Future Dental Journal 2 (2016) 22–27

Contents lists available at ScienceDirect

Future Dental Journal

journal homepage: http://www.journals.elsevier.com/locate/fdj

Capsaicin induced histological and ultrastructural changes in the submandibular salivary gland of albino rats

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ARTICLE INFO

Article history:
Received 19 January 2016
Accepted 4 April 2016
Available online 7 May 2016

ABSTRACT

Capsaicin is a pungent principle of hot red pepper. It is used in spices, food additives and drugs. In the present work, twenty rats were divided into two groups: control and capsaicin groups, each consisting of ten rats. The capsaicin group daily received a capsaicin dose equivalent to 0.1 mg/kg body weight dissolved in 0.5 ml distilled water by oro-oesophageal tube while the control group daily received 0.5 ml distilled water. After twenty one days, the submandibular salivary glands of both sides were excised, processed and examined histologically and ultrastructurally. Histological results revealed presence of pure mucous acini in the submandibular salivary gland. Some granular convoluted tubules showed degeneration while the excretory ducts showed loss of pseudostratification with the appearance of some flattened lining cells. Concerning the ultrastructural findings, some acinar cells showed dilated rough endoplasmic reticulum, other presented ultrastructural features similar to mucous acini. Granular convoluted tubules cells showed some irregular, shrunken nuclei with condensed chromatin. Their secretory granules were less electrondense than the control and presented ill-defined and fused outlines. Some of the excretory duct lining cells showed apically displaced irregular nuclei. One to two rows of flattened epithelial cells were observed apical to the lining cells. Vacuolizations, mitochondrial swelling and loss of cristae were detected in cells of some acini, granular convoluted tubules and excretory ducts. Most intercalated and striated duct cells showed ultrastructural features similar to that of control group. However, the basal part of some striated duct cells presented variable grades of mitochondrial affection.

From the present work, it could be concluded that chronic capsaicin intake was associated with noticeable histological and ultrastructural changes in acini, granular convoluted tubules and excretory ducts of the SMSG in albino rats.

1. Introduction

Natural flavors are widely used in various foods, cosmetic and pharmaceutical products. These kinds of additives are applied as colors, preservatives, aroma and tasting agents. The large-scale use of certain food flavors requires accumulation of data on these substances [17]. Capsaicin is a pungent principle of hot red pepper. It is used in spices, food additives and drugs [19]. Red pepper (Capsicum frutescens L.) is widely used as a spice for flavoring foods, particularly in South- East Asian and Latin-American countries and one of its major active ingredients is capsaicin [1,4]. It is thought that when red pepper is consumed in excessive amounts, it leads to “gastric ulcers” in view of its irritant and likely acid secreting nature. However, investigations carried out recently by [16] on gastric ulcers revealed that capsaicin is not the cause for ulcer formation but just a co-factor and it was found not to stimulate but on the contrary, to inhibit acid secretion. On the other hand, the same researchers reported that capsaicin stimulates alkali, mucus secretion and particularly gastric mucosal blood flow helping prevention and healing of ulcers. Capsaicin acts by stimulating afferent neurons in the stomach and signals for protection against injury causing agents [12]. reported that Capsaicin in low concentration range (1–8 μg/mL, 100 mL) given by nasogastric tube before gastric injuries induced by ethanol or indomethacin could

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http://dx.doi.org/10.1016/j.fdj.2016.04.002

protect the stomach and this was attributed to stimulation of the sensory nerve endings. The effects of dietary capsaicin on the rat submandibular gland secretion were investigated by [9,10]. The researchers reported the induction of cystatin S substance in submandibular saliva and its contribution in enhancing ingestion of the capsaicin diet. Furthermore, it was suggested that dietary capsaicin could induce salivary cystatin either by stimulating the reflex arc involving the glossopharyngeal nerve [9] or by irritation of the oral mucosa [10]. Data on the effect of capsaicin on the submandibular salivary gland structure was scarce. So, the present study aimed to investigate the effect of capsaicin on the submandibular salivary gland of the albino rats histologically and ultrastructurally.

2. Material and methods

Twenty adult male albino rats (weighing about 250 ± 20 g. each) were used in this study. Animals were recorded in "The Medical Research Center", Faculty of Medicine, Ain Shams University and were housed in wire mesh dated cages. They were fed certified pelleted diet and tap water ad libitum. Temperature and humidity conditions were controlled as possible on housing the animals during the experimental period. The capsaicin used in this study was purchased from Sigma chemical co., St. Louis, Mo, USA. The animals were divided into two groups: Control and capsaicin groups. The control group consisted of ten rats that received 0.5 ml distilled water daily by oro-oesophageal tube. The capsaicin group consisted of ten rats that received a daily capsaicin dose equivalent to 0.1 mg/kg body weight [8] (which is equivalent to the average consumption dose for capsaicin in people of Thailand) dissolved in 0.5 ml distilled water by oro-oesophageal tube. After twenty one days, all rats were killed by cervical dislocation, and the submandibular salivary gland of both sides were excised. Both glands of each animal were processed, one for light microscopic examination and the other for transmission electron microscopic examination.

2.1. For light microscopic study

The glands were fixed in 10% neutral buffered formalin for 24 h, washed, dehydrated and cleared to be embedded in paraffin. Then, sections (4–5 microns thickness) were sliced and stained by hematoxylin and eosin (H & E) for light microscopic examination.

2.2. For transmission electron microscopic study

The glands were sliced into fragments and primarily fixed in buffered formaldehyde- glutaraldehyde solution overnight at 4 °C, post fixed in buffered 1% osmium tetroxide solution for 1.5 h. The tissue samples were dehydrated in ascending concentrations of ethanol, and embedded in low viscosity resin (spur). Semi-thin sections were obtained, stained with toluidine blue and examined under light microscope to choose areas of interest. Ultra-thin sections were cut, stained with uranyl acetate and lead citrate to be examined by transmission electron microscope (TEM) in the electron microscopic unit of the Veterinary Hospital of Armed Forces.

3. Results

3.1. Light microscopic results

3.1.1. Control group

Examination of H&E stained sections of the SMSG of control rats revealed that the gland is predominantly formed of seromucous acini, granular convoluted tubules (GCTs), intercalated, striated, and excretory ducts, and finally connective tissue stroma. The acini appeared more or less spherical in shape and consisted of pyramidal cells having a moderately basophilic cytoplasm and rounded basally situated nuclei (Fig. 1A). The intercalated ducts were hardly encountered, they were lined by short cuboidal cells having basophilic cytoplasm and centrally placed large rounded nuclei (Fig. 1A). The granular convoluted tubules (GCTs) were lined with tall columnar cells having large rounded basally situated nuclei, and apical eosinophilic granules (Fig. 1B). The striated ducts were lined by columnar cells with centrally placed rounded nuclei and intensely eosinophilic cytoplasm with basal striations (Fig. 1A). The excretory ducts were lined by pseudostratified columnar epithelium and were surrounded by fibrous connective tissue stroma usually accompanied by variable sized blood vessels (Fig. 1C).

3.1.2. Capsaicin group

Histological sections of the capsaicin group glands presented acinar cells having morphological outline and staining almost identical to those of the control group (Fig. 1D). In other areas, some acini appeared smaller in size with darkly stained nuclei, while other acini presented variable sized cytoplasmic vacuoles (Fig. 1E). And still some acini presented histological features resembling mucous acini (Fig. 1D). GCTs showed two different histological patterns, few of them were normal with regular granular content and basally situated nuclei (Fig. 1D). However, most GCTs showed degeneration and ill-defined cells outline, increased eosinophilia and clumping of granular content were also detected (Fig. 1E). The intercalated and striated ducts presented almost normal features (Fig. 1D). The excretory ducts tended to present an irregular outline, their epithelial lining were frequently seen projecting towards the lumen. In most areas of these ducts, there was loss of pseudostratification with appearance of some flattened cells. The epithelial lining cells showed deformed and apically displaced nuclei. Areas of hydropic degeneration within the epithelial lining of these ducts were sometimes detected (Fig. 1F).

3.2. Electron microscopic results

3.2.1. Control group

Ultrastructural examination of the control SMSG revealed that the acinar end pieces appeared to be formed of pyramidal cells with spheroidal nuclei having prominent nucleoli with peripheral chromatin distribution (open faced nucleus). These cells showed parallel arrays of rough endoplasmic reticulum (RER) in their basal part, few oval mitochondria and Golgi complex. Membrane bounded secretory granules of variable sizes and electrondensities occupied the cytoplasm lateral and apical to the nucleus (Fig. 2A). The intercalated ducts were lined by cuboidal cells with large centrally placed nuclei and rounded lumen. Few cisternae of RER, scattered mitochondria, few secretory granules were sometimes encountered. The GCTs were lined by tall columnar cells in which the apical two thirds were occupied by numerous well circumscribed membrane bounded electrondense secretory granules of variable size. The basal part of these cells contained rounded electron lucent nuclei surrounded by numerous mitochondria that were arranged near the basal plasma membranes and few cisternae of RER (Fig. 2B). Cells of the striated ducts were tall columnar with large or rounded centrally placed nuclei surrounded by few RER and Golgi complex. The basal part of these cells presented deep infoldings of the plasma membrane that were almost parallel to the long axis of the cell. The cytoplasmic processes resulting from the basal infoldings contained numerous rod shape mitochondria (Fig. 2C). The excretory ducts were lined by pseudostratified epithelium consisting of tall columnar cells and basal cells that didn't reach the lumen. The columnar cells presented open faced