Spontaneous Ectopic Sebaceous Glands (Fordyce’s Granules) in the Oral Mucosa of Sprague-Dawley Rats

Masako Imaoka¹, Hiroshi Satoh¹, Kiyonori Kai¹, Tetsuyo Kajimura², and Kazuhisa Furuhama¹

¹Drug Safety Research Laboratory, Daiichi Pharmaceutical Co., Ltd., 1–16–13 Kitakasai, Edogawa-ku, Tokyo 134–8630, Japan
²Research Planning & Administration Department, Daiichi Pharmaceutical Co., Ltd., 1–16–13 Kitakasai, Edogawa-ku, Tokyo 134–8630, Japan

Abstract: Although Sprague-Dawley rats are most frequently used in numerous toxicological studies, there are only few reports dealing with the spontaneous occurrence of ectopic sebaceous glands in the oral tissue (Fordyce’s granules) so far. Hence, to clarify the morphological characteristics of Fordyce’s granules with their incidence in this strain, 110 male and 110 female CD(SD)IGS rats aged 19 to 112 weeks were examined microscopically. Fordyce’s granules were identified in the upper molar gingiva, and most of them were located around the first and third molars. The granules consisted of one or more sebaceous glands without hairs and hair follicles, and had some lobules comprising acini with adipose vacuoles. The short excretory duct was lined with stratified squamous cells and drained to the gingival surface. The sebum constituting disintegrated acinar cells in the duct was also noted. In some cases, the granules were accompanied by cystic dilatation in the ducts and/or inflammatory reactions, without neoplastic or pre-neoplastic lesions. The overall incidence (9.1%) of Fordyce’s granules in males was higher than that (0.9%) in females, and it somewhat increased with ascending ages, demonstrating sex- or age-specific difference. Based on the above findings, the nature of Fordyce’s granules seen in CD(SD)IGS rats was similar to those of other strains and humans.

(Key words: ectopic sebaceous gland, Fordyce’s granule, CD(SD)IGS, age or sex difference, spontaneous lesion)

Introduction

Fordyce’s granules were first reported as degeneration in the prickle cells of the oral epithelium in humans¹. At present, however, the granules are considered to be non-pathological ectopic sebaceous glands that are not associated with hair follicles²⁻³. Additionally, the granules are recognized to occur frequently and bilaterally in the buccal, labial, retromolar mucosa, and vermillion border of the lip²⁻⁶. In other species, Fordyce’s granules have been reported as ectopic sebaceous glands in the soft palate of the guinea pig⁷, the lip of the chimpanzee⁸, and the gingiva of rats⁹⁻¹². In rats, ectopic oral sebaceous glands have previously been reported in Holtzman, Long-Evans, Wistar, and F344 strains, and their morphological features closely resemble human Fordyce’s granules used as a common name. According to previous reports⁹⁻¹², Fordyce’s granules in rats have also been confirmed to emerge at high incidence in the first upper/lower molar of aged males. To our knowledge, however, morphological hallmarks of Fordyce’s granules with their incidence in the Sprague-Dawley strain, which is most frequently used in numerous toxicological studies, have not yet been investigated. In the present report, we provide information about the spontaneous occurrence of Fordyce’s granules in CD(SD)IGS (the Charles River international genetic standard strain) rats aged 19 to 112 weeks old.

Materials and Methods

Animals

A total of 110 male and 110 female Crj:CD(SD)IGS rats aged 5 weeks old (designated as week 1 of the study) were purchased from Charles River Japan Inc. (Kanagawa, Japan). They were housed one animal per wire-mesh cage in an air-conditioned room (temperature: 23 ± 2°C, relative humidity: 50 ± 10%) with a 12-hour light/dark cycle. Basal diet (F-2, Funabashi Farm, Chiba, Japan) and tap water were available ad libitum. The animal care and experimental procedures were performed in accordance with the
Guideline for Animal Experimentation issued by the Japanese Association for Laboratory Animal Science, and the protocol was approved by the Institutional Animal Care and Use Committee of Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan).

Histopathological examination

The animals from a non-treated group in a 2-year long-term study with CD(SD)IGS rats were divided into 4 groups, and they were euthanized by exsanguination under ether anesthesia on weeks 14 (n = 15 of each sex), 28 (n = 20 of each sex), 55 (n = 25 of each sex), and 106 to 107 (n = 50 of each sex). During weeks 56 to 106, when the animals were found under moribund conditions or found dead, they were immediately necropsied. The skull of all animals was removed, fixed in 10% neutral buffered formalin, and decalcified with 25% formic acid-sodium citrate solution. Then, trimming was done at the three routine levels of the nasal cavity: level 1: posterior part of the upper incisor teeth, level 2: incisive papilla, and level 3: anterior edge of the upper molar teeth. In addition to these levels, some specimens were excised to observe the regions of the first, second and third molar of the maxilla. Afterward, the respective samples were embedded in paraffin wax, cut at 4 µm in thickness, and stained with hematoxylin and eosin (H & E) for histological examination. The incidence of Fordyce’s granules in each sex was calculated at two points of weeks 19 to 60 and weeks 61 to 112.

Results

Morphological characteristics

Fordyce’s granules were not macroscopically observed. Histopathologically, the granules consisted of one or more sebaceous glands without hair and hair follicles, and had some lobules composed of acini with basal cells at the periphery (Fig. 1). Well-differentiated acinar cells with foamy cytoplasm had a round to oval centrally-located nucleus and prominent nucleolus. The granules located near the third molar were relatively large and possessed numerous glandular lobules as compared with those of other sites. Short excretory ducts were lined by stratified squamous cells and drained to the surface of the gingival mucosa. The sebum constituting disintegrated acinar cells in the duct was also seen (Fig. 1). The secretory status of Fordyce’s granules was likely to indicate the holocrine secretion. These ducts and acini were clearly circumscribed by the basal membrane and connecting tissue (Sharpey’s fibers), and closely mimicked sebaceous glands in other regions of the skin, except for the lack of hairs and hair follicles. No compression of the adjacent tissue and cellular pleomorphism was observed. Gingivitis, periodontitis and/or caries were observed in almost all of the animals, despite ages or sexes. Fordyce’s granules seen in one female exhibited marked dilation of the excretory duct and were located in the inner side of the second molar (Fig. 2). The glandular structure and acinar cell population were modestly developed in this case. In other cases, the granules were accompanied by cystic dilatation of the excretory duct, inflammatory cell infiltrations, and subsequently reactive ductal hyperplasia (Fig. 3). However, no age-specific difference was seen in the dimension or morphological characteristics of the granules.

Incidence

The microscopic incidence and location of Fordyce’s granules in CD(SD)IGS rats are summarized in Table 1. The incidence of Fordyce’s granules in weeks 19 to 60 and weeks 61 to 112 was 1.7% and 18.0% for males and 0% and 2.0% for females, respectively. The overall incidence on weeks 19 to 112 was 9.1% for males and 0.9% for females. Therefore, there was a sex- or age-specific difference. For males, the rank order of the incidence in the location of Fordyce’s granules (from highest to lowest) was the first ≥ third > second molar gingiva (no incidence).

Discussion

In rats, since the lips are haired with sebaceous glands formation unlike humans, their structures are considered normal. In humans, Fordyce’s granules are seen in the buccal, labial, retromolar mucosa, and the vermilion border of the lip, but gingiva is relatively rare. Differences in location of the granules between rats and humans may depend on the anatomical nature. Furthermore, the granules in humans begin to appear from 10 years of age, and increase in size and number is seen up to adulthood. Their incidence increases with ascending ages, and it is reported that 80% or more adults would own the granules.

The objective of this study is to assess the histopathological features of Fordyce’s granules with their incidence in Sprague-Dawley rats of both sexes. As results, the granules were more frequently observed in the upper first and third molar gingiva. Their morphological and secretory findings were closely similar to those of the sebaceous glands seen in the skin, except that they lacked hairs and hair follicles. In a previous ultrastructural study, it was found that the granules in rats remarkably resemble the sebaceous

Table 1. Microscopic Incidence and Location of Fordyce’s Granules in CD(SD)IGS Rats

<table>
<thead>
<tr>
<th>Weeks of age</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>60</td>
<td>50</td>
<td>60</td>
<td>50</td>
<td>110</td>
</tr>
<tr>
<td>Maxillary 1st Molar</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>2nd Molar</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3rd Molar</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Overall (%)</td>
<td>1 (1.7)</td>
<td>9 (18.0)</td>
<td>0 (0)</td>
<td>1 (2.0)</td>
<td>10 (9.1)</td>
</tr>
</tbody>
</table>

*: One animal had two Fordyce’s granules. Parentheses show the percent of incidence.
Fig. 1. Fordyce’s granules in the first molar and gingiva of a male CD(SD)IGS rat. The granule consists of sebaceous glands without hair and hair follicles (arrowhead). The short excretory duct is lined with stratified squamous cells, and the sebum constituting disintegrated acinar cells is also noted (arrow). H & E, × 80.

Fig. 2. Fordyce’s granules in the second molar of a female CD(SD)IGS rat. Marked dilation in the excretory duct is observed. H & E, × 80. Inset: Higher magnification of Fordyce’s granules. The granules exhibit poor development. H & E × 320.

Fig. 3. Fordyce’s granules with inflammation of a male CD(SD)IGS rat. Inflammatory cell infiltration and subsequently reactive ductal hyperplasia are seen in the granules (arrowhead). H & E, × 160.
glands in the skin, and, their morphological characteristics are also almost comparable to those in humans. Therefore, the granules in the rats were considered to be a heterogenous sebaceous gland in the oral mucosa.

In the present investigation, Fordyce’s granules possessed some morphological alterations such as cystic dilatation of the duct, inflammatory cell infiltrations, and subsequently reactive hyperplasia of the duct. In humans, Fordyce’s granules had the secreting function with true ducts, which were usually plugged with keratin. In F344 strain rats, the duct was reported to be occluded by sebum plugs, followed by the formation of cystic dilatation. Based on these data, cystic dilatation in the duct may be brought about by the presence of plugs derived from the sebum and/or food intake, leading to the cyst formation in the excretory ducts. Moreover, since gingivitis, periodontitis, and caries with pulp necrosis were frequently observed in either sex, inflammation around the granule may be present as a consequence of the progression of those lesions. Although neoplastic or pre-neoplastic lesions spontaneously arising from the Fordyce’s granule have been previously reported in humans, there has been no published information in other species including rats. In our investigation, neither neoplastic nor pre-neoplastic lesions of Fordyce’s granules were seen in any tissue examined. The reason may be in part explained by the fact that a morphological approach in this intraoral site is rarely allowed in routine, toxicological studies. Meanwhile, it has been reported that sebaceous metaplasia (de novo free sebaceous glands) in the gingiva is experimentally induced by treatment of rats with the carcinogen 4-nitroquinoline N-oxide (4-NQO), although the correlation between the neoplastic lesions and the Fordyce’s granules is still unclear.

In our work, there was an age- or sex-specific difference in the incidence of Fordyce’s granules. These results were essentially consistent with the data from previous investigators, suggesting that Fordyce’s granules are more extensively seen in the anterior portion of the molars of aged males. In Holtzman, Long-Evans, and Wistar strain rats, the locations of Fordyce’s granules have been reported to be only in the anterior gingiva of the first upper or lower molar. In contrast, the Fordyce’s granules examined under the conditions of this study were often noted in the upper third molar gingiva as well as the first molar. Interestingly, the presence of Fordyce’s granules in the third molar or posterior portion of the rat gingiva has not previously been reported (Table 2). In applying our trimming method, the skull is sectioned only at the three levels as mentioned above, leading occasionally to the loss of the granules located in the posterior portion. More comprehensive research may be required in the oral cavity of various strains of rats.

In F344 rats, Fordyce’s granules have been reported to be more frequent in the male incisor gingiva, and their incidence was in excess of 56.3% for males aged 112 weeks old. In our investigation, no granules were detected in the incisor gingiva. The difference in the incidence of Fordyce’s
granules between SD and F344 rats may be due to strain specificity.

The sebaceous glands play an important role in their development and proliferation dependent on the hormonal control, and the sebum production is increased by androgen stimulations. In particular, androgen is relevant to modulation of the sebum production and the development of the sebaceous glands in the skin. Based on both present and previous results, the high incidence and glandular formation of the granules were evidently observed in males. Accordingly, the high incidence of the granule in males is explained by the notion that the sebaceous glands are modulated by androgen. Taken together with all information, the possibility is raised that the ectopic sebaceous glands in the oral cavity would be widely present in other species. However, the biological and physiological roles of the granules in both rats and humans still remain unclear, and further investigations may be required to elucidate the underlying mechanism.

Acknowledgement: We thank Dr. K Yoshitomi, Pfizer Inc., and Dr. M Kato, Daiichi Pharmaceutical Co., Ltd., for their variable advice. We also thank Mr. Y Ozaki and Mr. T Enkawa, Technology Research Center, Daiichi Pharmaceutical Co., Ltd., for their technical assistance.

References
5. Gorsky M, Bachner A, Fundoiamu-Dayan D, and Cohen C.

---

Table 2. Summary of the Presence of Fordyce’s Granules Reported in Several Rat Strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Items</th>
<th>Incidence* (%)</th>
<th>Location</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>male</td>
<td>female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Holtzman9*</td>
<td>4/45</td>
<td>10/80</td>
<td>1st upper</td>
<td>Adult</td>
</tr>
<tr>
<td>Long-Evans10</td>
<td>8/734</td>
<td>3/722</td>
<td>1st lower</td>
<td>Adult</td>
</tr>
<tr>
<td>Wistar11</td>
<td>(8.9%)</td>
<td>(12.5%)</td>
<td>1st upper</td>
<td>60–110 days</td>
</tr>
<tr>
<td>F34412</td>
<td>(1.2%)</td>
<td>(0.4%)</td>
<td>1st upper</td>
<td>Adult</td>
</tr>
</tbody>
</table>

*: Reference Number
**: Number of animals with Fordyce’s granules/number of animals examined microscopically. Parentheses show the percent of incidence.


