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Comparisons of the effects of systemic administration of L-thyroxine and doxycycline on orthodontically induced root resorption in rats

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SUMMARY The aim of this study was to histologically evaluate and compare the effects of the systemic administration of L-thyroxine (TX) and doxycycline (DX) on orthodontically induced root resorption, Twenty-eight male 50- to 60-day-old Wistar rats were used. Seven rats served as the baseline control. Seven animals received TX (20 μg/kg bodyweight/day) and seven DX (1.2 mg/kg bodyweight/day), by means of a mini-osmotic pump implanted subcutaneously. Seven rats were separated as a sham, in order to evaluate the pure effect of the surgical procedure on the animals' health. Tooth movement (TM) was achieved with a continuous force of 50 g by placing Elgiloy coil springs between the right maxillary first molar and incisors for 14 days. The animals were sacrificed and specimens containing the appliance and maxillary tooth-bearing segments were processed for light microscopy. The surface area of root resorption lacunae was measured histomorphometrically using digital photomicrographs. To evaluate the resorptive changes on the molar root surface of each group, scanning electron microscopy (SEM) examinations were also carried out. Statistical evaluation of root resorption percentages was performed using Kruskal–Wallis analysis of variance test. Multiple comparisons were determined by the Student–Newman–Keuls method. The level of significance was set at $P < 0.05$.

Histomorphometric analysis of root resorption, expressed as a percentage, showed that the average relative root resorption affecting the maxillary molars on the TM side was 0.32 ± 0.25 in the TX and 0.26 ± 0.06 in the DX groups and 2.19 ± 0.86 in the control. Statistically significant inhibition of root resorption was determined both in the TX and DX groups ($P < 0.001$) on the TM side. There was no statistically significant difference in relative root resorption between the TX and DX groups. Systemic administration of TX and DX demonstrated similar effects on root resorption in rats and may have inhibitory effects on orthodontically induced resorptive activity.

Introduction

External apical root resorption is a common clinical complication of orthodontic treatment. It is the permanent shortening of the end of the tooth root that can be seen on routine dental radiographs (Hartsfield *et al.*, 2004). Some authors (Reitan, 1951; Rygh, 1977) consider it a side-effect of the cellular activity associated with the removal of necrotic tissue in an overcompressed periodontal ligament (PDL; Igarashi *et al.*, 1994; Ong *et al.*, 2000).

Treatment modalities, based on targeting osteoclasts, could have uses in clinical orthodontics. Potential effects of several pharmacological agents on orthodontically induced root resorption have been examined in several experimental studies and some of these agents have been applied clinically. The inhibitory effects of bisphosphonates and prednisolone, which putatively interfere with the function of clast cells and, hence, inhibit both bone and root resorption, have been shown in rats (Igarashi *et al.*, 1994; Ong *et al.*, 2000). Although Boekenoogen *et al.* (1996) reported that prostaglandin E_2 had no significant effect on the amount of orthodontically induced root resorption, Leiker *et al.* (1995) found that this agent increased root resorption. In a recent study, it was shown that systemically delivered echistatin (arginine–glycine–aspartic acid-containing peptide) significantly reduced root resorption induced by tooth movement (TM; Talic *et al.*, 2006).

Engström *et al.* (1988) demonstrated that although the level of parathyroid hormone in serum plays an important role in the regulation of the resorptive activity in bone, a change in serum calcium level is a determining factor for root resorption. In addition to parathyroid hormone, bone resorptive activity is also regulated by L-thyroxine (TX; Tapp, 1966; Persson *et al.*, 1989). Thyroid hormone plays a crucial role in normal growth and development of vertebrate bones (Vázquez-Landaverde

et al., 2002). The administration of high doses of TX in rats has been shown to increase bone resorption (Adams and Jowsey, 1967). Klaushofer *et al.* (1989) reported that thyroid hormones increase osteoclastic bone resorption in rats by stimulation of prostaglandin, especially prostacyclin synthesis. The effects of TX on root resorption are still controversial. Loberg and Engström (1994) showed that thyroid hormone administered in high-risk patients reduces orthodontically induced root resorption. They reported that treatment with the hormone produced no new root resorption, no worsening of the existing resorption, and no adverse effects. Similar inhibitory effects have been shown for the administration of low-dose TX and clinical application of this material has been attempted (Poumpros *et al.*, 1994; Shirazi *et al.*, 1999). However, Christiansen (1994) commenting on the study of Loberg and Engström (1994) criticized the fact that the authors did not clarify the mechanism by which the hormone acts; it is assumed that the hormone either increases the resistance of the cementum and dentine to clastic activity or increases the rate of alveolar bone resorption. Thus, Christiansen (1994) thought that the hormone enhanced TM as it indirectly reduced root resorption.

Tetracyclines have long been used as adjuncts in the treatment of periodontal disease (Grevstad, 1993). Although initially attributed to their antimicrobial properties, the clinical efficacy of tetracycline in periodontitis has recently been suggested to be due to its intrinsic anti-inflammatory activity (Walker *et al.*, 2000) since low doses of (sub-antimicrobial) doxycycline (DX) decrease attachment loss and excessive collagenase activity in the crevicular fluid of periodontitis patients (Thomas *et al.*, 2000). Inhibition of metalloproteinase activity would then be responsible for the protective effect of DX in periodontitis. DX has been shown to reduce the total number of osteoclasts and prevent root resorption and alveolar bone loss following mucoperiosteal flap surgery in rats (Grevstad and Bøe, 1995). In some re-implantation studies, a decrease in inflammatory root resorption has been reported (Selvig *et al.*, 1992). Clinically, it was found that low-dose DX substantially reduced collagenase activity in the gingiva and gingival crevicular fluid and prevented loss of attachment in adults with periodontitis (Schroeder *et al.*, 1990; Caton *et al.*, 2000). This Food and Drug Administration-approved treatment regimen (Ciancio and Ashley, 1998) was effective and safe: specific side-effects included gastrointestinal disturbance and emergence of tetracycline-resistant microorganisms (Schroeder *et al.*, 1990; Ciancio and Ashley, 1998; Skidmore *et al.*, 2003). Despite the protective effects of DX in periodontitis, there is only one study, which reported that the compounds effectively inhibit orthodontic root resorption (Mavragani *et al.*, 2005).

The above mentioned studies have indicated that systemical administration of a hormone, TX, and a pharmacological agent, DX, might have beneficial effects during orthodontic TM by reducing the amount of root resorption. The aim of this histological experimental study was to evaluate and compare the effects of the systemic administration of TX and DX on orthodontically induced root resorption.

Materials and methods

The experimental protocol was approved by the University of Erciyes Regional Animal Research Ethics Committee.

Animals and groups

Twenty-eight male 50- to 60-days-old Wistar rats weighing 132.53 ± 12.65 g (randomly separated into four groups of seven rats each) were used in this study. All animals were housed in polycarbonate cages and fed a standard pellet diet (Expanded pellets; Stepfield, Witham, Essex, UK) with tap water *ad libitum*.

Sham group. Seven rats were separated as sham, and mini-osmotic pumps were implanted in order to evaluate the pure effect of the surgical procedure on the animals' health. No appliances were placed.

Control group. Seven animals in this group served as the controls to the TX and DX groups. Appliances were inserted in the right maxillary segment and mini-osmotic pumps filled with physiological serum were placed. The maxillary right first molar was moved mesially using a standard procedure. The right maxillary side represented TM and the contralateral maxillary left side, which did not receive an appliance, represented no tooth movement (NTM).

L-thyroxine group. The animals received TX through mini-osmotic pumps. The right maxillary side represented TM. The contralateral maxillary left side did not receive a closed coil spring and represented NTM and physiological root resorption.

Doxycycline group. The animals received low-dose DX (Deva Pharmaceutical, Ankara, Turkey) through mini-osmotic pumps and the appliances were placed using the same procedure as for the TX group. Similar to the other experimental group, the contralateral maxillary left first molars without any appliance served as the NTM control and represented physiological root resorption.

Mini-osmotic pumps, implantation, and dosage

According to the manufacturer's instructions, the mini-osmotic pumps (Alzet® Mini-osmotic Pumps, Model No 1002; Alza Corporation, Palo Alto, California, USA) were placed subcutaneously in the mid-scapular region. Implantation and appliance insertion was undertaken on the same day under general anaesthesia [ketamine (60 mg/kg body weight; ketamine hydrochloride, Gedeon Richter Ltd, Budapest, Hungary) and Xylazine (10 mg/kg body weight; Rompoun, Bayer, Leverkusen, Germany)].

The animals in the TX group were given 20 μg TX/kg bodyweight/day (Sigma, St Louis, Missouri, USA) during the entire experimental period through the mini-osmotic pumps (Shirazi *et al.*, 1999).

The DX was released at a mean pumping rate of 0.50 μl/hour (±0.10 μl/hour) during the entire experimental period, which equals administration of 0.24 mg DX/day (1.2 mg DX/kg bodyweight/day; Mavragani *et al.*, 2005).

Appliance design

The appliance comprised a closed coil spring (Elgiloy spring, F-31 0.008 × 0.032 inches; Rocky Mountain Dental Products Co., Denver, Colorado, USA) and bands on the incisors. This orthodontic appliance was based on the modified technique described by Brudvik and Rygh (1993). Bands were manufactured and eyelets prepared from 0.5 inch diameter stainless steel wires which were welded on the bands. The coil springs were cut in to 5 mm sections and secured with 0.010 inch ligature wires (Dentaurum, Ispringen, Germany) distally to the upper first molars and anteriorly to the eyelets on the incisor bands. The force of 50 g was measured with a gauge at the time of insertion and was not reactivated until the end of the experimental period. A retractor was used to hold back the soft tissues and to hold the head securely (Houston, 1964).

Histological preparation

The animals were weighed and this was recorded twice a week. At the end of the experimental period of 14 days, the animals were killed with an overdose of anaesthetic [Fentanyl (Dormicum-F. Hoffmann-La Roche & Co. AG, Basel, Switzerland)/Fluanison Midazolam (Hypnorm-Janssen Pharmaceutical, Beerse, Belgium; 0.15–0.2 ml/100 g bodyweight)] which was injected subcutaneously. The rats were subsequently perfused through the left ventricle of the heart with McDowell's solution.

The appliance and tooth-bearing segments of the maxilla were dissected and kept in fixative for 24 hours at 4°C, rinsed in 0.1 M sodium cacodylate buffer containing 0.2 M sucrose, and decalcified in 0.25 M ethylenediaminetetraacetic acid (10 per cent) at 4°C for approximately 8 weeks. The specimens were embedded in paraffin and 5 μm para-sagittal sections were cut and stained with haematoxylin and eosin.

Histomorphometric evaluation

The slide showing the greatest length of the mesiobuccal root of the first molar and four adjacent slides were evaluated histomorphometrically. Each slide contained five sections. The histomorphometric evaluation design was adopted from Talic *et al.* (2006). For histomorphometric measurements, photomicrographs were taken digitally with ×4 objective lens with a microscope and digital camera system (Olympus CX41/DP25; Olympus Corp., Tokyo, Japan).

To calculate the percentage of root resorption, two reference points were selected that could be reliably identified on all sections: the cemento-enamel junction and the root bifurcation point. A line was drawn between the reference points. By tracing the pulp area, the computer image analysis software (AnalySIS 2.1; Soft-Imaging Software GmbH, Műnster, Germany) calculated the area (μm^2) of the pulp. The whole root was traced, and the area (square micrometres) of the whole root was also calculated by the software (Figure 1). To determine the percentage of root resorption for each root, the following formula was used: combined surface area of root resorption lacunae divided by the surface area of the whole root minus the surface area of the radicular pulp multiplied by 100. The measurements were taken from four sections at 25 μm intervals and the mean values were calculated.

Scanning electron microscopy examination

To investigate the resorptive changes on the molar root surface, scanning electron microscopy (SEM; Leo 440; M/s Leo Electron Microscopy, Cambridge, UK) examinations were performed on the mesial surfaces of the mesial root of the maxillary right first molars. The molar of the sham rat

Figure 1 Area of investigation: saggital section along the mesio-buccal root. Measurements of the area of root resorption and total root surface using an image analysis system. BP, bifurcation point; CEJ, cemento-enamel junction. Bar = 0.1 μm.

served as the normal root surface. One rat from each group was randomly selected. Each specimen (maxilla and corresponding teeth) was placed in a 4 per cent sodium hypochlorite solution for 1 week. The right upper first molar was then extracted, left to air dry for 1 day, and placed on the examination stub. Sites to be examined were coated with a layer of palladium–gold. Resorption patterns in the different study groups were observed and evaluated by SEM.

TM measurements

Orthodontic TM was measured, using the distance between the first and second molars, and the data were calculated morphometrically using an image analysis system (AnalySIS 2.1). The distance between the molars was measured between the cemento-enamel junction of the distal side of the first molar and the mesial side of the second molar in the most central section. The same operator (AB) performed all measurements.

Statistical analysis

All analyses were performed with SigmaStat for Windows Version 3.10 (Systat Software Inc., Richmond, California, USA). To determine the measurement error, 10 photomicrographs were randomly selected. The roots on these images were traced, and the surface area of each root was measured and documented with the AnalySIS software. Ten days later, a second measurement of the surface area of the roots was made. A paired *t*-test showed no statistically significant differences between the two measurements ($P = 0.660$). Statistical analysis used to investigate inter-observer method error also indicated no significant differences ($P = 0.870$).

For each animal, a mean value for the histomorphometrically evaluated sections was obtained. Statistical evaluation of root resorption percentages were performed using Kruskal–Wallis analysis of variance. Multiple comparisons were determined by Student–Newman–Keuls method. The level of significance was set at $P < 0.05$.

Results

Animals

The animals tolerated the appliance and the implanted subcutaneous mini-osmotic pump well. The incision wound from the implantation of the mini-osmotic pump was adequately healed on the day following surgery. For all animals, weight loss was observed for 1 week after appliance placement. In the second half of the experimental period, the weight increased. The sham group showed more weight gain for the 14 day experimental period.

Amount of resorption

The load delivered to the clinical crowns of the maxillary incisors and first molars with the closed coil spring caused uncontrolled tipping movement and created areas of compression and tension in the PDL. In the sections stained with haematoxylin and eosin, root resorption lacunae were seen on the root surface, with a few multinucleated odontoclasts adhering to resorption lacunae along the root surface, especially on the compression side. Osteoclasts were identified in all groups but not in all specimens of each group.

Descriptive statistical values of the relative root resorption expressed as a percentage are shown in Table 1. The Kruskal–Wallis test showed significant differences between the control and experimental groups ($P < 0.001$). Histomorphometric analysis showed that the average relative root resorption of the maxillary molars on the TM side was 0.32 ± 0.025 in the TX, 0.26 ± 0.06 in the DX groups and 2.19 ± 0.86 in the control group (Table 1, Figure 2). The average relative root resorption on the NTM side was 0.02 ± 0.59, 0.01 ± 0.64 and 0.01 ± 0.08, respectively. There was a significant difference in relative root resorption between the TM and NTM sides in the control groups ($P < 0.001$). There was a significant inhibition of root resorption in the experimental groups ($P < 0.001$) on the TM side. There was no significant difference in relative root resorption between the TX-TM and DX-TM groups. Furthermore, there was no

Table 1 Descriptive statistic values relative to root resorption expressed as percentages and the results of multiple comparison tests.

Groups	Mean	Standard deviation	Minimum	Maximum	Significance	Test*
	Percentage (%)					
Control TM	2.19	0.86	0.58	2.52	***$P < 0.001$	A
Control NTM	0.01	0.08	0.00	0.20		B
Thyroxine TM	0.32	0.25	0.16	0.76		C
Doxycycline TM	0.26	0.06	0.15	0.34		C
Thyroxine NTM	0.02	0.59	0.00	1.46		B
Doxycycline NTM	0.01	0.64	0.00	1.58		B

TM, tooth movement side; NTM, no tooth movement side.
*Groups with different letters are significantly different from each other.

significant difference in relative root resorption on the NTM side in the experimental and control groups.

SEM results

The mesial sides of the mesiobuccal roots were qualitatively evaluated by SEM and only subjective observations are reported. As only one sample was investigated for each group, the results should be interpreted with caution. Resorptive lacunae were observed on the mesial surface of all first molar mesial roots, even in the sham rat (the rat with no force application or TX/DX intervention). Root resorption at all TM and NTM sides was greater than in the control rat. Root resorption in the TX and DX groups was less than in the control group. Root resorption in the TX group was near the level observed in the DX group.

Resorption was evaluated according to Mavragani *et al.* (2004) (1) small isolated lacuna; (2) wide shallow resorption bays with no detectable dentinal tubules; (3) deep resorption lacuna extending into the dentine. Small isolated lacunaes and wide shallow bays were seen in all samples; occasionally in continuity with each other, and sometimes small isolated lacunaes encircled deep resorption bays (Figure 3). Deep resorption lacunaes were rarely detected (Figure 4). Undermining resorption detected as sharp edges were seen at the periphery of resorption bays.

Except for the appliance control group, resorption lacunae were localized in the cervical third of the root, whereas in the appliance control group resorption was seen in the middle third of the root (Figure 5).

Tooth movement

All molars on the TM side showed evidence of movement. No tooth movement was noted at the NTM sides. No significant difference was detected between the amount of TM in the control and experimental groups when considering the distance measured between the first and second molars (Table 2). The median values for the control, TX, and DX groups were 0.803 μm, 0.856 μm, and 0.667 μm, respectively.

Discussion

Orthodontically induced root resorption is a common but unpredictable consequence of orthodontic TM (Talic *et al.*, 2006). Foo *et al.* (2007) observed increases in resorption crater volumes in a TM group compared with the controls. Those authors reported that the incidence of root resorption increases significantly with, orthodontic TM. Identifying high-risk patients who may develop root resorption during orthodontic TM is a prerequisite for developing clinical or cellular treatment modalities to prevent or reduce the incidence of root resorption (Talic *et al.*, 2006).

The current study aimed to compare the influence of two different pharmacological agents on induced root resorption. An animal model was chosen to better control the variables and reduce other influencing factors. Wistar rats were chosen because they are bred to be genetically the same, thereby eliminating genetic variables. In the present study, the rats were approximately the same age (8 weeks) at the start of the experiment, and the development of their teeth and surrounding structures should have been complete (Matias *et al.*, 2003). The design and application of experimental orthodontic TM often shows shortcomings. As the effect of an applied force on the tissues is related to the size of the tooth involved, the magnitude of the applied force should be related to its root surface area (Isaacson

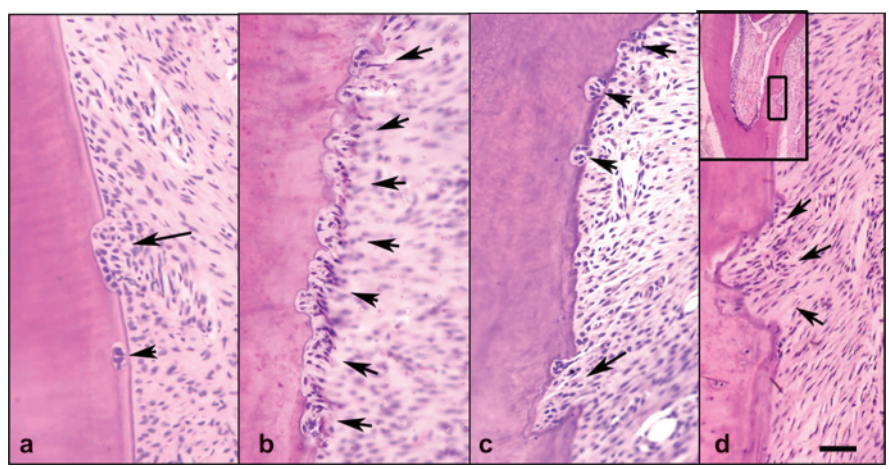

Figure 2 (a) Non-appliance control group: mild resorption of cement and dentine (original magnification ×20); (b) appliance control group: wide shallow resorption lacunae extending into the dentine (original magnification ×20); (c) doxycycline group: small isolated lacunae and deep dentine resorption (original magnification ×20); (d) L-thyroxine group: wide and deep dentine resorption (original magnification ×10). Bar = 0.1 μm.

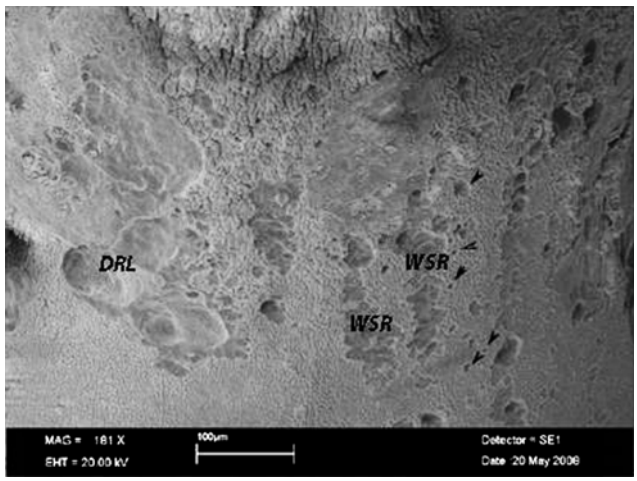

Figure 3 Doxycycline group: arrow heads indicate small isolated lacunae surrounding wide shallow resorption lacunae (WSR); DRL, deep resorption lacunae.

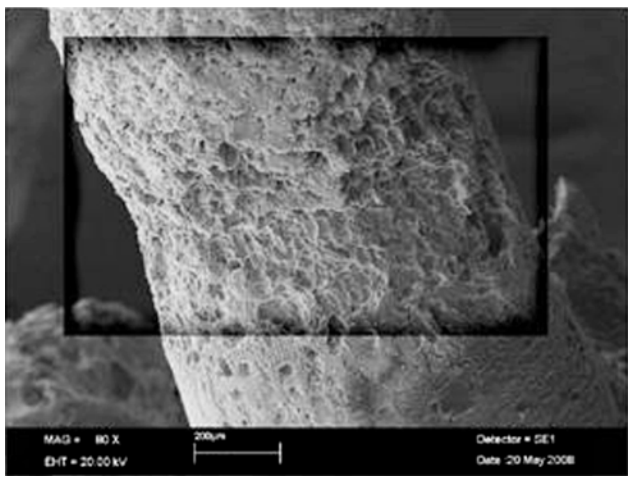

Figure 4 Severe dentine resorption in the appliance control group.

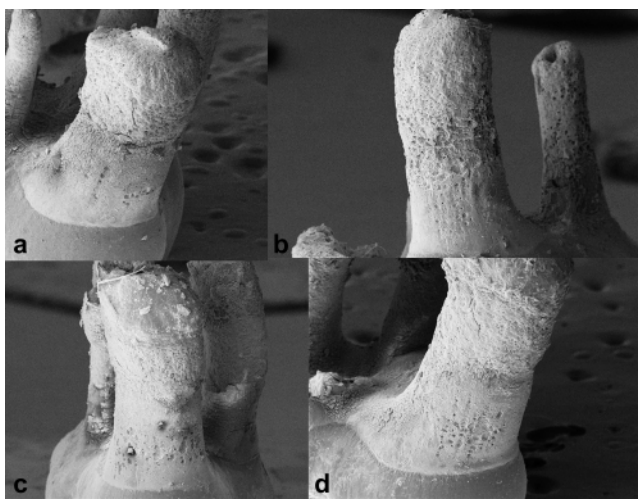

Figure 5 Scanning electron micrographs of (a) sham group; (b) appliance control group; (c) doxycycline group; (d) thyroxine group.

et al., 1993). Rat teeth are very thin (a rat molar is approximately 50 times smaller than a human molar), which complicates the design of an efficient orthodontic appliance that is suited to produce a constant and continuous force with an acceptable force range (Ren *et al.*, 2004).

The present orthodontic TM model was adapted from Ashizawa and Sahara (1998) but has also been used by Brudvik and Rygh (1993) and Mavragani *et al.* (2004, 2005). The appliance system was used to produce a continuous force over the experimentation period of 2 weeks (Melsen *et al.*, 1994). Both clinical and animal studies have shown that there are several phases in TM (Proffit and Fields, 2000). It takes from a few days to a few weeks to reach the so-called 'linear phase', where real TM through bone occurs. Therefore, studies aimed at describing the characteristics and biological response in the linear phase of TM should have an experimental period of at least 2 weeks. Orthodontic TM starts the process of orthodontically induced root resorption and, because of the continuous force applied, cementum repair should not occur (Foo *et al.*, 2007). Owman-Moll and Kurol (1998) showed cementum repair as early as 2 weeks after cessation of orthodontic TM. This methodology allowed the study of maximum resorptive sites after the 2 week experimental period in the present study, without the possibility of a reparative phase. The present appliance system elicited tissue reactions that included the recruitment of clast cells and their progenitors; this results in bone and root resorption. This was evident by the statistical difference in the extent of root resorption between the TM and the NTM sides of the control group.

In many experimental TM studies, non-standardized or unclearly explained springs or elastics have been used as the force delivery system. According to Ren *et al.* (2004), the force decay rate, the amount of force decay, and the dimensional changes of elastics during decompression have not yet been adequately investigated. Thus, standardized closed coil springs were preferred to produce a constant and continuous force over the experimental period.

In previous research, testing materials or pharmacological agents have been administered systemically through the drinking water of the experimental animals (Grevstad and Bøe, 1995). The implantation of mini-osmotic pumps offers a controlled way of continuous drug administration. However, it has been stated that the pumping rate becomes stable only several hours after implantation (Mavragani *et al.*, 2005). Therefore, pump implantation should, optimally, precede orthodontic appliance insertion by at least 1 day in order to establish a steady DX and TX serum level by the time of force application. In the present study, however, it was considered that two separate operations within a short interval might negatively affect the animals. Hence, the results should be interpreted carefully in the context of TX and DX levels during the first experimental day.

The measurement of root resorption craters was adopted from Talic *et al.* (2006) who used percentages

Table 2 Descriptive statistics for the tooth movement (TM) measurements and the results of multiple comparison tests.

Groups	*N*	Magnitude of TM (mm)			Significance	Multiple comparison		
		25%	Median	75%		Control TM	L-thyroxine TM	Doxycycline TM
Control NTM	7	0.263	0.278	0.373	***	*	*	*
Control TM	7	0.719	0.803	0.836			NS	NS
L-thyroxine TM	7	0.506	0.856	0.882				NS
Doxycycline TM	7	0.560	0.667	0.808				

NS, not significant. *$P < 0.05$.

instead of total resorption area in square micrometres. It was thought that the applied force could lead to more resorption of smaller teeth. Although the animals were nearly the same weight, individual differences may exist in the size of their teeth, similar to humans. Thus, the percentage of resorption areas to root was considered to be appropriate for assessment of root resorption instead of total resorption area.

The present results suggest that the TX-treated animals showed significantly less root resorptive lesions on the TM sides than the TM control group. These data corroborate the findings that administration of lower doses of TX reduces force-induced root resorption lesions (Poumpros *et al.*, 1994; Shirazi *et al.*, 1999). Vázquez-Landaverde *et al.* (2002) suggested that administration of low doses of TX might provide a protective role on the root surface during orthodontic TM, and in those patients that present spontaneous root resorption lesions.

DX was selected over other tetracyclines for this experiment because it has been shown to be a more potent collagenase inhibitor (Selvig *et al.*, 1992; Thomas *et al.*, 2000) and has demonstrated an inhibitory effect on root resorption and alveolar bone distraction in rats (Mavragani *et al.*, 2005). The current study demonstrated an inhibitory effect of DX on root resorption. Several pleiotropic and complex mechanisms have been proposed to explain the anti-resorptive properties of tetracyclines, primarily by the inhibition of several matrix metalloproteinases (Rifkin *et al.*, 1993). Matrix metalloproteinases are largely responsible for degrading constituents of connective tissues, not only during pathological tissue breakdown but also during normal remodelling (Greenwald *et al.*, 1998) and this phenomenon may partly explain the reduction in resorptive activity in the experimental animals.

In the present study, the aim was to determine whether DX, the most potent tetracycline with anti-collagenase activity commercially available, and a hormone, TX, could prevent root resorption in an experimental rat model. The results showed that systemic delivery of both agents significantly reduced orthodontically induced root resorption surface areas and it was found that there was no significant difference in relative root resorption between the DX-TM and TX-TM groups.

No statistically significant differences were found among the two experimental and one control group in the amount of TM. Because both osteoclasts and odontoclasts share numerous morphological and functional features, it is reasonable to assume that they would be similarly affected by the tested agents. Yet, in this study, a 14 day systemic administration of TX and DX significantly reduced orthodontically induced root resorption, but TM, which is linked to alveolar bone resorption, was not affected. This is contrary to the results of Shirazi *et al.* (1999) who found increased TM with TX. On the other hand, Poumpros *et al.* (1994) found that TM was not affected by low-dose TX and they interpreted the result as being due to a more efficient forced-induced remodelling process. Some reports have shown that administration of low-dose TX reduces bone resorption (Tapp, 1966; Adams and Jowsey, 1967) in contrast to high doses, which have been shown to increase bone resorption (Adams and Jowsey, 1967). Likewise, Mavragani *et al.* (2005), in their study on rats, noted decreased osteoclastic activity but no change in orthodontic TM compared with the control animals. The results were interpreted as normalization of bone loss with restoration of normal osteoblast structure and increased collagen synthesis (Sasaki *et al.*, 1991, 1992).

In a previous investigation where DX was shown to be effective in preventing root resorption in rats, the mean TM was 919.4 μm after a 14 day experimental period (Mavragani *et al.*, 2005). The difference in TM between the two studies may be due to the size of the animals. In the present study, 132.53 ± 12.65 g animals were used whereas this was 196 ± 10 g in the study of Mavragani *et al.* (2005). It can be considered that the use of larger animals results in increased TM as the gingival embrasures are larger.

SEM permits visualization of surface structures and is particularly valuable in recording the morphology of mineralized tissues (Boyde and Jones, 1968). This technique provides enhanced visual assessment of root surfaces, unattainable with histological models reconstructed from serial sections (Reitan, 1974). During specimen preparation

for SEM, the organic part was dissolved according to Mavragani *et al.* (2004), allowing alterations of the mineral component of the root surface and the 'footprints' of cells on the mineralized tissue to be observed. The area of cratering on the root surface was evaluated as an indicator of cell activity. Hellsing and Hammarstrom (1996), in a study using SEM, found definitive resorption sites forming within 1 week of appliance placement. The 2 week experimental period in the present investigation was sufficient to produce resorption sites for analysis.

Conclusions

Under the conditions of this experiment, TX and DX demonstrated inhibitory effects on root resorption in rats. However, the magnitude of TM, which is linked to alveolar bone resorption, was not affected.

Further, studies on the effect of low doses of DX and TX administration during orthodontic TM and the mechanisms involved have to be undertaken before the routine clinical application of these procedures can be recommended.

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