Loading Rate-Dependent Subchondral Bone Changes in a Mouse Model of Post-Traumatic Osteoarthritis

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Significance: In this study we determined tibial compression loading conditions necessary to create either an avulsion fractures or a mid-substance ACL tear in a previously described mouse model of post-traumatic osteoarthritis. We determined the subchondral bone adaptation response following these two injury modes, and found differential trabecular bone loss at 7 days post-injury, indicating possible differences in the early biological response to the two different injuries.

Introduction: Post-traumatic osteoarthritis (PTOA) is commonly a long-term consequence of traumatic joint injury, with approximately 50% of individuals with ACL rupture or meniscectomy developing PTOA within 10-20 years. In our lab we have developed a novel, non-invasive method for inducing knee injury in mice in vivo (Christiansen et al., Arthritis Rheum, submitted), in which tibial compression is used to induce ACL disruption via avulsion fracture. While this mouse model is able to rapidly and reproducibly disrupt the ACL leading to PTOA, the clinical relevance of this injury is still not ideal, since it involves avulsion fractures rather than mid-substance tears. Clinically, most ACL failures are primarily due to mid-substance tears, with avulsion fractures occurring in less than 10% of adult patients. In contrast, avulsion fractures are commonly present in ACL ruptures in pediatric patients. The ability to induce both avulsion fractures and mid-substance tears would allow us to investigate possible differences in the biological and mechanical response to these different injury modes.

Mechanical loading-induced failures at slower loading rates have the greatest probability of creating bony avulsion failures, while fast loading rates are more likely to lead to mid-substance tears (Crowninshield et al., J Trauma, 1976). Therefore, we sought to modify the loading rate of our tibial compression-induced ACL injury model to allow us to induce mid-substance tears in addition to avulsion fractures. We also sought to quantify the subchondral bone adaptation response to the two different injury modes.

Determination of Loading Rate-Dependent Injury Modes

Methods: Mice were anesthetized using isoflurane inhalation, then the right leg of each mouse was subjected to tibial compression-induced knee injury. A single dynamic axial compressive load was applied to a target displacement of 1.7 mm to induce knee injury. Our previous study used a loading rate of 1 mm/s to induce knee injury, which induced avulsion fractures in 100% of mice. In order to determine a loading rate that would induce mid-substance tears, mice (n = 6 per group) were loaded at 10, 50, and 500 mm/s. Mice were sacrificed immediately after injury, and knees were imaged with micro-computed tomography (SCANCO μCT 35, Bassersdorf, Switzerland).

Results: All loading rates created injuries of the ACL, determined by qualitative assessment of decreased stability in the injured knee, in particular increased anterior-posterior displacement and internal-external rotation. Knee injuries induced at loading rates of 10 or 50 mm/s resulted in avulsion fractures that could be detected by μCT similar to those observed previously for injuries created at 1 mm/s (Fig. 1). In contrast, for injuries created at a loading rate of 500 mm/s there were no avulsion fractures observed in injured knees, indicating injury to the soft tissues exclusively.

Subchondral Bone Adaptation Response to Different Injury Modes

Methods: The right knees of mice were injured at 500 mm/s loading rate as previously described. Mice were sacrificed 3, 7, or 14 days after injury (n = 4 per time point), and knees were analyzed with micro-computed tomography (SCANCO μCT 35, Bassersdorf, Switzerland). Trabecular bone parameters at the distal femoral epiphysis were quantified, and compared to previously reported results for mice injured at 1 mm/s.

Results: For mice injured at 1 mm/s (avulsion fracture) we observed a rapid loss of trabecular bone after injury, reaching a minimum near 7 days post-injury (-40% bone volume/total volume (BV/TV) compared to Baseline Control), followed by a partial recovery of trabecular bone volume by 14 days. In contrast, for mice injured at 500 mm/s (mid-substance tear) we observed a more gradual loss of trabecular bone after injury (-24% BV/TV at day 7, -32% BV/TV at day 14 compared to Baseline Control). The two groups were statistically different at day 7 (p < 0.05), while at the other time points they were not different. In the contralateral limb of both groups we observed a 7-10% loss of BV/TV at day 7 compared to Baseline Control, although these differences were not statistically significant.

Discussion: Similar to previous studies, we found that mechanical loading at a very high loading rate (500 mm/s) produced a mid-substance tear of the ACL, rather than avulsion fractures that were observed at slower loading rates. This is an important observation, because it gives us the ability to create an even more clinically-relevant knee injury in our established mouse model of PTOA. It also gives us the ability to investigate the differential response of musculoskeletal tissues to these different injury modes. This is an important tool that could be used to determine mechanistic pathways that lead to articular cartilage and subchondral bone degeneration following traumatic joint injury.

Using the two different loading rate-dependent injury modes, we investigated the subchondral bone adaptation response to avulsion fracture vs. mid-substance tears in our mouse model of PTOA. We found a differential bone response in the injured knee 7 days after injury, while at 3 days and 14 days post-injury, and at all time points in the contralateral knee there were no significant differences. This indicates that there may be differences in the biological response to the two different injury modes during the first week after injury. In mice that were injured at 1 mm/s loading rate, which created an avulsion fracture of the ACL, there was a more rapid and higher magnitude bone response by 7 days. This result is reasonable, since ACL disruption via avulsion fracture involves damage to the subchondral bone.

Figure 1: Transverse micro-computed tomography slice of a distal femur from a mouse injured at 1 mm/s loading rate. The presence of bone fragments in the joint space indicates injury via avulsion fracture of the ACL.

Figure 2: Subchondral bone adaptation at the distal femoral epiphysis following knee injury. Injury at 500 mm/s loading rate (mid-substance tear) resulted in a different subchondral bone response at 7 days post-injury than injury at 1 mm/s (avulsion fracture).