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Pain, motor and gait assessment of murine osteoarthritis in a cruciate ligament transection model

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SUMMARY

Objective: The major complaint of Osteoarthritis (OA) patients is pain. However, due to the nature of clinical studies and the limitation of animal studies, few studies have linked function impairment and behavioral changes in OA animal models to cartilage loss and histopathology. Our objective was to study surrogate markers of functional impairment in relation to cartilage loss and pathological changes in a post-traumatic mouse model of OA.
Method: We performed a battery of functional analyses in a mouse model of OA generated by cruciate ligament transection (CLT). The changes in functional analyses were linked to histological changes graded by OARSI standards, histological grading of synovitis, and volumetric changes of the articular cartilage and osteophytes quantified by phase contrast micro-computed tomography (μCT).
Results: OA generated by CLT led to decreased time on rotarod, delayed response on hotplate analysis, and altered gait starting from 4 weeks after surgery. Activity in open field analysis did not change at 4, 8, or 12 weeks after CLT. The magnitude of behavioral changes was directly correlated with higher OARSI histological scores of OA, synovitis in the knee joints, cartilage volume loss, and osteophyte formation.
Conclusion: Our findings link functional analyses to histological grading, synovitis, comprehensive three-dimensional assessment of cartilage volume and osteophyte formation. This serves as a reference for a mouse model in predicting outcomes of OA treatment.
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Introduction

Osteoarthritis (OA) is a joint disease characterized by articular cartilage loss, synovitis, subchondral bone remodeling and osteophyte formation[1]. Chronic disability imposes motor limitations on about 80% of OA patients and prevents about 25% from performing major activities of daily living[2]. The functional disability endured by patients with OA of the knee is among the highest, and comparable to heart disease, stroke, and depression[3]. The major complaint of OA patients is pain, which significantly affects motor function. Clinically, a diagnosis of OA is convincing when pain with joint movement correlates with pathology. However, many patients with pathologic and radiographic evidence of disease may not exhibit pain symptoms[4]. Mouse models of OA have recapitulated human joint pathology, but methods to assess function changes have not been well characterized.

Various OA mouse models are used to study disease mechanisms, test therapeutics, and implement behavior assays. Genetics, chemical, and surgical models are the main techniques used. The surgical model corresponds with the development of OA in humans after ligamentous knee injury, which makes it the most clinically relevant. The surgical destabilization of the medical meniscus (DMM) is a commonly used surgical model of OA[5]. Disruption of the medical meniscus leads to aberrant biomechanical loading patterns and a subsequent mild form of knee OA in mice. However, cartilage degeneration is predominantly on the medial condyle of the articular joint. In humans, although cartilage degradation is most apparent in the medial compartment, many combinations of compartments involvement are also present[6]. The location of disease is unpredictable due to differences in mechanism of injury and individual joint variation. For these reasons, cartilage changes occur in both the medial and lateral compartments[7,8]. Thus we chose to investigate an alternative cruciate ligament transection (CLT) model as described previously[9,10]. This model produces cartilage degradation that exhibits characteristics of human OA and disease progression on the microscopic level is well characterized.

Histopathology of the joint is commonly used to assess mouse OA models[11]. However, this method requires expertise and is prone

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to variability due to inter-observer subjectivity. More importantly, pain, motor dysfunction, and radiographic changes, and not histopathology, are used to clinically evaluate and diagnose OA in patients[1]. Recently, we applied novel methodology to image and quantify mouse articular cartilage using phase contrast, ultra-high resolution micro-computed tomography (μCT)[9], which combined the sensitivity to visualize the soft tissue of magnetic resonance imaging (MRI) with the resolution required to analyze small rodent models. While this method provided unparalleled quantification of mouse articular cartilage, it could not assess functional impairment.

Functional behavioral tests are frequently used in the neurological field[12,13]. They provide information about animal activity, learning, memory, sensitivity, pain, and motor function[14–22]. Here, we use a series of behavioral tests combined with histological and radiologic analysis to assess OA in a CLT mouse model. We show motor dysfunction, hyperalgesia, and altered gait using behavioral tests in a mouse model of OA induced by CLT. When compared to cartilage loss and pathological changes directly, these models would help unveil details about the clinical correlations between OA symptoms and radiographic disease. Taken together, pain, motor function, and imaging findings that are corroborated with histopathology would provide a comprehensive approach to study OA.

Material and methods

Animals

FVB/N mice were purchased from Jackson Laboratories (Bar Harbor, ME). All studies were performed with approval from the Baylor College of Medicine Institutional Animal Care and Use Committee (IACUC). All mice were housed under pathogen-free conditions. To avoid anxiety and possible behavior changes, every cage contained 4–5 mice. Mice had free access to feed and water. To avoid the effect of potential post-menopausal subchondral bone loss and possible differences in weight and activity, we used male mice in all our experiments. To avoid differences between different litters, we tested littermates in all experiments. And mice in different experimental groups are housed together.

CLT

Transection procedures are as previously described[9]. 8-week old male FVB/N mice were anesthetized and aseptically prepared for surgery. In the CLT group ($N = 17$-23 for each time point, three time points), the transection of the anterior and posterior cruciate ligament was made bilaterally instead of unilaterally as previously described. We chose to transect the limbs bilaterally because of two reasons: (1) From a local injury standpoint, the development of OA in a joint is similar regardless of a bilateral or unilateral transection and (2) it would be easier to interpret a functional change when mice were transected bilaterally. The success of the transection could be tested by valgus and varus laxity of the knee. In the sham group ($N = 17$–23 for each time point, three time points), no transection was made to either knee. Mice were allowed free cage activity immediately following recovery from surgery. Sutures were removed 2 weeks after surgery. Animals were randomly chosen from each group to perform the analyses.

Histology

Mice were euthanized and whole knee joints were fixed with 4% paraformaldehyde (Sigma–Aldrich) overnight in 4°C on a shaker. Whole knee joints were decalcified in 14% EDTA for 5 days in 4°C on a shaker. After dehydration by gradient alcohol and infiltration by xylene and paraffin, samples were embedded in paraffin. Paraffin-embedded joints were sectioned at 6 μm on a sagittal plane. Samples were stained with safranin O and fast green using standard protocols. $N = 8$ in each group.

Phase contrast μCT

Samples were prepared and analyzed as previously described[9].Whole joint knees were dissected and fixed with ruthenium hexamine tricholoride, glutaraldehyde, cacodylic acid, and osmium tetroxide based fixative buffers. The samples were then dehydrated and embedded in paraffin. Samples were scanned by Xradia (Xradia, California, US) microxCT. Data reconstruction was performed using Xradia software and articular cartilage and osteophytes were analyzed using TriBON software (RATOC, Tokyo, Japan). $N = 5$–6 in each group.

Hotplate nociception analysis

Mice were transferred to the room of analysis at least 30 min before experiment. Then, mouse was placed on the hotplate at 55°C one at a time (Columbus Instruments, Columbus, OH) [Fig. 1(a)]. The latency period for hind limb response (e.g., shaking, jumping, or licking) was recorded as response time. Each trial has a maximum time of 45 s. The mouse was removed from the hotplate immediately after a response was observed. $N = 12$–14 in each group.

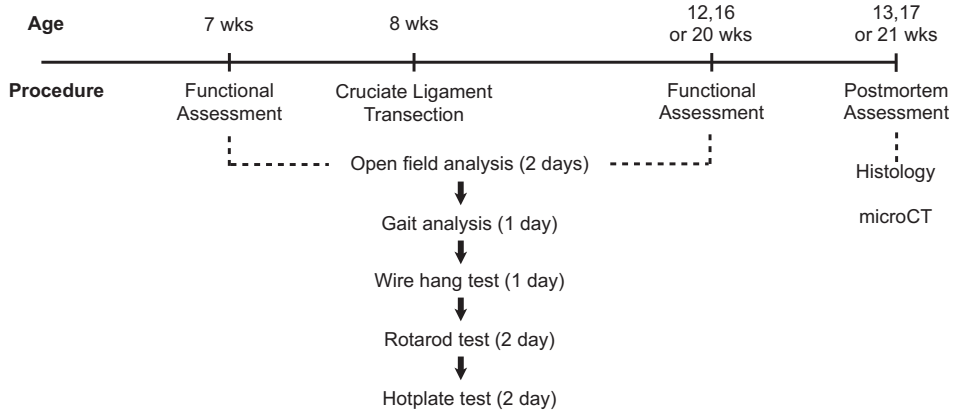

Fig. 1. Experiment scheme of functional change evaluation. We performed functional evaluation when the mice were 7 months old. This was followed by CLT and sham surgery. In different batches of mice, we waited 4, 8 or 12 weeks before another functional assessment. We performed postmortem assessment after the second and final functional analysis.

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