

Anti-Sclerostin Antibody Injections Promote Intervertebral Disc and Vertebral Bone Structure in Old Mice

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SIGNIFICANCE/CLINICAL RELEVANCE: Anti-sclerostin antibody injections promote vertebral bone structure in old mice¹ and could additionally be repurposed to augment the structure of aged intervertebral discs (IVD) that are prone to IVD degeneration and back pain.

INTRODUCTION: Aging is a major factor in IVD degeneration² which is a leading cause of lower back pain (LBP) and is macroscopically characterized by IVD height loss from extracellular matrix (ECM) breakdown and cellular maturation and apoptosis.³ There is a paucity of effective therapies for the IVD and the age-related replacement of IVD resident cells that regulate the ECM suggests that therapies may need to target molecular pathways involved in cellular differentiation and activity. Wnt signaling regulates nucleus pulposus cell differentiation and ECM maintenance⁴ is suppressed with aging and IVD degeneration⁵ suggesting its relevance in IVD homeostasis. Deletion of Wnt signaling cellular membrane receptor LRP5 induces IVD degeneration by reducing Wnt signaling⁶ and injection of anti-Wnt signaling inhibitors of sclerostin and dkk1, which bind LRP5, promote IVD structure in young-adult mice.(ref) Anti-sclerostin antibody (Scl-Ab) is an FDA-approved bone anabolic that activates Wnt signaling. Therefore, we hypothesized that injection of anti-sclerostin antibody will activate Wnt signaling to promote bone vertebral bone structure and improve the IVD structure in both ages.

METHODS: Female C57Bl/6 mice of either 6 months (adult) or 20 months (aged) of age were subcutaneously injected 2x/week for 6 weeks with either PBS (Vehicle, Veh, n=9/age), 25 mg/kg of monoclonal sclerostin antibody (Scl-Ab, n=9/age), 25 mg/kg of monoclonal dkk1 antibody (Dkk1-Ab, n=9/age) or 18.75/6.25 mg/kg of Scl-Ab/Dkk1-Ab (n=9/age). L1-3 were harvested and submerged in formalin for 48 hours, transferred to 70% ethanol, and scanned by microCT (Scanco) at a resolution of 9.6 μ m. Outcomes for the cortical bone included cortical thickness (Ct.Th) and tissue mineral density (TMD), and outcomes for trabecular bone included bone volume fraction (BV/TV) and volumetric bone mineral density (vBMD). For the IVD, the XY axis and YZ axis of the microCT reconstruction were used to analyze IVD height. All experiments were IACUC approved. Data were compared by a two-way ANOVA and a p-value of <0.05 was considered statistically significant.

RESULTS: Aging reduced cortical thickness (Ct.Th), trabecular bone volume fraction (BV/TV) and IVD height by 11%, 44% and 9.7%, respectively (Fig. 1A-D). Injection of Scl-ab increased cortical thickness (Ct.Th) by 35-55% and trabecular bone volume fraction (BV/TV) by over 130-170% in 6- and 22-month old mice (Fig. 1A, B). However, the injection-induced increase in Tb.BV/TV was less in old mice compared to young-adult mice (interaction, p<0.001). By contrast, scl-ab only increased IVD height in the aged group (interaction, p<0.001; Fig. 1C, D). Analysis for the dkk1 and the combinatorial injections is currently underway.

DISCUSSION: Injection of scl-ab increased the structure of cortical and trabecular bone in both ages, with a reduced benefit by Scl-ab in trabecular bone volume fraction of old mice. By contrast, scl-ab did not affect IVD height in young-adult mice and increased IVD height in the old mice. Our previous data indicated that injection of Scl-Ab in young-adult mice increases IVD height⁷ but we used the higher resolution technique of histology to make that determination and our current data will be corroborated using histology. Overall, these data suggest that the benefits of Scl-Ab to the structure of the IVD are not limited to the vertebral bone and may restore structural properties of the IVD lost with advanced aging.

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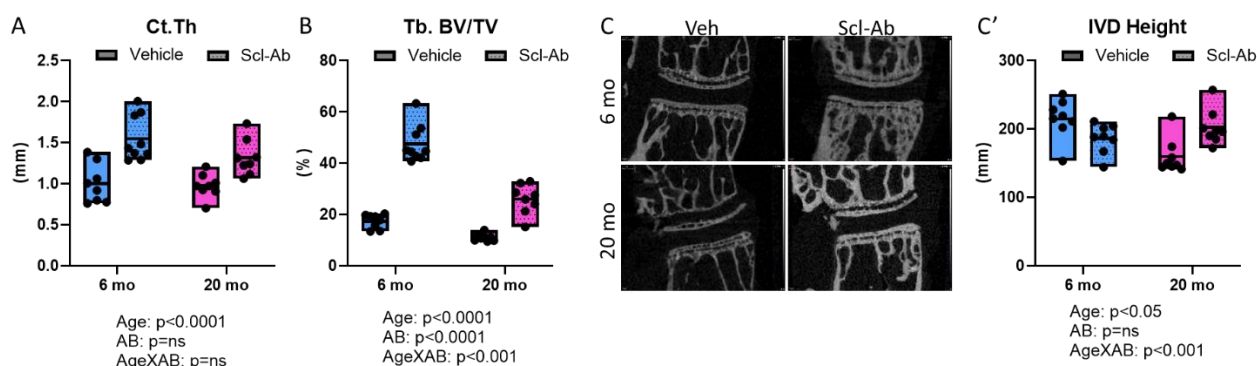


Figure 1. (A) Cortical thickness of 6 month and 20 month old vehicle and Scl-ab injected mice. (B) Trabecular bone volume fraction of 6 month and 20 month vehicle and Scl-ab injected mice. (C) μ CT representative images of motion segments. (C') Quantified IVD heights taken from μ CT images of 6 month and 20 month vehicle and Scl-ab injected mice. Scale bar: 100 μ m.