Original Research

An Experimental Study on the Behavior of Merkel Cells under a Denture Base

Ishizaki Ken*, DDS, Sakurai Kaoru*, DDS, PhD, Tazaki Yuki**, DDS, PhD,
Tazaki Masakazu**, DDS, PhD, Shimono Masaki***, DDS, PhD, Suzuki Yuta†, DDS,
Matsuzaka Kenichi††, DDS, PhD and Inoue Takashi††, DDS, PhD

**Department of Complete Denture Prosthodontics, Tokyo Dental College*

***Oral Health Science Center and Department of Physiology, Tokyo Dental College*

****Oral Health Science Center and Department of Pathology, Tokyo Dental College*

†Graduate School and Department of Crown and Bridge Prosthodontics, Tokyo Dental College

††Oral Health Science Center and Department of Clinical Pathophysiology, Tokyo Dental College

Clinical significance

The number of Merkel cells in the palatal rugae had decreased because of the covering by denture base, which may suggest that it is necessary to consider the design of prosthetic appliances at palatal area in terms of the sensory system.

ABSTRACT

Purpose : The purpose of this study was to investigate Merkel cell behavior under the condition of continuous pressure by a denture base.

Materials and Methods : A denture base made of 4-META/MMA-TBB adhesive resin was applied on the palatal rugae of golden hamsters, and the animals were sacrificed 2 and 3 weeks later.

Results : Immunohistochemically, an aggregation of CK 20-positive cells was seen at the tip of the rugae in the control groups. However, a significant decrease in CK 20-positive cells was observed in the experimental groups at each of the time periods.

Conclusion : These results suggest that the Merkel cells became apoptotic under continuous pressure and that the sensory system might have changed.

Key words

Merkel cell, palatal ruga, denture base

Corresponding author : Ishizaki Ken

1-2-2, Masago, Mihama-ku, Chiba 261-8502, Japan

Tel : +81-43-270-3933, Fax : +81-43-270-3935

E-mail : ishizaki@tdc.ac.jp

Received on March 11, 2003/Accepted on June 17, 2003

INTRODUCTION

Merkel cells were first identified in the epithelium of human skin by Merkel¹⁾ (1875), and are mechanoreceptors that perceive the sense of pressure by synaptic connections to nerve fibers. Merkel cells exist in the labial²⁾ or in the palatal mucosa³⁾, which are highly sensitive areas in the oral cavities of mammals. A particularly large number of Merkel cells are found in the palatal rugae⁴⁾ that participate in ingestion, mastication and articulation⁵⁾.

Currently, prosthetic appliances of the palate are usually selected from the viewpoint of stability. However, no studies have explained the behavior of Merkel cells of the palatal mucosa covered with a denture base.

The purpose of this study was to investigate Merkel cell behavior under the condition of continuous pressure from a denture base using immunohistochemistry.

MATERIALS AND METHODS

Twenty male golden hamsters, 80 g in body weight, were used in this study. All animal experiments conformed to the Guidelines for the Treatment of Experimental Animals at the Tokyo Dental College (approval number 01-11). Animals were anesthetized by intra-abdominal injection of 0.2 ml/100 g of sodium pentobarbital (Ketalar, Sankyo, Tokyo, Japan). 4-META/MMA-TBB adhesive resin (Super-Bond C&B, Sun Medical, Shiga, Japan) was applied to the surface of the palatal mucosa of each animal using the heaping up method and was also applied to the undercut around the buccal-gingival area to fix the resin on the palatal mucosa (Fig. 1). In order to apply continuous pressure to the mucosa, a 10 g weight was placed on the palatal base immediately after application

of the adhesive resin, and was removed after the resin was polymerized.

Six animals each were sacrificed at 2 and 3 weeks after the application of the resin plate. The palatal mucosa with the resin plate was removed and fixed in 10% neutral formalin solution for 3 days at room temperature. Subsequently, the specimens were decalcified with 10% tetrasodium ethylenediaminetetraacetate (EDTA, pH 7.2) for 2 weeks at room temperature before being embedded in paraffin. Serial paraffin sections (5 μ m thick) were cut at right angles to each of the rugae and were stained with hematoxylin and eosin.

For immunohistochemical staining, the streptavidin-biotinyl immunoperoxidase method was employed. Paraffin sections were cut in exactly the same manner as described above. Endogenous peroxidase was blocked with a 0.3% solution of hydrogen peroxide in methanol for 30 min. The sections were then incubated with a monoclonal mouse anti-CK 20 antibody, which reacts with 46-Kda cytokeratin (found in Merkel cells), at a dilution of 1 : 100 in normal goat serum. The sections were subsequently incubated for 30 min each with biotinylated secondary antibody and streptavidin peroxidase reagents. Finally, the presence of bound peroxidase was visualized by the diaminobenzidine reaction. A brown coloration indicated a positive reaction.

Fig. 1 a) Surface of the palatal mucosa
b) Covered by resin plate

Fig. 2 Merkel cell positive ratio was measured in the sections

Each section was photographed at a magnification of $40\times$ and was further enlarged $8.5\times$ with a Pictostat 200 (Fuji Photo Film, Tokyo, Japan). The numbers of basal cells and CK 20-positive cells in each palatal rugae were counted; the Merkel cell positive ratio = CK 20-positive cells/cells in the ruga $\times 100$ (Fig. 2). Data were analyzed statistically by the Levene test, and the criterion for statistical significance was $p < 0.05$.

RESULTS

Light Microscopic Observations

1. Control group

Merkel cells were recognized as clear cells in the basal layer of the stratified keratinized squamous epithelium, and were positive for CK 20. These CK 20-positive cells were aggregated in the tips of the rugae (Fig. 3). There were no inflammatory cell infiltrations in the underlying fibrous connective tissue.

2. Experimental group

Cells in the stratified keratinized squamous epithelium showed intra-cellular edema with a thickened keratinized layer at 2 and at 3 weeks after the application of the resin. The basal membrane was extremely flat with no epithelial ridges. The number of CK 20-positive cells had decreased, and positive reactions for CK 20 were weaker compared with the control groups (Fig.

Fig. 3 CK 20-positive cells aggregated in the tips of 4th ruga of control group

Fig. 4 Decrease in the number of CK 20-positive cells in 4th ruga 2 weeks after the experiment
Positive reactions for CK 20 antibody were weaker compared with the control groups

4). No inflammatory cell infiltration was observed in the underlying fibrous connective tissue with slight fibrosis.

Statistical Analysis (Fig. 5)

1. Control group

The Merkel cell positive ratio of the 1st ruga was 8.5 ± 0.5 , the 2nd ruga was 6.9 ± 0.5 , the 3rd ruga was 13.1 ± 7.8 , and the 4th ruga was 24.7 ± 0.9 . The Merkel cell positive ratio of the 4th ruga was significantly higher than the others. However, there were no significant differences among the 1st, 2nd and 3rd rugae.

2. Experimental group

Two weeks after the application of the resin plate, the Merkel cell positive ratio of the 1st

Fig. 5 Merkel cell positive ratio of each ruga for both control and experimental groups

ruga was 0.1 ± 0.03 , the 2nd ruga was 0.8 ± 0.1 , the 3rd ruga was 1.1 ± 0.2 , and the 4th ruga was 3.4 ± 0.7 .

Three weeks after the resin plate application, the Merkel cell positive ratio of the 1st ruga was 0.2 ± 0.1 , the 2nd ruga was 0.8 ± 0.3 , the 3rd ruga was 1.4 ± 0.5 , and the 4th ruga was 2.8 ± 0.3 .

The Merkel cell positive ratios of all rugae in both experimental groups were significantly lower than those of the control groups. However, there were no significant differences between any of the rugae at any of the time periods at 2 or at 3 weeks after the application of the resin plate.

DISCUSSION

The numbers of Merkel cells in the experimental groups were significantly lower than those of the control groups, which suggests that the continuous pressure of a palatal plate induces the death of Merkel cells. Apoptosis is known as an active type of cell death programmed genetically, and generally it is recognized to occur in incidence, differentiation, maturation,

and aging without local inflammatory reaction. Agar et al.⁶⁾ also showed that apoptosis was induced by ischemia, and by mechanical stimulation of various kinds of drugs and other stimuli.

In contrast, necrosis is defined as cell death that is generated during local inflammation. In our experiments, no inflammatory reactions were observed in the experimental group, and thus, we suggest that the Merkel cells became apoptotic.

Kurosumi et al.⁷⁾ reported that Merkel cells are nourished from nerves, because they decrease or disappear when nerves are cut. Suzuki et al.⁸⁾ suggested that Merkel cells do not regenerate during wound healing. This observation may mean that continuous mechanical stimulation with a palatal plate produces a circular disturbance that elicits nerve fiber necrosis, which may lead to the death of Merkel cells.

From the mention above, Merkel cell numbers decreased because of apoptosis and the sense of pressure at denture underlying tissue might have changed.

In daily clinical practice, impressions are commonly observed in the palatal mucosa, which is considered to be the result of long-term denture use. Based on the results obtained in this study,

it is possible to predict that continuous pressure over the palatal mucosa could lead to damage of the mechanoreceptors. It is necessary to keep in mind the importance of appropriate impression methods, denture design and maintenance of the denture.

REFERENCES

- 1) Merkel F. Tastzellen und Tastkörperchen. Bei den Haustieren und beim Menschen. Arch Microsk Anat 11 : 636-652, 1875.
- 2) Tachibana T, Nawa T. Merkel cell differentiation in the labial mucous epithelium of the rabbit. J Anat 131 : 145-155, 1980.
- 3) Garant PR, Feldman J, Cho MI et al. Ultrastructure of Merkel cells in the hard palate of the squirrel monkey (*Saimiri sciureus*). Am J Anat 157 : 155-168, 1980.
- 4) Liem RS, van Willigen JD, Jansen HW. Merkel cell neurite complexes in the hard palate of the wistar rat (*Rattus norvegicus*). Arch Oral Biol 29 : 249-251, 1984.
- 5) Luke DA. Development and growth of palatal rugae in the mouse. Acta Anat 133 : 41-44, 1988.
- 6) Agar A, Yip SS, Hill MA et al. Pressure related apoptosis in neuronal cell lines. J Neurosci Res 60 : 495-503, 2000.
- 7) Kurosumi K, Kurosumi U, Inoue K. Morphological and morphometric studies with the electron microscope on the Merkel cells and associated nerve terminals of normal and denervated skin. Arch Histol Jpn 42 : 243-261, 1979.
- 8) Suzuki Y, Sato T, Inoue T et al. A study on characteristics of the peri-implant epithelium in hamster palatine mucosa ; behavior of Merkel cell and nerve ending. Clin Oral Implants Res (submitted)