European Journal of Orthodontics 27 (2005) 215–225
doi:10.1093/ejo/cji015

Orthodontically induced root and alveolar bone resorption: inhibitory effect of systemic doxycycline administration in rats

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SUMMARY The aim of the present study was to investigate the effect of systemic administration of low-dose doxycycline (DC) on orthodontic root resorption. The effect on alveolar bone, the cell population involved, and the amount of tooth movement were also evaluated.

Fifty-six 40–50-day-old male Wistar rats were used. Six animals served as untreated controls. Six animals were only administered DC for 7 days, by means of a mini-osmotic pump implanted subcutaneously. In 44 animals the maxillary first molar was mesialized by a fixed orthodontic appliance exerting 50 g force upon insertion. In 28 of these animals DC was administered at the time of appliance insertion and throughout the experiment. The animals were sacrificed 7, 10 or 14 days after force application and block sections processed for analysis. An area including the mesial aspect of the distopalatal root and the adjacent inter-radicular alveolar bone was histomorphometrically evaluated. The root resorption area, absolute alveolar bone area, distance between first and second molars, number of odontoclasts, osteoclasts, mononuclear cells on the root, tartrate-resistant acid phosphatase (TRAP)-positive cells on the root, bone, and in the periodontal ligament (PDL) were compared between DC-treated and non-DC-treated animals.

The results revealed a significant reduction in root resorption, the number of odontoclasts, osteoclasts, mononuclear cells on the root surface, and TRAP-positive cells on the root and bone for the DC-administered group. The absolute alveolar bone area was greater, whereas the distance between the first and second molars did not differ between groups.

In conclusion, systemic administration of low-dose DC in rats may have an inhibitory effect on orthodontically induced resorptive activity.

Introduction

Orthodontically induced inflammatory root resorption is a common iatrogenic consequence of treatment. It has been considered a side-effect of the cellular activity associated with the removal of necrotic tissue in an over-compressed periodontal ligament (PDL) (Reitan, 1951; Kvam, 1973; Rygh, 1977).

The modifying effect of several pharmacological agents on orthodontic root resorption has been examined. Among them, L-thyroxine has been shown to have an inhibitory effect and clinical application has been attempted (Loberg and Engström, 1994; Poumpros *et al.*, 1994; Shirazi *et al.*, 1999). Similar effects have been shown for bisphosphonates and prednisolone in rats (Igarashi *et al.*, 1994, 1996; Ong *et al.*, 2000). Prostaglandin E_2 had no significant effect on the amount of orthodontically induced root resorption (Boekenoogen *et al.*, 1996; Seifi *et al.*, 2003), but has been shown to increase root resorption (Leiker *et al.*, 1995).

Tetracyclines, broad spectrum antibiotics, and their chemically modified analogues have been used as an adjunct in the treatment of periodontal disease. A series of studies has been published describing anti-inflammatory properties of tetracyclines unrelated to their antimicrobial effect (Golub *et al.*, 1983, 1984, 1985, 1987). Evidence has shown that tetracyclines inhibit the activity of metalloproteinases such as collagenase and gelatinase and can therefore prevent collagenolysis. The destruction of collagen, the principal structural protein of the body's connective tissues, is an essential step in the pathogenesis of a variety of diseases, including periodontal disease, rheumatoid arthritis, and osteoarthritis (Harris *et al.*, 1984).

Among the tetracyclines, doxycycline (DC) 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, 1993; Grevstad and Bøe, 1995). Experimental studies have also demonstrated the inhibitory effect of tetracyclines on degradation of the periodontium (Al-Ali *et al.*, 1989; Ramamurthy *et al.*, 1998), whereas Cvek *et al.* (1990) reported a decrease in inflammatory root resorption in re-implanted teeth in monkeys. Other re-implantation studies have shown improved periodontal repair only when the teeth are treated with both tetracycline and stannous fluoride (Bjorvatn *et al.*, 1989; Selvig *et al.*, 1992). In clinical trials, tetracycline and analogues, including low-dose DC, substantially reduced collagenase activity in the gingiva and the gingival crevicular fluid and prevented loss of attachment in adults with periodontitis (Golub *et al.*, 1983, 1984, 1985; Schroeder *et al.*, 1990, 1992; Thomas *et al.*, 1995; Caton *et al.*, 2000). DC has been administered in specially formulated capsules containing 20 per cent (20 mg DC/capsule) of the commercially available product as an adjunct in periodontal

treatment. This Food and Drug Administration approved treatment regimen was effective and safe: specific side-effects include gastrointestinal disturbance and emergence of tetracycline-resistant micro-organisms (Golub *et al.*, 1990a, 1998; Schroeder *et al.*, 1990, 1992; Ciancio and Ashley, 1998; Thomas *et al.*, 2000; Skidmore *et al.*, 2003).

These observations have indicated that tetracycline administration might have a beneficial effect during orthodontic tooth movement by reducing the amount of root resorption. The aim of the present investigation was to histologically evaluate the effect of the systemic administration of one semi-synthetic tetracycline, DC, on the tissues involved in orthodontic tooth movement with emphasis on root resorption.

Materials and methods

Animals and experimental procedure

The material comprised 56, 40–50-day-old, male Wistar rats (Mol:WIST Han) weighing 196 ± 10 g. All animals were housed in polycarbonate cages and fed a standard pellet diet (801157W expanded pellets, Stepfield, Witham, Essex, UK) with tap water *ad libitum*. The experimental protocol was approved by the Regional Committee for Animal Research Ethics, University of Bergen.

The animals were distributed into treatment groups as follows: the control group without an orthodontic appliance comprised 12 animals; six animals served as untreated controls (untreated control group), and the remaining six animals were administered DC for 7 days (doxy-control group). The experimental tooth movement group comprised 44 rats. Sixteen animals had only an orthodontic appliance inserted (ortho group), whereas 28 rats received both orthodontic force and DC (doxy-ortho group). The experimental periods were 7, 10, and 14 days. The number of animals per group is reported in Table 1.

The spring used was a closed coil spring (Elgiloy spring, F-31 0.008 × 0.032, Rocky Mountain Dental Products Co., Denver Colarado, USA) (Mavragani *et al*., 2004). The force was reduced to 27 g by day 7, 19 g by day 10 and 0 by day 14. Similar results were previously reported by Brudvik and Rygh (1995a). Animals scheduled for antibiotic treatment received 20 mg/ml Doxylin (Alpharma, Apothekernes Laboratorium AS, Oslo, Norway) by means of a mini-osmotic pump (alzet® mini-osmotic pump, model 2002, Alza Corporation, Palo Alto, California, USA) implanted subcutaneously on the back slightly posterior to the scapulae. Animals in the doxy-ortho group had the mini-osmotic pump implanted at the time of appliance insertion. Doxylin was released at a mean pumping rate of 0.5 (±0.1) µl/hour during the entire experimental period, which equals administration of 0.24 mg DC/day (1.2 mg DC/kg bodyweight/day).

The weight of the animals was recorded on the day of appliance insertion and before death. At the end of each experimental period the animals were killed with an overdose of anaesthetic, which was subcutaneously injected fentanyl (Dormicum-F. Hoffmann-La Roche & Co. AG, Basel, Switzerland)/fluanison midazolam (Hypnorm-Janssen Pharmaceutica, Beerse, Belgium) (0.15–0.2 ml/100 g bodyweight), and were subsequently perfused through the left heart ventricle with McDowell's solution. Following dissection, the right half of the maxilla, including the first, second and third molars, was 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 EDTA (10 per cent) at 4°C for approximately 6 weeks. The specimens were then embedded in paraffin and parasagittal sections of the teeth were cut at 6 µm.

Every fifth glass slide (five sections per slide) was stained with haematoxylin and eosin (H&E). The slide showing the greatest length of the distopalatal root and eight adjacent slides covering approximately 270 µm in a buccolingual direction were alternatively stained with H&E and tartrate-resistant acid phosphatase (TRAP) (Brudvik and Rygh, 1993a). On the nine slides, five H&E and four TRAP stained, every second section was evaluated histomorphometrically (a total of 23 sections per animal).

The area of investigation was the mesial aspect of the distopalatal root and adjacent structures, including the

Table 1 Values (median and range) for root resorption area (RR; µm²), absolute alveolar bone area (AVB; µm²), and distance between first and second molars (D; µm) for non-doxycycline-treated (Non-doxy) and doxycycline-treated (Doxy) animals by observation period.

	Non-doxy				Doxy				*P* values		
	n	RR	AVB	D	*n*	RR	AVB	D	RR	AVB	D
D0	6	344.5 44–1287	338 329 260 983–369 095	307.6 190.6–544.7	6	9.8 0–102.8	352 174 315 266–459 691	341.8 209.5–599.9	0.013	0.379	0.51
D7	4	6047.4 4946–9035	22 421 168 698–267 502	393.7 282.8–501.6	8	782.2 302–1874	306 824 254 317–470 844	466.6 322.0–644.3	0.009	0.034	0.391
D10	6	22 331 20 815–35 362	177 406 140 595–275 044	569.0 492.5–726.3	10	3408 1435–4877	290 763 251 084–447 996	566.1 401–1459	0.001	0.004	1.00
D14	6	16 139 136 24–20 182	186 582 146 569–250 160	919.4 648–1752	10	9969 6518–19 838	271 319 233 693–348 795	697.7 489.1–1010.2	0.093	0.008	0.203

D0 indicates rats in the doxy-control group.

inter-radicular alveolar bone septum. Under ×100 magnification, an area 1500 × 500 μm² was examined (Figure 1). The root resorption area, absolute alveolar bone area and the distance between the first and second molars were calculated morphometrically using an image analysis system (analySIS 2.1, Soft-Imaging Software GmbH, Műnster, Germany). The absolute alveolar bone area was considered as the bone tissue area excluding the marrow, lacunar and canalicular area. For estimation of the absolute bone area, the marrow and lacunar area were subtracted from the total bone tissue area, according to the definitions of Parfitt *et al.* (1987). Practically, the canalicular area cannot be assessed by light microscopy. The distance between the molars was measured between the cemento-enamel junctions of the distal side of the first molar and the mesial side of the second molar in the most central section examined.

Under higher magnification (×400) the following cell types were counted: odontoclasts, osteoclasts, mononucleated cells on the root surface, as well as TRAP-positive cells on the root, alveolar bone, and in the PDL. Multinucleated cells in contact with the root or bone, or residing in Howship's lacunae, were counted as odontoclasts or osteoclasts, respectively.

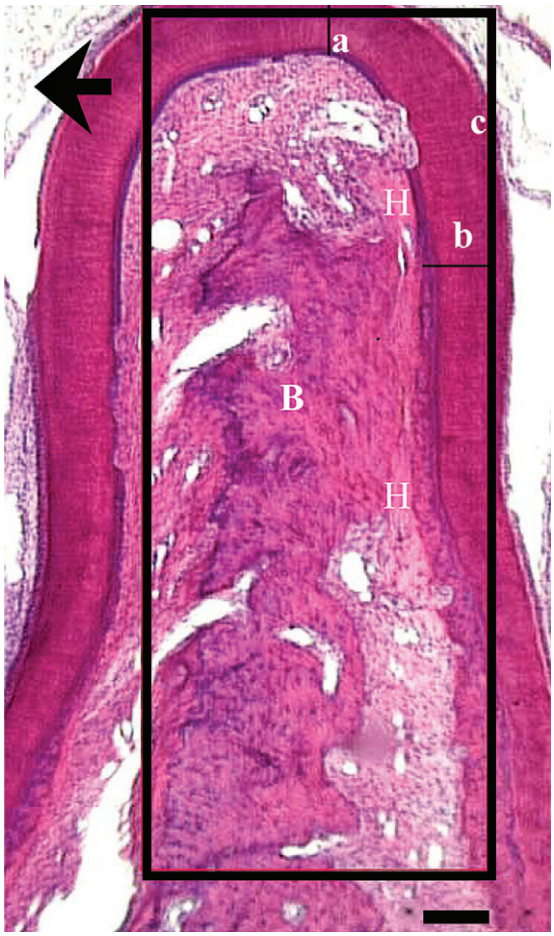

Figure 1 Area of investigation: a box 1500 × 500 μm² drawn with its long axis parallel to the root canal of the distopalatal root, the upper side positioned 60 μm from the highest point of the furcation (a) and the distal side extending 100 μm into the dentine (b) at a point 400 μm from the coronal side of the box (c). The arrow indicates the direction of the applied force. The specimen represents the ortho group, day 7. Marrow spaces and resorption defects are present in the inter-radicular bone (B). H, hyalinized tissue (bar = 100 μm).

Statistical methods

For all parameters considered, a mean value of the histomorphometrically evaluated sections was obtained per animal. A Mann–Whitney test was performed for each considered variable and observation period for comparison between DC-treated and non-DC-treated groups.

Thirty randomly selected sections were recounted within a 3 month interval, in order to test for intra-examiner error. The systematic error between the double measurements was then evaluated using the paired *t*-test, and the measurement error by the intraclass correlation coefficient, for each variable. No significant systematic differences were found and the measurement error was considered acceptable.

Results

Animals

The animals tolerated the appliance and the implanted mini-osmotic pump well. The incision wound from the implantation of the mini-osmotic pump was adequately healed by the day following surgery. At the end of each experimental period the animals had increased their bodyweight by a mean of 5, 20, and 35 g by days 7, 10, and 14, respectively.

After 7 days the mean remaining force was 27 g (range 25–28 g) and after 10 days 20 g (range 0–25 g) with no difference between DC- and non-DC-treated rats. No force remained after 14 days.

Control group

Resorption of the root surface was observed in the control animals. However, the control animals treated with DC for 7 days showed significantly less root resorption than the untreated controls (Table 1) (Figure 2a, b). Significantly fewer mononucleated cells were observed on the root surface of the doxy-control animals than in the untreated control rats. Furthermore, the doxy-control group showed significantly fewer osteoclasts than the untreated control group (Table 2). No TRAP-positive cells were observed in either of the control groups (Table 3).

Experimental group

Root.

Root resorption extending into the dentine was observed in all experimental tooth movement groups (Figures 3–5). The animals in the doxy-ortho group had significantly less root

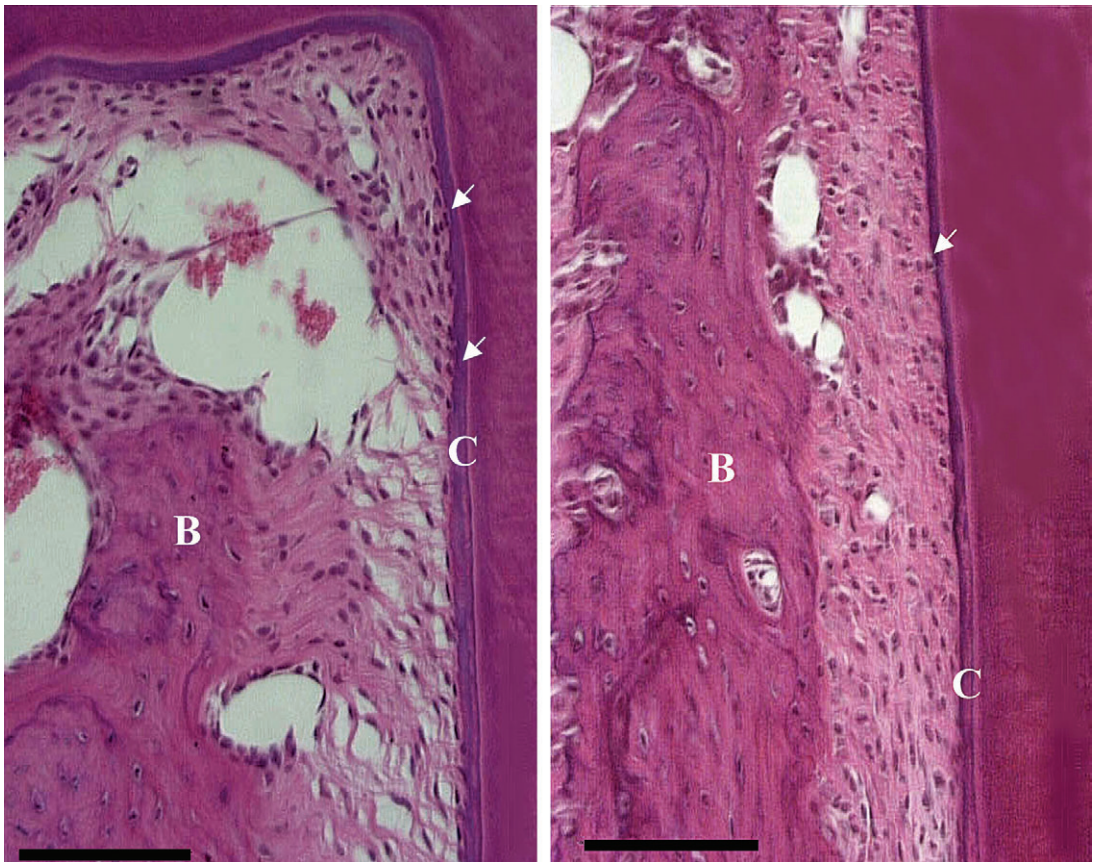

Figure 2 (a) Untreated control specimen. Arrows indicate the area of cementum resorption by mononuclear cells (bar = 100 μm). (b) Doxy-control specimen. The cementum surface appears intact with only a few mononuclear resorbing cells (arrow) (bar = 100 μm). B, inter-radicular bone; C, cementum.

Table 2 Values (median and range) for the number of odontoclasts (OD), osteoclasts (OCL) and mononucleated cells (MR) on the roots of non-doxycycline-treated (Non-doxy) and doxycycline-treated (Doxy) animals by observation period.

	Non-doxy				Doxy				*P* values		
	n	OD	OCL	MR	*n*	OD	OCL	MR	OD	OCL	MR
D0	6	0 0–0	6.34 3–11	17.83 8–33	6	0 0–0.33	2.25 1–3	5.00 4–6.5	0.987	0.007	0.005
D7	4	5.30 2.98–9.03	13.82 6.44–25.13	17.34 12.18–21.13	8	2.52 0.83–5.88	13.68 7.20–20.34	6.39 3.00–12.59	0.075	0.932	0.014
D10	6	10.80 6.50–15.00	18.54 13.67–21.00	6.87 4.00–9.22	10	5.35 3.40–8.00	12.60 7.83–18.00	6.50 4.00–11.40	0.006	0.011	0.515
D14	6	7.04 1.60–9.00	12.42 9.50–20.00	10.03 6.25–13.00	10	3.21 1.25–4.00	7.25 2.50–10.33	4.90 3.00–7.50	0.026	0.002	0.003

D0 indicates rats in the doxy-control group.

resorption than the animals in the ortho group at all experimental periods, except day 14 (Table 1). In the doxy-ortho group, the median value for root resorption increased throughout the experiment, while the ortho group revealed a reduction in the median value for root resorption by day 14.

No significant difference was found in the number of odontoclasts between doxy-ortho and ortho groups on day 7. However, the number of odontoclasts was significantly lower in the doxy-ortho group than in the ortho group on days 10 and 14. In both groups the number of odontoclasts presented a peak at day 10 and decreased towards day 14. Significantly fewer mononucleated cells were observed on the root surface of the doxy-ortho animals compared with the ortho group at days 7 and 14. The median number of

Table 3 Values (median and range) for the number of tartrate-resistant acid phosphatase-positive cells on root (TR), bone (TB) and in periodontal ligament (TP) in non-doxycycline-treated (Non-doxy) and doxycycline-treated (Doxy) animals by observation period.

	Non-doxy				Doxy				*P* values		
	n	TR	TB	TP	*n*	TR	TB	TP	TR	TB	TP
D0	6	0 0–0	0 0–0	0 0–0	6	0 0–0	0 0–0	0 0–0	1.000	1.000	1.000
D7	4	2.94 1.75–6.83	4.58 2.73–7.33	5.15 4.03–8.22	8	0.84 0.00–3.80	1.57 0.60–12.50	3.79 1.67–6.80	0.136	0.337	0.456
D10	6	7.75 2.00–10.00	14.50 2.50–21.00	8.50 1.00–15.00	10	1.63 0.00–2.67	1.29 0.00–3.00	5.84 0.00–13.00	0.013	0.008	0.306
D14	6	0.50 0.00–3.00	1.165 0.00–2.00	1.00 0.00–14.00	10	0.00 0.00–0.67	0.50 0.00–2.50	6.20 0.00–13.00	0.432	0.317	0.475

D0 indicates rats in the doxy-control group.

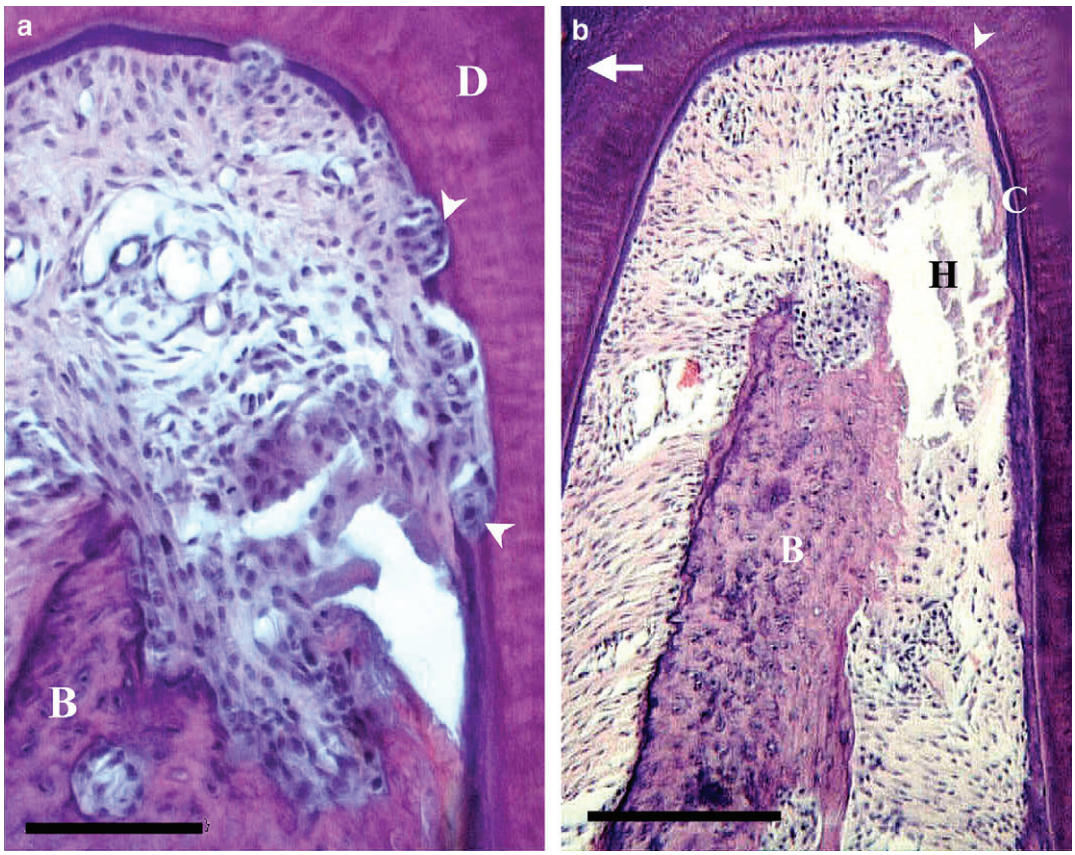

Figure 3 Day 7. (a) Ortho specimen. Multinucleated clast cells (arrows) in resorption lacunae (bar = 100 μm). (b) Doxy-ortho specimen. Rather low root resorption activity. A multinucleated cell in the resorption lacuna (small arrow). Alveolar bone retains its integrity. The arrow indicates the direction of tooth movement (bar = 200 μm). B, inter-radicular bone; C, cementum; D, dentine; H, hyalinized tissue.

mononucleated cells on the root decreased by day 10 in the ortho group and increased by day 14. In the doxy-ortho group the median for the number of mononucleated cells decreased from day 10 to day 14 (Table 2).

Similar to odontoclasts, the number of TRAP-positive cells on the root showed a peak at day 10 and decreased by day 14 in both experimental groups. However, the doxy-ortho group demonstrated significantly fewer TRAP-positive cells on the root surface than the ortho group after 10 days of force application (Figure 6a, b) (Table 3).

Alveolar bone.

The absolute alveolar bone area was significantly greater in the doxy-ortho group than in the ortho group at all observation periods. Until day 10, the alveolar bone area

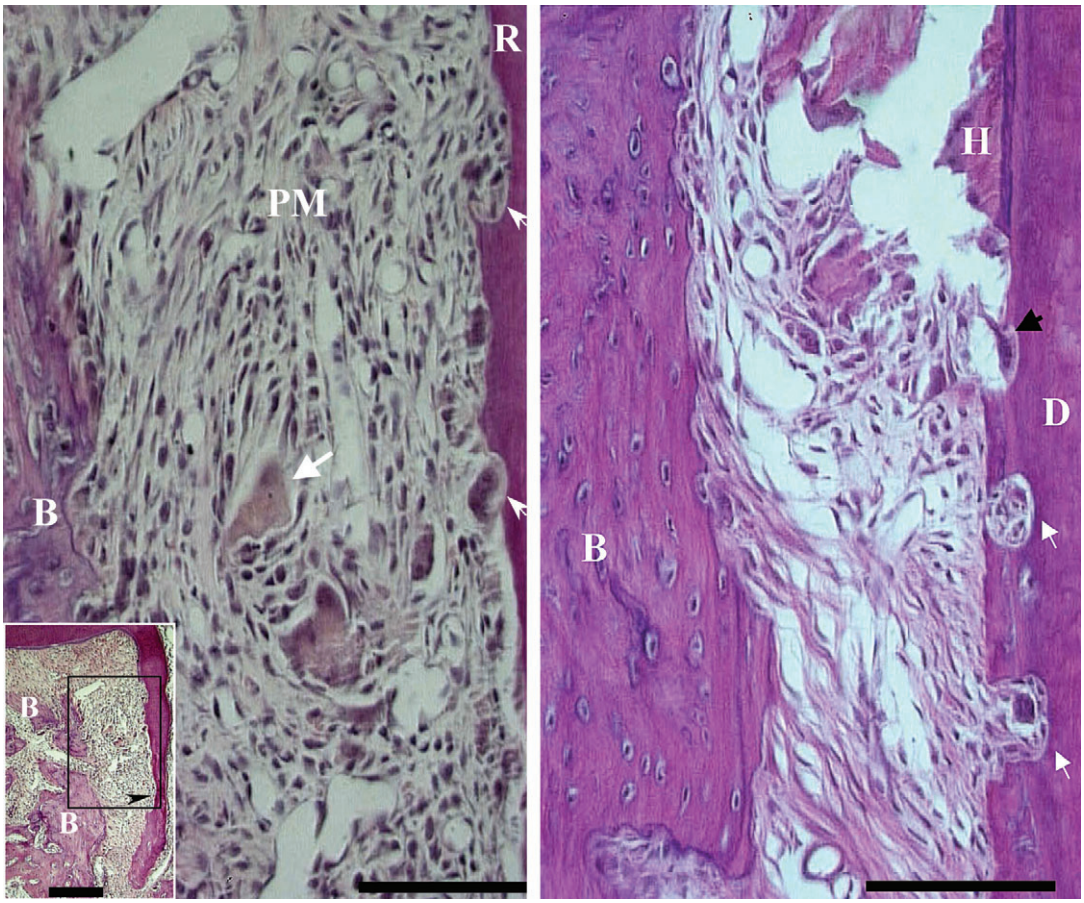

Figure 4 Day 10. (a) Overview and detail of ortho specimen. Periodontal ligament under repair. The large arrow indicates a remnant of hyalinized tissue being phagocytosed and small arrows indicate the transition to repair of resorption lacunae (bar = 100 μm). Inset: extensive resorption of the root (arrow) and distraction of the inter-radicular septum has occurred (bar = 200 μm). (b) Doxy-ortho specimen. White arrows indicate lacunae exhibiting the transition to repair. The black arrow indicates a rather flattened clast cell on a resorption lacuna (bar = 100 μm). B, inter-radicular bone; D, dentine; H, hyalinized tissue; R, root.

decreased in both groups (Table 1). In the doxy-ortho group the inter-radicular septum appeared more solid and the cancellous bone more dense (Figures 3b, 4b, 7a) than in the ortho group, where larger marrow spaces were present (Figures 1, 4a, 7b).

The number of osteoclasts was significantly lower in the doxy-ortho group than in the ortho group on days 10 and 14 (Table 2, Figure 7a, b). TRAP-positive cells were significantly fewer in the doxy-ortho group at day 10 (Figure 6a, b). Within the ortho group, the median for TRAP-positive cells on bone demonstrated a peak at day 10 and was reduced at day 14 (Table 3).

PDL–tooth movement.

The number of TRAP-positive cells in the PDL did not differ between the ortho and doxy-ortho groups. However, the number of TRAP-positive cells in the PDL was higher on day 14 in the doxy-ortho group than on days 7 and 10 (Table 3).

No significant difference was detected between the ortho and doxy-ortho groups at any time point considering the distance measured between the first and second molars (Table 1).

Discussion

DC was selected over other tetracyclines for this experiment because it has been shown to be a more potent collagenase inhibitor (Burns *et al.*, 1989; Golub *et al.*, 1990a, 1994). Yanagimura *et al.* (1989) found that DC, minocycline and tetracycline inhibited gingival crevicular fluid collagenase activity by 70, 45 and 23 per cent, respectively. A similar ranking was reported by Gabler and Creamer (1991) concerning the inhibition of human neutrophil function by tetracyclines.

In previous studies, DC has been administered systemically through the drinking water of the experimental animals (Grevstad, 1993; Grevstad and Bøe, 1995). The implantation of mini-osmotic pumps offers a controlled way of continuous drug administration. However, the

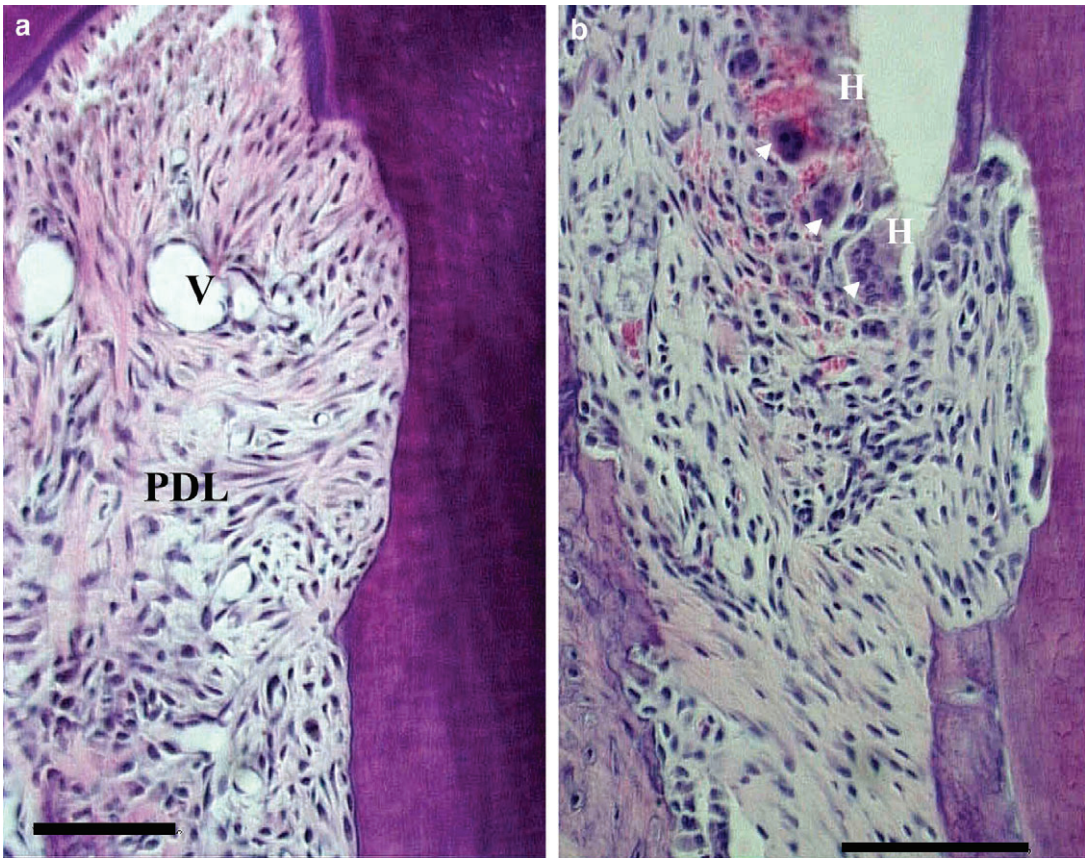

Figure 5 Day 14. (a) Ortho specimen. Extensive loss of root structure. Repair is taking place within a root resorption lacuna (bar = 100 μm). (b) Doxy-ortho specimen. Root resorption lacuna immediately apical to hyalinized tissue. Multinucleated cells (arrows) are actively resorbing hyalinized tissue remnants, while repair has started in the apical part of the adjacent resorption lacuna (bar = 100 μm). H, hyalinized tissue; PDL, periodontal ligament; V, vessel.

pumping rate becomes stable only several hours after implantation. Therefore, pump implantation should, optimally, precede orthodontic appliance insertion by at least 1 day in order to establish a steady DC 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 critically burden the animals. Hence, the results should be interpreted in the context of inadequate serum DC levels during the first experimental day. However, the inhibitory effect of DC on root resorption was still apparent. Taking into consideration that DC administration reduced the normal resorptive activity in the doxy-control animals, it can be hypothesized that early DC administration enhanced the inhibitory effect.

It was particularly interesting to test the effect of low-dose DC administration because of the recent clinical interest in treatment with tetracyclines at a sub-antimicrobial dosage. The suggested DC dosage for therapeutic use in rats is 2.5 mg/kg bodyweight/12 hours *per oss* (5 mg/kg bodyweight/day), which is virtually completely absorbed (Smith and Burgman, 1997). Ramamurthy *et al.* (1998) fed rats 5 mg DC/animal/day. In the present study, the subcutaneously delivered dose was only one-quarter of the suggested dose, yet the inhibitory effect on the resorptive process was distinct, as earlier speculated (Grevstad, 1993).

The distance between the first and second molars was measured as an indicator of possible tooth movement during the experiment. Preferably, a stable structure in the maxilla or cranium should have been chosen as a reference point for the movement of the first molar (Ashcraft *et al*., 1992). The primary aim of the present investigation, however, was the assessment of root resorptive activity and the use of a more sophisticated method for measuring tooth movement was beyond the scope of the study. No significant differences in distance were found between the two groups throughout the experiment, while the absolute volume of the alveolar bone was significantly higher for the experimental animals that received DC. The contradictory result could be explained by inaccuracy in tooth movement estimation or by the increased density of bone in the DC group.

The total observation period chosen for this study was 14 days, as repair processes dominate after that time (Brudvik and Rygh, 1995a, b). While root resorption declined in the ortho group from day 10 to day 14, the doxy-ortho group showed an increasing trend. It can be postulated that DC may delay the removal of necrotic tissue. Even though no

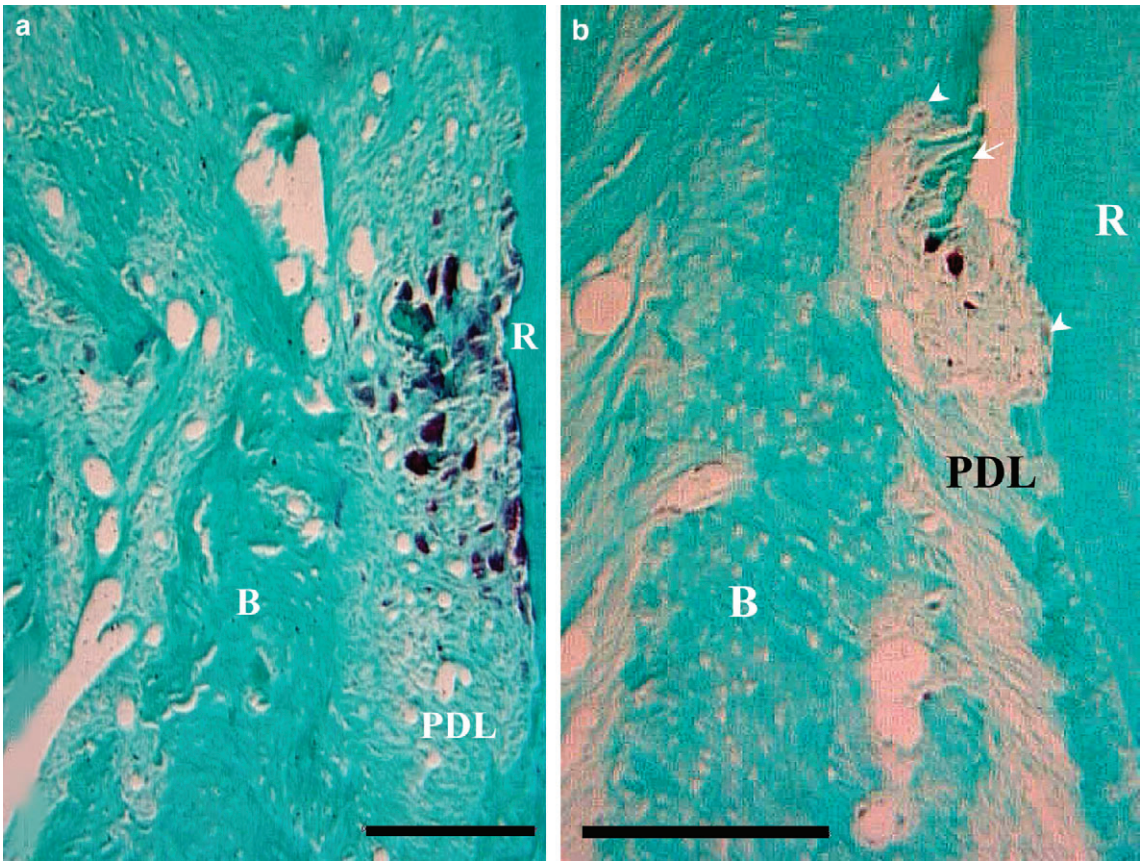

Figure 6 Day 10. (a) Ortho specimen. Intense tartrate-resistant acid phosphatase (TRAP)-positive staining. Several TRAP-positive cells remove necrotic tissue (bar = 100 μm). (b) Doxy-ortho specimen. Rather limited TRAP activity. TRAP-positive cells in the periodontal ligament (PDL) are removing necrotic material (long arrow). The lower intensity staining on the root and bone (short arrows) indicates relatively inactive clast cells (bar = 200 μm). B, inter-radicular bone; R, root.

significant difference in root resorption was detected between the groups, TRAP activity in the PDL seemed to remain rather high in the doxy-ortho group at day 14, indicating that a longer experimental period would have been of interest. However, a marked increase in root resorption would not be expected, as the number of odontoclasts was decreasing at the same rate in the two groups and there was no remaining active force after day 14.

This study demonstrated an inhibitory effect of DC on root resorption and alveolar bone loss. 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; Konttinen *et al.*, 1998). This may partly explain the reduction in resorptive activity in the control animals.

Tetracyclines can also down-regulate the expression of pro-inflammatory and autoimmune mediators, such as cytokine production including interleukin-1 and tumour necrosis factor (Shapira *et al.*, 1996), nitric oxide synthesis (Amin *et al.*, 1996), and phospholipase A_2 and arachidonic acid metabolism (Pruzanski *et al.*, 1992). The role of these mediators in connective tissue breakdown following orthodontic force application has been demonstrated (Yamasaki, 1983; Davidovitch, 1991; Saito *et al.*, 1991; Leiker *et al.*, 1995).

Orthodontic force stimulated odontoclast and osteoclast recruitment to an extent that was not affected by DC treatment on day 7. However, root and bone resorption were less in the doxy-ortho group. That may imply a reduced resorption capacity of the individual clast cell. It has been shown that tetracyclines can affect several parameters of osteoclast function, such as diminishing the secretion of lysosomal enzymes (Rifkin *et al.*, 1992). DC may also cause a significant decrease in the extracellular activities of cathepsin L and TRAP, two of the key osteoclast enzymes. A significant reduction in TRAP activity was shown on both bone and root surfaces on day 10. Osteoclast structure can also be affected by tetracyclines. Minocycline significantly inhibits the ruffled border, which is the active site of resorption. Additionally, podosomes, adhesion structures of osteoclasts, are reduced in tetracycline-treated

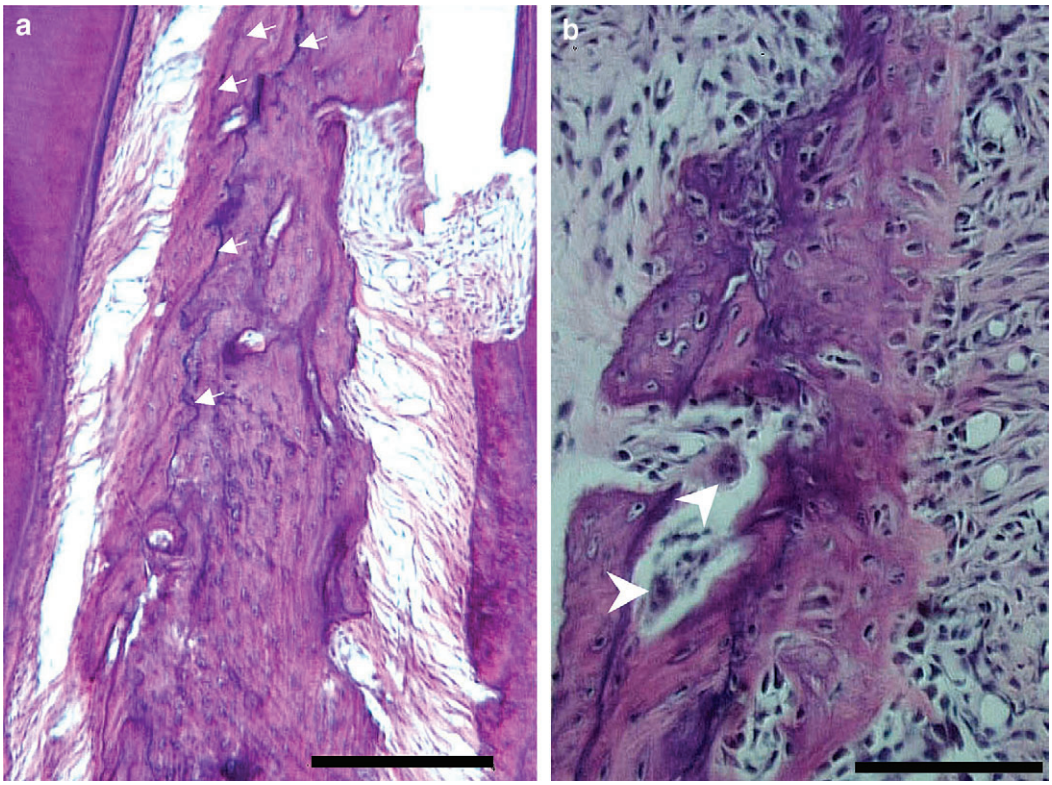

Figure 7 Day 14. (a) Doxy-ortho specimen. The alveolar bone septum seems quite dense in structure without large marrow spaces and maintains its integrity. Demarcation lines are indicated by small arrows (bar = 200 μm). (b) Ortho specimen. Bone resorption lacuna with residing osteoclasts (arrows) (bar = 100 μm).

cells, resulting in osteoclast retraction (Rifkin *et al.*, 1993, 1994, 1996).

The number of clast cells on the root and bone was significantly lower for the doxy-ortho group than the ortho group at days 10 and 14. This may reflect a change in the further recruitment and development of the cells, and/or their premature loss due to apoptosis. In the doxy-control animals, the lower number of osteoclasts can be explained by the induced apoptosis. The induction of apoptosis in mature terminally differentiated osteoclasts by DC can lead to a potent anti-resorptive effect (McGuire *et al.*, 1998; Vernillo and Rifkin, 1998).

A significant reduction in the number of mononucleated cells on the root surface was observed for the DC-treated animals. Such cells have been related to superficial root resorption, which often precedes the development of deeper lacunae (Brudvik and Rygh, 1993b; Mavragani *et al.*, 2004). At day 10, a large part of the root surface was occupied by deep lacunae, which may explain the reduction in the number of mononucleated cells. Tetracyclines affect the function of mononuclear cells such as polymorphonuclear neutrophil leukocytes and macrophages (Forsgren *et al.*, 1974; Martin *et al.*, 1974; Golub *et al.*, 1984, 1990a; Gabler and Creamer, 1991). The contribution of cementoblasts and osteoblasts in the degradation of the unmineralized osteoid as an initial step in root and bone resorption may further constitute a 'target' for inhibitory medication with tetracyclines (Chambers *et al.*, 1985; Ramamurthy *et al.*, 1990; Rifkin *et al.*, 1993; Vernillo *et al.*, 1994).

Although tetracyclines inhibit bone resorption by effects both on osteoclast function and collagenase activity, tetracyclines may also have pro-anabolic effects on bone metabolism, enhancing bone formation (Bjorvatn and Weiss, 1971; Golub *et al.*, 1990b; Williams *et al.*, 1996; Bain *et al.*, 1997). The normalization of bone loss, such as that observed in the present study, has been associated with the restoration of normal osteoblast structure and enhanced collagen synthesis (Sasaki *et al.*, 1991, 1992).

Conclusions

Under the conditions of this experiment, DC demonstrated an inhibitory effect on root resorption and alveolar bone distraction in rats. However, further studies on the effect of low-dose DC administration during orthodontic tooth movement and the mechanisms involved have to be undertaken before the clinical application of this concept is considered.

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Acknowledgements

The technical expertise of Mr Odd Lundberg, Ms Rita Greiner-Simonsen, Ms Anita Koldingsnes, Ms Marit Simonsen, Ms Randi Sundfjord and Ms Siren Østvold is gratefully acknowledged. We wish to express our gratitude to Dr Nils Jørgen Selliseth for methodological advice and Dr Olav Egil Bøe for statistical assistance. This project was partly supported by the Norwegian Dental Depot's Fund.

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