Electron Microscopy of the Canine Apocrine Sweat Duct

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ABSTRACT. Two different subunits were detected in the canine apocrine sweat duct by light and electron microscopy. One was the intradermal short duct composed of a single layer of inner cells and one or two layers of outer cells. These ductal cells contained only a few mitochondria, suggesting their low ability to reabsorb water and ions. The other was the intrafollicular duct surrounded with outer root sheath cells. In the lower portion of the intrafollicular duct, the outer cells contained many tonofibrils but no keratinization was noted. In the middle portion, the outer cells were keratinized with keratohyaline droplets and membrane coating granules, forming a keratinized band. In the upper portion, keratinization was seen in the inner as well as the outer cell layers. The inner cells with thickened plasma membrane contained abundant keratohyaline droplets but no membrane-coating granules. These keratinized inner and outer cells were considered to flow into the hair canal.

Many investigations on the apocrine sweat gland of the canine skin have been performed since it was first described by Gurlt [1]. Most of these studies have dealt with the structure and function of the secretory portions of this gland. However, its ductal portion is known to be composed of two or three layers with no myoepithelial cells.

The present report deals with light and electron microscopy of the canine apocrine sweat duct for clarifying its detailed structure.

MATERIALS AND METHODS

The skin tissues were taken from the dorsal hairy skin of an adult dog under anesthesia with sodium pentobarbital (25 mg/kg). For light microscopy, the skin was fixed in 10% neutral formalin and then embedded in paraffin. Thin horizontal serial sections (6 μ m) were made and stained with hematoxylin and eosin.

For electron microscopy, specimens were cut into small pieces and fixed in

2% glutaraldehyde-phosphate buffer for 2 h at 4°C and then with 1% osmium tetroxide for an hour. After dehydration with ascending concentrations of alcohol, the specimens were embedded in Epon 812. Horizontal serial ultrathin sections were prepared with a Porter-Blum MT-II ultramicrotome and doubly stained with uranyl acetate and lead nitrate. They were observed with a JEOL-100S electron microscope.

RESULTS

The canine apocrine sweat duct was divided into two distinct portions by light microscopy. One was the intradermal duct in the dermis situated between the secretory portion and the intrafollicular duct. It usually consisted of two to three cell layers with a relatively wide lumen (Fig. 1). The other was the intrafollicular duct surrounded with hair follicle cells and with an opening into the hair follicle at the level just above the sebaceous orifice (Figs. 2, 3 and 4).

I. Intradermal duct

(1) Light microscopy

The intradermal duct occupied the area from the uppermost portion of the secretory portion to the entrance of the hair follicle. The lumen of the intradermal sweat duct was surrounded with a concentric inner cell layer and one or two outer cell layers without myoepithelial cells.

(2) Electron microscopy

The inner and outer cells were flattened or cuboidal in shape. The plasma membrane of the inner and outer cells formed undulation or interdigitation with each other except between the outer cells. The lumen of the duct sometimes contained pinched-off villi and small vesicles. Short microvilli extended from the cell surface toward the lumen.

In the cytoplasm of the inner cells, mitochondria were usually small and few. Lysosomes with limiting membrane or myeline structure were sometimes observed as electron dense amorphous granules, 0.2–0.6 μm in diameter. Small Golgi elements were often seen near the nucleus of the inner cells.

The outer cells showed scarce desmosomes, and the adjacent cells formed slight undulation and interdigitation in plasma membrane. Mitochondria were also few and not accumulated in the outer cells. Numerous free ribosomes were seen in the cytoplasm. The inner cells of the upper part of the intradermal duct showed long microvilli protruding toward the lumen. The outer cells of the upper part had abundant tonofilaments in the cytoplasm, especially at the luminal side, which tended to form tonofibrils. Keratohyaline granules or membrane-coating granules were not found in this portion.

II. Intrafollicular duct

(1) Light microscopy

Light microscopy of horizontal serial

sections of the apocrine intrafollicular duct found three characteristic portions; the lower, middle and upper portions.

The inner and outer cells of the lower portion of the intrafollicular duct contained neither keratohyaline droplets nor keratohyaline granules. The middle portion was characterized by the keratinized material surrounding the inner cell layer. The outer cells contained abundant keratohyaline droplets (Fig. 3). The upper portion contained highly keratinized materials both in the inner and outer cells (Fig. 4).

(2) Electron microscopy

In the lower portion of the intrafollicular duct, obvious interdigitation of the plasma membrane was observed between the inner cells with numerous desmosome-tonofibril complexes. Cytoplasm of the inner cells contained abundant organella including fine filaments, polyosomes and rough endoplasmic reticulum. Mitochondria of various sizes were numerous in the duct cells of the intradermal duct. The inner cells frequently had vesicles with obvious membrane-containing fine granules. Numerous tonofibrils were dispersed throughout the cytoplasm of the outer cells. They were often aggregated and formed a circular band around the inner cells. In the middle portion, characteristic keratinized materials were detected in the outer cell layer. The outer cells of the middle portion contained keratohyaline droplets and numerous membrane-coating granules (Fig. 7). Such oval keratohyaline droplets were not identical to polygonal epidermal keratohyaline granules in the shape. No aggregation of tonofilaments was observed in the outer cells of the middle portion. The inner cells contained irregularly shaped keratohyaline droplets but no membrane-coating granules. In the upper portion, keratiniza-

tion of both the inner and outer cell layers was observed (Fig. 8). A non-keratinized outer cell layer was attached to the outer root-sheath cells containing irregularly shaped large keratohyaline granules. The thickened plasma membrane was the characteristic feature of inner cell keratinization.

DISCUSSION

Several studies have been conducted on the fine structure of the human normal apocrine and eccrine sweat ducts [3, 5, 6, 8]. However, the detailed structure of the canine apocrine sweat duct and its function have sufficiently been examined. Tani *et al.* [8] reported the fine structure of human apocrine acrosyringeal complex and classified the apocrine intrafollicular duct into three portions; the least keratinizing lower portion, the incompletely keratinizing middle portion, and the highly keratinizing upper portion.

The canine intrafollicular apocrine duct was also divided into three portions like those of man. In the lower portion of the intrafollicular duct, aggregated tonofilaments were detected both in the inner and outer cells; however, no keratinized materials were observed. The inner cells of the lower portion were not different from those of the intradermal duct except for prominent interdigitation in the former.

In the middle portion, the outer cells had begun to keratinize with abundant membrane-coating granules and keratohyaline droplets, while the inner cells contained neither of them. In the upper portion, the inner cells were keratinized following keratinization of the outer cells. The keratinized inner cells contained keratohyaline droplets but no membrane-coating granule. Thickened plasma membrane was a characteristic

feature of the keratinizing process of the inner cells. These findings suggest that the outer cells begin to keratinize in the middle portion and the inner cells in the upper portion.

Suzuki [7] suggested that the luminal cells of the intraepithelial duct of the mouse planter eccrine gland may begin to keratinize much earlier than do the surrounding epidermal keratinocytes. The luminal cells were keratinized incompletely without membrane-coating granules, while the peripheral cells keratinized like the epidermal keratinocytes with membrane-coating granules. On the contrary, keratinization of the canine apocrine sweat duct occurred at first in the outer cells, and the keratinization process seemed to be complete in the outer cells, while incomplete in the inner cells.

Oval keratohyaline droplets in the inner and outer cells of the canine apocrine intrafollicular duct were different in the shape from polygonal epidermal or infundibular keratohyaline granules. In man [8] and mice [7], keratohyaline droplets were observed particularly in the outer cell layer and not in the surrounding keratinocytes. Similarly, keratohyaline droplets were thought to be the characteristic feature of the canine apocrine duct cells.

Christophers *et al.* [1] showed that the "acrosyringeal basal cells" moved toward the acrosyringeal coiled lumen. However, upward moving of the ductal cells were suggested in our study as keratinization of the duct cells were observed in the middle and upper portions and not in the lower portion. Munger *et al.* [6] suggested that accumulation of mitochondria in the outer cell layer of human eccrine dermal duct was concerned with the reabsorption mechanism of water or ions in the duct. However Hashimoto [3]

or Kurosumi [5] failed to observe such mitochondrial accumulation in the outer cells of the eccrine dermal duct. In this study, the lack of accumulation of mitochondria in the outer cell and the shortness of the dermal duct and the weak activities of succinate dehydrogenase in the ductal cell [4] may not support the possibility of water and ion reabsorption in the canine apocrine sweat duct.

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EXPLANATION OF FIGURES

Fig. 1. An intradermal duct: the lumen surrounded with a layer of inner cells and two to three layers of outer cells.

ID: intradermal duct. $\times 400$.

Fig. 2. The lower portion of the intrafollicular duct: no keratinized materials neither in the inner nor outer cells.

IF: intrafollicular duct. $\times 400$.

Fig. 3. The middle portion of the intrafollicular duct: many keratohyaline droplets in the outer cell layer. Keratinized materials between the inner and outer layers.

KD: keratohyaline. $\times 400$.

Fig. 4. The upper portion of the intrafollicular duct: keratinized materials both in the intervene and inner cell layers, and keratohyaline droplets in the inner cell layer.

KI: keratinized inner cell; KO: keratinized outer cell. $\times 400$.

Fig. 5. The inner and outer cells of the intradermal duct: interdigitation of plasma membrane between the inner cells and lysosome-like dark granule (arrow) in the inner cell.

L: lumen; IC: inner cell; OC: outer cell. $\times 8900$.

Fig. 6. The lower portion of the intrafollicular duct: no keratinized materials either in the inner or outer cell layer, but a few membrane-coating granules and tonofibrils in the outer cells with strong interdigitation of plasma membrane of inner cells.

L: lumen; IC: inner cell; OC: outer cell; twin arrows: membrane coating granules. $\times 11000$.

Fig. 7. The middle portion of the intrafollicular duct: keratinized materials observed between the inner and outer cell layers, and keratohyaline droplets both in the inner and outer cells.

L: lumen; IC: inner cell; OC: outer cell; KD: keratohyaline droplet; KO: keratinized outer cell. $\times 8200$.

Fig. 8. The upper portion of the intrafollicular duct: keratinization of the inner cells following the appearance of the intervene keratinized material and the thickening of plasma membrane of the inner cells.

KI: keratinized inner cell; KO: keratinized outer cell; OC: outer cell; KD: keratohyaline droplets. $\times 6700$.

要 約

イヌアポクリン汗管の電子顕微鏡的観察: 岩崎利郎 (第一製薬 (株) 研究所) —— イヌの皮膚アポクリン汗腺の汗管を光学顕微鏡を用いて観察した。汗管は真皮内に存在する真皮内汗管と毛包内を走行する毛包内汗管に分けられた。真皮内汗管は1層の内周細胞とそれを取り囲む1~2層の外周細胞から構成され、周辺には筋上皮細胞はみられなかった。真皮内汗管はヒトのそれと比較して短かく、ミトコンドリアの集積もみられず、水分、イオン等の再吸収を暗示するような形態も認められなかった。毛包内汗管は毛根鞘細胞に取り囲まれていた。毛包内汗管の下部では外周細胞にトノフィブリルの集積はみられたが、角化の徴候は認められなかった。毛包内汗管中間部では外周細胞にケラトヒアリン滴, membrane-coating granule が出現すると共に外周細胞の角化が認められた。毛包内汗管上部では外周細胞の角化に続いて、内周細胞の角化が観察された。内周細胞の角化には membrane-coating granule の出現がなく、ケラトヒアリン滴を有し細胞膜の肥厚が認められた。角化した内周細胞および外周細胞は毛包腔内に脱落するものと考えられた。