THE EFFECTS OF SIALOADENECTOMY AND EPIDERMAL GROWTH FACTOR ON GINGIVAL TISSUE IN RATS: AN ULTRASTRUCTURAL STUDY

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ABSTRACT
Submandibular salivary glands (SMGs) synthesize, accumulate and secrete a large amount of epidermal growth factor (EGF) in rats. EGF stimulates cell proliferation and differentiation by binding to its reseptors (EGFR).
The aim of the present study was to mimic an endogenous epidermal growth factor deficiency through sialoadenectomy in parallel to exogenous EGF administration and to observe the ultrastructural changes in rat gingival tissue, employing electron microscopy.
Thirty adult female Wistar albino rats were divided randomly into three groups: a control group (n:10), a sialoadenectomy group (n:10) and a sialoadenectomy group to which an EGF was administered (n:10).
The experimental groups of rats were subjected to sialoadenectomy in order to create EGF deficiency. After 27 days both control and experimental rat groups were euthanized by pentobarbital and their gingival tissue was removed. Tissue samples from gingiva were processed for ultrastructural study.
Electron microscopic evaluation indicated that while in the control group gingival tissues showed a normal appearance changes were observed in the experimental groups. We observed a partial degeneration of the epithelial cell junctions. Widespread crystolisis was also observed in a group of mitochondria.
It is concluded that epidermal growth factor deficiency achieved by sialoadenectomy caused ultrastructural changes in gingival epithelium.

Keywords: gingival epithelium, rat, EGF

Introduction
Saliva plays an important role in maintaining oral health (15). A reduction of salivary secretion leads to altered physiologic functions of the saliva, such as reduced cleansing and lubricating the oral mucosa (21).

Submandibular salivary glands (SMGs) synthesize and accumulate a number of biologically active peptides that are released into both saliva and the bloodstream. One of these peptides is the epidermal growth factor (EGF) (3, 19).

Mouse submandibular gland is a rich source of epidermal growth factor, so mouse saliva contains a high level of EGF excreted from the submandibular glands (5, 6). Since EGF exerts a potent growth stimulating effect on various types of cells, it is supposed to play a role in the development and regeneration of salivary glands as well as oral mucosa (11, 17).

Epidermal growth factor (EGF) has been identified in gingival tissue (22) and gingival crevicular fluid (7). EGF is thought to mediate several of the responses observed during wound healing and inflammation, such as stimulation of cell proliferation and extracellular matrix turnover (16).

Several studies have suggested that EGF may regulate cell behavior during wound healing and chronic inflammation (8, 23). However, the cellular mechanisms involved in these responses are still unclear.

The present study aims to determine the electron microscopic changes in rat gingival tissue obtained from rats with artificial endogenous epidermal growth factor deficiency achieved by sialoadenectomy compared to sialoadectomied rats subjected to exogenous EGF administration.

Materials and Methods
Thirty adult female Wistar albino rats, 180-200 days old and 220-250g in weight were obtained from the Department of Medical Science Application and Research Centre of Dicle University (DÜSAM) - Diyarbakır. They were housed in individual cages in temperature-controlled enviroment (22°C) with a 12:12h light-dark cycle. All rats were fed standard pellet food and ad libitum tap water, which were performed according to the Declaration of Helsinki with the permission of the Governmental Animal protection committee.

Thirty Adult female Wistar albino rats were divided into three groups including a control and two experimental groups.

The animals from Group-I served as control. They were not subjected to sialoadenectomy (n:10).

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The rats in the second group Group-II were anesthetized with an intramuscular injection of Ketamine HCl (10mg/100g) and xylazine (0.8mg/100g). In order to remove the salivary glands, a 15 mm incision was made below the mandible and the submandibular glands were removed bilaterally (n: 10).

After 22 days from the sialoadenectomy operation, each rat from Group-III (n:10) was given drinking water to which was added 75 microgram/5 days exogenous EGF (Human Recombinant Epidermal Growth Factor, Sigma E9644).

After 27 days, rats from the control and experimental groups were euthanized by pentobarbital and their gingival tissue was removed. The tissues were fixed for 4 hours in 2.5% phosphate-buffered glutaraldehyde solution at pH 7 at 4°C. Post fixation was performed in 2% osmium tetroxide, followed by three times washing in phosphate buffer (pH 7.4) and dehydration in graded ethanol series. Sample tissues were embedded with Araldyte-Cy 212. Semi-thin sections were stained with Toluidine Blue. Thin sections were stained with Uranyl Acetate & Lead Citrate. The specimens were examined and photographed using transmission electron microscope (Carl Zais TEM).

Results and Discussion

Ultrastructural Findings

Control Group

We observed that the gingival epithelial cells of the rats of the control group were with normal appearance. It was seen that the junctional complex had a normal structure. The gingival epithelial cells were observed as having normal nucleus structure, but some had mitochondrial contractions and a view with dense matrix. GER tubulus were active (Fig. 1).

Fig. 1. Ultrastructural micrograph of the rat gingival epithelyum from the control group: N- nucleus, arrow indicates a junctional complex, m: mitochondria, GER: rough endoplasmic reticulum (Uranyl acetate-lead citrate X 7000)

Sialoadenectomy Group (SX)

Compared with the control group changes have occurred in the gingival epithelial cells of the SX. We observed partial degeneration of the epithelial cell junction. Widespread crystolysis was observed in a group of mitochondria (Fig. 2).

Sialoadenectomy Group (SX) + EGF

In the electron micrograph of SX+EGF group significant changes were observed. In this group, the gingival epithelium showed normal ultrastructure characteristics like the control group in terms of organelle content and nucleus characteristics. The examinations of the junctional complexes in this group, showed some similarities with the control group (Fig. 3).

Fig. 2. Ultrastructural micrograph of the rat gingival epithelyum from of the Sialoadenectomy group. N: Nucleus, arrow: junctional complex, m: mitochondria, GER: rough endoplasmic reticulum (Uranyl acetate-lead citrate X 14.000)

Fig. 3. Ultrastructural micrograph of the rat gingival epithelyum from of the Sialoadenectomy+ Exogenous EGF group. N: Nucleus, arrow: junctional complex, m: mitochondria, GER: rough endoplasmic reticulum ( Uranyl acetate-lead citrate X 7000)

The exogenous EGF had positive effects on the proliferation of the basal cells and the renovation of the other epithelial cells, improving the structure of the junction complexes among the cells.

Rats were selected as experimental animals in this study as their submandibular glands are rich in terms of epidermal growth factor (EGF) (5, 6). In order to understand better the effect of EGF, following the extraction of the submandibular glands one part of the experimental group was given EGF mixed with their water.

After the surgery, a close to normal appearance of the gingiva was observed in the group given EGF externally. In the group to which EGF was not administered we observed partial degeneration of the epithelial cell junction. Widespread crystolysis was observed in one group of mitochondria.
Some researchers have reported that EGF plays a role in epithelial cell proliferation and regeneration (1, 9). One group of researchers also reported that lack of EGF heightens the risk of gingival hyperplasia (7, 14).

Ketani et al. (12) reported a decrease in the thickness of the keratinisation layer and intracellular vacuole structure, and an irregularity and disappearance of the microscopic papilla in the sialoadenectomy rats.

Kilinc et al. (13) reported epidermal growth factor deficiency after sialoadenectomy to cause ultrastructural changes in dorsal tongue epithelium.

The epithelial cell degeneration observed in our study in the experimental group rats subjected to surgical extraction of the submandibular glands supports the above studies. We were unable to engage in a full discussion as we found no similar studies in the literature.

Most of the researches performed on animal models investigated the effect of EGF on wound healing, and an effect of wound healing acceleration has been experimentally observed in most studies (17, 18). Researchers report that EGF has an effect on the proliferation of fibroblasts (20) and keratinocytes (4).

Noguchi et al. (17) reported significant delays in the healing of wounds in the tongues of rats subjected to extraction of the submandibular glands, and concluded that EGF played a significant role in the healing process.

Bodner et al. (2) stated that when the submandibular glands were extracted, wound healing at the place of tooth extraction and palatal regions was delayed.

The changes determined by us in the gingival tissues of rats exposed to extraction of submandibular glands show that the submandibular glands and EGF play an important role in the maintenance of gingival integrity.

Girdler et al. concluded that EGF accelerated recovery when used as a mouthwash in the treatment of mouth ulcers (10).

Our study determined a close to normal ultrastructural appearance in the group subjected to sialoadenectomy to which EGF was administered externally. We therefore think that EGF plays a significant role in the protection of gingival tissue as the gingival surface is continuously bathed in saliva rich in epidermal growth factor (EGF). The gingival tissue is constantly subjected to mechanical and bacterial aggressions. However, the saliva, the epithelial surface through its degree of keratinisation, the turnover rate and the initial stages of the inflammatory response, all provide resistance to these actions. In addition to these, EGF secreted from submandibular glands stimulates epithelial proliferation and regeneration by attaching to specific receptors resulting in protection and regulation of gingival tissue.

Conclusions

According to the results of this study, ultrastructural changes (degeneration in epithelial cells and crystaliosis in the mitochondria) occur in the gingiva of rats subjected to surgical extraction of the submandibular glands (sialoadenectomy).

EGF plays a significant role in the protection and maintenance of the integrity of gingival tissue.

REFERENCES