of clastic cells and their precursors (Stark and Sinclair, 1987; Darendeliler *et al*., 1995).

Fibroblast-like cells located adjacent to the area of intense TRAP activity, on both sides of the PDL space in the appliance groups were positive for TRAP. Brudvik and Rygh (1993a,b) have reported that cells larger than fibroblasts stained positively for TRAP and that fibroblast-like cells were involved in the removal of the precementum layer. Therefore, in addition to synthesizing and releasing acid hydrolytic enzymes, as well as collagenolytic enzymes, during remodelling of PDL fibres (Ten Cate, 1971, 1972; Brudvik and Rygh, 1993b), these fibroblast-like cells may also release TRAP and be involved in hard tissue removal.

Until now, no study has investigated the distribution of GHR expression by cells in the PDL space during tooth movement. The expression of GHR of cells in the areas investigated showed no significant differences between groups M and NM, although some significant differences were observed compared with the non-appliance control side. It appeared that application of an orthodontic force caused a transient decline in GHR expression of cells in the PDL at day 3. With time, GHR expression was noted to increase to a similar or greater level to that recorded for the non-appliance control side. Individual cells that were positive for TRAP activity did not necessarily express GHR.

Decreased GHR expression, on the compression side, was observed 3 days after appliance activation. During differentiation, proliferation, and matrix synthesis, dental tissues tend to up-regulate the expression of GHR (Symons *et al*., 1994, 1996a,b; Young, 1995). The initial suppression of GHR immunoreactivity at day 3 may occur in response to the trauma of orthodontic force application, and the gradual increase in GHR immunoreactivity at days 7 and 14 suggests that cells have an altered function, possibly undergoing differentiation, proliferation and matrix synthesis.

Greater GHR immunoreactivity was observed in the PDL space compared with that on the mineralized tissue surfaces. The GHR immunoreactivity of fibroblast-like cells in the PDL space may result from an increase in cell proliferation during tooth movement. A similar observation was noted during the process of tooth eruption and root formation (Symons *et al*., 1994, 1996a,b). The increased GHR immunoreactivity by fibroblast-like cells in the

PDL space indicates that GH regulates their cellular activity either directly via its receptor or through the local production of IGF-I. The presence of a SMF did not alter the level of GHR immunoreactivity of cells during orthodontic tooth movement.

Conclusions

The effect of SMF exposure on orthodontic tooth movement of a finitely erupting maxillary rat first molar was examined. The results indicated that although exposure of 100–170 Gauss of a SMF produced a significantly greater degree of root resorption and a wider PDL space on the compression side at day 7, the on-going effects of a SMF were not sustained histologically. Exposure to a SMF of 100–170 Gauss did not significantly alter TRAP activity and GHR immunoreactivity of cells around the distal root of the rat maxillary first molar during tooth movement. However, a transient elevation of TRAP activity was observed in cells along the alveolar bone surface, on the compression side at day 7 and on the tension side at day 3 for group M. The results of this experiment question the benefits of SMFs in orthodontics. A closer investigation into the long-term effects of SMFs on cellular activity, particularly with respect to enzyme activity and the relationship of growth factors involved with bone remodelling following orthodontic tooth movement is warranted.

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References

Barker A T, Lunt M J 1983 The effects of pulsed magnetic fields of the type used in the stimulation of bone fracture healing. Review article. Clinical Physics and Physiological Measurement 4: 1–27

Bassett C A L, Pawluk R J, Pilla A A 1974 Acceleration of fracture repair by electromagnetic fields. A surgically non-invasive method. Annals of the New York Academy of Sciences 238: 242–262

Blechman A M, Steger E 1995 A possible mechanism of repelling, molar distalizing magnets. Part I. American Journal of Orthodontics and Dentofacial Orthopedics 108: 428–431

Blumenthal N C *et al*. 1997 Effects of low-intensity AC and/or DC electromagnetic fields on cell attachment and induction of apoptosis. Bioelectromagnetics 18: 264–272

Bondemark L 1994 Orthodontic magnets. A study of force and field pattern, biocompatibility and clinical effects. Swedish Dental Journal (Supplement) 99: 1–148

Brudvik P, Rygh P 1993a The initial phase of orthodontic root resorption incident to local compression of the periodontal ligament. European Journal of Orthodontics 15: 249–263

Brudvik P, Rygh P 1993b Non-clast cells star orthodontic root resorption in the periphery of hyalinized tissue. European Journal of Orthodontics 15: 467–480

Brudvik P, Rygh P 1994 Root resorption beneath the main hyalinized zone. European Journal of Orthodontics 16: 249–263

Brudvik P, Rygh P 1995 Transition and determinants of orthodontic root resorption-repair sequence. European Journal of Orthodontics 17: 177–188

Camilleri S, McDonald F 1993 Static magnetic field effects on the sagittal suture in *Rattus norvegicus*. American Journal of Orthodontics and Dentofacial Orthopedics 103: 240–246

Clearly S F 1993 A review of *in vitro* studies: low-frequency electromagnetic fields. American Industrial Hygiene Association Journal 54: 178–185

Colacicco G, Pilla A A 1984 Electromagnetic modulation of biological processes: influence of culture media and significance of methodology in the Ca-uptake by embryonal chick tibia *in vitro*. Calcified Tissue International 36: 167–174

Darendeliler M A, Sinclair P M, Kusy R P 1995 The effects of samarium cobalt and pulsed electromagnetic fields on tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics 107: 578–587

Darendeliler M A, Darendeliler A, Sinclair P M 1997 Effects of static magnetic and pulsed electromagnetic fields on bone healing. International Journal of Adult Orthodontics and Orthognathic Surgery 12: 43–53

Farndale R W, Murray J C 1985 Pulsed electromagnetic fields promote collagen production in bone marrow fibroblasts via athermal mechanisms. Calcified Tissue International 37: 178–182

Feinendiegen L E, Muhlensiepen H 1987 *In vivo* enzyme control through a strong stationary magnetic fields. The

case of thymidine kinase in mouse bone marrow cells. International Journal of Radiation Biology 52: 469–479

Gonzalez-Riola J *et al*. 1997 Influence of electromagnetic fields on bone mass and growth in developing rats: a morphometric, densitometric and histomorphometric study. Calcified Tissue International 60: 533–537

King G J, Fischlschweiger W 1982 The effects of force magnitude on extractable bone resorptive activity and cemental cratering in orthodontic tooth movement. Journal of Dental Research 61: 775–779

Kover K, Hung C H, Moore W V 1986 The characteristics of hGH binding to liver macrophages. Hormone and Metabolic Research 18: 26–30

Kvam E 1969 A study of the cell free zone following experimental tooth movement in the rat. Transactions of the European Orthodontic Society pp: 419–434

Leung D W *et al*. 1987 Growth hormone receptor and serum binding protein: purification, cloning and expression. Nature 330: 537–543

Liboff A E, Williams T, Strong D M, Wistar R 1984 Time varying magnetic fields on DNA synthesis. Science 223: 818–820

Loveridge N, Farquharson C, Palmer R, Lobley G E, Flint O J 1995 Growth hormone and longitudinal bone growth *in-vivo*: short term effect of a growth hormone antiserum. Journal of Endocrinology 146: 55–62

Luben R A, Cain C D, Chen M C-Y, Rosen D M, Adey W R 1982 Effects of electromagnetic stimuli in bone and bone cells *in vitro*: inhibition of responses to parathyroid hormone by low energy, low frequency fields. Proceedings of the National Academy of Sciences, USA 79: 4180–4184

Matthews L S, Enberg B, Norstedt G 1989 Regulation of rat GH receptor gene expression. Journal of Biological Chemistry 264: 9905–9910

McDonald F 1993 Effects of static magnetic fields on osteoblasts and fibroblasts *in vitro*. Bioelectromagnetics 14: 187–196

Mohan S, Baylink D J 1991 Bone growth factors. Clinical Orthopedics and Related Research 263: 30–48

Reitan K 1972 Mechanism of apical root resorption. Transactions of the European Orthodontic Society, pp. 363–379

Rodan G A, Bourret L A, Norton L A 1978 DNA synthesis in cartilage cells is stimulated by oscillating magnetic fields. Science 199: 690–692

Rygh P 1973 Ultra structural changes of the periodontal fibers and their attachment in rat molar periodontium incident to orthodontic tooth movement. Scandinavian Journal of Dental Research 81: 467–480

Stark T M, Sinclair P M 1987 Effects of pulsed electromagnetic fields on orthodontic tooth movement. American Journal of Orthodontics and Dentofacial Orthopedics 91: 91–104

Symons A L, Seymour G J 2000 A histological study of the effect of growth hormone on odontogenesis in the Lewis dwarf rat. Archives of Oral Biology 45: 123–131

Symons A L, Leong K, Zhang C Z, Young W G, Waters M J, Seymour G J 1994 The distribution of growth hormone receptor associated with tooth development in the rat. In: Davidovitch Z (ed.) Biological mechanism of tooth eruption, resorption and replacement by implants. Harvard Society for the Advancement of Orthodontics, EBSCO Media, Birmingham, Alabama, pp. 305–316

Symons A L, Leong K, Waters M J, Seymour G J 1996a The effect of growth hormone on root development in the rat mandibular molar. In: Davidovitch Z, Norton L A (eds) Biological mechanism of tooth movement and craniofacial adaptation. Harvard Society for the Advancement of Orthodontics, EBSCO Media, Birmingham, Alabama, pp. 449–458

Symons A L, MacKay C A, Leong K, Hume D A, Waters M J, Marks S C 1996b Decreased growth hormone receptor expression in long bone from toothless (osteopetrotic) rats and restoration by treatment with colony stimulating factor-1. Growth Factors 13: 1–10

Ten Cate A R 1971 Physiologic resorption of connective tissue associated with tooth eruption. Journal of Periodontal Research 6: 168–181

Ten Cate A R 1972 Morphological studies of fibrocytes in connective tissue undergoing rapid remodelling. Journal of Anatomy 112: 401–414

Young W G 1995 Growth hormone and insulin like growth factor-1 in odontogenesis. International Journal of Developmental Biology 39: 263–272

Zhang C Z, Young W G, Li H, Clayden A M, Garcia-Aragon J, Waters M J 1992 Expression of growth hormone receptor by immunohistochemistry in rat molar root formation and alveolar bone remodelling. Calcified Tissue International 50: 541–546