Influences of aging changes in proliferative rate of PDL cells during experimental tooth movement in rats

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The number of adult patients in most clinical orthodontic practices has increased in recent years. Because orthopedic jaw control through growth is impossible in adult patients and periodontal disease is more likely,[1] orthodontic tooth movement is more complex in adults than in adolescents. In particular, adults who have periodontal problems risk permanent damage to the periodontal tissues.[2] Thus, it is important to understand the differences in artificial tooth movement in young and adult subjects from clinical and histologic aspects.

Periodontal tissue, the periodontal ligament (PDL) in particular, plays a significant role in bone remodeling at the PDL-alveolar bone interface during tooth movement. Proliferative activity of fibroblast-like cells in the PDL decreases with age,[3] and faster or more efficient tooth movement can be achieved in younger individuals, both human[4] and experimental animals.[5] A hypothesis may be derived from these findings: The proliferative activity of PDL cells with aging influences experimental tooth movement. The validity of this hypothesis, however, has not yet been confirmed in biochemical or histochemical studies.

Various techniques have been developed for evaluating proliferative activity of the PDL cells. Autoradiography with tritium thymidine has been employed for this purpose in previous studies.[6,7] Meanwhile, an immunohistochemical technique with a specific antibody has proven more convenient and effective for the above-mentioned purpose than autoradiography.[8,9] The pur-

Abstract

This study was designed to investigate the influences of aging changes in the proliferative activity of PDL cells during experimental tooth movement in rats. Young (6-week-old) and adult (14-week-old) Wistar strain rats were used as experimental animals. Light (10g) or heavy forces (40g) were applied to the maxillary first molars for periods of 1, 3, 7, or 14 days. Proliferative activity of the PDL cells was evaluated immunohistochemically in terms of the ratio of the number of labeled cells to the total number of PDL cells (labeling index) or the number of labeled cells. In the controls, cellular activity was significantly greater in the young than in the adult group ($P<0.05$). Significant differences in the proliferative activity between young and adult groups were found in the tension and pressure areas during early stage of tooth movement ($P<0.05$), which indicated a delay of biologic responses to orthodontic stimuli in adult rats. It is shown that aging changes substantially influence proliferative activity of the PDL cells and subsequent tooth movement during the initial phase in particular.

Key Words

Aging • Tooth movement • Periodontal ligament • Bromodeoxyuridine • Immunohistochemistry

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Figure 1
Schematic representation of appliance for experimental tooth movement.
A: Orthodontic band; B: Main archwire; C: Cantilever spring with or without helix.

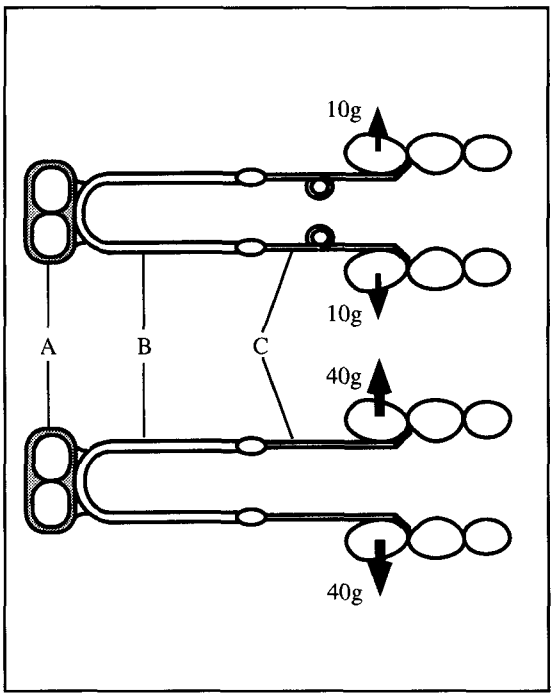

Figure 1

Figure 2
Pressure and tension areas in the PDL where cell proliferative activity was evaluated.

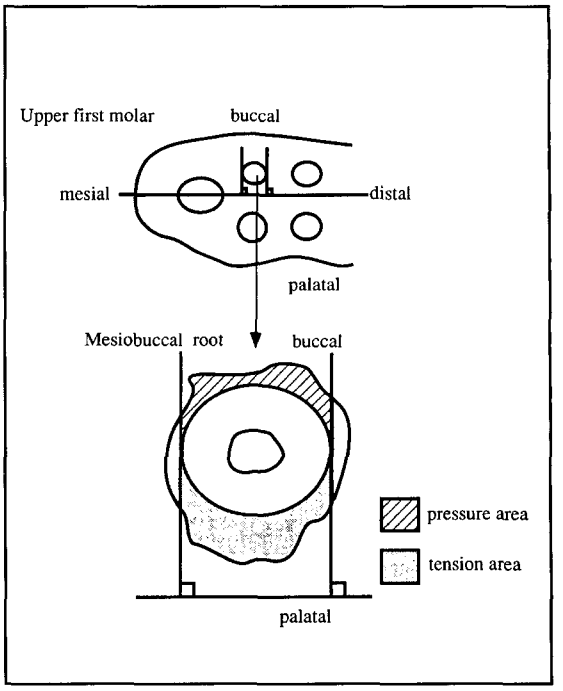

Figure 2

pose of this study was to examine this hypothesis by studying the biologic responses of the PDL cells to orthodontic stimuli in young and adult rats in terms of the proliferative rate with the immunohistochemical method.

Materials and methods

Thirty-six 6-week-old (young) and thirty-six 14-week-old (adult) Wistar strain male rats were used as experimental animals; four animals served as controls for each group. The experimental animals were further divided into subgroups according to the magnitude of force (light, 10 g; heavy, 40 g) applied to the maxillary first molars in the buccal direction. Hereafter, these groups are referred to as YL, YH, AL, and AH groups. All the animals were fed a standard laboratory diet consisting of pellets and water ad libitum. Body weight was measured daily during the experiment.

Experimental tooth movement

Experimental tooth movement was carried out using a fixed appliance (Figure 1) that was a modification of Reitan and Kvam's.[10] The appliance consisted of orthodontic bands on the maxillary incisors, a 0.7 mm diameter main archwire, and a 0.010 inch diameter cantilever spring with or without a helix, which was demonstrated in advance to exert an initial force of 10 g or 40 g, respectively. The duration of tooth movement was 1, 3, 7, and 14 days. The amount of tooth movement was determined as the difference in the distances between the mesial occlusal pits of the maxillary first molars before and after tooth movement and was measured using digital calipers (Mitsutoyo Co, Osaka, Japan).

Preparation of tissue sections for histologic observation

At the end of each experimental period, a labeling reagent with a concentrated aqueous solution of 5-bromo-2'-deoxyuridine (BrdU, 3.0 mg/ml, Amersham International plc, Buckingham Share, England) was injected intraperitoneally with a dose of 0.5 ml per 100 g body weight. One hour after injection, the maxillae were dissected under general anesthesia with pentobarbital, then fixed in 10% neutral buffered formalin for 24 hours. After fixation, the samples were decalcified in 14% EDTA for 2 weeks, dehydrated in ethanol, then embedded in paraffin. The embedded specimens were cut into horizontal sections 4.5 μm in thickness. Six sections separated from each other by a minimum distance of 9 μm, taken from the anatomic area ranging from the interradicular crest to one-fifth the root length to the apex. The sections were stained immunohistochemically and observed microscopically.

Immunohistochemical method

A cell proliferation kit (Amersham International plc, Buckingham Share, England) was used for immunohistochemical staining. After deparaffinization, anti-BrdU monoclonal antibody (primary antibody) was added to cover the specimen. The specimens were incubated for 90 minutes in a humidified box at room tempera-

ture, then treated with a secondary antibody, peroxidase anti-mouse IgG2a, and incubated for another 45 minutes. To detect the incorporated BrdU, the specimens were incubated with diaminobenzidine (DAB) (Vector Laboratories, Calif, USA) for 10 minutes. Between each step, the specimens were washed with phosphate buffered saline (PBS) (pH = 7.2).

After counterstaining with hematoxylin, photomicrographs of the periodontium around the root were used for the following evaluations.

Evaluations of proliferative rate of PDL cells

The proliferative rate of PDL cells was examined around the mesiobuccal root. The number of PDL cells, including fibroblast-like cells as well as miscellaneous cells with brown labeled nuclei by DAB, was counted for the palatal (tension) and buccal (pressure) areas on the photomicrographs (Figure 2).

For both areas in the control group and the tension area in the experimental group, the labeling index (percentage of the labeled cells) was determined by dividing the number of labeled cells by the total number of PDL cells. For the pressure area in the experimental group, the number of labeled cells observed in each section was used for the evaluation because the PDL cells numbered fewer than the 2,000 that are necessary for the above-mentioned evaluation with the labeling index.[11]

Statistical treatment

Means and standard deviations were calculated for each group. For values in the control group without tooth movement, Student's *t*-test was performed to examine the differences between young and adult groups. For the experimental groups, analysis of variance (ANOVA) was first performed to examine differences in variance among groups with different ages and force magnitudes at each experimental period. Pairwise comparisons (Scheffe) were then performed between groups when significant differences in variance were found ($P<0.05$). These calculations were performed with a statistical program, Stat View 4.11J, (Abacus Concepts Inc, Calif) on a Macintosh Quadra 650 computer (Apple Computer Corp, Cupertino, Calif). These methods are appropriate for comparing small groups.

Results

In general, young animals presented more substantial increases in body weight than adults (Figure 3). Body weight in the young experimental rats increased less than in the controls until 3 days after tooth movement, and then the rates of increase became similar. On the other hand,

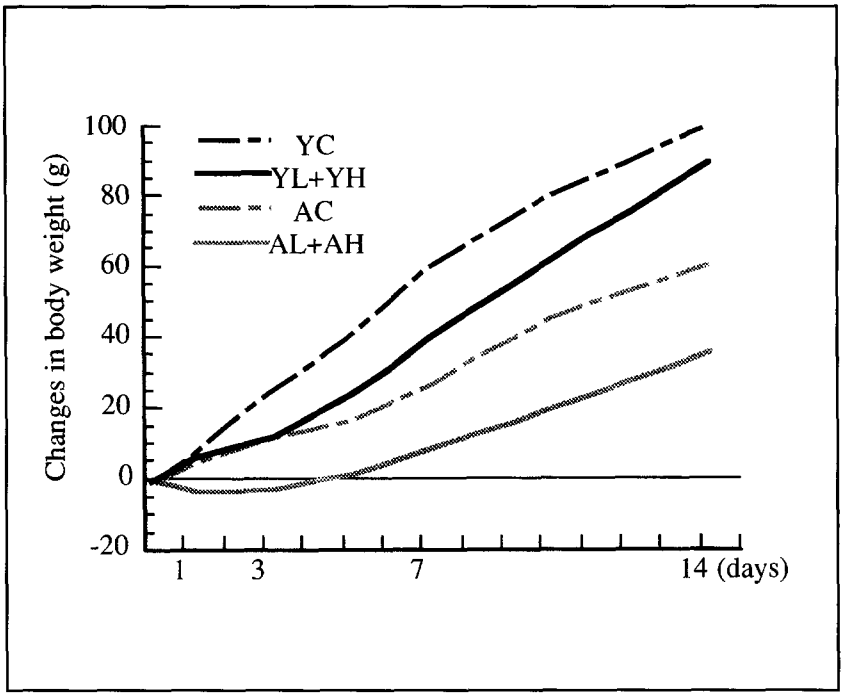

Figure 3

Figure 3
Changes in body weight during experiment. No significant differences were found between the experimental and control groups.

body weight in the adults decreased immediately after the initiation of tooth movement, and after 3 days increased at a rate similar to that of the controls. During the experimental period, no significant differences in body weight were found between control and experimental groups in either young or adult groups.

The amount of tooth movement was significantly greater in the young groups at 3, 7, and 14 days after the initiation of tooth movement. On the other hand, no significant differences were found between the light and heavy force groups (Table 1).

With respect to proliferative activity of the PDL cells, the labeling index was significantly greater in the young control animals than in the adults in both the palatal and buccal areas (Table 2), indicating a significant difference in the proliferative activity of PDL cells.

In the tension area, labeling indices were highest in all the experimental groups 1 day after the initiation of tooth movement, and significantly greater in the young group than in the adults at the 5% level of confidence (Table 3). At 3 days, labeling indices exhibited sudden decreases in all the experimental groups, and they continued to decrease 7 and 14 days after the initiation of tooth movement, indicating decreases in the proliferative activity of the PDL cells. On the other hand, no significant differences were found between the heavy and light force groups during the entire experimental period (Table 3).

In the pressure area, the number of PDL cells

Table 1
Amount of tooth movement during experimental period (n = 4)

Day	Experimental group				Differences between groups	
	Young Light (YL)	Adult Light (AL)	Young Heavy (YH)	Adult Heavy (AH)	YL,YH vs AL,AH	YL,AL vs YH,AH
1	0.42 ± 0.14	0.29 ± 0.08	0.51 ± 0.17	0.36 ± 0.15	NS	NS
3	0.68 ± 0.07	0.29 ± 0.12	0.76 ± 0.13	0.40 ± 0.10	*	NS
7	0.72 ± 0.22	0.35 ± 0.18	0.73 ± 0.16	0.42 ± 0.19	*	NS
14	0.98 ± 0.23	0.60 ± 0.12	1.21 ± 0.17	0.72 ± 0.27 (Unit : mm)	*	NS

NS: Not significantly different
*: Significantly different at 5 % level of confidence

Table 2
Mean and standard deviation of labeling index in the control groups (n = 4)

Location in the PDL	Young control group	Adult control group	Differences between groups
Palatal	1.50 ± 0.42	0.80 ± 0.23	*
Buccal	1.72 ± 0.16	0.77 ± 0.12 (Unit : %)	*

*: Significantly different at 5 % level of confidence

reduced 1 day after the initiation of tooth movement, and few labeled cells were found in the experimental groups (Table 4). After 3 days, a significantly greater number of labeled cells was observed in the YL group than in the YH or AL groups, demonstrating that age and force magnitude are essential factors for initial cellular activity under orthodontic stimuli. A substantial increase in proliferative activity was observed at 7 days, and a significant difference was found between the young and adult groups at the 5% level of confidence. After 14 days, however, no significant differences were found among the experimental groups.

Discussion

Changes in cell proliferative activity are considered pertinent to biologic phenomena associated with aging. Proliferative activities in the periodontal tissue generally decrease with aging.[3,12,13] In this study, the labeling index of PDL cells in the adult control group was almost half that of the young control group with a significant difference. These findings indicate that the proliferation of PDL cells is essentially affected by aging under physiologic conditions free of external stimuli to the periodontium. Meanwhile, little information is available in the literature about the association between changes in the proliferative activity of PDL cells that occur with aging and the nature of experimental tooth movement. Thus, the present study was designed to demonstrate this association or the relevant hypothesis by means of an immunohistochemical technique.

The immunohistochemical method with specific antibody for BrdU has been recognized as effective for the detection of replicating cells and is an alternative to autoradiography with tritium thymidine.[8,9] The measurement of cell activity in this study can be performed more easily and quickly than with autoradiography.

The amount of tooth movement was greater in the young groups than in adults during the experimental period. These findings seem to depend on differences in bone remodeling processes or tissue reactions to orthodontic stimuli between young and adult rats, as well as differences in bone density demonstrated in a previous study.[5] From these considerations, differences in cellular activity may be regarded as an essentially important factor for explaining the different rates of tooth movement.

With respect to cellular activity mediated by external mechanical stimuli, a marked increase in the proliferative rate of PDL cells was elucidated in the early stage of the tension area, which was similar to a previous finding.[6] The proliferative cells observed in this period are regarded as fibroblast-like cells including osteogenic cells.[7] The present study confirmed significant differ-

Table 3
Mean and standard deviation of labeling index in palatal (tension) side of the PDL space of experimental group (n = 4)

Day	Experimental group				Differences between groups	
	Young Light (YL)	Adult Light (AL)	Young Heavy (YH)	Adult Heavy (AH)	YL, YH vs AL,AH	YL, AL vs YH, AH
1	7.53 ± 0.80	5.22 ± 0.80	7.25 ± 1.57	5.68 ± 1.75	*	NS
3	1.78 ± 0.43	2.92 ± 1.17	2.61 ± 2.32	2.48 ± 0.84	NS	NS
7	1.11 ± 0.49	0.93 ± 0.35	1.41 ± 0.67	1.23 ± 0.53	NS	NS
14	1.00 ± 0.27	0.83 ± 0.45	1.21 ± 0.63	0.74 ± 0.20 (Unit : %)	NS	NS

NS: Not significantly different
* : Significantly different at 5 % level of confidence

Table 4
Mean and standard deviation of the number of labeled cells in buccal (pressure) side of the PDL space of experimental group (n = 4)

Day	Experimental group				Differences between groups	
	Young Light (YL)	Adult Light (AL)	Young Heavy (YH)	Adult Heavy (AH)	YL, YH vs AL,AH	YL, AL vs YH, AH
1	1.22 ± 1.07	1.43 ± 0.35	1.53 ± 1.39	0.80 ± 0.64	NS	NS
3	6.35 ± 1.86	2.09 ± 1.30	2.63 ± 2.49	1.31 ± 0.76	*	*
7	6.96 ± 0.47	5.26 ± 0.45	7.27 ± 2.71	4.26 ± 0.63	*	NS
14	4.02 ± 0.92	6.25 ± 1.82	5.58 ± 2.81	5.14 ± 0.50	NS	NS

NS: Not significantly different
*: Significantly different at 5 % level of confidence

ences in proliferative activity of these PDL cells between young and adult groups during the initial phase, suggesting that aging changes particularly affect initial cellular reaction in the periodontium subjected to external stimuli.

On the pressure side of the PDL, 3 days after the initiation of tooth movement, active bone resorption associated with osteoclasts was observed only in the YL group. A significantly higher rate of cell proliferation with labeled mononuclear cells adjacent to degenerative tissue or bone surface was also induced in the YL-group than in the remaining groups. Thus, quick cellular reaction was induced only in the YL group without excessive tissue damage, although such reactions were rarely found in adult rats. Tonna et al.[14] also reported that increases in cellular proliferative activities of injured periodontal tissues were delayed according to the degree of aging. From these findings, it is strongly emphasized that aging changes in the periodontium influence cellular activities and reactions in the PDL during the healing process, which are similar to changes on the pressure side of the PDL during tooth movement.

The present results suggest that appropriate and efficient tooth movement in adult patients can be achieved by applying low levels of force with appropriate intervals of reactivation, enough for delayed remodeling of the periodontal tissue.

Conclusions

The present study was conducted to investigate the influences of aging changes in proliferative activity of the PDL cells on experimental tooth movement in rats. The following results were obtained.

1. The amount of tooth movement was significantly greater in young animals than in adults 3, 7, and 14 days after the initiation of tooth movement.
2. Proliferative activity of the PDL cells was

significantly greater in young controls than in adults.

3. Significant differences in proliferative activity of the PDL cells were found between young and adult groups during the initial phase of tooth movement.

From these results, it is shown that aging changes substantially affect proliferative activity of the PDL cells during the initial reaction to orthodontic stimuli and subsequent tooth movement.

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