The impact of early intra-articular administration of interleukin-1 receptor antagonist on lubricin metabolism and cartilage degeneration in an anterior cruciate ligament transection model

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Objective: Study the impact of intra-articular interleukin-1 receptor antagonist (IL-1 ra) treatment on lubricin biosynthesis following anterior cruciate ligament transection (ACLT) in the rat and evaluate the effect of combined IL-1 ra and recombinant human lubricin (rhPRG4) treatments on chondrocyte apoptosis.

Methods: ACLT was performed in male Lewis rats. Treatments included IL-1 ra or vehicle (n = 36 in each group). IL-1 ra intra-articular dosing was performed on days 1, 3, 5 and 7 following ACLT using Anakinra (150 mg/ml; 40 μl). At 3 and 5 weeks, animals were sacrificed and RNA was isolated. Histological analyses included Safranin O and H&E. Lubricin synovial fluid (SF) lavage concentrations were determined at 5 weeks. ACLT animals were treated with a single injection of vehicle, IL-1 ra (75 mg/ml; 40 μl), rhPRG4 (200 μg/ml; 40 μl), or IL-1 ra + rhPRG4 (75 mg/ml + 200 μg/ml; 40 μl) (n = 6 in each group) on day 7 following ACLT and cartilage was probed for cleaved caspase-3 at 5 weeks.

Results: IL-1 ra treatment improved lubricin expression (P < 0.001) and lubricin SF lavage concentrations in the IL-1 ra group was higher (P = 0.005) than the vehicle. IL-1 ra treatment reduced cartilage and synovial scores (P < 0.001) compared to vehicle. IL-1 ra and rhPRG4 acted synergistically to reduce caspase-3 positive chondrocytes (P < 0.001) compared to individual treatments.

Conclusion: IL-1 ra treatment preserved lubricin following ACLT and a combined treatment of IL-1 ra + rhPRG4 may act synergistically to reduce cartilage catabolism.

Introduction

Lubricin/Proteoglycan 4 (PRG4) is a multifaceted glycoprotein, secreted from the superficial zone chondrocytes and synovium with boundary lubricating and chondroprotective properties. Lubricin gene expression is down regulated by pro-inflammatory cytokines e.g., interleukin-1 beta (IL-1 β) and tumor necrosis factor alpha (TNF-α) following an acute joint injury, lubricin synovial fluid (SF) concentrations are reduced and supplementation with either purified human lubricin, recombinant human lubricin (rhPRG4) or a truncated lubricin retards cartilage degeneration in pre-clinical surgically induced osteoarthritis (OA) models.

The objective of this study is to evaluate the impact of blocking the effects of IL-1 on lubricin biosynthesis, lubricin SF levels and its association with cartilage degeneration. We hypothesized that antagonizing IL-1, following anterior cruciate ligament transection (ACLT) in the rat, results in reversal of the down regulation of lubricin expression, restoring lubricin levels in the SF. This effect is associated with a reduction in chondrocyte apoptosis and the severity of cartilage degeneration. Additionally, we have explored the impact of simultaneously antagonizing IL-1 and supplementing lubricin with intra-articular delivery of full-length rhPRG4 on articular chondrocyte apoptosis, lubricin immunostaining in cartilage and matrix metalloproteinase-13 (MMP-13) cartilage distribution in a rat ACLT model. Justification for the second hypothesis...
comes from the fact that lubricin null (Prg4−/−) mice demonstrate chondrocyte apoptosis in the absence of inflammation.

Methods

ACLT in the rat

Transection of the ACL was performed in 9–10 weeks old male Lewis rats (n = 96). Following anesthesia with intraperitoneal Ketamine and Dexametomidine, the right knee joint skin was shaved, cleaned with povidone iodine topical antiseptic, and a lateral skin incision was made along the patellar tendon to access the joint capsule and expose the ACL. Severing the ACL was performed using a # 11 surgical blade and a positive anterior draw confirmed ACLT. Closure of the joint capsule and skin was performed using biodegradable PDSII sutures. In all animals, the right knee joint was the ACL transected joint. All surgeries were performed by KE and all required approvals were obtained from MCHS University IACUC committee. Control animals (n = 5) were age and sex-matched to the ACL transected animals and were used for comparison.

Intra-articular administration of interleukin-1 receptor antagonist (IL-1 ra), rhPRG4 or Phosphate Buffered Saline (PBS)

To evaluate the impact of IL-1 ra treatment on lubricin metabolism following ACLT, ACL transected animals were injected with 40 μl of recombinant human IL-1 ra (supplied as 150 mg/ml AnaKira, Amgen Inc, Thousand Oaks, CA) or PBS on days 1, 3, 5 and 7 following ACLT (n = 36 in each group). Injections were performed through the patellar tendon of the operated knee joint while the animal is receiving inhalational isoflurane. At 3 and 5 weeks post ACLT, animals were sacrificed and their joints were lavaged using PBS followed by joint harvest (n = 18 in each group at each time point). The harvested joints were used for RNA extraction and histological analyses as described below.

To study the impact of combining IL-1 ra and rhPRG4 on chondrocyte apoptosis, ACLT transected animals were injected with a single 40 μl dose of either rhPRG4 (200 μg/ml quantified using absorbance at 280 nm), 40 μl IL-1 ra (75 mg/ml) or a combination of rhPRG4 and IL-1 ra (40 μl) with a final rhPRG4 and IL-1 ra concentrations of 200 μg/ml and 75 mg/ml, respectively. rhPRG4 is a full-length product derived from Chinese Hamster Ovary (CHO) cells (Lubris, Framingham, MA). Briefly, the gene encoding the full length 1404 amino acid human PRG4 was inserted into plasmid vectors, commercially available at Selexis SA (Geneva, Switzerland), for enhanced gene expression in mammalian cells. Specifically, expression vectors encode the gene of interest under the control of the human elongation factor-1-alpha (hEF-1-alpha) promoter coupled to a CMV enhancer. Plasmids were verified by sequencing. CHO-M cell lines derived from CHO-K1 cells (ATCC) adapted to serum free cultivation conditions were used for the production of rhPRG4. rhPRG4 rich media was obtained from a shake culture with fed-batch cultivation (SMF4CHO medium, Hyclone) supplemented with 8 %, l-glutamine, hypoxanthine and thymidine (Life Technologies). For this study, conditioned culture media were subjected to ultrafiltration/diafiltration (UFDF) followed by a three-step chromatographic purification process followed by final UFDF into PBS. Concentration was determined by inhibition enzyme-linked immunosorbent assay (ELISA) using Mab 9G3 (EMDMillipore) against known concentrations of rhPRG4 determined by dry weight, and final purity was determined to be 96% based on residual host cell protein concentrations (ELISA, Cygnus Technologies). The injection was performed on day 7 post ACLT (n = 6 in each group) and these joints were harvested at 5 weeks following ACLT.

Quantitative lubricin expression

Immediately following joint harvest, tibial plateau cartilage was carefully dissected from the operated and contra-lateral knees and was immediately snap-frozen and stored at −80°C until RNA isolation (n = 9 in each group at each time point). Total RNA isolation followed by quantitative lubricin expression was performed as described previously in Ref. 12. Data was expressed as a ratio of PRG4 expression in the operated knee to that in the contralateral, standardized to GAPDH.

Lubricin quantitation in SF lavages and adjustment using urea

In a separate experiment, ACLT was performed on male Lewis rats (n = 16) as described above and animals were treated intra-articularly with 40 μl of recombinant human IL-1 ra (150 mg/ml) or PBS on days 1, 3, 5 and 7 following ACLT (n = 8 in each group). A total of six animals were used as controls. Lavaging of SF was performed at week 5 following ACLT at the time of sacrifice by injecting a total of 100 μl of PBS into the joint capsule followed by flexing and extending the joint for 10 times. We were successful in obtaining lavages in six animals that had ACLT + PBS, in six animals that had ACLT + IL-1 ra and six control animals. Additionally, sera were collected at the time of sacrifice. Approximately 20–30 μl of fluid was recovered per animal. Lubricin SF lavage concentrations were determined using an inhibition ELISA with lubricin-specific monoclonal antibody 9G312. High-binding 96-well plate (Corning, Sigma Aldrich) were coated with rhPRG4 at 5 μg/ml in PBS overnight (100 μl per well). Subsequently, wells were blocked using 2 % bovine serum albumin (300 μl per well) for 2 h at room temperature. An 8-point standard curve of rhPRG4 with 100 μg/ml as the highest concentration followed by two-fold dilutions or diluted SF lavages were mixed with Mab 9G3 (1:5000) in PBS and added to the ELISA plate and incubated for 60 min at room temperature. Following washing with PBS + 0.1% tween 20, goat anti-mouse IgG-HRP (ThermoScientific) at 1: 5000 dilution was added (100 μl per well) and the plate was incubated for 60 min at room temperature. Following washing with PBS + 0.1% tween 20, one-step Turbo TMB ELISA reagent (ThermoScientific) was added (100 μl per well) and incubated for 15 min at room temperature. Subsequently, 2N sulfuric acid was added (100 μl per well) and the absorbance was measured at 450 nm. The lower detection limit of the assay was approximately 1.5 μg/ml and the upper detection limit of the assay was approximately 50 μg/ml. Using high molecular weight hyaluronic acid (2 mg/ml; R&D systems) as a diluent, the recovery of rhPRG4 using this assay was calculated as 96.12 ± 5.69%.

Adjustment of lubricin lavage concentrations was performed using the urea adjustment approach19,20. Urea concentrations were determined in animal sera and SF lavages using a commercially available kit (Abcam). The ratio of urea concentration in the serum to that in the SF lavage was considered the dilution factor and was multiplied by the SF lavage concentrations to arrive at the urea-adjusted lavage concentrations.

Histological and immunohistochemical analyses

Paraffin-embedded coronal sections were taken from weight-bearing areas of the articular cartilage of ACL transected joints of each animal. Micromotomed sections were collected approximately every 250 μm to identify representative sections showing the femoral condyles, tibial plateau and both menisci. Histological stains included Safranin O/Fast green for histological scoring and assessment of sulfated glycosaminoglycans (sGAGs) and hematoxylin and eosin (H&E) for synovitis scoring. Immunohistochemical analyses included probing for lubricin using monoclonal antibody...
compared with 3 and 5 week ACLT joints treated with PBS (P < 0.001). mAb 9G312 at 1:200 dilution, cleaved caspase-3 antibody (Abcam) at 1:200 dilution and MMP-13 antibody (Abcam) at 1:200 dilution followed by developing using Vectastain ABC kit (Vector Laboratories).

**Histological scoring and semi-quantitative histological analysis**

A total of four histological sections were stained with Safranin O/FG for assessment of cartilage integrity and four adjacent histological sections were stained with H&E for synovitis scoring. Cartilage scoring was performed using the OA Research Society International (OARSI) modified Mankin scoring except for osteophyte formation21. Scoring was performed on the medial and lateral joint compartments of the harvested knees and an overall average was reported. Synovial histopathology scoring was performed using the criteria reported previously in Ref. 22 and included examination of intimal hyperplasia, inflammatory cell infiltration, subintimal fibrosis and vascularity with scores ranging from 0 to 3 for each criterion and a range of aggregate scores between 0 and 12. Quantitative estimation of caspase-3 positive chondrocytes was performed using four histological specimens per animal and the number of caspase-3 positive chondrocytes was determined in the medial and lateral compartments of the tibial plateau and expressed as a percentage of total chondrocytes visualized using adjacent sections stained with H&E.

**Determination of urinary CTXII (uCTXII) levels in controls, PBS and IL-1 ra-treated ACLT animals**

On day 34 following ACLT of animals receiving PBS or IL-1 ra, animals were housed in metabolic cages and 24-h urine collection was performed. Urine samples were centrifuged at 3000 rpm for 20 min and stored at −20 °C. The urinary concentration of CTXII neoeptopes was determined using the preclinical Urine Cartilag ELISA (Immunodiagnostics Systems). Urinary creatinine was determined using the Creatinine Assay Kit (Abcam) and the uCTXII concentrations were expressed as pg per mg creatinine per 24 h.

**Statistical analyses**

Variables in the relative lubricin expression, lubricin SF lavage concentrations, uCTXII levels, modified Mankin and synovial histology scores, and percentage of caspase-3 positive cells analyses were initially examined for equal variance and normality. Variables that satisfied both assumptions were analyzed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey’s test for pairwise comparisons. Variables that failed any of these two assumptions were analyzed using ANOVA on the ranks with Tukey’s test for pairwise comparisons. Statistical significance was set at α = 0.05 a priori. All statistical analyses were performed using Sigma Plot (version 11.0). Data are graphically presented as scatter

***Indicates that lubricin cartilage expression in 5 week ACLT joints treated with IL-1 ra was significantly higher compared with 5 week ACLT joints treated with PBS (P < 0.001). (B) mAb 9G3 immunostaining for lubricin from (A) control, (B) 3-week ACLT joints treated with PBS, (C) 3-week ACLT joints treated with IL-1 ra, (D) 5-week ACLT joints treated with PBS, and (E) 5-week ACLT joints treated with IL-1 ra. Arrows point to intense lubricin staining on the surface of articular cartilage and in superficial zone chondrocytes compared to 3 and 5 week ACLT joints treated with PBS. Scale = 50 μm. (C) Urea-adjusted lubricin SF lavage concentrations in control, 5-week ACLT joints treated with PBS, and IL-1 ra. Individual data points are presented and the median value is highlighted with an "X." Indicates that lubricin SF lavage concentrations in control animals were significantly higher compared with 5-week ACLT animals following treatment with PBS or IL-1 ra (n = 6 in each group). Individual data points are presented and the median value is highlighted with an "X." Indicates that lubricin SF lavage concentrations in control animals were significantly higher compared with 5-week ACLT joints treated with PBS (P < 0.001). **Indicates that lubricin SF lavage concentrations in 5-week ACLT joints treated with IL-1 ra was significantly higher compared with 5-week ACLT animals treated with PBS (P < 0.005).
and 7 (PBS) or IL-1 ra (IL-1 ra). The arrow points to synovial thickening and in animals that underwent ACLT followed by intra-articular injection of PBS on days 1, 3, 5 stained cartilage and HuCTXII release following ACLT in the rat. (A) Representative Safranin-O (top panel) Impact of IL-1 ra treatment on cartilage and synovial histopathologies and Fig. 2. & E (bottom panel) stained synovium from control animals and K.A. Elsaid et al. / Osteoarthritis and Cartilage 23 (2015) 114 & C treated synovial hyperplasia and in sGAG staining. Similarly, PBS-treated ACL transected joints exhibi- of lubricin on the surface of articular cartilage as well as widespread distribution in the superficial zone chondrocytes and less prevalence in the middle zone. At 3 and 5 weeks post-ACLT, PBS-treated joints displayed a progressive loss of surface deposition of lubricin and lack of lubricin staining in the superficial zone chondrocytes. On the contrary, IL-1 ra treatment appeared to preserve lubricin surface staining and superficial zone chondrocyte expression. Urea-adjusted lubricin SF lavage concentrations are presented in Fig. 1(C). Lubricin SF lavage concentrations in control animals were significantly higher (P < 0.001) than 5-week ACLT animals treated with PBS. Additionally, SF lavage concentrations in ACLT animals treated with IL-1 ra were significantly higher (P = 0.005) compared with ACLT animals treated with PBS. There was no significant difference in lubricin SF lavage concentrations between control animals and ACLT animals treated with IL-1 ra.

Impact of IL-1 ra treatment on lubricin metabolism following ACLT

The impact of IL-1 ra treatment on PRG4 gene expression, SF lavage concentrations and cartilage surface deposition is presented in Fig. 1. At 3 and 5 weeks following ACLT, lubricin gene expression was significantly reduced following PBS or IL-1 ra treatments compared to control animals (P < 0.001) [Fig. 1(A)]. At 3 weeks following ACLT, PRG4 gene expression in joints receiving IL-1 ra treatment was significantly higher compared with PBS treatment (P = 0.002). Similarly, lubricin gene expression in joints receiving IL-1 ra at 5 weeks post-ACLT was significantly higher than lubricin expression in joints receiving PBS (P = 0.001). There was no significant difference in lubricin expression between joints receiving IL-1 ra treatments at 3 and 5 weeks post-ACLT (P = 0.383).

Representative cartilage sections immunoprobed for lubricin is presented in Fig. 1(B). Control cartilage displayed intense staining for lubricin on the surface of articular cartilage as well as widespread distribution in the superficial zone chondrocytes and less prevalence in the middle zone. At 3 and 5 weeks post-ACLT, PBS-treated joints displayed a progressive loss of surface deposition of lubricin and lack of lubricin staining in the superficial zone chondrocytes. On the contrary, IL-1 ra treatment appeared to preserve lubricin surface staining and superficial zone chondrocyte expression. Urea-adjusted lubricin SF lavage concentrations are presented in Fig. 1(C). Lubricin SF lavage concentrations in control animals were significantly higher (P < 0.001) than 5-week ACLT animals treated with PBS. Additionally, SF lavage concentrations in ACLT animals treated with IL-1 ra were significantly higher (P = 0.005) compared with ACLT animals treated with PBS. There was no significant difference in lubricin SF lavage concentrations between control animals and ACLT animals treated with IL-1 ra.

Impact of IL-1 ra treatment on cartilage and synovial histopathology and uCTXII release following ACLT

The disease-modulating effect of IL-1 ra treatment is presented in Fig. 2. Representative cartilage and synovium sections from control animals and from ACLT animals receiving PBS or IL-1 ra are presented in Fig. 2(A) and (B). ACLT animals receiving PBS displayed loss of superficial zone cartilage, surface fibrillation and loss of sGAG staining. Similarly, PBS-treated ACL transected joints exhibited synovial hyperplasia and infiltration of inflammatory cells. IL-1 ra treatment preserved the articular cartilage integrity and sGAG plots with the median values highlighted. Additionally, percentage caspase-3 positive chondrocytes across different experimental groups are reported in the text using the mean along with 95% confidence interval (CI).

**Results**

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Impact of recombinant lubricin (rhPRG4) and IL-1 ra treatments on chondrocyte apoptosis, lubricin and MMP-13 immunostaining following ACLT

Representative cartilage sections probed for cleaved caspase-3, lubricin and MMP-13 are presented in Fig. 3(A). Control joints lacked cleaved caspase-3 and MMP-13 staining and displayed lubricin deposition on the surface of articular cartilage and widespread presence in and around superficial zone chondrocytes. In PBS-treated ACL transected joints, cartilage exhibited intense cleaved caspase-3 and MMP-13 staining with lack of cartilage lubricin staining. IL-1 ra and rhPRG4 treatments re-established lubricin immunostaining in the superficial layer of articular cartilage and qualitatively further reduced cleaved caspase-3 and MMP-13 staining. Compared to PBS-treated ACL transected joints, animals receiving IL-1 ra + lubricin had reduced cleaved caspase-3 and MMP-13 staining.

The mean percentage of caspase-3 chondrocytes in control joints was 9.7, 95% CI (4.7–14.7) compared to ACL transected joints receiving PBS 76.2, 95% CI (62.6–89.9), IL-1 ra 51.8, 95% CI (40.4–63.2), rhPRG4 50.0, 95% CI (41.2–58.7) and 34.0, 95% CI (22.0–46.0) in the IL-1 ra + rhPRG4 treatment group. The percentage of chondrocytes positive for caspase-3 positive in the PBS-treated animals was significantly higher than in controls (P < 0.001), IL-1 ra (P = 0.003), rhPRG4 (P = 0.001) and IL-1 ra + rhPRG4 treated groups (P < 0.001) [Fig. 3(B)]. The percentage of caspase-3 positive chondrocytes in the IL-1 ra-treated animals was significantly higher than percentage of caspase-3 positive chondrocytes in controls (P < 0.001) and the IL-1 ra + rhPRG4 treated group (P = 0.039).

Discussion

ACL injury is a significant risk factor for the development of posttraumatic OA (PTOA)23–26, with current treatment modalities continuing to provide suboptimal outcomes27–29. In the acute phase following a joint injury, pro-inflammatory cytokines e.g., IL-1, TNF-α and IL-6 were shown to be elevated in the SF predisposing articular cartilage to cytokine-mediated degradation30–32. Blocking the effects of these key pro-inflammatory cytokines may prove useful in retarding the onset of cartilage degeneration and eventual PTOA. In this study, early high-dose treatment with IL-1 ra was

treated animals was significantly higher than percentage caspase-3 positive cells in controls (P < 0.001), IL-1 ra (P = 0.003), rhPRG4 (P = 0.001) and IL-1 ra + rhPRG4 (P < 0.001). *Indicates that percentage caspase-3 positive cells in the IL-1 ra-treated animals was significantly higher than percentage caspase-3 positive cells in controls (P < 0.001) and IL-1 ra + rhPRG4 (P = 0.039).
shown to reduce the extent of cartilage degeneration and preserved lubricin in a time-dependent manner. The protective effect of IL-1 ra on lubricin was evidenced by an observed recovery in lubricin expression as early as 2-weeks following the conclusion of drug dosing coupled with an increase in lubricin SF lavage concentrations at 5 weeks post ACLT. Lubricin expression levels were not fully restored to control values with IL-1 ra treatment. This partial effect can be rationalized by the fact that lubricin is also down regulated by TNF-α, that is also up regulated in this model. The reported lubricin SF lavage concentrations in this work are significantly higher than earlier reports due to the urea adjustment effect, which we did not employ previously. Additionally, the reported urea-adjusted SF lavage lubricin concentrations in control rats were higher than what has been reported for lubricin concentrations in the SF from contralateral uninjured joints of patients with a unilateral ACL injury. This may be attributed to inter-species variability, the use of different detection antibodies in the ELISA assays as well as the use of rhPRG4 as a standard in this study as opposed to the use of purified human lubricin in the earlier report. Furthermore, lavaging the knee joint may artificially elevate SF lubricin concentration by solubilizing cartilage and synovium-bound lubricin. Interestingly, urea adjustment did not alter observed differences among the different experimental groups. For the unadjusted SF lavage concentrations, control levels were higher than ACLT animals that received PBS, while IL-1 ra treatment increased lubricin SF lavage concentration compared to PBS.

Concomitant with restoration of lubricin expression was a reduction in the extent of cartilage degeneration, reflected by a lowering of OARSI scores, a reduction in the extent of synovial hyperplasia and inflammation and in uCTXII levels. A reduction in synovial inflammation is an important disease-modifying effect as chronic synovial inflammation contributes to sustained cartilage degradation. Even with a lowering of IL-1 ra dosing and reduced dosing frequency, important disease-modifying effects were still observed. Most significantly, a single dose of IL-1 ra resulted in preserving superficial and middle zone chondrocytes’ ability to synthesize lubricin and reduced the number of chondrocytes undergoing apoptosis. Chondrocyte apoptosis is a key contributor to cartilage degeneration following an acute joint injury and inhibition of apoptosis was shown to be efficacious in reducing the extent of OA in a surgically induced OA animal model. IL-1 β activates the nuclear factor κB (NFκB) signaling pathway with down-stream effects of promoting inflammation, increase expression of proteolytic enzymes e.g., MMP-13, and activation of effector caspases e.g., caspase-3. The reduction in caspase-3 activation, shown with IL-1 ra treatment is due to the prevention of IL-1β binding to the IL-1 receptor and down-stream activation of the NF-kappa B pathway. While we have observed a significant reduction in the percentage of articular chondrocytes with activated caspases-3, we were not able to observe any difference in the extent of MMP-13 staining between PBS and IL-1 ra treatment. Our findings support the disease-modifying effects of intra-articular IL-1 ra treatment and confirm previous findings that IL-1 ra treatment is beneficial in retarding cartilage damage in PTOA animal models.

A single-dose rhPRG4 treatment also significantly reduced the activation of caspase-3 in articular chondrocytes, as demonstrated by a reduction in percentage of caspases-3 positive chondrocytes. The ability of native human lubricin to reduce caspases-3 activation in chondrocytes has been previously documented in an inflammatory PTOA animal model. Purified human synovioocyte lubricin also prevented caspases-3 activation ex vivo when used as a lubricant between two bovine cartilage explants compared to either using PBS or a lubricin-deficient SF. Additionally, lubricin null (Prg4−/−) mice displayed increased caspases-3 activation in articular cartilage compared to age-matched heterozygous or wild type animals which express Prg4. The majority of the caspases-3 positive chondrocytes were located in the superficial and upper middle zone. While the median percentage of caspase-3 positive cells in cartilage of ACL transected joints was 79%, the median in rhPRG4-treated animals was 49%, reflecting a 38% reduction in caspase-3 positive chondrocytes. Interestingly, the abundance of cells that lacked caspase-3 staining with rhPRG4 treatment was in the superficial and middle zones of articular cartilage. In addition to preserving chondrocytes’ ability to synthesize lubricin, rhPRG4 treatment has reduced the extent of MMP-13 staining in articular cartilage compared to PBS-treated animals.

The PTOA disease process is complex to the extent that a treatment with a single mechanism of action may not confer the optimal chondroprotective effect to reduce the progression of cartilage degeneration. The use of combined treatment modalities with different mechanisms of action may exert a synergistic effect leading to a better control of the PTOA disease progression. IL-1 ra and lubricin exert their chondroprotective effect via different mechanisms and have individually shown promise as anti-PTOA treatments. We aimed to explore the impact of combining the two treatments on chondrocyte apoptosis, MMP-13 and lubricin expression in articular cartilage following ACLT. The combination of IL-1 ra and rhPRG4 has resulted in a synergistic effect on caspase-3 activation in chondrocytes, with the median percentage caspase-3 positive chondrocytes following IL-1 ra + rhPRG4 treatment of 34% compared to a median of 52% and 49% following IL-1 ra and rhPRG4 treatments, respectively. This represents a 57% reduction in percentage of caspase-3 positive chondrocytes compared to a 34% and 38% reduction following PBS and IL-1 ra treatments. Similarly, IL-1 ra + rhPRG4 treatment has reduced MMP-13 staining in articular cartilage to a greater extent compared to either IL-1 ra or rhPRG4 treatments. Taken together, these effects may demonstrate the benefit of combining a biological response modifying agent (Anakrina) with a tribosupplement (rhPRG4) which employ different therapeutic mechanisms. The full anabolic potential of this approach has not been maximized since the relative timing of the administration of both biologics may be important in lieu of co-administration.

In conclusion, our study has evaluated the impact of blocking the effects of IL-1 on lubricin metabolism and biosynthesis following a traumatic joint injury in the rat. IL-1 ra antagonized the IL-1 mediated reduction in lubricin expression and this effect occurred at an earlier stage prior to any appreciable cartilage damage. Individually, IL-1 ra and rhPRG4 intra-articular treatments reduced chondrocyte apoptosis following ACLT in the rat. Combining IL-1 ra and rhPRG4 resulted in a synergistic anti-apoptotic effect and may provide a potential therapeutic strategy to treat PTOA.

Author contributions statement

Elsaid: Study conception and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript and final approval of the submitted version. Dr Elsaid holds the ultimate responsibility for the integrity of the work.

Zhang: Acquisition of data, analysis of data, drafting and revision of the manuscript, and final approval of the submitted version.

Shaman: Acquisition of data, analysis and interpretation, manuscript drafting, and final approval of the submitted version.

Patel: Acquisition of data, data interpretation, drafting and revision of manuscript and final approval of the submitted version.

Jay: Study design and conception, data interpretation, drafting and revision of manuscript and final approval of submitted version.
Conflict of interest
Both CJ and TS have a financial interest in, and are named inventors on issued patents held by a commercial entity developing rhPRG4 for therapeutic uses.

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