Mechanical Properties of the Periodontal Ligament in the Incisor Teeth of Rats from 6 to 24 Months of Age

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Mechanical properties of the periodontal ligament of the incisor have been examined in sections of the mandibles of rats from 6 to 24 months of age. Mechanical measures estimated from the load-deformation and stress-strain curves did not show age-related changes, although the body and mandible weights increased gradually during the experimental period. It is suggested that qualitative and quantitative changes in the supporting tissues/unit area of the ligament are minor at a restricted region examined.

The ultimate load required to extract the rat incisor from its socket increased gradually with age from 4 to 24 weeks [Chiba et al., 1980]. A similar tendency was also estimated from the load-deformation curves when the tooth was pushed out of its surrounding alveolar bone in sections of the mandibles of rats from 3 to 24 weeks of age [Yamane, 1990]. However, age-related changes were not appreciable in all mechanical measures estimated from the stress-strain curves in the latter experiment.

It has been suggested that increases in rat periodontal mechanical strength with age are related to those in the size of teeth and/or that area of the periodontal ligament facing cementum [Chiba et al., 1980; Yamane, 1990]. Furthermore, the qualitative and quantitative changes per unit area of the ligament are minor from 3 to 24 weeks of age [Yamane, 1990].

Whether or not marked changes in the mechanical properties of the periodontal ligament occur in rats older than 24 weeks of age has not yet been studied. Therefore, the effect of age on the mechanical properties of the periodontal ligament was examined by analyzing load-deformation and stress-strain curves obtained from the transverse section of the mandibular incisor of rats from 6 to 24 months of age.

MATERIALS AND METHODS

Thirty-one male rats of the Wistar strain were used. They were fed a pellet diet (CE-2, Clea Japan, Tokyo, Japan) with water available ad libitum during the experimental period. Groups of 10–11 rats were killed by decapitation under ether anaesthesia at 6, 12 and 24 months of age. Immediately after death, the left mandibles were dissected and adherent soft tissues removed. A transverse section of the mandible was cut through an axis near the mesial side of the mandibular first molar [Komatsu, 1988]. Experimental techniques and procedures were basically the same as those described in the previous experiment by Yamane [1990].

Mechanical properties of the periodontal ligament were examined by a method described by Komatsu [1988]. The load and deformation were recorded on chart paper by a pen-recorder at 50 mm/min. The extension rate of a tensiometer was 5 mm/min. The direction of loading was intrusive.

Radiographs of the transverse sections of the mandible were processed in an image analyser (Luzex 500, Nihon Regulator, Tokyo, Japan); the perimeters of the cementum and socket wall between the enamel/cementum junctions on both sides of the tooth and the sectional area of the lingual periodontal ligament were measured [Komatsu, 1988]. The area of the periodontal ligament facing cementum and the average width of the periodontal ligament were also calculated [Komatsu, 1988].

The load was plotted against the deformation from the original pen-recorded traces. Maximum shear load, maximum deformation, elastic stiffness and failure energy in shear were estimated from the load-deformation curve [Haut, 1986; Komatsu, 1988; Mandel et al., 1986]. Then the load-deformation curve was transformed into a stress-strain curve [Komatsu, 1988]. Maximum shear stress, maximum strain, elastic stiffness and failure strain energy density were estimated from the stress-strain curve.

The differences of the mean values were examined by the t-test. The differences among the age groups were examined by the analysis of variance.

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RESULTS

General Observations

Figure 1 shows the changes in the body (Figure 1a) and mandible weights (Figure 1b) during the experimental period. The average body weight increased with age from 6 to 24 months of age. The differences were significant between 6 and 12 months ($p < 0.001$) and between 12 and 24 months ($p < 0.001$). The average body weight at 24 months was 1.60 times greater than that at 6 months. The mandible weight also increased with age from 6 to 24 months. The differences were significant between 6 and 12 months ($p < 0.001$) and between 12 and 24 months ($p < 0.001$). The average mandible weight at 24 months was 1.39 times greater than that at 6 months. Figure 2 shows photographs of rat mandibles at 6, 12 and 24 months of age. Increases in the size of incisors and mandibles with age were appreciable from 6 to 24 months.

Radiographic Observations

The average perimeters of the cementum and socket wall increased with age from 6 to 24 months (Table 1). The differences of the perimeter of the cementum were significant between 6 and 12 months ($p < 0.001$) and between 12 and 24 months ($p < 0.001$). The difference of the perimeter of the socket wall was significant between 6 and 12 months ($p < 0.001$) but not between 12 and 24 months. The perimeter of the cementum in sections from 24-month-old animals was 1.18 times greater than that from 6-month-old animals; and that of the socket wall, 1.13 times greater. The average sectional area of the periodontal ligament and the area of the periodontal ligament facing cementum also increased with age from 6 to 24 months (Table 2). The sectional area of the periodontal ligament at 24 months was 1.18 times greater (but not significant) than that at 6 months. The area of the periodontal ligament facing cementum at 24 months was 1.26 times greater ($p < 0.001$) than that at 6 months; the difference was also significant between 12 and 24 months ($p < 0.05$). The average width of the periodontal ligament did not show significant differences between any two groups (Table 2).
TABLE 1
Thickness of Sections and the Perimeters of Cementum and Socket Wall in Transverse Sections of Rat Mandibles in Each Group

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Thickness of Sections (mm)</th>
<th>Perimeter of Cementum (mm)</th>
<th>Perimeter of Socket Wall (mm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.610 ± 0.037</td>
<td>3.72 ± 0.14</td>
<td>4.76 ± 0.20</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>0.615 ± 0.076</td>
<td>4.11 ± 0.11***</td>
<td>5.26 ± 0.24***</td>
<td>10</td>
</tr>
<tr>
<td>24</td>
<td>0.648 ± 0.043</td>
<td>4.40 ± 0.17***</td>
<td>5.37 ± 0.18***</td>
<td>9</td>
</tr>
</tbody>
</table>

Perimeters were measured on the lingual aspect between the enamel/cementum junctions on both sides of the tooth section. Mean ± 1 SD of the population is shown in each group. n, number of rats. Significant differences from the 6-month group. ***p < 0.001.

TABLE 2
Sectional Area of the Periodontal Ligament, the Area of Periodontal Ligament Facing Cementum and the Average Width of Periodontal Ligament in the Transverse Sections of Rat Mandibles at Various Ages

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Sectional Area of Periodontal Ligament (mm²)</th>
<th>Area of Periodontal Ligament Facing Cementum (mm²)</th>
<th>Average Width of Periodontal Ligament (mm)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.985 ± 0.064</td>
<td>2.27 ± 0.21</td>
<td>0.233 ± 0.019</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>1.094 ± 0.114*</td>
<td>2.53 ± 0.32*</td>
<td>0.233 ± 0.023</td>
<td>10</td>
</tr>
<tr>
<td>24</td>
<td>1.166 ± 0.275</td>
<td>2.85 ± 0.23***</td>
<td>0.238 ± 0.053</td>
<td>9</td>
</tr>
</tbody>
</table>

All measurements were made on the lingual aspect between the enamel/cementum junctions on both sides of the tooth section. Mean ± 1 SD of the population is shown in each group. Area of periodontal ligament facing cementum = thickness of section × perimeter of cementum. Average width of periodontal ligament = sectional area of periodontal ligament/(perimeter of cementum + perimeter of socket wall)/2. n, number of rats. Significant differences from the 6-month group. *p < 0.05, ***p < 0.001.

FIGURE 3. Load-deformation curves obtained from the transverse sections of rat mandibles at 6, 12 and 24 months of age. The load was plotted against the deformation at intervals of 25 μm. Each point and vertical bar represent the mean and ± 1 SD of the population. N, newtons.
FIGURE 4. Stress-strain curves obtained from the transverse sections of rat mandibles at 6, 12 and 24 months of age. The end point of each curve represents the maximum shear stress and strain. The means of 9-10 animals are shown. N, newtons.

FIGURE 5. Changes in the maximum shear load (a) estimated from the load-deformation curves and the maximum shear stress (b) estimated from the stress-strain curves in groups of rats at 6, 12 and 24 months of age. Each point and vertical bar represent mean and ±1 SD of the population.

FIGURE 6. Changes (plotted as in Figure 5) in the maximum deformation (a) estimated from the load-deformation curves and the maximum strain (b) estimated from the stress-strain curves in groups of rats at 6, 12 and 24 months of age.
STRENGTH OF PERIODONTUM: OLDER RATS

FIGURE 7. Changes (plotted as in Figures 5 and 6) in the elastic stiffness (a) estimated from the load-deformation curves and the elastic stiffness (b) estimated from the stress-strain curves in groups of rats at 6, 12 and 24 months of age. Significant difference from the 6-month group, *p < 0.05.

Load-deformation and Stress-strain Curves

Figure 3 shows load-deformation curves obtained from the rat mandibles at 6, 12 and 24 months of age. The time between killing of animals and mechanical testing ranged from 31 to 74 min. When the surrounding alveolar bone was broken during the mechanical testing, data from such samples were excluded. With increase of distance pushed, the load increased steeply until it reached the maximum point and fell gradually in all cases. Figure 4 shows stress-strain curves obtained from the rat mandibles at 6, 12 and 24 months of age. Here the mean values of 9-10 samples are shown. The end point of each curve represents the maximum shear stress and strain. The curves in all groups are sigmoid in shape. The general appearances of the load-deformation and stress-strain curves were similar between the age groups.

Mechanical Measures at Various Ages

Maximum shear load and maximum shear stress. Figure 5 shows changes in the maximum shear load estimated from the load-deformation curves (Figure 5a) and the maximum shear stress estimated from the stress-strain curves (Figure 5b). Neither maximum shear load nor maximum shear stress showed significant differences between any two groups. The average maximum shear load ranged from 2.56 to 2.87 N. The average maximum shear stress ranged from 0.97 to 1.13 N/mm². Significant differences among the age groups were not found in both cases by the analysis of variance.

Maximum deformation and maximum strain. Figure 6 shows changes in the maximum deformation estimated from the load-deformation curves (Figure 6a) and the maximum strain estimated from the stress-strain curves (Figure 6b). Neither maximum deformation nor maximum strain showed significant differences between any two groups. The average maximum deformation ranged from 0.219 to 0.260 mm. The average maximum strain ranged from 0.95 to 1.08. Significant differences among the age groups were not found by the analysis of variance.

Elastic stiffnesses. Figure 7 shows changes in the elastic stiffnesses estimated from the load-deformation curves...
(Figure 7a) and from the stress-strain curves (Figure 7b). The average elastic stiffness estimated from the load-deformation curves at 12 months was only significantly greater ($p < 0.05$) than that at 6 months, but the differences were not significant between any other two groups. The elastic stiffness estimated from the stress-strain curves did not show significant differences between any two groups except between 12 and 24 months ($p < 0.05$). A significant difference among the age groups was only found in the elastic stiffness estimated from the stress-strain curves by the analysis of variance ($p < 0.05$).

Failure energy in shear and failure strain energy density. Figure 8 shows changes in the failure energy in shear estimated from the load-deformation curves (Figure 8a) and the failure strain energy density from the stress-strain curves (Figure 8b). Neither failure energy in shear nor failure strain energy density showed significant differences between any two groups. Significant differences among the age groups were not found in both cases by the analysis of variance.

DISCUSSION

In the present experiment, a transverse section was obtained from each mandible through an axis near the mesial side of the mandibular first molar in all rats as in the previous experiment using younger animals [Yamane, 1990]. Although there are regional differences in the mechanical properties of the ligament along the long axis of the tooth [Chiba et al., 1990], age-related changes in the mechanical properties of the ligament could be compared at a restricted region.

The time between killing of animals and mechanical testing was longer in the present (31–74 min) than in the previous (13–18 min, Yamane, 1990) experiments. It is believed that the effects of post mortem changes on the mechanical strength of the ligament were minor within that time range, as it was found that storage of transverse sections of the rat mandible in saline for a day at room temperature did not significantly change the maximum load and stress of the periodontal ligament of the rat incisor [Chiba et al., unpublished data]. Care was also taken to diminish tissue damage due to sectioning by cooling the jaws with saline during cutting, and to avoid drying of specimens by immersing them in saline before and during the mechanical testing.

The direction of loading was intrusive in the present experiment, whereas it was extrusive in the previous study using younger animals [Yamane, 1990]. The difference was not intentional but accidental. It has been reported that loading of the human or macaque periodontal ligament for either extrusion or intrusion did not appear to change its behaviour [Picton, 1986; Ralph, 1982]. In addition, it was found that direction of loading did not cause significant changes of the mechanical strength of the periodontal ligament in both impeded and unimpeded incisors when sections of mandibles were used for mechanical testing [Chiba et al., unpublished data]. Therefore, it is assumed that the difference of the direction of loading between the present and previous [Yamane, 1990] experiments does not substantially affect the mechanical measures obtained and subsequent interpretations of the results.

It has been reported by Chiba and Ohkawa [1980] that the ultimate load required to extract the rat mandibular first molar from its socket did not differ significantly between rats given powdered or pellet diets for 17 days. They suggested that it would be necessary to compare the effects of different diets on the growth of jaws and the mechanical properties of the periodontal ligament following feeding of a much harder diet for a longer period of time. In the present experiment, the animals had been fed a pellet diet for a considerably longer period of time. It is conceivable that the difference in the shape of the diet may induce different masticatory behaviours in animals and thus affect the growth of jaws and teeth and the mechanical properties of the periodontal ligament. However, it should be possible to compare age-related changes in the mechanical properties of the ligament among groups of rats fed a diet with the same shape.

Mechanical measures obtained in the present study did not distinctly show age-related changes from 6 to 24 months (Figures 5–8), although the body and mandible weights continued to increase (Figure 1). Significant differences found in the elastic stiffnesses (Figure 7a, b, and results) do not appear to relate to the aging process. It is possible that age-related changes in the mechanical properties of the periodontal ligament were hidden by the wide variation of readings. Yamane [1990] has suggested that the mechanical strength per unit area of the periodontal ligament did not change much from 3 to 24 weeks. Similarly, it is feasible that changes in the mechanical properties of the periodontal ligament of the rat incisor are much less marked from 6 to 24 months.

Age-related changes in the tensile strength per unit cross-sectional area in rat skin, tail tendon and aorta [Fry et al., 1964; Svendsen & Thomson, 1984; Vogel, 1978] have been shown. Reductions in periodontal matrix formation, in periodontal bone formation and in the rates of turnover in gingival fibres with increasing age have been found in the molar teeth of mice [Stahl & Tonna, 1977; Tonna, 1976; Tonna et al., 1980]. Unlike other connective tissues, the periodontal ligament of continuously growing rat incisors does not seem to show age-related changes after the tooth has attained functional occlusion, as suggested by Yamane [1990].

It is likely that qualitative and quantitative changes in the periodontal supporting tissue per unit area of the ligament is minor in both growing younger rats from 3 to 24 weeks [Yamane, 1990] and in older rats from 6 to 24 months. However, this does not necessarily mean that the periodontal ligament of the rat incisor is a stable tissue. In fact, it has been shown that the periodontal ligament responds very sensitively to various internal and external factors such as drugs [Chiba et al., 1981; Ohshima, 1982] and occlusal conditions [Komatsu, 1988]. It is plausible that dynamic changes in the quality and quantity of the ligament are continuously occurring along the
long axis of the incisor [Chiba et al., 1990] throughout the animal’s life in association with the eruptive movement of the tooth. The final stage of the maturation of the ligament is fairly uniform among animals of different ages, as seen in the present and previous [Yamane 1990] experiments.

REFERENCES


