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Ultrastructural Differences in Cranial Cruciate Ligaments from Dogs of Two Breeds with a Differing Predisposition to Ligament Degeneration and Rupture

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Summary

Cranial (anterior) cruciate ligament (CCL) samples were obtained from dogs of the Labrador retriever (LR) and greyhound (GH) breeds, of which the former but not the latter is predisposed to CCL rupture. Electron microscopy revealed that the collagen fibril diameters of GHs were larger than those of LRs ($P=0.03$). Histological examination revealed a "fibrocartilaginous" appearance of CCLs in seven of eight GHs, and, to a lesser extent, in three of eight LRs. The formation of fibrocartilage is clearly not a disadvantage to the healthy racing GH, and cannot be regarded as a pathological degeneration in this breed. It is suggested that fibrocartilage is formed as a beneficial physiological adaptation to the compression of CCLs caused by tensile stress as a result of the tightening of two twisted bands. Fibrocartilage would appear to protect CCLs in the GH, but it may be indicative of a mild degenerative change, which may eventually lead to rupture in the LR.

Keywords: collagen fibrils; cranial cruciate ligament; dog; fibrocartilage; ligament

Introduction

Non-contact cranial (anterior) cruciate ligament (CCL) injury occurs commonly in dogs (Whitehair *et al.*, 1993) and risk factors that play a role in canine CCL degeneration include breed, age, bodyweight (Vasseur *et al.*, 1985), immobilization (Laros *et al.*, 1971) and a stenotic distal femoral intercondylar notch (Aiken *et al.*, 1995). Certain breeds such as the Labrador retriever and Rottweiler (Whitehair *et al.*, 1993) are predisposed to CCL rupture whereas others, such as the greyhound, are at low risk of ligament failure (Whitehair *et al.*, 1993).

Light and electron microscopy have been used to examine the structural features of the cruciate ligaments in many species, and also the morphology of collagen fibrils. These techniques can provide indications of tissue integrity, ageing, turnover, tensile strength and exercise status (Parry *et al.*, 1978; Cherdchutham *et al.*, 2001). Research in this area has centred on medial collateral ligaments (MCLs) and CCLs (Amiel *et al.*, 1991; Cunningham *et al.*, 1999; Provenzano *et al.*, 2002), and anterior cruciate ligaments in man (Danylchuk *et al.*, 1978; Neurath *et al.*, 1994; Shino *et al.*, 1995; Murray *et al.*, 2004;), with the aim of clarifying the pathogenesis of ligament rupture and its management. There are conflicting views on the contribution of collagen fibril diameter to ligament status and function, but it is generally agreed that small fibrils are found in immature tissues and those undergoing remodelling (increased turnover) (Parry *et al.*, 1978).

The histological appearance of normal and damaged canine CCL has been described in several studies (Paatsama, 1952; Vasseur *et al.*, 1985; Narama *et al.*, 1996; Murray *et al.*, 2004). The severity of ligament degradation was graded from I to III in an extensive study on canine CCLs by Vasseur *et al.*

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(1985). More recently, loss and altered phenotype of ligament fibroblasts, and a reduction of crimp (where the collagen fibres have a periodic wave pattern which responds to the initial laxity in ligaments and tendons under tensile loading as the fibres straighten) was noted in ruptured CCLs (Hayashi *et al.*, 2002). Scanning electron microscopy was used by Yahia and Drouin (1989) and Clark and Sidles (1990) to examine the normal CCL and its fascicle morphology in dogs. Zachos *et al.* (2002) reported the small diameter of collagen fibrils in posterior cruciate ligaments from canine stifles with ruptured CCLs, suggestive of increased remodelling and turnover. However, there are few published data on the diameter and distribution of collagen fibril in dogs of breeds predisposed to CCL disease and rupture.

The aim of the present study was to investigate the features (histological and ultrastructural) of macroscopically normal CCLs in dogs of two breeds, namely, the Labrador retriever, which shows a predisposition to CCL disease, and the greyhound, which does not.

Materials and Methods

Samples

With the owners' consent, mid-CCL samples were obtained *post mortem* from the anatomically, macroscopically and radiologically normal stifle joints of Labrador retrievers (LRs) and greyhounds (GHs), details of which are given in Table 1. The animals had been humanely destroyed for reasons other than musculoskeletal disease. The numbers of specimens for light microscopy and electron microscopy were eight and seven respectively (LRs), and eight and five respectively (GHs).

Transmission Electron Microscopy (TEM)

Sample preparation. The samples of ligament (Table 1) were trimmed to approximately 5 mm^3 blocks and fixed in glutaraldehyde (2.5%) in 0.1 M sodium cacodylate buffer (pH 7.4) at 21 °C. The tissue blocks were then rinsed several times in 0.1 M sodium cacodylate buffer and stored at 4 °C in 0.1 M sodium cacodylate buffer containing sodium azide (1%) until required. The fixed tissue blocks were then processed by routine methods for TEM (Decker *et al.*, 1991).

Table 1
Details of dogs from which CCL samples were examined

	Details of dogs of the stated breed from which CCLs were examined by			
	light microscopy		*electron microscopy*	
Observation	*LR*	*GH*	*LR*	*GH*
Gender (and *n*)	M (2) F (6)	M (6) F (2)	M (3) F (4)	M (4) F (1)
Age (years)*	4.8±3.4	5.6±2.9	5.6±3.0	5.2±1.9
Bodyweight (kg)*	28.2±5.7	28.9±2.2	28.7±6.8	30±4.0

LR, Labrador retriever; GH, greyhound; M, male; F, female.
*Mean±SD.

Data analysis. Photographic negatives of each section were obtained at a magnification of ×22 000. The negatives were enlarged (×2.5) photographically to A4 paper size and digitally scanned, with an accompanying metric scale, by means of a flatbed scanner (Precision Scan Pro; Hewlett Packard, Bracknell, Berkshire, UK). The cross-sectional area of each fibril in the scanned image was measured by tracing around it with appropriate software (Image Tool; Cornell University, Ithaca, New York, USA). The fibril diameter was then obtained from the measured circular area by the formula Area$=\pi r^2$, after correcting for magnification. For each ligament sample, the fibril diameters were classified according to size, in 10 nm bands (10–20 nm, 20–30 nm etc). The results for each band were expressed as a percentage of the total number of fibrils examined.

Statistical analysis. The mean fibril diameters were examined by a non-parametric test (Mann Whitney test), with statistical software (Instat 3; GraphPad, San Diego, CA, USA). Differences between the populations of fibril distributions were analysed by a two-sample *t*-test (Kolmogarov-Smirnov test), with statistical software (Genstat for Windows, VSN International, Hertfordshire, UK).

Light Microscopy (LM)

Sample preparation. Cross-sectional portions (1–2 mm thick) from the mid-zone of each CCL sample were fixed in 10% buffered formalin until required. They were then processed by routine methods to paraffin wax, sectioned at 4 μm and stained. A transverse and longitudinal section of each ligament sample were obtained. The stains used were haematoxylin and eosin (HE) for general assessment, and Alcian-blue periodic acid-Schiff stain (AB-PAS) for mucopolysaccharides.

Data analysis. The sections were examined microscopically by two independent observers (E.C. and A.W.), without knowledge of the sample origin. Sections were compared with the normal description of canine ligament given by Van Sickle *et al.* (1993), and any differences were graded I to III according to the scale used by Vasseur *et al.* (1985). Grade I changes consisted of rounded