PATHOLOGICAL STUDY OF FLUOROQUINOLONE-MEDIATED CHONDROARTHROPATHY IN JUVENILE DOGS

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ABSTRACT

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INTRODUCTION

In 1743, Hunter stated, "From Hippocrates to the present age it is universally allowed that ulcerated cartilage is a troublesome thing and when once destroyed it is not repaired."[1] From his century, to ours, his words ring true, as researchers investigate the complex and seemingly mysterious mechanisms of cartilage pathophysiology.

Recent reports of cartilage destruction associated with the oral administration of fluoroquinolone analoges have baffled investigators.[2,3] These broad-spectrum antibacterial agents, composed of a 4-quinolone nucleus and a carboxylate substituent at position 3, have been used successfully against many systemic infections. However, high-level oral dosages with prolonged therapy induced articular cartilage changes in immature animals. This effect has been reported in immature rabbits,[4] dogs,[3] and monkeys.[5] Articular cartilage had previously been considered resistant to adverse effects from systemically administered drugs.[6]

Typically, the lesions appeared grossly as blister-like elevated vesicles, on the articular surface of major joints. The vesicles coalesced, increasing their diameter and fragility, which led to tearing and focal articular cartilage effacement. Despite the multiple locations, and activity-enhanced lesion-severity, the dogs remained weight-bearing and active. Microscopically, the findings included foci of chondromalacia, chondrocyte clusters,

fibrillation, and clefting.[2,3]

This study evaluates lesions produced in puppies after high level and prolonged duration oral fluoroquinolone treatment. The objective of this study is to illustrate the cartilagenous lesion progression initiated by oral fluoroquinolone administration.

I. REVIEW OF LITERATURE

LITERATURE REVIEW

Articular cartilage is a highly specialized form of connective tissue well-suited for its dual role as a shock absorber and weight bearing surface in a moveable joint. The composition of articular cartilage suggested to early investigators that it was an inert hypometabolic connective tissue structure. The opposite has since proved true.

Articular cartilage is the workhorse of diarthroidal joints, where it provides almost frictionless movement of the articulating surfaces as well as necessary resiliency. Cartilage is firmly attached to the underlying subchondral cortical bone, and measures less than 5 mm in thickness in mammalian joints, though species, joint, and site variations occur.[7,8] Surprisingly, articular surface is not smooth.[9] Electron microscopy illustrated gentle undulations and irregular depressions. Constituents of cartilage include cells, collagen, and a gel-like ground substance whose combined properties lend resiliency and structure.

Cellular Component

The cellular component of cartilage may be divided into 4. Zone 1, referred to as a tangential or gliding zone, contains elongated cells with their long axes parallel to the surface residing in a less developed proteoglycan matrix.

Merging into this region is zone 2, or transitional zone,

where randomly distributed chondrocytes are round or ovoid. Zone 2 is the most metabolically active layer of articular cartilage. Electron microscopy of chondrocytes demonstrated extensive networks of rough endoplasmic reticulum, dilated cisternae, vacuoles and Golgi apparatus, suggesting protein synthesis and matrix production.[10] Radioactive isotope studies illustrated chondrocytic synthesis of macromolecular proteoglycan and collagen component portions, assembled intracellularly and rapidly extruded into the surrounding matrix.[11-17] The secretions envelope chondrocytes which become isolated in their lacunae. Chondroblasts maturing into chondrocytes undergo cellular hypertrophy and enlargement of the flattened lacunae to an ovoid angular shape.

Zone 2 gives way to the columnar zone 3, or radial zone. The cells are lined up in short, irregular columns, which become longer and the cells larger in the deeper part of the cartilage. Clusters of chondrocytes as isogenous groups or cell nests occur. The cell nests represent daughter cells of a single chondrocyte that underwent mitosis (interstitial growth).

The calcified zone of zone 4 contains matrix and cells heavily encrusted with hydroxyapatite. Zone 4 is separated from the radial zone by an irregular bluish line, the "tidemark" and on its deep surface the zone merges with the end-plate of the underlying bone.[18,19]

Matrical Component

Aside from the metabolically active cellular component of articular cartilage, there is the chondrocyte-generated matrix. Its constituents are macromolecular organic solids consisting equally of randomly interwoven fibrous type II collagen and gel-like proteoglycan ground substance.[20,21]

To picture the gel-like matrix, imagine a bottle brush representation of cartilage proteoglycans, with a protein core and a large number of attached glycosaminoglycan chains of chondroitin sulfate.[22] Chondroitin-6-sulfate is the principal glycosaminoglycan, accounting for 45 to 75% of the sugar component of hyaline cartilage and roughly 15% of its dry weight.[23] In immature articular cartilage, chondroitin-4-sulfate is more prevalent, and decreases in concentration with age, to the adult level of less than 5% of the total glycosaminoglycan concentration.[23] Concomittantly, a third type of glycosaminoglycan, keratin sulfate, increases with animal age. Mourao et al. found that chondroitin-4-sulfate seems to be related to ossification, whereas chondroitin-6-sulfate assumes a major role in the maintenance of articular surfaces.[24]

Assessing metabolic turnover of proteoglycans is difficult as metabolic "pools" of proteoglycans exist in the matrix.[25] Research suggests considerable variation occurs in turnover rates of glycosaminoglycans corresponding with the depth in articular cartilage in between different joints. Age also appears to generally decrease the turnover of proteoglycans.[26] The collagen component is also metabolically active, but turnover occurs at a

much slower rate.[27]

Cartilage collagen, categorized as type II, differs from type I collagen of skin or bone. Type II collagen consists of 3 identical $_1$II chains in a helical arrangement, however type I contains 2 $_1$ I, $_2$ chains.[28-31] Type II collagen chains differ from those of type I principally in the increased quantity of hydroxylysine and excessive glycosylation.[29,30]

Collagen fibers assume an arcade-like arrangement in articular cartilage, spanning 3 architectural zones. The deepest zone, found adjacent to subchondral bone, help to secure cartilage to bone. This radial zone incorporates collagen fibers oriented perpendicular to the subchondral bone.

An intermediate zone assumes a haphazard formation, similar to randomly arranged zone 2 chondrocytes. The most superficial layer lies parallel to the articular surface an comprises the tangential zone. This arcade formation aids cartilage compressibility and elasticity.[32,33]

Articular cartilage is a hyperhydrated tissue with water content estimates ranging as high as 80% in the immature animal.[34,35] Water content is higher near the surface, with zone 4 cartilage containing the least water.[36] This unique hydrophilic property is established by the extensive negative field generated from the polyionic glycosaminoglycan configuration. The negative charges repel one another, causing the macromolecule to rest stiffly extended in its electrostatic "domain". Linkage of glycosaminoglycans occurs via hyaluronate and two glycoproteins,

forming a stiffly extended hydrophilic macromolecule bonded to collagen molecules.[37-40] Such architecture increases the density of the matrix and the aggregates provide three significant matrical functions; stability, volume definition, and compressive properties.

Stability is derived from linkage of proteoglycans to hyaluronic acid and collagen fibers in a unique spatial arrangement, allowing maximum surface area for entrapping interstitial fluid. Efficient fluid absorption ability of proteoglycans enables remarkable cartilage volume definition, and is also responsible for cartilage hydrodynamics. Proteoglycan aggregates are resistant to compressive forces. Applied pressure will decrease their volume proportionally to the amount of force and upon removal of pressure, the macromolecules return to their original volume. Charge densities also play a role in hydrodynamics. As the volume of proteoglycan aggregates decrease, the charge density and mobility restraints of these polyions increase.

The essential hydrodynamic properties are lost if the synthesis or proper formation of the proteoglycan aggregates is compromised. Such a change would alter the effective fluid entrapment abilities of the cartilagenous matrix and increase cartilage compressibility. Tissue damage increases with the loss of hydrodynamic cartilage resistance.

Articular Cartilage Nutrition

Articular cartilage is isolated in the sense that it lacks direct vascularity, lymphatics and nerves. Nutrients must pass through a double diffusion barrier in order to reach the cells. In the mature adults, all nutrients must first diffuse out of the synovial vascular plexus, traverse the synovial membrane to enter synovial fluid, and pass through the dense hyaline matrix of cartilage to reach the chondrocytes.[41]

In immature animals, nutrient diffusion from underlying bone vasculature to the basilar layer of cartilage can occur, in addition to nutrition from synovial fluid.[42] The source of nutrients is the plexuses of vessels in the subchondral cancellous tissue and vessels of the joint capsule.[43]

Exercise plays an integral role in pumping nutrient-rich synovial fluid through cartilagenous matrix to the chondrocytes. The rate-limiting feature of nutrient diffusion into articular cartilage is the cartilagenous matrix pore size, theoretically established as 6.9 nm (68A).[41,42,44,45] Even low molecular weight proteins diffuse slowly across this barrier. The facilitation for nutrient diffusion is exercise, which plays an integral role in pumping nutrient-rich synovial fluid through cartilagenous matrix to chondrocytes.

Because of its compressability, cartilage under pressure, can behave like a sponge.[46] During pressure fluid is expressed, and when pressure is released, fluid is reabsorbed. Numerous repetitions of such impact cycles favor local penetration by molecules otherwise considered too large to make their way

unaided into the dense structure of normal cartilage. Research has shown that without exercise the cells atrophy, lacunae merge to form cysts, surface integrity is lost and disintegration of the articular plate occurs.[43]

Synovial fluid functions to lubricate articular cartilage surfaces, provide nutrition and protect joint surfaces. This super blood dialysate incorporates glycosaminoglycans, predominatly hyaluronic acid, to provide viscosity. Other components of synovial fluid include degraded products of cartilagenous matrix and synovial lining cells, lysosomal enzymes, and articular cartilage constituents that leached or "wept" into synovial fluid.

Growth and Remodeling of Articular Cartilage

Most critical when discussing healing abilities is the potential of chondrocytes to reproduce. Cell replication is evident in immature cartilage, but even in young animals, mitotic activity is not uniform. Two distinct zones of replication occur, both in the middle regions of cartilage.[47,48] The first lies subjacent to the articular surface for which it account for the region's cellular growth. Located deeper in this area is the second zone of cells whose narrow band resembles the proliferative zone of a bone's microepiphyseal plate.[48]

The pattern changes as the animal ages and approaches maturity. The mitotic index decreases, and only one zone remains, located above the zone of vascular invasion in the deeper layers of cartilage.[49] At skeletal maturity, which

generally coincides with epiphyseal closure, mitotic activity appears to cease. The unanswered question was whether this apparent inability to replicate was reversible, allowing DNA synthesis and cell replication under certain circumstances. Studies indicated termination of DNA synthesis was due to replicatory apparatus suppression rather than permanent structural damage or change to the synthesis mechanism.[6] Thus chondrocytes can, under appropriate conditions, display replicative abilities.

Previously regarded "inert" cartilage initiates a constant rate of synthesis and degradation of its matrical components. Articular chondrocytes are responsible for proteoglycan synthesis, a small portion of which rapidly turns over.[50-53] Collagen is also partly synthesized by local chondrocytes, but is more stable than the proteoglycan component.[27,56] Cartilage incorporates an active internal remodeling system through autodegradative lysosomal enzymes that act upon proteoglycan.[54,55] The specific enzymes involved remain unidentified although cathepsin D and a neutral proteoglycanase are likely.[25,57,58] A collagenase which degrades cartilage collagen has been found in tissues from osteoarthritic human joints, but not in normal articular cartilage.[59]

Response of Articular Cartilage to Injury

Since Hunter's observation in 1743, researchers have found that injuries confined exclusively to cartilage of adult animals

fail to repair effectively. A major limitation is lack of an inflammatory element in the avascular cartilage meshwork. Repair is independent of extent or depth of the lesion, and is characterized by minimal attempts on the part of the cartilage to add cellular and matrix elements.[60-62].

Immediately after superficial injury, a burst of mitotic activity is detected adjacent to the damaged sites, which correlated with increased synthesis of matrix components.[62] Unfortunately, the reparative effort is short-lived and falls back to normal levels one week post-injury.[62]

Partial thickness defects in articular cartilage of rabbits were produced by Fuller and Ghadially in their cartilage research.[63] They found no significant repair reaction, even in young animals. Through electron microscopy, they recorded cell death at the wound margins and increased cellular activity from surviving chondrocytes. Nuclear hypertrophy, increased quantities of rough endoplasmic reticulum, and occasional increased numbers of Golgi complexes, were noted after injury, but by 6 months, the defect remained and healing processes had ceased.[63] Similar long-term research reported the lesions remain stable and osteoarthritic changes occurred only occasionally.[64-66]

In contrast, deeper lesions involving subchondral vasculature, initiate an inflammatory response. Initially, fibrous tissue fills the defect, which becomes progressively chondrified to form a fibrocartilagenous mass. However, a 12-month study by Mitchell and Shepard, found fibrocartilagenous material became

more fibrous with time.[52] The surface layers became fibrillated, although the deep, cartilage-filled defect remained unchanged, and function seemed unimpaired.

Degenerative Cartilage Changes

Degenerative cartilage lesions attributed to osteroarthrotic conditions differ from superficial lacerative injuries. Primary degenerative joint disease is attributed to trauma or constant wear on a joint surface. One theory is that trauma to articular surfaces allows an increased amount of synovial fluid to penetrate the articular matrix. The hyaluronidase faction begins to break down the proteoglycan matrix.[67] Stress on the now weakened cartilage induces cracks and fissures, which in turn allows more synovial fluid to enter.[68]

Another popular theory implies indirect degradation occurs by factors, which stimulate chondrocyte production of matrix-degrading enzymes. Fell and Jubb first demonstrated that live synovial tissue induced cartilage matrix depletion when co-cultured in the same dish, though not in contact with live cartilage explants, whereas no depletion was found with dead cartilage.[69] Dingle and Dingle have shown that matrix degrading activity occurred only in the pericellular area in cartilage explants, close to chondrocyte membranes.[70] Current in vitro studies of articular cartilage explants, cultured with blood mononuclear cell supernates, elicited degradation of matrix proteoglycan and collagen.[71]

Grossly, the degenerated articular surfaces are yellowish and less elastic than normal, with roughened foci. Chondromalacia is an early sign of weakened cartilage matrix due to proteoglycan loss.[72-76] The softened cartilage is unable to support the collagen framework, a situation aided by weight-bearing stress. Fibrillation, the term describing exposure of the collagen arcade through the loss of ground substance results, as well as irregular surface depressions.[75,76]

Typically, the microscopic appearance of chondromalacia is a notable loss in metachromasia. The articular cartilate is pale, hypocellular, and pink with H & E stain. Clustering of chondrocytes, or "chondrones" are found, and believed to represent an abortive repair effort.[76] As fibrillation continues, the more severe fissuring, or vertical cleft formation is noted, extending into deeper zones. Erosion may continue to subchondral bone, where mechanical forces and synovial fluid act to polish its surface, by a process termed eburnation.[32,77]

One biochemical change early in degeneration is an increase in cartilage water content. One explanation is increased bonding of water to collagen molecules occurs,[78] or it is the result of damaged collagen network failing to oppose swelling pressure of proteoglycans.[79] The unfolding proteoglycans were able to bind 9% more water.[78]

Research also detected a significant increase in synthetic activity in the early stages of osteoarthritis, prior to gross disruption of the cartilage surfaces.[80] In effect, the cells

attempt to replenish their matrix in the face of increased degradation.

Responding to this increased demand, the matrix components undergo change. A significant increase in chondroitin-4-sulfate occurs, and a decrease in keratin sulfate.[23] This pattern is attributed to chondrocytes responding by producing younger cartilage, or reverting to a chondroblastic phase.

Collagen synthesis, too, is varied. Degenerative cartilage manufactures Type I collagen, found in bone or skin, rather than the normal Type II collagen.[81]
of transcription, DNA repair, recombination, and transposition.

Fluoroquinolone Family of Antibacterial Agents

In recent years, newly developed 4-quinolone-3-carboxylates have generated attention for their scope and effectiveness against Gram-negative bacilli and cocci in vitro, and their capacity to control experimentally-induced systmeic bacterial infections when given orally.[82] The family of fluoroquinolone antibiotics include new additions of norfloxacin, ofloxacin, ciprofloxacin, amifloxacin, enoxacin and pefloxacin, as well as nalidixic and oxolinic acids, among others.

The fluoroquinolone mechanism of action is directed against bacterial DNA gyrase, an essential bacterial enzyme that maintains DNA superhelical twists.[23,84,85] DNA gyrase introduces negative superhelical twists into double-stranded DNA, which allows unwinding of the double strands. Double-stranded breaks are made in DNA by DNA gyrase, another DNA duplex passes

through the break, and cut strands are released. By these methods, DNA gyrase is essential for DNA replication, and certain aspects of transcription, DNA repair, recombination, and transposition.

Each chromosomal domain is independently supercoiled by DNA gyrase, and gyrase action upon each domain occurs at a single specific site. Quinolones can affect gyrase action by preventing sealing of the single-strand gaps, which inhibits DNA supercoiling, thereby increasing the spatial requirements within the bacterial cell.[86] Researchers have found that bacteria treated with quinolone elongate abnormally.[86]

The exact mechanism of action by fluoroquinolones has yet to be fully clarified. Recent results suggest that norfloxacin and other quinolones bind to purified DNA rather than to purified DNA gyrase.[87] The exact bactericidal mechanism is not known,[88,89] but may involve cleavage of bacterial chromosomal DNA by DNA gyrase. Quinolones kill bacteria rapidly, with as much as a thousand fold decrease in viability in 1 to 2 hours of drug exposure at 1 to 4 times the Minimum Inhibitory Concentration (MIC).[88,90-96]

Toxicity trials have shown quinolone antibacterials are generally well tolerated, with the most notable toxic effect observed being erosion of articular cartilage in immature animals. This effect has been reported for naladixic, oxolinic, cinoxacin, pipemidic acid and piromidic acid. It was shown to occur in rabbits,[4] dogs,[3] and monkeys,[5] and only in immature

animals.[53] Reported clinical side effects include dizziness, visual disturbances, hemolytic anemia, photosensitivity, and intracranial hypertension.[97]

Previous In Vivo Studies

Several reports of pharmaceutical trials testing oxolinic and pipemidic acid in juvenile dogs indicated fluoroquinolone-mediated articular cartilage erosion. Gough et al. tested 4 groups of 3-month-old beagles given oxolinic acid at 500 to 100 mg/kg, or pipemidic acid at 500 mg/kg, orally dosed twice daily.[3] A similar study by Ingham et al. tested 4 12-month-old beagles, orally dosed with 200-1,000 mg/kg per day of pipemidic acid for period of 1-15 days.[2] The dogs were necropsied from 1-87 days after completion of the dosage regimen. Other groups of 4 beagles each, were treated daily for 6 months with 0, 50, 100, or 200, increasing to 350 mg/kg pipemidic acid. The dogs were necropsied immediately following treatment.

Gough and coworkers prepared a grading criteria of gross and microscopic lesions for evaluation[3]

Grade	Gross	Microscopic
0	No lesions seen	No lesions detected
1	1 small, 1-3 mm diameter vesicle, on articular surface	Discrete matrix loss, cartilage fibrillation, and chondrocyte clusters.
2	1-2 vesicles or bulla, more than 3 mm diameter	Fissuring of matrix, and microcavitations not seen grossly.

3	More than 2 vesicles or bullae	Zone 2 cavitations, and cartilage surface elevations correlating with gross.
4	Surface erosion & cartilage effacement	Large cavities, matrix loss, irregular fragmentation.

Clinically, Gough et al. observed increased motor activity in the 500 mg/kg oxolinic acid treated group, characterized by intense pacing from day 1 to 11. The dogs showed reluctancy to rise, hindquarter stiffness, and stilted gait on day 12, which disappeared once pacing began. Dogs dosed with 100 mg/kg oxolinic acid had mildly accentuated pacing, with no sign of lameness. Pipemidic acid inhibited spontaneous motor activity and the dogs were unable to stand, and moved with obvious pain.

At necropsy, the carpal, humeroradial, tarsal, scapulohumeral, coxofermoral and femorotibial joints were examined. All dogs had varying degrees of articular cartilage vesiculation, surface detachment, and complete erosion. The joints from pipemidic acid-treated dogs contained large amounts of blood-tinged synovial fluid.

Histopathology revealed early focal loss of cartilagenous matrix, which evolved into cavitations. Cartilage fibrillation and chondrocyte clustering were pronounced within the intermediate zone. Several pipemidic acid-treated dogs developed synovial membrane changes.

Pipedimic acid-treated dogs incurred the highest gross and microscopic incidences, 4 times higher when compared with the oxolinic acid-treated dogs at the same dose. Lesion severity was

dose-related between the two oxolinic acid-treated groups.

Results from Ingham's experiment concluded that clinically, the age was of greater importance than the dosage of pipemidic acid, as generally, the younger the dog, the earlier the onset of arthropathy[2]. He found similar severe changes, gait ataxia, stiffness, and inability to rise. However, in several cases, even with continued medication, clinical recovery began 7-10 days after onset of signs, and was complete within 2-3 weeks.

Lesions at necropsy were most frequently located on the humeral head, glenoid cavity of the scapula, the head and trochlea of the femur, and the trochlear surface of the patella. The joints from clinically lame dogs contained large amounts of clear synovial fluid. There was no microscopic evidence of lesion resolution in 3 dogs necropsied 22, 26, and 87 days after medication withdrawal, although they were no longer lame prior to necropsy.

In a 6-month study, half of the dogs in the two highest does rates became lame, having clinical signs after 3-14 days of treatment. All afflicted dogs had clinically recovered by the end of the first month. Necropsy revealed blistering of the articular surfaces in 15 out of 16 dogs. Microscopic findings in both cases were similar to those found by Gough et al.; however, there were no changes in the synovial membrane of either group. Another pipemidic acid study with beagles 2 years or older did not produce gross or microscopic lesions.[2]

Cinoxocin, another fluoroquinolone, was tested in 2-3

month-old beagles given 250 mg/kg/day.[93] Clinical signs of arthropathy were seen after 2-7 doses. Gross and microscopic lesions characterized the previously described "blistering" and cartilagenous matrix loss. Lesions were not observed grossly or histologically in dogs 8 months old or older.

Patella

The patella is pivotal for effective stifle mechanics. Two forces act on the patella during joint movement, a patellofemoral compresssion force and a quadriceps muscle tension force. Studies of human patellar bio-mechanics find that patello-femoral compression rises sharply after 30 knee flexion.[99] This is approximates the body weight. At 60 flexion, it is 4 times the body weight.

In daily activities, this force is estimated to be 1.5 times the body weight for walking, 3.3 times the body weight climbing stairs, and nearly 8 times the body weight on squatting. Quadriceps tension forces also rise after 15 knee flexion. Force estimates by Kettelkamp are 1 times body weight for walking, 3.4 times body weight for climbing stairs, and 5 times body weight for squatting.[99]

Human medicine has documented the syndrome chondromalacia patellae, which refers to softening of the articular cartilage of the patella, and commonly begins in adolescents and young adults. Classic arthritic signs of joint effusion and limited range of movement are lacking, and the syndrome does not necessarily

lead to patello-femoral osteoarthritis in later years. Macroscopically, fibrillation, fissuring, and erosion of the articular cartilage occur, and may be categorized into 4 grades.[100]

I: Localized softening, swelling or fibrillation of the articular cartilage.

II: Fragmentation and fissuring in an area 1.3 cm. or less in diameter.

III: Fragmentation and fissuring in an area greater than 1.3 cm. in diameter.

IV: Erosion of articular cartilage down to subchondral bone.

Research of chondromalacia patellae, conducted by Goodfellow et al., divided the pathology of patellar articular cartilage into age-dependent surface degeneration and basal degeneration.[101] Age-dependent surface degeneration is commonly encountered in its advanced form in middle-aged joints. The articular surface is affected by fibrillation and erosion, which progressively deepens to the subchondral bone plate. Contact studies of articular surfaces have illustrated areas most frequently afflicted. Surprisingly, habitually non-contract areas undergo degeneration even in youth, and high contact areas are les commonly affected. This is believed to be due to lack of pressure allowing nutrients into the dense cartilage meshwork.[102] Once degeneration begins, tension forces transmitted across the articular surface are disrupted and adjacent areas breakdown.[103]

Basilar degeneration, noted in young, athletic adults, initially involved the deep cartilage zones, and only later

affected the articular surface. In the early stages, the surface is smooth, though the cartilage has a spongy consistency and "pitting edema". Fasiculation of the zone 4 region is believed to occur, which eventually leads to surface fibrillation. Histologically, the basilar collagen fibers were thick, and embedded in a pale-staining ground substance. Chondrocyte proliferation occurred within the frond-like projections in advanced lesions. "Blistering" was also noted, though the bullae contained a plug of unorganized fibrous tissue.

Basilar degenerative lesions developed in a different area than normally observed for the age-dependent, surface degenerative patellar lesions. The lesions occurred in an area of heavy compression loading, and also subjected to shearing forces, as the patella glides off the patellar facets and onto the femoral condyles and back again.

Cartilage Staining Characteristics

Due to the presence of sulfate groups on proteoglycans, the acidic ground substance matrix stains basophilic, though rarely does cartilage stain uniformly. Staining properties are conferred by the acidophilic nature of collagen and the basophilic carboxylate and sulfate groups of proteoglycans. Stain intensity and variation may be associated with age or metabolic activity of cartilage. Young cartilage may be acidophilic, however perilacunar matrix of old cartilage may stain strongly basophilic. Acidophilia of younger cartilage stems from collagen

dominating the small number of basophilic sulfate groups present. Generally, the basophilic components predominate in routine hematoxylin and eosin (H & E) staining.

Metachromatic stains, such as toluidine blue or Alcian blue, are useful for demonstrating glycosaminoglycans. Other nonspecific glycosaminoglycans of cartilagenous matrix are periodic acid schiff (PAS) negative, however, the cartilagenous matrix stains positively with PAS. The constituents responsible for this staining reaction remain unidentified, though they are probably collagen and other structural glycoproteins.[104]

Research by Scott and Dorling undertaken to differentiate between carboxyl and sulfated groups in glycosaminoglycans, resulted in the technique of altering $MgCl_2$ molarities while using Alcian blue.[105] The technique is based on the assumption that electrolytes ($MgCl_2$) when incorporated into the Alcian blue staining solution, will compete with Alcian blue molecules for the reactive constituents of the acid mucosubstances. Below .06 mol/l $MgCl_2$, both carboxylated and sulfated mucosubstances will stain; above .3 mol/l, only sulfated mucosubstances will stain with Alcian blue. The anticipated results are as follows: carboxyl and sulfated mucosubstances–positive with .06 M $MgCl_2$., weakly and strongly sulfated mucosubstances–positive with .3M $MgCl_2$., strongly sulfated mucosubstances–positive with .5M $MgCl_2$., highly sulfated connective tissue mucins–positive with .7M $MgCl_2$., and keratin sulfate only–positive with .9M $MgCl_2$. Criticism of this technique stems from false negatived resulting from poor

penetration by Alcian blue, and poor tissue fixation will result in inadequate substrate with which Alcian blue can react.[106]

Glossary

Cartilage Fibrillation - Exposure of collagen framework composing hyaline cartilage, due to degradation of proteoglycan matrix.

Chondromalacia - Softening of cartilage as determined by loss of sulfated proteoglycans in cartilagenous matrix. Loss of staining on H & E.

Chondrones or chondrocyte clusters - Cellular nests that represent chondrocyte mitosis in abortive or ineffective attempts at repair.

Clefting - Horizontal clefts found in zone 2, surrounded by cartilage undergoing chondromalacia.

Fissuring - Vertical clefts in articular cartilage.

Flaking - Cracks or fissures in the surface layer of the articular cartilage.

Matrix Rarefaction - Loss of matrix density as indicated by pale stain uptake.

Mesochondrium - The matrix in which are embedded the cellular elements of hyaline cartilage.

Territorial Matrix - Refers to the lacunar margins of individual or groups of chondrocytes. Strongly basophilic on H & E.

Interterritorial Matrix - Lighter staining region of matrix not directly adjacent to chondrocytes.

Superficial or Surface Layer (Zone 1) - Small, flattened chondrocytes, their long axes parallel to articular surface.

Intermediate or Transitional Layer (Zone 2) - Round cells in various stages of maturation.

Deep Zone (Zone 3) - Mature and hypertrophic cells.

Mineralization Zone (Zone 4) - Point of union of articular cartilage with underlying compact epiphyseal bone.

Tidemark - Junction between noncalcified and calcified portions of cartilage.

Bulla - A large vesicle, usually 2 mm or greater in diameter.

Vesicle - A blister less than 2 mm in diameter.

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II. PATHOLOGICAL STUDY OF FLUOROQUINOLONE-MEDIATED CHONDROARTHROPATHY IN JUVENILE DOGS

INTRODUCTION

Recent development of the promising fluoroquinolone antibiotic family has initiated considerable toxicity-level investigation. Prolonged administration of high oral dosages resulted in articular cartilage changes in immature animals. This effect, by an unknown mechanism, has been reported in rabbits,[1] dogs,[2] and monkeys,[3] though only in immature animals.[2,3] Researchers have suggested various pathogenetic mechanisms for the articular changes, but the histologic initiator remains undetermined.

This report documents the gross and microscopic development of articular cartilage lesions associated with prolonged, high level oral fluoroquinolone administration in immature dogs. Illustration of progressive articular changes initiated by matrix depletion, may help clarify the degenerative mechanism involved.

MATERIALS AND METHODS

Thirty dogs were given a fluoroquinolone antibiotic at the proposed use level and elevated doses, for 3 times the proposed treatment duration. In the first trial, 15 Labrador puppies, 19-28 weeks old, were divided into 4 groups, 3 test groupings containing 4 each and 1 control of 3 pups. Fluoroquinolone was administered orally as follows:

Group	Daily dosage level, 30 consecutive days
I	Nontreated controls
II	Proposed use level
III	3 x proposed use level
IV	5 x proposed use level

Following the thirtieth treatment, half of the pups were euthanized using a sodium pentobarbitol solution (Sleep-Away) and necropsied, with the remainder examined the following week.

In the second trial 15 Labrador pups, 12-15 weeks old, were divided into 5 groups of 3, 4 test and 1 control. The method of selection was random, by weight.

Group	Daily dosage level, 30 consecutive days
I	Nontreated controls
II	.5 x proposed use level
III	1.5 x proposed use level
IV	2.5 x proposed use level
V	3 x proposed use level, for 15 days

After the thirtieth day of treatment, 2 puppies from groups I and V, and 1 from each of the other groups were euthanized with sodium pentobarbitol and necropsied. The remaining pups were

necropsied 9 days post-treatment.

For histopathological examination, the coxofemoral, carpal, and stifle joints were removed and fixed in 10% buffered, neutral formalin (BNF). After 2 weeks, the joints were washed, and placed in formic acid-sodium citrate solution for 10 days.[4] The solution was changed several times during the decalcification process. Following routine processing and embedding, the tissues were cut at 5 microns and stained with hematoxylin and eosin,[4] (H & F), and 2 concentrations of Alcian blue, at .5M $MgCl_2$, and .9M $MgCl_2$.[5] Another mucopolysaccharide sensitive stain, Azure A,[4] was also tried on several sections. The tissues were evaluated by light microscopy.

Sequential development of gross and microscopic lesions of the acetabulum, femoral head, patella, distal femur, and proximal tibia, were examined by the following criteria:

RESULTS

I. Clinical Observations

In trial 1, 3 of the 4 dogs treated at 5 times the proposed dosage level developed hindquarter weakness within 7 days of treatment. All 3 dogs in trial 2, receiving 3 times the proposed dose level, had hindquarter weakness and stiffness by day 11 of treatment. Signs of this weakness had clinically resolved 9 by days post-treatment. In both cases, the hindquarter weakness did not appear to be painful.

II. Gross Observations

In trial 1, groups 1 and 2, the articulating sufaces of the stifle were bluish to white, smooth and glistening (Fig 1 and 2). Stifle cartilage changes were first detected in group III, dosed at 3 times the proposed use level, in which 3 or 4 older puppies developed lesions. The common lesions included surface roughening, characterized by a dull, granular, grayish-yellow appearance, and erosion, which was a deeper, ulcerative surface defect. The distal patella, medial and lateral articular surfaces of the tibia, and femoral condyles, were roughened and eroded.

A 3 x 4 mm bulla was found on the lateral trochlear ridge, and another, 5 x 8 mm, was found on the lateral condyle of the same femur. The vesicular lesions projected 1-2 mm above the surrounding cartilage and were circular, unless located on the trochlear ridges where they were elongated appearance. The

larger vesicles or bullae were rarely intact, having been torn or ruptured, producing large flaps of loose, smooth-surfaced, thin, cartilage. The underlying eroded surface was roughened and dull, with ragged edges, Subchondral bone was never exposed.

All 4 older pups, dosed at 5 times the proposed use level, had stifle lesions. The least affected case developed a 4 x 5 mm trochlear erosion and accompanying roughening of the tibial articular surface. Of the remaining 3 cases, the patella of 1 had a prolapsed, 6 x 6 mm "blister" and associated erosion of its distal surface (Fig. 25). Erosions and cartilage tags were present on the other patellar surfaces (Fig. 4 and 6).

The femoral trochlea had either 10 x 10 mm of surface roughening, (Fig. 5 and 6), 8 x 12 mm focal erosion with cartilagenous tags, (Fig. 3 and 4), or a 12 x 13 mm prolapsed bulla (Fig. 24 and 25). In all cases, the lateral and medial condyles developed roughened or eroded articular cartilage (Fig. 3, 5, and 24).

The younger puppies in trial 2 had stifle changes beginning in group III, at 1.5 times the proposed use level, where 2 of 3 developed lesions. In both pups, the lateral tibial surface was roughened, and in addition, one had a 9 x 11 mm focal erosion with cartilage detachment. The lateral condyle from the same stifle developed a 6 x 9 mm erosion with cartilage tags.

At 2.5 times the proposed therapeutic dose, all 3 puppies had stifle lesions. The medial condyles of each puppy developed erosions, and the articular tibial surfaces, both medial and

lateral sides, were roughened and/or eroded.

Stifles examined from puppies treated at 3 times the proposed usage level ranged from articulaIn one pup, two intact vesicles were located in the intercondylar area. The same femur had a trochlear ridge blister measuring 17 x 7 mm. Another puppy had 3 vesicles, elliptically arranged, on its tibial articular surface, an area not subject to direct appositional forces.

III. Microscopic Observation

Microscopically, the articulating cartilage of group I, in both trials, had a smooth, undulating surface composed of the flattened chondrocytes in zone 1, underwhich lay the randomly oriented, well-dispersed chondrocyte population of zone 2, followed by zone 3 chondrocyte columns and the calcified junction found at zone 4 (Fig 7-9, 13, 19 and 33). The only interruption in the normally smooth surface was due to the interwoven fibrous attachment of ligaments or the synovial membrane (Fig 10-12).

Stifles which developed macroscopic lesions had chondromalacia as a common microscopic lesion. Characterized by loss of matrix stain uptake, resulting in a pale, often eosinophilic appearance (Fig 14 and 15). Chondromalacic areas generally had reduced numbers of chondrocytes, whith those present often being hypertrophied or atrophied, with cytoplasmic vacuolation.

The more advanced lesions with roughened and eroded

surfaces developed cartliage fibrillation. Cartilage fibrillation and chondromalacia were pronounced in the intermediate zone (zone 2), (Fig 21-23), and also in foci of surface erosion (Fig 15-18, and 34).

Chondrocytes residing in areas of chondromalacia and commonly encircling vesicles, formed clusters or "chondrones". Chondromes were typically nests of 4 or more chondrocytes, with their nuclei arranged in annular formation (Fig. 16). More severe vesicular lesions, or cartilage tags, contained large, mature chondrones, composed of up to 20 or more chondrocytes. These clusters were the main cellular components wi cartilagenous fronds, often containing multiple chondrones (Fig. 17 and 18). The roughened articular surface of the tibia had characteristic fissuring and fibrillation.

Cavitations of the intermediate zone developed most frequently within the stifles of the higher dosage groups. Chondrones, and disrupted, thickened, collagen fibers framed these zone 2 cavities, under which often lay disorganized zone 3 chondrocyte columns (Fig. 20 and 26).

The macroscopic "blisters" corresponded to enlarged cavitations, whose cartilagenous "roof" projected above the surface. The thin, protective, outer layer was pale staining, and hypocellular, except for chondrocyte clusters, which were more prominent along the sides and underlying borders (Fig. 32). Several cavities contained small amounts of pale, eosinophilic material, while other, larger vesicles, had some internal

basophilic material, and cellular debris. Occasionally, once the intervening thin, intact, articular surface had worn away, the underlying cartilage was exposed, fibrillated and fissured, with chondrones present along he lesion edge (Fig. 27).

Although, the Azure A stained slides did not contrast strongly enough for good evaluation, the tissues stained by the two Alcian Blue solutions vividly illustrated chondromalacia (Fig. 14 and 18). The proteoglycan-depleted matrix was pale, with hypocellular foci, compared with a matrix normally deep blue in color. Chondrones contrast with their background, as the glycosaminoglycan-producing clusters stain blue, nestled in a pale, wispy matrix.

Discussion

The development of articular changes was dose and age dependent, although there were variations of lesion severity and incidence among each group. Clinical observations did not necessarily correspond with lesion severity. Examination of the joints from those pups whose clinical signs of hindquarter stiffness and weakness resolved, had marked gross and microscopic lesions. One evident difference in results between the 2 trials was a total lack of patellar lesion development in trial 2, which was composed of younger puppies.

The grossly evident cartilage lesions had as their source a common cascade of histological events. Chondromalacia, a key

feature in pathogenesis, resulted from decreased proteoglycan content, the ground substance for the cartilagenous matrix. The softened cartilage is mechanically inadequate to support the collagen framework, which becomes distorted, thickened, and degenerated. In an emergency repair effort in the face of increased destruction,[6] clusters of chondrocytes form to replenish the matrix with chondroitin-4-sulfate. This is verified by their deep blue appearance when stained with metachromatic Alcian blue. The anticipated evaluation of the type and amount of chondroitin sulfates present, based on subtle .5M and .9M Alcian blue concentration differences, was not possible due to poor color differentiation. However, over all proteoglycan depletion was evident.

Normal articular hydrodynamics and resiliency are lost in the collapsing, overly-hydrated matrix.[7] Unable to withstand appositional pressures and shearing forces, the collagen framework separates, resulting in internal cavitations. These may evolve later into surface vesicles, and if ruptured, result in marked focal cartilage effacement. Synovial fluid, with its hyaluronidase component, gains wide access to the internal cartilage, which enzymatically further degrades the matrix.[8]

Results of research by Gough et al. on a similar fluoroquinolone analogue study, were evaluated primarily by vesicular and/or bullae formation within articular cartilage.[2] Modifications of this evaluation scheme were made to accommodate the milder lesion of surface roughening, which was commonly

observed in this study. Also, by separating the subtle microscopic changes, the lesions' pathogenesis were more thoroughly traced.

A theory derived from previous studies of fluoroquinolone-mediated cartilage changes, suggested surface crystalline formation. The crystals, when combined with appositional forces, allegedly initiated articular cartilage destruction. In this study lesions often developed within the metabolically active zone 2, independent of surface influence (Fig. 20, 21, and 28).

Yet to be answered, is how the cascade of events is initiated. Recent studies revealed two types of cartilage degradation have been reported: (a) direct degradation by enzymes, for instance, those released by synovial tissue; (b) indirect degradation by factors, such as retinol and mononuclear cell factor, which stimulate chondrocytes to produce matrix degrading enzymes.[8-10] The results from these in vitro studies mirror the cartilage changes produced from prolonged high levels of fluoroquinolone. Mononuclear cell factor is believed to play a major role in rheumatoid arthritis[11,12] and osteo-arthritis.[13] These substances stimulate chondrocytes to release enzymes, which focus their digestive abilities on the proteoglycan matrix set the stage for further destruction.

Lesions produced in the present study were dosage dependent, and their pattern and severity were reflective of the dog's age. Why patellar lesions did not occur in younger puppies but were consistently observed in slightly older pups, is

unknown. Assuming the lesions are initiated by proteoglycan loss, resulting in a weakened matrix, then lesion severity would be influenced by increased physical activity and weight on the the articular surfaces. Slightly older pups would be expected to apply more stress through physical activity and increased body mass, than smaller, less active, 3 month old pups.

Several studies reported that this antibiotic family did not induced cartilage lesions in mature animals.[2,3] Other research comparing responses by young and old cartilage to various substances tried to explain why differences occur.[14] Huber-Bruning *et al.* conducted studies with retinol and mononuclear cell factor on human cartilage *in vitro* from children ($\leq$ 2-years-old) and adults ($\geq$ 40-years-old). They found proteoglycan content shifts in both groups, though more severely in the young than the old. Older cartilage tends to balance proteoglycan release with greatly increased proteoglycan synthesis, so total proteoglycan content remained unchanged.[16] Proteoglycan content of young cartilage dropped due to enhanced release from cartilage into the media, and suppression of proteoglycan synthesis. Huber-Bruning concluded that naturally occurring mononuclear cell factor can only exert a destructive effect on cartilage that is actively synthesizing proteoglycan, a condition met in older people with osteo-arthritis.[15] More studies are required to explain some of these questions. The fluoroquinolone antibiotic family offers great therapeutic promise, as well as opportunities for future study.

SUMMARY

Two groups of 15 puppies, each of different age ranges, were histopathologically evaluated following various dosages of a fluoroquinolone antibiotic. The stifle joints were examined for macroscopic and microscopic articular cartilage changes. Grossly, the lesions varied from surface roughening and erosion to vesiculation and deep focal erosion and cartilage effacement. Microscopic changes progressed from matrix depletion and cartilage fibrillation to matrix fissuring, accented by chondrone formation, zone 2 cavitations, surface vesiculation, and irregular foci of cartilage fragmentation. The histological cascade which produced the lesions began with destruction of the proteoglycan matrix. Loss of matrix metachromasia when stained with Alcian blue, demonstrated proteoglycan depletion. Cartilage changes were dose and age dependent, though lesion severity and incidence varied among groups. Notably, younger puppies did not develop patellar lesions, as did older pups.

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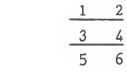


Fig 1 - Normal articular surface of the distal femur and proximal tibia.

Fig 2 - Normal femoral trochlea and patella.

Fig 3 - Roughened and eroded tibial articular surface. Erosion and vesiculation of condylar and trochlear surfaces from the stifle of a pup administered 5 times the therapeutic dose.

Fig 4 - The same stifle as in Fig 9. Deep focal trochlear erosion with associated cartilagenous tag. Note focal patellar erosion.

Fig 5 - Stifle from a pup given 5 times the proposed usage level. Roughened and eroded articular surface. Foci of deep condylar erosion with a small vesicle noted on the trochlear ridge.

Fig 6 - The same stifle pictured in Fig 11. Marked patellar erosion with an associated cartilagenous tag. Deep, circumscribed erosion of the proximal trochlear surface. Small vesicle on lateral trochlear ridge.

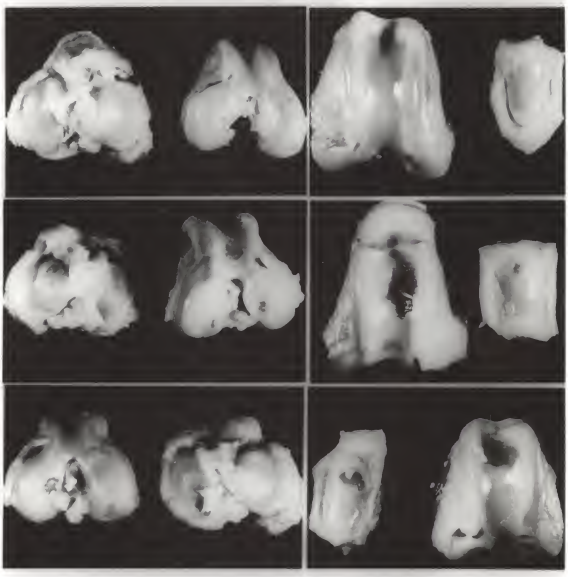


Fig 7 - Photomicrograph of normal cartilage of the femoral condyle. H&E

A - Surface layer (zone 1)
B - Intermediate layer (zone 2)
C - Deep zone (zone 3)
D - Mineralization zone (zone 4)

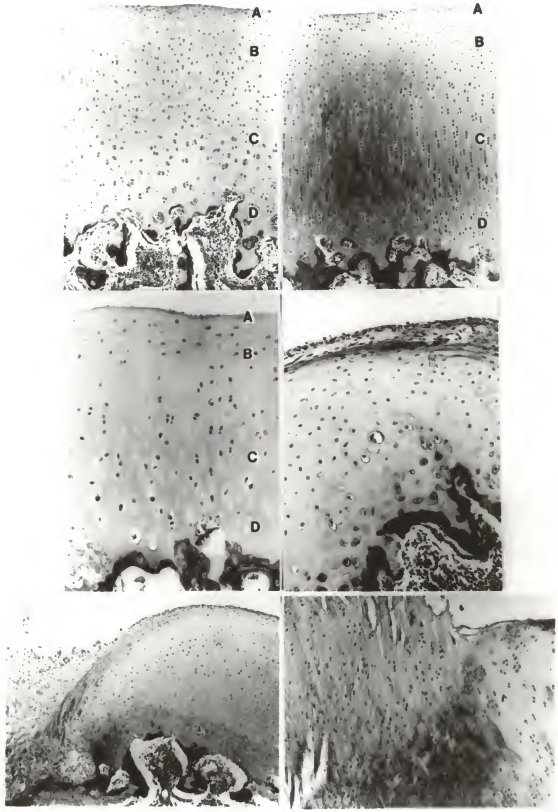
Fig 8 - Photomicrograph of normal articular cartilage from the patella. Note well-defined zone 3 (C) chondrocyte columns. H&E.

Fig 9 - Closer view of cartilage layers in Fig 1. Zone 1 (A) contains small, flattened chondrocytes. Zone 2 (B) chondrocytes are round and in various phases of maturation. Zone 3 (C) has mature and hypertrophic cells. Isogenous cell or cell nests are present. Zone 4 (D) represents the junction between the noncalcified and calcified portions of cartilage. H&E

Fig 10 - Photomicrograph of normal interwoven attachment of the fibrous synovial membrane and nonweight-bearing portion of the articular surface. H&E

Fig 11 - Photomicrograph of normal blending of synovial membrane with the nonweight-bearing articular cartilage surface of the femoral head. The perichondral ring surrounds the peripheral limits of the growth plate at the transition point between articular cartilage and periosteum. H&E

Fig 12 - Photomicrograph of normal femoral head at the point of round ligament attachment. H&F


A
B
C
D
A
B
C
D
A
B
C
D

13 14

15 16

17 18

Fig 13 - Photomicrograph of normal articular cartilage. H&E

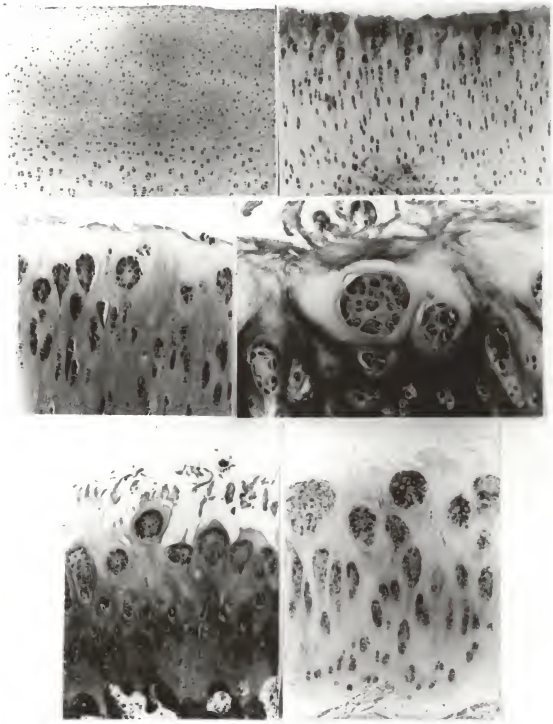
Fig 14 - Photomicrograph of Alcian blue stain demonstrating moderate chondromalacia of the femoral condyle. Pitted, roughened surface lacks a normal zone 1. Multiple, active chondrones reside within zone 2. This area stained darkly as compared to the rest of the pale matrix due to proteoglycan production by the chondrones.

Fig 15 - Photomicrograph of marked chondromalacia of the femoral condyle. Note pale, uneven, hypocellular zone 1 and multiple, large chondrones reside within zone 2. H&E.

Fig 16 - Photomicrograph of large chondrones present in fibrillated matrix from the tibial articular surface. H&E

Fig 17 - Photomicrograph of severe chondromalacia, fibrillation and fissuring of the femoral condyle. Large chondrones present in the projecting, pale, surface. H&E

Fig 18 - Photomicrograph of Alcian blue stain of the same tissue in Fig 17. The territorial matrix, adjacent to chondrocytes composing the chondrones, stained darkly, indicating presence of proteoglycans. The interterritorial matrix is pale, indicating proteoglycan depletion and chondromalacia.



19 20

21

22 23

Fig 19 – Photomicrograph of normal articular cartilage from the femoral condyle. H&E

Fig 20 – Photomicrograph of zone 2 cavity in the femoral condyle. Note multiple, large chondrones encircling the matrix defect. The cartilage is hypocellular, fibrillated and pale-staining along the cleft edges. H&E

Fig 21 – Photomicrograph of early cavity formation. The collagen meshwork is distorted and the disrupted fibers have separated. Medium-sized chondrones line the zone 2 defect. H&E

Fig 22 – Photomicrograph of Alcian blue stain of early cleft formation within a femoral condyle. The darker areas correspond to chondrone, or chondrocyte locations. the pale area lacks metachromasia, indicating proteoglycan depletion.

Fig 23 – Polarization of the same area in Fig 22. The mid-zonal, light area, represents collagen fibers. Their wavy, distorted, architecture highlights the defect. H&E

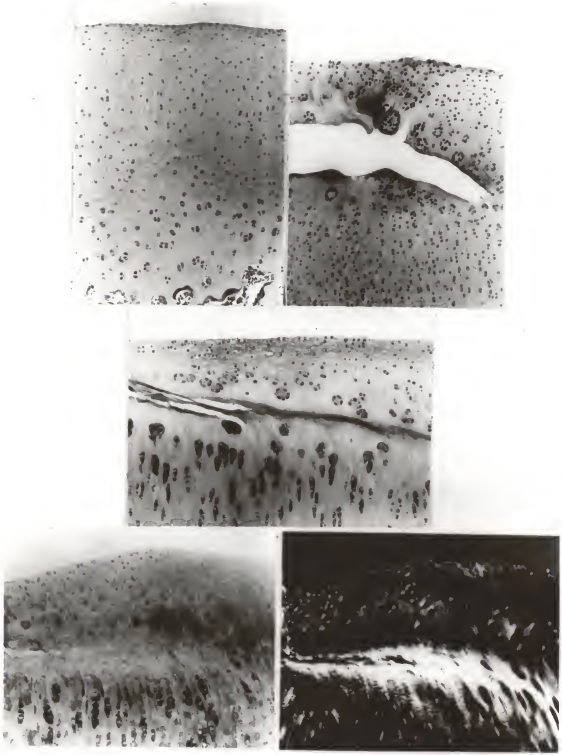


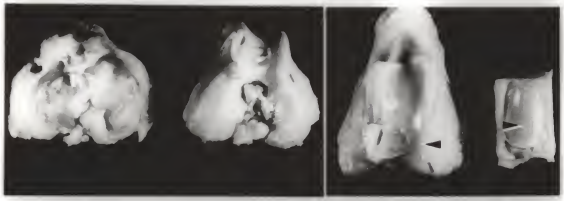
Fig 24 – Stifle from a pup dosed at 5 times the proposed therapeutic level. Note cartilage erosion of the tibial surface. A prolapsed bulla is loosely attached to the lateral trochlear ridge.

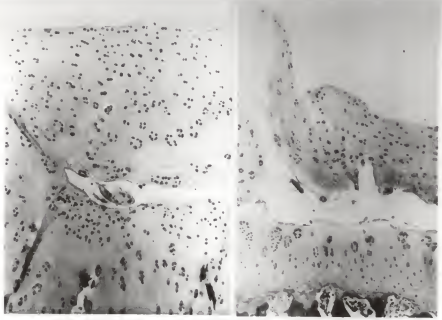
Fig 25 – Another view of loose, folded appearance to the smooth-surfaced trochlear lesion. Bulla formation of the articular patellar surface.

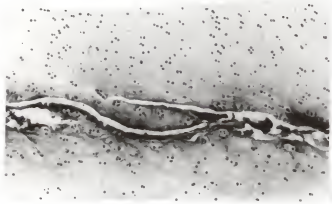
Fig 26 – Photomicrograph of a zone 2 cavity in the femoral condyle in Fig 25. Note chondromalacia, fibrillation, and chondrone formation associated with the cavity. H&E

Fig 27 – Photomicrograph the roughened tibial surface in Fig 24 and 25. Note cartilage tags, fissuring, and zone 2 cavitation. Chondrones in a pale-staining matrix accentuated the cavity. H&E

Fig 28 – Photomicrograph of the femoral condyle had extensive zone 2 clefting underlying the smooth articular surface prior to the large, prolapsed bulla. H&E







29 30

31 32

33 34

Fig 29 - Roughened and eroded tibial surface from a pup administered 3 times the proposed therapeutic dose. A small, intact bulla lies on the medial trochlear ridge (arrow).

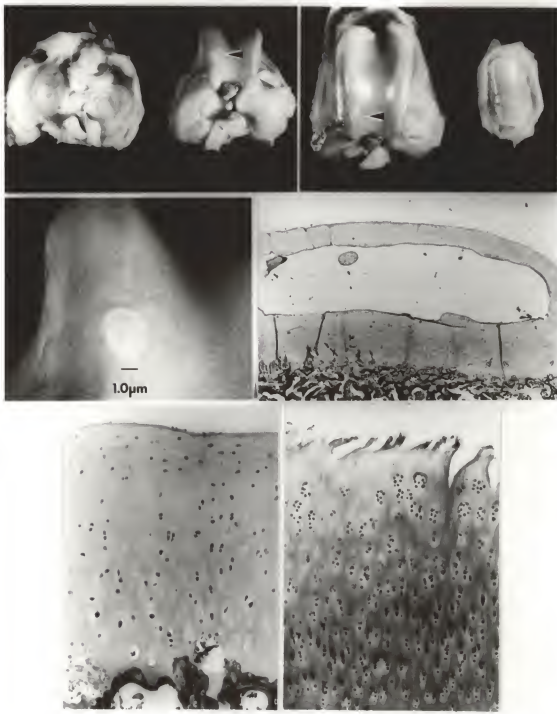
Fig 30 - Focal patella roughening and erosion. Intact bulla on trochlear ridge (arrow).

Fig 31 - Higher magnification of the 3 x 4 bull in Fig 30.

Fig 32 - Photomicrograph of intact bulla. Two masses of cellular debris are present inside the cavity. H&E.

Fig 33 - Photomicrograph of normal articular cartilage.

Fig 34 - Photomicrograph showing marked chondromalacia, fibrillation, fissuring and chondrone formation of the patellar surface. H&E


1.0μm

APPENDIX A: Gross and Microscopic Lesions of Individual Pups.

TRIAL ONE

Dog A . Group I Nontreated controls

Gross lesion	coxofemoral: acetabulum NVL	coxofemoral: head NVL	stifle: femur NVL	stifle: tibia NVL	stifle: patella NVL
Surface roughening					
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bulla					
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion	X	X	X	X	X
Discrete areas of matrix loss					
Cartilage fibrillation					
Chondrocyte clusters (chondrones)					
Fissuring of matrix					
Intermediate (zone 2) cavitations					
Elevation of cartilage surface correlating with gross					
Large cavities in cartilage					
Irregular fragmentation and loss of matrix					

Dog B. Group I Nontreated controls

Gross lesion	coxofemoral: acetabulum NVL	coxofemoral: head NVL	stifle: femur NVL	stifle: tibia NVL	stifle: patella NVL
Surface roughening					
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bulla					
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion	X	X	X	X	X
Discrete areas of matrix loss					
Cartilage fibrillation					
Chondrocyte clusters (chondrones)					
Fissuring of matrix					
Intermediate (zone 2) cavitations					
Elevation of cartilage surface correlating with gross					
Large cavities in cartilage					
Irregular fragmentation and loss of matrix					

Dog C. Group I Nontreated controls

	coxofemoral			stifle		
	acetabulum	head	femur	tibia	patella	
Gross lesion	NVL	NVL	NVL	NVL	NVL	
Surface roughening						
1 or 2 vesicles and/or bulla						
More than 2 vesicles and/or bulla						
Deep erosion(s) and cartilage effacement						
Microscopic						
No significant microscopic lesion	X	X	X	X	X	
Discrete areas of matrix loss						
Cartilage fibrillation						
Chondrocyte clusters (chondrones)						
Fissuring of matrix						
Intermediate (zone 2) cavitations						
Elevation of cartilage surface correlating with gross						
Large cavities in cartilage						
Irregular fragmentation and loss of matrix						

Dog A. Group II: Proposed use level and 3 x duration of treatment

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	NVL	NVL	NVL
Surface roughening					
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bulla					
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion	X	X	X	X	X
Discrete areas of matrix loss					
Cartilage fibrillation					
Chondrocyte clusters (chondrones)					
Fissuring of matrix					
Intermediate (zone 2) cavitations					
Elevation of cartilage surface correlating with gross					
Large cavities in cartilage					
Irregular fragmentation and loss of matrix					

Dog B. Group II: Proposed use level and 3 x duration of treatment

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	NVL	NVL	NVL
Surface roughening				x	
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bulla					
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion	x	x	x		x
Discrete areas of matrix loss				x	
Cartilage fibrillation				x	
Chondrocyte clusters (chondrones)					
Fissuring of matrix					
Intermediate (zone 2) cavitations					
Elevation of cartilage surface correlating with gross					
Large cavities in cartilage					
Irregular fragmentation and loss of matrix				x	
Microcavitations not seen grossly				x	

Dog C. Group II: Proposed use level and 3 x duration of treatment

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	NVL	NVL	NVL
Surface roughening				x	
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bulla					
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion	x	x	x	x	x
Discrete areas of matrix loss					
Cartilage fibrillation					
Chondrocyte clusters (chondrones)					
Fissuring of matrix					
Intermediate (zone 2) cavitations					
Elevation of cartilage surface correlating with gross					
Large cavities in cartilage					
Irregular fragmentation and loss of matrix					

Dog D. Group II: Proposed use level and 3 x duration of treatment

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	NVL	NVL	NVL
Surface roughening		x			
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bulla					
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion	x		x	x	
Discrete areas of matrix loss		x			
Cartilage fibrillation		x			x
Chondrocyte clusters (chondrones)		x			x
Fissuring of matrix					
Intermediate (zone 2) cavitations					
Elevation of cartilage surface correlating with gross					
Large cavities in cartilage					
Irregular fragmentation and loss of matrix		x			
Microcavitations not seen grossly		x			x

Dog A. Group III: 3 x proposed use level and 3 x duration of treatment

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	NVL	NVL	NVL
Surface roughening		x			
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bulla					
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion	x	x	x	x	x
Discrete areas of matrix loss					
Cartilage fibrillation					
Chondrocyte clusters (chondrones)					
Fissuring of matrix					
Intermediate (zone 2) cavitations					
Elevation of cartilage surface correlating with gross					
Large cavities in cartilage					
Irregular fragmentation and loss of matrix					

Dog B . Group III: 3 x proposed use level and 3 x duration of treatment

	coxofemoral				stifle			
	acetabulum		head		femur		tibia		patella	
Gross lesion	NVL		NVL		NVL		NVL		NVL	
Surface roughening	x		x		x		x		x	
1 or 2 vesicles and/or bulla										
More than 2 vesicles and/or bulla					x					
Deep erosion(s) and cartilage effacement			x		x		x			
Microscopic										
No significant microscopic lesion									x	
Discrete areas of matrix loss	x		x		x		x			
Cartilage fibrillation			x		x		x			
Chondrocyte clusters (chondrones)			x		x					
Fissuring of matrix			x		x		x			
Intermediate (zone 2) cavitations			x							
Elevation of cartilage surface correlating with gross			x				x			
Large cavities in cartilage			x							
Irregular fragmentation and loss of matrix			x		x		x			

Dog C. Group III: 3 x proposed use level and 3 x duration of treatment

	coxofemoral				stifle			
	acetabulum		head		femur		tibia		patella	
Gross lesion	NVL		NVL		NVL		NVL		NVL	
Surface roughening	x		x				x		x	
1 or 2 vesicles and/or bulla										
More than 2 vesicles and/or bulla										
Deep erosion(s) and cartilage effacement			x				x		x	
Microscopic										
No significant microscopic lesion	x				x					
Discrete areas of matrix loss			x				x		x	
Cartilage fibrillation			x				x		x	
Chondrocyte clusters (chondrones)			x				x		x	
Fissuring of matrix			x				x		x	
Intermediate (zone 2) cavitations			x							
Elevation of cartilage surface correlating with gross			x							
Large cavities in cartilage										
Irregular fragmentation and loss of matrix			x				x		x	

Dog D. Group III: 3 x proposed use level and 3 x duration of treatment

	coxofemoral				stifle					
	acetabulum		head		femur		tibia		patella	
Gross lesion	NVL		NVL		NVL		NVL		NVL	
Surface roughening		x		x		x		x		x
1 or 2 vesicles and/or bulla										
More than 2 vesicles and/or bulla										
Deep erosion(s) and cartilage effacement		x		x		x		x		x
Microscopic										
No significant microscopic lesion										
Discrete areas of matrix loss		x		x		x		x		x
Cartilage fibrillation				x		x		x		x
Chondrocyte clusters (chondrones)				x		x				
Fissuring of matrix				x		x				x
Intermediate (zone 2) cavitations				x		x				x
Elevation of cartilage surface correlating with gross				x		x				
Large cavities in cartilage										
Irregular fragmentation and loss of matrix				x		x				

Dog A. Group IV: 5 x proposed use level and 3 x duration of treatment

	coxofemoral				stifle					
	acetabulum		head		femur		tibia		patella	
Gross lesion	NVL		NVL		NVL		NVL		NVL	
Surface roughening				x		x		x		x
1 or 2 vesicles and/or bulla										
More than 2 vesicles and/or bulla										
Deep erosion(s) and cartilage effacement				x		x				
Microscopic										
No significant microscopic lesion		x								x
Discrete areas of matrix loss				x		x		x		
Cartilage fibrillation				x		x		x		
Chondrocyte clusters (chondrones)				x						
Fissuring of matrix				x		x		x		
Intermediate (zone 2) cavitations				x		x		x		
Elevation of cartilage surface correlating with gross				x		x		x		
Large cavities in cartilage										
Irregular fragmentation and loss of matrix				x		x		x		

Dog B. Group IV: 5 x proposed use level and 3 x duration of treatment

	coxofemoral				stifle			
	acetabulum		head		femur		tibia		patella	
Gross lesion	NVL		NVL		NVL		NVL		NVL	
Surface roughening	x		x		x		x		x	
1 or 2 vesicles and/or bulla										
More than 2 vesicles and/or bulla					x					
Deep erosion(s) and cartilage effacement			x		x		x		x	
Microscopic										
No significant microscopic lesion										
Discrete areas of matrix loss	x		x		x		x		x	
Cartilage fibrillation	x		x		x		x		x	
Chondrocyte clusters (chondrones)	x		x		x		x		x	
Fissuring of matrix			x		x		x		x	
Intermediate (zone 2) cavitations			x		x		x		x	
Elevation of cartilage surface correlating with gross			x		x		x		x	
Large cavities in cartilage										
Irregular fragmentation and loss of matrix			x		x		x		x	

Dog C. Group IV: 5 x proposed use level and 3 x duration of treatment

	coxofemoral				stifle			
	acetabulum		head		femur		tibia		patella	
Gross lesion	NVL		NVL		NVL		NVL		NVL	
Surface roughening	x		x		x		x		x	
1 or 2 vesicles and/or bulla										
More than 2 vesicles and/or bulla										
Deep erosion(s) and cartilage effacement			x		x				x	
Microscopic										
No significant microscopic lesion	x									
Discrete areas of matrix loss			x		x		x		x	
Cartilage fibrillation			x		x		x		x	
Chondrocyte clusters (chondrones)			x		x		x		x	
Fissuring of matrix			x		x		x		x	
Intermediate (zone 2) cavitations			x		x		x		x	
Elevation of cartilage surface correlating with gross			x		x		x		x	
Large cavities in cartilage										
Irregular fragmentation and loss of matrix			x		x		x		x	

Dog D. Group IV: 5 x proposed use level and 3 x duration of treatment

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	NVL	NVL	NVL
Surface roughening	x	x	x	x	x
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bulla					
Deep erosion(s) and cartilage effacement			x	x	x
Microscopic					
No significant microscopic lesion	x	x			
Discrete areas of matrix loss			x	x	x
Cartilage fibrillation			x	x	x
Chondrocyte clusters (chondrones)			x	x	x
Fissuring of matrix			x	x	x
Intermediate (zone 2) cavitations			x	x	x
Elevation of cartilage surface correlating with gross			x	x	x
Large cavities in cartilage					
Irregular fragmentation and loss of matrix			x	x	x

Dog A. Group I Nontreated Controls

TRIAL TWO

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	NVL	NVL	NVL
Surface roughening					
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bullae					
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion	x	x	x	x	x
Discrete areas of matrix loss					
Cartilage fibrillation					
Chondrocyte clusters (chondrones)					
Fissuring of matrix					
Microcavitations					
Intermediate (zone 2) cavitations					
Elevation of surface = gross					
Large cavities in cartilage					
Irregular fragmentation & loss of matrix					

Dog B. Group I Nontreated Controls

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	NVL	NVL	NVL
Surface roughening					
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bullae					
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion	x	x	x	x	x
Discrete areas of matrix loss					
Cartilage fibrillation					
Chondrocyte clusters (chondrones)					
Fissuring of matrix					
Microcavitations					
Intermediate (zone 2) cavitations					
Elevation of surface = gross					
Large cavities in cartilage					
Irregular fragmentation & loss of matrix					

Dog C. Group I Nontreated Controls

	coxofemoral		stifle			
	acetabulum	head	femur	tibia	patella	
Gross lesion	NVL	NVL	NVL	NVL	NVL	
Surface roughening						
1 or 2 vesicles and/or bulla						
More than 2 vesicles and/or bullae						
Deep erosion(s) and cartilage effacement						
Microscopic						
No significant microscopic lesion	x	x	x	x	x	
Discrete areas of matrix loss						
Cartilage fibrillation						
Chondrocyte clusters (chondrones)						
Fissuring of matrix						
Microcavitations						
Intermediate (zone 2) cavitations						
Elevation of surface = gross						
Large cavities in cartilage						
Irregular fragmentation & loss of matrix						

Dog A. Group II 0.5 x proposed use level for 30 consecutive days

	coxofemoral		stifle			
	acetabulum	head	femur	tibia	patella	
Gross lesion	NVL	NVL	NVL	NVL	NVL	
Surface roughening						
1 or 2 vesicles and/or bulla						
More than 2 vesicles and/or bullae						
Deep erosion(s) and cartilage effacement						
Microscopic						
No significant microscopic lesion	x	x	x	x	x	
Discrete areas of matrix loss						
Cartilage fibrillation						
Chondrocyte clusters (chondrones)						
Fissuring of matrix						
Microcavitations						
Intermediate (zone 2) cavitations						
Elevation of surface = gross						
Large cavities in cartilage						
Irregular fragmentation & loss of matrix						

Dog B. Group II 0.5 x proposed use level for 30 consecutive days

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	NVL	NVL	NVL
Surface roughening					
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bullae					
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion	x	x	x	x	x
Discrete areas of matrix loss					
Cartilage fibrillation					
Chondrocyte clusters (chondrones)					
Fissuring of matrix					
Microcavitations					
Intermediate (zone 2) cavitations					
Elevation of surface = gross					
Large cavities in cartilage					
Irregular fragmentation & loss of matrix					

Dog C. Group II 0.5 x proposed use level for 30 consecutive days

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	NVL	NVL	NVL
Surface roughening					
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bullae					
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion	x	x	x	x	x
Discrete areas of matrix loss					
Cartilage fibrillation					
Chondrocyte clusters (chondrones)					
Fissuring of matrix					
Microcavitations					
Intermediate (zone 2) cavitations					
Elevation of surface = gross					
Large cavities in cartilage					
Irregular fragmentation & loss of matrix					

Dog A. Group III 1.5 x proposed use level for 30 consecutive days

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	yes	yes	NVL
Surface roughening			x	x	
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bullae					
Deep erosion(s) and cartilage effacement			x	x	
Microscopic					
No significant microscopic lesion	x	x			x
Discrete areas of matrix loss			x	x	
Cartilage fibrillation			x	x	
Chondrocyte clusters (chondrones)			x	x	
Fissuring of matrix			x	x	
Microcavitations			x	x	
Intermediate (zone 2) cavitations			x		
Elevation of surface = gross			x	x	
Large cavities in cartilage					
Irregular fragmentation & loss of matrix					

Dog B. Group III 1.5 x proposed use level for 30 consecutive days

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	NVL	NVL	NVL
Surface roughening					
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bullae					
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion	x				x
Discrete areas of matrix loss		x	x	x	
Cartilage fibrillation		x		x	
Chondrocyte clusters (chondrones)					
Fissuring of matrix					
Microcavitations					
Intermediate (zone 2) cavitations					
Elevation of surface = gross					
Large cavities in cartilage					
Irregular fragmentation & loss of matrix					

Dog C. Group III 1.5 x proposed use level for 30 consecutive days

	coxofemoral		stifle			
	acetabulum	head	femur	tibia	patella	
Gross lesion	NVL	NVL	NVL	yes	NVL	
Surface roughening				x		
1 or 2 vesicles and/or bulla						
More than 2 vesicles and/or bullae						
Deep erosion(s) and cartilage effacement						
Microscopic						
No significant microscopic lesion	x	x	x	x	x	
Discrete areas of matrix loss						
Cartilage fibrillation						
Chondrocyte clusters (chondrones)						
Fissuring of matrix						
Microcavitations						
Intermediate (zone 2) cavitations						
Elevation of surface = gross						
Large cavities in cartilage						
Irregular fragmentation & loss of matrix						

Dog A. Group IV 2.5 x proposed use level for 30 consecutive days

	coxofemoral		stifle			
	acetabulum	head	femur	tibia	patella	
Gross lesion	NVL	NVL	yes	yes	NVL	
Surface roughening			x	x		
1 or 2 vesicles and/or bulla						
More than 2 vesicles and/or bullae						
Deep erosion(s) and cartilage effacement			x	x		
Microscopic						
No significant microscopic lesion	x	x			x	
Discrete areas of matrix loss			x	x		
Cartilage fibrillation				x		
Chondrocyte clusters (chondrones)				x		
Fissuring of matrix				x		
Microcavitations				x		
Intermediate (zone 2) cavitations						
Elevation of surface = gross				x		
Large cavities in cartilage						
Irregular fragmentation & loss of matrix						

Dog B. Group IV 2.5 x proposed use level for 30 consecutive days

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	yes	yes	yes	NVL
Surface roughening		x	x	x	
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bullae					
Deep erosion(s) and cartilage effacement			x	x	
Microscopic					
No significant microscopic lesion	x				x
Discrete areas of matrix loss		x	x	x	
Cartilage fibrillation		x	x	x	
Chondrocyte clusters (chondrones)		x	x	x	
Fissuring of matrix		x	x		
Microcavitations		x	x	x	
Intermediate (zone 2) cavitations		x	x	x	
Elevation of surface = gross		x	x	x	
Large cavities in cartilage			x		
Irregular fragmentation & loss of matrix		x	x	x	

Dog C. Group IV 2.5 x proposed use level for 30 consecutive days

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	yes	yes	yes	NVL
Surface roughening		x	x	x	
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bullae		x	x	x	
Deep erosion(s) and cartilage effacement					
Microscopic					
No significant microscopic lesion					x
Discrete areas of matrix loss	x	x	x	x	
Cartilage fibrillation		x	x	x	
Chondrocyte clusters (chondrones)			x	x	
Fissuring of matrix			x	x	
Microcavitations			x	x	
Intermediate (zone 2) cavitations			x	x	
Elevation of surface = gross			x	x	
Large cavities in cartilage			x		
Irregular fragmentation & loss of matrix			x	x	

Dog A. Group V 3 x proposed use level for 15 consecutive days

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	yes	yes	NVL
Surface roughening			x	x	
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bullae					
Deep erosion(s) and cartilage effacement			x	x	
Microscopic					
No significant microscopic lesion	x				x
Discrete areas of matrix loss		x	x	x	
Cartilage fibrillation		x	x	x	
Chondrocyte clusters (chondrones)			x	x	
Fissuring of matrix			x	x	
Microcavitations			x	x	
Intermediate (zone 2) cavitations			x	x	
Elevation of surface = gross			x	x	
Large cavities in cartilage					
Irregular fragmentation & loss of matrix			x	x	

Dog B. Group V 3 x proposed use level for 15 consecutive days

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	yes	yes	yes	NVL
Surface roughening			x	x	
1 or 2 vesicles and/or bulla		x			
More than 2 vesicles and/or bullae			x		
Deep erosion(s) and cartilage effacement			x	x	
Microscopic					
No significant microscopic lesion					x
Discrete areas of matrix loss	x	x	x	x	
Cartilage fibrillation		x	x	x	
Chondrocyte clusters (chondrones)			x	x	
Fissuring of matrix			x	x	
Microcavitations			x	x	
Intermediate (zone 2) cavitations			x	x	
Elevation of surface = gross			x	x	
Large cavities in cartilage			x		
Irregular fragmentation & loss of matrix			x	x	

Dog C. Group V 3 x proposed use level for 15 consecutive days

	coxofemoral		stifle		
	acetabulum	head	femur	tibia	patella
Gross lesion	NVL	NVL	yes	yes	NVL
Surface roughening			x	x	
1 or 2 vesicles and/or bulla					
More than 2 vesicles and/or bullae				x	
Deep erosion(s) and cartilage effacement				x	
Microscopic					
No significant microscopic lesion	x	x	x		x
Discrete areas of matrix loss				x	
Cartilage fibrillation				x	
Chondrocyte clusters (chondrones)				x	
Fissuring of matrix				x	
Microcavitations				x	
Intermediate (zone 2) cavitations					
Elevation of surface = gross				x	
Large cavities in cartilage					
Irregular fragmentation & loss of matrix					

APPENDIX B: Continuing *in vitro* Research on Fluoroquinolone-mediated Articular Changes

INTRODUCTION

In vitro degradation of cartilage has been induced by retinol and mononuclear cell factor.[1,2,3] Cartilagenous changes occur through stimulated degradative activities of chondrocyte-produced enzymes. Indirect degradation demonstrated by these experiments paralleled closely the described articular cartilage lesion pathogenesis from prolonged high level fluoroquinolone antibiotic administration in immature dogs. Based upon this similarity, *in vitro* studies using living and dead cartilage explants, was performed to see if degradative changes, produced indirectly, would occur in cartilage removed from all pressure and weight-bearing demands.

MATERIALS AND METHODS

Tissues:

Normal articular cartilage was obtained aseptically from the distal femurs of a 11 kg, 4 1/2-months-old, Chow cross pup.

Tissue Culture:

Slices of sterile articular cartilage, varying in size from 5-8 mm by 2-4 mm, and of full cartilage thickness, (range 2-3 mm) were placed individually, and at random, in a multiwell tissue culture plate (6-well, 35 x 14 mm. Falcon 3046: Becton Dickenson Labware, Oxnard, CA). There were 5 test groups, with two pieces

of cartilage per group. The pieces were placed in media with a pure fluoroquinolone antibiotic.

Group	Fluoroquinolone Concentrations
C	Control
A	.5 proposed usage level
B	1.5 x proposed usage level
D	2.5 x proposed usage level
DD	Killed cartilage, bathed in 2.5 x proposed usage level

The basic culture media consisted of: 100 ml Dulbecco's modified Eagle's medium (DMEM), 10 ml inactivated fetal calf serum, 12 mg/100 ml ascorbic acid, 1 ml .5g/100 ml gentamycin, .25 ml fungizone. The antibiotic calculations were based on a 10 kg dog having 7.1 water and 4 ml media/well. The two dead cartilage explants were thrice frozen and thawed in liquid nitrogen.

The tissue plates were placed in a moist chamber, gassed with a mixture of 20% O_2, 75% N_2, and 5% CO_2, and incubated at 35 C. Media and antibiotic solutions were changed twice, at every fourth day. On the eighth day of culture, the cartilage pieces were prepared for transmission electron microscopy and routine histology. The media which had bathed the explants and been drawn off twice, was frozen for future proteoglycan content analysis.

RESULTS

Tissue examination under light microscopy using H&E stain:

Group	Matrix	Chondrocytes	Clefting
C	Strong, sharp & eosinophilic. Even cellular distribution	Round & darkly nucleated	None
A	Pale, pink & hypocellular zone 2. Few empty lacunae	Cytoplasmic vacuolization. Some stained eosinophilic	Present
B*	Pale, pink & hypocellular. Some empty lucunae	Zones 1 & 2 have pyknotic, dark nuclei & cytoplasmic vacuolization	Present
D	Pale, pink & hypocellular. Empty lacunae	Eosinophilic with small pyknotic nuclei, or eosinophilic nuclei	Present
DD	Eosinophilic, well-defined empty lucunae	Shrunken, pyknotic chondrocytes	Present

*One sample became contaminated by fungal growth.

In groups A, B and D, diagonal to horizontal clefting was associated wit a pale-staining, hypocellular matrix, composed of prominent collagen fibers and eosinophilic chondrocytes, often containing dark, pyknotic nuclei. Ocassionally, a pale, basophilic, acellular substance lined some clefts.

Preliminary examinations by electron microscopy indicated chondrocytes in groups A, B, and D, had undergone marked cytoplasmic vacuolization. The extent of the changes appeared to be dose related, with group A chondrocytes less affected than those in group D. Further results are pending.

REFERENCES

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Fig 1 - Photomicrograph of chondrocyte from the control cartilage explant x 5,000.

Fig 2 - Higher magnification photomicrograph of the cytoplasmic organelles in Fig 1. Note well-defined mitochondria, lysosomes and Golgi apparatus x 20,000.

Fig 3 - Photomicrograph of a chondrocyte bathed in media containing .5 times proposed usage level of fluoroquinolone. Note vesiculated cytoplasm x 15,000.

Fig 4 - Photomicrograph of a chondrocyte exposed to 1.5 times proposed usage level of fluoroquinolone. Note "softened" outline and vesiculated cytoplasm containing electron-dense, amorphous material x 20,000.

Fig 5 - Photomicrograph of a chondrocyte bathed in media with 2.5 times proposed usage level. Membrane outlines are indistinct and multifocal to coalescing electron-dense amorphous material fills the vesiculated cytoplasm x 5,000.

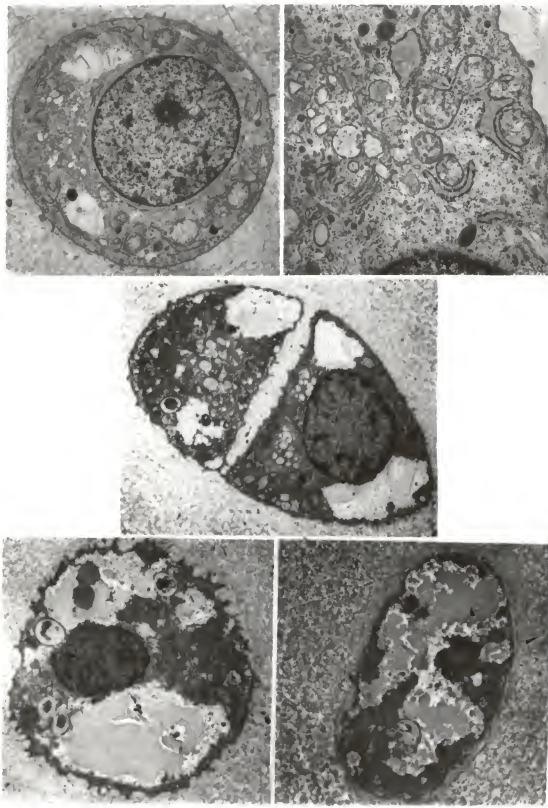

Fig 6 - Photomicrograph of a chondrocyte exposed to 2.5 times proposed fluoroquinolone usage level. Note the vesiculated cytoplasm x 5,000.

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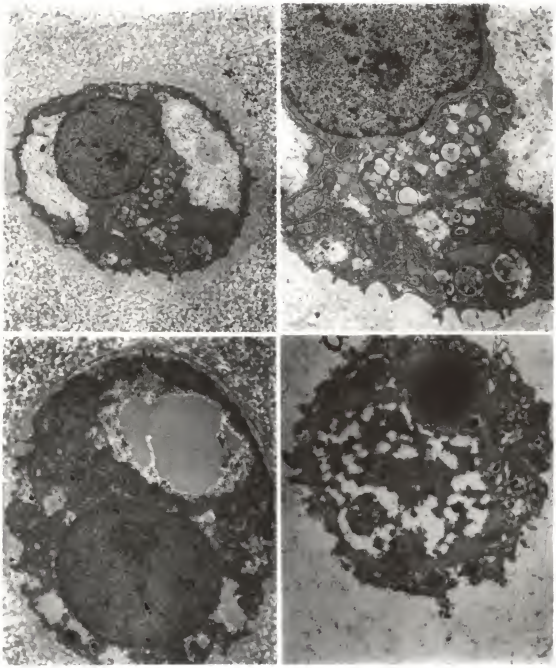

PATHOLOGICAL STUDY OF FLUOROQUINOLONE-MEDIATED CHONDROARTHROPATHY IN JUVENILE DOGS

by

LINDA JOSEPHINE KASSEBAUM

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AN ABSTRACT OF A THESIS

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Department of Pathology

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Two groups/of 15 dogs each, were histopathologically evaluated following 30-day pharmaceutical trials. In group 1, 5-7-months-old pups were orally treated either at the proposed usage level, or 3 or 5 times that dosage, for a period amounting to 3 times the proposed treatment duration. In the second group, 3-4-months-old puppies received daily oral doses .5, 1.5, 2.5, or 3 times the proposed usage level, for 30 days, or 3 times the proposed treatment duration.

Grossly, typical coxofermoral and stifle lesions found in groups 1 and 2 pups, which received the high dosages, included roughening, "blistering", or deep erosion of the articular joint surface. The vesicular lesions were characterized by a smooth, thin, cartilagenous surface, which had frequently torn to, reveal focal cartilage effacement. Accompanying microscopic changes were typified by proteoglycan loss, best illustrated by Alcian blue staining, progressing to collagen fibrillation, chondrocyte clusters, clefts within zone 2, and vesicular formation. Notably, group 1 puppies developed ulcerative lesions of the patella, which were not observed in the younger, group 2 pups.

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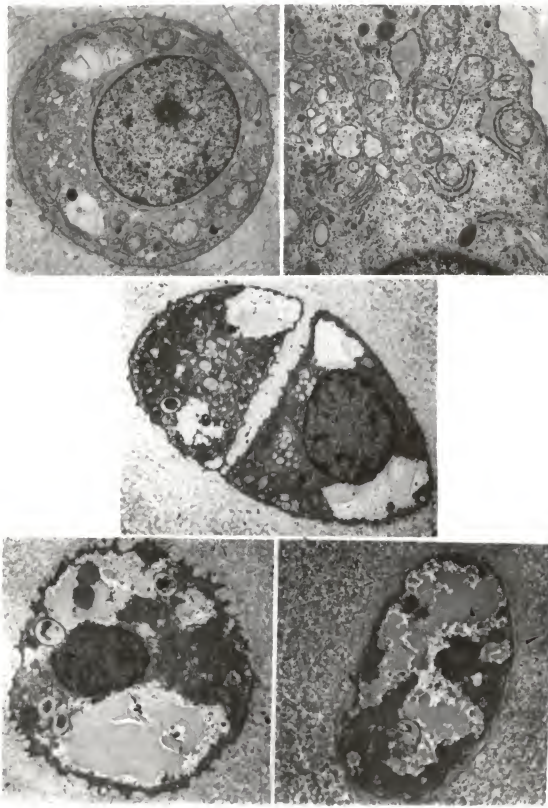

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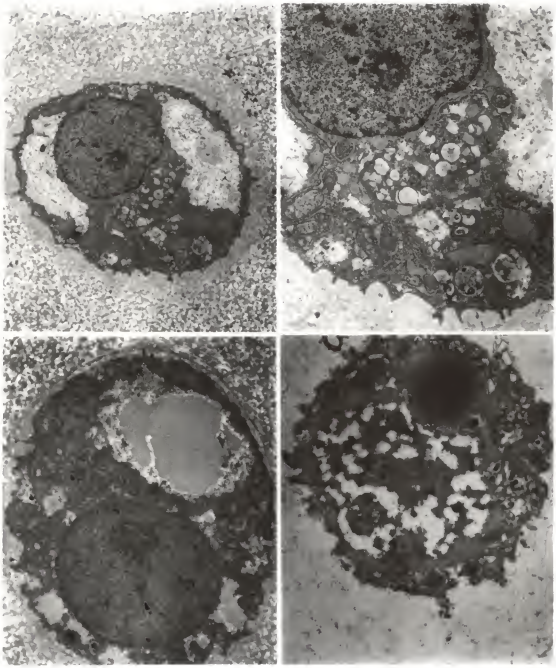

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