The effects of epidermal growth factor deficiency on rat gingival epithelia

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ABSTRACT

Epidermal growth factor (EGF) is a low molecular mass polypeptide with 53 amino acids and is known to stimulate cell proliferation and differentiation in a wide range of tissues. EGF is thought to have important functions in epithelial growth and differentiation and in wound healing. In the present study, the teratogenic effects of sialoadenectomy on rat gingival epithelia were investigated histologically. Twenty adult female Wistar albino rats were divided into two groups (N = 10), a control and an experimental group. The experimental group was subjected to sialoadenectomy in order to create EGF deficiency. After 60 days of sialoadenectomy (EGF deficiency), control group rats were killed using pentobarbital and their maxilla removed. The sections were stained with haematoxylin-eosin and Mason triple for evaluation by using a light microscope. A statistically significant reduction in body mass was noted in rats in the experimental group when compared to the control group. Decreasing the thickness of keratization layer, irregularity and disappearance of microscopic papilla, intraepithelial focal cystic lesions resulting from EGF deficiency, and a decrease in saliva, were noted. As a result, epidermal growth factor deficiency performed by sialoadenectomy caused body mass reduction and gingival epithelia abnormalities.

Key words: epidermal growth factor, sialoadenectomy, gingival epithelia

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Introduction

Taste buds on the dorsal tongue surface and gingival epithelium are continually bathed in saliva rich in epidermal growth factor (EGF).

Saliva has multiple functions in the oral cavity. It is a protective cleanser (with antibacterial activity), a buffer (inhibiting demineralisation), lubricant, digestive necessity, and transport media to taste sensors. When xerostomia ensues the above functions are seriously altered. Signs and symptoms include intraoral dryness or burning, alterations in tongue surface, dysphagia, cheilosis, alterations in taste, difficulty with speech and development of tooth caries.

EGF is a mitogenic polypeptide hormone which, in vivo, stimulates ectodermal (epithelia) and endodermal cell growth and in vitro growth of epithelial cell and fibroblasts (COHEN, 1983). However, transcripts of EGF have not been epidermis. EGF is formed in salivary glands, kidney tubules and intestinal tract and occurs in nanogram quantities in plasma. EGF is a small, 53-amino acid, single chain polypeptide that is found in the highest concentrations in salivary glands (TSUTSUMI et al., 1986; ELDER et al., 1978).

EGF plays a role in a variety of biological actions, including promotion of epidermal development, wound healing, eruption of the incisors, activation of various transport systems and changes in cellular metabolism, in addition to mitogenesis, stimulation of pituitary secretion of ACTH and GH, and inhibition of gastric and thyroid hormone secretion. Moreover, most evidence indicates that it is an important hormone in the male reproductive system (LIU et al., 1994).

The submandibulary gland in mouse is a rich source of epidermal growth factor, and there is at least ten times as much EGF in the submandibulary glands of male mice as in female mice. After sialoadenectomy, plasma EGF decreased rapidly and was undetectable by week 3, indicating that the submandibular gland is a major source of circulating EGF (ARANCIBIA and ASSENMACHER, 1985; GUBITS et al., 1986; KURACHI and OKA, 1985).

In the same studies, the effect of sialoadenectomy and treatment with EGF antiserum on epidermis was investigated. The thickness of epidermis was also noted after EGF antiserum administration in sialoadenectomized

mice. No appreciable change was observed in the dermis and subcutaneous tissue in this study (TSUTSUMI et al., 1987).

TGFα (Transforming Growth Factor α) is homologous to EGF in sequence, has identical activity to EGF and is also produced in the salivary glands, although in a lesser amount (SHULTZ et al., 1991; HUMPERYS-BEHER et al., 1994). The effects of both EGF and TGFα are triggered through their binding to membrane receptor, EGFR, which activates an intrinsic tyrosine kinase in the cytoplasmic domain of the receptor (YARDEN and ULLRICH, 1988). The significance of the large amount of excess EGF in saliva is not known. It has been shown that salivary EGF affects wound closure, mediated through licking (HUTSON et al., 1979).

It has been suggested that the males of certain species produce more salivary EGF as an evolutionary response to increased fighting, resulting in a higher frequency of wounding. Indeed, EGF/ TGFα can stimulate many of the essential processes in wound healing, including neo-vascularization, chemotaxis of wound cells and keratinocyte proliferation and maturation (SHULTZ et al., 1991). However, another possibility is that an excess of EGF in the saliva is necessary to maintain the integrity and normal functioning of the oral cavity.

Previous studies have shown that in rat and human skin, EGF receptors are presented on basal epidermal cells and that they decrease in number and ability to bind EGF with increasing age (TSUTSUMI et al., 1987; SHULTZ et al., 1991).

EGF stimulates the proliferation and keratinisation of cells in oral epithelium (STEIDLER et al., 1980). In addition to its effect on cellular proliferation EGF has a variety of other cellular functions.

EGF exerts its action by binding to a specific cell surface receptor at the targetcell (CARPENTER and COHEN, 1979).

Target cells responsive to EGF include those of both epithelial and mesenchymal origin. The effect of EGF on these target cells is varied and includes stimulation of DNA synthesis, protein synthesis and cell motility (CARPENTER and COHEN, 1979; HATA et al., 1988).

The purpose of the present study was to investigate histologically the teratogenic effects of sialoadenectomy on rat gingival epithelia (oral epithelium).

Materials and methods

Twenty adult female Wistar albino rats, 180-200 days old and 225-250 g in mass, were obtained from the Department of Medical Science Application and Research Centre of Dicle University (DÜSAM). They were housed in invidual cages in a temperature-controlled environment (22° C) with a 12:12 h light-dark cycle. All rats were fed with standard pellet food and tap water was provided *ad libitum*, all conditions according to the Helsinki Declaration and with the permission of the Governmental Animal Protection Committee. The rats were divided into the following groups: the first group of rats was not subjected to sialoadenectomy (control group, n = 10).

The second group of rats was anaesthetized with an intramuscular injection of Ketamine HCl (50mg/kg) and xylazine (10 mg/kg) (Tuffery, 1987). To remove the salivary glands, a 15 mm incision was made below the mandible, and bilaterally submandibular glands removed (n = 10).

After 60 days of sialoadenectomy (EGF deficiency), control group rats were killed using pentobarbital and their maxilla were removed. The tissues were fixed in 10% neutral formalin and decalcification 5% nitric acids for 7 days at 4 °C. The tissues were embedded in paraffin and cut into 4-5 micrometers (μm) longitudinally. The sections were stained with haematoxylin-eosin and Mason triple for evaluation by using a light microscope. Statistical validation (body mass) of significant sialoadenectomy effects was accomplished using the Two Sample t-Test. All parameters were compared between sialoadenectomy groups and control groups (SAUNDERS and TRAPP, 1994).

Results

Body mass. Mean body masss are presented in Table 1 and Figure 1. Initial masss of control and sialoadenectomized animals were essentially identical

Table 1. Statistical value

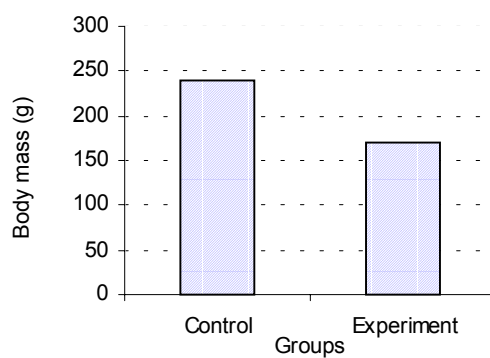| Groups	n	Mean	SD
Control	10	235.085	8.904
Experiment	10	170.065	1.620

Fig.1. Body mass of control and sialoadenectomized rats in the end of experimental period

on the day of surgery. However, by the time preference studies were initiated, control animals gained significantly more mass than did sialoadenectomy rats, and maintained this differential until the end of the studies (day 60).
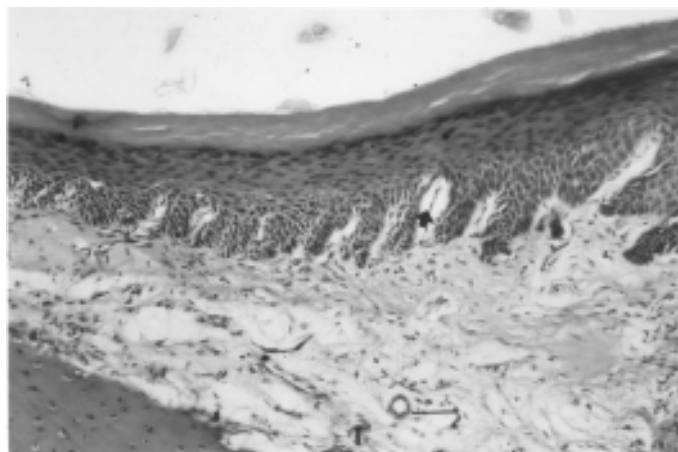

Fig. 2a. Histological appearance of control groups. Normal microscopic papilla (arrows) and gingival epithelia (oral epithelium). H&E; ×100; scale bar = 10 μm.

A statistically significant (P <0.001) reduction in body mass was noted in rats in the experimental group when compared to those in the control group.

Histological changes. We observed no pathology in the oral part of gingival epithelium in control group rats (Fig. 2a).

The gingiva is stratified squamous epithelium and is either fully or partially keratinised. Deep in the epithelium is a dense, irregular collogenous connective tissue, whose collagen fibres form a principal fibre group that resemble those of the periodontal ligament. The junctional epithelium forms a robust barrier between the bacteria-laden oral cavity and gingival connective tissue. The region of the gingival epithelium that attaches to the enamel surface is known as the junctional epithelium, which forms a collar around the neck of tooth (Fig. 2b).

We observed a decrease in the thickness of the keratinisation layer and intraepithelial vacuole structure, and an irregularity and disappearance of microscopic papilla in the sialoadenectomy groups (Fig. 3a).
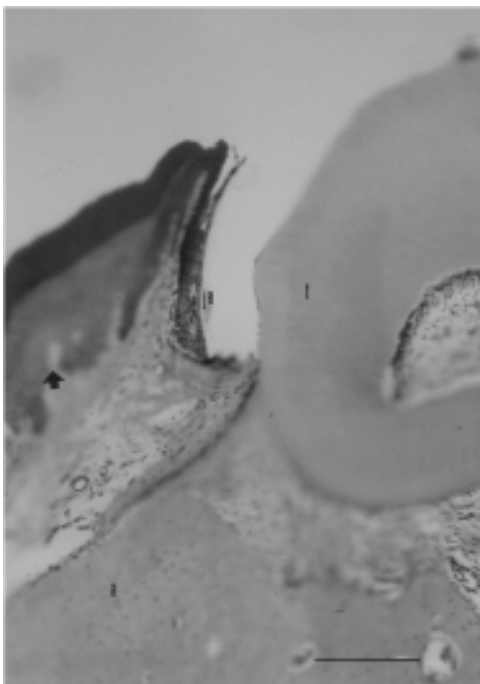

Fig. 2b. Histological appearance of control groups. Normal microscopic papilla (arrow), junctional epithelium (je), tooth (t) and alveolar bone (a) are present. Mason triple; ×200; scale bar = 24 μm.
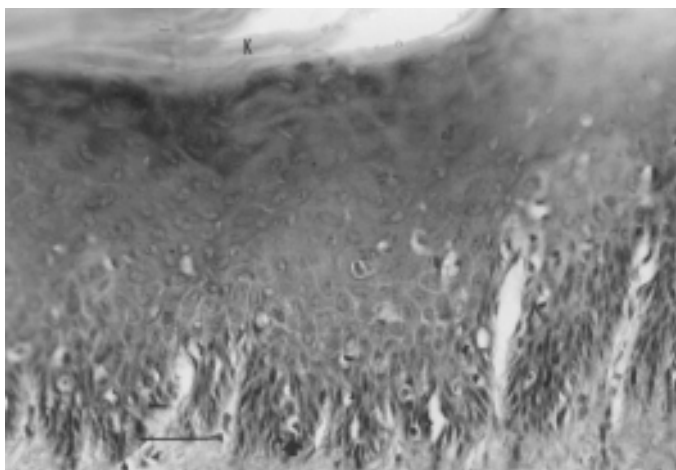
Fig. 3a. Histological appearance of exposed to sialoadenectomy. Decrease keratinisation (K), intraepithelial vacuole (v) and disappearance of microscopic papilla (arrow) are seen. H&E; ×400; scale bar = 20 µm.
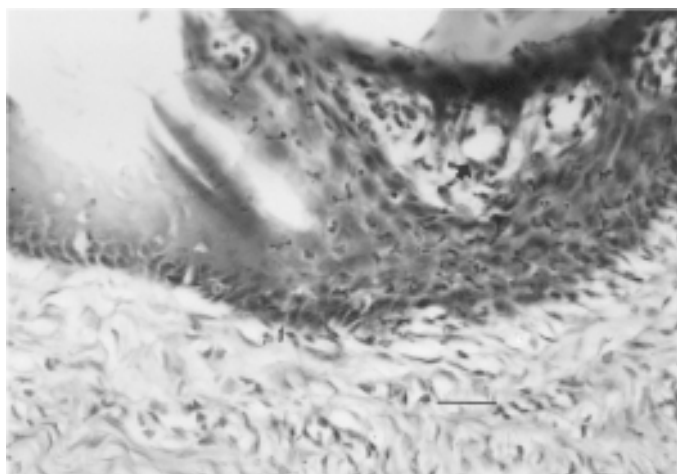

Fig. 3b. Histological appearance of exposed to sialoadenectomy. Mixed type inflammatory cell infiltration in the gingival epithelium (arrow) and disappearance microscopic papilla (d) are present. (H&E; ×400; scale bar = 30 µm.

We also observed disappearance of microscopic papilla, disorders of the epithelial layer and mixed-type inflammatory cell infiltration in the gingival epithelium in sialoadenectomy groups (Fig. 3b).

Fig. 3c. Histological appearance of exposed to sialoadenectomy. Decrease keratinisation (K), intraepithelial vacuole (v) and disappearance of microscopic papilla (arrow) are seen. H&E; ×400, scale bar = 20 μm.

We clearly observed intraepithelial focal cystic lesions (depending on degeneration), decrease in keratinisation and normal lamina propria in sialoadenectomized rats (Fig. 3c).

Discussion

The gingiva tissue is constantly subjected to mechanical and bacterial aggressions. The saliva, epithelial surface and the initial stages of the inflammatory response provide resistance to these actions. The role of the epithelium is through its degree of keratinisation and turnover rate.

The oral epithelium undergoes continuous renewal. Its thickness is maintained by a balance between new cell formation in the basal and spinous layers and the shedding of old cells at the surface.

EGF and TGF-α may affect increased epithelial proliferation through binding to their receptor EGFR. EGFR has been shown to be linked to the mitogenic pathway and the expression of EGFR has been shown to be enhanced in proliferation cells (NANNEY et al., 1984). In adults, tissues that are continuously undergoing proliferation and replacement, such as skin and oral and intestinal mucosa, are thought to depend on the interactions of EGF/TGF-α with EGFR to provide the mitogenic signal (YARDEN and ULLRICH, 1988).

Mitotic activity exhibits a 24-hour periodicity, with the highest and lowest rates occurring in the morning and in the evening.

We determined thickness of keratinisation layer in sialoadenectomized rats. This result also was compatible with STEIDLER and READE, (1980).

The mitotic rate is higher in nonkeratinized areas and is increased in gingivitis. The mitotic rate in experimental animals varies in different areas of the oral epithelium in the following descending order: buccal mucosa, hard palate, sulcular epithelium, junctional epithelium, outer surface of the marginal gingiva and attached gingiva (CARRANZA, 1990).

A statistically significant reduced body mass was noted in rats in the experimental group when compared to those in the control group. Therefore, the results mentioned here are also supported by NADANA and CATALINOTTO (1981).

TAJIMA et al. (1992) have demonstrated the EGF is selectively localized in junctional epithelial cells of adult rat gingival (TAJIMA et al., 1992).

THESLEFF (1987) reported that EGF receptors were concentrated in the epithelial cell rests of Malassez. The basal cells in oral and gingival epithelia are also suggested to be receptor sites (THESLEFF, 1987; NORDLUND et al., 1991). It has been generally accepted that Malassez epithelial cells, when activated and proliferated, contribute to dental cyst formation (FREMAN, 1989).

Salivary secretions are protective in nature because they maintain oral tissues in a physiologic state. Saliva exerts a major influence on plaque by mechanically cleaning the exposed oral surface, by buffering acids

produced by bacteria, and by controlling bacterial activity. Removal of the salivary glands in experimental animals significantly increases the incidence of periodontal disease and delays the healing of wounds (SHEN et al., 1979).

Our results are in contrast to those reported by NADANA and CATALINOTTO (1981) who reported that sialoadenectomy results in increased keratosis of the dorsal tongue epithelium.

In general, growth factors are needed for normal cell proliferation, but the physiological roles of EGF and TGF-α are not yet fully understood. EGF and TGF-α stimulate cell proliferation in several tissues and accelerate wound healing (SHULTZ et al., 1991). The proliferation and migration of sulcular epithelium can be considered as an attempt to restore tissue wounded by inflammation and could thus be analogous to the healing of cutaneous wounds. Factors controlling this epithelial proliferation and migration are not known, but soluble mediators probably participate in the process (MACKENZIE, 1988). Normally, EGF and TGF-α could function in the maintenance of epithelial tissue and in the repair of small injuries in the oral cavity.

The findings in this study of microscopic papilla irregularity and disappearance, and intraepithelial cyst structure, show that EGF deficiency can affect the gingival epithelium of a normal structure. These results were not mentioned in previous studies.

Many of the changes observed in these long-term studies may have been the result of continuous deficiency salivary factors, including EGF, necessary for epithelial maintenance, and the accompanying desiccation of the gingival epithelium. In this study we showed that sialoadenectomy (EGF deficiency) caused reduction in body mass and gingival epithelia abnormalities.

In conclusion, in order to determine mechanisms for understanding the effects of sialoadenectomy on gingival epithelia and mucosa, more extensive molecular and immunohistochemical studies should be carried out.

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SAŽETAK

Epidermalni faktor rasta jest niskomolekularni peptid, koji se sastoji od 53 aminokiseline, odgovoran za proliferaciju i diferencijaciju stanica različitih tkiva, a poznato je da ima i važnu ulogu u cijeljenju rana. U ovom istraživanju teratogeni učinak sijaloadenektomije istražen je na štakorima, i to praćenjem tjelesne mase i promjenama na gingivalnom epitelu. Dvadeset albino Wistar štakora svrstano je u dvije skupine po 10 životinja, pokusnu na kojoj je provedena sijaloadenektomija i na kontrolnu. Šestdesetog dana nakon sijaloadenektomije štakori su eutanazirani pentobarbitalom, nakon čega su im uklonjene maksile. Daljnji je postupak proveden bojenjem histoloških rezova gingivalnog epitela hematoksilin-eozinom te metodom bojanja po Masonu. U štakora pokusne skupine utvrđeno je statistički značajno smanjenje tjelesne mase u odnosu na kontrolnu. Kao rezultat deficita faktora rasta utvrđeno je smanjenje debljine keratinizirajućeg sloja, nepravilnost i odsutnost papila, intraepitelne žarišne cistične lezije i smanjena količina sline.

Ključne riječi: epidermalni faktor rasta, sijaloadenektomija, gingivalni epitel