THE HISTOLOGICAL STRUCTURE OF CIRCUMVALLATE PAPILLAE OF THE PUPPY RATS WHICH WERE BORN FROM THE RATS GIVEN EGF AFTER SIALOADENECTOMY

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ABSTRACT
The papillaries on the dorsal surface of tongue are constantly washed by saliva rich in Epidermal Growth Factor (EGF). After the complete removal of submandibular gland surgically (Sialoadenectomy), morphological alterations, and the effects of EGF given orally on these alterations were investigated. In the study, 30 adult female Spraque-Dawley rats were used, which were divided into three groups: Control group (n=10); sialoadenectomy group (SX, n= 10); sialoadenectomy + epidermal growth factor group (SX+EGF, n=10). Following a three week period of recovery, the rats were mated. On days 16-19th of pregnancy, a total of 5 microgram (µg) EGF was given to the animals in SX + EGF group with orogastric tub as 1.25µg daily to each animal. The fetuses born following pregnancy were kept to grow up until the 28th day. The puppy rat of 28 days in all groups were sacrificed. Tongues of all tree group were dissected and specimens separately put into %10 neutral buffered formalin solution. The paraffin section obtained through routine histological methods were stained with Hematoxylin–Eosine (H.E), Methyl blue- Basic Fuchsin, and were examined under light microscope. No pathology could be observed on the circumvallate papillae of control group. The observation revealed that the circumvallate papillae of SX group usually displayed an atrophic situation, and there was perforation, particularly in the apical epithelial. The degeneretion in the epithelial made itself felt on the opposite wall of the trench. The trench surrounding the papilla was noticeably blocked in patches, and its depth and the surface area was diminished considerably when compared to the control group. A significant increase in the keratinization was detected on the surface epithelial of the circumvallate papilla. On the microscopic examination of the circumvallate papillae of the SX+EGF group rats, it was observed that these papillae had acquired to be similar that of the control group.

Introduction
The tongue papillae were first identified by Malpighi in 1664 (1). Taste buds found in humans and mammals are the receptors for the sensation of taste, and in rats there are three types of taste bud-bearing tongue papillae, which are fungiform papilla, circumvallate papilla, and foliate papilla, respectively. While the fungiform papillae are on the anterior portion of the tongue and the foliate papillae on the rear-lateral edges, the circumvallate papilla, which is singular, is placed more posteriorly on the midline of the tongue (2).

In rats the circumvallate papilla begins to take shape by its penetration into the epithelium on the 15th embryonic day. The penetrated epidermal ring separates from the external trench wall of papillae. The interior wall of trench limits the center of papillae, excluding the anterior edge. The downward protrusion of a single ring on the 15th day of pregnancy should be regarded as the first sign of the papilla cir-
cumvallata development. On the 21st day of the pregnancy, a trench starts to form (3).

In the study that they had described the morphogenesis of three types of lingual papilla in rats under Scanning Electron Microscopy (SEM) (2), Iwasaki and co-workers stated that non-fully-developed circumvallate papillae and fungiform papillae appeared earlier than the filiform papillae, and this was related to the perception of taste. In studies performed, the length and width of the papilla and the depth of the trench were reported to increase with age. The taste buds of the circumvallate papilla are located in the epithelial of the trench wall. In mammals, the taste buds were first detected by Schwalbe (1867) and Loven (1868) (4). In rats the taste buds, which are the intraepithelial sense organs that render the function of taste perception, start to develop on the 17th day of the pregnancy in the circumvallate papilla (5).

The objective of this study is to determine the potential effects of tongue sialoadenectomy on tongue papillae and the role of exogenous Epidermal Growth Factor (EGF) administration.

Materials and Methods

Animals

This study was performed in the Practice and Research Center of Medical Sciences in Dicle University. 30 adult female Sprague-Dawley rats weighing about 250-300 g were used in our research.

The female rats were divided into three groups in equal numbers (n=10) as control, sialoadenectomy (SX), and sialoadenectomy + Epidermal Growth Factor (EGF) groups, respectively.

Surgical Procedures

Control: Female rats of this group were let to mate without any pre-operation. The first day of the pregnancy was determined by the microscopic examination of vaginal smear. On the 28th day following their birth, tongues of all neonate rats were fixed in a 10% neutral buffered formalin solution.

Sialoadenectomy (SX): Female Sprague-Dawley rats were put under general anesthesia by intramuscular administration of Ketamine + Xylazine. A transversal incision was performed at the neck region and the submandibular glands were totally extracted (6). For three weeks following the operation it was waited for the EGF level to drop. Then the test animals were mated, and the first day of the pregnancy was determined through a microscopic examination of vaginal smears. On the 28th day following their birth, the tissues of neonate rats were fixed in a 10% neutral buffered formalin solution for the histological examination.

Sialoadenectomy + Epidermal Growth Factor (SX+EGF): As in Group 2, the submandibular glands of female rats were extirpated by a sialoadenectomy operation, three weeks after which, they were allowed to mate and the starting day of their pregnancy were determined again by microscopic examination of vaginal smears. During the four days between 16th and 19th days of pregnancy, a 1.25 μg of EGF (Human Recombinant EGF, Sigma) per subject was administered daily to pregnant rats by orogastric probe, making a sum of 5 μg per subject in total. On the 28th day following their birth, tongues of neonate rats were fixed in a 10% neutral buffered formalin solution.

Histological Method

On the 28th day following the birth, tongues of neonate rats of all three groups were dissected and cut into two from the line determining the anterior 2/3 portion and the posterior 1/3 portion. The specimens were separately put into 10% neutral formalin solutions for 24 hours for fixation, and then rinsed with running water for an other 24 hours. Then, they were dehydrated through an ethanol series, made transparent in xylene, and blockaded in fresh paraffin through a paraffin bath. The sections that were taken transversally and coronally from those paraffin blocks with a thickness of 4-6 μm with the aid of a sliding microtome were stained with Haematoxylin-
Results and Discussion

Microscopic Results of Tongue Papillae

Control Group

No pathology could be observed on the circumvallate papilla (Fig. 1).

Sialoadenectomy Group (SX)

The investigation of differently stained serial sections taken from the circumvallate papillae of the SX group rats led to the following microscopic results: The observation revealed that the circumvallate papillae of this group usually displayed an atrophic situation, and there was perforation, particularly in the apical epithelial. The degeneration in the epithelial made itself felt on the opposite wall of the trench also (Fig. 2). The trench surrounding the papilla was noticeably blocked in patches, and its depth and the surface area was diminished considerably when compared to the control group. A significant increase in the keratinization was detected on the surface epithelial of the circumvallate papilla. It was discovered when the taste bud cells of circumvallate papilla were examined that dark, fair, and basal intermediary cells that make up of the buds could not be distinguished from each other, unlike the control group; on the contrary, degradation in the cytoplasm and the nucleus and a vague appearance was all observed (Fig. 3).

Sialoadenectomy + Epidermal Growth Factor Group (SX+EGF)

On the microscopic examination of the circumvallate papillae of the SX+EGF group rats, it was observed that these papillae had acquired an appearance close to that of the control group (Fig. 4). In their study, Nanda et al (8) reported that the EGF concentration was reduced considerably, and as a result of this, the healing process of tongue of wounds was retarded in mice and rats subjected to sialoadenectomy. It is known that the orally administered EGF afterwards, which does not increase the EGF level of portal and peripheral blood, enhances the healing process of tongue scars. Besides it was reported to cause mouth dryness in mice, as the amount of saliva in the mouth was diminished by the extraction of main saliva source, i.e., submandibular gland. In mice, the inadequacy of EGF is the major cause of retardation observed in the recovery of wounds. It was discovered that the recovery rate of wounds reached usual levels when exogenous EGF was introduced. As quite well-known, the tongues of mice are covered with a stratified squamous epithe-
Fig. 3. SX Group: The contraction in patches in the trench surrounding the circumvallate papilla, the degeneration in the taste bud (t), and the increase in the keratinization (k) (Methylene blue-Basic fuchsin, original magnification X 41).

Fig. 4. SX+EGF group: The panoramic view of the circumvallate papilla which displays a close appearance to that of the control group (Methylene blue-Basic fuchsin, Original magnification X 41).

lium; and since the EGF found in saliva cannot be absorbed from the lingual epithelial under normal condition, it is reported not to affect the basal epithelial cells. However, if the lingual epithelial is impaired due to certain chemical or mechanical agents, the EGF in the saliva can easily access the basal epithelial cells and contributes to the healing of wound. It was declared that this mechanism usually works quite efficiently in the recovery of wounds that come into contact with extracts like tear, urine, and duodenal liquid, which are rich in EGF. The healing process of wounds in mice with sialoadenectomy was reported to exhibit a two-day with respect to normal healthy mice (8).

It should be noted that, in our study, the pathology emerged in the circumvallate papillae of the rats with sialoadenectomy due to the EGF deficiency (Fig. 3) had undergone a significant recovery (Fig. 4) when EGF was orally administered. The improvement observed in the circumvallate papilla, of course, has a connection with the oral administration of EGF.

The EGF in the saliva may at the same time indicate the maturation and, related to this, the activity of taste bud cells. The distribution of EGF, TGFα, and EGFRα in the taste bud cells and their locations in the dorsal epithelial of the tongue has no connection with sialoadenectomy, and this implies that the local settlement is not arranged by the EGF in saliva. The outcomes of a study that investigated the activity of EGF applied on wound healing of tongue also support the argument that endogenous EGF levels are not affected by sialoadenectomy.

Sialoadenectomy has no impact on the number and the morphology of taste buds in circumvallate papilla. This situation may possibly be indicated by the immunofluorescent staining, or it may be related to the high concentration of EGF and TGFα in taste buds of circumvallate papilla. Few number of other cell types accompany to the high EGF, TGFα, and EGFRα levels detected in circumvallate taste buds. The immunostaining revealed that the level of these factors were higher than those detected in the fungiform taste bud cells.

The presence and the availability of TGFα which is synthesized by the acinar cells found in the glands of Von Ebner adjacent circumvallate papillae may serve as a factor that suppresses the impact of extraction of saliva glands on circumvallate papillae. The obtained results conforms with the outcomes of Nanda and Catalino (8), who reported that sialoadenectomy...
leads to the increase of keratosis in the dorsal epithelial of tongue and causes disorganization by the damage occurred in the circumvallate taste bud cells. However, these researchers had observed the postoperative effects of surgical sialoadenectomy on rats during a very long time period like 95 to 110 days. Most of the changes encountered during these longer observations end up with the drying of lingual epithelial due to the permanent deficiency of EGF in saliva.

In our work, circumvallate papilla of the SX group has usually displayed an atrophic situation; degeneration and perforation in apical epithelial in patches was detected. Furthermore, the trench surrounding the papilla was observed to get narrower, even blocked, in some areas. The depth of the trench was obviously reduced comparing with the control group, and an increase in the keratinization on the surface epithelial was observed (Fig. 3). Unlike in the control group, a clear distinction cannot be made among the cell population that constitutes the taste buds. Besides, a flu appearance together with some degradation was witnessed in the nucleus and cytoplasm of those cells. The remarkable histological findings regarding the circumvallate papilla display a correlation with the studies of Nanda and co-workers (8).

In an experimental study concerning the circumvallate papilla, Morris-Wiman et al. (9) claimed that a morphological differentiation did not arise in the circumvallate papilla and its taste buds. The results of our study are in opposite with the works of these researchers. Consequently, the structural changes observed as a result of sialoadenectomy in the circumvallate papilla demonstrate a significant improvement with the orally administration of EGF.

REFERENCES