


Oral Administration of a Chemically Modified Curcumin, TRB-N0224, Reduced Inflammatory Cytokines and Cartilage Erosion in a Rabbit ACL Transection Injury Model

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Abstract

Objective. To evaluate the effects of TRB-N0224, a chemically modified curcumin (CMC) with zinc binding properties and improved pharmacokinetics, in a rabbit anterior cruciate ligament (ACL) transection injury–induced model of osteoarthritis (OA). **Design.** Thirty-eight skeletally mature New Zealand white rabbits were studied in 4 groups: a sham with arthrotomy ($n = 6$), control with ACL transection ($n = 6$), and 2 treatment groups with ACL transection and administration of TRB-N0224 at low (25 mg/kg/day) ($n = 13$) and high (50 mg/kg/day) ($n = 13$) doses. After euthanization at 12 weeks, outcomes were measured by post-necropsy gross morphology, biomechanics, and cartilage and synovium histology. Rabbit blood ELISA quantified cytokine and matrix metalloproteinase (MMP) concentrations at 0, 4, 8, and 12 weeks. **Results.** Both treatment doses had fewer distal femoral condyle erosive defects than the control; the low dose demonstrated a mean 78% decrease ($P < 0.01$). Histologically, the low- and high-dose treatment groups had fewer cartilage pathologic changes and less severe synovitis than the control. CMC alone did not have a major effect on the biomechanics of healthy cartilage or cartilage in the ACL transection model, as demonstrated in 5 of the 6 measured properties/regions ($P < 0.05$). ELISA results suggested that the key mediators of OA, (interleukin) IL-1 β , IL-6, TNF α (tumor necrosis factor- α), MMP-9, and MMP-13, had decreased concentrations with TRB-N0224 treatment at different time points between weeks 4 to 12 ($P < 0.05$). **Conclusions.** In the pathogenesis of OA, an imbalance exists between catabolic and anabolic mediators. These results suggest the potential of TRB-N0224 to modulate MMP and cytokine levels, slowing the macroscopic and histopathological progression of OA.

Keywords

osteoarthritis, chemically modified curcumin, MMP inhibitors, inflammation, cytokines, DMOAD

Introduction

The pathophysiology of osteoarthritis (OA) involves the dysregulated production of inflammatory mediators IL-1 β (interleukin), IL-6, and TNF α (tumor necrosis factor- α) by activated chondrocytes, synoviocytes, and mononuclear cells.^{1,2} IL-1 β stimulates the activation of matrix metalloproteinases (MMPs), suppresses synthesis of type II collagen and aggrecan (aggregating proteoglycan), and induces chondrocyte apoptosis.^{3,4} IL-6 has a similar role, activating MMPs and reducing type II collagen expression.⁵ TNF α plays an additive part by inducing production of IL-6, while also stimulating the activation of MMPs and suppressing type II collagen and aggrecan synthesis.^{4,6} MMPs are zinc-dependent enzymes that degrade aggrecan and collagen, the principle components of articular cartilage, by enzymatic cleavage.¹ Inducible, unbalanced MMPs can reach

pathological levels leading to chondrocyte apoptosis and plays a central role in OA.^{7,8}

TRB-N0224 is a chemically modified curcumin (CMC) that combined the known effects of tetracyclines and curcumin, a component of turmeric, on inflammatory mediators in OA. Tetracyclines inhibit MMPs by binding zinc in

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the enzyme's catalytic site.⁹ Periostat and Oracea are non-antimicrobial doses of doxycycline, approved for the treatment of periodontal disease and rosacea by inhibiting MMPs. However, the dosage of these drugs is limited by their side effects and the potential development of antibiotic resistant bacteria.¹⁰⁻¹² Curcumin has been shown to have anti-inflammatory properties through suppression of the NF κ B pathway.¹³ However, unmodified curcumin has poor solubility, rapid metabolism, and low biological activity resulting from the low plasma and tissue levels.^{14,15} To address these limitations, a 20% curcuminoid, compared to the natural 5% of turmeric, curcumin-phosphatidyl choline complex called Meriva, was developed.^{14,16} A study of 50 OA patients found Meriva improved patient reported outcomes (WOMAC scores) by 58%, decreased joint pain and improved function at 3 months and 8 months, and decreased IL-6 and IL-1 β plasma levels.¹⁷

TRB-N0224 (CMC) is a novel and proprietary phenyl amino carbonyl analog of curcumin with the zinc-binding features of tetracyclines. Curcumin was selected as a parent structure for 2 reasons: it has a 1,3-diketone moiety akin to tetracyclines and the inherent anti-inflammatory properties of curcumin itself. The pharmacokinetic properties of curcumin were improved by addition of an electron-withdrawing group that increased the acidic character of the enolic system. This significantly improved the solubility and decreased the metabolism rate of CMC with no observed toxicity to date. In our preliminary studies, CMC was shown to have pleiotropic anti-inflammatory effects, most likely as a MMP modulator and cytokine inhibitor. CMC was studied *in vitro* for chondroprotective effects against IL-1 β and Oncostatin M-induced chondrolysis in a bovine femoral condyle articular cartilage model by measuring ³⁵S sulfate release at 24, 48, and 72 hours.¹⁸ CMC demonstrated a chondroprotective effect, as evidenced by a lowered release of ³⁵S at all time points and improved histological outcomes.¹⁸

Hypothesis

Our aim was to evaluate the efficacy of CMC in an *in vivo* rabbit injury-induced model that closely resembled the human OA disease progression. We hypothesized that administration of CMC will lower tissue and serum levels of pro-inflammatory cytokines and MMPs, and that decreased levels of these inflammatory mediators will prevent OA progression macroscopically, histologically, and biomechanically.

Methods

Animals and Surgical Model

The Institutional Animal Care and Use Committee (IACUC) (Protocol #2014-013) approved this study. Thirty-eight

Table 1. Representation of the 4 Groups Studied.

Groups	n	ACL Transection	TRBN0224 (CMC)
Control	6	Yes	No
Sham	6	No	No
High dose	13	Yes	50 kg/mg/day
Low dose	13	Yes	25 kg/mg/day

ACL = anterior cruciate ligament; CMC = curcumin.

skeletally mature male New Zealand white rabbits (4.5 kg average weight) were randomized into 4 groups. These included one sham group with arthrotomy to anterior cruciate ligament (ACL) exposure without transection ($n = 6$) and 3 groups with ACL transection: a control without additional treatment ($n = 6$), a low-dose (25 mg/kg/day) CMC ($n = 13$) treatment group, and a high-dose (50 mg/kg/day) CMC ($n = 13$) treatment group (**Table 1**).

Rabbits were given a pre-anesthetic injection of ketamine (26 mg/kg), xylazine (0.78 mg/kg), and maintained on isoflurane (1.5% to 3%). Cephalotin (13 mg/kg) was given pre-surgery and post-surgery for 2 days at 12-hour intervals with butorphanol (0.1 to 0.5 mg/kg) at 4-hour intervals. The ACL was exposed through an aseptic, medial para-patellar arthrotomy with lateral patellar dislocation^{19,20} and transected approximately mid-ACL with a number-11 scalpel blade. The sham operation exposed the ACL identically to transection groups, but without ligament transection. The joint capsule and skin was closed in layers with 4-0 vicryl resorbable sutures. No significant complications occurred during surgery or post-surgery. CMC was administered systemically by daily oral gavage at 2 doses (25 or 50 mg/kg); the control and sham rabbits did not receive daily oral gavage. Rabbits were euthanized 12 weeks post-surgery.

Macroscopic Examination

The rabbit distal femoral condyles and tibial plateaus were exposed and photographed post necropsy. The articular surfaces were analyzed for osteophyte formation, erosions, and other abnormalities of the total condyle surface area. The program Image J from the National Institutes of Health was used to quantify the percentage of surface area containing erosions.

Biomechanical Testing

For biomechanics, rabbit condyles were thawed at room temperature and were kept hydrated in a phosphate-buffered saline bath during testing. The femur epiphyses were cut flat with a band saw, and the cut surface was mounted to a custom sample holder using cyanoacrylate glue. Positioning of the cartilage surface was adjusted to achieve perpendicular contact between indenter and the

cartilage surface using a ball and socket tilt stage mounted on top of an x,y -micromanipulator linear stage (Newport). Indentation testing was performed at the central part of both the lateral and medial femoral condyles on regions of intact cartilage, to ensure full contact with the indenter. Indentation tests were performed using a spherical stainless steel indenter (1.5 mm radius) attached to a material test frame (Instron 5566) through a custom holder, and loads were measured with a 10 N load cell. After establishing contact with the articular surface, an instantaneous step indentation of 100 μm was applied, and the resulting load was measured until equilibrium. Force and displacement data were analyzed using Hertzian biphasic theory (HBT), adapted from approach by Moore *et al.*, to simultaneously quantify cartilage contact (compressive) modulus (E_Y^-), tensile modulus (E_Y^+), and permeability (k).²¹ Tissue thickness was then measured at the site of indentation using a needle indenter. Tissue thickness was determined from the force versus needle distance curve, which has sharp peak corresponding to the subchondral bone as the needle indenter is pushed through the cartilage layer. The indentation and thickness measurement was performed on the medial and lateral femoral condyles, while randomizing the order of testing. Analysis of the contralateral knees was also performed to determine the effects of the systemic oral CMC administration (labeled “drug”) on the noninjured joints.

Histologic Analysis

Histological preparation followed ORS international guidelines.²² After the necropsy at 12 weeks, 2 samples from the control and sham groups as well as 5 samples from the high- and low-dose groups were used for cartilage histology. The whole knee specimen was fixed in 10% formalin and subsequently decalcified in 5% formic acid. Five-micrometer-thick sections of the tibial femoral condyle were stained with safranin-O/fast green. Magnification digital images (10 \times) were captured on an Olympus BX-60 microscope. The histological progression was graded in a blinded fashion according to the Mankin scale for OA, a 14-point score involving cellular changes, architectural changes, and presence of safranin-O matrix staining.²²⁻²⁴ One sample of synovium was taken from the control sham group, and 2 samples from the high- and low-dose groups for synovium histology. Each sample was also fixed and stained with hematoxylin-eosin. The synovium was graded in a blinded fashion for severity of synovitis, taking into account lining cell layer hyperplasia, resident cellular density, and leukocyte infiltrate.^{25,26}

ELISA

Rabbit blood was collected and analyzed by the RayBio Rabbit ELISA Kit for Cell Culture Supernatants, Plasma, and Serum samples. The analytes quantified using ELISA were IL-1 β , IL-6, TNF α , MMP-9, and MMP-13. Blood was

drawn at 0, 4, 8, and 12 weeks; week 0 was immediately prior to surgery. Whole rabbit blood was collected from each animal into a BD vacutainer without additives, kept at room temperature for approximately 20 minutes, and then centrifuged for 10 minutes at 3,000 rpm. Aliquots of the serum were either used immediately or stored at -80°C and brought to room temperature (18-25 $^\circ\text{C}$) along with the reagents before use. The serum samples were diluted 2-fold and 5-fold, and were run in duplicate. All reagents, samples, and standards were prepared as per manufacturer’s instructions. Briefly, 100 μL standard or sample was added to each well and incubated for 2.5 hours at room temperature. One hundred microliters of prepared biotin antibody was then added and the samples incubated an additional hour at room temperature. Next, 100 μL of prepared streptavidin solution was added and the samples incubated for another 45 minutes at room temperature. Last, 100 μL TMB One-Step Substrate Reagent was added and the samples incubated for 30 minutes at room temperature. Fifty microliters of Stop Solution was then added to each well and absorbance measured at 450 nm immediately by spectrophotometry.

Statistical Analysis

For the macroscopic results, a one-way ANOVA was used to test for differences in osteophytes and erosive lesions between the 4 groups followed by the Bonferroni post hoc test. Significance was set at $P < 0.05$, and statistical analysis was performed in SPSS.

For biomechanics, we did not observe a dose response of CMC on measured properties; therefore, the low- and high-dose groups were pooled together. A 2-way ANOVA was performed to determine effect of injury (ACL transection) and effect of treatment (CMC) on mechanical properties and tissue thickness. Group comparisons were performed with using LSD post hoc test, with $P < 0.05$ considered significant. Statistical analysis was performed in Statistica.

Descriptive statistics alone, the mean and standard deviation, were compared for histological outcomes, as a result of the small sample size.

A 2-way repeated-measures ANOVA with estimated marginal means was employed to evaluate the effect of CMC and time on cytokine and MMP inhibitor concentrations between the treatment groups. A Bonferroni multiple comparisons post hoc analysis was subsequently used to determine differences between groups with significance set at $P < 0.05$. Statistical analysis was performed in SPSS.

Results

Macroscopic Features of Osteoarthritis

Macroscopic measurements of OA progression included gross fibrillation, osteophyte formation, and bone eburnation (**Fig. 1**). The control group exhibited a significantly

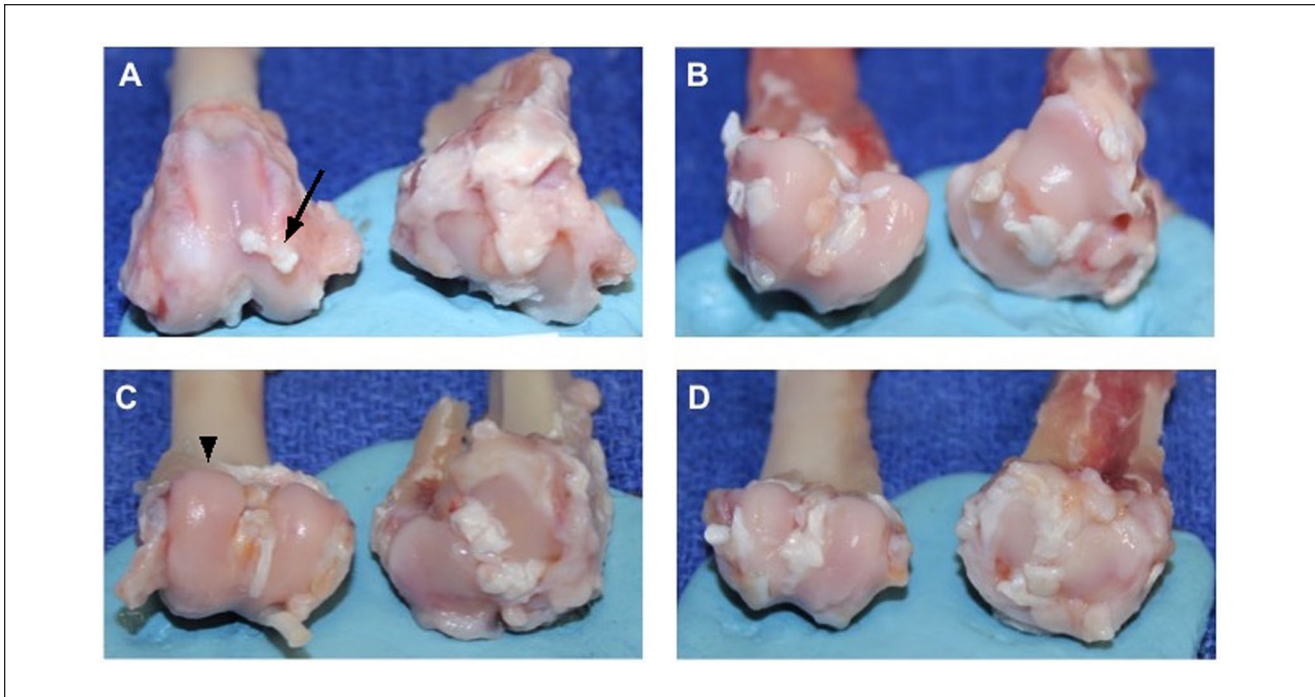


Figure 1. Exposed distal femoral condyles (left) and tibial plateau (right). **(A)** Control demonstrates cartilage damage, osteophytes (arrow), and eburnated bone on medial tibial plateau. **(B)** The sham cartilage appears smooth and glistening (arrow head) with no fibrillations on both articular surfaces compared to control. **(C)** The low-dose and **(D)** high-dose treatment groups exhibit fewer focal areas of fibrillation and osteophytes than control.

higher percentage of surface erosions on the distal femoral condyle as compared to the sham and low-dose groups (**Fig. 1A** vs. **B, C**, respectively). The high-dose group exhibited a similar trend to the low-dose group (**Fig. 1D** vs. **C**). After 12 weeks, the low-dose group had a mean of 77.8% fewer articular cartilage erosive lesions on both the femoral condyle and tibial plateau than the control. The articular surfaces in the high- and low-dose treatment groups specifically exhibited fewer osteophytes than the control group, but this finding did not reach statistical significance (**Fig. 2**).

Altered Cartilage Biomechanics

The HBT analysis yielded high-quality fits of the measured biomechanical data ($R^2 = 0.85$ to 0.9). The cartilage thickness was found to be similar across all groups in both the lateral and medial femoral condyles, with a mean thickness of 0.76 ± 0.33 mm and 0.91 ± 0.26 mm in sham groups, respectively (**Fig. 3**). In evaluating E_y^+ , a significant effect of ACL injury ($P = 0.007$) and treatment ($P = 0.017$) was observed in the lateral compartment, but not the medial femoral condyle. E_y^+ was significantly lower in the control lateral condyle (of the joints that received ACL transection) versus sham group. Oral administration of CMC reduced lateral E_y^+ compared to sham, even in

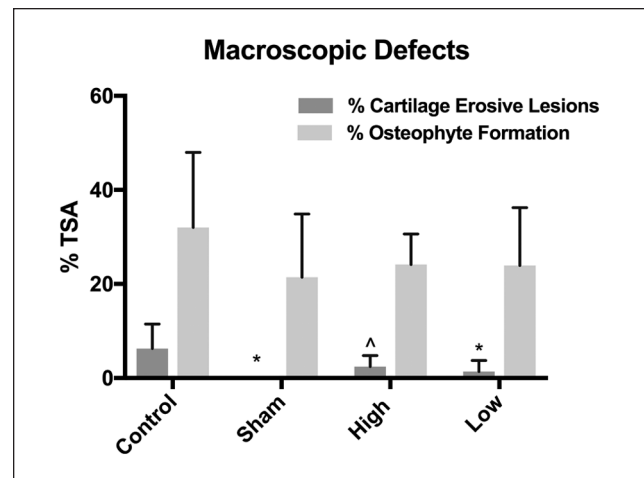


Figure 2. Differences in cartilage erosive lesions and osteophyte formation between groups. Each data set is presented normalized (percentage) relative to the total surface area (TSA) of the distal femoral condyle. *Signifies $P < 0.01$ versus control group, and ^ indicates $P = 0.0595$ (trend).

the presence of ACL transection (**Fig. 4A**). In the medial compartment, E_y^+ in ACLT+CMC (TRB-N0224) treated groups was lower than control (ACLT only) group (**Fig. 4B**). In evaluating E_y^- , a significant effect of ACL injury

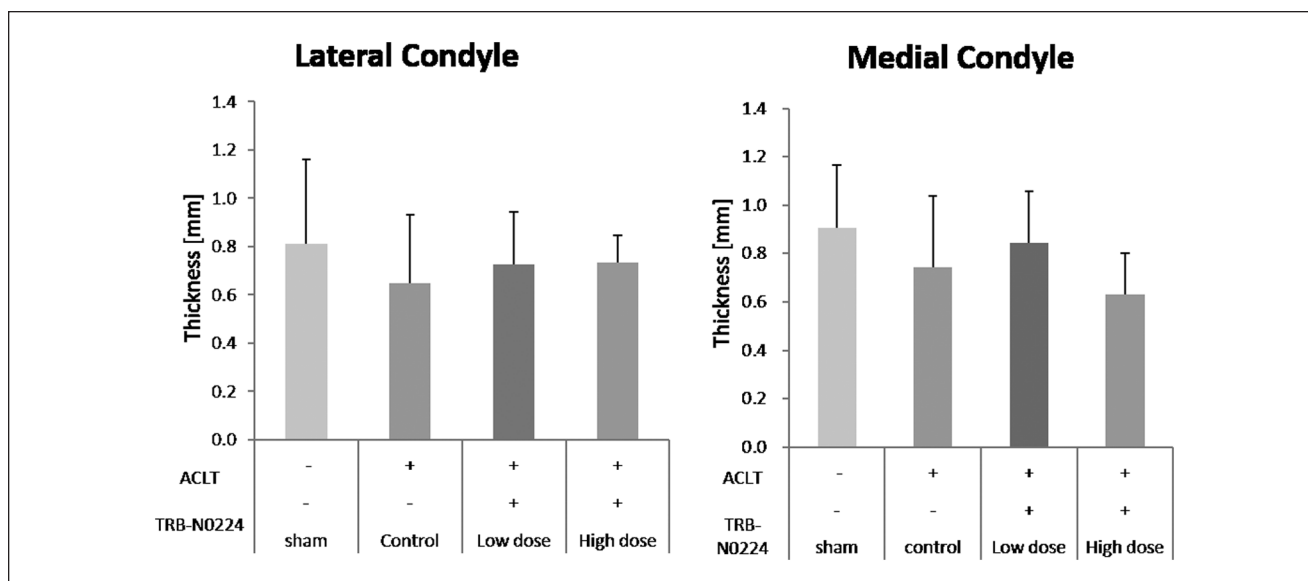


Figure 3. Cartilage tissue thickness in lateral and medial femoral condyles at site of indentation. No significant effect of surgery or treatment administration was observed on cartilage thickness.

was observed in the lateral ($P = 0.0016$) and medial ($P = 0.038$) condyles. In the lateral condyle, administration of CMC resulted in significantly higher E^- compared to control (Fig. 4c). In evaluating permeability (k), group comparisons indicated that ACLT + CMC groups had higher permeability than drug administration alone (Fig. 4E and F).

Histopathologic Features

Control synovium samples had a higher-grade synovitis than the high- or low-dose treatment groups. The control group exhibited a mean score of 3, while the high- and low-dose groups resulted in mean scores of 2 and 1.50, respectively. The histopathological changes seen in the control specimens included moderate reactive synovial hyperplasia of the cell lining layer and an increase in density of the resident cells (Fig. 5A), as compared to a mild increase in cell lining layer and local inflammatory cell density in the low- and high-dose groups (Fig. 5B and C).

The articular cartilage histopathology showed similar results. The Mankin scale for histopathological grading found lower mean scores in the low (2.60 ± 0.548) and high (1.25 ± 1.258) dose treatment groups as compared to the control (3.5 ± 0.707). The control group exhibited a greater number of features of OA progression, including fissuring at the articular surface extending down to the subchondral plate, decreased safranin-O staining indicative of decreased aggrecan concentration, and other structural abnormalities (Fig. 6). The sham group did not exhibit these histopathological features of OA (0 ± 0.0).

The Effect of CMC on Cytokines and Matrix Metalloproteinases

At week 0, no significant inflammatory mediator concentration differences existed between groups. At week 4, MMP-13, IL-6, and IL-1 β showed significantly decreased and trends of decreased concentrations in the sham, high-, and low-dose groups as compared to the control (Fig. 7). The concentration of IL-6 from weeks 4 to 12 was lower in treatment groups than the control, with levels similar to the sham, although no difference existed between dosages. No statistically significant difference in any inflammatory mediator existed between the low- and high-dose groups at a single time point. In addition, both treatment groups and the sham group did not have significant differences in concentrations of any inflammatory mediators, except for increased levels of TNF α in the sham as compared to the treatment groups at week 4.

IL-1 β concentrations were decreased in treatment groups at weeks 4 and 8, but this finding lost significance at week 12. TNF α levels in the sham hardly varied between weeks. The control group also exhibited similar concentrations in week 4 as in week 12. At week 12, TNF α concentration in the treatment groups and sham was significantly less than the control.

MMP-9 had less consistent sham concentrations at week 4, but this resulted in significantly decreased MMP-9 in the sham and low-dose groups compared to the control at week 12. The MMP-9 concentration in the high-dose group was not significantly decreased from the control. The opposite occurred in MMP-13, where only the high-dose group had significantly decreased concentrations at weeks 4 and 12.

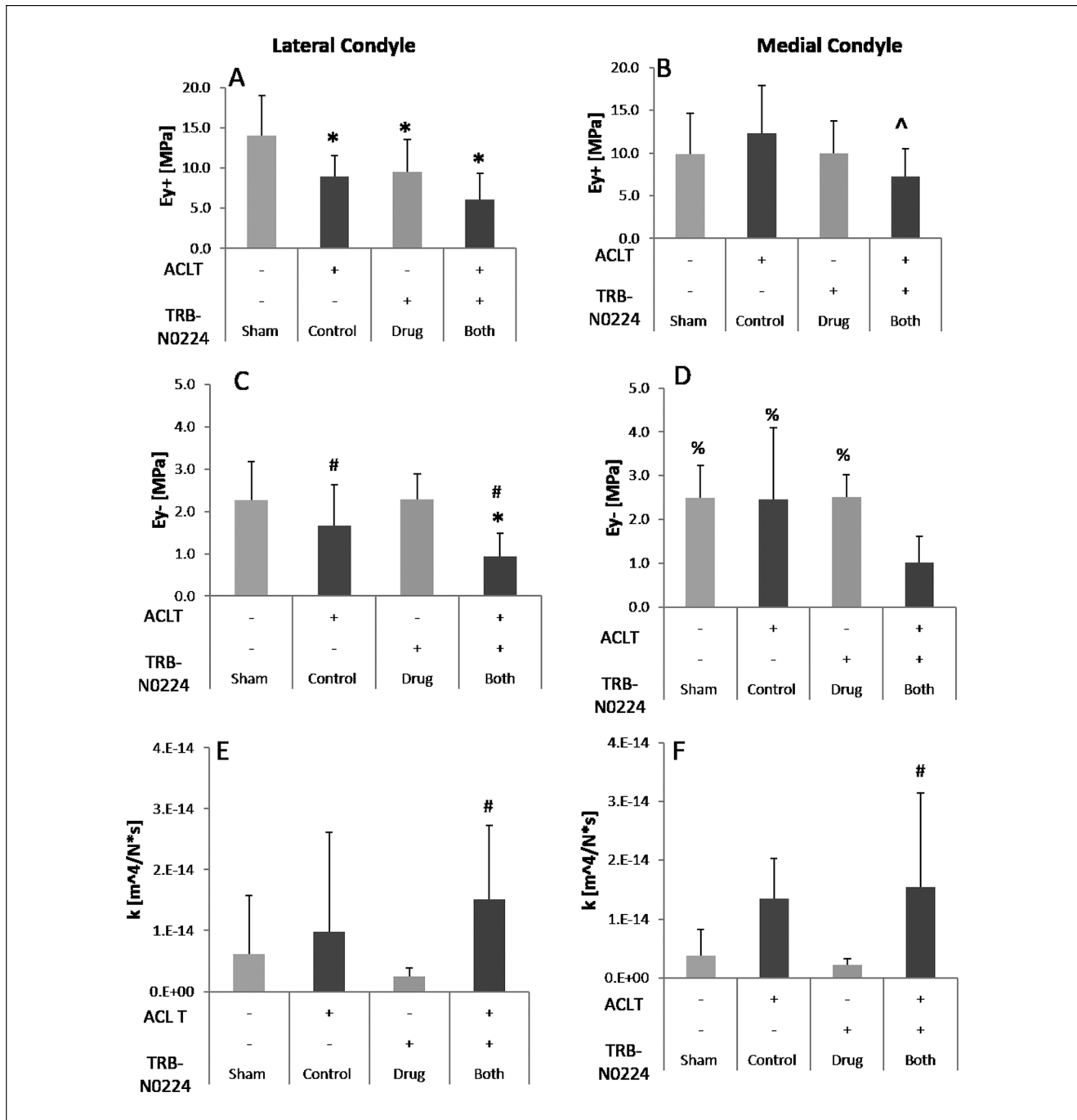


Figure 4. Indentation testing of the lateral (left) and medial (right) femoral condyle cartilage. (A, B) Tensile modulus (E_y^+); (C, D) Compressive modulus (E_y^-); (E, F) Permeability (k). Lateral condyle had more sensitivity to biomechanical changes compared to medial condyle. A significant effect of surgery was observed in lateral E_y^+ (A: $P < 0.006$), as well as lateral and medial E_y^- (C: $P < 0.002$; D: $P < 0.05$). A significant effect of treatment was observed in lateral E_y^+ (A: $P < 0.02$) and medial E_y^- (D: $P = 0.053$). *Denotes $P < 0.05$ when compared to “Sham”; # signifies $P < 0.05$ compared to “Drug”; ^ signifies $P < 0.05$ compared to “Control”; % signifies $P < 0.05$ compared to “Both.”

Discussion

Current treatment options for OA manage daily pain and inflammation without prevention of OA progression.

Innovative therapeutic options are needed to decrease symptom severity and protect articular cartilage from further damage.¹⁴ The results of this study suggest that CMC modulated pro-inflammatory cytokine and MMP concentrations

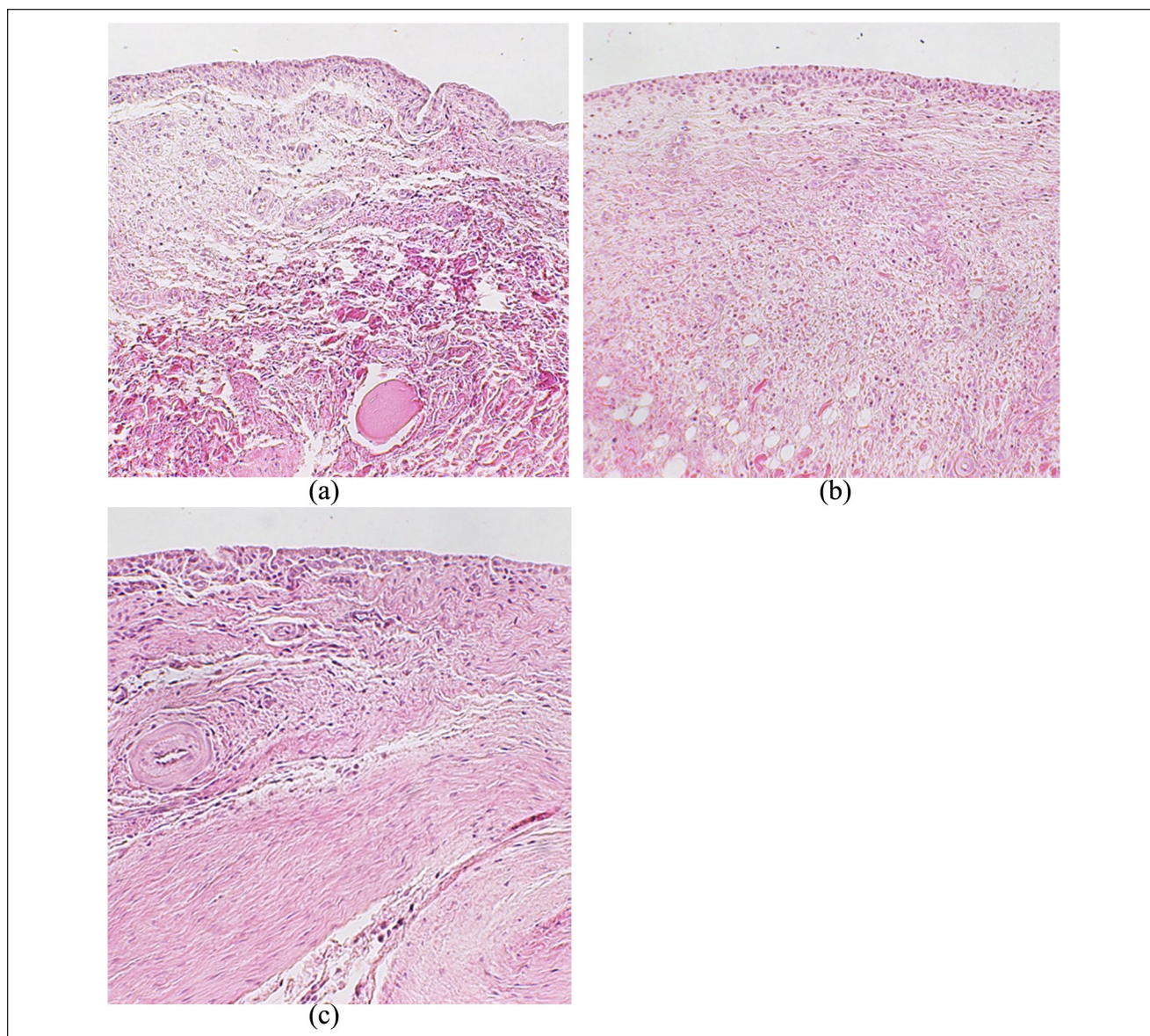


Figure 5. Synovium histopathological samples at 10 \times magnification. The control group (A) had enlargement of the synovial lining cell layer and increased density of resident cells. The low-dose group (B) demonstrated mild reactive synovial hyperplasia. The high-dose group exhibited the lowest grade synovitis, with a single cell lining layer to the right (C).

resulting in decreased articular cartilage erosive lesions and histopathological OA progression. The outcomes of this study are impactful because they demonstrate that CMC combined the positive effects of curcumin and tetracyclines, 2 innovative potential treatments for OA, to suppress the systemic expression of 5 inflammatory mediators of OA. Additionally, CMC was successful as an oral, systemic medication rather than local injection treatment. These findings support the notion that CMC offers a step toward a DMOAD that alters the natural progression of OA.

Curcumin has been shown to lower levels of IL-1 β ,^{27,28} IL-6,²⁹ TNF α ,²⁸ and MMP-9,³⁰ and MMP-13²⁸ likely through suppression of the NF κ B pathway.³⁰ For CMC, this quality

was enhanced by its zinc-binding capabilities. CMC decreased the concentrations of all 5 studied inflammatory mediators at variable time points between weeks 4, 8, or 12, and at no point did the concentration increase relative to the control. This result is highly indicative of CMC exhibiting an inhibitory effect on inflammation. The lack of significant differences between the treatment and sham groups suggests CMC lowered concentrations to levels seen in uninjured rabbits. The close relationship of the high- and low-dose groups implies that increasing, or nearly doubling, the dosage had little effect on CMC's modulation capabilities. We believe this is due to the increased bioavailability offered by the modified drug. It is of interest that the

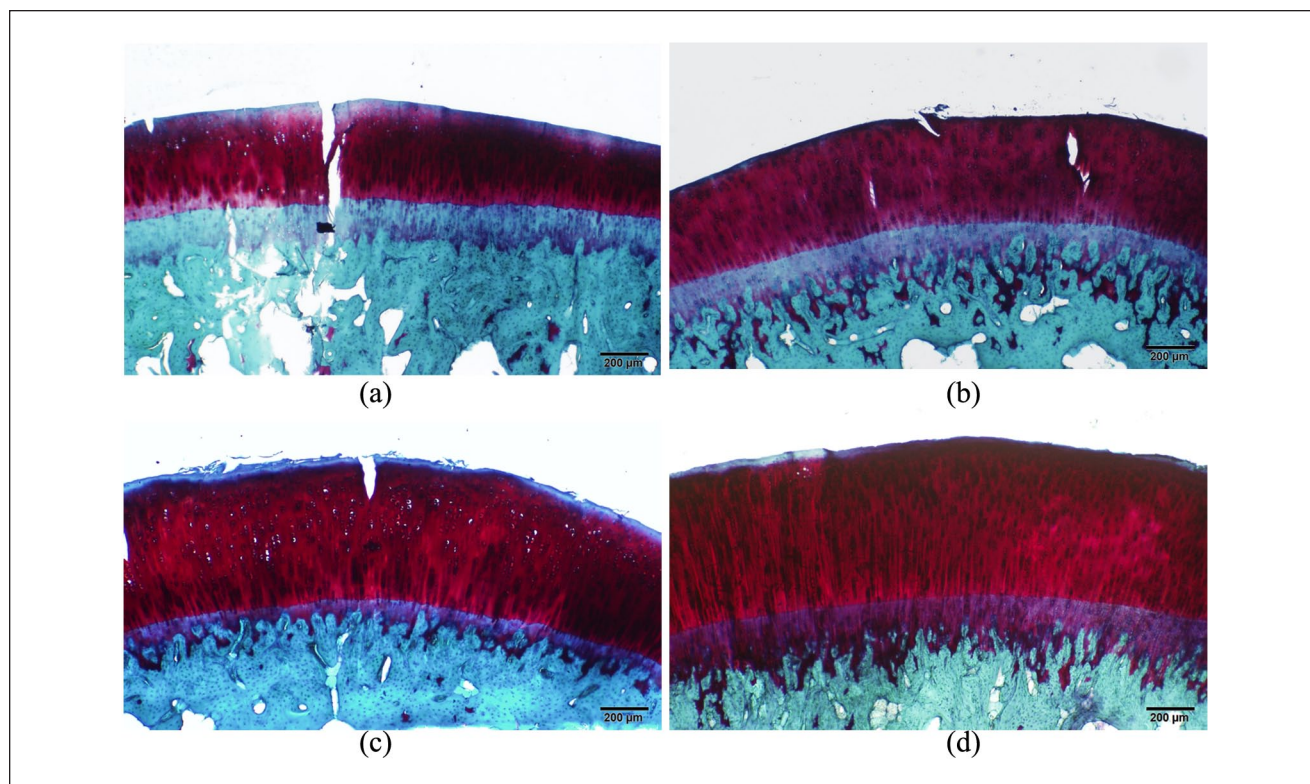


Figure 6. Histopathological images of the tibial femoral condyle cartilage: The control (A) demonstrated fissuring and reduction of safranin-O staining. The sham (B) and high-dose (D) groups were nearly indistinguishable, representing normal articular cartilage. The low-dose group (C) exhibited mild fissuring.

cytokine concentrations in the sham group also increased post arthrotomy without ACL transection. This could be attributed to increase in inflammatory mediators from arthrotomy alone. However, this increase in cytokines did not translate to significant macroscopic or histologic fractures of OA, mostly likely because the cytokines were at significantly lower levels than the other 3 groups. Perhaps more global histopathology would reveal pathologic features. High doses of curcumin have been shown to induce generation of reactive oxygen species, which can lead to myocardial tissue damage.³¹ This is concerning for having a counterproductive effect, and emphasizes the importance of utilizing the lowest possible effective dose. Although neither dose was associated with toxicities, the lowest effective dose of CMC is preferable to prevent potential adverse effects.

The results of the biomechanical testing did not correlate with the other measured parameters. This could be attributed to the constraints of testing at a single time point and from one location of the femoral condyle that was suitably covered with intact articular cartilage required for testing. The presence of surface erosion is an obstacle for indentation testing, because full contact between the indenter and the cartilage surface is required, and therefore testing could not be performed in eroded tissue regions. Nevertheless, the

ACL transection was found to diminish the tensile modulus of cartilage, and induced a higher cartilage permeability. However, the compressive modulus was less sensitive to the transection injury at the 12-week time point. Analysis of the contralateral limbs indicates that administration of CMC alone did not have a major effect on the biomechanics of healthy cartilage or the ACL transected joints, as demonstrated in 5 of the 6 measured properties/regions. Results show that tensile modulus was most sensitive to injury and treatment, which is consistent with the qualitative histological changes in collagen deposition observed in the study (Fig. 6). This is consistent with quantitative results of several other studies have found that administering curcumin increased collagen deposition.^{32,33} Previous studies also show that greater alterations in the collagen orientation angle occurs with ACLT and such alterations extend deeper into the cartilage layer, which may be contributing to the measured altered biomechanics.³⁴ Interestingly, the HBT indentation approach showed more sensitivity to detecting differences in cartilage permeability than previously reported.³⁵

An important novelty of our study is that as an oral agent CMC had systemic effects and modulated the disease progression in the articular cartilage and synovium. While the sensitivity of blood cytokine measurement for detection of

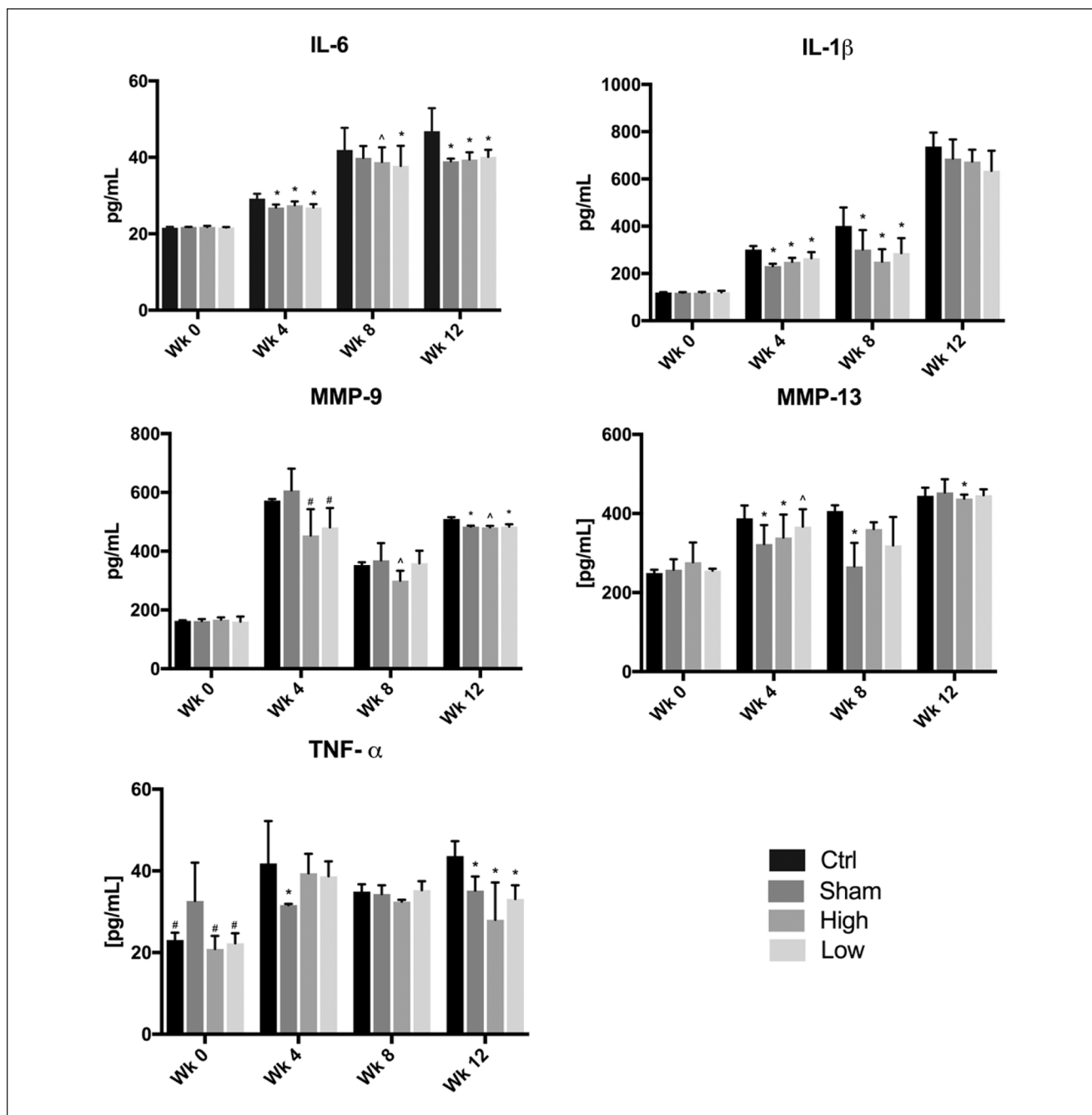


Figure 7. Concentration results from pro-inflammatory cytokine and MMP ELISA. *Indicates $P < 0.05$ compared to control group; ^denotes a trend, or $P = \sim 0.06$, compared to the control; #signifies $P < 0.05$ compared to sham group.

joint injury remains to be fully evaluated, several recent studies have shown that both systemic and local inflammatory mediators are involved in the animal ACL transection model of OA, predominantly in rats.³⁶⁻³⁸ The significant differences in pro-inflammatory cytokine and MMP levels between the sham and ACL transection control groups in our study further demonstrate that systemic changes in serum cytokine concentrations result from rabbit ACL

injury and associated OA progression. In addition, other studies have also found that systemic agents, delivered orally or by intramuscular injection, modulated both serum and joint tissue inflammatory cytokine concentrations in the rat ACL transection model indicating that a local therapeutic effect occurs with systemic drug agent.³⁹⁻⁴⁵ Fewer such studies exist in rabbits; previous studies of oral agents in the rabbit ACL transection model primarily quantified cartilage

cytokine levels.⁴⁶⁻⁴⁸ To our knowledge, only one other study correlating the effects of oral treatment and serum cytokine levels in rabbits exists, making our study of serum cytokine concentrations unique.⁴⁹ In the current study, we chose to measure blood cytokine concentrations to investigate if oral drug administration led to systemic immunomodulatory effects, seeing that the therapy was an oral agent rather than local intra-articular injection, as most past studies of DMOADs have been. Quantifying the immunomodulatory effects of CMC on synovial fluid is an important future direction for our study.

One limitation of the study is that joint analysis was performed at 12 weeks post-surgery. Other prior studies have shown changes in response to rabbit ACLT from 4- to 9-week time frame. Moreover, the temporal analysis of cytokine levels in the blood of IL-1 β , IL-6, and MMP-9 were found to be elevated in injured animals at 4 and 8 weeks, but that resolution of cytokine levels occurred by 12 weeks. Another limitation is the small sample size, in particular for the histopathology. Future studies would require the use of more rabbits, so that additional cartilage and synovium could be used for analysis.

Nevertheless, in the pathogenesis of OA, an imbalance exists between catabolic and anabolic mediators. CMC seems to offset this imbalance by reducing pro-inflammatory and catabolic mediators. Studies have shown that decreasing IL-1 β concentration inhibited the destructive articular cartilage process and allowed for collagen deposition to occur.³ Despite the small sample size and power, this shift from catabolism to anabolism resulted in fewer erosive lesions and preserved cartilage and synovial histology in our samples. The treatment groups both exhibited fewer features of OA macroscopically and histologically, with no significant differences between dosages.

In summary, this study built upon the previous knowledge of CMC's chondroprotective effects *in vitro*¹⁸ and further demonstrated these chondroprotective effects *in vivo* in a rabbit injury-induced model of OA. These results indicate the potential of CMC to modulate concentrations of several pro-inflammatory cytokines and MMPs leading to reduced inflammation, preserved joint cartilage, and slowed OA progression. CMC should continue to be studied as a potential therapeutic oral DMOAD in OA.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics Approval

Ethical approval for this study was obtained from the Institutional Animal Care and Use Committee (IACUC) at The Feinstein Institute (protocol #2014-013).

Animal Welfare

The present study followed international, national, and/or institutional guidelines for humane animal treatment and complied with relevant legislation.

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