Archives of Oral Biology 96 (2018) 39–45

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Recovery of atrophic parotid glands in rats fed a liquid diet by switching to a pellet diet

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ARTICLE INFO

Keywords:
Parotid gland
Recovery
Liquid diet
Atrophy

ABSTRACT

Objective: In this study, we aimed to clarify how parotid glands, made atrophic by a liquid diet, recover after diet change.

Design: Seven-week-old male Wistar rats were fed a pellet (control group) or a liquid diet (experimental group) for the first 14 days. Thereafter, all animals were fed a pellet diet for up to 14 days (days 0–14). The parotid glands were removed, weighed and examined histologically and ultrastructurally. Immunohistochemistry was performed for BrdU, a marker of proliferating cells, and Casp-3, a marker of apoptotic cells.

Results: Feeding of a liquid diet for 14 days induced atrophy of the parotid glands. Histologically, acinar cells were small on day 0, compared with the control group. After changing the diet from liquid to pellet form, acinar cells increased in size over time, recovering nearly fully by day 7. Many BrdU-positive acinar cells were observed in the glands in the experimental group on days 1 and 3. Although more acinar cells were Casp-3-positive compared with the control group on day 0, there was no difference between the two groups after the diet change. Ultrastructurally, the cellular organelles did not exhibit a substantial alteration, except for an increase in secretory granules following diet change.

Conclusions: Our findings suggest that atrophic parotid glands are able to recover to their normal size by switching the diet from liquid to pellet form and that an increase in both the size and number of acinar cells plays an important role in this recovery process.

1. Introduction

Many experimental studies have shown that continuous intake of soft food, which is currently a popular dietary trend, negatively influences oral maxillofacial regions such as the jaw bones (Bozzini, Picasso, Champin, Bozzini, & Alippi, 2015; Hichijo et al., 2014; Shimizu et al., 2013), masticatory muscles (Fujishita et al., 2015; Kawai et al., 2010; Kitagawa et al., 2004) and temporomandibular joints (Kato, Takahashi, & Domon, 2015; Kiliaridis, Thilander, Kjellberg, Topouzelis, & Zafiriadis, 1999; Uekita, Takahashi, Domon, & Yamaguchi, 2015). The effect of a liquid diet on salivary glands has been investigated in animals in several reports. These studies have revealed differences in the response to a liquid diet among the three major salivary glands. While a liquid diet induces strong atrophy of the parotid glands (Hall & Schneyer, 1964; Kurahashi & Inomata, 1999; Kurahashi, 2002; Nakamura, 1997; Scott & Gunn, 1991; Takahashi et al., 2012; Takahashi, Uekita, Kato, Inoue, & Domon, 2015; Wilborn & Schneyer, 1970), its effect on the submandibular and sublingual glands is negligible or very limited (Kurahashi & Inomata, 1999; Kurahashi, 2002; Nakamura, 1997; Scott & Gunn, 1991; Takahashi et al., 2014; Takahashi, Uekita, Taniwaki, & Domon, 2017). It has been reported that acinar cell shrinkage (Hall & Schneyer, 1964; Hand & Ho, 1981; Scott & Gunn, 1991; Scott, Berry, Gunn, & Woods, 1990; Takahashi et al., 2012; Wilborn & Schneyer, 1970), reduction of proliferative activity of acinar cells (Takahashi et al., 2012) and an increase in apoptotic acinar cells (ElGhamrawy, 2015; Takahashi et al., 2012) play important roles in parotid gland atrophy.

As salivary glands are important for the health of the oral cavity, an important question from the standpoint of clinical dentistry is whether atrophic parotid glands are able to recover following a change from a liquid to a pellet diet. However, there are only a few reports addressing this question (Hall & Schneyer, 1964; Nakamura, 1997; Schneyer & Hall, 1975). Of these, Hall and Schneyer (1964) and Nakamura (1997) found that the atrophic parotid glands recovered their weight after diet

Abbreviations: HE, hematoxylin and eosin; PAS, periodic acid Schiff; BrdU, 5-bromo-2′-deoxyuridine; Casp-3, cleaved caspase-3

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https://doi.org/10.1016/j.archoralbio.2018.08.015
Received 4 July 2018; Received in revised form 22 August 2018; Accepted 24 August 2018
0003-9969/

change. Hall and Schneyer (1964) and Schneyer and Hall (1975) counted acinar cells per a field under a light microscope and found that the number of acinar cells per a field decreased after changing the diet from liquid to pellet form, suggesting that acinar cells become larger following diet change. They also counted the mitotic figures of acinar cells and reported that they were most numerous on day 2 after diet change (Schneyer & Hall, 1975). These observations suggest that acinar cells recover their size and increase in number after diet change.

The methods used in the above studies were appropriate in those days, and their observations were valuable. However, there seems to be some problems requiring additional examinations from the point of view of the present studies. In these older studies, the area of the individual acinar cells was not measured, and it is difficult to exclude completely the area of other parenchymal cells and stromal components from the observed field. Therefore, the measurement of the individual area of the acinar cells with an image analysis system is necessary to confirm the recovery of acinar cells in size. In addition, mitotic figures are difficult to quantify because the proliferative stage (M-phase) is very short (Yu, Woods, & Levison, 1992). Currently, immunohistochemical methods that identify the cycling cells in other phases of the cell cycle are generally used to assess proliferation (Hall & Woods, 1990; Liu & Klein-Szanto, 2000; Yu et al., 1992).

It has been reported that apoptosis takes place in progressive (Fan, Kren, & Steer, 1998; Taira, Hiroyasu, Shiraishi, Muto, & Koji, 2001; Takahashi, Nakamura, Domon, Yamamoto, & Wakita, 2005) as well as in regressive (Takahashi et al., 2000; Walker, 1987; Walker, Bennett, & Kerr, 1989) changes of some exocrine glands. Because apoptosis is an important factor in the regulation of cell populations (Kerr, Wyllie, & Currie, 1972), it is desirable to examine apoptosis in acinar cells in atrophic parotid glands undergoing recovery after diet change.

In the present study, we aimed to clarify how atrophic parotid glands in rats fed a liquid diet by switching to a pellet diet recovered. For this purpose, those parotid glands were examined using histological analysis, histomorphometric analysis with an image-analysis system, immunohistochemical analysis with BrdU, a marker of proliferating cells, and Casp-3, a marker of apoptotic cells, and ultrastructural analysis with a transmission electron microscope.

2. Materials and methods

2.1. Animals

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals of Hokkaido University and was approved by the Laboratory Animal Committee of Hokkaido University (Approval No.13-0206).

Forty male Wistar rats aged 7 weeks (CLEA Japan Inc., Tokyo, Japan) were maintained in a temperature-controlled room (about 22 °C) with a 12 h light/dark cycle, with ad libitum access to drinking water. The animals were weighed, and their health status was checked daily by visual observation during the course of the experiments.

2.2. Experimental protocol

The animals were divided into control (n = 20) and experimental (n = 20) groups. For the first 14 days, rats in the control group were given a pellet diet (25 g/day; Labo MR Standard, Nosan Corp., Yokohama, Japan), while those in the experimental group were given a liquid diet prepared daily by mixing 25 g of the powdered form of the pellet diet with 50 mL water. Subsequently, both groups were fed a pellet diet for 0 (day 0), 1, 3, 7 or 14 days (n = 4 for both groups at each time point). For each time point, the rats were fasted for 12 h at night to synchronize the state of storage of acinar secretory granules and then perfused with 4% formaldehyde prepared by dissolving paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) under pentobarbital anesthesia, 1 h after administration of BrdU at a dose of 25 mg/kg body weight by intraperitoneal injection (Sigma-Aldrich, St. Louis, MO). Then, the right parotid glands were excised and weighed. A small portion of each gland was set aside for transmission electron microscopy, and the major part of each gland was used for histological and immunohistochemical examination.

2.3. Histological analysis

The samples were placed in the 4% formaldehyde prepared by dissolving paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 24 h, routinely processed, and embedded in paraffin. Then, 4 μm sections were cut, stained with HE and PAS, and observed with a light microscope.

Histomorphometric analysis was performed to determine individual acinar cell area. Three HE-stained sections from each animal were used, and approximately 1000 acinar cells were measured in each section by the image-analysis system (DS-L2, Nikon, Tokyo, Japan). The mean of the data from 3 sections was calculated, and used as the representative value for that animal (n = 4).

2.4. Immunohistochemical analysis

Deparaffinized sections were immersed in 0.3% hydrogen peroxide in methanol to inhibit endogenous peroxidase. The sections for BrdU staining, to detect proliferating cells, were incubated with 0.1% trypsin for 20 min at 37 °C and later with 3 N HCl for 10 min at 37 °C. After pretreatment, the sections were reacted with anti-BrdU mouse monoclonal antibody (Bu-20a, DakoCytomation, Glostrup, Denmark) at a dilution of 1/50 for 2 h, and then with biotinylated rabbit anti-mouse polyclonal antibody (DakoCytomation) for 1 h at a dilution of 1/100, in turn. The sections for Casp-3 staining, to detect apoptotic cells, were boiled in 10 mM Tris/1 mM EDTA buffer (pH 8.8) for 15 min. After antigen retrieval, the sections were reacted with anti-Casp-3 rabbit polyclonal antibody (Asp 175, Biocare Medical, Concord, CA) at a dilution of 1/20 overnight at 4 °C, and then with biotinylated swine anti-rabbit polyclonal antibody (DakoCytomation) at a dilution of 1/100 for 1 h. Then, the sections for both BrdU and Casp-3 staining were incubated with peroxidase conjugated streptavidin (Histofine, Nichirei Bioscience, Tokyo, Japan) for 30 min. Peroxidase activity was visualized with 3, 3′-diaminobenzidine, and lightly counterstained with Mayer's hematoxylin. For the respective negative controls, normal mouse serum was used in place of the anti-BrdU primary, while rabbit serum was used in place of the anti-Casp-3 primary.

After immunohistochemical staining, approximately 1000 acinar cells were observed to calculate the percentages of BrdU and Casp-3-posive acinar cells. The mean of the labeling indices of three sections was taken as the representative value for that animal (n = 4).

2.5. Statistical analysis

Quantitative data such as body weight, parotid gland weight, individual acinar cell area and labeling indices for BrdU and Casp-3-posive acinar cells, were expressed as medians and ranges in box plots for four control and four experimental animals. Differences between control and experimental groups were assessed with the Mann-Whitney U-test (Ystat2008, Igakutosho, Tokyo, Japan). $P < 0.05$ was considered to indicate a significant difference.

2.6. Ultrastructural analysis

The specimens for transmission electron microscopy were immersed in 2% formaldehyde prepared by dissolving paraformaldehyde-1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 h, and then post-fixed in 1% osmium tetroxide for 2 h. After fixation, the tissues were stained *en bloc* with 4% uranyl acetate, and embedded in Epon 812. Semithin sections were cut and stained with toluidine blue to