Establishment Of Surgical Destabilization Model Of Mouse Ankle Osteoarthritis

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Introduction: Incidence rate of ankle osteoarthritis (OA) is much lower than that of knee OA. Previous studies have described the reasons for the difference in susceptibility to cartilage destruction of the two joints from various study fields including anatomy, biomechanics, biochemistry and molecular biology; (1) the weight bearing contact area in ankle is narrower than that in knee, resulting in more force per square millimeter in the former, (2) the ankle acts mainly as a rolling joint with high congruency while the knee deals with higher shear forces by acting more as a rolling, sliding and rotating joint, (3) the middle and deep zones in ankle cartilage are thinner than those in knee cartilage, and (4) the extracellular matrix of the ankle has greater ability to withstand compressive loads. In addition, recent studies displayed different gene expression profiles of the two joint cartilages. However, in vivo experiments to confirm the association of these factors with the susceptibility have not been performed due to lack of a proper animal model of ankle OA.

To address this issue, we aimed to develop a surgical destabilization model of ankle OA in mice. In the present study, we created two different destabilization models, and compared the histological changes and scores of ankle joints in the two models.

Methods: 8-week-old C57BL/6 mice were used. Anatomy of tendons and ligaments of the ankle was shown in Figure 1. For the medial model, tibialis posterior tendon (TP), deltoid ligament (DL) and medial ankle capsule (MAC) were resected in 13 mice (7 males and 6 females). For the mediolateral model, TP, DL, MAC, anterior talofibular ligament (ATFL), calcaneofibular ligament (CFL), peroneus longus tendon (PL), peroneus brevis tendon (PB) and lateral ankle capsule (LAC) were resected in 12 mice (6 males and 6 females). Mice were sacrificed 8 weeks after the surgery, and the ankle joints were fixed in 4% paraformaldehyde for 24 hours. Whole joints were decalcified in EDTA for 5 days on a shaker. Joints were then embedded in paraffin and 4 µm frontal sections were taken through the entire joint. Slides were stained with Safranin-O and Fast green. Each ankle yielded 5-10 slides for scoring using a semi-quantitative scoring system recommended by Glasson in 2010 for the mouse knee OA model, where 0 represented normal cartilage; 0.5, loss of Safranin-O with no structural lesions; 1, small fibrillations without loss of cartilage; 2, vertical clefts down to the layer immediately below the superficial layer and some loss of surface lamina; 3, vertical clefts/erosion to the calcified cartilage extending to 75% of the articular surface. Histological scoring was made of the tibiotalar joints (sum of tibial and talar surface scores) and the subtalar joints (sum of talar and calcaneal surface scores). Results were expressed as the mean ± standard error of the mean (S.E.M.) of the maximum score.

Results: Representative sections of the normal, the medial model and the mediolateral model mice were shown in Figure 2. The tibiotalar joint cartilage was degenerated in both the medial and mediolateral models. In both models, OA lesion increased from center to medial part of the tibiotalar joint. Subtalar joint cartilage degeneration was more severe in the mediolateral model than in the medial model. The summed tibiotalar joint scores were 6.8 ± 0.9 in the medial model, and 6.9 ± 1.0 in
the mediolateral model, while the summed subtalar joint scores were 2.6 ± 1.3 in the medial model, and 4.8 ± 1.0 in the mediolateral model with significant difference between two groups. When we sequentially sacrificed the medial model mice at 2, 4, 6, 8, and 12 weeks after the surgery, the OA lesion gradually progressed throughout the period, while the cartilage destruction was complete by 6-8 weeks after the surgery.

**Discussion:** Considering that human ankle OA is mostly caused by posttraumatic factors, our mouse models may be relevant to a human ankle OA study. The medial model (resection of TP, DL and MAC) gradually accelerated degeneration of the tibiotalar joint, while the mediolateral model (resection of TP, DL, MAC, ATFL, CFL, PL, PB and LAC) accelerated degeneration of both the tibiotalar and subtalar joints. The present data imply that the two models may be useful for research on various subtypes of human ankle OA.

We had previously established surgical destabilization models of knee OA in mice, which have accelerated the research on molecular pathophysiology of knee OA. However, the surgical protocol is technically demanding and requires researcher training. In contrast, the present method for the ankle OA models is more simple and easier; it does not include intra-articular management such as the meniscus resection in the knee OA model. The simplicity of the protocol will reduce the risk of joint surface damage by the surgical procedure, and provide high reproducibility even for beginners. We believe that our ankle OA models will contribute to further understanding of ankle OA pathophysiology.

**Significance:** In the present study, we established two surgical destabilization models of ankle OA in mice for the first time: the medial model and the mediolateral model.
Figure 1.
The left ankle anatomy of a mouse. Red double line shows tendons and ligaments for resection in our destabilization models. Tibialis posterior tendon (TP) and deltoid ligament (DL) in the medial side (upper left panel). Anterior talofibular ligament (ATFL, upper right panel), calcaneofibular ligament (CFL, below left panel), peroneus longus tendon (PL, below right panel) and peroneus brevis tendon (PB, below right panel) in the lateral side.

Figure 2.
Coronal sections of the mouse ankle joint with Safranin-O staining. The normal (left panel), the medial model (middle panel) and the mediolateral model (right panel) 8 weeks after the surgical induction. Black arrows show OA lesion of tibiotalar joint. Yellow arrow shows OA lesion of subtalar joint.

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