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Effects on Osteoclast in Periodontal Ligament Space by Denerveation of Inferior Alveolar Nerve in Young and Adult Rats

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Osteoclast action is necessary for alveolar bone remodeling in orthodontic tooth movement. The nervous system has also been reported to be associated with bone remodeling. This study was aimed to investigate the changes of osteoclasts in the periodontal ligament (PDL) space after surgical resection of the inferior alveolar nerve (IAN). Experimental rats were divided into young and adult groups. A surgical resection procedure of the IAN was carried out in the left side of the mandible and a sham operation in the right side of the mandible. The number of osteoclasts on the bundle bone surface and the resorption activity of the osteoclasts were histomorphometrically measured. The changes in distribution of substance P (SP) immunoreactive (IR) nerve fiber were evaluated in the PDL and pulp. SP-IR nerve fiber was depleted in both the PDL and pulp of the IAN resection side in both groups, which confirmed the resection of IAN to be successfully conducted. The number of osteoclasts in the IAN resection side was significantly reduced in both the young and adult groups ($p<0.01$ and $p<0.05$), whereas the resorption activity of osteoclasts did not show any significant difference between the IAN resection side and the sham operation side in both groups ($p>0.05$ and $p>0.05$). The adult group showed that the number of osteoclasts reduced significantly ($p<0.01$) and the resorption acitvity didn't change in comparison with the young group ($p>0.05$). These results suggest that surgical resection of the IAN and aging reduce the population of the recruited osteoclasts within the PDL, but don't affect on the osteoclastic resorption activity.

Key words : Osteoclast, Inferior alveolar nerve, Denervation, Periodontal ligament space

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Bone resorption and deposition particularly in alveolar bone are necessary to allow orthodontic tooth movement with remodeling of the periodontal ligament (PDL). Bone remodeling requires the action of osteoblasts and osteoclasts. The interest in the role of the nervous system related to the action of osteoblasts and osteoclasts has recently increased. The sensory portion of the PDL and pulp in the mandible is supplied by the inferior alveolar nerve (IAN) which releases the sensory neuropeptide like substance P (SP), calcitonin gene-related peptide (CGRP), etc produced in the trigeminal ganglion.[1-7] These sensory neuropeptides have been reported to play a role in the control of osteoblast and osteoclast action in bone remodeling.[8-15]

SP was first introduced by von Euler and Gaddum[16] and has been peripherally identified in afferent C- and A delta fibers to relate the noxious stimuli.[17,18] SP containing nerve fibers have been considered to be reduced or depleted in the PDL and pulp after denervation of the IAN.[1,5,6]

Experiments of denervation of the IAN were performed with neurotoxic agent like capsaicin[5,6] and surgical resection[7,19] to assess the relationship between sensory denervation and bone remodeling. When capsaicin was given to neonatal rats and the change of bone resorption in the periosteal area evaluated at the adult stage by extraction of opposing maxillary teeth, minor effects on bone resorption has been reported.[5] However another similar experiment where capsaicin treatment and the evaluation of bone resorption were carried out at the adult age resulted in the denervation of the IAN which lead to a significant reduction of bone resorption.[6] On the other hand, surgical resection of the IAN was reported to have no effect on the osteoclast number and resorption surface when the marrow cavity of the alveolar bone was observed.[7]

This study was aimed to identify whether surgical denervation of the IAN has an effect on the number and resorption activity of osteoclasts in the PDL space of young and adult rats. In order to confirm that surgical resection of the IAN was properly executed, the change in distribution of SP containing nerve fibers was evaluated.

MATERIALS AND METHODS

Materials

This experiment was carried out in 20 Sprague-Dawley (SD) male rats. The animals were divided into young and adult groups. The young group included 10 rats weighing 160-170g at 6 weeks of age. The other 10 rats weighing 300-320g at 10 weeks of age were classified as the adult group.

Surgical procedure

All rats were anesthetized with an intraperitoneal (I.P.) injection of sodium pentobarbital (25mg/kg body weight). Skin incision and blunt dissection of the masseter muscle were performed to make an approach to the inferior alveolar nerve canal, followed by uncovering of the buccal cortical plate of the canal. On the left side, the exposed IAN was ligated with a suture silk and severed. The right side was processed in the same way without resection of the IAN as the sham operation side. In order to give sufficient time for nerve degeneration,[20,21] rats were maintained healthy for 7 days with a standard rodent diet and water ad libitum after resection of the IAN.

Tissue preparation

Rats were deeply anesthetized with sodium pentobartal (50mg/kg body weight, I.P.) and perfused transcardially with saline and 4% paraformaldehyde (PFA). Left and right hemi-mandibles were then extracted and post-fixed in 4% PFA for 1 day at 4%. After trimming the hemi-mandible, decalcification was processed with 10% ethylene diamine tetraacetate (EDTA) solution for 2 weeks. Each specimen was cut at the middle portion of the second molar along the long axis of the tooth to divide them into mesial and distal parts. The mesial part including the first molar and half of the second molar was embedded in paraffin for staining to identify the tartrate resistant acid phosphatase (TRAP) activity of

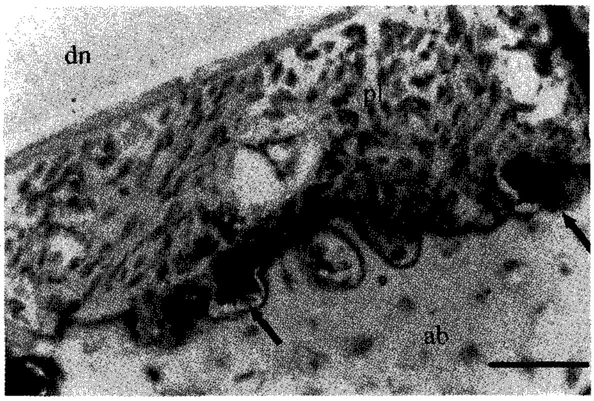

Fig 1. The tartrate resistant acid phosphatase(TRAP) positive osteoclast in periodontal ligament space. Arrow indicates the osteolcast placed directly on the bundle bone surface. ab: alveolar bone; pl: periodontal ligament; dn: root dentin; Scale bar = 50 ㎛.

osteoclasts. The distal part where half of the second molar and third molar were included was prepared for immunohistochemical processing for identifying the SP containing nerve fiber.

Histomorphometric analysis for TRAP positive osteoclast

The mesial portion of the specimen was cut to 10 ㎛ thickness from the tooth furcation area parallel to the occlusal plane of the first molar. Five consecutively sliced sections were selected to be placed on a slide. The sections were processed to detect TRAP. 50mM tartrate (Sigma Chemical Co., U.S.A.) in aceton buffer (Ph5.0) combined with Naphtol As-Mx (Sigma Chemical Co., U.S.A.), N.N Dimethylamide (Sigma Chemical Co., U.S.A.) and fast red violet LB (Sigma Chemical Co., U.S.A.) was applied. Afterward they were counter-stained with methyl green. TRAP positive cells with a large cytoplasm and multinucleus were identified as osteoclasts which was found to be settled in the Howship's lacuna of bundle bone (Fig 1). These sections were morphometrically examined with a semi-automatic image analyzer. The image at a constant microscope magnification (X 200) was transferred into a computer (Power Macintosh, Apple Inc.) with a video camera installed on the microscope and analyzed with the software (NIH image ver 6.0). The length of the bundle bone surface associated with the periodontal ligament in all roots of the first molar was measured to be expre-ssed as a total measured bone surface (TMBS). The TRAP positive osteoclasts situated directly on the bundle bone surface was identified around all roots of the first molar.

The number of osteoclasts were counted and recor-ded as cell numbers per mm of TMBS. The surface length of the osteoclast contacting with bundle bone to make the sealing zone was measured and divided by the number of osteoclasts to produce an average resorption surface length of an osteoclast. This measurement meant the average resorption activity of the osteoclast.

The obtained data were statistically analyzed with the statistical software (SPSS-PC ver10.0, SPSS Inc., U.S.A.) so as to compare the value of measurements between the IAN resection side and the sham operation side in both young and adult groups. Paired t-test was performed for each group. Two factors, age and surgi-cal resection of the IAN, were the criteria for data comparison. Therefore two-way analysis of variance (ANOVA) was accomplished to evaluate the effect of two factors on the measurements. The difference was considered significant as the p-value was below 0.05.

Immunohistochemical processing

In order to evaluate the distribution of SP containing nerve fiber in the PDL and pulp, the specimens from the distal part were immunohistochemically examined. These were cut on a cryostat to 25 ㎛ thickness in the mesiodistal direction along the long axis of the tooth. Two sliced sections from each specimen where the PDL and pulp of the distal root of the second molar that were completely included were placed on a slide. The sections were washed in 0.05M phosphate buffered saline (PBS) at Ph 7.4 and incubated for 30 min. with 0.3% H_2O2 in 0.05M PBS. They were then rinsed with 0.05M PBS. After rinsing once more with 0.3% triton in 0.05M PBS, they were incubated with 4 % normal goat serum (NGS)

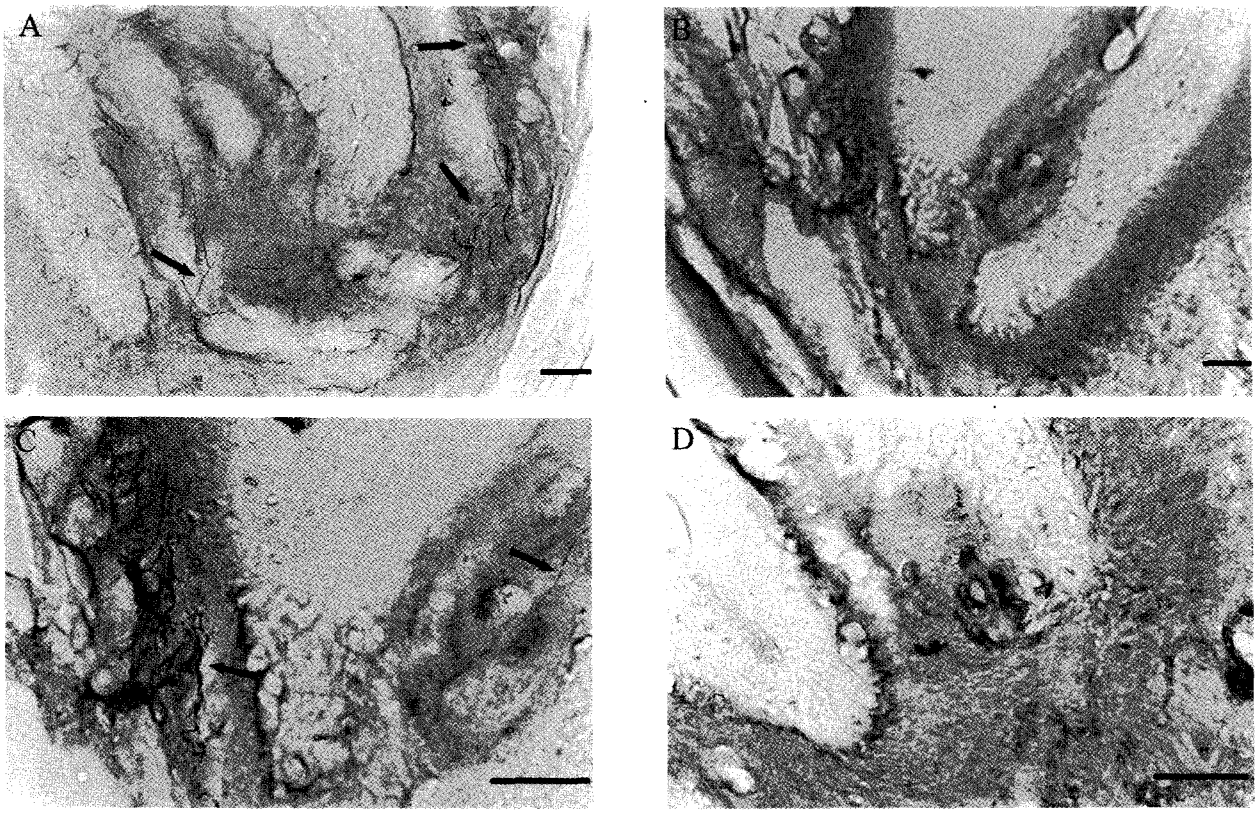

Fig 2. The distribution of substance P(SP) immunoreactive(IR) nerve fiber in periodontal ligament and pulp of distal root of second mandibular molar. Arrow indicates SP-IR nerve fibers. A,C: sham operation side; B,D: the inferior alveolar resection side; Scale bar = 100 μm.

in 0.3% triton in 0.05M PBS for 2 hours at room temperature and then incubated with primary antibody rabbit anti-substance P (dilution 1:5000; Genosys, U. K.) for 1 hour at room temperature and incubated overnight at 4%. They were washed with 1% NGS in 3% triton in 0.05 M PBS and incubated with secondary anti-rabbit IgG (dilution 1:200) for 2 hours at room temperature. After washing with 1% NGS in 0.3% triton in 0.05 M PBS, secondary antibody was coulpled to avidine biotin-peroxidase (Vectastain ABC Kit, Vector Laboratories, U.S.A.) for 2 hours at room temperature, after which they were washed with 0.05 M PBS. Diaminobenzidine (DAB, Sigma Chemical Co., U.S.A.) was treated with 3% H_2O_2 to express the antibody reaction. SP immunoreactive (IR) fiber was observed in the PDL and pulp of the distal root of the second molar under the microscope (X200 and X400).

RESULTS

Distribution of SP immunoreactive nerve fiber

In the sham operation side, SP-IR nerve fibers in the PDL of the young and adult group were sparsely distributed along blood vessels, more visualized in the area of the root apex. The PDL showed fewer numbers of SP-IR fibers than pulp. In contrast, SP-IR nerve fiber in the IAN resection side of both groups was absent in the PDL and pulp (Fig 2).

Changes in osteoclast

The osteoclast number was significantly reduced in both the young ($p<0.01$) and adult groups ($p<0.05$) after resection of IAN (Table 1). The number of osteoclasts

Table 1. The change of osteoclast action in the periodontal ligament space after surgical resection of inferior alveolar nerve

	Young group		Adult group	
	IAN resection side		IAN resection side	
Number (number /mm)	0.37 ± 0.03 **	0.59 ± 0.04	0.19 ± 0.03 *	0.28 ± 0.04
Resorption activity (㎛)	27.93 ± 1.73	31.1 ± 0.81	33.80 ± 1.86	31.98 ± 1.95

Osteoclast number per mm of total measured bundle bone surface is shown. Resorption activity is the average length of osteoclast to contact with the bundle bone surface. The data is given as mean and standard error of mean. *p<0.05 versus the sham operation side; **p<0.01 versus the sham operation side.

Table 2. Effect of age and the inferior alveolar nerve resection factor on the measurements by two way analysis of variance (ANOVA)

	Age	IAN resection	interaction
Number	.00	.00	.12
Resorption activity	.49	.66	.14

Osteoclast number per mm of total measured bundle bone surface is shown. The resorption activity is the average length of osteoclast to contact with bundle bone surface. The p-value where below 0.05 is considered to have a significant effect

was decreased by 37% in the young group and by 32% in the adult group. On the other hand, the average resorption activity of the osteoclasts did not show any significant difference between the IAN resection side and the sham operation side in both groups (p>0.05, Table 1).

In the two way ANOVA as to the effect of 2 factors on the measurements, age factor and IAN resection factor showed significant effects on the measurements of the osteoclast number (p<0.01, Table 2). Both the two factors did not have an effect on the resorption activity of osteoclasts (p>0.05 , Table 2).

DISCUSSION

SP-IR nerve fibers in the sham operation side were sparsely found around blood vessels in the PDL of young and adult groups. The apical area showed more density in the number of SP-IR nerve fiber than other areas of the PDL. The pulp showed more SP-IR nerve fibers than the PDL. On the other hand, the IAN resection side showed depletion of SP-IR nerve fibers in the PDL and pulp. Sensory innervation of the PDL and pulp for the mandibular molar comes from the trigeminal ganglion.[2] Therefore depletion of SP-IR nerve fibers in the PDL and pulp of the second molar results in the disturbance of sensory conduction in the IAN. The distribution of SP-IR nerve fibers in the side of sensory denervation with capsaicin treatment were reduced in periosteum[5] and dental pulp.[6] In the experiment of surgical resection for denervation of the IAN, SP-IR nerve fibers disappeared in both pulp and PDL.[1] In the present study, depletion of SP-IR nerve fibers in PDL and pulp confirmed to successfully perform surgical denervation of the IAN.

In the young group, at 6 weeks of age showing high bone metabolism by growth, the number of osteoclasts was significantly reduced after resection of the IAN (p<0.01). In the adult group where growth was complete it also appeared that the osteoclast significantly reduced in number after resection of the IAN (p<0.05). Hill et al. (1991)[5] reported when percentage of mandibular periosteal bone surface occupied by osteclast was evaluated at the site opposite to the extraction site of maxillary molar in the male SD rats whose sensory nerve were treated with capsaicin at thirty six and forty eight hours after birth, it was reduced by 21% at 7weeks of age.[5] A similar experiment was carried out in the adult rat to which capsaicin treatment was given for denervation of the IAN.[6] In that experiment the size of the population and resorption activity of the osteoclasts were not modified but the resoprtion surface was reduced by 40% and the number of actively resorbing the osteoclasts were significantly reduced due to the induced bone growth. Thus in past studies for IAN denervation with capsaicin treatment there was a little difference in the amount of reduction as well as in the items of measurement selected for analysis. It could also be pointed out that the age in which they gave the capsaicin treatment was different. On the other hand, in the model of surgical resection of IAN, it was reported that there was no significant difference in the osteoclast number and the osteoclast surface occupying the bone surface measured at the medullary cavity in the adult rat.[7] They suggested that the sensory nerve in the normal physiologic state had little influence on alveolar bone metabolism.[7] Because they observed the osteoclasts in the medullary cavity, there was a difference in the tissue where the measure was performed compared with this study.

Osteoclast is formed by the fusion of mononucleated osetoclast precursor cells derived from hamatopoetic tissue.[22,23] The osteoclasts make access to the bone surface and function by resorbing bone through the formation of a sealing zone and ruffled border.[24] In the present study the reduction in the number of osteoclast of the young and adult group by sensory denervation could mean that the recruitment of osteoclasts into the PDL space was affected. However the average length of osteoclast to contact with the bundle bone surface, which means the osteoclastic resorption activity, was not disturbed by sensory denervation in both groups. Therefore the osteoclastic resorption activity was not dependent on the action of the sensory nerve.

Physiologic processes for bone remodeling, tooth eruption and calcium homeostasis requires osteoclast action. The bone remodeling process leads to the growth of bone, with the amount depending on age. In order to evaluate the effect of age factor and IAN resection factor used as the criteria to compare data, two way ANOVA was statistically performed. The results showed that the age factor showed a significant effect on the number of the osteoclasts ($p<0.01$), not on the resorption activity of the osteoclast ($p>0.05$). Eventually the age difference in the rat between 6 weeks and 10 weeks of age made a difference in the population of the recruited osteoclasts. Because osteolcast action for alveolar bone remodeling in orthodontic tooth movement is necessary, the decrease in osteoclast population with age results in difficulty of tooth movement at the adult age.

On the other hand, the IAN resection factor showed an effect on the number of osteoclasts ($p<0.01$), not on the resorption activity of the osteoclasts ($p>0.05$).

Conclusively surgical resection of the IAN and aging affected the recruitment of the osteoclasts in the PDL space. However the resorption activity of the osteoclast was not affected by denervation of the sensory nerve and aging.

CONCLUSION

The aim of this study was to investigate the changes of osteoclast action in the periodontal ligament (PDL) space after surgical resection of the inferior alveolar nerve (IAN). The number and resorption activity of osteoclasts on bundle bone were histomorphometrically analyzed in the young and adult rats. After resection of IAN in the young and adult rat, the osteoclast population was decreased without change the osteoclast resorption activity. This might mean that denervation of the sensory nerve has an affect on the recruitment of osteoclasts within tissue.

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국문초록

하치조 신경 절단이 치주인대공간에서 파골세포에 미치는 영향

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본 연구는 하치조신경의 수술적 절단이 치주인대공간에서의 파골세포에 미치는 영향을 평가하기 위하여 시행하였다. 이를 위하여 실험동물을 젊은 쥐 군 과 성 쥐 군으로 구분하여 좌측 하악골에는 하치조신경절단을 시행하여 실험측으로, 우측하악골은 가수술측으로 사용하였다. Bundle 골 표면에 위치한 파골세포의 수 그리고 파골세포의 골 흡수 활성도를 조직 형태적으로 측정하였다. 또한 Substance P 면역반응 신경섬유의 분포 변화를 치주인대와 치수조직에서 평가 하였다. Substance P 면역반응 신경섬유는 양군 실험측에서 고갈됨으로서 하치조신경의 수술적 절단이 성공적이었다는 것을 확인 할 수 있었다. 실험측에서 파골세포의 수가 젊은 쥐 군 과 성 쥐 군 모두에서 유의하게 감소하였다($p<0.01$ 과 $p<0.05$). 그러나 파골세포의 골 흡수 활성도는 양군 모두에서 실험측과 가수술측 사이에 차이를 나타내지 않았다($p>0.05$). 성 쥐 군에서 파골세포의 수가 젊은 쥐 군에 비해 유의하게 감소 되었으며 ($p<0.01$), 파골세포의 골 흡수 활성도에서는 변화가 없었다($p>0.05$). 이들 결과를 통하여 감각신경인 하치조신경의 절단과 연령증가는 치주인대 공간에서 Bundle골 표면의 파골세포의 수를 감소시켰으며 골 흡수 활성도에는 영향을 주지 않는 것으로 나타났다.

주요 단어 : 파골세포, 하치조신경, 신경절단, 치주인대공간

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