Age-dependent external root resorption during tooth movement in rats

YIJIN REN1, JAAP C. MALTHA2, ROBERT S. B. LIEM3, IETSE STOKROOS3 & ANNE MARIE KUIJPERS-JAGTMAN2

1Department of Orthodontics, University Medical Centre Groningen, University of Groningen, The Netherlands, 2Department of Orthodontics and Oral Biology, Radboud University Nijmegen Medical Centre, The Netherlands and 3Department of Cell Biology, Section Electronmicroscopy, University Medical Centre Groningen, University of Groningen, The Netherlands

Abstract

Objective. To investigate the effect of age on root resorption and distribution along different parts of the root during prolonged light force application. Material and methods. Orthodontic appliances were placed in two groups of 30 rats (one group 6 weeks old, the other 9–12 months old), with contralateral sides as controls. Groups of animals were killed at 1, 2, 4, 8, and 12 weeks. At the study site, incidence was counted as either 1 (with resorption) or 0 (without); severity was measured as the summed length of all resorption lacunae as a percentage of study total root length. Results. Young and adult rats had the same incidence of root resorption in the early phase (<4 weeks), and both increased in the late phase (4–12 weeks) to the same level. Severity of resorption increased with prolonged tooth movement only in adult rats. However, there was no age-related difference in either the early or the late phase. In both groups, the middle part of the root had the highest incidence of resorption; the most severe resorption occurred exclusively at this part. Conclusion. Orthodontic intervention even with light forces increased both the incidence and severity of root resorption, the more so in the middle part of the root. Adult rats had increased incidence and severity with prolonged tooth movement.

Key Words: Age, orthodontics, rats, root resorption, tooth movement

Introduction

External root resorption has been recognized as a frequent iatrogenic consequence of orthodontic treatment [1]. Although it also occurs in permanent teeth under physiological conditions, the incidence is higher among treated individuals [2,3]. The risk factors related to higher susceptibility are generally categorized as patient-related and treatment-related factors, among which age of the patients, force regimen, and treatment duration are closely involved [4,5].

Age has been suggested as a predisposing factor for root resorption [6,7]. Increased age is concomitant with differences in bone density and capacity for bone remodelling [8,9]. A common perception has been that adults may be at greater risk of root resorption [3,10]. However, some studies have demonstrated that orthodontic treatment does not place adults at greater risk for root resorption and that it is the periodontal condition at the start of treatment that merits attention [11–13]. To date, no experimental study has investigated quantitively the impact of age on root resorption during prolonged tooth movement.

In previous clinical studies, the focus has mainly been apical root resorption – this partly attributable to the fact that on radiographs only shortening of root length can be recognized. These studies showed increased incidence of apical resorption in orthodontic patients [6,14]. Histology studies in patients have shown more root resorption in the apical region after tipping tooth movement [15,16]. These findings concur with those of Follin et al. [17], in whose study localization of root resorption appeared to be related to type of movement. Localization might be explained by the differences in stress/strain distribution in the periodontal ligament [1]. Studies on beagle dogs have shown more resorption in the middle and cervical parts of the root after bodily
tooth movement [18,19]. However, quantitative experimental data are lacking on the distribution of root resorption over the root in standardized conditions and on whether this distribution pattern is age-related.

The present study was designed to address the above-mentioned unexplored areas and to overcome a number of the drawbacks of previous experimental studies, i.e. a mixing-up of the incidence and severity of resorption [19], investigation only of the pressure surfaces [18], no discrimination of the pressure and tension regions [19], and application of heavy forces [20]. Our aim was to test the following hypotheses over a 12-week observation period with a light continuous force in rats: orthodontic tooth movement increases the incidence and severity of root resorption; both incidence and severity are higher in adult rats than in young rats and higher in the middle part of the roots than in the apical and cervical parts.

Material and methods

Experimental tooth movement

Two groups of 30 male Wistar rats, aged 6 weeks and 9–12 months, respectively, were used. The animals were acclimatized for 2 weeks before the start of the experiment. They were housed under normal laboratory conditions and fed powdered laboratory rat chow (Sniff, Soest, The Netherlands) and water ad libitum. Ethical permission was obtained from the Radboud University Nijmegen Medical Centre, The Netherlands.

The appliance design has been described in detail in a previous publication [20]. Briefly, a split-mouth design was used with the experimental side chosen randomly, and with the contralateral side as the control. An orthodontic appliance was placed in the experimental side after general anesthesia (Figure 1). At 1, 2, 4, and 8 weeks, 5 or 6 rats from each group were killed, and at 12 weeks also the remaining animals.

Material preparation

The rats were given an overdose of anesthetic prior to being killed. They were then perfused with 4% formaldehyde solution at 37°C. The maxillae were dissected and immersed in the same fixative for 24 h at 4°C. After decalcification in 10% EDTA and paraffin embedding, serial parasagittal 7 μm sections were cut. Every 25th section was collected on SuperFrost/Plus slides (Menzel-Gläser, Braunschweig, Germany) and stained with H&E.

Registration of root resorption

Three roots (two from the 1st molar, i.e. the middle and distal roots, and one from the 2nd molar, i.e. the mesial root) per section and three sections per study side were selected. Total root length (RL), measured as the distance along the long axis of the root from the cemento-enamel junction to the apex, was divided equally into cervical, middle, and apical parts (Figure 2). The experimental and control sides each had mesial and distal regions, which, depending on the direction of tooth movement, were pressure or tension regions; each had three areas (cervical, middle, and apical). Only dentin resorption was taken into consideration. In each region/area, incidence was counted as either 1 (with resorption) or 0 (without resorption); severity was measured as the summed length of all resorption lacunae as a percentage of RL or 1/3 RL (Figure 2). For average severity, the mean of all 9 measurements (3 roots × 3 sections) per region/area was calculated; for maximal severity, the largest measurement was selected as representing the most severe resorption in the region/area per animal. All resorption lacunae were directly registered in the microscope.

Statistics

Medians were calculated for each region/area. First, comparisons across time were performed using the

---

Figure 1. The orthodontic appliance. A transverse hole was drilled through the alveolar bone and maxillary incisors at mid-root level. A preformed ligature wire enclosing all three molars was bonded. A coil spring was attached to a ligature wire through the hole to move the molars mesially with a 10 cN force. At the control side, molars drifted distally, thus the distal and mesial regions were pressure and tension regions. The opposite was the case at the experimental side, where molars moved mesially.
Kruskal-Wallis non-parametric (ANOVA) test followed by the Tukey-Kramer multiple comparison test. Based on our previous study [20], tooth movement was divided into early phase (weeks 1, 2) and late phase (weeks 4, 8, 12). As no time-dependent difference was found within the early and late phase groups, the data were pooled and Mann-Whitney tests were used to compare the two phases. The same tests were used for comparisons between the experimental and control sides, and between young and adult rats. Individual animals were the unit for calculations in all statistics. Differences were considered significant if \( p < 0.05 \). Data from the early and late phases were pooled for a general distribution pattern of the incidence and severity of root resorption per area.

**Results**

At control, side teeth had drifted distally, and thus the distal and mesial could be considered as pressure and tension regions. During orthodontic intervention, teeth had moved mesially; the pressure region was thus at the mesial and the tension region at the distal (Figure 1). No difference was found between weeks 1 and 2 or between weeks 4, 8, and 12 for any of the study parameters in any region or in either age group. The data were therefore pooled for the early and late phases.

**Incidence (Figure 3)**

The two age groups showed similar changes in incidence, which increased from the early to the late phase only in the experimental mesial regions \( (p < 0.01) \). Incidence in these regions in the late phase was higher than in the experimental distal and control mesial regions in both age groups \( (p < 0.01) \). There was no difference between young and adult animals in any region or in either phase of tooth movement (Figure 3a,b). In almost all regions, the middle part of the root had a higher incidence than the apical and cervical parts (Figure 3c).

**Average severity (Figure 4)**

Only in adult rats was there any increase in severity from the early to the late phase in the experimental mesial region \( (p < 0.01) \), where severity was significantly higher than in experimental distal regions in the late phase in both age groups \( (p < 0.01) \). It was also higher than the control counterpart \( (p < 0.05) \) and both phases \( (p < 0.01) \) in young rats, and only in the late phase \( (p < 0.01) \) in adult rats. There was no difference between young and adult animals in any region or in either phase (Figure 4a,b).

**Maximal severity (Figure 5)**

Figure 4 shows the maximal severity of root resorption per region (Figure 5a,b) and per area.
(Figure 5c,d). Similar to the average severity, only in adult rats was there a significant increase from the early to the late phase in the experimental mesial region ($p < 0.01$). Maximal severity in this region was significantly higher than that in experimental distal regions in the late phase in both age groups ($p < 0.01$). It was also higher than the control counterpart in the early ($p < 0.05$) and late ($p < 0.01$) phases in young rats, but only in the late phase ($p < 0.01$) in adult rats. There was no difference between young and adult animals in any region or in either phase (Figure 5a,b).

In experimental mesial regions, the middle part of the root had a higher severity than the cervical part ($p < 0.05$), which counts for both age groups (Figure 4c,d); in the middle part, the level was higher in the mesial than in the distal region ($p < 0.01$). In young rats, the control distal region also showed a higher severity in the middle part ($p < 0.01$); the experimental mesial region had a higher level than the control counterparts in middle ($p < 0.01$) and apical ($p < 0.05$) areas in young rats, and in all areas in adult rats ($p < 0.05$ cervical, $p < 0.01$ middle and apical). There was no difference between young and adult animals in any area. In both age groups, the most severe resorption occurred in the middle part.

**Discussion**

The present study confirms that orthodontic intervention increases both the incidence and severity of root resorption in pressure regions, which is in line with previous studies [22–24]. The results indicate that root resorption is a common phenomenon accompanying tooth movement even with light forces. We acknowledge that root resorption is a three-dimensional phenomenon, and that two-dimensional measurements may underestimate its incidence and severity. The maximal severity measurements were certainly less than or, at most, close...
to the most severe situations. Nevertheless, with a sufficient number of study roots and sections it may provide a good representation of the three-dimensional situation and reliable measurements [25]. Severity indicators appeared non-different using length, relative length, depth and surface area of the resorption lacunae when the same study roots were used [17].

The most important finding of the present study was that prolonged tooth movement increased the incidence of root resorption in both age groups; however, only in adult rats did it increase severity. Limited experimental data are available on age-dependent root resorption to compare with our study. Data on humans show that adults are at no greater risk for root resorption [11–13]. The results of studies on the effects of treatment duration in humans are controversial [15,26–30], which may be explained by the following aspects. First, apical resorption is related to the total distance the apex moves and the time it takes [31], not to the duration of general treatment, which does not reflect the amount of apex displacement. Second, the ages of the study subjects were not considered, which may have been important. The present study showed that a short period of tooth movement is not particularly disadvantageous to adult rats. Prolonged movement, however, significantly increased the severity of resorption in older animals but not in younger animals. Previous studies have suggested that tooth movement and root resorption are related to bone turnover rate and functional state of the periodontium [23,32]; that higher bone turnover increases tooth movement without affecting root resorption; and that decreased bone turnover increases the risk of resorption [19]. This may partly explain the age-related differences in our study, since bone turnover rate decreases with age [33].

Although the severity of resorption increased from early to late phases in adult rats, there was no difference between young and adult animals in either phase. These findings may indeed suggest that both the average and the most severe situation of orthodontically induced root resorption are comparable in young and adult animals. On the other hand, they might be related to the large individual variations which existed in both age groups. Previous studies with standardized set-ups using animals from the same genetic strains have also reported large inter-individual differences in the velocity and amount of tooth movement [21,34]. It is possible that susceptibility to root resorption correlates to the velocity or amount of tooth movement at the individual level. This aspect requires further investigation.

Another important finding is that in both age groups the middle part of the root hosted the highest incidence and severity of root resorption. In a beagle dog with prolonged bodily movement, root resorption appeared exclusively in the middle part [19], which was explained by the sand-glass shaped periodontal space thinnest in the middle part. Another study on beagles [18] for a similar observation period showed that in bodily movement root resorption was also predominantly in the middle and cervical parts. Together with the results of the present study this indicates that at the microscopic level the middle part of the root is most vulnerable for root resorption. Clinical studies, limited in their measurement methods, cannot detect resorption at the cervical or middle part, and may therefore come...
to a biased conclusion. On the other hand, one has to bear in mind that histological and clinical observations are at different levels, and comparisons need to be made with caution.

To our knowledge, this is the first study describing the age effects on root resorption as well as its distribution along different parts of the root. The results confirm that orthodontic experimental intervention even with light forces increases both the incidence and severity of root resorption, which were highest along the middle part of the root. Adult rats had an increased incidence and severity with prolonged tooth movement, but neither was different from the youngsters.

References