The Aryl Hydrocarbon Receptor (AhR) antagonist BAY2416964 prevents age-related muscle and cortical bone loss in female C57Bl/6 mice

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INTRODUCTION: The aryl hydrocarbon receptor (AhR) is a cytosolic nuclear hormone receptor proposed to mediate the effects of the tryptophan metabolite kynurenine (KYN) and a variety of other ligands in vivo [1]. KYN increases with age in mice and humans, is associated with musculoskeletal frailty in humans, and has been identified as a likely causative factor for musculoskeletal decline, as administration of exogenous KYN in young adult mice promoted muscle atrophy, BMSC senescence, osteoblast and osteoclast dysfunction, and bone loss that together mimic a musculoskeletal aging phenotype [2-6]. This raises the possibility that therapeutically targeting the AhR could be beneficial for musculoskeletal health. A novel AhR inhibitor called BAY 2416964 (BAY) is currently in clinical trials as an anti-cancer agent. We recently reported results from preliminary studies demonstrating that BAY improved muscle endurance and grip strength in young (4 month old mice), with positive effects more pronounced in female as compared to male mice [7], but its effects on the aging musculoskeletal system have not yet been reported. The goal of the current study was to test whether BAY treatment could abrogate musculoskeletal decline in aging female mice.

METHODS: All experiments followed NIH guidelines and were approved by the Institutional Animal Care and Use Committee at Augusta University. Female C57BL/6 mice (18 months old; n=40) were obtained from the NIA rodent colony. Two mice died prior to completion of studies due to unknown causes, and two additional mice were excluded for non-study-related aging pathologies. Mice were treated with vehicle (VEH; Ethanol 10/Solutol 40/Water 50) or BAY2416964 (BAY; 30 mg/kg, Targetmol T10270) via daily oral gavage 5 d/wk for 8 wks (VEH: n=18, BAY: n=20). Skeletal muscle endurance was assessed via hang-time testing and forelimb skeletal muscle grip strength was assessed via grip strength meter (Bioseb BIO-GS3) at 0 (baseline, pre-treatment), 4, and 8 wks of treatment; one mouse was lost from muscle assessments due to non-compliance with testing protocols. DXA scans (Kubtec Parameter 3D x-ray cabinet and Digimus software) were performed at 0 and 8 wks of treatment to assess body composition and bone density. Skeletal muscles (TA, EDL, Soleus, Quads) and long bones were collected at sacrifice. Muscle fiber size was measured from H&E-stained sections of the TA. The left tibia and femur from each mouse were used for static and dynamic histomorphometry, respectively. Femurs were scanned by micro-computed tomography (Skyscan 1272, 9-micron resolution) to measure cortical and trabecular bone architecture. Primary BMSC were isolated from the right tibia and femur from each mouse and were cultured in BMSC or osteogenic medium as previously described [2, 6] and stained at D7 and D14 to assess BMSC colony formation and D21 to assess mineralized matrix production by alizarin red staining. Serum P1NP (bone formation; IDS #AC-33F13) and TRAcP5b (bone resorption; IDS #SB-TR103) were quantified via ELISA, and inflammatory cytokines were measured using a Legendplex panel (Mouse Inflammation 13-Plex). Groups were compared by unpaired t-tests.

RESULTS: Body mass and body composition (%lean mass and %fat mass, as measured by DXA) were unchanged over the 8-week study in either treatment group (p>0.200). With regards to skeletal muscle, VEH-treated animals demonstrated a significant age-related decline in forelimb grip strength during the study, represented as a negative percent change in grip strength between 0 and 8 weeks of treatment, but this decline was prevented by BAY treatment (p <0.0001; Figure 1A). BAY-treated mice had significantly greater grip strength (p<0.0001) and grip strength normalized to body mass (p=0.0004) as compared to VEH-treated mice at 8 wks of treatment. Hang time testing of muscle endurance and analysis of muscle fiber size in the TA revealed no differences between groups.

With regards to bone, while DXA analyses did not demonstrate any differences in whole body BMD or site-specific BMD in the femoral mid-diaphysis or lumbar vertebrae (p>0.172), higher-resolution microCT measurements of cortical bone architecture in the left femur demonstrated that BAY-treated mice had a subtle but statistically significant increase in cortical bone thickness (p=0.025) and cortical bone volume (p=0.043) as compared to VEH-treated mice (Figure 1B). Other bone architectural properties, including trabecular bone properties in the distal femoral metaphysis, were not different between groups (p>0.205). Histomorphometry studies in cortical and trabecular bone compartments are ongoing. Interestingly, while we observed an increase in the serum bone formation marker P1NP in younger BAY-treated animals [7], serum remodeling and inflammatory markers were largely unchanged in the aged BAY-treated mice. However, consistent with the observed increase in cortical bone mass, BMSC isolated from bone marrow of BAY-treated mice showed enhanced production of mineralized matrix via alizarin red staining on D21 in culture (Figure 1C). Moreover, BMSC isolated from the BAY treated mice demonstrated a propensity for enhanced proliferation, as colony formation visualized by crystal violet staining was enhanced in cultures derived from the BAY-treated mice at both D7 and D14 of culture (Figure 1C).

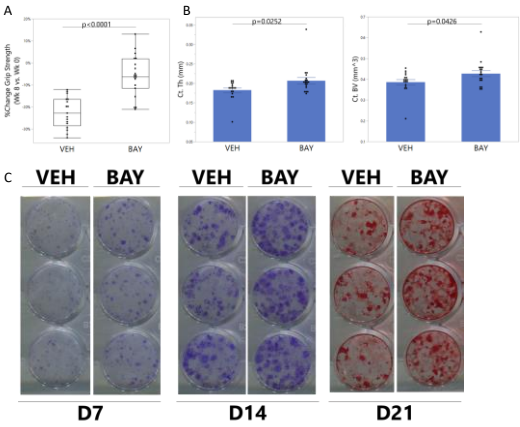

Figure 1: A) Grip strength declined from 18-20 months of age in VEH-treated, but not BAY-treated, mice. B) Cortical bone architecture was subtly but significantly greater in BAY-treated mice. C) BMSC from BAY-treated mice demonstrated enhanced proliferation by crystal violet staining, and produced more mineralized matrix as seen by alizarin red staining in osteogenic culture.

DISCUSSION: These data suggest that inhibiting AhR via BAY2416964 may prevent age-related declines in bone and muscle function. While grip strength was preserved in BAY-treated mice, no changes were seen in hindlimb muscle fiber size. Further investigations into muscle quality, such as exploring NMJ (neuromuscular junction) morphology and mitochondrial functionality, are ongoing to define the mechanism of the muscle preservation in the BAY-treated mice. The administration of BAY demonstrated a positive impact on cortical bone, although no such effect was observed on trabecular bone; this discrepancy could be attributed to the relatively low abundance of trabecular bone present in the distal femoral metaphysis in 18-month-old female mice at the onset of treatment. Analyses of trabecular bone architecture in a more robust site, such as the lumbar vertebrae, as well as experiments testing the efficacy of initiating treatment at a younger age, are warranted in future studies. SIGNIFICANCE/CLINICAL RELEVANCE: These data support AhR as a therapeutic target for improving musculoskeletal health during aging.

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