Original

Experimental Alveolar Bone Resorption in Hamsters

— Morphologic Measurement Using Two-Dimensional Analysis —*

ハムスターの実験的歯槽骨吸収の観察

—二次元的形態計測法による解析—

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概要: 小動物における歯槽骨吸収の進行過程を, 同一個体により経日的に観察することを目的として, 軟X線口内撮影装置を考案した。本装置を用いて, ハムスターの歯槽骨吸収の経日的進行を観察するため, 実験動物として15週齢のゴールデンハムスター雄10匹を使用し, 対照群と実験群をそれぞれ5匹の2群に分け, 実験群のハムスターの下顎右側 M₁-M₂ 間歯槽骨部に100g 荷重のゴム糸結紮処置を行い, 実験的に歯槽骨吸収を惹起させ, 同部位を長さおよび面積により計測を行ったところ, 以下の成績が得られた。

- 1) 歯槽骨の長さおよび面積のいずれの計測値も対照群に比較して有意な減少が認められた。なかでも, 面積計測は最も高い減少率が認められた。
- 2) 長さの計測法は, 経日的観察で順次有意に減少することが認められた。

Key words: Soft X-ray, Alveolar bone resorption, Intraoral radiographing device

索引用語: 軟X線, 歯槽骨吸収, 口内撮影装置

Introduction

To observe the daily progression of resorption of alveolar bone in hamsters, we devised an intraoral soft radiographic device¹⁾. This allows us to attach soft X-ray film close to the mandibular molar region on the lingual surface, and makes it possible to take radiographs of the alveolar bone, crown, and root of the molar region.

Conventional methods to induce experimental resorption of the alveolar bone include bacterial periodontitis after a high sucrose diet²⁻⁴⁾, direct inoculation with bacteria^{1,4,5)}, and traumatic occlusion⁶⁾.

However, because these are models of resorption complicated by inflammation, the experimental

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term is long, and morphologic changes of alveolar bone due to dramatic bone resorption are not seen. Therefore, to induce resorption of alveolar bone in a short period by local stimulation only, we used rubber thread ligation and daily, standardized radiography.

Various methods have been used to measure morphologic changes of small animal alveolar bone *in vivo*. However, the measurement methods are limited by the need to avoid killing the experimental animals. In the present study we measured daily morphologic changes of the alveolar bone.

Materials and Methods

Ten 15-week-old male golden hamsters were divided into two groups of 5 animals each, a control group and an experimental group. As shown in Fig. 1, in the experimental group, according to Yokota's method⁷⁾. In the control group, a sham operation was performed in which the same procedure was carried out until immediately before the application of the rubber thread. Powdered feed and water freely available during the experimental period. The right mandibular alveolar bone region was observed by radiography with a soft X-ray projector (SOFRON TYPE SRO-M50S) after placement of the intraoral radiographic device under general anesthesia. The radiographic conditions were 25 KVp of accelerating voltage, 5 mA and 60 sec. and the developmental conditions followed conventional methods¹⁾. To measure morphologic changes of the alveolar bone from soft X-ray film, the following method was used.

For the length of the alveolar bone, the distance between M_1 and M_2 was measured¹⁾. The standard for the measurement followed the method described by Schei et al.⁸⁾, and the measurement using the soft X-ray film was done directly, with a transmission illumination-type stereoscopic microscope (SZH, OLYMPUS) connected to a digital ocular micrometer (OSM-D4, OLYMPUS). This value was then converted into actual length. Area of the alveolar bone between M_1 and M_2 was measured as follows: The soft X-ray film was first projected at 30 $\times$ magnification. The image was traced manually using a digitizer (MITABLET DT-1000, WATANABE) connected to a computer (PC-9801, RA2, NEC), which then computed the area.

Results

1. Soft radiogram findings

Fig. 2 and 3 show soft X-ray pictures from representative cases during the experimental period; the control group is shown in Fig. 2, and the experimental group is shown in Fig. 3. On radiograms immediately before the ligation with rubber thread, both groups showed normal alveolar bone. Although the control group did not show changes at 3 days after the procedure, the experimental group showed some resorption of the upper alveolar bone. In the experimental group at 7 days after the treatment, there was vertical resorption. The M_1 side of the upper region of the alveolar bone was resorbed in the direction

Fig. 1 A rubber thread of about 100g in weight was inserted for ligation of the alveolar bone in the region.

Fig. 2 Soft X-ray pictures from representative cases in the control group during the experimental period.

- (a) before the ligation
- (b) 5 days after the procedure
- (c) 14 days after the procedure

Fig. 3 Soft X-ray pictures from representative cases in the experimental group during the experimental period.

- (a) before the ligation
- (b) 5 days after the treatment
- (c) 14 days after the treatment

of the root apex. There was separation of M_1 , which appeared to be due to pressure stress from the rubber thread. The control group did not show changes at 14 days after treatment, but the experimental group showed marked resorption of the alveolar bone and separation of the crown.

2. Morphologic measurement of the alveolar bone region

Table 1 shows the lengths of the alveolar bone region in each group. The length in the control group ranged from 2.471 to 2.473 mm, and did not change. The length in the experimental group was 2.473 mm before the treatment, and 2.439 mm at one day after the treatment, a significant reduction. In the experimental group, there was gradual reduction, and the bone was 2.180 mm at 14 days. Comparison between the control and experimental groups showed significant differences ($P < 0.001$), except before the

Table 1 The lengths of the alveolar bone region in each group.

Period days	Cont. group	Exp. group	Significance
before	2.471±0.001	2.473±0.005	NS
1	2.471±0.001	2.439±0.004	p<0.001
3	2.472±0.001	2.378±0.001	p<0.001
5	2.471±0.002	2.318±0.004	p<0.001
7	2.473±0.001	2.270±0.007	p<0.001
10	2.472±0.001	2.232±0.005	p<0.001
14	2.471±0.001	2.180±0.005	p<0.001

Mean±SD,mm

Table 2 The areas of the alveolar bone region in each group.

Period days	Cont. group	Exp. group	Significance
before	1.081±0.107	0.921±0.128	NS
1	1.071±0.045	0.830±0.065	p<0.01
3	1.054±0.044	0.887±0.188	NS
5	1.066±0.062	0.812±0.117	p<0.05
7	1.068±0.012	0.738±0.107	p<0.01
10	1.084±0.048	0.692±0.116	p<0.01
14	1.197±0.179	0.638±0.067	p<0.01

Mean±SD,mm²

treatment. Table 2 shows the areas of the alveolar bone region. No change in area was seen in the control group (1.054–1.197 mm²). However, in the experimental group the area gradually decreased. In addition, in comparison between corresponding groups, except for levels at 3 days, there was significant difference before the treatment. The percent reductions in length and area are shown in Fig. 4. The greatest reduction in area was 30.7%, and in length it was 11.9%.

Discussion

Since conventional methods to experimentally induce resorption of alveolar bone¹⁻⁶⁾ entail inflammation, the experimental period is long, and morphologic changes from dramatic bone resorption are not seen. Thus, to induce reproducible resorption of the alveolar bone in a short time by local stimulation only, we used ligation with rubber thread. Although rats^{7,10)} and cats¹¹⁾ have been used as experimental animals, we used hamsters in this study because the jaw opens about 180°, so it is easy to treat and observe the oral cavity¹⁾.

The conventional method of observing morphologic changes of the alveolar bone of small animals entails extraction of the jaw bone after the animal is killed. However, in order to observe daily morphologic changes without killing the experimental animals, it is necessary to use standardized radiography with a soft X-ray intraoral radiographic device^{1,4,9,12)}, and an appropriate analytic method to evaluate the soft X-ray film. We have already reported that there is no significant difference between this intraoral standardized radiography and values measured conventionally from extracted jaw bone¹²⁾.

What is problematic in daily observation by soft X-ray is the impairment of normal physiological function due to irradiation. Therefore, the radiation dose was measured by water with a thermoluminescence-dosimeter (MSO-S, Kyokko). The soft X-ray energy per radiograph was 12.07 R, and there were no direct effects on the experiment. However, to reduce the dose, appropriate standardized radiography using an open-end cone was conducted in which the field at the cone edge was 2 cm.

Fig. 4 Comparison of rate of change (%) in the measured value between the length and area.

- : length of the control group
- : length of the experimental group
- △ : area of the control group
- ▲ : area of the experimental group

This experiment was conducted to compare methods of measuring the length and area of the alveolar bone directly from soft X-ray film.

To standardize the site and the measurement, there is a method in which the standard is obtained from a line tangential to the M_1 distal root edge and the M_2 mesial root edge. However, in this experimental system, M_1 shifts mesially because of the rapid resorption of the alveolar bone, so the tangential line also moves. Therefore, a line directly connecting the mesial and distal root edges of M_2 was used as the standard.

In the experimental group, the greatest reduction in length was 11.9 %, and the greatest reduction in area was 30.7 % (Fig. 4). Lengths and areas in the control group either did not change or increased. However, the measured area tended to vary greatly early in the experiment. The reproducibility of the measurements might have been reduced because of the complex morphology of the alveolar bone. Thus, this technique may not reveal subtle changes in area occurring soon after the beginning of resorption. Measurements of length are probably more accurate.

Conclusion

Using a soft X-ray intraoral radiographic device, we measured experimentally induced alveolar bone resorption in hamsters, between right mandibular M_1 and M_2 . The length and area at the site were measured, giving the following results:

- 1) There was significant reduction in both length and area. The highest reduction was in the measured area.
- 2) Gradual, significant reduction in length was recognized by daily observation.

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