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Full Length Research Paper

The role of sialoadenectomy and epıdermal growth factor (EGF) in skin development

Mehmet İrfan Karadede[1], Yusuf Nergiz[2] and Ayfer Aktaş[2]

[1]Department of Orhodontics, Faculty of Dentristry, University of Dicle, 21280, Diyarbakır/Turkey.
[2]Department of Histology and Embryology, Medical School, University of Dicle, 21280, Diyarbakır/Turkey

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In this study, the effect of epidermal growth factor (EGF) on skin development was investigated. A total of 24 adult female Spraque-Dawley rats were used in the study. The rats were divided into 3 equal groups as control, sialoadenectomy (SX) and sialoadenectomy+epidermal growth factor (SX+EGF). Sialoadenectomy was performed on the other groups except control. After a 21-day recovery period, subjects were made to mate. SX+EGF group rats were also given 12.5 µg/day EGF for each animal orally in the 16, 17th, 18th and 19th days of pregnancy. After pregnancy, the offsprings obtained were grown until the 28th day. All 28-day-old offsprings in all groups were weighed and sacrificed. Skin samples from interscapular region were examined under light and electron microscope. In light and electron microscopy, skin sections of SX group, atrophy in epiderm, hyperkeratosis, decrease in hair follicles and sebaceous glands, along with local thinning of basal membrane, hemidesmosome loss and necrotic cells were seen. In skin sections of SX+EGF group, the view was similar to controls. As a result, epidermal growth factor was concluded to have an important role in skin development.

Key words: Epidermal growth factor, rat skin, sialoadenectomy.

INTRODUCTION

Epidermal growth factor (EGF), which was first isolated from submandibular gland of male rats, is a mono-chain polypeptid hormone that weighs 6000 kilodalton (kDa). It was recognized by early opening of eye lids and early coming out of incisor teeth in new-born rats injected with submandibular saliva content (Cohens, 1962).

Many researchers have reported on the important role of EGF on healing of skin and gastric mucosa wounds (Olsen and Nexo, 1983; Nıall et al., 1982; Olsen et al., 1984). Olsen and Nexo (1983) pointed out that skin wounds healed more rapidly with the effect of EGF. The saliva is known to contain many substances that accelerate healing of wounds, as well as EGF, but it is a well-known fact that topical application of EGF on skin accelerates wound healing (Nıall et al., 1982).

In rats on which sialoadenectomy was performed, it was reported that healing delayed gastric ulcer wounds, but that positive responses were achieved after intra-gastric EGF application (Olsen et al., 1984; Konturek et al., 1988).

Epidermal cells in basal layer of normal skin constantly reproduce and provide source for keratinized cells. EGF is necessary for proliferation of epidermal cells *in vivo*. In *in vitro* media, however, EGF stimulates epidermal cell proliferation (Bem and Rıchardson, 1971). In new-born rats administered with EGF, increase in epiderm thickness was noted, in contrast to the epiderm of adult rats (Cohen and Elıot, 1963; Tsutsumı et al., 1986).

Tsutsumı et al. (1987) studied the possible effect of sialoadenectomy on epiderm and the role of EGF and antiserum EGF in prevention of this effect. As a result of this study, they reported that EGF is important physio-logically in maintaining normal structures of epidermal cells.

EGF, which is known to be a mitogenic polypeptid hormone, stimulates ectodermal (epithelial) and endo-dermal cells in *in vivo* media and fibroblasts in *in vitro* media (Cohen,1983).

It has been observed that submandibular glands of male rats produce more EGF than those of female rats and that they are at very low level at the end of the 3rd

*Coresponding author. E-mail: m.irfan.karadede@gmail.com. Tel: 90 412 248 81 00. Fax: 90 412 248 81 01.

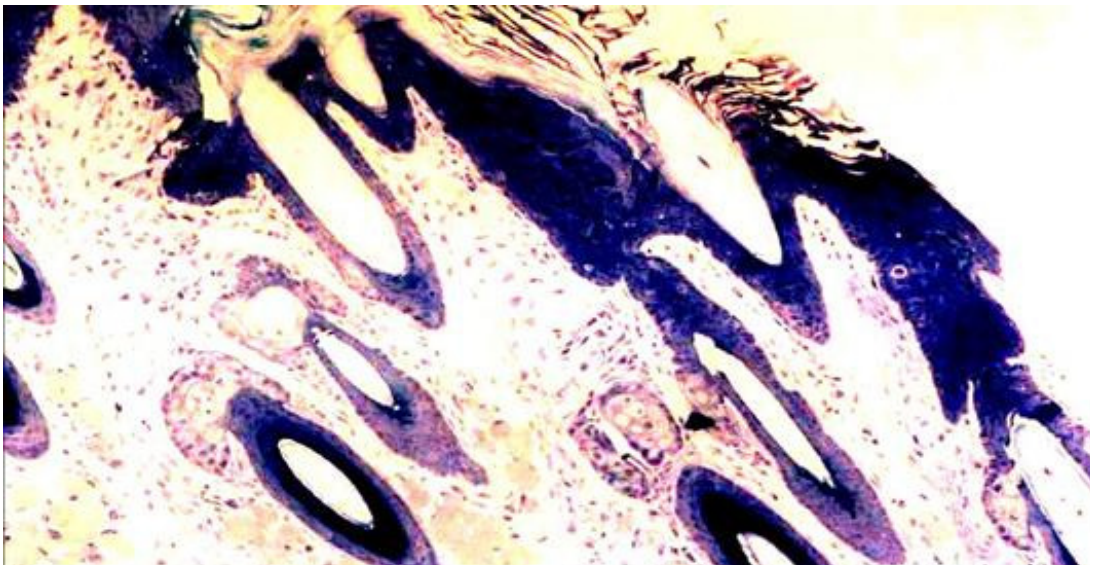

Figure 1. Control group: histological view of rat skin (Toluidine Blue, Original magnification X41).

week. These figures show that submandibular gland is the source of EGF in circulation. Even though Lui et al. (1994) pointed out that EGF in saliva is responsible for maintaining normal taste bud morphology, it has not been possible to attain clues as to how this is accomplished. The main function of EGF, although it is known to be effective on various cell types, is epitel growth and maturation, as its name indicates.

MATERIALS AND METHODS

Animals model

This study was carried out in health sciences application and research center Dicle university (DÜSAM) after approval of Dicle university ethics committee.

A total of 24 virgin female Spraque-Dawley type rats were used in the study. The rats were divided into 3 equal groups (n=8) as control (C), sialoadenectomy (SX) and sialoadenectomy+epidermal growth factor (SX+EGF). Test animals were kept at 22°C optimal temperature for 12 h in light and 12 h in dark. Rats kept in 45% relative humidity during experiment, were given water and pellets.

Surgical procedure

Control group

Female rats in this group were allowed to mate without any procedure. The first day of pregnancy was detected with microscopic examination of vaginal smear. On day 28 after birth, body weights of offsprings were measured. Biopsy samples from back skin in interscapular region were taken and fixed with 2.5% buffered gluteraldehite for electron microscopic examination.

Sialoadenectomy group

After general anesthesia on rats with Ketamine+Xylazine combination, submandibular glands were totally removed by transversal incision in cervical region. After 21 days following the operation, plasma EGF levels were decreased. Then, experimental animals were allowed to mating, and the 1st day of pregnancy was detected by microscopic examination of vaginal smear. On day 28 after birth, body weights of offsprings were measured. Under general anesthesia, skin samples from interscapular region were taken and fixed in 2.5% buffered gluteraldehite.

Sialoadenectomy + epidermal growth factor group

As in SX group, sialoadenectomy operation was performed on female rats and their submandibular glands were extirpated. After 21 days following sialoadenectomy, the first day of pregnancy of mated test animals was detected.

On the 16, 17, 18, and 19th days of pregnancy, 12.5 µg/day (50 µg in total) of EGF (Human Recombinant EGF. Sigma) was given to each rat by orogastric probe (Noguchi et al., 1991).

Histological method

On day 28 after birth, biopsy samples from interscapular region of back skin from offsprings in all 3 groups were taken under general anesthesia. The obtained biopsy samples were taken under examination by routine electron microscopy technique (Reynolds, 1990).

RESULTS

Light microscopic findings of skin

Control group

Keratinized squamous cells at the surface of epidermis and 4 - 5 lines of epidermal cells under these were monitored. Basal cell layer was observed as cubiodale view, and epidermal formations as normal view (Figure1).

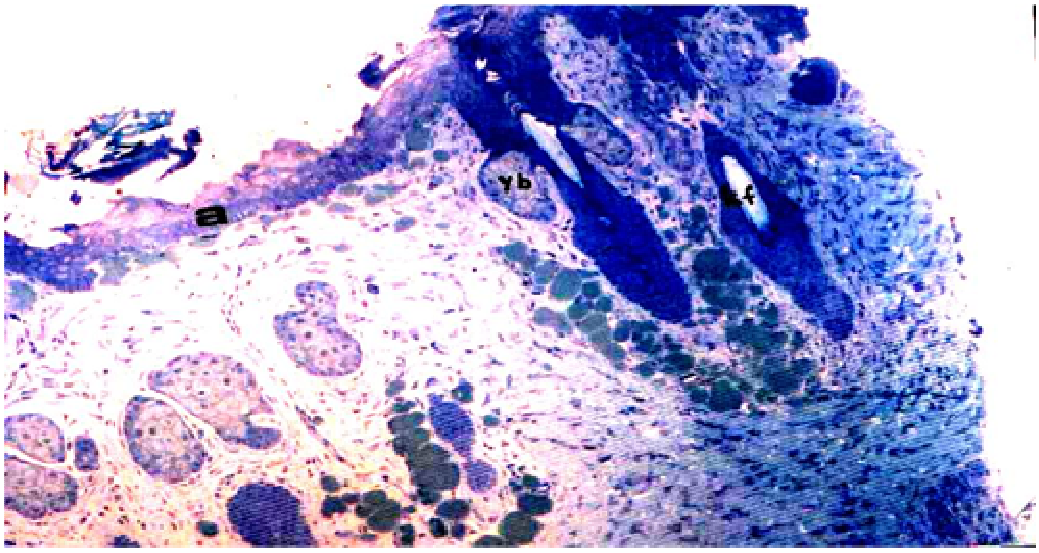

Figure 2. SX group: epiderm: relative decrease in hair follicles and glands in number, and atrophy are noticed. a, Atrophy; hf, hair follicle; lg, sebaceouse gland (Toluidine blue, original magnification X 41).

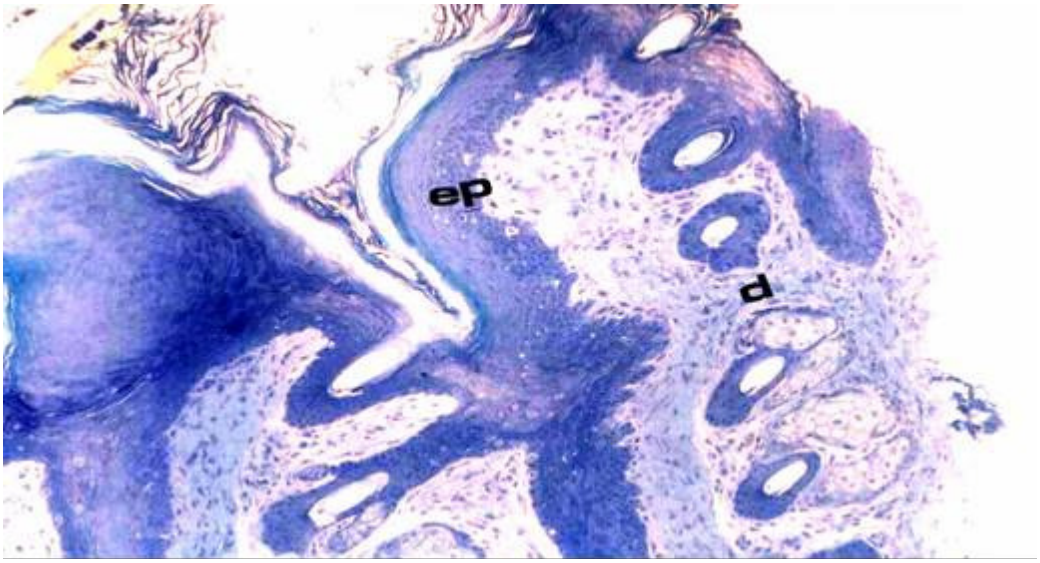

Figure 3. SX+EGF group: Similar view of epidermis and dermis to that in control group. ep, Epidermis; d, dermis (Toluidine blue, Original magnification X 41).

Sialoadenectomy group

In general, skin sections showed atrophy, along with decreased hair follicles and sebaceouse glands. Straighteningin epidermal protrusions, atrophy in epidermis, hyperkeratosis and/or parakeratosis are seen (Figure 2).

Sialoadenectomy+epidermal growth factor group

In this group, the view of hair follicles and sebaceouse glands in both epidermis and dermis was similar to that of controls (Figure 3).

Electron microscopic findings of the skin

Control group

Other members of the epidermis and dermis, collagen content, basal membrane thickness, junctional complexes between hemidesmosomes and epidermal cells were

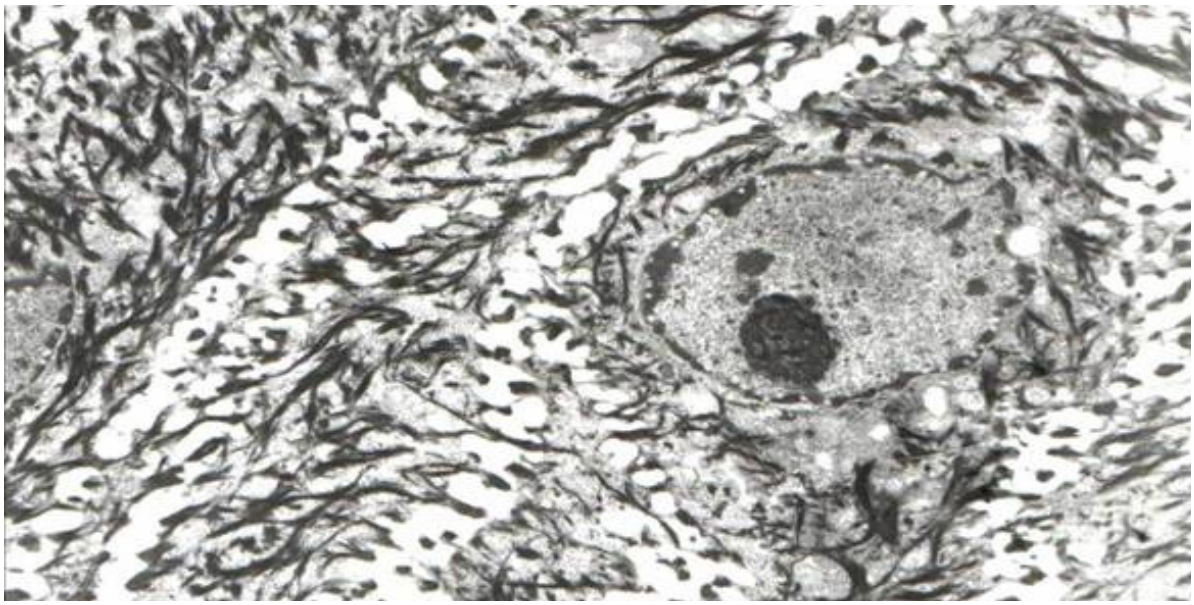

Figure 4. Control group: Normal view of junctional complexes between epidermal cells (Uranyl acetate-lead citrate, X 3000).

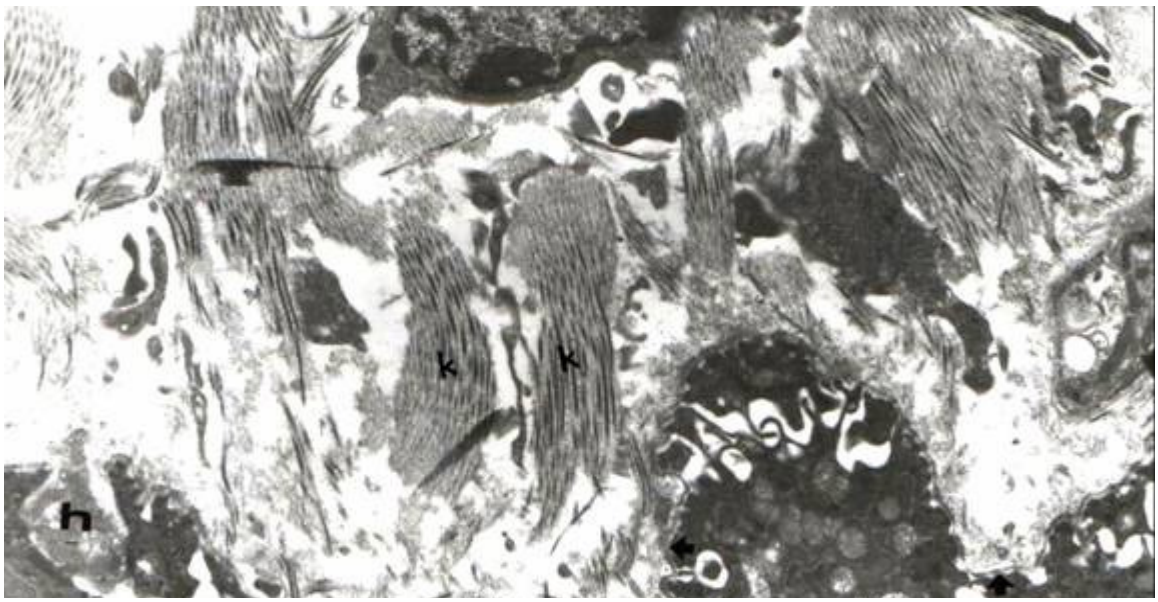

Figure 5. Control group: Basal membrane (thick arrow) thickness, hemidesmosome (h) and collagen (c) content is viewed in the normal structure (Uranyl acetate-lead citrate X 4400).

viewed to be of normal structure (Figure 4).

Sialoadenectomy group

Thinning of epidermis basal membrane in parts induced hemidesmosome loss and breakage in junctional complexes between epidermal cells resulting in wide openings between cells and some necrotic cells are seen (Figures 6-7).

Sialoadenectomy + epidermal growth factor group

Epidermis basal membrane showed the same thickness everywhere and hemidesmosomes were arranged with regular spaces between them (Figure 8). Junctional complexes between epidermal cells were of normal

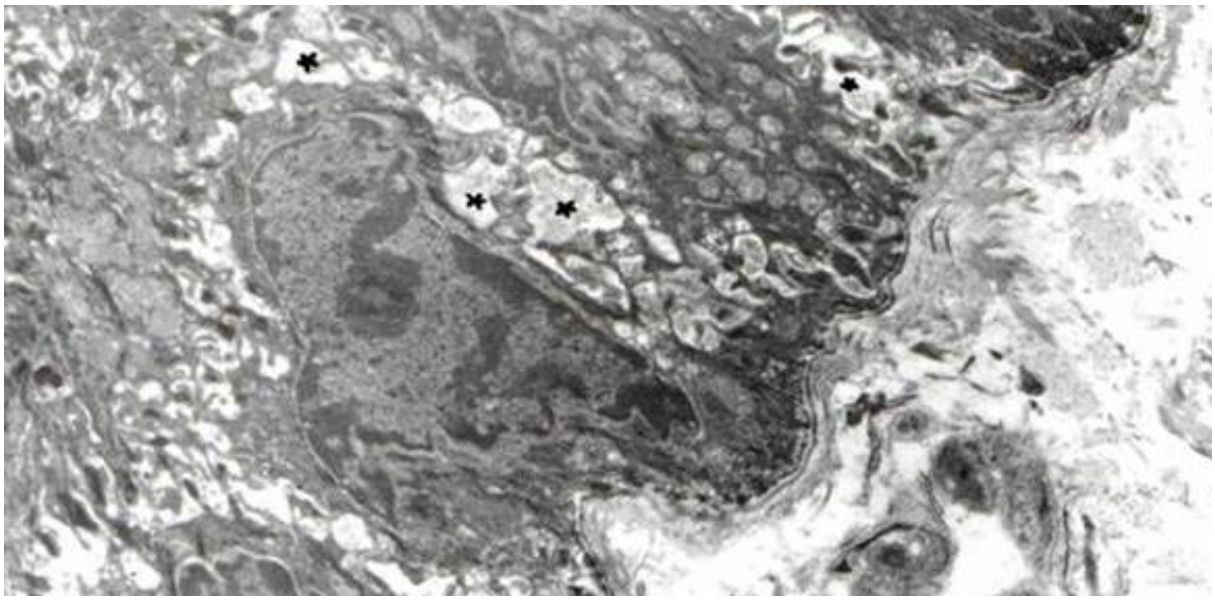

Figure 6. SX group: Wide openings between cells due to breakage in junctional complexes between epidermal cells (*) is seen (Uranyl acetate – lead citrate X 4400).

Figure 7. SX group: Necrotic cell (nc) is noticed among normal epidermal cells (Uranyl acetate – lead citrate X 3000).

structure, and necrotic cells were not seen (Figure 9).

DISCUSSION

In earlier studies on rats and mice, it was reported that sialoadenectomy decreases plasma EGF levels and that submandibular gland is the only source of EGF (Tsutsumı et al., 1986).

In the experimental study by Tsutsumi et al. (1987) with sialoadenectomy and antiserum EGF, it was determined that epidermis became significantly thinner after sialoadenectomy, while significant changes in dermis and hypodermis did not occur. However, in our study, along with atrophic epidermis, both atrophy and decrease in the number of hair follicles and sebaceous glands in the sialoadenectomy group were seen (Figure 2), while SX+EGF group rat skins did not reflect the same figure.

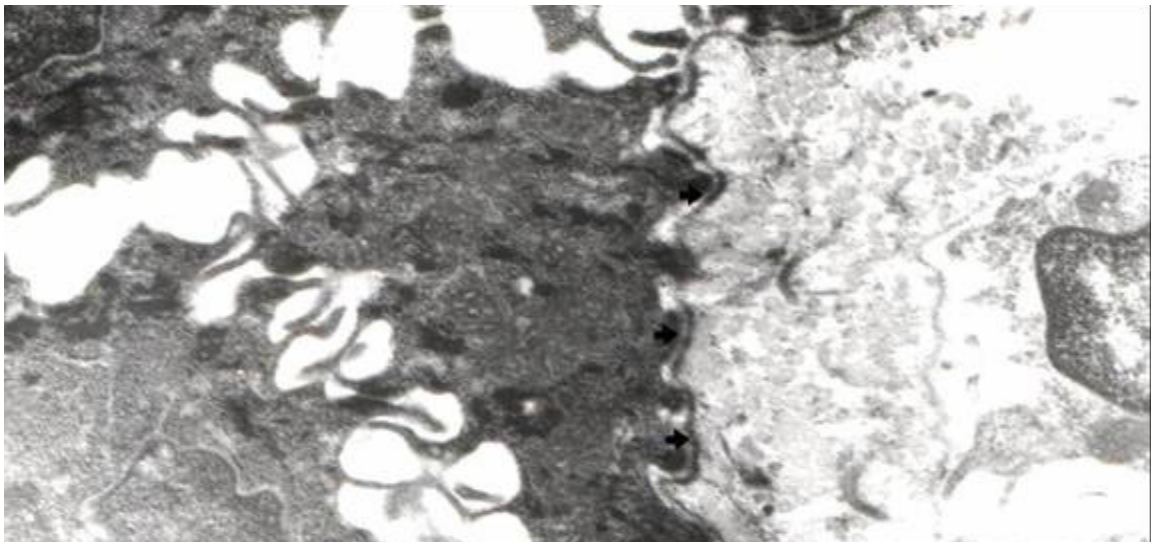

Figure 8. SX+ EGF group: Epidermis basal membrane (arrow) thickness is the same everywhere and arrangement of hemidesmosomes is regular (Uranyl acetate – lead citrate X 12000).

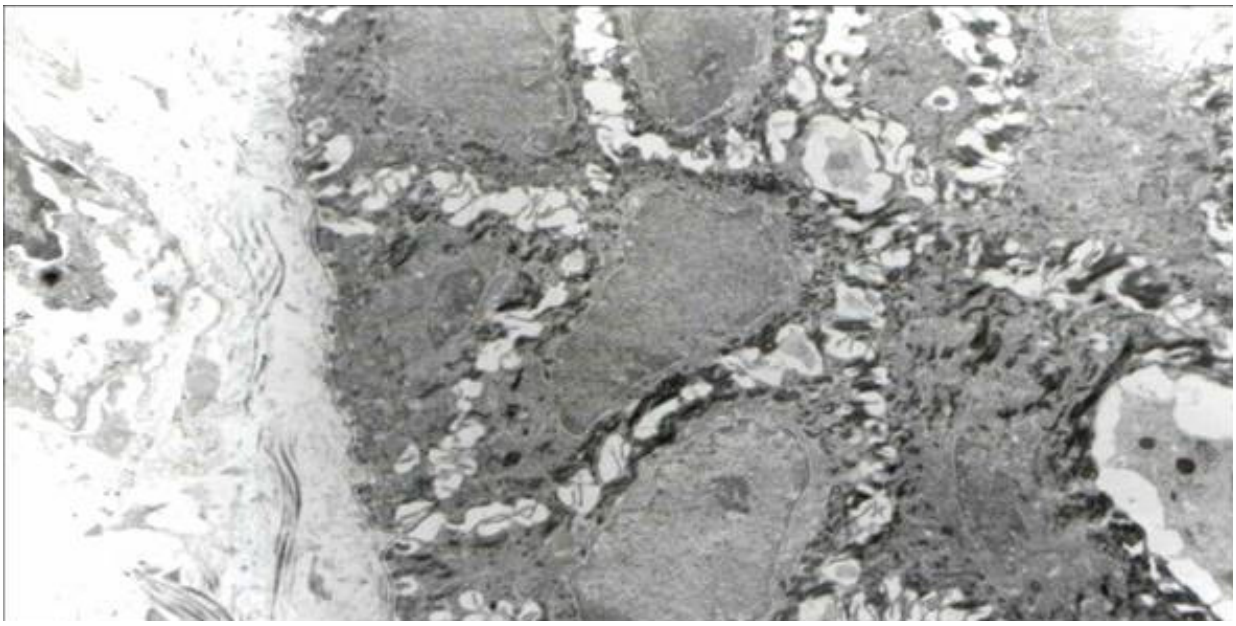

Figure 9. SX + EGF group: Junctional complexes units between epidermal cells is seen in the normal structure (Uranyl acetate – lead citrate X 3000).

On the other hand, Hutson et al. (1979) recorded a remarkable finding in skin healing of sialoadenectomy and normal rats. According to their observations, wound healing in the skin is slower than that in organs covered with mucosa.

The main reason for this is that the skin is covered with keratinized epithelium. The rapid healing in gastroduodenal ulcers might be due to frequent regeneration of mucosa epithelium by the effect of EGF.

Tsutsumi et al. (1987) reported that they found thinning in the epiderm of rats administered with EGF immediately after sialoadenectomy, and that this improved depending on the dose. They observed that atrophy in epidermis could be prevented through daily application of 1 - 5 µg EGF, while it was not effective in 0.2 µg application. The same researchers emphasized in another study that 3-weeks after sialoadenectomy, epidermis became very thin, and then as a result of 3-week exogenic EGF replacement, epidermis reached its normal view, and histologically, it could not be differentiated from normal

mouse skin.

Even though atrophy in epidermis of the experimental group of our study, on which sialoadenectomy was performed, was in parallel with the findings of Tsutsumi et al. (1987) decrease in the number of hair follicles and sebaceous glands was in contrast.

Recently, it has been suggested that epithelial cells in the basal layer of epidermis have EGF receptors, but higher layers are deprived of EGF receptors. Accordingly, it was reported that epithelial cells that form the basal layer show proliferative activation, but epithelial cells in other layers develop towards diversification (Steıdler and Reade, 1980).

Epidermal growth factor increases mitotic activity in epidermal basal cells, and also plays a role in wound healing (Nıall et al., 1982). These findings indicate that EGF supports proliferation in epidermis basal cells.

In the electron micrographs of the sialoadenectomy group of our study; thinning of basal membrane in parts, hemid-esmosome loss, wide openings in junctional complexes between epidermal cells and presence of necrotic cells were noticeable (Figures 6 and 7). In contrast, these structural failures were not seen in epidermis of SX+EGF group (Figures 8 and 9).

Cohen (1983) indicated that epidermal thickness in mice with sialoadenectomy is composed of one or two lines of cells. This view, which results from EGF deficiency, can be corrected by EGF replacement, and normal epidermis is attained. Since EGF deficiency does not affect keratinization in epidermal cells, stratum corneum preserves its normal view. Also, epidermal formations in the dermis and hypodermis were reported to maintain their normal morphological appearance. These results show that EGF, which originates from submandibular gland, has a significant physiological role in maintaining epidermis basal cell layer.

Ketani et al. (2001) showed a decrease in the thickness of the keratinisation layer and intraepithelial vacuole structure, and an irregularity and disappearance of the microscopic papilla in the sialoadenectomy rats.

Kilinc et al. (2006) reported epidermal growth factor deficiency after sialoadenectomy to cause ultrastructural changes in dorsal tongue epithelium.

Dag et al. (2008) observed that epidermal growth factor deficiency achieved by sialoadenectomy caused ultra-structural changes in gingival epithelium. 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 comlexes among the cells.

Conclusion

It was concluded that epidermal growth factor, which is secreted from the submandibular gland has an important role in the development and protection of the skin.

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