A salivary mucocele is defined as an accumulation of leaking salivary secretion in single or multiloculated cavities in the connective tissue of the mouth or neck contiguous to a salivary gland or duct. In general, it can be observed in the form of a cyst lined with granulation tissue and the absence of any epithelial lining. A salivary mucocele can occur in several kinds of animals including dogs of any breed. The cause is generally not identified; however, blunt trauma, foreign body and sialolith have been suspected as major causes of salivary mucocele. It arises most commonly from the sublingual salivary gland, either from individual units of the polystomatic portion or from the duct of the monostomatic portion in dogs. Saliva usually leaks from the torn portion, and accumulates in the adjacent tissue. Consequently, the accumulated saliva induces an inflammatory response. A wall of granulation tissue is gradually developed in response to the inflammation. The diagnosis is usually made based on the history, physical examination by palpation and cytological examination after aspiration of the cyst and can be confirmed by histopathological examination.

In a toxicological study, we encountered a laboratory beagle with a salivary mucocele characterized by lining epithelial-like cells. It was considered to be a spontaneous lesion because there were no similar lesions in the other animals. Unexpectedly, there are only a few reports focused on the histological features of salivary mucoceles in animals. Therefore, we describe the histopathological characteristics of this lesion.

The animal was a male beagle (Kitayama Labes Co., Ltd., Yamaguchi, Japan) treated with a compound in a 2-week repeated-dose toxicity study with a 4-week recovery period. The experiment procedures were approved by the Animal Ethics Committee of Takeda Pharmaceutical Company Limited. No abnormality was observed in the appearance of the neck and mandible or in the clinical pathology. The animal was sacrificed at 14 months old by exsanguination under thiopental sodium anesthesia and subjected to a complete necropsy. At necropsy, a pale yellowish cyst, 10 × 40 × 12 mm in size, containing frothy mucus was observed under the mandibular skin. The proximal edge of the cyst was not clearly identified because it was buried between the digastric and mylohyoid muscles. The other organs had no abnormal gross findings. The cyst was fixed in 10% neutral buffered formalin solution. Routine paraffin embedded sections were stained with hematoxylin and eosin (HE). Alcian blue (pH 2.5)-periodic acid-Schiff (AB-PAS) double staining and immunohistochemical staining for human cytokeratin (hCK), which identifies cytokeratin 5, 6, 8, 17 and 19, and macrophage scavenger receptor A (MSR-A) were performed. The antibodies used for this study were as follows: anti-human cytokeratin mouse monoclonal antibody
(diluted 1:500, clone: MNF116, Dako Cytomation, Tokyo, Japan) and anti-human macrophage scavenger receptor A mouse monoclonal antibody (diluted 1:100, clone: SRA-E5, Transgenic Inc., Kobe, Hyogo, Japan). For electron microscopy, small pieces of the cyst wall, originally fixed in 10% neutral buffered formalin solution, were postfixed in osmium tetroxide, dehydrated and embedded in epoxy resin. Ultrathin sections were cut and stained with lead citrate and uranyl acetate and examined using a transmission electron microscope.

Light microscopically, the cyst was encapsulated by dense connective tissue and numerous villous projections arose from the internal surface of the cyst (Fig. 1a). Villous projections had fibrovascular stalks with lymphoplasmacytic cells and pigmented macrophages and were lined by stratified epithelial-like cells (Fig. 1b). The epithelial-like lining cells had round to oval nuclei and slightly eosinophilic and foamy cytoplasm. The lumen of the cyst was filled with eosinophilic amorphous material with a few desquamated cells (Fig. 1b). Some multinucleated giant cells were also observed on the surface of the lining cells (Fig. 1c). The sublingual gland (Fig. 1d) and a ruptured sublingual interlobar duct (Fig. 1e) connected to the cyst were observed in the surrounding connective tissue. The amorphous material within the cyst showed a positive reaction for AB-PAS with a bluish or reddish violet coloration (Fig. 1f). The epithelial-like cells also had AB-PAS-positive reddish violet colored granules in their cytoplasm (Fig. 1g). As expected, secretory granules of the sublingual gland and saliva in the ducts showed a similar positive reaction for AB-PAS (Fig. 1h).

Immunohistochemically, the epithelial-like cells were positive for MSR-A, a macrophage marker in dogs (diluted 1:100, clone: SRA-E5). For electron microscopy, small pieces of the cyst wall, originally fixed in 10% neutral buffered formalin solution, were postfixed in osmium tetroxide, dehydrated and embedded in epoxy resin. Ultrathin sections were cut and stained with lead citrate and uranyl acetate and examined using a transmission electron microscope.

In the present case, fortunately, we found a ruptured sublingual interlobar duct that could be a cause of this lesion. However, it is not adequate to allege that the ruptured duct was the primary site of leakage. It is reported that salivary mucoceles sometimes have more than one communication between the sublingual gland and the cyst. In view of the orientation of the cyst, it was speculated that the primary leakage probably occurred in the individual units of the polystomatic portion or the duct of the monostomatic portion.

The histological appearance of salivary mucoceles varies greatly depending on the stage of development. This case was recognized as a relatively long-standing lesion because the cyst was circumscribed by mature dense connective tissue with abundant vessels. On the other hand, the lumen of the cyst was filled with eosinophilic amorphous material and desquamated cells, suggesting that sublingual secretion could still be leaking out consistently and that this lesion would be in the process of formation. These prolonged irritations might induce accumulation of a large number of macrophages and influence its morphological changes, leading to an epithelial-like appearance.

We believe this case report helps to understand a diversity of the background findings in beagles used in toxicity studies.

Acknowledgment: The authors would like to thank Mr. Ryo Fukuda, Mr. Ryotaro Hori and Mr. Yoshiyuki Furukawa for their support during this work.
Fig. 1. Histopathology of the cyst in the laboratory beagle. (a) The cyst was circumscribed by mature dense connective tissue, and the wall frequently projected into the lumen with fibrovascular connective tissue stalks. (b) The lining cells were morphologically similar to epithelial cells and projected up toward the lumen. The epithelial-like cells, lining cells, had round to oval nuclei and slightly eosinophilic and foamy cytoplasm. Granulation tissue, abundant vessels with fibroblasts, lymphocytes, pigmented macrophages, and plasma cell infiltrations were observed inside of the villous projection. The lumen of the cyst was filled with eosinophilic amorphous material with a few desquamated cells. (c) Some multinucleated giant cells (arrows) were also observed in the lumen side. (d) Normal sublingual gland tissue (asterisk) was observed in the connective tissue near the cyst (arrows). In the upper right side, the normal oral mucosa was observed (arrowhead). (e) A ruptured sublingual interlobar duct connected to the lumen was observed in the peripheral connective tissue. (f) The amorphous material showed a positive reaction with a bluish or reddish violet coloration. (g) The epithelial-like cells had reddish violet colored granular staining in their cytoplasm. (h) In the nearby normal sublingual gland, the cytoplasm of the mucous cells and the luminal side of the serous cells showed violet colored granular staining. The secretion in the lumen showed a positive reaction with a bluish or reddish violet coloration. HE: ×11 (a), ×206 (b), ×110 (c), ×17 (d), ×55 (e). AB-PAS double stain: ×17 (f), ×432 (g), ×103 (h).
Fig. 2. Immunohistochemical staining for MSR-A and hCK. (a-b) The epithelial-like cells were positive for MSR-A. Multinucleated giant cells were negative for MSR-A (b: Inset). (c) The epithelial-like cells were negative for hCK. (d) The epithelial cells of the interlobar duct were strongly positive for hCK. Immunohistochemistry, counterstained with hematoxylin, ×17 (a, c), ×41 (d), ×103 (b) (Inset: ×450).

Fig. 3. Electron microscopy of the epithelial-like cells. (a) The epithelial-like cells had numerous vacuoles containing electron-lucent material, which was presumed to be lysosomal in origin. (b) The epithelial-like cells had pseudopods (arrows) on their cell surfaces interdigitating with those on the adjacent cells. Scale bars: 2 mm (a), 0.5 mm (b).
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