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Oral administration of undenatured native chicken type II collagen (UC-II) diminished deterioration of articular cartilage in a rat model of osteoarthritis (OA)

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SUMMARY

Objective: The aim of this study was to determine the ability of undenatured native chicken type II collagen (UC-II) to prevent excessive articular cartilage deterioration in a rat model of osteoarthritis (OA).
Methods: Twenty male rats were subjected to partial medial meniscectomy tear (PMMT) surgery to induce OA. Immediately after the surgery 10 rats received vehicle and another 10 rats oral daily dose of UC-II at 0.66 mg/kg for a period of 8 weeks. In addition 10 naïve rats were used as an intact control and another 10 rats received sham surgery. Study endpoints included a weight-bearing capacity of front and hind legs, serum biomarkers of bone and cartilage metabolism, analyses of subchondral and cancellous bone at the tibial epiphysis and metaphysis, and cartilage pathology at the medial tibial plateau using histological methods.
Results: PMMT surgery produced moderate OA at the medial tibial plateau. Specifically, the deterioration of articular cartilage negatively impacted the weight bearing capacity of the operated limb. Immediate treatment with the UC-II preserved the weight-bearing capacity of the injured leg, preserved integrity of the cancellous bone at tibial metaphysis and limited the excessive osteophyte formation and deterioration of articular cartilage.
Conclusion: Study results demonstrate that a clinically relevant daily dose of UC-II when applied immediately after injury can improve the mechanical function of the injured knee and prevent excessive deterioration of articular cartilage.

Introduction

Osteoarthritis (OA) is a complex multifactorial disease process involving over time all of the tissues within and surrounding the synovial lined joints. Progression of the disease leads to disability associated with joint pain and dysfunction[1,2]. Epidemiologic studies have determined that risk factors for the progression of OA include aging, over- or non-physiological loads, obesity, trauma, hormonal disorders or a combination of several factors. While the exact etiology of OA in not yet known, injury to the articular cartilage over time results in changes in both the chondrocyte and synoviocyte metabolism such that inflammatory cytokines that are produced impair the chondrocytes ability to restore the cartilage matrix[3]. The search for effective therapies that attenuate joint degradation, improve joint flexibility and relieve joint pain has been challenging and current therapies to treat OA include acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs)[4].

Because collagen is the most prevalent component of the solid phase of articular cartilage, collagen supplementation has been considered a key treatment option to prevent damage to the articular cartilage over time and support the healing process following the onset of OA. Several hypotheses have been proposed to elucidate the exact mechanisms by which collagen derivatives improve the health of the articular cartilage[5]. Currently, glucosamine and chondroitin are the two most commonly used nutraceuticals that provide medicinal, therapeutic, and health benefits to arthritic patients[6,7]. For example, treatment with collagen

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derivatives has been proposed to provide an adequate supply of nutrients required for cartilage repair and maintenance[8,9], improve and preserve the quality of the subchondral bone[10,11], and maintain the overall health of articular cartilage and subchondral bone[12,13]. Over the past several years, a novel nutraceutical undenatured type II collagen (UC-II) from chicken sternum cartilage has been studied in knee OA subjects[14–16]. *In vivo* animal studies have reported that UC-II acts via specific regulatory T cells (Tregs) in the gut that migrate and concentrate in areas of inflammation upon stimulation, where they modulate local immune responses in an antigen-specific manner[17,18]. Irrespective of the actual mechanism of action, collagen derivatives seem to improve the health of the articular cartilage and are safe for patients and therefore, should be considered for the prevention or treatment of OA as a sole therapy or in combination with other drugs[15,19]. UC-II is derived from chicken sternum cartilage and is being marketed as a powdered, shelf-stable ingredient that at daily dose of 40 mg demonstrated clinical benefit by improving joint comfort, flexibility and mobility in OA patients.[20,21]

Commonly used method to induce OA in rodents is unilateral medial meniscal tear (MMT) method resulting in rapid progression of degenerative changes in the articular cartilage of the medial tibial plateau including fibrillation of articular cartilage, osteophyte formation and a loss of chondrocytes[22,23]. The medial meniscotibial ligament anchors the medial meniscus to the medial tibial plateau to ensure high congruency between articular structures and the transfer of weight-bearing loads during locomotion. Because cartilage degeneration develops rather rapidly in rats, evaluating drugs aimed to protect articular cartilage using the MMT model is challenging. The partial medial meniscectomy tear (PMMT) method is deemed less invasive than the complete medial meniscectomy model and is thus considered a more suitable model to test the ability of UC-II products to prevent the deterioration of cartilage degeneration and improve the healing of damaged articular cartilage.[24]

The present study tested the ability of undenatured native chicken type II collagen administered orally at the time of cartilage injury imposed by PMMT to prevent the excessive deterioration and improve the healing of articular cartilage.

Method

Test article

UC-II (InterHealth, Benicia, CA) consists of undenatured native chicken type II collagen (collagen 263.0 mg/g, hydroxyproline 32.9 mg/g). UC-II was manufactured from chicken sternum cartilage in a GMP-certified facility using a patented, low-temperature manufacturing process that ensures a particular level of UC-II collagen. UC-II was formulated in 0.5% methyl cellulose suspension and administered orally at 0.66 mg/kg/day for a period of 8 weeks. The rat 0.66 mg/kg/day UC-II dose was chosen because it is equivalent to the 40 mg/day UC-II used in clinical studies for a 60 kg human. The vehicle (0.5% methyl cellulose) was dosed orally at 5 ml/kg/day 7 days per week.

Animals and management

Male, 4 months old Lewis rats (Charles River Laboratories, Portage, MI) weighing 350 g at the beginning of the experiments were used in this study. All *in vivo* procedures were approved by the Institutional Animal Care and Use Committee (IACUC) and were performed in accordance with the US National Institutes of Health (NIH) Publication No. 85–23, revised 1996[25]. The rats were pair housed in a temperature- and humidity-controlled room on a regular 12 h light/dark cycle. Irradiated LabDiet™ 5053 (Purina, Richmond, IN) and water were provided *ad libitum*. The animals were acclimated for 1 week and were allocated to study groups based on their body weight the day before surgery. A group of 10 naïve rats were used as an intact control (Naïve), and another 10 rats received sham surgery (Sham). Additionally, 20 rats received the PMMT surgery and were allocated to receive vehicle treatment (PMMT/veh) or a UC-II (PMMT/UC-II) treatment. The ARRIVE guidelines was used to ensure the rigor of study conduct ad reporting of the data.

Surgery

Surgeries are performed in a dedicated rodent surgical facility at Pfizer consisting of an animal preparation room and recovery room, surgeon preparation room and a surgical suite. To minimize variations, only one surgical research specialist with extensive experience in performing the PMMT surgery was certified by the Academy of Surgical Research and have had his surgical skills and knowledge assessed by a designated subject matter expert (Global Trainer or Global Surgeon) approved to perform surgery. The rats were induced and maintained under anesthesia using isoflurane. One dose each of carprofen (Pfizer Animal Health, Florham Park, NJ) and sustained-release buprenorphine (Zoopharm, Windsor, CO) were administered prior to surgery to ensure analgesia. Rats in the surgery groups were subjected to a partial medial meniscal tear (PMMT) surgery[24]. Briefly, the medial meniscus was freed from its attachments to the margin of the medial tibial plateau prior to grasping the meniscus with forceps and transecting one-third of the medial collateral ligament and medial meniscus. In the sham surgery rats, the medial meniscus was visualized but not transected. The surgical incisions were closed in two layers using absorbable sutures.

Body weight, tissue collection and serum analyses

The body weight was recorded twice weekly throughout the study. At the end of the study rats were euthanized and the entire right hind limb was harvested and carefully cleaned of the soft tissue. The limbs were wrapped in saline-soaked gauze and frozen at −20°C for the *ex vivo* imaging and histological analyses of the tibial articular cartilage and bone. Blood was collected 8 weeks after surgery by jugular venipuncture under isoflurane anesthesia. The serum was stored at −20°C and used to run the standard chemistry panel and biomarkers of bone and cartilage metabolism[26] (see Supplemental Material for details).

Dynamic weight bearing (DWB)

DWB measurements were obtained before surgery, 6 days after surgery, 4 weeks after surgery and before euthanasia to assess the effects of surgery on the weight-bearing capacity of the hind and front legs. The DWB system (Bioseb, software 1.3.; Boulogne, France) is non-invasive method for measuring the weight and surface area of all four feet in a freely moving animal[27,28]. Zone parameters were set for the analysis as follows: ≥4 g for one sensor or a minimum of three adjacent sensors ≥2 g (in order to be considered a valid zone). For each time segment that was stable for more than one second, zones that meet the above criteria were validated and assigned as either right or left and front or rear. A mean value for the weight and area of each zone were calculated over the entire testing period, based on the length of time of each validated segment. For each testing period, the animals were placed into the chamber and allowed 20–30 s to explore prior to data collection. The following parameters were measured over a 3-min