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Evidence to suggest that cathepsin K degrades articular cartilage in naturally occurring equine osteoarthritis[1]

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Summary

Objective: The mechanisms leading to degeneration of articular cartilage in osteoarthritis (OA) are complex and not yet fully understood. Cathepsin K (CK) is a cysteine protease which can also cleave the triple helix of type II collagen. This exposes a neoepitope that can now be identified by specific antibodies. The aim of this study was to obtain evidence suggesting a role for CK in naturally occurring equine OA in both lesional and peri-lesional regions.

Methods: Articular cartilages ($n = 12$ horses; 5 healthy, 7 OA) were harvested from animals postmortem. A gross macroscopic examination, histologic (Safranin O-Fast Green and Picrosirius red staining) and immunohistochemical evaluation were performed. Samples were divided into normal appearing cartilage, peri-lesional and lesional cartilage. Cartilage degradation in the samples was graded histologically and immunohistochemically. CK and possible CK cleavage were detected immunohistochemically with specific anti-protein and anti-neoepitope antibodies, respectively. A comparison of CK neoepitope (C2K) production with the collagenase-generated neoepitope produced by matrix metalloproteinases (MMP)-1, 8 and 13 (C2C) was also assessed immunohistochemically.

Results: CK and CK cleavage were significantly more abundant in OA cartilage (both peri-lesional and lesional) when compared to remote cartilage within the sample joint or cartilage from healthy joints. The immunohistochemical pattern observed for CK degradation (C2K) was similar to that of collagenase degradation (C2C). Macroscopic cartilage changes and histologic findings were significantly correlated with immunohistochemistry results.

Conclusion: The data generated suggests that CK may be involved in cartilage collagen degradation in naturally occurring osteoarthritis.

Key words: Horse, Cartilage, Cathepsin K, Osteoarthritis, Type II collagen.

Introduction

The progressive degeneration of articular cartilage is a hallmark feature in the pathogenesis of osteoarthritis (OA), and leads to a loss of joint function and to debilitating pain[1]. The molecular mechanisms that lead to degeneration of the extracellular matrix of articular cartilage are complex and a number of enzymes and their interrelated pathways have been studied extensively[2]. However, the molecular characterization of OA remains incomplete.

The principal molecules of the extracellular matrix of articular cartilage are type II collagen and the proteoglycan aggrecan, together comprising 95% of its dry matter[3]. Type II collagen, a major structural component of the extracellular matrix, is composed of three identical α-helical chains arranged in a triple helical structure[4]. The triple helix is cleaved by collagenases (MMPs) or matrix metalloproteinases at a specific site to produce 3/4 and 1/4 fragments. MMP-1, 8, 13 and 14 are all capable of this cleavage but MMP-13 is currently believed to be the key player in type II collagen degradation in OA[5]. These MMPs have been the focus of most of the protease-related studies of type II collagen cleavage in OA research in recent years[6].

Cathepsin K (CK), a cysteine protease, is synthesized by healthy and OA chondrocytes. It is now believed that this enzyme could play an important role in the breakdown of the cartilage collagen network in OA[7]. Evidence from a study of the effects of an anti-inflammatory drug in an experimental model of OA also suggests an important role for CK in addition to the well-established role of MMPs, in cartilage degradation[8]. CK, like MMP collagenases, is capable of cleavage of the type II collagen triple helix. It subsequently cleaves at multiple sites within the triple helical region of both type I and II collagens. The primary specific cleavage site close to the N-terminus of its triple helix has been identified[9,10] and a polyclonal antibody was raised against the C-terminus of this neoepitope termed C2K[7]. The latter authors also showed that this neoepitope cannot be generated by collagenases and that CK cannot generate the collagenase neoepitope.

Recent data has shown that CK is expressed in OA cartilage[11]. Transgenic Del1 mice models of OA exhibit up-regulation of CK expression in OA cartilage[12]. This

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animal model harbours six additional copies of the 39 kb murine gene for type II collagen (*Col2a1*) carrying a short deletion mutation removing exon 7 and intron 7. This results in production of shortened proα1 (II) collagen chains incapable of fibril formation *in vivo* and consequently a greatly reduced collagen network in the cartilaginous extracellular matrix. Upon ageing the Del1 mice develop early-onset OA. The investigators observed CK in articular chondrocytes near sites of cartilage matrix destruction in the OA mice[12].

CK is now a target for disease modifying OA drugs (DMOADS). We hypothesized that if CK is involved in cartilage degeneration then its expression and involvement in matrix degradation would correlate with the severity of cartilage degradation in naturally occurring OA, which would provide further evidence for its role in this disease.

Our objectives were to study CK expression and cleavage of type II collagen in healthy and naturally occurring equine OA cartilage, and compare this with type II collagen cleavage mediated by collagenases.

Materials and methods

CARTILAGE SPECIMENS

Articular cartilage was aseptically harvested from the metacarpophalangeal joints (MCP) of 10 horses either euthanized for reasons unrelated to joint disease at the Veterinary Teaching Hospital, University of Montreal (2 horses with clinical signs compatible with OA) or from a local abattoir (8 horses). The age and sex was recorded in the medical record or the age of the animals was assessed by teeth examination[13]. The joints were visually assessed, palpated and abnormalities noted. In addition to the animals from the abattoir and clinic, material was harvested from 2 patients with OA diagnosed on a clinical examination (carpi and interphalangeal joint).

SPECIMEN COLLECTION

The joints were opened and imaged with high-resolution digital photography (digital D1 Nikon camera, Tokyo, Japan) to document the macroscopic distribution of cartilage lesions. The lesions were also manually mapped and the joints were assessed for lesion extent with an assigned numeric score for severity, based on visual assessment (Table I). A joint was considered an OA joint for the purposes of this study with a score ≥ 1. Macroscopic signs of OA including synovitis and osteophytosis were semi-quantitatively assessed (presence or absence) and recorded for each joint.

Full depth articular cartilage (to the calcified zone) was removed with a scalpel from the articular surfaces, within 5 h of death. A schematic drawing was made of the areas from which the cartilage was removed. Cartilage was harvested from the lesion (L), the area surrounding the lesion (perilesion, PL), and an area remote from the lesion, corresponding to an area of healthy appearing cartilage (remote, R) (Fig. 1). Cartilage was also harvested from corresponding areas of macroscopically healthy (lesion-free) joints.

PREPARATION OF SECTIONS

Cartilage was embedded in optimal cutting temperature compound (OCT) (Tissue Tek, Sakura Finetek USA, Inc. Torrance, CA, USA) and stored at $-80\,^{\circ}C$ until analysis. Full depth cartilage sections of 5 μm thickness were cut with a cryostat (Leica CM 3050S). The cartilage sections were placed on slides coated with silane (Scientific Device Laboratory, Des plaines, IL, USA) which provided optimal adhesive qualities for performing immunohistochemical staining techniques. Samples from healthy cartilage were placed singly on each slide. When samples came from OA joints, the lesion, perilesion and remote specimens were all placed on the same slide allowing a direct comparison of stain uptake between sites, within the same joints.

Table I
Macroscopic grade

Normal	0
Fibrillation	1
Erosion	2
Ulceration	3

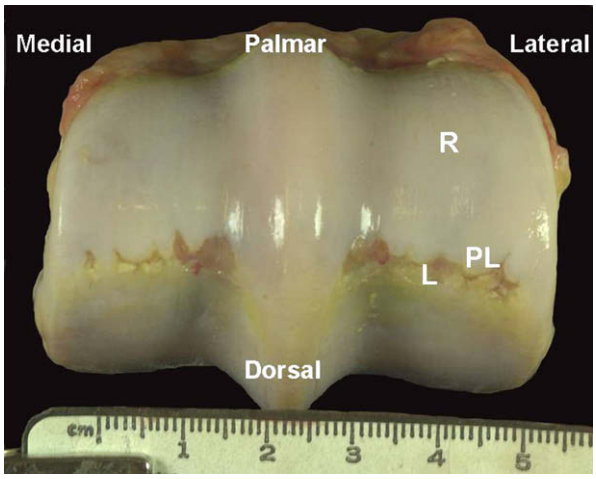

Fig. 1. Representative OA lesion on the distal metacarpus illustrating 3 sites from which cartilage was harvested postmortem: L = lesion, PL = adjacent to the lesion, and R = remote from the lesion.

HISTOLOGIC GRADING

The sections were stained with Safranin O-Fast Green (SOFG), which stains glycosaminoglycans (GAGs), and graded semi-quantitatively for degenerative changes with a modified Mankin grade[14] (Table II). Following grading, the joint compartment with evidence of most severe histological disease was selected for subsequent study and analysis. Sections were selected from the sites with the most severe lesions in the joints with OA confirmed on macroscopic examination.

Picrosirius red was employed to stain type II collagen in the samples. Briefly, it enhances collagen birefringence, and thus reveals the orientation of the collagen fibers in the various zones of the cartilage. Packing of collagen molecules and fiber thickness determine the polarization colours of Picrosirius red stained collagens; thin fibers are green to yellowish-green and thicker fibers are yellowish-orange to red[15]. All frozen sections were stained as described previously[16]. The Picrosirius red stained sections were graded semi-quantitatively for degenerative changes employing polarized light microscopy to assess collagen structure (Table III). A loss of integrity represented a loss of the normal architecture of the samples when compared to the normal articular cartilage. The cartilage sections were examined with a Leica DM4000B polarized light microscope (PLM) equipped with interference filters and compensator plates mounted with a Leica DFC320 video camera.

Table II
Modified Mankin grade (SOFG)

Structure	
Normal	0
Surface irregularities	1
Fibrillation or clefts	2
Erosion up to 1/3 of cartilage thickness	3
Erosion up to 2/3 of cartilage thickness	4
Erosion $>$ 2/3 of cartilage thickness (Including complete ulceration)	5
Cells	
Normal	0
Clusters	1
Hypocellularity	2
Safranin O staining	
Normal	0
Slight reduction	1
Moderate reduction	2
Severe reduction	3