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SHORT COMMUNICATION

Restoring knee joint kinematics after anterior cruciate ligament injury might inhibit synovial membrane inflammation

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Abstract
We developed a novel controlled abnormal joint movement (CAJM) model that controls instability after traditional anterior cruciate ligament transection (ACL-T). We evaluated whether joint instability damping suppresses synovial inflammation in osteoarthritis patients using our new CAJM rat model. We found that joint instability in rats differed between the ACL-T and CAJM models. Joint instability might contribute to synovitis and inhibit osteoarthritis.

Keywords Joint kinematics · Synovitis · Knee · Rat model

Abbreviations

OA	Osteoarthritis
ACL	Anterior cruciate ligament
ACL-T	ACL transection
CAJM	Controlled abnormal joint movement
MMP	Matrix metalloproteinase
ADAMTs	A disintegrin and metalloproteinase with thrombospondin motifs

Introduction

The anterior cruciate ligament (ACL) plays an important role in stabilizing the motion of the knee, and ACL injury induces anterior instability of the tibia. Currently, ACL reconstruction is frequently performed; however, many elderly people often do not choose to undergo surgery. In these cases, there are abnormalities in joint kinematics, such as joint instability induced by the damage to the ACL. Such dysfunction is one of many types of injuries that may impact joint kinematics.

Our previous work indicated the process by which knee joint tissue heals in animal model with controlling of abnormalities kinematics in knee joint. First, we modified the intra-articular condition by controlled abnormal joint motion after ACL ruptured, which led to spontaneous ACL healing [1]. Second, articular cartilage is generally thought to have a limited regeneration capacity, and abnormal joint movement after ACL injury aggravated the progression of osteoarthritis (OA) [2].

Therefore, although abnormal joint kinematics is a risk factor of OA progression, the relationship between synovitis and joint kinematics is not well-understood. Because intra-articular conditions, such as an ACL tear or meniscus injury, involve joint invasion, it is not clear whether injury-related primary inflammation or joint instability-related, secondary mechanical stress leads to cartilage degeneration.

In our previous study, we developed a novel controlled abnormal joint movement (CAJM) rat model to restore the knee joint's kinematics. Studies on traditional models, such as an ACL transection model, failed to reveal associations between the joint's kinematics and OA progression [2]. Using the CAJM model, we hypothesized that restoring the knee joint's kinematics after ACL injury would change intra-articular synovial inflammation, and we examined the contribution of joint instability to synovitis.

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Materials and methods

Animals and surgical procedures

All procedures were approved by the Ethics Committee of Saitama Prefectural University (approval number: 27-6). 15 Wistar rats, aged 6 months, were used. We randomly divided the 15 rats into three groups and histologically analyzed them: the ACL-T model [(ACL-T); anterior tibia joint instability induced by ACL transection], CAJM (anterior tibia joint instability was limited for restoring knee joint kinematics) according to our previous studies, and INTACT (no surgery). The surgical procedure was described previously [2]. After the rats were anesthetized, the medial capsule of the right knee joint was exposed without disrupting the patellar tendon, and the ACL was completely transected. After anterior instability of the tibia was confirmed, the surgical incision was closed in rats in the ACL-T group. CAJM was achieved by creating a bone tunnel along the anterior aspect of the proximal tibia, through which a nylon thread was passed and tied to the posterior aspect of the distal femur. Abnormal joint movements were dampened without intra-articular suturing of the ligament (as performed in ACL reconstruction). All rats were housed in and maintained on a 12-h light–dark cycle, and had free access to food and tap water.

Histology of the synovial membrane

12 weeks postoperatively, the animals were sacrificed, and their right knee joints were dissected ($n = 5$ for each group) and fixed in 4% paraformaldehyde solution for 2 days. We decalcified the samples in 10% EDTA solution for 48 days and embedded them in an optimal cutting temperature compound (Sakura Finetek Co., Ltd., Tokyo, Japan). We cut the specimens at 14 μm in the sagittal plane and stained them using safranin-O fast green. We scored the synovial membrane histological analysis using the synovial membrane inflammation score. The score characterizes inflammation based on increased numbers of cell layers in the synovial lining, synovial tissue proliferation, and inflammatory cell infiltration. A total score of 4 indicates severe synovial inflammation; a score of 0 indicates normal tissue. The osteophytes were evaluated semi-quantitatively using osteophyte formation scores consisting of two factors: size and maturity. We used the Shapiro–Wilk test to evaluate the normality of data distribution, the Kruskal–Wallis test for group differences, and the Mann–Whitney *U* test for post hoc analyses.

Immunohistochemical examination of cytokine mediators

We visualized the inflammation mediators of OA, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, using immunohistochemistry. We blocked sections with normal goat serum and incubated them overnight at 4 °C with rabbit polyclonal anti-TNF-α (dilution: 1:250; Bioss Antibodies, Woburn, MA, USA) and anti-IL-1β antibodies (dilution: 1:250; Bioss, Woburn, MA, USA). We performed the streptavidin–biotin-peroxidase complex technique at room temperature using an avidin–biotin complex kit (Vector Laboratories, Burlingame, CA, USA) and visualized the specimens using diaminobenzidine. We determined the synovial membrane staining visually and stratified them into five categories (0–4), referring to a previous study: 0 = negative, 1 = weakly positive, 2 = positive, 3 = strongly positive, and 4 = high staining intensity.

Results

Degeneration of the articular cartilage was more prominent in ACL-T rats compared with CAJM rats. In particular, we observed cluster cells and attenuation of staining in rats in the ACT-T (Fig. 1a: filled upward triangle). Regarding the synovial membrane, fibrous layer thickening occurred in rats in both the ACL-T and CAJM groups; however, fibroblasts on the synovial membrane's surface were aggregated in rats in the ACL-T group. Osteophyte formation, which was the most prominent in the ACL-T group, was observed in the posterior tibia.

The synovial membrane inflammation score is presented in Fig. 1b. Anteriorly, the score was significantly higher in the ACL-T group than in the INTACT group [$p = 0.018$, INTACT, 1 (0–1); CAJM, 1 (1–3); ACL-T, 2 (1–3)]. However, there were no differences between the CAJM and INTACT groups ($p = 0.143$). Posteriorly, the score was significantly lower in the INTACT group than in the CAJM and ACL-T groups [INTACT, 1 (1–1); CAJM, 2 (2–3.5); ACL-T, 4 (4–4)]. However, there were no differences between the CAJM and INTACT groups ($p = 0.239$). On the other hand, the osteophyte score was significantly higher in the ACL-T group than in the CAJM and INTACT groups [INTACT, 0 (0–0); CAJM, 1 (0–2); ACL-T, 5 (4.5–5)].

The immunohistological findings of synovial TNF-α and IL-1β are shown in Fig. 2. In rats in the ACL-T group, we confirmed cell proliferation at the synovial margin and observed deep staining on immunohistochemical staining. In rats in the INTACT group, the fat and synovial layers

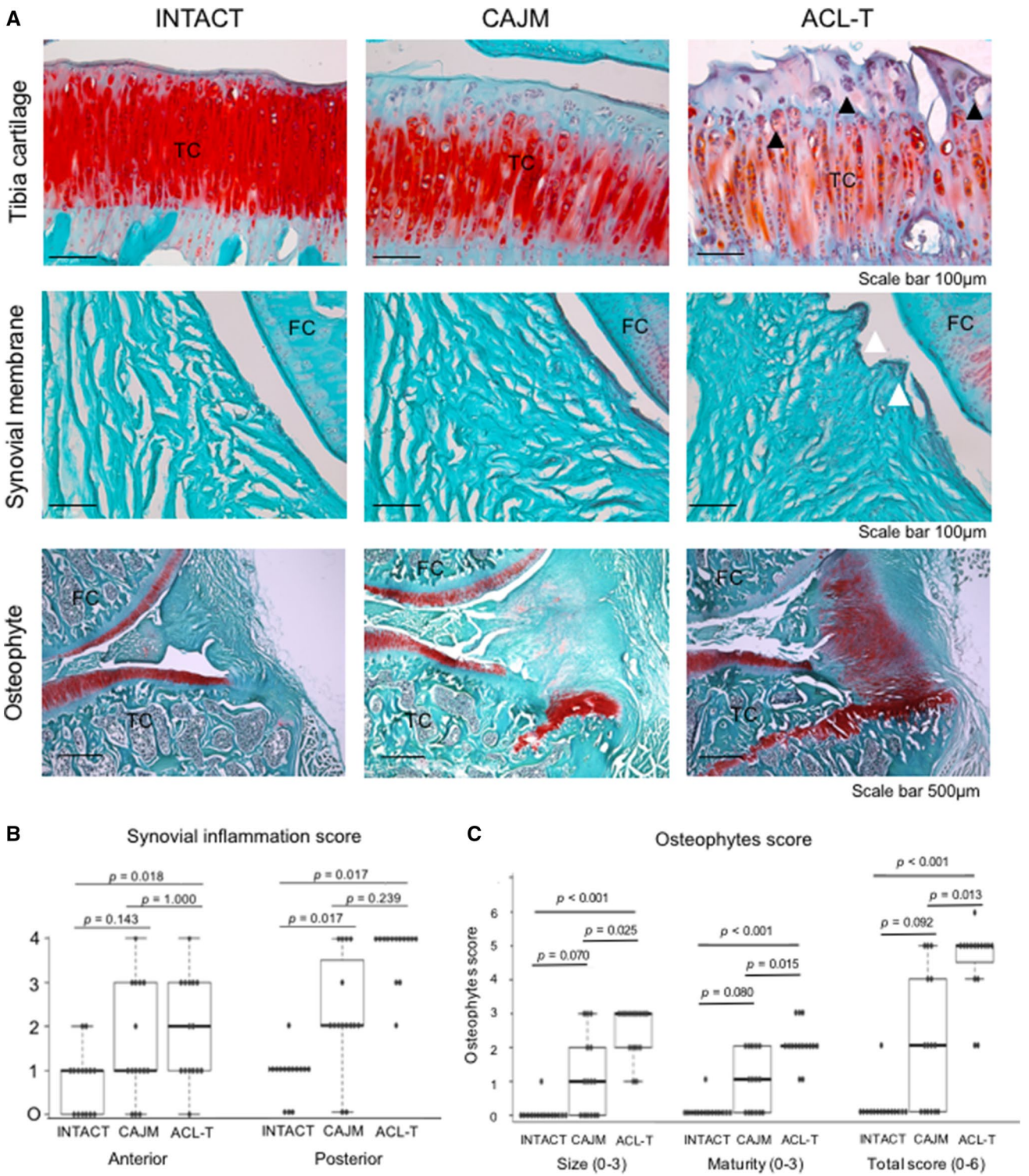

Fig. 1 **a** Histological findings of the articular cartilage, synovial membrane, and osteophyte formation using safranin O and fast green at 12 weeks. **b** The synovial membrane histological score indicated the influence of controlling abnormal joint movements. **c** The osteophyte score was increased in the ACL-T group compared with the CAJM and INTACT groups. *ACLT-T* anterior cruciate ligament transection, *CAJM* controlled abnormal joint movement, *INTACT* intact group

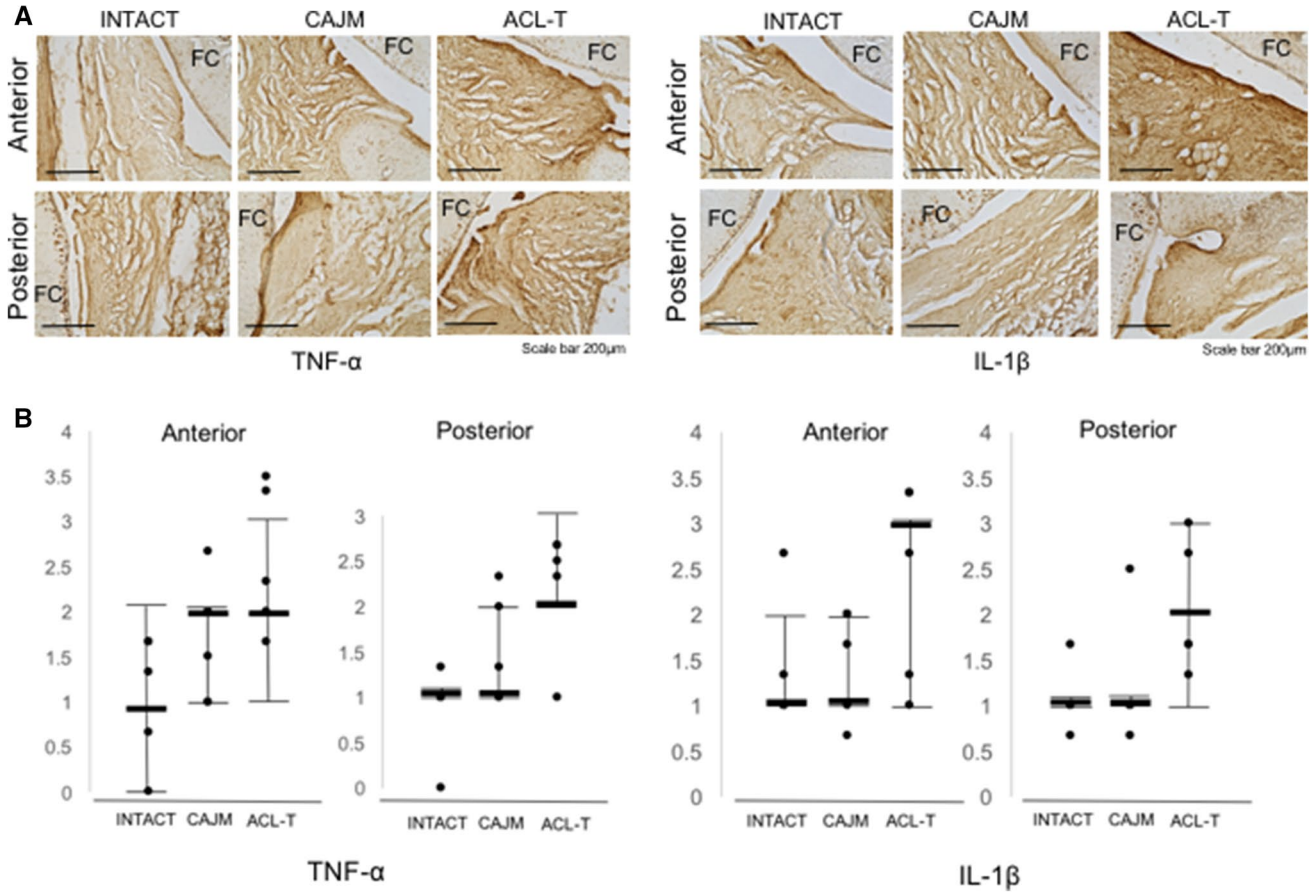

Fig. 2 Immunohistochemical staining sections of IL-1β and TNF-α. **a** The synovial membrane staining of IL-1β and TNF-α were compared among the groups (INTACT, CAJM, and ACL-T) at 12 weeks. **b** The anterior and posterior semi-quantitative scores are shown. In the INTACT group, the fat and synovial layers were present, but synovial layer thickening appeared in rats in the ACL-T and CAJM groups. The data are presented as the median with interquartile range. *IL* interleukin, *TNF* tumor necrosis factor, *INTACT* intact group, *CAJM* controlled abnormal joint movement, *ACLT-T* anterior cruciate ligament transection

were present, but synovial layer thickening appeared in rats in the ACL-T and CAJM groups.

Discussion

We found that change in knee joint kinematics after ACL injury induced changes in synovitis, which we assessed using the histological characteristics of fibroblast proliferation and inflammatory mediator staining. These results might aid in preventing OA in the future.

OA progression is multifactorial, and synovitis is involved [3]. The inflammatory reactions during synovitis induce intra-articular catabolic enzymes that destroy proteoglycan and collagen type-2 (e.g., matrix metallopeptidase-13 and a disintegrin and metalloproteinase with thrombospondin motifs) [4]. In addition, lubricin and hyaluronic acid, which lubricate cartilage, are decreased due to synovitis. Therefore, although synovitis might cause cartilage deterioration in the knee joint, whether synovitis is a secondary response to mechanical factors or a primary response of cartilage degeneration is still unclear.

Our CAJM model, which experimentally reproduced different forms of joint instability after ACL transection, might address this problem. The traditional ACL-T model was inadequate to evaluate joint instability because intra-articular conditions, such as ACL-T or meniscus tear, involves joint invasion. The traditional ACL-T model involves complete transection of the ACL, which results in excessive anterior translation of the tibia on the femur. This results in in abnormal joint kinematics, which is a defining feature of joint instability [2]. To mitigate anterior translation of the tibia on the femur following ACL transection in the CAJM model, a bone tunnel was created in the anterior portion of the proximal tibia, and secured to the posterior aspect of the distal femur. The nylon thread, therefore, had the same orientation as the native ACL, which provided a posteriorly directed traction force on the tibia to resist anterior motion

over the condyles of the femur. Recent reports have reported that joint instability induces synovitis and meniscal position abnormality progresses OA [5, 6]. Other data support a role for chondro/osteophytes in joint restabilization after injury, and provide crucial insight into the time course and pathology of joint degeneration during OA development in the mouse [7]. From these backgrounds, we believe that meniscal or ligament dysfunction causes kinetic abnormalities in the knee joint and causes excessive stress on synovial membrane and articular cartilage. In our previous results, cartilage was significantly degenerated in rats in the ACL-T group, and osteophyte formation was also promoted to the marginal part of the cartilage. Although no statistically significant difference was observed, the synovial score was more prominent in the ACL-T group than in the CAJM group. Therefore, restoring knee joint kinematics after ACL injury may decrease mechanical stress and suppress secondary synovitis.

Importantly, secondary mechanical stress, such as joint instability, may also cause synovitis and OA progression. Most OA research has indicated that gait dynamic changes occur at severe stages of OA progression in animal models. In humans, high adduction movements in OA patients (lateral thrust) are also associated with abnormal joint instability, thereby contributing to compression stress [8]. OA patients feel joint instability during gait in self-reported joint instability evaluations. Therefore, even if there is no kinematic change, such as the angle, finer changes in articulation may be affected.

Conclusion

Using our novel CAJM model, we found that restoring the knee joint kinematics might influence intra-articular conditions such as synovitis and might inhibit OA in the future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval The Ethics Committee of Saitama Prefectural University approved all procedures (approval number: 27-6). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Informed consent Informed consent is not applicable for this study.

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