Angiokeratoma with lysosomal dilatation in keratinocytes in a dog: a case report

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ABSTRACT: Herein, we report the histopathology of angiokeratoma characterised by non-invasive, proliferative, ectatic vascular malformations accompanying lysosomal dilation in the canine skin. Two cutaneous angiokeratomas were diagnosed in a six-year-old spayed female Pekinese dog. Physical examination of the skin revealed two small erythematous papules on the axillary and abdominal regions. The masses were approximately 2–3 mm in diameter, well-circumscribed, purple to red in colour, and slightly elevated above the skin surface. The two small masses had similar histopathological features, characterised by exophytic proliferation of a mixture of multiple vascular channels resembling a cavernous haemangioma on the superficial dermis. The entire lesion was well circumscribed and the overlying epidermal hyperplasia formed a collarette. Ultrastructural analysis with transmission electron microscopy revealed electron-lucent lysosomal dilatation in the keratinocytes of the irregular hyperplastic epithelial trabeculae like rete pegs.

Keywords: angiokeratoma; haemangioma; lysosomal storage disease; transmission electron microscopy

Haemangiomas are benign tumours of the vascular endothelium and are relatively common in dogs, but rare in other domestic animals. In dogs, haemangiomas occupy 2–3% of skin and usually arise on the leg, flank, neck, face, and eyelid (Vala and Esteves 2001; Meuten 2002).

Angiokeratoma is an uncommon variant of dermal haemangioma and has both a vascular and epithelial component. An angiokeratoma is a small, well-circumscribed, elevated dermal mass with histological characteristics of benign proliferations of dilated thin-walled blood vessels in the upper dermis with overlying epidermal hyperplasia that appears to have invaded the vascular component. Hyperkeratosis can also be observed (Yager and Wilcox 1994; Gross et al. 2005). This tumour type occurs most often on the eyelid and conjunctiva but can also arise in the superficial dermis at any site (Vala and Esteves 2001).

Occasionally haemangioma, especially verrucous haemangioma which is a structural variant of capillary or cavernous haemangioma has similar macroscopic features to angiokeratoma, although the histological appearance and clinical behaviour are different. Therefore, distinguishing these lesions is important in histological study (Imperial and Helwig 1967).

In human haemangioma cases, several studies have suggested that angiokeratoma, in particular angiokeratoma corporis diffusum, should raise the suspicion of various lysosomal storage diseases such as Anderson-Fabry's disease, β-galactosidosis, fucosidosis, aspartylglucosaminuria, galactosialidosis, β-mannosidosis and Schindler/Kanzaki disease (Ozdemir et al. 2004).

Lysosomal storage diseases are inherited metabolic disorders that result from an enzymatic deficiency within the lysosomal catabolic pathway (Skelly and Franklin 2002). The lysosome contains more than 60 kinds of acid hydrolases and a deficiency in these enzymes results in excessive storage of their substrates, leading to progressive cell damage and organ dysfunction (Nagayasu et al. 2008). Thus, electron-dense lysosomal deposits or electron-lucent lysosomal vacuoles are observed depending on the accumulated substrates (Kanda et al. 2002; Kanitakis et al. 2005; Molho-Pessach et al. 2007; Albano et al. 2010).

Herein, we report the histopathologic and ultrastructural analysis of an angiokeratoma char-

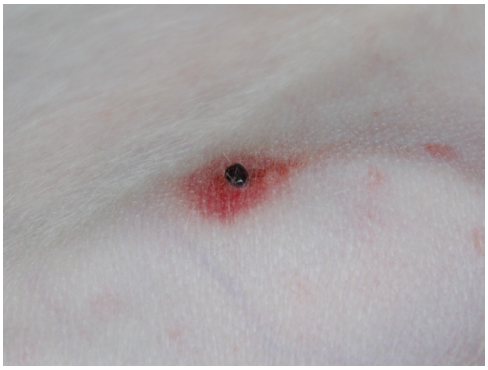

Figure 1. The wart-like mass on the abdominal skin of a Pekinese was 2 mm in diameter, well-circumscribed, and purple to red

acterised by miscellaneous benign hypervascular lesions accompanying lysosomal dilatation in the epidermal collarette.

Case description

A six-year-old spayed female, Pekinese dog was presented to the veterinary clinic with two masses on the axillary and abdominal skin without any other significant signs. The dog had a history of continuous intervertebral disc disease since four years of age and auricular haematoma.

General physical examination revealed two erythematous papules that were 2–3 mm in diameter, ovoid in shape, well-circumscribed, and slightly elevated above the skin surface on the axillary and abdominal region (Figure 1).

The masses were surgically removed, fixed in 10% neutral buffered formalin (BBC; Mount Vernon, WA, USA) and embedded in paraffin for light microscopic evaluation. The paraffin-embedded tissue was sectioned at 4 µm, and stained with haematoxylin and eosin formalin (BBC; Mount Vernon, WA, USA). For ultrastructural analysis, a piece of the mass (1 × 1 × 1 mm in dimension) was fixed in 2.5% glutaraldehyde (Sigma-Aldrich; St. Louis, MO, USA) at 4 °C for 24 h. The samples were then post-fixed in osmium tetroxide (OsO_4; Merck, Darmstadt, Germany), dehydrated and embedded in Epon-812 (Electron microscopy sciences; Hatfield, PA, USA). Semithin sections were stained with 2% toluidine blue and examined to locate areas of interest within the block. Ultrathin sections were cut using an Ultracut E microtome (Reichert-Jung; Depew, NY, USA) and stained with 1.0% uranyl acetate and 1.0% lead citrate. Transmission electron microscope (TEM) (H-7650; Hitachi, Ontario, Canada) examination was performed with a standard square mesh (GG200-Ni; Electron microscopy sciences) for calibration.

Microscopic examination revealed both the axillary and abdominal masses to be well-circumscribed, ulcerated, and exophytic nodules consisting of irregular hyperplasia of the overlying epidermis with hyperkeratosis (Figure 2). The superficial corium was thickened by dilated, blood-filled vascular spaces identical to those in cavernous haemangioma. Beneath the nodule, the dermal layer showed oedematous collagen connective tissue and compressed skin adnexal structures (Figure 2). Hyperplastic epithelium with spongiosis

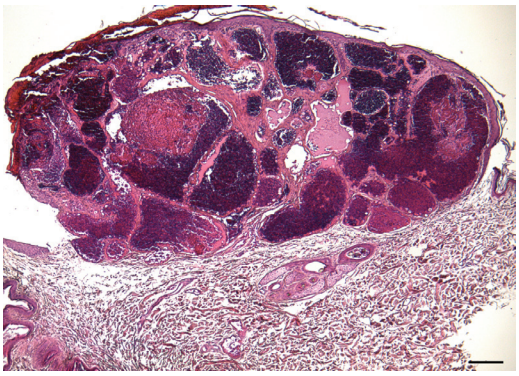

Figure 2. The well-circumscribed, ovoid shaped exophytic nodule consisted of rete peg like hyperplasia of the overlying epithelium and enclosed vascular spaces. The vascular spaces were filled with blood and fibrin thrombus. Beneath the nodule, the dermal layer showed oedematous collagen fibres and compressed skin adnexal structures (H&E × 40, bar = 100 µm)

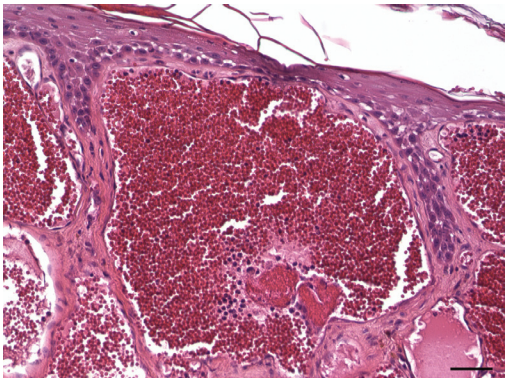

Figure 3. The overlying epithelium showed irregular hyperplasia without dysplasia and cytoplasmic vacuolisation of basal cells. The hyperplastic epithelial trabeculae invaded the vascular component and partially surrounded the vascular structures. The blood-filled vascular structures were lined by a single layer of mature endothelial cells (H&E × 200, bar = 50 µm)

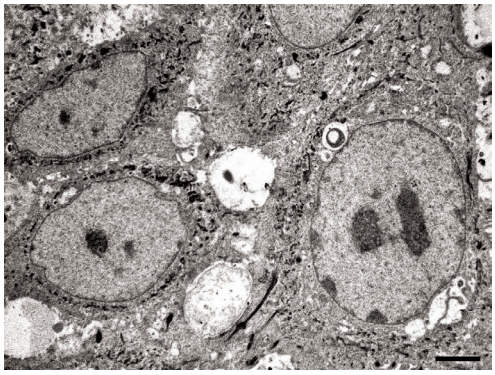

Figure 4. The electron-lucent vacuoles within keratinocytes (× 5000, bar = 2 μm)

invaded the vascular component and partially surrounded the vascular structures (Figure 3).

The axillary mass was identical to the abdominal mass with hyperplasia of overlying epidermis and vascular spaces in the superficial corium. Because of the identical nature of the two masses, only the abdominal mass was selected for electron microscopic examination.

TEM showed cytoplasmic vacuoles within the keratinocytes that had invaded the vascular spaces. The vacuoles were electron-lucent and limited by a single membrane (Figure 4).

In summary, in the presented dog axillary and abdominal lesions were diagnosed as angiokeratoma of the skin. In addition, the presence of electron-lucent lysosomal dilatation raised the possibility of concomitant lysosomal storage disease.

DISCUSSION AND CONCLUSIONS

This report describes a case of angiokeratoma with suspicion of lysosomal storage disease in a dog. Angiokeratoma is a rare variant of dermal haemangioma.

Generally, haemangiomas are classified as being of the capillary or cavernous type depending on the size of the vascular spaces and the amount of intervening fibrous tissue (Scott et al. 2001). Verrucous haemangioma, a structural variant of capillary and cavernous haemangioma, has similar macroscopic and clinical features to angiokeratoma, with epidermal acanthosis, papillomatosis, and hyperkeratosis developing secondarily. However, angiokeratoma involves only the papillary dermis and is located directly under the hyperplastic epidermis, whereas verrucous haemangioma involves the dermis and subcutaneous fat (Imperial and Helwig 1967). Therefore, not only the characteristics of cellular components, such as epidermal hyperplasia, hyperkeratosis, and dilated vessels and a surrounding of fine stroma, but also the structural characteristics of angiokeratoma, such as the location of the lesion, involvement of the dermis and subcutaneous tissue, and margins of the lesion, could be useful in the differential diagnosis of haemangioma variants (Imperial and Helwig 1967). An accurate diagnosis based on the histopathological features of the lesion is important for complete surgical excision, to make a prognosis and to predict possible complications (Mani and Feierabend 1982).

There are several clinical variants of angiokeratomas in humans. Local angiokeratoma comprises: 1. Angiokeratoma of Mibell; 2. Angiokeratoma of Fordyce; 3. Solitary and multiple popular angiokeratoma; 4. Angiokeratoma circumscriptum. Systemic angiokeratoma is described as angiokeratoma corporis diffusum, with widespread papules, and is a manifestation of one of several inherited lysosomal storage diseases such as Anderson-Fabry's disease or fucosidosis (Ozdemir et al. 2004; Siponen et al. 2006).

A correlation between lysosomal storage disease and angiokeratoma has been described in several studies on human subjects (Kanitakis et al. 2005; Molho-Pessach et al. 2007). According to a previous study, angiokeratoma corporis diffusum, which is a generalised systemic form that is usually associated with a metabolic disorder, was present in 52% of fucosidosis patients (Kanitakis et al. 2005). Because lysosomal storage disease is hereditary and has no definitive clinical or histological features, taking a family history for identification of genetic factors is important. However, it is difficult to obtain the family history of a dog from a pet shop. Thus, finding screening markers for lysosomal storage disease in pet animals is more important than for human cases.

Unlike most lysosomal storage disease cases, the presented dog showed just two masses of solitary angiokeratoma, i.e. not angiokeratoma corporis diffusum, and consequently more studies regarding this kind of presentation are required. Unfortunately, due to the limited circumstances including the absence of non-fixed material for enzyme analysis, a lack of family history and the owner's opposition to further examination, only a histopathological analysis was performed. The presence of electron-lucent lysosomal dilatation suggested the possibility of a lysosomal storage disease other than Anderson-Fabry's disease which is characterised by electron-dense lysosomal deposits. However, to confirm a diagnosis of lysosomal storage disease, lysosomal enzyme analysis with molecular genetic testing,

pathology examination and other novel diagnostic techniques are required (Skelly and Franklin 2002).

Generally, angiokeratoma is a benign tumour and when growth is only local, surgical excision may be curative (Yager and Wilcox 1994; Ozdemir et al. 2004). In the case of angiokeratoma with lysosomal storage disease, the treatment of the angiokeratoma should be accompanied by control of the lysosomal storage disease. Treatment for lysosomal storage disease directed at a single enzyme includes enzyme replacement, bone marrow transplantation, and gene replacement protocols (Ohshima et al. 1999; Skelly and Franklin 2002).

The present study shows a rare case of two angiokeratomas with a histological description and attempted ultrastructural analysis of canine angiokeratomas presenting with electron-lucent lysosomal dilatation, suggestive of lysosomal storage disease. Although this study was limited in scope, nevertheless, these results suggest the necessity of more detailed histological diagnosis of vascular tumours in domestic animals and further studies on the correlation of lysosomal storage disease with angiokeratoma.

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