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Canine aural cholesteatoma: A histological and immunohistochemical study

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ABSTRACT

Canine aural cholesteatoma is an epidermoid cyst that forms in the middle ear cavity as a rare complication of otitis media but the aetiopathogenesis remains controversial. In the present study, 13 cases of canine aural cholesteatoma were investigated histologically and immunohistochemically and compared with cases of chronic otitis. The immunohistochemical investigation was performed using the following monoclonal antibodies: anti-cytokeratins (CK) 14, 16, 8/18, and 19, and anti-Ki67. The proliferative indexes (PIs) of cholesteatomata and otitis epithelium were calculated as the percentage of Ki67 positive nuclei/total nuclei. Histologically, the cholesteatomata were composed of a hyperplastic, hyperkeratotic epithelium (matrix) resting on a fibrous perimatrix, infiltrated by inflammatory cells and devoid of cutaneous adnexa. Immunohistochemically, the cholesteatoma epithelium was CK14- and CK16-positive, and CK8/18- and CK19-negative. A similar pattern of CK expression was found in otitis externa. In otitis media, ciliated epithelium stained CK8/18- and CK19-positive in all layers, CK14-positive in the basal layers, and CK16-negative. The mean PIs in cholesteatomata and otitides were 18.8 and 17.8, respectively. The immunohistochemical pattern of CK expression in cholesteatomata, when compared with chronic otitis, was suggestive of hyperproliferative epithelium, but its origin could not be demonstrated. Comparable PI values were obtained in cholesteatoma and in chronic otitis, which confirmed that Ki67 is a valuable indicator of a hyperproliferative state, but not a predictor of aggressiveness.

Introduction

Aural cholesteatoma is an epidermoid cyst that forms in the middle ear and is composed of hyperplastic, hyperkeratotic squamous epithelium surrounding a core of keratin debris. It has been described as 'skin growing in the wrong place' (Strunk, 1993) and the term 'cholesteatoma' itself, coined by Müller in 1838, is currently considered a misnomer because it is neither a granulomatous lesion or a neoplasm (Friedmann and Arnold, 1993; Strunk, 1993).

In humans, aural cholesteatoma is classified as congenital or acquired (Persaud et al., 2007). Congenital cholesteatoma develops beneath an intact tympanic membrane in patients whose clinical history does not include an obvious ear infection or auditory tube dysfunction (Strunk, 1993). It is hypothesised to originate from embryonic epidermoid remnants that fail to involute (Koltai et al., 2002). Acquired cholesteatoma is a complication of chronic otitis and it is thought to originate either as a result of squamous metaplasia of the middle ear epithelium or from the migration of external ear canal epithelium through a tympanic perforation (Sadé, 1980; Semaan and Megerian, 2006). Other workers have suggested that it could originate from an invagination of a portion of the tympanic membrane into the epitympanum, secondary to a Eustachian tube dysfunction (retraction pocket theory; Sadé, 1980; Olszewska et al., 2003). To date, the origin of the epithelium of cholesteatoma remains uncertain.

In dogs, cholesteatoma is mostly considered an acquired disease and a severe complication of otitis media, with or without concurrent rupture of the tympanic membrane (Little et al., 1991a, 1991b; Venker-Van Haagen, 2005; Harran et al., 2012). To our knowledge, congenital cholesteatoma has not been reported in dogs.

In human medicine, several immunohistochemical studies have been performed in an attempt to identify the origin of cholesteatoma epithelium (Bujía et al., 1993; Kuijpers et al., 1996; Olszewska et al., 2005). More specifically, the expression of cytokeratins, the intermediate filaments of epithelia, has been extensively investigated, since the pattern of cytokeratin expression in a particular epithelium varies with its anatomical location, developmental stage,

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Table 1
Details of antibodies employed in immunohistochemistry (antigen retrieval, dilution and sources).

Antibodies	Clone	Antigenic retrieval	Dilution	Source
CK14	LL002	Pressure cooker, 10 min, citrate buffer (pH 6)	1 : 2000	NeoMarkers
CK16	LL025	Pressure cooker, 10 min, citrate buffer (pH 6)	1 : 200	Novocastra
CK8/18	5D3	Pepsin[a], 37 °C, 14 min	1 : 200	Zymed
CK19	B170	Pepsin[a], 37 °C, 14 min	1 : 200	Novocastra
Ki67	MIB-1	Pressure cooker, 18 min, citrate buffer (pH 6)	1 : 600	Dakocytomation

[a] Zymed.

and state of differentiation. However, conflicting results have been reported. To elucidate the mechanisms of the progressive growth that characterise cholesteatomata, numerous studies in human medicine focused on the proliferative capability of lining epithelium (Strunk, 1993; Gersdorff et al., 2006). Investigations used MIB 1 (Ki67) immunostaining, in an attempt to characterise the aggressiveness of the cholesteatoma and the likelihood of recurrence (Bujía et al., 1996; Mallet et al., 2003).

To the best of our knowledge, detailed histological and immunohistochemical characterisations of canine middle ear cholesteatoma are not currently available in the veterinary literature. Therefore, the aim of the current study was to characterise canine aural cholesteatoma, comparing epithelial immunophenotypes (cytokeratins) and proliferative activity (Ki67) in cholesteatomata, in normal auditory canals, and in otitis externa and otitis media.

Materials and methods

Thirteen aural cholesteatomata from 11 dogs were reviewed; two cases were recurrences. Specimens were obtained by total ear canal ablation and lateral bulla osteotomy (TECALBO) or ventral bulla osteotomy (VBO), fixed in 10% neutral buffered formalin, and submitted to the Department of Veterinary Science and Public Health (DIVET) of Milan for histopathology. In all cases, clinical, radiological (computed tomography scan, CT), and video-otoscopy findings were consistent with the diagnosis of aural cholesteatoma (Little et al., 1991b; Travetti et al., 2010; Greci et al., 2011). Signalment, clinical signs, and results of middle ear swabs for bacteriological culture were recorded for each dog. Follow-up consisted of a clinical examination. Ten histological specimens of chronic otitis externa or media from 10 dogs and 1 normal canine external meatus auditory canal specimen were retrieved from DIVET archives and were included in the study.

All histological specimens were routinely processed, paraffin embedded, and stained with haematoxylin and eosin (HE). Serial sections were obtained and immunolabelled using the standard avidin–biotin–peroxidase complex (ABC) procedure (Hsu et al., 1981) and the following antibodies: anti-cytokeratin 8/18, 14, 16, and 19, and anti- Ki67 protein. Primary antibodies and antigen retrieval methods used are listed in Table 1. A section of normal dog skin served as a positive control. For negative controls, the primary antibody was replaced by serum from a clinically healthy horse. Cytokeratin immunostaining was quantitatively interpreted by light microscopy (positive and negative). When a positive result was obtained, immunostaining was scored as faint, moderate, or intense. The expression of Ki67 (MIB-1) was quantitatively assessed using an automatic image analysis system (Image Pro Plus 4.5, Media Cybernetics). The relative percentage of immunostained nuclei in 10 high power fields was calculated and recorded as a proliferative index (PI).

Results

Signalment and clinical features of dogs with aural cholesteatoma

Signalment and follow-up data for dogs with cholesteatoma are summarised in Table 2. Thirteen cases of aural cholesteatoma were diagnosed in 11 dogs. Two cases (numbers 3 and 10) were recurrences. The mean age at diagnosis was 7 years (range, 5–10 years); eight dogs were males (one neutered) and there were three females (one spayed). A variety of breeds were represented. All dogs had a history of chronic recurrent otitis externa that was unresponsive to topical or systemic therapy over the previous 3–30 weeks. The main clinical signs recorded were head shaking, otodynia, and head tilt. The main findings on computed tomography (CT) were severe expansion and osteolysis of the tympanic bulla with loss of air contrast. Partial or total occlusion of the horizontal canal (end-stage otitis) was observed on video-otoscopy in all cases. Eight cases had rupture of the tympanic membrane, and amorphous, pearly material in the middle ear cavity was evident in nine cases.

The middle ear content was cultured in each of the 13 cases. *Staphylococcus intermedius* was isolated in three cases, *Proteus mirabilis* in two cases, *E. coli* in one case, *Pseudomonas aeruginosa* and *P. mirabilis* in one case and *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in another case. No bacteria were recovered in five cases.

Histopathology

Normal external auditory meatus

The normal meatus was lined by a thin squamous epithelium, one to two cell layers thick, resting on a derma containing hair follicles, sebaceous and ceruminous glands, and supported by annular cartilage.

Cholesteatoma

Ten cases of aural cholesteatoma were composed of segments of the wall of a cystic lesion that contained abundant amorphous lamellar keratin debris. The cysts were lined by a multi-layered keratinising squamous epithelium (matrix) resting on a stroma (perimatrix) composed of dense fibrovascular connective tissue devoid of adnexa (Fig. 1). The epithelium was intensely hyperplastic (5–25 layers thick), with severe orthokeratotic hyperkeratosis, hypergranulosis and mild parakeratosis. Keratinocytes showed progressive maturation; no mitotic figures were observed. Mild multifocal intracellular oedema and minimal neutrophilic exocytosis were present. The perimatrix immediately beneath the epithelium was composed of loosely arranged collagen fibres expanded by moderate oedema; the deepest layers of the perimatrix were densely cellular, composed of fibroblasts and fibrocytes embedded in abundant collagenous stroma. There was moderate to severe stromal inflammation, consisting of numerous perivascular lymphocytes and plasma cells, scattered haemosiderin-laden macrophages and a few

Table 2
Signalment data of dogs with cholesteatoma and mean values of proliferative index (PI) of the epithelium as obtained by immunolabelling with monoclonal antibody MIB1.

Case no.	Signalment	PI	Recurrence
1	Pug, male, 8 years	–[a]	
2	Mixed breed, male, 5 years	12.45	
3	Mixed breed, male, 6 years	14.12	Recurrence of case 2
4	Flat coated retriever, male, 10 years	20.51	
5	Poodle, castrated male, 5 years	–[a]	
6	Afghan hound, male, 8 years	17.35	
7	Weimaraner, female, 9 years	8.42	
8	American cocker spaniel, male, 6 years	–[a]	
9	Schnauzer , male, 5 years	31.79	
10	Poodle, castrated male, 5 years	15.90	Recurrence of case 5
11	Mixed breed, spayed female, 9 years	34.81	
12	Labrador, male, 9 years	15.74	
13	Golden retriever, female, 5 years	16.93	

[a] Specimen could not be processed as it constituted only keratin debris.