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Comparison of the chondroprotective effect of a novel hydrogel compound and traditional hyaluronate on rat cartilage in a papain-induced osteoarthritis model

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ABSTRACT

Purpose: The aim of this experimental study is to evaluate the efficacy of a novel intraarticular drug in a papain induced osteoarthritis (OA) rat model and compare with the traditional hyaluronat (HA) visco supplementation.
Methods: An early stage OA model was induced by the intra-articular injection of papain enzyme in the right knee joints of 44 Sprague-Dawley rats. Eleven rats (eleven right knees: papain group, 11 left knees: control group) were chosen randomly 28 days after the last injection and sacrificed for verifying OA. The remaining rats (n = 33) were randomly divided into 3 groups. Group 1 was injected 0,2 mL of sterile saline solution (0,9%), group 2 was injected 0,2 mL HA and the group 3 was injected 0,2 mL of HA-CSNAG (hyaluronat, chondritin sulfate, N-acetyl-D-glucosamine) combination in the right knees. Injections were performed on the 35th, the 42nd and the 49th days consecutively. Two weeks after the last injection, all groups were sacrificed to evaluate the severity of OA according to Mankin system.
Results: Early stage of OA was verified regarding total Mankin scores ($p < 0.05$). There was statistically significant difference between Group 1 and Group 2 ($p < 0.05$), between Group 1 and Group 3 ($p < 0.05$) on the 63th day regarding total Mankin scores. Group 3 showed statistically significant improvement in terms of proteoglycan content of matrix when compared to Group 2 ($p < 0,05$).
Conclusion: HA-CS-NAG compound in hydrogel form is more chondroprotective to rats' cartilage when compared to HA during the early stages of OA.
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Introduction

Osteoarthritis (OA) is one of the most prevalent and debilitating joint disease associated with reduced quality of life and increased healthcare costs. With respect to recent applications of histopathological and imaging techniques, OA is now considered a heterogeneous chronic disease that may affect all tissues of the synovial joint. A large cascade of events lead to the breakdown and loss of articular cartilage, resulting in the damage of all structures of the joint including subchondral bone change, varying degrees of osteophyte formation, synovitis and adjacent supporting connective tissue elements.[1]

Available medical therapies, including traditional analgesics and non-steroidal anti-inflammatory drugs (NSAIDs) for OA are ineffective at altering or slowing down the disease progression and rather alleviate the symptoms by reducing pain and improving the joint mobility. Additionally, their chronic use has been limited by their deleterious side effects.[2,3] However, various agents including cartilaginous matrix precursors (hyaluronat [HA], chondroitin sulfate [CS], glucosamine [GA]) and cytokine modulators are currently being investigated for their effects on both symptoms and joint structural degradation. These molecules are expected to limit the progression of the disease by affecting the pathological changes in

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OA and are classified as structure-modifying drugs.[4] Due to its positive effects in pain relief and joint functional improvement, the use of viscosupplementation (HA, CS and GA) to treat OA is growing worldwide.[5] In addition to HA having potentially chondroprotective effects within the joint, CS and GA play an important role in maintaining joint homeostasis[6] and they both have oral and intra-articular injectable forms. Some authors make a point of low bioavailability and potential side effects, including systemic toxicity, impaired glucose tolerance and gastric ulceration of these nutraceuticals when taken orally.[7,8] Moreover, the limitations of these drugs, such as protein hypersensitivity of the synovium may have a negative effect on the intra-articular route. Also, the drugs that are injected intra-articularly may be rapidly cleared of the synovial fluid, thus obviating the advantage of direct delivery. Ideally, the drug should be delivered directly inside the affected joint in order to achieve a high intra-articular concentration and have limited systemic toxicity.[3]

For this purpose, many pharmacologically active drug molecules are under investigation. Recently, an intra-articular injectable hydrogel form of a compound of HA (36 mg/2.25 mL), CS (45 mg/2.25 mL) and N-Acetylglucosamine (NAG) (9 mg/2.25 mL) has been introduced, to achieve long-term drug exposure and low-protein concentration in order to avoid synovial hypersensitivity reaction when delivered intra-articularly.

The purpose of this study was to evaluate the efficacy of this novel intra-articular drug as a structure-modifier and a chondroprotective agent in a papain-induced OA rat model and to compare it to the traditional HA viscosupplementation.

Materials and methods

Study design

All animal experimental research protocols were approved by the Animal Research Committee and the study was supported by our Hospital Research Fund. A total of 44 adult female Sprague-Dawley rats with a mean age of 12 months and weighing between 250 and 350 g were used. Animals were housed (5 rats/cage) under standard management conditions. Room temperature and humidity were maintained at 20–24 °C and at 50–60%, respectively. The light cycle was fixed at 12 h and the animals were fed a standard rat diet with water *ad libitum*.

Osteoarthritis was induced by intra-articular injection of papain enzyme (Sigma-P3125; Sigma–Aldrich Corp., St. Louis, MO, USA) at a dose rate of 10 mg in 0.05 M sodium acetate (pH: 4.5) with enzymatic activity of 31 IU/mg in the right knee joints of the 44 Sprague-Dawley rats on the 1st, 4th and 7th days of the study as described by Murat et al[9] (Fig. 1). On the papain injection days, all rats were injected with 0,2 mL of sterile saline solution (0.9%) in the left knee joints to serve as the control group. On the 35th day, 28 days after the last injection, 11 rats were chosen randomly and sacrificed with high-dose thiopental (200 mg/kg) given intraperitoneally. Both the right (papain group) and the left (control group) knee joints were extra-articularly removed to evaluate the severity of OA according to histological scoring system.[10]

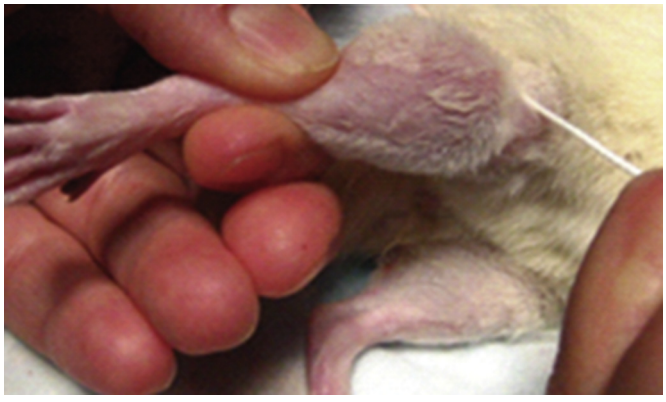

Fig. 1. Injection to the knee.

On the 35th day, the remaining experimentally OA-induced rats (n = 33) were randomly divided into three groups, and were given injections in their right knees as follows; 0,2 mL of sterile saline solution (0.9%) in Group 1, 0,2 mL of HA (Ostenil®; TRB Chemedica AG, Munich, Germany) with a mean molecular weight of 1.2 MDa in Group 2, and 0,2 mL of HA-CS-NAG combination (sodium hyaluronic acid [36 mg/2.25 mL], sodium chondroitin sulfate [45 mg/2.25 mL] and NAG [9 mg/2.25 mL] (Genvisc™ gplus; Phibio GmbH, Frankfurt, Germany)) in Group 3. The injections were given again on the 42nd and 49th days to mimic the clinical use. Two weeks after the last injection, all groups were sacrificed with high dose of intraperitoneal thiopental (200 mg/kg).

Tissue preparation and histological grading

On each scarification day, the tibiofemoral joints of the rats were separated by cutting the tibia and femur, and the attaching ligaments and tendons were removed with a surgical blade. Femoral condyles and tibia samples were fixed at 10% buffered formalin and decalcified in 8% formic acid. After dehydration through a graded series of ethanol solutions, the specimens were embedded in paraffin. The frontal parts were sectioned at 4 μm thickness. Each specimen was evaluated using the modified Mankin's histological grading system.[11] Hematoxylin and eosin stains were used to evaluate the structure, the chondrocytes, tidemark and pannus formation. Loss of proteoglycan staining intensity was assessed by Toluidine blue staining. Samples were examined and assessed independently by two blinded investigators.

Statistical analysis

Interobserver reliability was evaluated using two-way random, absolute agreement method. Data were evaluated using the SPSS for Windows 21.0 (SPSS Inc., Chicago, IL, USA) software. Before starting the study, a power analysis using pilot data was performed. This analysis determined that with a 95% confidence interval and a power of 80%, 11 animals per group would be required. The Kruskal–Wallis and Dunn tests were used for the comparisons of the parameters. An alpha level of 0.05 was chosen to judge statistical significance.

Results

Statistically significant differences were found between the control group (11 left knees) and papain-injected group (11 right knees) regarding total Mankin scores (control: 3.91, papain: 14.0; $p < 0.001$) (Table 1). Representative histological lesions in the cartilage of OA-induced rats were; moderate and severe surface irregularities, cleft formation in the transitional and radial zone, hypercellularity and disrupted columns of chondrocytes and multilayered tidemark. On the 63rd day, lesions were not reduced and got worse in Group 1. Representative histological lesions in Group 1 were; loss of severe matrix loss, moderate pannus formation, all of the above and large patches of cell death and sloughing of layers till mid-zone (Figs. 2 and 3). On the 63rd day, there was a statistically significant difference between Group 1 and Group 2, between Group 1 and Group 3 regarding total Mankin scores (Group 1: 20.27, Group 2: 10.2, Group 3: 7.18) (Figs. 4 and 5).

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