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Intra-articular therapy with recombinant human GDF5 arrests disease progression and stimulates cartilage repair in the rat medial meniscus transection (MMT) model of osteoarthritis

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SUMMARY

Objective: Investigation of osteoarthritis (OA) risk alleles suggests that reduced levels of growth and differentiation factor-5 (GDF5) may be a precipitating factor in OA. We hypothesized that intra-articular recombinant human GDF5 (rhGDF5) supplementation to the OA joint may alter disease progression.
Methods: A rat medial meniscus transection (MMT) joint instability OA model was used. Animals received either one intra-articular injection, or two or three bi-weekly intra-articular injections of either 30 μg or 100 μg of rhGDF5 beginning on day 21 post surgery after structural pathology had been established. Nine weeks after MMT surgery, joints were processed for histological analysis following staining with toluidine blue. Control groups received intra-articular vehicle injections, comprising a glycine-buffered trehalose solution. OA changes in the joint were evaluated using histopathological end points that were collected by a pathologist who was blinded to treatment.
Results: Intra-articular rhGDF5 supplementation reduced cartilage lesions on the medial tibial plateau in a dose-dependent manner when administered therapeutically to intercept OA disease progression. A single 100 μg rhGDF5 injection on day 21 slowed disease progression at day 63. A similar effect was achieved with two bi-weekly injections of 30 μg. Two bi-weekly injections of 100 μg or three bi-weekly injections of 30 μg stopped progression of cartilage lesions. Importantly, three biweekly injections of 100 μg rhGDF5 stimulated significant cartilage repair.
Conclusions: Intra-articular rhGDF5 supplementation can prevent and even reverse OA disease progression in the rat MMT OA model. Collectively, these results support rhGDF5 supplementation as an intra-articular disease modifying OA therapy.

Introduction

Growth and differentiation factor-5 (GDF5) is a bone morphogenic protein (also known as BMP-14 and CDMP-1) and a member of the transforming growth factor-β (TGF-β) superfamily[1]. GDF5 is a developmental marker for early joint formation in the embryo, and is involved in the maintenance and repair of bone and cartilage in the adult[2,3]. The importance of GDF5 in synovial joint development is well established[2], as is the importance of GDF5 for normal synovial joint function[4]. Mutations in GDF5 also cause chondrodysplasias that prevent normal skeletal development and are associated with severe articular abnormalities[5]. Interestingly, single nucleotide polymorphisms (SNPs) in the human GDF5 gene have been identified by genome-wide association studies (GWAS) as significant genetic risk factors associated with osteoarthritis (OA) by different investigators studying distinct populations of OA

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patients[6–10]. For example, the rs143383 SNP that resides in the 5′ untranslated region (UTR) of the GDF5 promoter results in a C–T transition substitution that was originally identified in a GWAS cohort of Asian descent[6]. The association of this rs143383 SNP with OA was subsequently replicated in a cohort of European descent[7]. On a mechanistic level, the risk associated "T" allele has been shown to have significantly reduced transcriptional activity compared to the "C" allele in chondrocytes, the resident cells that are responsible for synthesizing and maintaining cartilage[11]. Therefore, the increased OA susceptibility associated with the rs143383 SNP is believed to be mediated through reduced expression of GDF5[11–13].

The therapeutic potential of GDF5 in connective tissue (fibrous and cartilaginous) healing has been demonstrated in several preclinical model systems. For example, stimulatory effects of exogenous GDF5 on matrix synthesis in human articular chondrocytes have been demonstrated *in vitro* using cells harvested from healthy individuals as well as OA patients[14,15]. In addition, animal studies have shown that exogenous GDF5 treatment can stimulate rodent tendon healing[16,17] and can positively impact the degeneration of intervertebral discs in mouse, rabbit and bovine models[18–20]. Collectively, this foundation of evidence supports our hypothesis that intra-articular supplementation of recombinant human GDF5 (rhGDF5) in an osteoarthritic joint may provide a therapeutic means to prevent disease progression and potentially activate anabolic responses leading to cartilage repair. Therefore, we studied the effect of intra-articular GDF5 supplementation in the rat medial meniscus transection (MMT) model of progressive degenerative OA. A distinct benefit of the MMT model is that it is a well-established model for studying OA structural changes where the kinetics of disease progression are highly reproducible[21,22]. We found that therapeutic intra-articular supplementation of rhGDF5 beginning on day 21 post MMT surgery slowed disease progression with a single 100 μg injection, whereas two bi-weekly 100 μg injections arrested disease progression and three bi-weekly 100 μg injections stimulated cartilage repair. The effects were dose-dependent, as two bi-weekly 30 μg injections were necessary to achieve protective effects similar to those observed for a single 100 μg injection, and three bi-weekly 30 μg injections were necessary to provide levels of disease arrest similar to those observed with two bi-weekly 100 μg injections. Taken together, these data support the therapeutic potential of intra-articular supplementation with rhGDF5 as a disease modifying therapy for OA.

Materials and methods

rhGDF5 preparation

Bulk rhGDF5 was obtained from Biopharm GmbH at a concentration of 3.5 mg/ml in 10 mM HCl in a frozen format stored at −80°C. The frozen bulk protein was thawed overnight at 2–8°C prior to formulation. Bulk rhGDF5 was dialyzed against a 5 mM glycine-HCl buffer overnight through a 3000 MW cutoff membrane at 2–8°C. Dialysis resulted in an increase in concentration to 3.8 mg/ml rhGDF5. This rhGDF5 solution was then diluted to a final concentration of 0.5 mg/ml rhGDF5 in a 5 mM glycine-HCl buffer containing 5% trehalose (W:V) at pH 3.0. This solution was then passed through a 0.22 micron filter and further diluted with sterile 5 mM glycine-HCl buffer with 5% trehalose at pH 3.0 to obtain working concentrations for injection. One ml of each formulation was dispensed directly into vials for lyophilization to achieve the indicated treatment dosages when the lyophilized cake is resuspended in 1 ml of water for injection. Vials from sample lots were tested at random after reconstitution with water for injection to confirm concentration, stability, and potency of rhGDF5 prior to initiation of animal studies using OsteoArthritis Research Society International (OARSI) recommended methodologies described in detail elsewhere[23], with minor modifications to enhance the accuracy of the measurement of collagen matrix loss (parameter #1 in the OARSI guidelines). Rather than employing the broad the 0, 50% and 100% loss scaling outlined by Gerwin, Bendele, Glasson, and Carlson[21], a more sensitive range of loss approach was used in order to provide a more specific and detailed look at the depths of degeneration and/or repair. This modification includes tibial cartilage micrometer measurements for minimal damage (superficial, affecting upper 10% only), mild damage (extending through 11–30% of the cartilage thickness), moderate damage (extending through 31–60% of the cartilage thickness), marked damage (extending through 61–90% of the cartilage thickness), severe damage (total or near total loss of collagen to tidemark, >90% thickness), and any damage (fibrillation ranging from superficial to full thickness loss). "Any damage" corresponds to the 0% measure in the OARSI Guidelines, and "severe damage" corresponds to the 100% measure. This expanded scaling provides the sensitivity to express the micrometer measurements either individually or in sum combinations to examine potential effects on cartilage sparing or repair in a therapeutic setting. None of the un-injected control animals at day 63 displayed lesions more shallow than the range of "moderate damage" (31–60% of the cartilage thickness), therefore the analysis for this parameter was focused on the sum of cartilage degeneration including moderate, marked, and severe damage so that this parameter indicates beneficial treatment effects on matrix loss extending through 31% or greater of the cartilage thickness.

Animals

This study was carried out in strict accordance with the standards set forth by the Animal Welfare Act and the recommendations in 'Guide for the Care and Use of Laboratory Animals' (HHS Publication (NIH) No. 85-23), The protocol was approved by the Institutional Animal Care and Use Committee at Bolder BioPATH, Inc (Protocol# BBP-008). All surgery was performed under isoflurane anesthesia, and all efforts were made to minimize suffering with pre and post-operative analgesia. Male Lewis rats weighing approximately 300 g (~12 weeks upon arrival) (Charles River, Wilmington, MA) were housed three per cage in shoe-box polycarbonate cages with wire tops in sanitary ventilated animal rooms with temperatures ranging between 67 and 76 °F and relative humidity between 30 and 70%. Automatic timers provided 12 h of light and 12 h of dark. Cages contained wood chip bedding and suspended food (Rodent chow, Harlan Teklad, Indianapolis, IN) and access to water bottles *ad libitum*. Animals were acclimated for 7 days prior to being randomized into groups. An attending veterinarian was on site or on call during the live phase of the study.

Induction of OA

Animals were anesthetized with isoflurane and the right knee area was shaved and scrubbed in preparation for surgery. A skin incision was made over the medial aspect of the knee and the medial collateral ligament (MCL) was exposed by blunt dissection, and then transected. The medial meniscus was reflected medially to prevent damage to the articular cartilage, then cut through its full thickness with small surgical scissors to simulate a complete meniscal tear. Skin and subcutis were closed with 4-0 VICRYL™ suture. All animals resumed weight bearing immediately post-surgery upon recovery from anesthesia and there was no evidence of excessive post-operative swelling indicative of joint infection.