Characterizing osteochondrosis in the dog: potential roles for matrix metalloproteinases and mechanical load in pathogenesis and disease progression


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Summary

Objective: To address possible roles of matrix metalloproteinases (MMPs) and mechanical stress in the pathogenesis of osteochondrosis (OC).

Methods: Naturally-occurring canine OC lesions ($n = 50$) were immunohistochemically analyzed for MMP-1, -3, and -13, and normal canine articular cartilage explants ($n = 6$) cultured under 0-, 2-, or 4-MPa compressive loads (0.1 Hz, 20 min every 8 h up to 12 days) were compared to OC samples ($n = 4$) biochemically and molecularly.

Results: MMP-1 and -3 immunoactivities were readily detected in both OC samples and control tissues obtained from age-matched dogs ($n = 11$) whereas MMP-13 was only detectable in OC samples. MMP-13 gene expression as determined by real-time reverse transcription polymerase chain reaction was elevated in OC samples and cartilage explants cultured without mechanical stimuli (0 MPa groups) compared to normal cartilage (day 0 controls). Glycosaminoglycan content (per weight) in cartilage explants cultured under no load was significantly ($P < 0.05$) lower on day 12 than in the day 0 controls. Gene expression levels of aggrecan and type II collagen in OC samples were lower than those in the day 0 controls. High levels of aggrecan and collagen II expression were seen in the 2 MPa groups.

Conclusions: These findings imply that impaired biochemical characteristics in OC-affected cartilage may be attributable to decreased extracellular matrix production that may stem from disruption of normal weight bearing forces.

Key words: Osteoarthritis, Matrix metalloproteinase, Compressive load, Dog.

Introduction

Osteochondrosis (OC) is a developmental orthopedic disease commonly diagnosed across species. Although many factors including genetics, nutrition, hormonal disturbances, trauma and ischemia are thought to play roles in OC development, the disease mechanisms are not fully understood. Since the pathophysiology of the disease has high similarities between humans and animals, studies of OC using the dog as a model may deepen our insights into the disease and contribute to elucidating its pathogenesis in both humans and animals.

Tomlinson et al. reported that glycosaminoglycan (GAG) content and immunoreactivity of collagen types I, II, and X were different between cartilage affected by OC and normal cartilage in dogs. Alterations in the biochemical characteristics of cartilage extracellular matrix in OC lesions have also been reported in other species. Findings of these studies suggest that disruption of the integrity of cartilage extracellular matrix may play a role in the disease development. The question remains whether abnormalities in the extracellular matrix of cartilage affected by OC are the result of decreased production of integral matrix molecules, increased loss of these molecules, or both. A recent study from our laboratory showed that cellular viability and matrix composition in three-dimensional chondrocyte cultures from OC lesions were inferior to those from normal cartilage. The study indicated that at least a portion of matrix alterations in cartilage affected by OC can be attributed to disruption of matrix turnover by chondrocytes. However, whether these changes represent a cause or an effect of the disease is still unknown. In addition to impaired production of matrix macromolecules by chondrocytes, abnormal remodeling and degradation of extracellular matrix may also contribute to alterations in the biochemical characteristics of cartilage affected by OC.

Matrix metalloproteinases (MMP) are known to play important roles in articular cartilage extracellular matrix turnover. In diarthrodial joints, MMP-1 (interstitial collagenases), MMP-3 (stromelysin-1), and MMP-13 (collagenase-3) have been given great attention because of their known ability to degrade cartilage-specific proteoglycans and collagens. However, little information as to the expression of these MMPs in naturally-occurring OC lesions is present in the literature. Inappropriately high MMP levels would imply increased protease activity as a cause of abnormal extracellular matrix in cartilage affected by OC, whereas...
inappropriately low MMP levels would suggest extracellular matrix abnormalities result from retarded matrix turnover.

Douglas and Rang\(^2\) have reported that traumatic mechanical stress on joints plays an important role in the pathogenesis of OC. Since biomechanical forces have been incriminated as a possible etiologic factor in OC, further investigation involving precisely controlled mechanical forces on articular cartilage may help to delineate the roles of mechanical force in disease development. Recently, a computer-driven dynamic pressure transmitter system has been used to investigate the effects of mechanical forces on cultured cells and tissues making this type of investigation possible\(^8\)–\(^11\). Therefore, the objectives of the present study were to determine immuno-reactivity of MMP-1, -3, and -13 in naturally-occurring OC of dogs and to delineate the effects of compressive forces on cellular and extracellular matrix characteristics of canine cartilage explants. The hypotheses of the present study were that altered expression of MMP-1, -3, and -13 would be detected in naturally-occurring OC lesions of dogs and that repetitive high compressive loads on normal canine articular cartilage explants in vitro would alter characteristics of chondrocytes and extracellular matrix similar to those of naturally-occurring canine OC.

**Materials and methods**

**SAMPLE COLLECTION**

For the MMP immunohistochemical study, detached articular cartilage flaps (n = 50) from OC lesions of the humeral head were collected from 39 client-owned dogs (range 4–30 months of age, mean 10.4 months) either arthroscopically or after arthrotomy. Grossly normal, full-thickness articular cartilage samples (n = 11) were obtained from the caudodorsal humeral head of 11 young adult dogs (range 7–18 months of age, mean 15.4 months) immediately after euthanasia performed for reasons unrelated to this study. Cartilage samples were placed in 10% buffered formalin for routine histologic processing and 5-micron sections were obtained.

For the in vitro mechanical loading study, full-thickness articular cartilage slices were aseptically obtained from the caudodorsal portion of the humeral head of six young adult canine cadavers (range 12–24 months of age) via arthrotomy performed immediately after euthanasia. The dogs were euthanatized for reasons unrelated to this study and were apparently healthy with grossly normal humeral head cartilage. Using a 4-mm dermal punch, uniform-sized full-thickness cartilage explants were obtained from each dog. Cartilage samples collected immediately after euthanasia served as day 0 controls for analyses of histology, biochemistry, and gene expression. Remaining cartilage explants from each dog were placed in 6-well BioFlex™ plates (Flexcell International, Hillsborough, NC) with one explant per well. Cartilage explants from each dog were prepared in triplicate for histology, biochemistry, and gene expression analyses and were bathed with 3 ml of Dulbecco’s modified eagle medium supplemented with 10% fetal bovine serum, antibiotics (100 μg/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/ml amphotericin B), and ascorbate (50 μg/ml). A FX-4000C Flexercell Compression Plus™ Unit (Flexcell International) was used to apply cyclic compressive loads on cartilage explants. Cyclic compression was driven by air pressure, which was monitored with an in-line manometer and controlled by solenoid valves using FlexSoTM software (Flexcell International). Load was applied at a frequency of 0.1 Hz at 5.6 lb or 11.2 lb for 20 min, every 8 h through the BioFlex plate piston (Flexcell International). Substantial loads applied on cartilage explants were calculated based on the formula: pressure = force/area. With a 4 mm diameter explant in each well, the pressures applied in the present study: 5.6 lb and 11.2 lb correspond to loads of 2 MPa and 4 MPa applied onto the cartilage, respectively. Cartilage explants cultured in the identical environment with no compressive load applied served as 0 MPa groups. The explants were incubated at 37°C with 5% CO₂ and 95% humidity. The explants were collected on days 6 and 12 and the media were collected and refreshed every 3 days for evaluation of the effects of compressive loading.

**MMP IMMUNOHISTOCHEMISTRY**

Immunohistochemistry was performed on ex vivo cartilage explants (naturally-occurring OC and normal cartilages) as well as cartilage explants undergoing mechanical loading using mouse monoclonal antibodies against human MMP-1, -3, and -13 (Oncogene Research Products, Cambridge, MA) as previously described\(^12\). The immunostained sections were subjectively evaluated for the presence and localization of staining and scored by two investigators who were blinded to sample group on the basis of the presence (1) or the absence (0) of positive staining.

**CELL VIABILITY**

Chondrocyte viability in cartilage explants was determined with use of ethidium homodimer-1 (13 μl/ml) and calcein AM (0.4 μl/ml) fluorescent stains (Live/Dead Viability/Cytotoxicity Kit, Molecular Probes, Eugene, OR) and a confocal laser microscopy (BioRad Radiance 2000 confocal system coupled to an Olympus IX70 inverted microscope) equipped with Krypton–Argon mixed lasers and a red diode laser. Approximately 1.5 mm thickness cartilage cross-sections were incubated with the stains in phosphate buffered saline for 30 min at room temperature. The number of calcein AM stained live cells (green fluorescence) and ethidium homodimer-1 stained dead cells (red fluorescence) of all explants were objectively evaluated using image analysis software (Image-Pro Plus, MediaCybernetics, Carlsbad, CA).

**EXTRACELLULAR MATRIX EVALUATION**

To evaluate proteoglycan distribution within the tissue matrix, samples were processed using routine histological procedures and 5 μm sections were stained with toluidine blue (T-blue). In addition, GAG in cartilage explants and in liquid media were quantitated by DMMB assay\(^13\). Briefly, collected cartilage explant samples were lyophilized (Free-Zone, Labconco, Kansas City, MO), weighted (dry weight), and digested in 1 ml of 0.5 mg/ml papain (Sigma, St. Louis, MO) in 20 mM sodium phosphate buffer (pH 6.8) containing 1 mM EDTA at 60°C overnight. A 5 μl aliquot of the digest solution was assayed for total GAG content (GAG/dry weight) by addition of 245 μl of DMMB solution and spectrophotometric determination of absorbance at 525 nm with known concentrations of chondroitin sulfate as standards. Results were standardized to correct for differences in sample weight. Total GAG content for samples was reported in micrograms per milligram. GAG content in liquid medium samples (5 μl) were assayed by
addition of 245 μl of DMBB solution and spectrophotometric
determination of absorbance at 525 nm. GAG loss from the
explant was determined by calculating GAG content in the
liquid media as a percentage of total GAG in the explant for
each sample.

For collagen matrix evaluation, total collagen contents of
the cartilage explants were determined by measuring
hydroxyproline (HP) using a colorimetric procedure14 as
previously described15. Presence and distribution of type I,
II, and X collagens in cartilage explants were subjectively
determined with use of an avidin–biotin immunohistochem-
tical technique as previously described16.

**REAL-TIME REVERSE TRANSCRIPTION POLYMERASE
CHAIN REACTION (REAL-TIME RT-PCR)**

Total RNA was extracted from naturally-occurring OC
lesions of the humeral head collected from four client-
owned dogs (n = 4, range 6–7 months of age, mean 6.4
months) and the cartilage explants from the in *vita*
mechanical loading study and analyzed by RT-PCR using
primers (Table I) corresponding to cDNA sequences for
structural matrix macromolecules (type I and II collagens,
aggrecan, and decorin), proteinases (MMP-3 and MMP-13),
proteinase inhibitors (TIMP-1, TIMP-2, and TIMP-3), and
the housekeeping gene glyceraldehyde-3-phosphate de-
hydrogenase (GAPDH).

Snap frozen cartilage samples were powdered, transferred
to a 0.5 ml screw cap tube filled with 1.0 mm diameter
Zirconia Beads (BioSpec Products, Bartlesville, OK) and
Trizol® reagent (Invitrogen, Carlsbad, CA), and homoge-
nized using a mini-bead beater (BioSpec Products) at
5000 rpm for 2 min. RNA was extracted from the homoge-
nates using the TRIspin method as described16. RNA was
denatured in a hydrosheath (GAPDH).

Primers correspond to cDNA sequences deposited in GeneBank. Col 2 = collagen II, Col 1 = collagen I.
0 controls with respect to each assay at different collection times were analyzed in a similar manner. Significance was established at $P < 0.05$.

**Results**

**MMP IMMUNOHISTOCHEMISTRY FOR NATURALLY-OCcurring OC**

In normal cartilage controls ($n = 11$), MMP-1 and -3 were detected in eight samples. When detected, MMP-1 was identified in chondrocytes in the middle and deep zones [Fig. 1(A)], whereas MMP-3 was diffusely distributed in interterritorial regions of each zone of the matrix, except for the zone of calcification [Fig. 1(B)]. A weak, positive correlation ($P = 0.03$, $r = 0.28$) was found between the presence of MMP-1 and -3 in controls. No MMP-13 staining was present in control samples [Fig. 1(C)]. In OC samples ($n = 50$), 22 specimens stained positive for MMP-1 and 20 specimens were positive for MMP-3. When present, distribution of MMP-1 [Fig. 1(D)] and MMP-3 [Fig. 1(E)] in OC samples was similar to those in controls. MMP-13 was detected in 21 OC-affected cartilage samples [Fig. 1(F)]. There was a statistically significant difference between groups with significantly ($P < 0.001$) more cartilage affected by OC staining positive for MMP-13 than controls.

**CELL VIABILITY**

The effects of *in vitro* compressive loading of cartilage explants were evaluated with confocal laser microscopic analysis for cell viability. The majority of chondrocytes in cartilage explants in the 0 MPa and 2 MPa groups [Fig. 2(A,B)] emitted green fluorescence at each sample collection time indicating the maintenance of cell viability throughout the study period. In contrast, many chondrocytes in explants from the 4 MPa group emitted red fluorescence at each sample collection time indicating cell death.

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![Fig. 1](image1.png)  
Fig. 1. Representative photomicrographs of MMP-1 (A, D), MMP-3 (B, E), and MMP-13 (C, F) immunostaining in controls (A, B, C) and in OC-affected cartilages (D, E, F). MMP-1 was identified in the cytoplasm of chondrocytes in the middle and deep zones (A, D), whereas MMP-3 was diffusely distributed in interterritorial regions of each zone of the matrix, except for the calcification zone (B, E). Note the intensity of immunostaining in OC-affected cartilages (D, E) was apparently stronger than those in controls (A, B). No MMP-13 staining was detectable in control samples (C), while it was detectable in OC-affected cartilages (F). Avidin–biotin–peroxidase stain; original magnification = 20×.
fluorescence indicating reduced cell viability [Fig. 2(C)]. Analysis of chondrocytes emitting green fluorescence or red fluorescence in each sample [Fig. 2(D,E)] showed that cell viability in the 4 MPa group was significantly ($P < 0.05$) lower than in the 2 MPa group on day 6 and the 0 MPa group on day 12 [Fig. 3(A)]. To verify the findings of cell viability evaluated by confocal laser microscopy, diphenylamine assay was conducted to quantify total DNA content in cartilage explants, as described previously. Similar to the results shown by confocal laser microscopy, DNA content in the 4 MPa group was decreased during the study period and the reduction on day 6 and day 12 was significant ($P < 0.05$) compared to day 0 controls [Fig. 3(B)]. These results demonstrated that cell viability could be maintained well under regimens of 0 MPa and 2 MPa compressive loads at 0.1 Hz for 20 min, every 8 h, while the 4 MPa regimen led to significant cell death in this model.

EXTRACELLULAR MATRIX EVALUATION

The effects of different compressive loads on cartilage explant extracellular matrix macromolecules were evaluated. Compared to the day 0 controls, GAG content was significantly ($P = 0.036$) decreased in the 0 MPa group on day 12 (Fig. 4). In addition, T-blue histochemical analysis showed the presence of decreased stained areas in the superficial to middle layers in the 0 MPa group (Fig. 5). These findings corresponded well with each other and indicated that the lack of mechanical stimuli on cartilage leads to depletion of proteoglycans. Moreover, aggrecan gene expression was apparently higher in the day 0 controls compared to OC samples and the gene expression was better preserved in the 2 MPa group (Table II). Gene expression of decorin between OC samples and the controls was unchanged (Table II).

In contrast to GAG content, collagen content in the 0 MPa group as determined by the HP assay was minimally affected during the study period (Fig. 6). A significant
(P < 0.05) decrease in collagen content for the 4 MPa group was found on day 6 compared to the 0 MPa group and the day 0 controls. Immunohistochemical analyses for type I, II, and X collagens were conducted to further elucidate the effects of compressive loads on collagen extracellular matrix. Type II collagen expression was diffusively present throughout the tissue with more intense immunostaining in surface layers in the day 0 controls [Fig. 7(A)]. Although all cartilage samples were positive for type II collagen [Fig. 7(B–D)], immunoreactivity was subjectively less intense in all treated groups compared to the day 0 controls. In addition, collagen II gene expression was higher in the day 0 controls compared to OC samples and collagen II gene expression was best preserved in the 2 MPa groups among the three treatment regimens (Table II). Type X collagen immunohistochemistry showed collagen X to be concentrated in the territorial and interterritorial regions of chondrocytes in superficial to middle layers [Fig. 7(E)] and immunostaining was well preserved in those samples cultured under compressive loads [Fig. 7(F–H)]. No cartilage explants were positive for type I collagen (data not shown) and collagen I gene expression was significantly (P < 0.05) higher in OC samples than the day 0 controls. None of the treatment regimens induced collagen I gene expression (Table II).

MMP EVALUATION

In order to investigate whether MMPs play a role in the alterations noted in the matrix molecules of loaded cartilage explants, immunohistochemistry for MMP-1, -3, and -13 and real-time RT-PCR for MMP-3 and -13 were performed. Similar to those data seen in the MMP immunohistochemistry on normal cartilage samples, some cartilage samples in day 0 controls were positive for MMP-1 (5/6) and MMP-3 (2/6) and none of day 0 controls stained positive for MMP-13 (0/6). When MMP staining was detected, staining patterns were similar to those seen in the first study (Fig. 1) and the presence and distribution of each MMP were not significantly varied throughout the culture period (data not shown). Interestingly gene expression of MMP-13 was apparently higher in OC samples compared to the day 0 controls and its expression was higher in the 0 MPa groups than the day 0 controls with a significant (P < 0.05) difference on day 6 (Table II).

In contrast, MMP-3 gene expression in OC samples and the day 0 controls was not significantly different. The significantly (P < 0.05) lower levels of TIMP-2 and TIMP-3 gene expression in OC samples compared to the day 0 controls implies imbalance between MMP and TIMP in cartilage affected by OC (Table II).

Discussion

Only a limited number of studies have looked into potential roles for MMPs in the pathophysiology of OC. Braman et al.\textsuperscript{20,21} reported that MMPs in OC-affected equine synovial fluid were not significantly different from those in synovial fluid obtained from normal horses and proposed that MMP-mediated extracellular matrix destruction is not of major importance in OC. Similarly, a study of naturally-occurring canine OC showed that concentrations of MMP-3 in synovial fluid obtained from OC-affected joints were not
Table II

<table>
<thead>
<tr>
<th>Gene</th>
<th>Day 0 (controls)</th>
<th>Day 6</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggrecan</td>
<td>0 MPa</td>
<td>2 MPa</td>
<td>4 MPa</td>
</tr>
<tr>
<td></td>
<td>0.6 (± 0.1)</td>
<td>4.7 (± 0.2)</td>
<td>3.5 (± 0.2)</td>
</tr>
<tr>
<td>Decorin</td>
<td>2.5 (± 0.1)</td>
<td>3.0 (± 0.1)</td>
<td>0.4 (± 0.1)</td>
</tr>
<tr>
<td>Col 2</td>
<td>36.3 (± 6.3)</td>
<td>155.0 (± 21.0)</td>
<td>5.7 (± 1.0)</td>
</tr>
<tr>
<td>Col 1</td>
<td>3.8 (± 0.1)</td>
<td>0.3 (± 0.1)</td>
<td>0.1 (± 0.0)</td>
</tr>
<tr>
<td>MMP-3</td>
<td>0.1 (± 0.0)</td>
<td>0.2 (± 0.0)</td>
<td>0.1 (± 0.0)</td>
</tr>
<tr>
<td>MMP-13</td>
<td>1.1 (± 0.2)</td>
<td>6.8 (± 1.2)</td>
<td>0.2 (± 0.1)</td>
</tr>
<tr>
<td>TIMP-1</td>
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<td>0.6 (± 0.1)</td>
<td>0.1 (± 0.0)</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>1.1 (± 0.2)</td>
<td>5.5 (± 1.1)</td>
<td>0.1 (± 0.0)</td>
</tr>
<tr>
<td>TIMP-3</td>
<td>1.7 (± 0.3)</td>
<td>1.6 (± 0.3)</td>
<td>0.1 (± 0.0)</td>
</tr>
</tbody>
</table>

*Significant (*P < 0.05) difference compared to the day 0 controls.

Fig. 6. Mean (± S.E.M.) hydroxyproline (HP) content (HP/dry weight) in articular cartilage explants. HP content was significantly decreased in the 4 MPa group on day 6 compared to the 0 MPa group and the day 0 control (*P < 0.05).

In humans, normal physiological stresses on hip joint cartilage have been reported to be as high as 18 MPa. Although pressures in canine shoulder joints under physiological stresses are not known, it can be speculated that 2 MPa falls within the range of physiological pressures, while 4 MPa may approach supraphysiological pressures based on the weight of these dogs (between 25 and 30 kg),
distribution of weight bearing to the forelimbs in dogs, and data from the human literature. The present in vitro study clearly demonstrated that compressive loading of 4 MPa on canine articular cartilage explants with a frequency of 0.1 Hz for 20 min every 8 h causes increased cell death, especially in more superficial zones. Although cartilage necrosis is a common pathological feature associated with OC in dogs, the area of necrosis is usually a focal area within the intermediate and deep zones of developing articular or epiphyseal cartilage. Therefore, the mechanism causing cell death associated with high compressive loading is likely different from that seen in naturally occurring OC.

Destruction of proteoglycan matrix as determined by GAG loss to the media was not significantly different among groups throughout the study period and this finding was consistent with a recent study in which no evidence of increased proteoglycan degradation was observed in equine OC. In addition, the apparent decrease in aggrecan gene expression levels in OC samples compared to controls was in accordance with previous studies reporting decreased proteoglycan content in OC-affected cartilage. Preservation of GAG content despite increased cell death in explants in the 4 MPa group and decreased GAG content despite well preserved cell viability in the 0 MPa group may be explained by differential effects of compressive load on chondrocytes based on phenotype and location as previously reported. In fact, GAG levels in the 4 MPa group normalized by DNA content were the highest among the three treatment groups followed by the 2 MPa group (data not shown). These data suggest that physical destruction of cells and matrix in the superficial zone of loaded explants in this in vitro system with concurrent stimulus to the middle and deep zones appropriate for proteoglycan synthesis may occur. It then follows that disruption of proteoglycan matrix integrity in OC-affected cartilage reported in previous studies may be attributable to a lack of normal weight bearing stimuli secondary to OC. Further evidence for incriminating a lack of normal weight bearing stimuli as a cause for diminutive proteoglycan content in OC-affected cartilage is present in the proteoglycan depletion in articular cartilage seen with prolonged unloading of a joint.

Decreased collagen content in cartilage explants under 4 MPa dynamic compressive loads and maintenance of collagen content in the 0 MPa group throughout the study period despite increased MMP-13 gene expression levels indicates that this alteration more likely stems from mechanical destruction rather than enzymatic degradation. The differential effects on collagen content and proteoglycan content in this study may be explained by the structure of articular cartilage in which dense collagen fibers are distributed in the superficial layer, which has a relatively low amount of proteoglycan. It can be speculated that the compressive loading associated with direct contact of cartilage and the bioreactor in this system had a negative impact on the collagen-dense superficial zone, resulting in prominent reduction of collagen content. The result of collagen II gene expression is suggestive of the importance of mechanical stimuli for transcriptional regulation of type II collagen expression, and further, both lack of load and excess load may cause alterations in the biochemical characteristics of cartilage extracellular matrix.

Increased type I collagen gene expression in OC samples verified our previous study in which 28 of 42 OC-affected canine cartilage samples were positive for collagen type I while normal cartilage had no detectable type I collagen as determined by immunohistochemistry. Although the present study indicated that compressive loads alone do not affect this shift in collagen synthesis, future research is needed to determine whether type I collagen expression is a cause or an effect of the disease. Type X collagen immunoreactivity was well preserved for cartilage explants cultured under compressive forces. Avidin–biotin–peroxidase stain; original magnification = 20×.

Fig. 7. Representative photomicrographs of cross-sections of articular cartilage explants harvested on day 0 (A, E) and cultured under 0 MPa (B, F), 2 MPa (C, G), and 4 MPa (D, H) for 12 days. Sections were stained with antibodies against type II collagen (A–D) or type X collagen (E–H). Type II collagen (A–D) was detected in the territorial and interterritorial regions of cartilage matrix and its immunoreactivity was subjectively less intense in all treatment groups (C, D, E), especially samples from the 4 MPa group compared to the day 0 controls (A). Type X collagen (E–H) was detectable in the territorial and interterritorial regions in the superficial to middle zones. Note type X collagen immunoreactivity was better preserved in explants cultured under compressive forces (G, H).
present study. Although type X collagen has been considered as a specific product of hypertrophic chondrocytes at sites of endochondral ossification, it has also been identified in the surface of normal articular cartilage in various species including adult dogs. The observation of type X collagen in superficial to middle zones and the persistence of its expression in cartilage explants cultured under compressive loads in this study may support previous studies that indicated that type X collagen could contribute to the structural integrity of articular cartilage and that expression of this collagen might be regulated by mechanical forces. A previous study showed that OC-affected cartilage in dogs had significantly less type X collagen than did normal cartilage. Further investigation including gene expression analysis is necessary to elucidate whether the decrease in type X collagen is relevant to the disturbance of endochondral ossification or due to the disruption of normal weight bearing secondary to OC.

The unknown duration of the disease at the time of surgical intervention is always a major issue in the investigation of OC using naturally-occurring samples. The median age for the onset of lameness associated with humeral head OC in canines has been reported to range between 5 and 6 months. The initiation of the disease process would occur even earlier than notable lameness. The time lag from the onset of disease, to the diagnosis, and subsequent surgical intervention may conceal critical pathologic alterations in OC cartilage samples. OC is a very common cause of secondary OA in dogs. In addition, disruption of the normal weight bearing forces on OC-affected articular cartilage is likely to cause tissue atrophy. Future investigation using OC cartilage samples should attempt to adapt a rigorous scientific method to select samples which represent the early stages of the disease processes.

Although in vitro compressive loading did not alter the gene expression profiles in cartilage explants similar to those in OC samples, our findings suggested that the extracellular matrix composition and integrity altered by OC and loads appears to be related to viability and synthetic capabilities of chondrocytes, perturbations of cell phenotype, and alterations and imbalances in MMP and TIMP synthesis. Further defining the in vivo processes of disease and refinement of this model may allow for attainment of a representative in vitro model for the study of OC. Development of an OC model would then allow for directed research into the mechanisms of disease and provide avenues for optimal prevention and treatment across species.

Acknowledgments

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