Osteoarthritis and Cartilage 25 (2017) 297–308

Osteoarthritis and Cartilage

Controlling joint instability delays the degeneration of articular cartilage in a rat model

K. Murata † ‡, N. Kanemura ‡ *, T. Kokubun ‡, T. Fujino †, Y. Morishita †, K. Onitsuka †, S. Fujiwara †, A. Nakajima †, D. Shimizu ‡, K. Takayanagi ‡

† *Graduate Course of Health and Social Services, Graduate School of Saitama Prefectural University, Saitama, Japan*
‡ *Department of Physical Therapy, School of Health and Social Services, Saitama Prefectural University, Saitama, Japan*

ARTICLE INFO

Article history:
Received 13 July 2016
Accepted 10 October 2016

Keywords:
Osteoarthritis
Articular cartilage
Joint instability
Tumor necrosis factor alpha
Caspase-3

SUMMARY

Objective: Joint instability induced by anterior cruciate ligament (ACL) transection is commonly considered as a predisposing factor for osteoarthritis (OA) of the knee; however, the influence of re-stabilization on the protection of articular cartilage is unclear. The aim of this study was to evaluate the effect of joint re-stabilization on articular cartilage using an instability and re-stabilization ACL transection model.

Design: To induce different models of joint instability, our laboratory created a controlled abnormal joint movement (CAJM) group and an anterior cruciate ligament transection group (ACL-T). Seventy-five Wistar male rats were randomly assigned to the CAJM ($n = 30$), ACL-T ($n = 30$), or no treatment (INTACT) group ($n = 15$). Cartilage changes were assessed with soft X-ray analysis, histological and immunohistochemistry analysis, and real-time polymerase chain reaction (PCR) analysis at 2, 4, and 12 weeks.

Results: Joint instability, as indicated by the difference in anterior displacement between the CAJM and ACL-T groups ($P < 0.001$), and cartilage degeneration, as evaluated according to the Osteoarthritis Research Society International (OARSI) score, were significantly higher in the ACL-T group than the CAJM group at 12 weeks ($P < 0.001$). Moreover, joint re-stabilization maintained cartilage structure (thickness [$P < 0.001$], surface roughness [$P < 0.001$], and glycosaminoglycan stainability [$P < 0.001$]) and suppressed tumor necrosis factor-alpha (TNF-α) and caspase-3 at 4 weeks after surgery.

Conclusion: Re-stabilization of joint instability may suppress inflammatory cytokines, thereby delaying the progression of OA. Joint instability is a substantial contributor to cartilage degeneration.

Introduction

Osteoarthritis (OA) is a common cause of chronic pain, impairment in activities of daily living, and disability. Multiple factors have been identified as contributors to the development of knee OA, including aging, sex, obesity, trauma, muscle weakness, and inflammation[1–7]. In particular, mechanical stress has been proposed to play a critical role in the progression of articular cartilage degeneration. Recently, joint instability has been identified as one of the important factors for OA progression. Stability at the knee joint is provided by static structures, such as muscles, ligaments, menisci, and the joint capsule, in combination with the coordinated activation of dynamic movement; together, these factors produce appropriate loading of the articular cartilage in response to knee movement. However, instability induced by anterior cruciate ligament (ACL) transection alters kinematics, resulting in abnormal loading during weight-bearing activities[8–11]. Therefore, the ACL transection model has been widely used as an animal model to investigate the pathomechanics of OA.

However, the influence of stability on OA remains unclear. Movement with adequate stability is a positive mechanical stress, and articular cartilage requires mechanical loading to maintain homeostasis. On the other hand, increases in joint instability are considered a negative mechanical stress in the pathogenesis of OA. Previously, animal studies have shown that OA progression increases at a rate proportional to instability, resulting in significant changes in knee joint loading and gait performance[12–14]. In a

* Address correspondence and reprint requests to: N. Kanemura, Department of Physical Therapy, School of Health and Social Services, Saitama Prefectural University, Sannomiya 820, Koshigaya, Saitama, Japan. Fax: 81-48-973-4123.
E-mail address: kanemura-naohiko@spu.ac.jp (N. Kanemura).

http://dx.doi.org/10.1016/j.joca.2016.10.011
1063-4584/

human study, self-reported knee instability was associated with significant alterations in knee joint function during the stance phase of gait in patients with OA[15,16]. Moreover, high adduction moments during weight-bearing activities were associated with OA progression, and were noted to result in significant knee instability[17–19]. For this reason, joint instability is a major contributing factor to the progression of OA in both human and animal models, and results in abnormal mechanical loading on the affected knee.

Therefore, we hypothesized that providing biomechanical control following ACL transection may result in the inhibition of articular cartilage degradation. Intra-articular surgery, such as that performed in the ACL transection, is insufficient to evaluate the relationship between joint instability and OA progression. Because intra-articular surgery is invasive, it cannot be determined whether inflammation and secondary instability is a product of ACL transection or other mechanisms[20]. Thus, an animal model is required that can replicate the intra-articular instability condition of ACL transection and provide control over abnormal instability using an extra-articular bracing system.

The purpose of our study was to examine the effect of providing control of instability after ACL transection on the progression of OA. Consequently, it was necessary to develop a novel controlled abnormal joint movement (CAJM) rodent model. Histological and immunofluorescence analyses were performed as a means of examining cartilage structure and pro-inflammatory responses.

Material and methods

Animals and experimental design

For this study, a novel protocol was devised according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines[21]. In addition, all methods and procedures were approved by the Animal Research Committee of Saitama Prefectural University (approval number: 27-6). The experimental design is presented in Fig. 1. Wistar male rats (Clea Japan, Tokyo, Japan), aged 6 months and weighing 320–380 g, were used in our study. A total of 75 rats were randomized into three group: no surgery group (INTACT, $n = 15$; without intervention), CAJM group (CAJM, $n = 30$; external support provided to stabilize the knee joint after ACL injury), and ACL transection group (ACL-T, $n = 30$; the ACL was surgically transected). All rats were housed in polycarbonate cages, with two animals per cage. The room had a 12/12 h light–dark cycle and was maintained at a constant temperature of 23°C. Rats were permitted unrestricted movement within the cage and had access to food and water freely.

Surgical procedures

In the ACL-T and CAJM groups, ACL transection was performed to introduce joint instability. In the CAJM group, a second procedure was performed to restore biomechanical function following ACL transection using a nylon suture, placed on the outer aspect of the knee joint [Fig. 2(A)]. After a 1-week acclimatization period, animals in the CAJM and ACL-T groups underwent surgery. Under anesthesia with pentobarbital (1.0 ml/kg), the right knee joint was exposed via the medial capsule [Fig. 2(B-a)] without disruption of the patellar tendon, and the ACL was completely transected [Fig. 2(B-b)]. Transection caused excessive anterior translation of the tibia on the femur, resulting in abnormal joint kinematics, a defining feature of joint instability. To mitigate anterior translation of the tibia on the femur following ACL transection in the CAJM group, a bone tunnel was created in the anterior portion of the proximal tibia [Fig. 2(B-c)]. Subsequently, a nylon thread was passed through the tunnel [Fig. 2(B-d)], and secured to the posterior aspect of the distal femur [Fig. 2(B-e)]. The nylon thread, therefore, had the same orientation as the native ACL, providing a posteriorly directed traction force on the tibia to resist anterior motion over the condyles of the femur [Fig. 2(B-f,g)].

Soft X-ray radiography

To evaluate knee instability and OA change, rats were sacrificed at 4 and 12 weeks after surgery. Joint instability was evaluated by anterior traction using a constant force spring (0.2 kgf) and soft-X ray radiography (M-60; Softex Co., Tokyo, Japan). To assess OA change, limbs were dissected free of all soft tissues and positioned with 90° of flexion at the knee joint. Frontal and sagittal radiographs were taken. Soft X-ray radiography was performed at 28 kV and 1 mA for 1 s, and imaged using a NAOMI digital X-ray sensor (RF Co. Ltd., Nagano, Japan).

Histological analysis

Rats ($n = 30$, each group $n = 10$) were sacrificed at 4 and 12 weeks, and the knee joints were fixed in a 4% paraformaldehyde/phosphate-buffered saline (PBS) solution for 48 h at 4°C. The knees were then

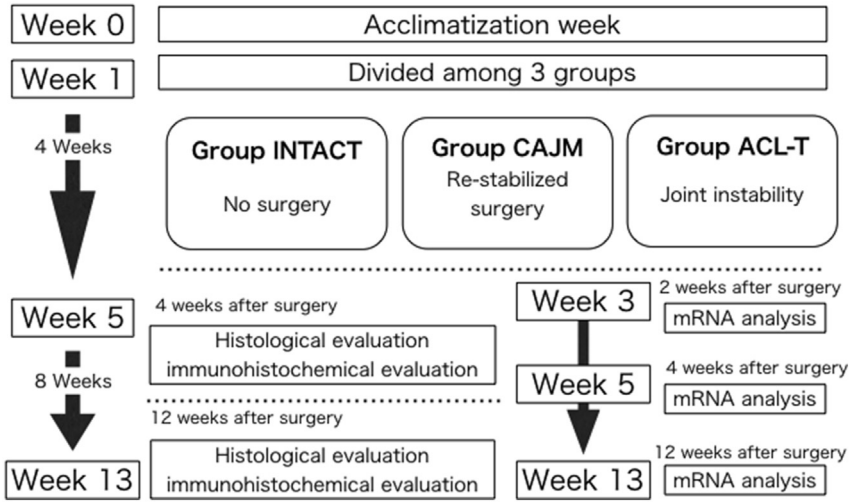

Fig. 1. Schematic of the experimental protocol.