Traumatic Fracture Healing in Geriatric Mice Shows Decreased Callus Formation with Associated Deficiencies in Cell Cycle and Immune Cell Function

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Purpose: Geriatric fragility fractures often display reduced healing, which leads to substantial morbidity, mortality, and cost to the patient as well as cost to society. An improved understanding of the fundamental biological deficiencies of geriatric fracture healing will provide mechanistic insight into this disordered healing and provide rationally selected therapeutic targets. The purpose of this study was to develop a truly geriatric fracture model based on chronological age.

Methods: Traumatic, prestabilized, transverse, 3-point-bend tibia fractures were created in 5-month-old (m/o) mature young adults and 25-m/o geriatric C57BL/6 mice from the National Institute of Aging (NIA) colonies. Fracture calluses were harvested at 0, 5, 10, 15, 20, 25, 30, and 40 days post fracture (dpf) for analysis with micro-CT (vivaCT 40), histomorphometry (Safranin-O, Masson's Trichrome), immunohistochemistry (IHC; anti-Proliferating Cellular Nuclear Antigen [PCNA]), quantitative real-time polymerase chain reaction (qPCR), and microarray. Microarray data was uploaded into DAVID bioinformatics for gene set enrichment analysis (GSEA) and Cell Type Enrichment Analysis for Microarray Data (CTen) to evaluate cell populations.

Results: Geriatric mice produce a significantly reduced healing response. As early as 10 dpf, geriatric mice produced less cartilage and total callus. This blunted response in conjunction with delays in endochondral ossification led to diminished bone and callus formation throughout fracture healing (Figs. 1A and B). Despite the reduced total cartilage and bone produced by geriatric mice, the ratio of cartilage and bone relative to total callus produced was not significantly altered (Fig. 1C). Reflective of this, the relative expression of chondrogenic and osteogenic genes was similar. Staining for PCNA revealed decreased proliferation in mesenchymal stem cells in 25-m/o mice. Global gene expression analysis revealed differences in aged and young healing profiles most strikingly related to cell cycle and immune function. Cell cycle genes are highly upregulated in young mice at 0, 5, and 10 dpf, but upregulated in geriatric mice at 20 dpf.

Conclusion: Overall, the fracture-healing template appears to be intact in geriatric mice, much as it is in geriatric humans, but callus expansion is significantly hindered with additional temporal delay. Gene expression based analyses of cell populations demonstrate a reduced proliferative capacity of progenitor cells, which also highlighted differences in genes related to the cell cycle and immune response. Further exploration of the difference in progenitor and fracture callus cell populations and the healing environmental milieu is warranted to identify therapeutically targetable deficiencies in geriatric fracture healing.

- The FDA has not cleared this drug and/or medical device for the use described in this presentation (i.e., the drug or medical device is being discussed for an "off label" use). For full information, refer to page 600.

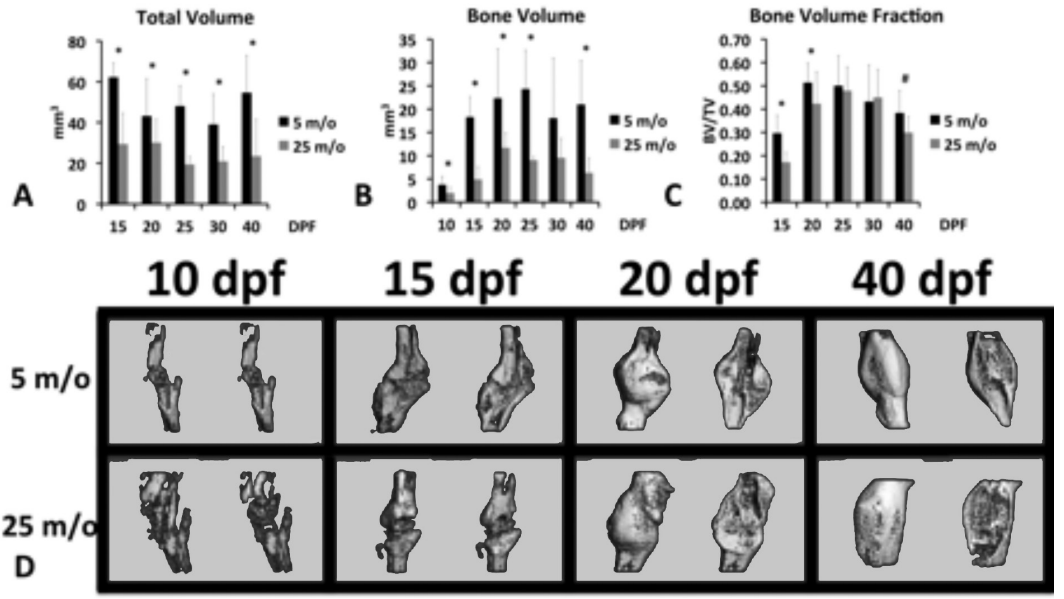

Fig 1. Micro-CT measurements of (A) total volume, (B) bone volume, and (C) bone volume fraction. (D) Representative whole callus and midcoronal plane images.

See pages 99 - 147 for financial disclosure information.