

Gross and Microscopic Pathologic Changes in Cranial Cruciate Ligament Deficient Dogs: Effectiveness of an Enzyme Inhibitor of Cartilage Breakdown

Summary: This study compared the gross and microscopic changes that occur in dogs that underwent cranial cruciate ligament transection (as a model for osteoarthritis) after administration of a synthetic oral MMP inhibitor compared to a placebo. Overall, the results of this study did not demonstrate significant gross or microscopic differences in overall cartilage degeneration, osteophyte formation, or meniscal damage post CCL transection in dogs administered a synthetic MMP inhibitor compared to those administered a placebo. This suggests that the synthetic MMP inhibitor administered in this study is not protective of cartilage degeneration that occurs with osteoarthritis development in this model.

Osteoarthritis (OA) is a major cause of morbidity and expense for all species. OA is usually initiated by a traumatic incident that may lead to instability of the joint creating inflammation within the joint. This leads to upregulation and activation of degradative enzymes, called matrix metalloproteinases (MMPs), as well as related enzymes (ADAMTS's) called aggrecanases. These enzymes have the capability of degrading articular cartilage, ultimately leading to breakdown of the joint. Research has been conducted for many years trying to control the progression of the disease as well as the morbidity it causes. A novel therapeutic approach to the treatment of OA is the use of a synthetic MMP inhibitor. These inhibitors bind to the MMPs so that they are unable to degrade the cartilage. Therefore, this therapy should reduce the degree of cartilage degeneration that develops over time, improving the patient morbidity. Many different animal models of OA have been used to mimic the early stages of OA in humans and dogs, in an attempt to be able to test different treatment methods. One of the most common models used is the cranial cruciate ligament (CCL) transection model in the dog. Transection of the CCL in the dog causes instability, leading to radiographic evidence of arthritis and ultimately results in damage to the articular cartilage. Therefore, Dr. Troy Trumble, along with Drs. A. M. Bendele, Clark Billingham and Wayne McIlwraith, compared the gross and microscopic changes that occur in dogs that underwent cranial cruciate ligament transection after administration of a synthetic oral MMP inhibitor compared to a placebo.

Forty mature male Walker Hounds free of orthopedic disease had their right CCL transected arthroscopically. Twenty dogs were randomly assigned to a control group and administered an oral placebo and 20 dogs were administered the oral MMP inhibitor (treatment group) daily throughout

the study, starting at 14 days post-transection. The dogs were exercised for 30 minutes a day, 5 days a week, starting two days post-operatively to insure consistent exercise for the induction of OA. All dogs were humanely euthanized at 126 days post CCL transection. The care and housing of the dogs was approved and in direct compliance with the Animal Care and Use Committee of Colorado State University.

Immediately after sacrifice, the right and left stifles of all dogs were opened and the lesions were described, measured, and photographed by a board certified pathologist (AMB). All of the gross and microscopic evaluations were done without knowledge of treatment group status. The evaluations were divided into two basic compartments: tibial plateaus and femoral condyles. For each compartment, gross pathology was subjectively graded for cartilage degeneration and osteophyte formation. The degree of medial and lateral meniscal damage was also graded. The degree of cartilage damage was subjectively graded and summed for each compartment using a scale of 0-4 (0 = normal, and 4 = severe deep lesions). In addition, the cartilage degeneration was also objectively measured (length x width x depth). Meniscal damage was graded from 0-3 (0 = none, 3 = severe fraying without significant repair), and osteophyte formation was also graded from 0-3 (0 = none, 3 = large osteophyte present). After gross examination, the tissues were fixed in formalin and decalcified for later microscopic examination. Each compartment was divided into three sections (cranial, middle, and caudal). Cartilage depth and severity of the microscopic lesions were graded from 0-5 (0 = normal, 5 = extends the entire depth of the cartilage) for each section. Osteophytes were again graded from 0-3, based on size (μm). The menisci were not examined microscopically. Upon completion of the analyses, the treatment group designations were revealed for

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data comparison. The mean \pm standard error (SEM) for each graded parameter for each compartment was determined by summing the values for each animal, arriving at a total tibial, total femoral and total joint score for each animal. The mean \pm SEM for each score was determined for the dogs in each treatment group and compared using the student's t-test with significance set at $p \leq 0.05$.

Gross and microscopic pathologic findings were similar. All but one dog had complete transection of the cranial cruciate ligament. Data from this animal was excluded from further analyses. Most dogs had moderate to severe thickening and fibrosis of the synovium and joint capsule. Meniscal damage was greatest medially, with the lateral menisci having minimal to no damage present. Damage ranged from mild fraying to near complete destruction, but with no difference between treatment groups for degree of changes or location of changes.

Osteophytes were largest and most numerous in the patellar groove, but they were also commonly present on the back and medial aspect of the medial tibial plateau, and edge of the lateral tibial plateau. In addition, osteophytes were common on the inside of the lateral femoral condyle, which was often associated with bone resorption of the non weight-bearing area of the lateral femoral condyle. There was no difference between the location and degree of osteophyte formation between treatment groups.

Cartilage lesions on the femoral condyles and tibial plateaus were in predictable locations when present. There was a common lesion on the non weight-bearing junction between the medial femoral

condyle and the patellar groove that had focal, small but deep lesions. This tended to coincide with the non weight-bearing cranial region of the medial tibial plateau, which tended to have severe degenerative changes present (Figure 1). When present medially, this also tended to be present laterally, but to a lesser degree. Subjective and objective cartilage degradation scores were highest for this cranial medial portion of the tibia (Figure 2), but there was no difference between treatment groups.

The central weight-bearing regions of the medial femoral condyle were at risk for development of larger lesions than those described on the non weight-bearing regions. The severity ranged from diffuse superficial roughening to severe extensive cartilage damage. The lateral femoral condyles had lesions in similar locations, but were always less severe. The central weight-bearing regions of the medial and lateral tibial plateaus not protected by the menisci had superficial areas of cartilage degeneration in operated and normal stifles. No differences were present between treatment groups in either the femoral condyles or tibial plateaus.

Expressing the data as a sum of calculated gross scores for the tibia or femur indicated that there was greater cartilage damage present on the tibial plateaus than on the femur. Again, however, there was no difference between treatment groups. Adding the tibial and femoral scores together to produce a total joint score also yields no difference between the treatment groups.

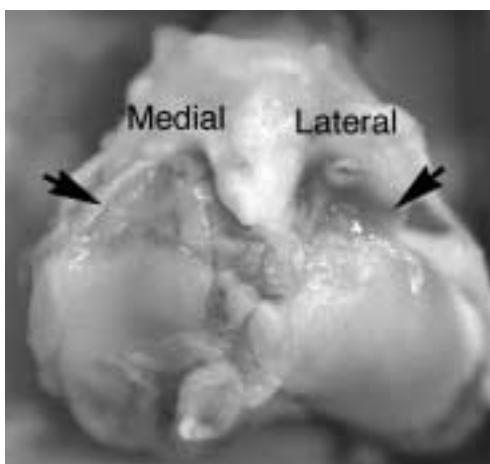
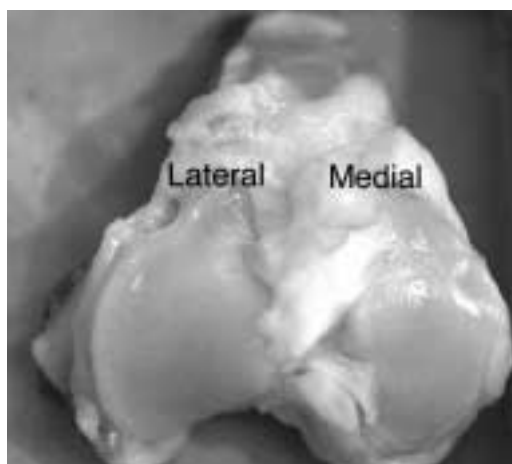


Figure 1: Disarticulated tibial plateau from a normal left non-operated stifle (left) and one from a CCL transected stifle (right). Medial and lateral for each stifle is labeled accordingly. Notice the severe articular cartilage degeneration in the cranial medial and lateral region (black arrows) in the operated stifle compared to the normal stifle.

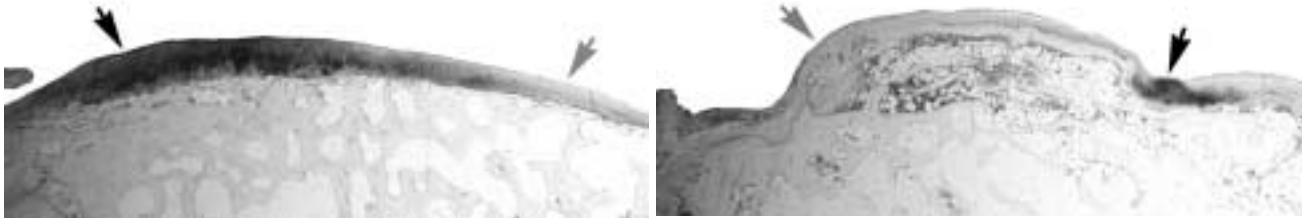


Figure 2: Microscopic section of the cranial section of the medial tibial plateau from the normal left unoperated stifle (left), and the CCL transected stifle (right) depicted in figure 1. The section on the left shows the normal appearance of this region, with thicker more cartilaginous areas (black arrow) and thinner more fibrous areas (gray arrow). The section from the operated limb on the right depicts a highly irregular surface with fibrous areas (grey arrow) as well as residual fibrocartilage areas (black arrow).

The gross and microscopic subjective and objective pathological scores were similar between the dogs treated with the MMP inhibitor and the control dogs, no matter what compartment was being examined. This is also similar for osteophyte formation, suggesting a consistent variability in this model, regardless of treatment effect. However, in general, the lesions present were highly variable between dogs with respect to the incidence, severity, and type, especially when comparing meniscal damage. The lack of treatment effect on the degree of meniscal damage is likely reflective of individual animal load bearing and activity response to the instability created by surgical transection of the CCL. This may also partially explain why most cartilage damage was present on traditionally non weight-bearing surfaces (cranial surface of the medial tibia). In other words, transection of the CCL may change the load bearing so that the front of the medial tibial plateau and the front surface of the medial femoral condyle become more involved in

load bearing and are therefore subjected to mechanical forces that would not be present in the normal CCL intact dog.

Overall, the results of this study did not demonstrate significant gross or microscopic differences in overall cartilage degeneration, osteophyte formation, or meniscal damage post CCL transection in dogs administered a synthetic MMP inhibitor compared to those administered a placebo. This suggests that the synthetic MMP inhibitor administered in this study is not protective of cartilage degeneration that occurs with osteoarthritis development in this model. At the same time, the severity of the changes associated with this model calls into question its appropriateness in assessing a disease modifying agent.

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