Bone Morphological Changes Correlate with Reduction in PTA after Articular Fracture in the MRL/MpJ Mouse

Kelly A. Kimmerling, MEng1, Bridgette D. Furman, B.S.², Tyler J. Vovos, BS1, Janet L. Huebner, MS³, Virginia B. Kraus, MD, PhD², Farshid Guilak, PhD², Steven A. Olson, MD².
¹Duke University, Durham, NC, USA, ²Duke University Medical Center, Durham, NC, USA, ³Duke Molecular Physiology Institute, Durham, NC, USA.

Disclosures: K.A. Kimmerling: None. B.D. Furman: None. T.J. Vovos: None. J.L. Huebner: None. V.B. Kraus: 5; Bioiberica. F. Guilak: 3A; Cytex Therapeutics Inc.. 4; Cytex Therapeutics Inc.. 7; Elsevier Ltd. S.A. Olson: 3B; Bioventis. 5; Synthes USA, Bioventis.

Introduction: Post-traumatic arthritis (PTA) occurs after joint trauma, such as articular fractures, but the mechanism is not well-understood [1, 2, 3]. While PTA can occur rapidly after moderate to severe articular injuries, not every patient will go on to develop this condition. Currently, there are no effective screening methods to determine who is at risk. Characterizing degenerative changes in joint tissues following articular fracture in an animal model provides an opportunity to study the pathology of joint injury and the development of PTA. In previous studies, we have shown that C57BL/6 mice exhibit arthritic changes following articular fracture, whereas the MRL/MpJ strain is protected from PTA [1, 4]. The objective of this study was to identify differences in acute joint pathology and degeneration in C57BL/6 and MRL/MpJ mice as measured in the articular cartilage, synovium, and periarticular bone following articular fracture.

Methods: All animal procedures were performed in accordance with an IACUC-approved protocol. Male C57BL/6 and MRL/MpJ mice (n=83) were subjected to an articular fracture at 16 weeks of age using an established model [5]. Six mice from each strain did not receive a fracture and served as pre-fracture controls. Mice were sacrificed at 0, 1, 7, 14, and 56 days after fracture (n=6-11 per strain per time point). The left (fractured) and right (non-fractured) limbs were harvested, formalin fixed and scanned with microCT to assess bone morphology in the tibial epiphysis and metaphysis and femoral condyles. Histology sections (FFPE, 8µm thick in coronal plane) of all limbs were assessed for cartilage degeneration in the lateral and medial femoral condyles (LF, MF) and lateral and medial aspects of the tibial plateau (LT, MT) using a modified Mankin score, synovial inflammation using a modified synovitis score with semi-quantitative scales, and osteophyte score [1,5,6,7,8]. Parametric analyses were performed for bone morphological measures and histological assessment.

Results: Mankin scores of cartilage degeneration were significantly greater in the C57BL/6 strain compared to the MRL/MpJ strain in both the lateral femur (p=0.0204) and the medial tibia (p=0.0015). No strain differences were seen in the lateral tibia, where the fracture occurred, or in the medial femur. Synovial inflammation did not differ by strain; however, there was a significant increase in the fractured limb compared to the control limb at 1 and 2 weeks post-fracture (p<0.05). Osteophyte scores did not show any trends, but were present in both strains at 7 and 14 days and were not present at 56 days. Subchondral bone thickening was significantly increased in the C57BL/6 mice compared to the MRL/MpJ mice in the medial femur (p=0.0278) and the medial tibia (p=0.0077), but not on the lateral side.
Bone morphological changes in response to fracture were significantly different between the two mouse strains. In the fractured limbs, bone mineral density (BMD), bone volume (BV), and bone fraction (BV/TV) in both the tibial epiphysis and metaphysis were significantly greater (p<0.0015) in the MRL/MpJ strain compared to the C57BL/6 strain (p<0.001). However, in the femoral condyles, both BMD and cancellous bone fraction (BV/TV) were significantly increased in the C57BL/6 strain compared to the MRL/MpJ strain (p=0.0001).

Correlations of the histological parameters with the bone morphological parameters showed that in tibial metaphyseal region, the Mankin total joint score negatively correlated with both the BMD (rs=-0.453, p=0.0298) and BV/TV (rs=-0.437, p=0.0370) in the MRL/MpJ strain, but did not correlate with any bone parameters in the C57BL/6 strain. The synovitis total joint score in the fractured limb positively correlated with both the BMD (rs=0.658, p=0.0001) and BV/TV (rs=0.662, p=0.0001) in the C57BL/6 strain, but not in the MRL/MpJ strain.

**Discussion:** Analysis of both histologic and bone morphologic measures in this study suggest that the differences between the C57BL/6 and MRL/MpJ strains may be associated with bone morphological changes. Despite the lack of difference in synovial inflammation between strains, cartilage degradation and bone parameters were significantly different, which may account for the altered healing response after articular fracture [4]. MRL/MpJ mice are reported to have increased levels of TGF-β1, which may contribute to the enhanced bone response following fracture found in this study [9]. Interestingly, synovitis in the C57BL/6 mice was associated with greater bone changes. Previous reports have shown that C57BL/6 mice have elevated levels of pro-inflammatory cytokines IL-1 and TNF-α following joint injury [1]. An increased local inflammatory environment may contribute to altered bone morphology and subsequent degenerative changes in the joint tissues. The difference in these arthritic profiles indicates that there may be a benefit to focusing first on fracture healing, then following up with suppression of the pro-inflammatory environment that leads to subsequent degradation of the joint. Clinically, surgical restoration of the articular surface is the only treatment for articular fractures. To date, there is no method of identifying patients that are at risk for developing PTA. In addition to measures of joint pathology, serum and synovial fluid from both strains of mice will be analyzed for biomarkers. The comparison of early imaging or biochemical biomarkers between mice strains and humans, which are predictive of PTA, may be useful in assessing clinical risk in articular fracture patients.

**Significance:** By characterizing degenerative changes in the C57BL/6 and MRL/MpJ strains, key factors that contribute to the development of PTA can be identified. By understanding what drives disease progression, potential screening methods may be developed to identify patients at high risk of developing PTA.
Figure 1
Cartilage degeneration assessment using a modified Mankin joint score for each quadrant shown for 7, 14, and 56 days post-fracture. Quadrants include the lateral femoral condyle (LF), lateral tibial plateau (LT), medial femoral condyle (MF), and medial tibial plateau (MT). Mean and standard deviation reported (n=6-11 per strain per time point). * denotes significance between strains by a repeated measures ANOVA (p=0.0204 in LF; p=0.0015 in MT).
Figure 2

Correlations between Mankin total joint score, synovitis total joint score, and bone fraction (BV/TV) of the tibial metaphysis for both the MRL/MpJ and C57BL/6 strains. Values are displayed as a rank order within strain for each outcome measure (n=21-28 per strain). R, values indicate Spearman correlation coefficient for each strain. (A) Mankin correlated with BV/TV ($r_s$=0.437, p=0.0370) in the MRL/MpJ strain only. (B) Synovitis correlated with BV/TV ($r_s$=0.662, p=0.0001) in the C57BL/6 strain only. (C) Representative images of the tibial metaphysis for the C57BL/6 strain at each time point. Scale bar is 0.5mm. (D) Representative images of the tibial metaphysis for the MRL/MpJ strain at each time point. Scale bar is 0.5mm.