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The comparison of the effects of a novel hydrogel compound and traditional hyaluronate following micro-fracture procedure in a rat full-thickness chondral defect model

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ABSTRACT

Purpose: The aim of this experimental study was to investigate the impact of HA–CS–NAG compound (hyaluronate, sodium chondroitin sulfate, N-acetyl-D-glucosamine) on the quality of repair tissue after micro-fracture and to compare it with HA (hyaluronat), in a rat full-thickness chondral defect model.
Methods: Full-thickness chondral defects were created in a non-weight bearing area by using a handle 2.7-mm drill bit, in the right knees of 33 Sprague–Dawley rats. Each specimen then underwent micro-fracture using a needle. Two weeks after surgery, 3 groups were randomly formed among the rats ($n = 33$). In Group 1, 0.2 mL of sterile saline solution (0.9%) was injected. In Group 2, 0.2 mL HA with a mean molecular weight of 1.2 Mda was injected. In Group 3, 0.2 mL of HA–CS–NAG compound (hyaluronate, sodium chondroitin sulfate, N-acetyl-D-glucosamine) was injected. The injections were applied on the 14th, the 21st and the 28th postoperative days. All rats were sacrificed on the 42nd postoperative day. Histological analysis of the repair tissue was performed for each specimen by two blinded observers using Wakitani scoring system.
Results: There was significantly improved repair tissue in both Group 3 and Group 2 when compared with Group 1. Group 3 showed statistically significant improvement in terms of 'cell morphology' and 'integration of donor with host' when compared to Group 2 ($p < 0.001$).
Conclusion: Intra-articular injection of HA–CS–NAG compound after micro-fracture results in significantly improved repair tissue in rats' chondral defects when compared to HA regarding the donor integration and cell morphology.

Introduction

Full-thickness cartilage defects are common and related to trauma or overuse. Due to low cellular activity of chondrocytes and the absence of vascular response, they have very limited capacity for self-repair. An ideal treatment for cartilage lesions aims to recreate a healthy hyaline cartilage in the area of the defect that is integrated with surrounding normal cartilage and has similar mechanical properties.[1]

Micro-fracture is one of the marrow stimulation techniques and often used to treat focal full-thickness cartilage defects (2.5 cm>) due to simple and minimally invasive application properties, lower morbidity and cost-effectiveness.[2–4] The short-term results are satisfying with this technique.[5] Although some long-term studies have reported good results and little decline in the functional improvement seen after micro-fracture[2,3,6] some other studies reported long term results to be poor and resulting in osteoarthritis

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regardless of the defect size.[5,7–9] Therefore there is still a concern about the durability and longevity of the fibrocartilaginous repair tissue.[5,10–12] The main nature of repair tissue after micro-fracture is fibrocartilage, which primarily contains type I collagen and can't fully replicate the biomechanical features of hyaline cartilage, and probably this would be the reason for long term failure of micro-fracture technique.[13,14] Thus, with the aim of generating pure quality of hyaline cartilage without fibrous and hypertrophic tissues, the management of long-term functional healing still remains challenging and many pharmacologically active drug molecules are under investigation in order to increase the quality and the endurance of the fibrocartilaginous repair tissue obtained by the micro-fracture procedure.[15]

HA supplementation has been suggested as a supportive agent after micro-fracture.[14,16] However, supplementation with HA combined with structure modifying drugs including CS and glucosamines which are thought to be potentially effective as chondroprotectives by altering the catabolic and anabolic balance of the joint[17] has not been evaluated yet. These agents are candidates for intra-articular use, due to either their poor oral bioavailability or concern for systemic toxicity.[18] In a previous study, it is found out that a recently introduced intra-articular injectable hydrogel form of a compound of HA (hyaluronate) (36 mg/2.25 mL), CS (chondroitin sulfate) (45 mg/2.25 mL) and NAG (N-acetyl-D-glucosamine) (9 mg/2.25 mL) is more chondroprotective to rats' cartilage when compared to HA during the early stages of osteoarthritis with respect to preserving and healing articular surfaces, cellular abnormality and proteoglycan content.[19] Despite of the presence of many studies on the intra-articular use of HA, oral NAG and CS combination, there is no experimental data to evaluate the intra-articular application following micro-fracture application and the effects on hyaline cartilage of these drugs. The purpose of this experimental study was to compare the effects of intra-articularly injected HA and intra-articular injectable hydrogel form of a compound of HA, NAG and CS which are applied following micro-fracture procedure in an full-thickness chondral defect model in rat, through the histopathological parameters.

The purpose of the present study was to investigate the impact of HA–CS–NAG compound viscosupplementation on the quality of repair tissue after micro-fracture and to compare with the traditional HA in a rat full-thickness chondral defect model.

Materials and methods

Study design

The animal experiment was approved by the Institutional Animal Care and Use Committee. Thirty three adult female Sprague–Dawley rats (approximately 250–350 g, 12 months old) were used. All animals were housed in standard cages, five animals per cage at 20–24 °C and at 50–60%, and a standard 12-h light and 12-h dark cycle was used after the operation. The animals were fed with rat chow and water ad libitum.

The rats were anesthetized with a combined intra-peritoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The right limbs of the rats were shaved. A medial para-patellar incision was used in the right knees of the rats under sterile conditions. After the lateral dislocation of the patella, the articular cartilage of the patellar grove and the femoral condyles were exposed. Full-thickness chondral defects in the articular cartilage of the intercondylar notch were created without violation of the subchondral bone, using a handled 2.7 mm diameter drill bit. The defects were created in the articular cartilage of the intercondylar notch as this area is a non-weight bearing region of the distal femur. A non-weight bearing region was choosen because the rats were left free to move freely inside the cages, in the postoperative period. The drill tip contacted the surface vertically, cutting lips of the drill directly engaging the chondral surface. Rotational movements with slight pressure on the drill bit was applied to create a defect with a diameter of approximately 3 mm. High speed drilling was not applied to avoid thermal damage. Three holes were made using a needle in each defect as more number of holes would lead to violation of the neighboring holes. Needle tapped into the subchondral bone, avoiding thermal damage to the defects, to a depth of approximately 3 mm, until bleeding from the hole was present (Fig. 1a). The debris was completely removed. Then, the patella was reduced, and joint capsule and subcutaneous tissue were closed in anatomical layers reduced, and joint capsule and subcutaneous tissue were closed in anatomical layers with 4/0 vicryl after the operation. The same environmental and dietary conditions were provided for all animals, during the study.

Two weeks after surgery, rats (n = 33) with experimentally created full-thickness chondral defects were randomly divided into 3 groups as follows and the injections were performed in the operated knees:

Group 1: 0.2 mL of sterile saline solution (0.9%) to serve as the control group.
Group 2: 0.2 mL HA with a mean molecular weight of 1.2 Mda (Ostenil; TRB Chemedica AG, Munich, Germany).
Group 3: 0.2 mL of HA–CS–NAG compound [sodium hyaluronic acid (36 mg/2.25 mL), sodium chondroitin sulfate (45 mg/2.25 mL) and N-acetyl-D-glucosamine (9 mg/2.25 mL) (Genvisc Gplus, Phibio GmbH, Frankfurt am Main, Germany).

Injections were performed on the 14th, the 21st and the 28th days consecutively under clean conditions (Fig. 1b). All rats were sacrificed with high dose of intraperitoneal thiopental (200 mg/kg), on the 42nd postoperative day (two weeks after the last injection).

Tissue preparation and histological grading

After the sacrification, the tibia and the femur were cut in order to separate the tibio-femoral joints of the rats. Tendons and the attaching ligaments were removed using a surgical blade. Specimens were fixed at 10% buffered formalin and decalcified in 8% formic acid. The specimens were placed in paraffin after dehydration process performed through graded series of ethanol solutions. Five micrometer sections were cut through the micro-fractured osteochondral defects sagittally, perpendicular to the defect. The specimens were stained by hematoxylin and eosin. The defects to be examined were numbered randomly and noted elsewhere. Histologic analysis of the repair tissue was performed for each specimen by two blinded observers using Wakitani scoring system which is a well-detailed histologic grading system.[20] The scoring system is composed of five categories, including cell morphology, matrix staining, surface regularity, thickness of cartilage and integration of donor with host, and assigns a score ranging from 0 to 14 points.

Statistical analysis

The adjustment between observers was evaluated using reliability statistics two-way random absolute agreement method. Data were evaluated using SPSS for windows 21.0 software (SPSS Inc, Chicago, IL). A power analysis using pilot data was performed before beginning the study. This analysis determined that with a 95% confidence limits and a power of 80%, 11 animals per group would be required.

Descriptive statistics were calculated as frequency and percentage for categorical variables and as mean, standard deviation

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