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Original Article

Cartilage repair and inhibition of the progression of cartilage degeneration after transplantation of allogeneic chondrocyte sheets in a nontraumatic early arthritis model

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ABSTRACT

Introduction: Using a rat model of nontraumatic early arthritis induced by intra-articular administration of low-dose monoiodoacetic acid (MIA), we transplanted allogeneic chondrocyte sheets and examined the effects on tissue repair.

Methods: MIA (0.2 mg/50 μl) was injected into the right knee of 20 male Wistar rats. Four weeks later, rats were randomly allocated into three groups: Group A was examined 4 weeks after administration of MIA; Group B, 8 weeks after MIA injection and chondrocyte sheet transplantation, and Group C, 8 weeks after MIA injection but without chondrocyte sheet transplantation. Allogeneic chondrocyte sheets were transplanted into the right knee of Group B rats. Pain was assessed as the weight distribution ratio of the damaged to undamaged limb. The OARSI score was used for histological scoring.

Results: The limb weight distribution ratio indicated significantly less pain in Group B. Histological scoring showed significant differences in cartilage repair and inhibition of the progression of cartilage degeneration between Groups B and C, but not between Groups A and B, or Groups A and C.

Conclusions: These findings suggest that, in this rat model of nontraumatic early arthritis induced by low-dose MIA injection, allogeneic chondrocyte sheet transplantation induces cartilage repair and suppresses cartilage degeneration.

1. Introduction

Osteoarthritis (OA) is one of the most prevalent joint diseases, especially in mature women [1]. The condition is characterized by degenerative changes in articular cartilage, which cause pain and malfunctions as it progresses [2,3]. Structural changes include loss of articular cartilage, subchondral bone sclerosis, and formation of cysts and osteophytes [4]. The low cellularity and avascular nature of articular cartilage means that its capacity to self-regenerate after injury or degeneration is limited [5]. Mild OA is treated conservatively with oral administration of nonsteroidal anti-inflammatory drugs, intra-articular injection of corticosteroid [6] and/or hyaluronic acid [7], and exercise therapy [8]. However, surgical treatment may be required as the disease progresses. The main treatments are high tibial osteotomy and total knee arthroplasty.

Various arthritis models, such as surgical intervention and intra-articular drug injection, have been used to study cartilage regeneration. The anterior cruciate ligament resection model is the surgical intervention model used most frequently. In this model, anterior cruciate ligament resection triggers cartilage degeneration, subchondral bone sclerosis, and osteophyte formation, which mimic the pathological changes observed in human OA [9–11].

Abbreviations: AB, Antibiotic-antimycotic solution; Acan, Aggrecan; Col1A1, Collagen type Ialpha 1; Col2A1, Collagen type IIalpha 1; Comp, Cartilage oligomeric matrix protein; DMEM, Dulbecco's modified Eagle's medium; DPBS, Dulbecco's phosphate-buffered saline; EDTA, Ethylenediaminetetraacetic acid; FBS, Fetal bovine serum; IFP, Infrapatellar fat pad; ITGa10, Integrin alpha-10; MIA, Monoiodoacetic acid; Mmp13, Matrix metalloproteinase-13; OA, Osteoarthritis; OARSI, Osteoarthritis research society international; PVDF, Polyvinylidene difluoride; qPCR, Quantitative real-time polymerase chain reaction.

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Intra-articular injection of monoiodoacetic acid (MIA) is one method for inducing OA experimentally. MIA inhibits glyceraldehyde-3-phosphate dehydrogenase activity in chondrocytes and causes cell death and decreased proteoglycan content. The reaction is simple, rapid, and reproducible [12–15]. Some reports indicate that the OA caused by MIA-induced chemical induction is different from human OA [16,17]. On the other hand, Sangeetha et al. and others have reported histological and morphological changes in the MIA model similar to those in human OA [18–23]. Udo et al. [24] reported that the changes caused by MIA depend on the dose and timing, and that low-dose administration is useful for evaluating the pathology of OA, pain mechanisms, and therapeutic effects. Mohan et al. [25] reported that the low-dose MIA-induced arthritis model is suitable for studying nontraumatic OA and the therapeutic effects on cartilage and bone.

We have transplanted chondrocyte sheets into areas showing traumatic changes in an articular cartilage damage model in various animals and have confirmed its effectiveness [26–28]. The purpose of this study was to determine whether the repair or inhibition of the degeneration of damaged cartilage can be obtained by transplanting allogeneic chondrocyte sheets in a nontraumatic arthritis model of OA induced by intra-articular injection of low-dose MIA.

2. Materials and methods

All procedures using animals in this study were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised 1978) published by the National Institutes of Health, USA, and the Guidelines of Tokai University on Animal Use. This animal experiment was approved by the Animal Committee of Tokai University (permission number 171059).

2.1. Animals

Twenty male Wistar rats (CLEA Japan Inc., Tokyo, Japan) at 8 weeks of age and three male Lewis rats (Charles River Japan Inc., Kanagawa, Japan) at 6 weeks of age were used for the study. The animals were housed under normal conditions for 2 weeks before the start of the experiments to acclimate them to the environment. One to two rats were housed per cage in sterile conditions, and rodent chow and water were allowed ad libitum.

2.2. Chondrocyte sheets

2.2.1. Harvest of chondrocytes and fabrication of chondrocyte sheets

To prepare allogeneic cell sheets for transplantation, three 6-week-old male Lewis rats were used. The rats were sacrificed by an overdose of 50% isoflurane. A medial parapatellar incision was made in both legs, each patella was dislocated laterally, and the collateral ligaments and the anterior and posterior cruciate ligaments were severed. The joint capsule on the hip joint side was incised, and the femur was extracted. The articular cartilage tissue was collected with a scalpel from the bilateral femoral head and bilateral femoral condyle. Cells were isolated from the tissue sample using an enzymatic procedure and then incubated in Dulbecco's modified Eagle's medium/F12 (DMEM/F12; Gibco, Grand Island, NY, USA) supplemented with 20% fetal bovine serum (FBS; AusGeneX, Molendinar, Australia), 1% antibiotic–antimycotic solution (AB; Gibco), and 5 mg/ml collagenase type 1 (Worthington Biochemical, Mannheim, Germany) for 3 h at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. The cell suspension was washed and passed through a 100 μm strainer (BD Falcon, Franklin Lakes, NJ, USA).

The collected cells were seeded at a density of 1×10^4 cells/cm^2 in a 150 cm^2 culture flask in DMEM/F12 supplemented with 20% FBS and 1% AB, and incubated at 37 °C. After 3 days, 100 μg/ml ascorbic acid (Nissin Pharmaceutical, Yamagata, Japan) was added to the medium, and the medium was replaced every 3 or 4 days. Cells were passaged once when they reached confluence and then cryopreserved.

To fabricate cell sheets, cells were thawed, passaged once, and then seeded onto UpCell® inserts (CellSeed Inc., Tokyo, Japan) at 1×10^4 cells/cm^2. Ascorbic acid was added, the medium was replaced every 3 or 4 days, and the cells were cultured for 14 days. To harvest the cells using the characteristics of the UpCell insert, the culture temperature was lowered to <25 °C, which caused the chondrocytes to detach, after which they were preserved for further analysis.

2.2.2. Cell counting

The cell sheets were washed in Dulbecco's phosphate-buffered saline (PBS; Gibco). The sheets were then incubated in TripLE Express® (Gibco) at 37 °C for 40 min and centrifuged at 1500 rpm for 5 min. The cell sheets were resuspended in 0.25 mg/ml collagenase P (Roche, Basel, Switzerland) at 37 °C for up to 5 min and then centrifuged at 1500 rpm for 5 min. Finally, the isolated cells were resuspended in DMEM/F12, and the cells were counted using a trypan blue exclusion assay.

2.2.3. Histological staining

The collected cell sheets were fixed with 20% formalin (Wako Pure Chemical, Japan) and embedded in paraffin wax. The tissue was cut into 3 μm sections, which were then deparaffinized according to standard procedures, and stained with hematoxylin–eosin. The sections were stained with Safranin O in a 0.08% fast green aqueous solution and 0.1% Safranin O aqueous solution. The sections were also stained with a 0.05% toluidine blue solution.

2.2.4. Immunohistochemical staining

Paraffin-embedded tissue was sectioned at 3 μm and deparaffinized using standard procedures. The sections were stained for collagen type I with goat anti-type I collagen-UNLB (Southern Biotech, USA). The sections were washed with PBS and processed using an ImmPRESS Anti-goat Ig Reagent Kit (Vector, USA) and the slides were sealed. Sections for collagen type II staining were deparaffinized and then reacted with Anti-hCL (II) purified IgG (Daiich Fine Chemical, Japan). The sections were washed with PBS and processed using an ImmPRESS Anti-mouse Ig Reaction Kit (Vector), and the slides were sealed.

2.2.5. RNA isolation, cDNA synthesis, and quantitative real-time PCR

Three samples of passage 1 chondrocytes and three samples of cultured cartilage cell sheets were analyzed. The recovered chondrocyte sheets were crushed at 1500 rpm for 3 min using SHAKE Master Neo (Bio Medical Science, Japan). Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Next, first-strand cDNA was synthesized using 1 μg of total RNA and the enzyme, buffer, and primer of a QuantiTect Reverse Transcription Kit (Qiagen) and elongated using a GeneAmp PCR system 9600 (Thermo Fisher Scientific, USA) at 42 °C for 15 min and 95 °C for 3 min. Quantitative real-time polymerase chain reaction (qPCR) was performed using an Applied Biosystems 7300 Real-Time PCR system (Applied Biosystems, USA). The primers were as follows for TaqMan® Gene Expression Assays (Applied Biosystems, USA): *Col1A1* (Rn01637087_m1), *Col2A1* (Rn01463848_m1), *Sox9* (Rn01751070_m1), *Acan* (Rn00573424_m1), *ITGa10* (Rn01533928_m1), *Mmp13* (Rn01448194_m1), *Lect1*