Modification of the Gut Microbiome Protects Against Age-Related Subchondral Bone Changes in Mice

Cameron X. Villarreal[1], Noah A. Jasinkiewicz[1], Chongshan Liu[2], Erika L. Cyphert[2,3], Christopher J. Hernandez[2,3], Deva D. Chan[1]
[1]Purdue University, West Lafayette, IN. [2]Cornell University, Ithaca, NY. [3]University of California San Francisco, San Francisco, CA.
villarcc@purdue.edu

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INTRODUCTION: Osteoarthritis (OA) is a debilitating joint disease hallmarked by tissue degeneration, joint pain, and altered mobility and function. OA phenotypes are highly heterogeneous, and the disease has been associated with various risk factors and comorbidities, among these prior joint injury, diabetes and other metabolic syndromes, and aging. Notably, there is a profound overlap in these OA-related factors and alterations of the microbe communities residing in the gut. The gut microbiome has been implicated in various multi-organ crosstalk [1, 2], including measures of bone quality [3-5] and the progression of post-traumatic OA in mice [6, 7]. However, it is unclear whether prolonged manipulation of the gut microbiome alters the development and trajectory of primary OA. C57Bl/6J mice – an inbred mouse background commonly used in orthopedic research – are known to develop spontaneous OA with age, with an incidence of 80% by 16 months of age and advancing severity with age [8]. In this study, we evaluate subchondral bone morphology in aged mice with and without continuous dosing of antibiotics to manipulate the microbiome. We hypothesized that gut microbiome manipulation through prolonged antibiotic treatment will mitigate subchondral bone changes associated with age-related, primary OA.
METHODS: Under an institutionally approved protocol (Cornell University, protocol #2019-0078), antibiotics (1.0 g/L Ampicillin + 0.5 g/L Neomycin, Amp+Neo) were administered to male C57Bl/6J mice in drinking water from wean to 22 months of age. The Amp+Neo group (n = 7) and age-matched untreated control mice (n = 7) were housed in the same facility and were permitted *ad libitum* access to water and chow. At the endpoint of the experiment, mice were humanely euthanized, and the left stifles were promptly dissected for formalin fixation. Fixed hindlimbs were stored in 70% ethanol at 4°C until microcomputed tomography (Bruker SkyScan 1272) with 0.5 mm aluminum filter, 6µm isometric voxel size, 0.4 step size, and 2 averages. Images were reconstructed using manufacturer's software prior to manual segmentation of the proximal subchondral plate and proximal epiphyseal trabecular space in the tibiae (3D Slicer). Reconstruction parameters were Gaussian smoothing of 2, ring artifact correction of 8, and beam hardening correction of 20. Segmented regions of interest were visualized and analyzed using standard subroutines (Dragonfly) for subchondral plate thickness and porosity and trabecular bone volume fraction (bone volume divided by tissue volume), number, and thickness. Continuous dosing groups were compared to age-matched controls using unpaired t test, under assumptions of equal variance. Data are reported as mean ± standard deviation, and p-value threshold of $\alpha = 0.05$ was used to evaluate statistical significance.
RESULTS: Proximal tibial subchondral bone was imaged with microCT and successfully segmented, enabling quantification of bone parameters. Subchondral plate (SP) and epiphyseal trabecular (Tb) bone parameters were compared between Amp+Neo and control mice (Figure 1). Subchondral plate thickness (SP.Th) significantly differed (p = 0.036) in Amp+Neo mice (0.14 ± 0.02 mm) compared to control (0.18 ± 0.04 mm). Subchondral plate porosity (SP.Po) was 0.54 ± 0.08 in Amp+Neo and 0.42 ± 0.06 in control (p = 0.008). Trabecular number (Tb.N) was significantly different (p = 0.002) – 11.7 ± 3.5 in Amp+Neo compared to 4.7 ± 3.3 in control – but not thickness (Tb.Th) or bone volume fraction (BV/TV).
DISCUSSION: Microbiome modification with continuous Amp+Neo dosing showed a protective effect from aging-related subchondral plate changes that were observed in control aged mice. Primary OA is associated with both bone and cartilage changes in both human patients and preclinical models. In this study, mice were aged to 22 months of age (equivalent to ~65 years old in humans [9]), ~6 months past the age when at least 80% of the mice would be expected to show incidence of spontaneous OA [8]. Subchondral plate thickness was lower in Amp+Neo mice than controls. Subchondral plate sclerosis occurs before joint space narrowing, marking earlier degenerative changes in human knee OA [10]. Similarly, in a senescence accelerated mouse (SAM) model of aging related OA, subchondral bone thickness was greater than in senescence resistant mice [11]. With spontaneous OA in a guinea pig model, subchondral plate demonstrated increased thickness and lower porosity [12]. In this work, we also observed greater subchondral plate porosity with continuous antibiotics dosing. Interestingly, in a small study of primary OA patients, higher subchondral plate porosity in the tibial plateau distinguished patients with hypertension or type 2 diabetes mellitus from those with no comorbidities [13]. Thus, while subchondral bone may be protected against age-related changes, gut microbiome manipulation could be expected to impact other complex, systemic mechanisms and physiologies yet to be uncovered. We focused herein on the proximal tibiae, where the greatest bone changes were expected with murine spontaneous OA. However, it remains to be seen whether these effects exist in other compartments of the joint and whether protection against histological OA will be significant. Continuing work includes evaluation of bone parameters in tibial metaphyseal and all femoral compartments, as well as OARSI scoring of joint pathology. Future work will incorporate sex as a biological variable with the inclusion of age-matched female mice and altered timing for Amp+Neo dosing.
SIGNIFICANCE: Better understanding of the cross-system effects of microbiome changes on the synovial joint – and their role in modifying the development and progression of primary osteoarthritis will provide insight into the relationship between gut and joint health. Modification of the microbiome has potential as an affordable and readily implementable approach to reducing risk of osteoarthritis and its comorbid conditions.
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