Osteoarthritis and Cartilage 24 (2016) 1918–1927

Osteoarthritis and Cartilage

Articular cartilage degeneration following anterior cruciate ligament injury: a comparison of surgical transection and noninvasive rupture as preclinical models of post-traumatic osteoarthritis

T. Maerz † ‡ §, M.D. Newton †, M.D. Kurdziel † ‡, P. Altman ||, K. Anderson ‡ ||, H.W.T. Matthew § ¶, K.C. Baker † ‡ *

† *Orthopaedic Research Laboratory, Beaumont Health, Royal Oak, MI, USA*
‡ *Department of Orthopaedic Surgery, Oakland University – William Beaumont School of Medicine, Rochester, MI, USA*
§ *Department of Biomedical Engineering, Wayne State University, Detroit, MI, USA*
|| *Department of Orthopaedic Surgery, Beaumont Health, Royal Oak, MI, USA*
¶ *Department of Chemical Engineering & Materials Science, Wayne State University, Detroit, MI, USA*

ARTICLE INFO

Article history:
Received 11 March 2016
Accepted 13 June 2016

Keywords:
Post-traumatic osteoarthritis
Anterior cruciate ligament rupture
Articular cartilage morphology
Contrast-enhanced μCT

SUMMARY

Objective: Post-traumatic osteoarthritis (PTOA) is commonly studied using animal models. Surgical ACL transection is an established model, but noninvasive models may mimic human injury more closely. The purpose of this study was to quantify and compare changes in 3D articular cartilage (AC) morphology following noninvasive ACL rupture and surgical ACL transection.

Methods: Thirty-six rats were randomized to uninjured control, noninvasive ACL rupture (Rupture), and surgical ACL transection (Transection), and 4 and 10 week time points ($n = 6$ per group). Contrast-enhanced micro-computed tomography (CE-μCT) was employed for AC imaging. Femoral and tibial AC were segmented and converted into thickness maps. Compartmental and sub-compartmental AC thickness and surface roughness (S_a) were computed. OARSI histologic scoring was performed.

Results: In both injury groups, zones of adjacent thickening and thinning were evident on the medial femoral condyle, along with general thickening and roughening of femoral and tibial AC. The posterior tibia exhibited drastic thickening and surface degeneration, and this was worse in Transection. Both injury groups had increased AC thickness and S_a compared to Control at both time points, and Transection exhibited significantly higher S_a in every tibial compartment compared to Rupture. Histologic score was elevated in both groups, and the medial femur exhibited the most severe histologic degeneration.

Conclusions: This is the first 3D quantification of preclinical AC remodeling after ACL injury. Both injury models induced similar changes in AC morphology, but Transection exhibited higher tibial S_a and a greater degree of posterior tibial degeneration. We conclude that AC degeneration is a time-, compartment-, and injury-dependent cascade.

Introduction

Anterior cruciate ligament (ACL) rupture is a common sporting injury and a major risk factor for the development of post-traumatic osteoarthritis (PTOA)[1,2]. Long-term clinical studies have indicated the risk for the development of PTOA after ACL rupture to be as high as 100%[3–6]. Surgical reconstruction represents the gold-standard treatment to alleviate pain and restore knee stability, but long-term studies have demonstrated that surgical reconstruction does not lower the risk for PTOA[6–10]. Initial joint trauma, chronic joint destabilization, chronic inflammation, and imperfect restoration of native joint kinematics following reconstruction are all known contributors to the onset of PTOA[1], but its full etiology remains largely unknown.

Animal models are frequently employed to study both the acute and chronic response to injury, and numerous knee PTOA models

* Address correspondence and reprint requests to: K.C. Baker, 3811 W Thirteen Mile Road, Royal Oak, MI 48073, USA. Tel: 1-248-551-9177; Fax: 1-248-551-0191.
E-mail address: kevin.baker@beaumont.edu (K.C. Baker).

http://dx.doi.org/10.1016/j.joca.2016.06.013
1063-4584/

have been described, including surgical destabilization[11–18], chemical injection[19], and mechanical loading[20–22]. In the rat, surgical transection of the ACL[13–18] is commonly-employed, but it fails to fully mimic the mechanism of human injury and introduces unknown but potentially confounding biological and biomechanical factors due to surgical trauma, the introduction of foreign bodies within the joint, and suturing of the joint capsule[23,24]. Furthermore, this model lacks biomechanical trauma that natively occurs during ACL injury. Therefore, it is unknown whether this animal model overestimates or underestimates the PTOA-related articular cartilage (AC) response following injury.

Models of noninvasive injury utilizing mechanical joint loading represent a more clinically-relevant mode of studying PTOA[23,24]. Only a few groups have, however, utilized noninvasive ACL injury models: Christiansen *et al.* utilized a tibial compression overload model in mice and demonstrated increased histologic score, increased serum-level cartilage oligomeric matrix protein (COMP) concentration, and synovial hyperplasia[21,22]. Onur *et al.*[25] utilized cyclic tibiofemoral compression to noninvasively induce ACL rupture and demonstrated histology-based progression of OA-like changes in the knee, but no quantitative assessment of AC morphology was performed. Xue *et al.*[26] developed a rotational model to noninvasively induce ACL rupture in the rat to study matrix metalloproteinase (MMP) expression following injury, but this study also did not perform quantitative assessment of AC. Recently, our group implemented and biomechanically characterized the tibial compression injury model in the rat[27]. This model results in a complete ACL injury with significant increases in anterior-posterior (AP) and varus laxity. Subsequent comparisons of subchondral and epiphyseal bone remodeling following this noninvasive rupture and surgical ACL transection demonstrated injury-dependent differences between the two models[28], but, to date, no studies assessing quantitative AC morphology in the setting of degeneration and remodeling have been performed.

The time- and injury-dependent cascade of AC remodeling remains poorly understood. The purpose of this study was to quantify and compare femoral and tibial AC degeneration and remodeling between surgical ACL transection and noninvasive ACL rupture as animal models of PTOA. We hypothesized that compared to surgical ACL transection, the biomechanical trauma of the noninvasive ACL rupture model would result in a greater degree of AC degeneration as evidenced by the assessment of AC morphology and histology.

Methods

Treatment groups and procedures

After institutional animal care and use committee (IACUC)-approval, 36 female Lewis rats aged 14 weeks, ~200 g (Charles River Laboratories, Wilmington, MA, USA) were randomly assigned to a control group (Control), noninvasive ACL rupture (Rupture), or surgical ACL transection (Transection) ($n = 12$ rats per group), and further randomized to either 4 or 10 week endpoints ($n = 6$ rats per group). Randomizations were performed using a computer algorithm. Sample size was determined based on previous studies of cartilage and bone changes in similar injury models. Randomizations were performed using computer software. Rats used in the present study are the same rats used in a previous investigation characterizing subchondral and epiphyseal bone remodeling following ACL rupture and surgical transection[28].

Approximately one hour prior to surgery, a non-steroidal, anti-inflammatory drug (Carprofen, 5 mg/kg) was administered subcutaneously to each animal. Anesthesia was induced by intraperitoneal ketamine and xylazine injections and maintained with 0.5–1.5% inhaled isofluorane. Subcutaneous buprenorphine injections were administered for post-operative analgesia.

Rats in the Rupture group were subjected to the following ACL injury protocol established by Maerz *et al.*[27] Briefly, rats were mounted to a materials testing system utilizing custom fixtures (Insight 5, MTS Systems, Eden Prairie, MN, USA) [Fig. 1(A)]. The rat was positioned prone on a heated bed with the right knee in 100° of flexion in a 3 mm deep trough to restrict medial and lateral translation. The right paw was mounted in 30° of dorsiflexion in a fixture constraining all motion except flexion-extension. After a preload, 10 preconditioning cycles, and a secondary preload ramp to 15N, a displacement of 3 mm was applied to the paw fixture at a rate of 8 mm/s. Rapid axial tibial displacement causes anterior tibial subluxation and subsequent failure of the ACL, as previously characterized[27]. ACL rupture was confirmed using an anterior drawer test [Fig. 1(B), (C)].

Rats in the Transection group underwent surgical transection of the ACL, as previously described[29–31]. Briefly, following the same anesthetic protocol described above, a midline knee incision was made using a number 15 scalpel blade followed by medial parapatellar arthrotomy and lateral patella subluxation. Using a Size 0 micro-scalpel (Biomedical Research Instruments, Silver Spring, Maryland, USA), a complete mid-substance transection of the ACL was performed, with care taken to avoid contacting cartilaginous surfaces. ACL rupture and PCL integrity were verified by anterior and posterior drawer testing, respectively. The knee was lavaged with sterile saline and the arthrotomy closed using non-resorbable sutures in an interrupted pattern. Skin was closed with resorbable suture.

In the Control group, rats received the same anesthetic and analgesic protocol described above, with no subsequent injury or surgery. Postoperatively, rats were allowed normal diet and *ad libitum* cage activity in a light/dark cycle facility until sacrifice at 4 or 10 weeks by CO_2 asphyxia. Rats were housed individually for 1 week postoperatively and then in groups of 4–6 until sacrifice.

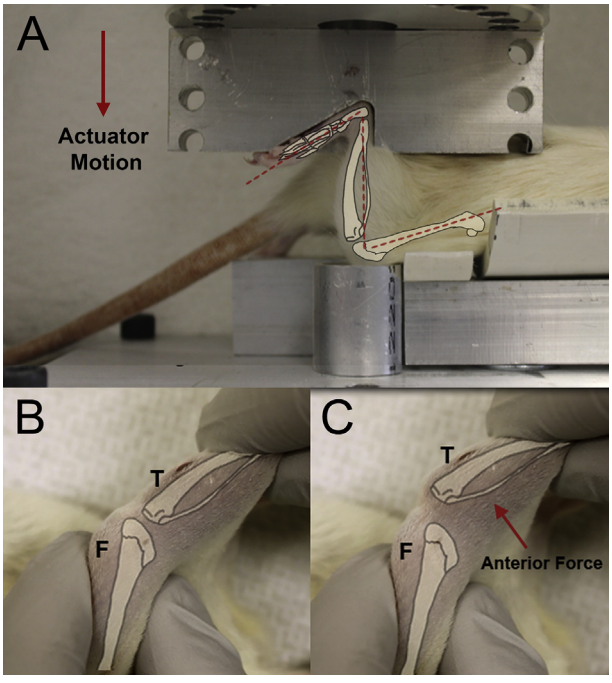

Fig. 1. Injury loading fixture and animal positioning to induce ACL rupture (A). Laxity was confirmed via an anterior drawer test (B, C). T = tibia; F = femur.