Effect of duration of force application on blood vessels in young and adult rats

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Introduction: Age effects on orthodontically induced periodontal vascular reactions have not been studied. The aim of the present study was to test the hypothesis that prolonged tooth movement induces age-related increases in periodontal vascularity. Methods: A standardized orthodontic appliance was placed in 2 groups of 30 rats aged 6 weeks and 9 to 12 months. At 1, 2, 4, 8, and 12 weeks, animals were killed. Blood vessels (BV) were identified based on their morphology and by immunohistochemical staining for alpha-smooth muscle actin. At each study region, surface areas (SA) of the periodontal ligament space and each BV were measured; BV mean SA, BV relative SA (the summed BV SA as a percentage of the periodontal ligament SA), and BV numbers were calculated. Results: Pressure and tension regions showed similar vascular changes. Young rats had lower BV relative SA and BV mean SA in the early phase of force application (<4 weeks); this increased in the late phase, reaching the same level as adult rats. In the late phase (4-12 weeks), young rats had increases of both small- and large-sized BV that did not affect the BV mean SA; adult rats had an increase of small-sized BV only; this resulted in decreased BV mean SA. Conclusions: The hypothesis was confirmed that prolonged tooth movement increases periodontal vascularity, which is age related. These results suggest that clinicians should consider age-related difference in tissue reactions during orthodontic tooth movement. (Am J Orthod Dentofacial Orthop 2008;133:752-7)

The blood vessels (BV) in the periodontal ligament (PDL) are involved in the regulation of tissue remodeling during orthodontic intervention. In areas of tension, PDL vascularity increases; subsequently, osteoclast precursors migrate from the PDL capillaries and produce various signaling molecules involved in force-induced tissue remodeling.1-3 In areas of compression, the PDL shows leakage of blood constituents into the extravascular space, gradual obliteration of the BV, and breakdown of the walls of veins.4-6 Regarding the timing of vascular reactions, packing of erythrocytes in dilated blood vessels occurred within 30 minutes, fragmentation of erythrocytes after 2 to 3 hours, and disintegration of BV walls and extravasation of their contents after 1 to 7 days.1 Studies longer than 1 week showed increased vascular activity,7 sprouting of microvessels,8,9 and increased BV density10,11 at both regions. Observations on vascular changes during tooth movement longer than 2 to 3 weeks are lacking. The PDL BV showed dynamic changes in response to force regimen11: the vascular density at both tension and pressure areas increased after force application, decreased on force removal, and increased again during distal movement of the molars (distal drift is a normal physiologic phenomenon in rats).9 This responsiveness of the vasculature to changes in tissue strain caused us to look into vascular reactions during prolonged force administration after initial adaptation of the periodontal microenvironment. Moreover, our previous studies showed an age-related pattern in both the rate of tooth movement and osteoclast recruitment over time.12,13 However, age-related vasculature reactions have not been quantified.

Therefore, the aim of this study was two-fold: (1) to evaluate changes in vasculature with short- and long-term orthodontic force application, and (2) to analyze the change in the nature of blood vessels in young and adult rats with orthodontic force application. Because the recruitment of osteoclasts depends on vasculature, and prolonged tooth movement requires continuous...
recruitment of osteoclasts, we hypothesized that prolonged force application induces long-lasting increased periodontal vascularity that is age related.

MATERIAL AND METHODS

Two groups of 30 male Wistar rats aged 6 weeks (150-250 g) and 9 to 12 months (400-550 g) were used.

The animals were acclimatized for 2 weeks before the experiment. They were housed under normal laboratory conditions and fed powdered rat chow (Sniff, Soest, The Netherlands) and water ad libitum. Ethical permission was obtained from Radboud University Nijmegen Medical Centre, The Netherlands.

A split-mouth design was used with the experimen-
tal side randomly chosen and the contralateral side as the control. An orthodontic appliance was placed only on the experimental side after general anesthesia with an intraperitoneal injection containing fentanyl citrate (0.079 mg per milliliter), fluanisone (2.5 mg per milliliter), and midazolam (2.5 mg per milliliter) (Janssen, Beerse, Belgium) in a dosage of 2.7 mL per kilogram of body weight. The appliance was described extensively elsewhere.\textsuperscript{15} Briefly, a transverse hole was drilled through the alveolar bone and the maxillary incisors at the midroot level. A preformed ligature wire enclosing all 3 molars was bonded on the experimental side. A coil spring was attached to a ligature wire through the hole to move the molars mesially with a force of 10\textsuperscript{2}cN.\textsuperscript{12} At 1, 2, 4, and 8 weeks, 5 or 6 rats from each group were killed, and, at 12 weeks, the remaining animals were killed.

The rats received an overdose of anesthetic before they were killed. They were then perfused with 4\% formaldehyde solution at 37°C. The maxillae were dissected and immersed in the same fixative for 24 hours at 4°C. After decalcification in 10\% EDTA and paraffin embedding, serial parasagittal 7-\mu m sections including all 3 molars were cut. Every 25th section was collected on slides and stained with hematoxylin and eosin.

BVs were identified by their morphology and by immunohistochemical staining for alpha-smooth muscle actin. Before staining, the sections were deparaffinated and rehydrated. Sections were preincubated with 0.1\% trypsin in Tris/HCL buffer for 10 minutes at 37°C for antigen retrieval. They were pretreated with 3\% hydrogen peroxide followed by incubation with 5\% PBSA. Subsequently, the sections were first incubated with mouse \(\alpha\)-SMA monoclonal antibody (R&D, Minneapolis, Minn) overnight and then with biotin-SP-conjugated affnipure donkey anti-mouse IgG (Jackson, Westgrove, Pa) for 45 minutes. The sections were then treated with ABC-peroxidase (Vector, Burlingame, Pa). The staining was enhanced by incubating the samples with 0.5\% copper sulphate in a 0.9\% sodium chloride solution. Immunohistochemical controls included replacement of the primary antibody with PBS.

Three roots per section and 3 sections near the largest longitudinal root surfaces were selected to represent each animal. The sections were digitized, and the borders of the BV and the PDL were highlighted and measured (Quantimet 520, Cambridge, England). Twenty sections were randomly chosen from both age groups. The same observer (Y.R.) made the measurements twice on these 20 sections with a 3-week interval. Intraobserver agreement was tested by kappa statistics (\(\kappa = 0.91\)). The upper border of the PDL space was defined as the cementodentin junction. At each region, surface areas (SA) of the PDL space and each BV were measured, and BV mean SA (the average SA of all BV), BV relative SA (the summed BV SA as a percentage of the PDL SA), and BV numbers (the counts of all BV; small-sized BV: SA \(<500\ \mu \text{m}^2\), and large-sized BV: SA \(\geq 500\ \mu \text{m}^2\)) were calculated (Fig).

### Statistical analysis

For each region, the mean of the 9 measurements (3 roots \(\times\) 3 sections) of each variable was calculated, representing 1 animal. Medians were calculated for each region with the number of animals as units. Comparisons across time were done with the Kruskal-Wallis nonparametric (ANOVA) test. Because there were no differences in the early (weeks 1 and 2) and the late (weeks 4, 8, and 12) groups, tooth movement was divided into early (<4 weeks) and late phases (4-12 weeks),\textsuperscript{12} and Mann-Whitney tests were used to compare early and late phases, young and adult groups, and experimental and controls sides. Differences were considered significant if \(P < 0.05\).

### RESULTS

For all 3 variables, the pressure and tension regions showed similar vascular changes with time in both age groups. The changes described below refer to both regions unless specified.

### Table II. BV mean SA during tooth movement (medians and ranges)

<table>
<thead>
<tr>
<th>BV mean SA ((\mu \text{m}^2))</th>
<th>Early phase</th>
<th>Late phase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pressure</td>
<td>Tension</td>
</tr>
<tr>
<td>Young</td>
<td>451\textsuperscript{†} (341-1096)</td>
<td>670\textsuperscript{†} (314-1500)</td>
</tr>
<tr>
<td>Adult</td>
<td>459 (231-724)</td>
<td>514 (207-1198)</td>
</tr>
</tbody>
</table>
In the BV relative SA (Table I), no time-related differences existed in the control samples of either group. In young rats, the BV relative SA was lower than in adults in both early and late phases \((P < 0.05)\). At the experimental sides, it increased from early to late phases \((P < 0.05)\) only in the young rats; a difference between the 2 age groups existed only in the early phase \((P < 0.05)\). Differences between the experimental and control sides existed only in late phase at pressure regions in young rats and at both regions in adult rats \((P < 0.05)\).

In the BV mean SA (Table II), at the control sides, no difference existed between the 2 phases in either age group. The BV mean SA was lower in youngsters than in adults in both early and late phases \((P < 0.05)\). At the experimental sides, it increased from early to late phases \((P < 0.05)\) only in the young rats; a difference between the 2 age groups existed only in the early phase \((P < 0.05)\). Differences between the experimental and control sides existed only in late phase at pressure regions in young rats and at both regions in adult rats \((P < 0.05)\).

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In the BV mean SA (Table II), at the control sides, no difference existed between the 2 phases in either age group. The BV mean SA was lower in youngsters than in adults in both phases \((P < 0.05)\). At the experimental sides, the BV mean SA in the early phase was higher in adults than in youngsters \((P < 0.05)\), and it decreased in the late phase \((P < 0.05)\) only in the adults. A significant difference between experimental and control sides existed in the late phase only in the adult group \((P < 0.01)\).

Neither tooth movement-related nor age-related changes of the BV numbers existed at control sides (Table III). At the experimental sides, the total number increased from early to late phases at the pressure region in both groups \((P < 0.05)\). The BV numbers in the late phase were higher than those at the control sides \((P < 0.05)\). The numbers of small-sized BV showed the same changes as the overall BV numbers. The numbers of large-sized BV increased from early to late phases only in young rats \((P < 0.05)\). In the early phase, it was lower in youngsters than in adults \((P < 0.05)\), and in late phase it was higher than the control side only at the pressure region in young rats \((P < 0.05)\).

**DISCUSSION**

Orthodontically induced periodontal vascular reactions, previously reported, focused mainly on the early phase.\(^6\,7\,15\) Moreover, age effects on vascular changes during tooth movement have never been investigated. Our study provides a quantitative representation of the PDL vascular reactions in rats and confirms the hypothesis that prolonged force application in rats resulted in increased vascularity, which is age related. We acknowledge that the variables in our study are affected by the orientations of periodontal vasculature and histologic sections. However, this is a point for discussion at all regions, and it might be considered random error equally distributed in all samples.

At the physiologic state, the general vascular relative SA and average BV size were larger in adult rats,
but the BV numbers did not differ with age. Prolonged force application induced a significant increase of the BV relative SA only in youngsters, reaching the same level as in adults, and induced a significant decrease of the BV mean SA only in adult rats. The differences could be explained by the BV numbers, which showed apparent age-related and size-related alterations: youngsters had increases in both small and large BV, and adults had an increase only in small BV. Therefore, in youngsters, the BV relative SA increased without affecting the mean SA. In adult rats, it seems contradictory that increased BV did not increase its relative SA; it was even decreased compared with the controls. This might be explained by increased BV permeability. The BV of the rat PDL is characterized by many fenestrations. Mechanical loading induces increased numbers and sizes of these fenestrae, and BV respond with increased permeability, which enhances extravasation of fluid into the interstitial tissue. The leakage might have reduced the relative BV SA in adult rats.

Previous studies showed that, at the start of force application, the periodontal vasculature at the pressure and tension regions reacted differently, and, in the first weeks, the trend of vascular changes was similar under pressure and tension. This study demonstrated that this similarity exists also in the late phase. Prolonged force altered PDL vascularity for both tissue deposition and resorption; this gives rise to an interesting question: why did bone resorption occur and continue at 1 side and bone formation at the other? Mechanically, the first reaction to orthodontic force application is alteration in the strain-stress distribution in the periodontium; this triggers the reaction of the PDL cells. Strain is postulated as the major biomechanical factor influencing cell behavior. It is plausible that vascular reactions at the onset of force application alter local stress and strain in the PDL. This triggers differential activations of osteoblasts and osteoclasts to initiate bone resorption and apposition, respectively. Once tooth movement starts, vasculature remodeling is not dictated by stress or strain in the PDL.

CONCLUSIONS

Prolonged tooth movement induces increases in periodontal vascularity that is age related. These results suggest that clinicians should consider the age-related differences in tissue reactions during orthodontic tooth movement. These results might also suggest that the vasculature changes in the periodontium can influence the time-related biomechanical properties of the PDL. This should be taken into account in future finite element modeling studies.

REFERENCES


