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Analysis of age-related changes in the functional morphologies of salivary glands in mice

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ABSTRACT

Background and Objectives: Salivary glands in the elderly commonly exhibit salivary dysfunction resulting dry mouth, poor oral hygiene, and dental caries. However, in vivo changes of salivary glands during aging have not been well documented in the literature. This study was undertaken to determine age-related morphometric and functional changes of salivary glands using an aging mouse model.

Methods: Male C57BL/6 mice were divided into three groups, group A (10 weeks old; $n = 10$), group B (30 weeks old; $n = 10$), and group C (90 weeks old; $n = 10$). Body weights, salivary gland weights, salivary flow rates, and salivary lag times were measured and compared. Histomorphometric examinations and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays were performed. In addition, changes in salivary uptake and excretion were observed by single-photon emission computed tomography (SPECT).

Results: Body and gland weights increased with age. Gland weight was significantly higher in group B than in groups A and C. Salivary lag time was significantly greater in group C than in groups A and B, and salivary flow rate was significantly greater in group B than in groups A and C. Histologic evaluations exhibited acinar cell atrophy, cytoplasmic vacuolization, lymphocyte infiltration, small mucin component and more periductal fibrosis in salivary glands of group C. TUNEL assays revealed that apoptotic salivary epithelial cells were significantly more numerous in group C than in groups A and B. ^{99m}Tc-pertechnetate excretion rate was significantly lower in group C than in groups A and B in SPECT.

Conclusion: Various morphometric and histopathological changes were observed in the salivary glands of aging mouse as well as relevant functional alterations, such as, decreased saliva production and excretion. Increased number of apoptotic salivary epithelial cells may contribute to the observed functional deterioration.

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1. Introduction

The function of the salivary glands is to produce saliva, which is crucial for digestion, taste, the maintenance of tooth integrity, oral clearance, lubrication, and protection against infection. Ageing is a normal physiological phenomenon that affects almost all organs including the glands.[1] Furthermore, it is known that age-related morphometric and functional changes in salivary glands reduce salivary functions. Reduced salivation leading to complaints of dry mouth (xerostomia) is common in older people. In fact, 25∼30% of the elderly (aged > 65 years) suffer from a dry mouth (xerostomia).[2] Age-related salivary gland dysfunction also commonly involves sialadenitis, stomatitis, oral candidiasis, and dental caries, which severely impair quality of life.[3] Age related changes in the glands have been studied in humans[4–11] and animals[5,12,13], but it has not been fully determined how ageing influences the function of salivary glands. Some literature on human salivary glands was obtained from post mortem studies.[4,6,8–10,14] In these studies, histologic examinations demonstrated that during ageing, the parenchyma of salivary glands is gradually replaced by fat, connective tissue, and oncocytes.[4,6,15,16] Syrjänen also reported that histologic examinations of labial salivary glands in healthy elderly individuals showed acinar atrophy, ductal dilatation, fatty change, fibrosis, periacinar callus formation and inflammatory infiltration.[17] Some functional studies on healthy individuals show that ageing does not diminish the ability of salivary glands to produce saliva.[2] On the other hand, some studies show that there may be a progressive, though minor loss, in salivary flow from the glands.[15,18] To date, this apparent paradox regarding morphometric and functional changes has not been explained. Furthermore, the effects of ageing on mouse salivary glands are unclear, and no age-related morphologic evaluation or functional study has been conducted. Therefore, the aim of this study was to evaluate age-related morphometric and functional salivary gland changes in a murine model, and to provide further baseline information for preclinical research on salivary gland dysfunction related to ageing.

2. Materials and methods

2.1. Animal model

10- to 90-week-old male C57BL/6 mice were purchased from the Research Model Producing Centre (Orient Bio, Gyeonggi-Do, Korea) for the study. Animals were housed in a temperature, humidity, and light controlled environment on a standard mouse diet with free access to water. Mice were allocated to three groups: group A (the young group, 10 weeks old; $n = 10$), group B (the adult group, 30 weeks old; $n = 10$) and group C (the old group, 90 weeks old; $n = 10$). The protocol used in this study was approved by the Animal Ethics Committee of Inha University Hospital.

2.2. Measurements of mouse body weights, salivary gland weights, salivary lag times, and salivary flow rates

Mice were premedicated with xylazine (10 mg/kg) and anesthetized with an intraperitoneal (i.p.) injection of ketamine (110 mg/kg). Each mouse was positioned upside down after stimulation with pilocarpine (2 mg/kg, i.p.). When the floor of mouth was filled with saliva, we collected the saliva using a micropipette for 5 min and placed in preweighed 0.75 ml Eppendorf tubes. To reduce the effects of diurnal variation, saliva was collected at 2:00 p.m. Salivary flow rate (μl/min) was calculated as total saliva weight (mg) divided by the collection time (min) (saliva has a specific gravity of 1 mg/ml). We defined 'lag time' as the time from pilocarpine administration until saliva flow began. Salivation was determined by visual observation of the floor of the mouth, and 'lag times' were measured in seconds using a stopwatch. After saliva collection, mice were humanely euthanized and skins were removed. Submaxillary glands located at both sides of the trachea were surrounded by fat and connective tissues. Fatty lymph nodes and sublingual glands were located over the submaxillary gland. Laterally, parotid glands were observed above the external jugular vein. Because of their light weights, submaxillary glands were harvested carefully and weighed using a semi-micro analytical balance.

2.3. Morphological analysis of tissues and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay

Salivary glands were immediately placed in 4% paraformaldehyde, embedded in paraffin, sectioned at 4 μm, and stained with haematoxylin and eosin (H–E), alcian blue, and Masson's Trichrome (MT) using standard pathology department protocols.

Apoptosis in these gland tissues was determined using a TUNEL assay using an ApopTag Plus In Situ Apoptosis Kit (Chemicon Int., Temecula, CA). Briefly, after deparaffinization and rehydration, slides were incubated with a TUNEL reaction mixture containing TdT enzyme for 1 h at 37 °C and then with anti-digoxigenin fluorescein for 30 min at room temperature. Nuclei were visualized with DAPI. TUNEL-positive cells were detected. Two examiners unaware of experiment details independently counted absolute numbers of apoptotic cells in three random fields per tissue section.

2.4. Single-photon emission computed tomography (SPECT) protocol

Technetium pertechnetate (55.5 MBq, [^{99m}Tc] TcO_4^-; New Korea Industrial) was administered i.p. to anesthetized mice. Mice were maintained in an unconscious state using isoflurane gas (2 vol % in air) during the entire imaging protocol. Whole-body SPECT imaging was started immediately after [^{99m}Tc]TcO_4^- administration and repeated every 5 min up to 100 min (NanoSPECT, Bioscan, USA). Overall, 21 images were obtained per mouse. Pilocarpine (2 mg/kg body weight, i.p.) was administered 60 min after SPECT.

Whole body SPECT images of each mouse were obtained using a large field-of-view rotating gamma camera equipped with four multi-pinhole collimators (Fig. 1A). The acquisition parameters used were; 24 projections over 360°, circular orbit, and a total acquisition time of 6 min (4 s per projection). Tomographic images were reconstructed using an iterative reconstruction algorithm).[19,20]