

## Effects of tooth movement on the alveolar bone in osteoporotic rats

Yukiko OIKAWA, Akihide YOSHIDA, Takashi MORIOKA, Lan Hua SHEN,  
Yukio SEINO and Hiroyuki MIURA

Department of Orthodontics, School of Dentistry, Iwate Medical University

(Chief : prof. Hiroyuki MIURA)

[Received : February 26, 2004 ; Accepted : March 18, 2004]

**Abstract :** With recently prevailed knowledge and information on orthodontic treatment as well as improved awareness about dental health and aesthetic concerns, the proportion of adult female orthodontic patients has tended to increase yearly. The decreases of bone density in females forty and older are known. Although several reports have investigated the possible relationship between osteoporosis and the alveolar bone, we could not find a report concerning the tooth movement in relation to this disease. In this study, we confirmed that tooth movement was experimentally induced by inserting a gum ring for orthodontic treatment onto the molar site of normal and osteoporotic rats 12 weeks postpartum. The histological study of the alveolar bone was performed with immunohistological staining using TGF- $\beta$  1 antibody as a primary antibody. As a result, we found that TGF- $\beta$  1 is induced by orthodontic force and acts on osteoblasts and osteoclasts in the alveolar bone and ultimately induces a reconstruction of alveolar bone through activation of both bone formation and resorption. This also occurred even in the case of osteoporosis, however, an enhanced bone resorption and delayed initiation of bone formation was suggested in the osteoporotic group.

**Key words :** tooth movement, osteoporotic rats, alveolar bone, histochemistry

### Introduction

Current knowledge and information of orthodontic treatments are made available and is well known to the general population. The demand for such orthodontic treatments for the purpose of aesthetics, improved oral hygiene, etc., is increasing among middle to advanced aged people<sup>1)</sup>. The female proportion is statistically high<sup>2)</sup>. However, female bone density is apt to

decrease on and after forty years of age, probably due to hormonal disorder<sup>3)</sup>. When an orthodontic treatment is initiated in this group, therefore, the bone density might be decreased even though they were not diagnosed with osteoporosis.

In the dental field, it is often found that the amounts of alveolar bone is significantly decrease in females as compared to males with periodontal disease of a similar degree<sup>4)</sup>. Female patients with a progressed

---

Effects of tooth movement on the alveolar bone in osteoporotic rats

Yukiko OIKAWA, Akihide YOSHIDA, Takashi MORIOKA, Lan Hua SHEN, Yukio SEINO and Hiroyuki MIURA

Department of Orthodontics, School of Dentistry, Iwate Medical University

1-3-27 Chuo-dori, Morioka, Iwate 020-8505, Japan

岩手県盛岡市中央通1丁目3-27 (〒020-8505)

*Dent. J. Iwate Med. Univ.* 29 : 93-102, 2004

periodontal disease may have osteoporosis<sup>5</sup> and have a different curing process after tooth extraction<sup>6</sup>. From these findings, osteoporosis is considered to have a close correlation with alveolar bone tissue.

Since the tooth movement due to orthodontic force is generated as a result of activated reconstruction of alveolar bone by a mechanical stress, an inflammatory remodeling may occur consequently, the type of which is different from a physiological remodeling in the periodontal tissue. For this reason, the orthodontic force is often listed as a factor to aggravate these symptoms when the periodontal disease is not adequately controlled<sup>7</sup>. In general, a local producing factor (cytokine) seems to play an important role in the metabolism of alveolar bone where an orthodontic force is added, suggesting a possible effect of orthodontic force on the local productivity of cytokine. If there is a causal relationship between the curing process of periodontal disease or extracted cavity and osteoporosis, it is thus considered important to determine the change in the alveolar bone in response to the orthodontic force by means of a menopausal model before an orthodontic treatment is initiated in adult females.

In the present study, a tooth movement was made in rats that received an ovariectomy or laparotomy alone to examine a possible change in the alveolar bone during the early stage of movement and to determine the influence of osteoporosis on the alveolar bone, especially focusing on TGF- $\beta$  1 having a potent modulating activity on the bone metabolism.

## Material and Methods

### 1. Experimental animals and tooth move-

ment technique

Twenty female Sprague-Dawley rats (8 weeks postpartum, 150-170g of body weight) were divided into 2 groups, a control group (Sham group) received laparotomy alone and an osteoporotic group (OVX group) received ovariectomy. At 12 weeks of age in both Sham (200-210g of body weight) and OVX (230-240g of body weight) groups, a gum ring for orthodontic treatment (1/4 L, 3 M Unitek Co. Ltd., USA) was inserted into the space between the left maxillary 1st and 2nd molar according to Waldo's method<sup>8</sup> and a tooth movement in a mesiodistal direction was made on 1, 3, 5 and 7 days. Since rat molars are physiologically moved in a distal direction, a state without tooth movement was also observed (0 day).

### 2. Immunohistological staining

After perfused fixation of rats with 4% paraformaldehyde dissolved in 0.1 M phosphate buffer (pH 7.4), alveolar bone and right maxillary molar was extracted from each animal and further immersed in the same fixative for a subsequent 14 days at 4°C. After fixation, decalcification was performed with 10% formic acid-sodium citrate decalcifying solution (pH 7.4) followed by dehydration in an elevated alcoholic system and paraffin embedding according to a routine procedure.

Horizontal sections of 4 $\mu$ m thickness were prepared and pasted on a glass slide pretreated with silane. According to a routine procedure, these sections were immersed in methanol solution containing 3% hydrogen peroxide for 30 minutes after removal of paraffin, and intrinsic peroxidase was inactivated. Then these sections were washed with 0.01M phosphate buffered

saline and an immunohistological staining was performed by avidin-biotin-peroxidase complex method (ABC method) using Vectastain Elite kit<sup>®</sup> (Vector Co. Ltd., USA). As a primary antibody, a 100-fold diluted rabbit TGF- $\beta$  1 polyclonal antibody (R & D System Co. Ltd., USA) was used. The TGF- $\beta$  1 antibody used in this study is an antibody for detecting an activated type alone. The specificity of the antibody was confirmed by the absence of reaction in the staining procedure using normal rabbit serum (Wako Pure Chemical Industries, Ltd., Osaka) instead of the primary antibody. Then, the sections were reacted with biotin labeled anti-rabbit IgG goat serum, being a secondary antibody. After coloring with DAB reaction, a nuclear staining was performed with hematoxylin followed by dehydration according to a routine procedure and embedding for observation under light microscopy.

### Results

On 0 day without tooth movement, a weak positive TGF- $\beta$  1 reaction was observed in osteoblasts on the marginal alveolar bone and in a part of fibroblasts of the periodontal ligament in both Sham and OVX groups (Fig. 1 a, b). In addition, no polynuclear osteoclasts were observed on the alveolar bone (Fig. 2 a, b).

On the 1st day of tooth movement, as compared to 0 day, however, a strong positive TGF- $\beta$  1 reaction was observed in osteoblasts on the marginal alveolar bone and in a part of fibroblasts in the periodontal ligament (Fig. 1 c, d). On the pressure side, osteoclasts existing in the Howship's lacuna and a part of fibroblasts in the periodontal ligament showed a strong positive reaction

(Fig. 2 c, d).

In the Sham group, a strong positive reaction was observed in osteoblasts on the marginal alveolar bone on the tension side on the 3rd day of movement, as well as in a part of fibroblasts in the periodontal ligament (Fig. 1 e, f). On the pressure side, the number of osteoclasts with a strong positive TGF- $\beta$  1 reaction increased in both Sham and OVX groups (Fig. 2 e, f). In the OVX group, the numbers of osteoclasts were slightly greater as compared with the Sham group.

On 5th day of movement, a positive reaction was observed in osteoblasts on the marginal alveolar bone on the tension side and a strong positive reaction in fibroblasts in the periodontal ligament, however, its reaction in the Sham group was slightly weak as compared with that observed on the 3rd day (Fig. 1 g) with a decreased number of osteoclasts on the pressure side (Fig. 2 g). In the OVX group, a maximally strong positive TGF- $\beta$  1 reaction was still observed in osteoblasts on the marginal alveolar bone and in a part of fibroblasts in the periodontal ligament (Fig. 1 h), but no noticeable change was evident in the number of osteoclasts as compared with that on the 3rd day (Fig. 2 h).

On the 7th day of movement, a positive TGF- $\beta$  1 reaction was observed in the osteoblasts of an almost round shape on the marginal alveolar bone and in a part of fibroblasts on the periodontal ligament on the tension side (Fig. 1 i, j). On the compressed side, polynuclear osteoclasts showing a positive TGF- $\beta$  1 reaction were still observed (Fig. 2 i, j).

In the Sham group, the number of TGF- $\beta$  1 positive cell increased on the 3rd day of

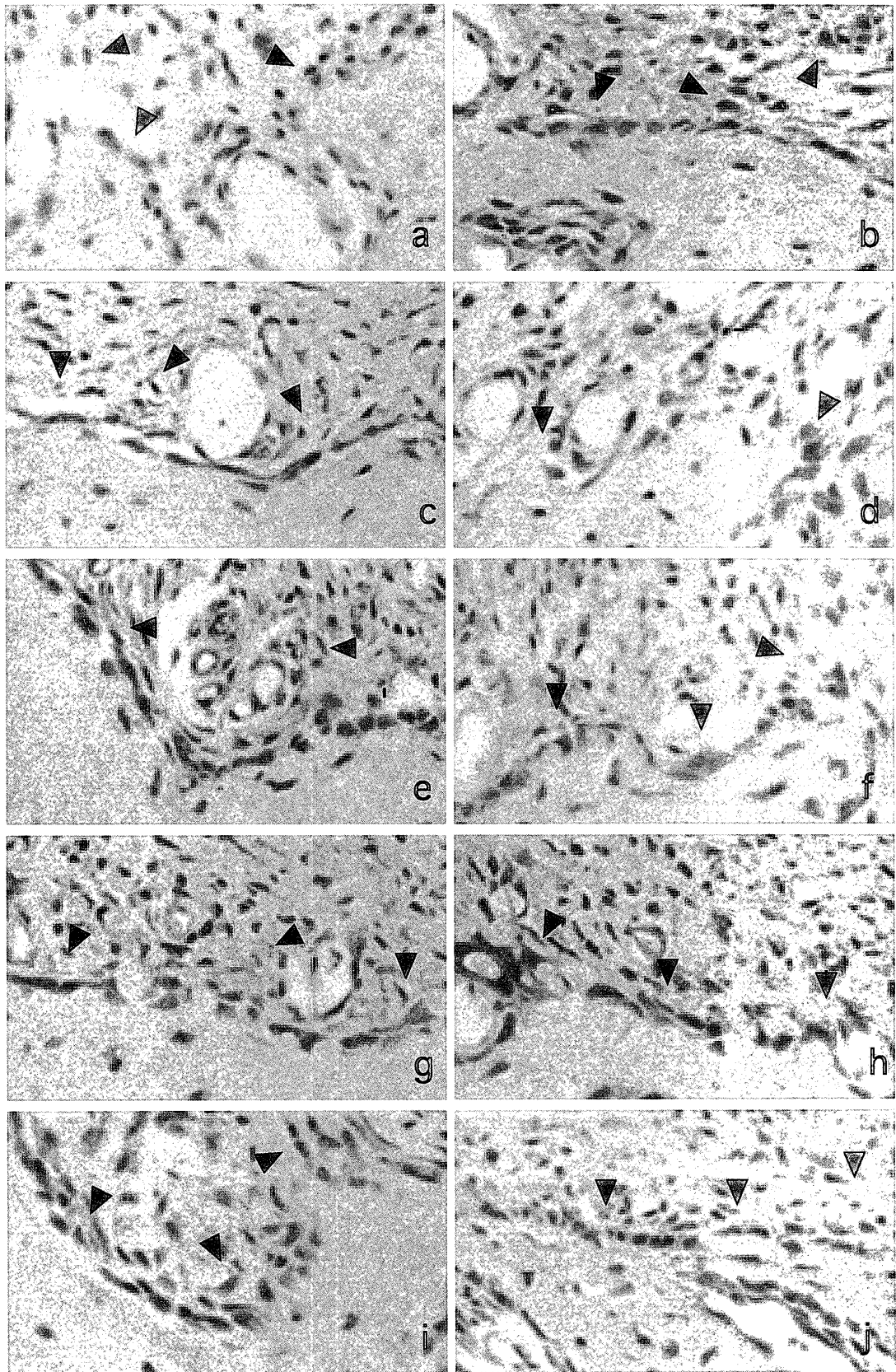


Fig. 1. Tension side ( $\times 250$ )  
a : 0 day : Sham group b : 0 day : OVX group c : 1 day : Sham group d : 1 day : OVX group  
e : 3 day : Sham group f : 3 day : OVX group g : 5 day : Sham group h : 5 day : OVX group  
i : 7 day : Sham group j : 7 day : OVX group

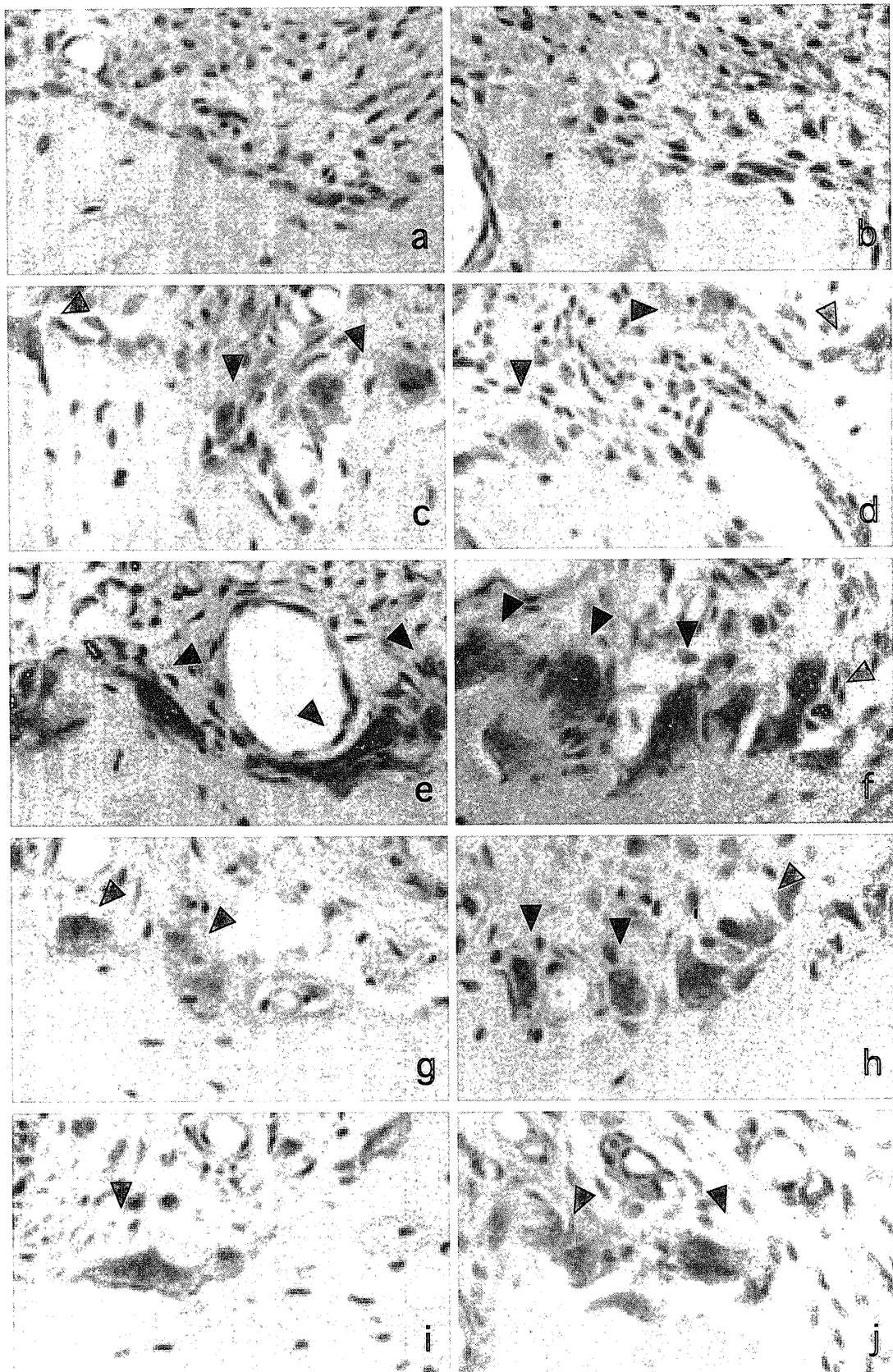


Fig. 2. Pressure side ( $\times 250$ )

a : 0 day : Sham group    b : 0 day : OVX group    c : 1 day : Sham group    d : 1 day : OVX group  
 e : 3 day : Sham group    f : 3 day : OVX group    g : 5 day : Sham group    h : 5 day : OVX group  
 i : 7 day : Sham group    j : 7 day : OVX group

movement on both tension and pressure sides showing the strongest staining reaction, but the degree of staining for these positive cells slightly and gradually decreased on the 5th to 7th days. In the OVX group, on the other hand, the number of TGF- $\beta$  1 positive cell increased considerably on the 5th day of movement on the tension side and on the 3rd day of movement on the compressed side with the strongest degree of staining which was, however, slightly diminished thereafter.

TGF- $\beta$  1 positive reaction was observed on the cytoplasm and cell membrane at osteoclasts, in addition to resorption lacunae on the marginal alveolar bone. On the other hand, TGF- $\beta$  1 positive reaction was observed on the cytoplasm of osteoblasts.

### Discussion

In general, human bone volume increases up to 30 years of age and gradually decreases on and after 40 years, showing a physiological aging change in the bone mass. Osteoporosis is a pathologic state with a disturbed balance in the bone mass, which is classified into 2 types: a high-rotation type with normal bone formation but excessive bone resorption and a low-rotation type with both decreased bone formation and resorption<sup>3)</sup>. The former is said to abruptly occur after menopause, while the latter is said to occur gradually with advancing of age<sup>3)</sup>. Though several reports have studied periodontal disease or curing state of extracted cavity in relation to osteoporosis<sup>4,6)</sup>, almost no report is available for the orthodontic treatment in these patients.

Alveolar bone which is a bone region for supporting teeth is a specific bone region

with high bone density, caused by receiving an occlusive force constantly due to mastication, etc. And thus, dissimilar to other long bones, layer of proper alveolar bone surrounding the periodontal ligament and cortical bone maintaining the external form of mandibular bone still remain and resist mechanical stimulation even when an active bone resorption occurs, and exhibits a characteristic mode of bone resorption not to lose its function as a supporting tissue for teeth. In addition, a bone remodeling by orthodontic force presents a different form from a physiological bone remodeling in nature, and it differs from remodeling of other long bones in osteoporosis.

Except for ovariectomy as a method for preparation of rat osteoporotic model, OCIF knockout mouse by a genetic procedure or immobilization, restricted calcium uptake and some drug administration are listed. In this study, the animals were ovariectomized at their age of 8 weeks postpartum and a histological observation was performed on and after 12 weeks of age to serve as an osteoporotic model of high-rotation type after menopause. The body weight at 12 weeks of age ranged from 200 to 210g in the Sham group and 230 to 240g in the OVX group with some difference between the two groups, which was probably attributable to a temporary but rapidly increased body weight due to disturbed hormonal balance after ovariectomy. According to Kanai *et al.*<sup>10)</sup>, a promoted bone resorption was observed around 4 weeks after ovariectomy, indicating a pathologic state of osteoporosis. Though no histological features were presented in this study, rats with a confirmed rough state of long bone density as compared with the Sham group were



selected as a test item. In addition, rats with no visual and histological changes such as periodontal inflammation were qualified in the two groups.

In addition to systemic factors such as calcitonin, PTH and Vitamin D<sub>3</sub>, cytokines which are produced locally in the bones or cartilages are involved in both bone formation and resorption<sup>11</sup>. Among them, TGF- $\beta$  1 is one of cytokines having a potent modulating action on bone metabolism and is reported to be expressed strongly in the bone tissue in response to a mechanical stress<sup>12</sup>. The authors had also reported previously that TGF- $\beta$  1 is a modulator which plays an important role in the remodeling system<sup>13, 14</sup>. Cytokines may be induced in the alveolar bone affected by orthodontic force and TGF- $\beta$  1 among them may activate the reconstruction of alveolar bone, ultimately causing a tooth movement. In the present study, the appearance of cytokine in the alveolar bone in response to tooth movement was examined in both Sham and OVX groups by focusing on TGF- $\beta$  1.

In consequence, localization of strong immunoreactivity of TGF- $\beta$  1 protein was confirmed in osteoblasts on the tension side on the 3rd day of tooth movement in the Sham group and on the 5th day in the OVX group as well as in osteoclasts on the compressed side on the 3rd day of tooth movement in both Sham and OVX groups. The action of TGF- $\beta$  1 is exhibited through a release of inactivated TGF- $\beta$  1 existing in the calcified bone substrate by some acids driven from outside the substrate and its activation, or through production of activated TGF- $\beta$  1 from cells such as osteoclasts or osteoblasts<sup>14</sup>. Since TGF- $\beta$  1

antibody used in this study is an antibody for detecting an activated type alone, TGF- $\beta$  1 detected on the sections concerned can be said to be of an activated type.

In the OVX group, as compared to the Sham group, the number of osteoclasts were slightly increased with a prolonged duration of existence. During the curing process, after implantation or tooth extraction, formation of new bone or osteoblasts were suggested to occur relatively slowly with contrarily rapid and prolonged bone resorption<sup>6, 15, 16</sup>. A similar trend was also observed in the present study. Almost no histological changes suggestive of bone formation or resorption were evident in the alveolar bone and no TGF- $\beta$  1 positive cells were also detected on 0 day with no treatment. While no TGF- $\beta$  1 was observed in cells probably being an interphase squamous osteoblast among those arranged on the bone surface on the tension side of tooth movement, localized TGF- $\beta$  1 was evident in round-shaped osteoblasts during the bone formation period. Since the positive reaction of osteoblasts against TGF- $\beta$  1 were the strongest on the 5th day in the OVX group, the reaction to bone formation seems to progress mildly as compared with the Sham group. Though these results indicate that TGF- $\beta$  1 participates not only in the bone formation by osteoblasts, but also in the bone resorption by osteoclasts at the site of tooth movement in both groups, a gap in the timings of bone formation and resorption was suggested from the result of TGF- $\beta$  1 expression.

In the case of osteoporosis of high-rotation type, some authors have stated that the bone formation is generally normal or active but the bone density is reduced due to the bone

resorption exceeding the formation level, while others have emphasized that no difference is observed in the formation of new bone but the decreased mechanical strength of bone is induced due to subsequently delayed calcification<sup>3)</sup>. From the present results observed during the early stages of tooth movement, no difference was evident in the calcification or in the morphology of osteoblasts, but a variation was observed in the expression of TGF- $\beta$ 1, suggesting a possible difference in the activity of bone formation and some influence of osteoporosis on the alveolar bone as well.

Based on the evidences mentioned previously, it is clear that TGF- $\beta$ 1 is induced by orthodontic force even in the case of osteoporosis and that TGF- $\beta$ 1 acts not only on osteoblasts but also on osteoclasts in the alveolar bone and induces a reconstruction of alveolar bone through activation of both bone formation and resorption with a possible minimal difference in these epochs. Since qualitative changes of TGF- $\beta$ 1 were followed in the alveolar bone during the early stages of tooth movement in this study, a distinct difference was hardly indicated, but a tooth movement was suggested to be practicable even in the case of osteoporosis. This finding may provide a useful fundamental knowledge for orthodontic practice.

#### *Acknowledgement*

The authors deeply thank Prof. T. Nawa of the 2nd Department of Oral Anatomy for his kind instructions and advice on this study. We also thank doctors in the Department of Orthodontics for their cooperation in performing this study. This

study was supported in part by a Grant-in-Aid for Open Research Project for private universities from the Ministry of Education, Science and Technology of Japan.

#### **Reference**

- 1) Oikawa, Y., Miura, H., Seino, Y., Yamauchi, K. and Morioka, T. : The case of skeletal anterior cross bite with periodontitis in her sixties. *Orthod. Waves.* 61 : 96-102, 2002.
- 2) Murata, M., Seino, Y. and Miura, H. : An investigation on orthodontic patients at iwate medical university dental hospital in recent years-from 1996 to 1998. *Jpn. Soc. Dent. Prac.* 34 : 197-203, 2000.
- 3) Roschger, P., Rinnerthaler, S., Yates, J., Rodan, G.A., Fratzl, P. and Klaushofer, K. : Alendronate increases degree and uniformity of mineralization in cancellous bone and decreases the porosity in cortical bone of osteoporotic women. *Bone.* 29 : 185-191, 2001.
- 4) McCann, AL. and Bonci, L. : Maintaining women's oral health. *Dent. Clinics of North Am.* 45 : 571-601, 2001.
- 5) Ootogoto, J., Watanabe, H., Ono, M., Higaki, K., Sato, T., Shiina, N., Iyoda, H., Wen, C., Kamijyo, H., Sakamoto, H., Kawatani, K., Ito, S. and Ota, N. : Correlation between periodontal disease and osteoporosis using panoramic radiographic parameters to diagnose osteoporosis. *J. Jpn. Soc. Period.* 43 : 13-24, 2001.
- 6) Shimizu, M. and Sasaki, T. : Structural changes of maxillary bone after molar extraction in ovariectomized aged rats. *J. Showa Univ. Dent. Soc.* 20 : 435-448, 2001.
- 7) Beppu, R., Noda, K., Yoshii, T., Shimpo, S., Oikawa, T., Nakamura, Y. and Kuwahara, Y. : Characteristics of osteoclasts in the periodontal tissue during experimental tooth movement in aged rats with periodontitis. *Turumi Univ. Dent. J.* 27 : 337-345, 2001.
- 8) Waldo, C.M. and Rothblatt, J.M. : Histologic response to tooth movement in the laboratory rat. *J. Dent. Res.* 33 : 481-486, 1954.
- 9) Yamamoto, M. and Izumi, Y. : Tooth loss and osteoporosis. *Clinical Calcium.* 12 : 987-991, 2002.
- 10) Kanai, Y., Kawamoto, M., Endo, H., Higashino, H. and Taniguchi, N. : Histological study in experimental osteopenia rats. *Orthope. and Traumatology.* 52 : 154-157, 2003.
- 11) Suda, T., Udagawa, N., Nakamura, I., Miyaura, C. and Takahashi, N. : Modulation of osteoclast differentiation by local factors. *Bone.* 17 : 87-91, 1995.



- 12) Sugiyama, T. : Involvement of interleukin-6 and prostaglandin E 2 in periarticular osteoporosis of postmenopausal women with rheumatoid arthritis. *J. Bone & Min. Metab.* 19 : 89-96, 2001.
- 13) Oikawa, Y. : Effects of bisphosphonate (pamidronate) on the osteoclast during the experimental tooth movement. *Orthod. Waves.* 57 : 307-317, 1998.
- 14) Yoshida, A. : Change of expression and localization of transforming growth factor- $\beta$  1 and its receptor during the experimental tooth movement. *Jpn. J. Oral Biology.* 40 : 611-621, 1998.
- 15) Roberts, WE. : Adjunctive orthodontic therapy in adults over 50 years of age clinical management of compensated, partially edentulous malocclusion. *J. Indiana Dent. Assoc.* 76 : 33-41, 1997.
- 16) Ashcraft, MB., Southard, KA. and Tolley, EA. : The effect of corticosteroid-induced osteoporosis on orthodontic tooth movement. *Am. J. Orthod. & Dentof. Orthop.* 102 : 310-319, 1992.

## 骨粗鬆症ラットにおける歯槽骨に対する歯の移動の影響

及川由紀子, 吉田 彰英, 森岡 尚, 沈 蘭花, 清野 幸男, 三浦 廣行

岩手医科大学歯学部歯科矯正学講座

(主任：三浦 廣行 教授)

(受付：2004年2月26日)

(受理：2004年3月18日)

**抄録：** 歯科矯正治療についての知識や情報が一般的に知られ、歯の健康に対する意識や審美的意識の向上により、なかでも成人女性の矯正治療を希望する割合は年々増加する傾向にある。しかし、女性は40歳をさかいに骨密度が減少するとされている。骨粗鬆症と歯槽骨の関係に関してはいくつかの報告があるが、歯の移動に関する報告はほとんどない。本研究では、12週齢の正常ラットと骨粗鬆症ラットの臼歯部に、矯正治療用ゴムリングを挿入し歯の移動を行った。歯槽骨の組織学的観察は一次抗体に TGF- $\beta$  1 抗体を用いた免疫組織染色法にて行った。その結果、骨粗鬆症であっても矯正力で TGF- $\beta$  1 は誘導され、歯槽骨骨梁の骨芽細胞ならびに破骨細胞に作用し、骨形成と骨吸収の両作用を活性化することにより歯槽骨の改造を引き起こすが、骨粗鬆症群では骨吸収の亢進と骨形成開始の遅延が生じている可能性が示唆された。

**キーワード：** 歯の移動, 骨粗鬆症ラット, 歯槽骨, 組織化学