

Effects of orthodontic magnets on cutaneous epithelial thickness and tibial bone growth in rats

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The use of permanent magnets in orthodontic treatment has increased significantly over the past years, triggered by the introduction of rare earth magnetic alloys. Concerns about possible side effects have been expressed relating to their use in orthodontic treatment. In previous experimental studies evidence of a reduction in bone formation and epithelial turnover close to rare earth magnets has been presented. The aim of the present study was twofold: to confirm earlier results and to study whether the observed changes are reversible. One hind leg in each of 45 rats was fitted with aluminum rings with Co₅Sm magnets, whereas the other hind leg was fitted with sham rings. After 8 weeks 25 rats were killed, and the rings in the remaining rats were removed. The latter group was killed after another 11 weeks. The epithelial and bone reactions were evaluated histomorphometrically. Previous results showing thinner epithelium and retarded rate of bone formation close to Co₅Sm magnets were confirmed. These effects were also shown to be largely reversible. It was concluded on the basis of this and the magnitude of the effects that any local side effects from orthodontic rare earth magnets appear negligible when extrapolated to the clinical situation. □ *Bone; epithelium; magnets; morphometry; orthodontics*

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The use of permanent magnets in orthodontic treatment has increased significantly over the past years, triggered by the introduction of rare earth permanent magnetic alloys (1). Most reports in the dental literature have dealt with clinical and technical issues relating to their uses and found significant advantages in certain clinical situations (for review, see Refs. 2–7). However, reports on possible biologic effects from static magnetic fields have been published (8–13), and some of these deal specifically with orthodontic magnets (14–16). Qualitative results pertaining to implanted rare earth magnets have indicated no adverse effects (17, 18). Nevertheless, with regard to their use in orthodontic treatment two main concerns about possible side effects have been expressed: possible 'toxic' effects of corrosion products following contact with the oral environment (15, 16, 19–24) and possible direct effects of the static magnetic field on cells (20, 25, 26; own unpublished observations).

According to Autian (27), testing of dental materials should include three levels of testing: a screening phase in which pre-clinical tests and studies are performed in animals and in vitro; an 'in-use' test in animals at sites where the material is to be used, and in which the test procedure should duplicate as much as possible the procedure to be used when the material is to be used in patients; and clinical trials in humans.

The different levels of testing and their increasing complexity are intended to identify possible therapeutic

and adverse effects for further testing at the next level. Thus, identifying biologic effects of orthodontic rare earth magnets in a simple animal model relating to the first level of testing is intended to aid design and evaluation of future 'in-use' tests in animals and clinical trials in humans. However, the clinical significance of the present results can only be fully assessed following clinical trials.

In a previous report on the effect of rare earth permanent magnets (24) we found that exposure of the proximal tibia of young rats to cobalt-samarium (Co₅Sm) magnets resulted in a statistically significant reduction of total and cortical bone thickness during an 8-week observation period. The ratio between cortical and total bone width was also significantly reduced under the magnets, indicating an inhibition of bone formation (24). However, it has also been reported that resorbing surfaces increase progressively after stimulation with static rare earth magnets (22). Furthermore, a reduced thickness of the epithelium under the magnets has been reported (22). Although these effects were statistically significant, their magnitude and the number of observations were limited. In the present study we have therefore repeated parts of these experiments, using both female and male rats and the magnets fitted to either the left or right leg. Furthermore, and more important, the question whether the observed changes are reversible was

addressed in the present study relating to test level 1 in accordance with Autian (27).

Materials and methods

Experimental procedure

Forty-five young Sprague-Dawley rats with an initial weight of 150 g were used in the experiments. The proximal tibial region of one of the hind legs in each of the rats was fitted with individually adjusted aluminum rings (I. Ö. Mekaniska HB, Bankeryd, Sweden) to which two Co₅Sm magnets (Medical Magnetics Inc., Ramsey, N.J., USA) were attached, medially and laterally. The other leg in each rat was similarly fitted with an aluminum ring, although without magnets, as previously described (22, 24). The 45 rats were subsequently divided into 4 different groups:

Group A consisted of 15 female rats. In each of 10 of these rats a ring fitted with magnets was placed around the right hind leg and a control ring placed around the left hind leg. Each of the remaining five rats had a ring fitted with magnets placed around the left hind leg and a control ring placed around the right hind leg. All rats were injected with 33 mg/kg b/w doxycycline (Vibramycine®, Pfizer, Amboise, France) at the start of the experiment and were killed after 8 weeks.

Group B consisted of 10 female rats. In each of five of these rats a ring fitted with magnets was placed around the left hind leg and a control ring placed around the right hind leg. Each of the other five rats had a ring fitted with magnets placed around the right hind leg and a control ring placed around the left hind leg. All rats were injected with 33 mg/kg b/w doxycycline at the start of the experiment and after 8 weeks, on removal of the rings. The animals were killed after another 11 weeks.

Group C consisted of five male and five female rats. In each of the five male rats a ring fitted with magnets was placed around the left hind leg and a control ring placed around the right hind leg. The five female rats had a ring fitted with magnets placed around the right hind leg and a control ring placed around the left hind leg. All rats were killed after 8 weeks.

Group D consisted of five male and five female rats. In each of the five male rats a ring fitted with magnets was placed around the right hind leg and a control ring placed around the left hind leg. The five female rats had a ring fitted with magnets placed around the left hind leg and a control ring placed around the right hind leg. All rings were removed after 8 weeks, and the animals were killed after another 11 weeks.

The rats were checked daily and the rings adjusted when needed as described previously (22, 24). They were fed a standard laboratory diet consisting of pelleted food and water ad libitum and were killed by inhalation of CO₂ for 5 min followed by cervical dislocation. The parts of the tibias located inside the rings were removed

immediately after death and were then prepared for histologic and histomorphometric evaluation.

Histotechnical preparation

Groups A and B. The tibias were fixed in 5% neutral-buffered formalin and gently freed from the soft tissues. The skin immediately under the rings was processed for paraffin sectioning perpendicular to the long axis of the tibias. The sections were cut at a thickness of 8 µm and stained with hematoxylin and eosin. The remaining mineralized parts of the specimens were dehydrated and embedded in a low-viscosity embedding medium (Spurr, Agar Aids Ltd., Stansted, England) and cut into 50-µm slices with a diamond saw (Exakt Apparatebau, Otto Herrmann, Norderstedt, Germany) perpendicular to the long axis of the tibias. The sections were mounted in a synthetic mounting medium (Eukitt, O. Kindler, Freiburg, Germany).

Groups C and D. The specimens were divided transversally in the middle, immersed for 3 days in McDowell's fixative (28), rinsed in 0.1 M sodium cacodylate buffer with 0.2 M sucrose at 4°C, demineralized for 6 weeks in a 0.2 M ethylenediaminetetraacetic acid (EDTA) solution and embedded in paraffin admixed with beeswax (42 g beeswax/kg paraffin). The paraffin blocks were serially sectioned, producing sections both in a distal and proximal direction (24). All sections were taken perpendicular to the long axis of the tibias at a thickness of 8 µm. For each specimen the exact mid-section under the magnets was defined in accordance with histologic reference levels previously described (29). The sections were mounted on slides with albumin-glycerin and stained with hematoxylin and eosin.

Histologic and histomorphometric evaluation

Groups A and B. Ten consecutive serial sections from the central part of the experimental area of each piece of skin were examined in a light microscope (Aristoplan, Ernst Leitz GmbH, Wetzlar, Germany), and the number of cells constituting the thickness of the epithelium was determined in three locations under both the medial and lateral magnets and corresponding sites in the control specimens. The measurements were repeated by the same investigator at a 2-week time interval. Intra-examiner correlation was determined.

The five central-most sections in relation to the magnets on each leg in accordance with the mapping made by Linder-Aronson & Rygh (29) were evaluated under incident ultraviolet and transmitted polarized light in a microscope equipped with computerized image analysis facilities (Argus-50, Hamamatsu, Hamamatsu City, Japan), to visualize tetracycline (TC) deposits. Measurements under the medial magnets were made of the total bone width and the following distances within the bone tissue (Fig. 1): from the bone surface in the

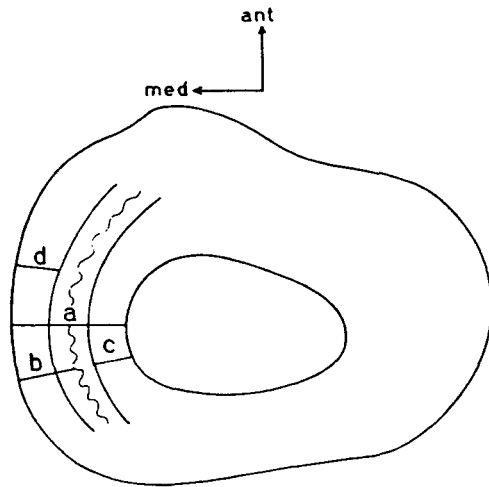


Fig. 1. Schematic drawing of a cross-sectioned tibia in the area selected for evaluation. The following measurements in relation to the tetracycline (TC) lines were made: total bone width (a) and cortical bone width (b) and distances from the bone surface in the marrow cavity to the innermost TC line (c) and from the outermost TC line to the outer bone surface (d). The bone maturation index is defined as the ratio between measurements *b* and *a* × 100.

marrow cavity to the innermost TC line and from the outermost TC line to the outer bone surface.

Groups C and D. Every 25th section, 200 µm apart in an area 1 mm wide, for a total of 5 slides around the designated mid-section corresponding to reference level 5 (29), was evaluated histomorphometrically. The total bone width and the cortical bone width of the medial part of the tibia closest to the medial magnets were measured, using computerized image analysis (Image-Pro II 2.00.06, Media Cybernetics Inc., Silver Spring, Md., USA). The cortical bone width was expressed as the mean value of the minimum and the maximum measurements from the outer medial border to a line bordering cartilage remnants between the cancellous and the cortical bone. Total bone width and cortical

bone width were compared intra-individually. The ratio of cortical bone width to total bone width was calculated and defined as the bone maturation index (24) (Fig. 1).

Statistical analyses

Wilcoxon's sign rank test for matched pairs (30) was used to test the significance of differences between test and control groups for all variables. The results were consistently analyzed as matched pairs within the same animal. Differences were considered significant at *p* < 0.05.

Results

The rats appeared to tolerate the experimental devices well, and no altered behavior was noted during the observation periods. This was confirmed by a regular increase in weight during the observation periods. However, for histotechnical reasons two epithelial specimens and four bone specimens were excluded from the analysis (Tables 1 and 2). The remaining specimens were carefully examined for signs of inflammation on a histologic level. One such reaction was found in a magnet specimen; this specimen was consequently excluded (Table 1).

No statistically significant differences with regard to histomorphometric recordings of tissue reactions were observed between the female and male rats, nor were any statistically significant differences noted between right and left legs regardless of their use as experimental or control legs. The results of male and female test and control specimens were therefore pooled, and no distinction will be made between right or left leg. A detailed summary of means from the morphometric measurements and results from the statistical analyses can be found in Tables 1 and 2.

Epithelial thickness

The epithelium under the magnets in both groups A

Table 1. Means with standard deviations (SD) of the histomorphometric measurements after 8 weeks of magnetic application and significance levels (*p*) for differences between test and control. The groups correspond to those described in Materials and methods. *N* denotes original number of rats, whereas *n* denotes the number of rats for paired analysis after exclusions due to macroscopic evidence of irritation from the rings

Group	<i>N</i>	Variable	Unit	<i>n</i>	Control		Magnet		<i>p</i>
					Mean	SD	Mean	SD	
A	15	Epithelial thickness	No. of cells	12	3.00	1.16	2.28	0.76	<0.01
A, C	25	Total bone width (a)	µm	23	642	42.7	615	46.8	<0.05
C	10	Cortical bone width (b)	µm	8	271	148	258	117	>0.05
C	10	Bone maturation index (b)/(a) × 100	%	8	41.6	21.7	41.4	16.9	>0.05
A	15	Bone width measurement (c)	µm	15	166	50.0	130	48.6	<0.05
A	15	Bone width measurement (d)	µm	15	79.5	32.8	90.5	25.5	>0.05

Table 2. Means with standard deviations (SD) of the histomorphometric measurements after 8 weeks of magnet application followed by 11 weeks without appliances. *P* denotes significance levels for differences between test and control. The groups correspond to those described in Materials and methods. *N* denotes original number of rats, whereas *n* denotes the number of rats for paired analysis after exclusions due to macroscopic evidence of irritation from the rings

Group	<i>N</i>	Variable	Unit	<i>n</i>	Control		Magnet		<i>p</i>
					Mean	SD	Mean	SD	
B	10	Epithelial thickness	No. of cells	10	1.85	0.15	1.70	0.17	<0.01
B, D	20	Total bone width (a)	µm	18	620	84	611	86.6	>0.05
D	10	Cortical bone width (b)	µm	8	126	86.5	161	67.8	>0.05
D	10	Bone maturation index (b)/(a) × 100	%	8	21.3	10.5	29.4	9.0	>0.05
B	10	Bone width measurement (c)	µm	10	166	35.6	155	45.1	>0.05
B	10	Bone width measurement (d)	µm	10	119	22.0	128	23.3	>0.05

and B was significantly thinner than in the paired control leg (Tables 1 and 2), although the absolute difference had decreased after the 11-week period without rings (Table 2). The intra-examiner correlation was 0.98.

Bone measurements

After 8 weeks the mean total bone width under the magnets in all groups was significantly smaller than in corresponding areas under the control rings (Table 1). Significantly less bone had, furthermore, formed along the bone surfaces in the marrow cavity (Table 1). However, after another 11 weeks without rings no significant differences remained in either location (Table 2). Neither cortical bone width nor bone maturation indices differed significantly after any observation time as calculated for groups C and D (Tables 1 and 2). Similarly, for none of the other measurements were the means found to differ significantly between control and test groups.

Discussion

The present study has shown a significant but largely reversible effect on bone and epithelium by externally placed Co₅Sm magnets. The nature of these changes and their possible causal relationship with the magnets will therefore be the focus of this discussion. However, first some methodologic issues need to be addressed.

The experimental and evaluation methods used in the present study have been used previously and their suitability analyzed in detail (22, 24). In addition, test and control legs were consistently paired within the same rat, to avoid inter-individual variation. The results thus obtained were analyzed with the Wilcoxon sign rank test, a non-parametric test based on matched pairs. Furthermore, previously defined landmarks were used to enable identification and analysis of comparable sections from test and control specimens (23). Reliability was further shown by a high intra-examiner correlation

for the measurements of epithelial thickness. Since previous results may have been biased by the use of male rats only (22, 24, 29), the present study used groups of rats of both sexes, and the experimental and control legs were varied within the groups. However, no differences were observed with regard to sex or choice of left or right leg as experimental leg. Hence, we conclude that results from the present study can be compared with previous results (22, 24, 29). Our results confirm some previous findings with regard to reduction in epithelial thickness and bone width immediately after a period of magnets applied to rat tibia (22, 24). Although the means for epithelial thickness differ between the studies, comparisons of differences between and within studies are justified since they are based on matched control/experiment pairs.

The epithelium under the magnets was significantly thinner than in controls, although the mean difference was less than one cell layer after 8 weeks. It is not unlikely that this is the result of a reduced mitotic activity in the epithelium adjacent to the magnets. The turnover period of the epidermis is 15 to 30 days (31, 32) depending on localization, which makes the 8-week period of exposure to magnets sufficient for any effect thereof to become evident in the thickness of the epithelium. With the present experimental design it cannot be determined whether the effect was due to the magnetic field or the magnetic material itself. However, recent *in vitro* results have indicated that static magnetic fields have a direct inhibitory effect on mitotic activity (unpublished observations). Although the epithelium in the present study was still significantly thinner after an additional 11-week period without magnets, the difference as compared with control had decreased, indicating that the effect is reversible. Thus, extrapolated to a clinical orthodontic situation, any possible side effect from rare earth magnets may be negligible with regard to inhibition of epithelial turnover, although this remains to be determined in 'in-use' tests.

A significantly reduced total bone width (bone measurement *a* in Fig. 1) was observed under the magnets after 8 weeks, in accordance with a previous study

(24). This reduction in bone thickness appeared to be the result of reduced bone formation within the marrow cavity, as seen in a significantly thinner bone measurement c (Fig. 1). This indicates an inhibitory effect on bone growth by the magnets and may be in accordance with the above discussion on an inhibitory effect of mitotic activity in the epithelium, although the possibility of a direct effect on rate of bone formation cannot be excluded. The effect appeared, however, to be reversible, since no significant differences were found 11 weeks after removal of the magnets. Furthermore, the effect on bone tissue appeared to be very subtle, since no effect was observed on the outer cortical bone surface. It is not unlikely that the reason for this is the comparatively larger bone surface area in the marrow cavity and, thus, a higher responsiveness in this area. This is supported by the fact that a previously recorded effect on bone maturation as defined by the ratio between cortical and total bone width could not be confirmed (24).

In conclusion, the present study has largely confirmed previously observed effects of rare earth magnets on bone growth and epithelial thickness (22–24; own unpublished observations) and, in addition, shown that any inhibitory effect on these tissues appears reversible. Extrapolated to the clinical situation, any local side effects from orthodontic rare earth magnets appear negligible.

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