Original

Experimental Periodontal Disease in Hamsters
Induced by Ligation of the Salivary Glands
and a Sucrose-poor Diet

唾液腺結紮および低蔗糖食餌によるハムスターの
実験的歯周疾患に関する研究

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概要: ゴールデンハムスターを用いた高蔗糖食餌による Keyes らの実験系は現在でもよく使用されている。しかし、それに用いる Diet#2000は蔗糖が56%含有されているので、歯周疾患誘発性食餌として適しているかどうか検討する必要があると考えられる。そこで、食餌の低蔗糖化と唾液腺の分泌導管部の結紮および摘除処置により、歯周疾患を誘発させる実験モデルの基礎的検討と歯槽骨吸収の経目的観察を軟X線口内規格撮影装置にて行い、以下の結論を得た。

1) 唾液腺の分泌導管部の結紮および摘除処置 (Ⅲ, Ⅳ, Ⅴ群) により、低蔗糖食餌による歯周疾患の発症が可能となった。

2) 処置による実験動物の発育状況は、Ⅲ, Ⅳ, Ⅴ群は一時的に体重の減少傾向が認められたが、Ⅴ群を除いて群間に有意差は認められなかった。

3) 処置による唾液流出量の影響は、対照群のⅠ, Ⅱ群とⅣ群およびⅤ群間、Ⅲ群とⅤ群、Ⅳ群とⅤ群間に有意差が認められ、飲水量についてはⅤ群が他の群の約2倍の飲水量であった。

4) 短期実験系においては、両側耳下腺分泌導管部結紮および顎下腺、舌下腺摘除処置のⅤ群が、長期にわたる実験系では、両側耳下腺分泌導管部結紮および舌下腺摘除処置のⅣ群が適切と考えられた。

Key words: Hamsters, Periodontal disease, Ligation of salivary glands

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Introduction

In past studies of the etiology and pathophysiology of periodontal diseases, rodents such as rats and

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hamsters were frequently used as experimental animals³⁾. Among other models, the experimental system of Keyes *et al.*²⁾, using golden hamsters given a sucrose-rich diet (diet #2000²⁾), has been used particularly frequently. However, because the diet #2000 was originally developed as a caries-inducing diet, it is not clear whether this diet is appropriate in inducing periodontal diseases in experimental animals.

When the sucrose content in a diet was reduced to 15–16 %, abundant plaque attachment to the teeth was observed^{3,4)}. However, no method has been established for experimentally inducing marked bone resorption in a short period. Paying attention to the effect of saliva on the oral environment, we recently suppressed the cleansing and buffering effect of saliva by ligating or resecting the secretory ducts of salivary glands⁵⁾. In these animals, we conducted a basic study of experimental induction of periodontal diseases using a sucrose-poor diet, accompanied by intraoral soft radiographic assessment⁶⁾ of alveolar bone resorption, which is a terminal symptom of periodontal diseases.

Materials and Methods

1. Experimental animals

Fifty 4-week-old male golden hamsters (Nippon Seibutsu Zairyo Center, Tokyo) were used. They were bred in plastic cages with bedding in a room where the temperature was set at 20–24°C and the relative humidity at 40–60 %. Until 6 weeks of age, they were given a commercial solid diet and allowed free access to purified water. Weight measurement was begun at the age of 4 weeks. The animals were then divided into 5 groups (10 animals/group): Group I contained animals which were given a powdered diet for hamsters and underwent a sham operation similar to the operative steps used before ligation of the secretory ducts of the salivary gland. Group II contained animals which were given a sucrose-poor diet, i.e., diet #2000 after reducing the sucrose content from 56 % to 10 %, and which underwent the same sham operation as Group I animals. Group III contained animals which were fed a sucrose-poor diet and underwent bilateral resection of the submandibular glands. Group IV contained animals which were fed a sucrose-poor diet and underwent bilateral ligation of the secretory ducts of the parotid glands and resection of the bilateral sublingual glands. Group V contained animals which were fed a sucrose-poor diet and underwent bilateral ligation of the secretory ducts of the parotid glands and resection of the submandibular and sublingual glands.

All operations were carried out at the age of 5 weeks under general anesthesia with intraperitoneal pentobarbital 2 ml/100 g (Nembutal® Dainabot, Osaka).

2. Salivary outflow and water consumption

To confirm that ligation or resection of the salivary gland ducts had been successfully carried out, the animals were treated with 10 mg/kg of pilocarpine hydrochloride under intraperitoneal anesthesia one week after operation. In these animals, salivary outflow was determined for 30 minutes after pilocarpine treatment.

Salivary outflow was measured by subtracting the weight of a cotton ball after insertion into the oral cavity from the predetermined weight of the same cotton ball. Water consumption was monitored for 5 weeks after operation by determining the decrease of water in the bottle on each occasion of water replenishment using a 50 ml Terumo syringe® (Terumo, Tokyo).

3. Macroscopic observation of periodontal tissue

With the animal's mouth kept open with a small animal mouth opener (Yamaura, Tokyo), the oral cavity was photographed with a Medical Nikkol (Nikon, Tokyo). The picture was macroscopically evaluated for plaque and calculus attachment and inflammation-associated morphological changes in the gum. Based on these observations, the degree of periodontal tissue destruction was assessed.

Table 1 Salivary flow (1 week after operation)

Group	Ligation of the salivary glands	Salivary outflow (30 min. collection) Mean $\pm$ Standard Deviation (gram)
I + II	non	0.968 $\pm$ 0.243
III	SM	0.793 $\pm$ 0.138 *
IV	PT, SL	0.542 $\pm$ 0.081
V	SM, PT, SL	0.035 $\pm$ 0.016

SM : Submandibular glands, SL : Sublingual glands, PT : Parotid glands

* : $P < 0.05$, ** : $P < 0.01$, *** : $P < 0.001$ (t -test)

4. Soft radiography

Before and 1, 3, 5 and 7 weeks after ligation or resection of the salivary gland ducts, intraoral soft X-ray pictures of the lower right alveolar bone were taken with a SOFRON type SRO-M50S device under general anesthesia (40 kVp, 2.5 mA and 20 sec.). Films were developed by the routine method⁶⁾.

5. Morphological analysis of the alveolar bone

Morphological analysis was done for the alveolar bone between the first premolar (M1) and the second premolar (M2).

From the alveolar crest, a line perpendicular to the tangent line of the M2's mesiodistal root was drawn, and its length was measured⁷⁾.

Results were expressed as the mean $\pm$ standard deviation and comparisons were made by Student's t -test.

Results

1. Body weight

One week after the operation, body weight of Groups I and II differed significantly from that of Group V ($P < 0.05$). 2 weeks after the operation, weight differed significantly between Group IV and V ($P < 0.05$). 4 weeks after the operation, the weight in Group I and II differed significantly from that in Group III ($P < 0.05$). No other significant inter-group difference in weight gain was observed.

2. Salivary flow and water consumption

Table 1 shows salivary flow in each group 1 week after salivary gland ligation. Significant difference in salivary flow was observed between Group I or II and Group IV ($P < 0.05$), between Group I or II and Group V ($P < 0.001$), between Group III and V ($P < 0.01$), and between Group IV and V ($P < 0.001$). The total water consumption during the 5 weeks after the operation was 968 ml for Group I, 980 ml for Group II, 714 ml for Group III, 962 ml for Group IV and 1869 ml for Group V. Thus, water consumption in Group V was about twice that in any other group.

3. Macroscopic observation of the periodontal tissue

Throughout the study period, no change in the periodontal tissue was observed in Groups I and II. In Groups III and IV, on the other hand, plaque attachment to 1/3 of the crown was seen 3 weeks after operation and redness and swelling of the interdental papilla and marginal gingiva were seen 5 weeks after operation (Fig. 1 and 2). In Group V, plaque attachment to about 2/3 of the crown was observed one week after the operation, calculus attachment to the toothneck and redness swelling of the interdental papilla and marginal gingiva at 3 weeks, inflammatory edema accompanied by redness at 5 weeks, and gingival retraction at 7 weeks (Fig. 3).

4. Soft X-ray findings

Fig. 1 Lower right molars in group III (5 weeks after operation).
Note redness and swelling of the interdental papilla and marginal gingiva (arrow).

Fig. 2 Lower right molars in group IV (5 weeks after operation).
Note redness and swelling of the interdental papilla and marginal gingiva (arrow).

Soft X-ray films, taken before and 7 weeks after the operation in each group, are shown in Fig. 4. In each group, clear soft X-ray pictures with a relatively high diagnostic value were obtained. In Groups I and II, no marked change was observed in the alveolar crest, and the trabecular bone of the interalveolar septum always showed a regular distribution of shadow. In Groups III, IV and V, trabecular bone thinning in the alveolar crest between M1 and M2 and in the alveolar crest mesial to M1 were observed 3 weeks after the operation. These three groups showed a more marked tendency of trabecular thinning in the alveolar crest at 5 weeks. Seven weeks after the operation, bone resorption affected about 1/3 of the alveolus in Group III, about 1/2 in Group IV and 2/3 in Group V.

Fig. 3 Lower right molars in Group V (7 weeks after operation).
Note gingival retraction (arrow).

5. Morphological analysis of the alveolar bone

Table 2 shows the values of the morphological parameters examined for each group. Before the operation, no significant inter-group difference was observed in the parameters. One week after the operation, the parameters differed significantly between Group I (2.166 mm) and Group V (2.380 mm) ($P < 0.05$). Three weeks after the operation, the parameters in Groups I (2.307 mm) differed significantly from that in Group IV (2.114 mm) and Group V (2.329 mm) ($P < 0.05$). Five weeks after the operation, a significant difference was observed between Group I (2.316 mm) and Group IV (2.094 mm) or Group V (2.318 mm) ($P < 0.01$). Seven weeks after the operation, the parameters differed significantly between Group I (2.358 mm) and Group IV (2.064 mm) ($P < 0.05$) and between Group I or Group II (2.393 mm) and Group V (2.300 mm) ($P < 0.01$).

No other significant inter-group difference was observed.

Fig. 5 shows percent variance of the parameter in each group relative to the value in Group I. One week after the operation, a slight increase was observed in Group II (+13.5%) and Group III (+35.1%), whereas a slight decrease was seen in Group IV (-0.5%) and Group V (-11.3%). Three weeks

Fig. 4 Soft X-ray films before (L) and 7 weeks after operation (R).
(a) Group I, (b) Group II, (c) Group III, (d) Group IV, (e) Group V

Table 2 Morphological parameters of the alveolar bone on different occasions

Period weeks	before	1 week	3 weeks	5 weeks	7 weeks
Group I	1.944±0.157	2.166±0.002	2.307±0.170	2.316±0.114	2.358±0.108
Group II	2.186±0.002	2.189±0.059	2.239±0.285	2.306±0.190	2.303±0.075
Group III	2.217±0.244	2.295±0.244 *	2.313±0.313 *	2.314±0.287 *	2.243±0.237 *
Group IV	2.119±0.143	2.118±0.190	2.114±0.139	2.094±0.045	2.064±0.178
Group V	2.405±0.125	2.380±0.074	2.329±0.140	2.318±0.102	2.300±0.013

Mean ± S.D. (mm)

* : $P < 0.05$, ** : $P < 0.01$ (t -test)

after the operation, the percentage was +14.6 % in Group II, +24.6 % in Group III, -1.4 % in Group IV and -20.9 % in Group V. Five weeks after the operation, it was +32.3 % in Group II, +17.5 % in Group III, -6.7 % in Group IV and -23.4 % in Group V. Seven weeks after the operation, it was +50.0 %, +6.3 %, -13.3 % and -25.4 %, respectively.

Discussion

A variety of basic studies in small animals have been carried out for the purpose of clarifying the etiology and pathophysiology of periodontal diseases^{6,8-11)}. Among others, the experimental system of Keyes *et al.*, which involves feeding golden hamsters with sucrose-rich (56 %) diet #2000²⁾, has been frequently used as a caries-inducing diet procedure. However, it is questionable whether or not this diet is valid as a caries-inducing diet, for it has been reported that a diet containing 15-16 % sucrose induced abundant plaque attachment, accompanied by inflammatory reaction and alveolar bone resorption^{3,4)}. In view of these reports, it seems possible to induce periodontal diseases by using a diet containing sucrose.

When we compare the past methods of inducing periodontal diseases in experimental animals, many methods induced periodontal diseases by causing an infection with a particular pathogen. In practice, however, periodontal diseases are not attributable to a single extrinsic pathogen but to an imbalance in the bacterial flora in individual animals. In this sense, the past experimental methods need to be reviewed.

In the present study, we focused on the effect of saliva on the oral environment and suppressed the cleansing and buffering functions of saliva by ligating or resecting the salivary gland ducts. In animals after such operations, periodontal diseases were induced with a sucrose-poor diet (sucrose content : 10 %).

Aging is a factor involved in the progression of periodontal diseases. In this connection, Tsurumoto *et al.*¹¹⁾ reported that an increase in bone resorption at the age of 17-25 weeks is explained by an age-related decrease in resistant factors and formation of pockets. In this age range, alveolar bone growth reaches a peak, and physiological bone resorption begins. Therefore, if hamsters in this age range are affected by periodontal diseases, alveolar bone resorption will be accelerated. This view is supported by long-term observation of the alveolus of the same hamsters, in which alveolar bone growth reached a peak

Fig. 5 Percent variance in the morphological parameters relative to the value for Group I on different occasions.
Symbols : Group II (-O-), Group III (-●-), Group IV (-△-), Group V (-▲-)

at age about 23 weeks of age and decased thereafter. Therefore, this factor should also be considered in studying age-related progression of periodontal diseases.

When the influence of salivary gland ligation or resection on body weight, salivary secretion and water consumption was examined in the present study, the operated animals (Groups III, IV and V) showed a transient tendency for weight loss, which was significant for Group V.

In analysis of the influence of operations on salivary secretion, salivary outflow was reduced by about 17.7 % in Group III (after resection of the submandibular gland) and by about 44 % in Group V (after parotid gland ligation and sublingual gland resection) as compared to Groups I and II (sham operation groups).

These results may be explained by the anatomical features of the hamster salivary gland system, which consists of three pairs of large and small salivary glands, i.e., the parotid glands (serous), the submandibular glands (sero-mucous) and the major sublingual glands (mucous). It is likely that the action of pilocarpine on the parasympathetic nerves stimulated the secretory activity of the serous salivary glands, and that the decreased saliva secretion from the operation glands was partially compensated for by the other large salivary glands.

Analysis of the influence of the operations on water consumption revealed an approximately doubled water consumption in Group V as compared to the other groups. This increase in water consumption in Group V may be interpreted as a behavior to compensate for the decreased salivary secretion. In this group, macroscopic observation of the oral cavity revealed abundant plaque attachment and signs of periodontal inflammation, and soff radiography disclosed marked bone resorption. These findings suggest that the increase in water consumption does not greatly affect oral cavity condition. Throughout the study period, no caries developed in any group.

From these results, we conclude that ligation of the bilateral parotid gland ducts and resection of the submandibular and sublingual glands (as done in Group V) are most appropriate for a short-term experimental study of alveolar bone resorption, and that ligation of the bilateral parotid gland ducts and resection of the sublingual gland are optimal for a long-term experimental study of alveolar bone resorption.

In the future, the time course of normal microbial flora and alveolar mineral content need to be studied in relation to bone resorption.

Conclusion

In animals after ligation or resection of the salivary glands, we attempted to induce periodontal diseases with a sucrose-poor diet, and observed by observation of the time course of alveolar bone resorption intraoral soft radiography. The following results were obtained.

1. After ligation and resection of salivary gland ducts, periodontal diseases could be induced with a sucrose-poor diet.
2. After the operation, temporary weight loss was observed in Groups III, IV and V, although this change was only significant in Group V as compared to the sham operation groups.
3. After the operation, salivary outflow differed significantly between Group IV and Group I or II, between Group III and V and between Group IV and V. In Group V, water consumption after the operation was about twice that for any other group.
4. These results indicate that ligation of the bilateral parotid gland ducts and resection of the submandibular and sublingual glands (as done in Group V) are useful for a short-term experiments on alveolar bone resorption, and that bilateral ligation of the parotid gland ducts and resection of the sublingual gland are useful for a long-term experimental study of alveolar bone resorption.

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