Intravenous, short-term tail vein injections of raloxifene enhances the axial mechanical stiffness of the intervertebral disc
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SIGNIFICANCE/CLINICAL RELEVANCE: More direct injections of raloxifene to improve the mechanical stiffness of the intervertebral disc (IVD) may facilitate usage of a lower dosage and limit the iatrogenic effects of raloxifene. In mice, this approach facilitates the use of tail disc degeneration models.

INTRODUCTION: IVD degeneration is the leading cause of back pain, which incurs a cost of approximately $100 billion in the United States 1,2. Pre- and post-menopausal women exhibit greater IVD degeneration than age-matched men and experience pain more frequently and at higher intensities 3-5. Raloxifene is an FDA-approved selective estrogen receptor modulator (SERM) prescribed to postmenopausal women to reduce the risk of vertebral fracture, that also relieves self-reported back pain 6 and increases IVD height 3-5. We find that subcutaneous (SQ) injection of raloxifene to mice improves lumbar IVD/bone structure but not the same in tail IVDs 7-9. In addition, long-term injection of raloxifene increases the risk of thromboembolism and stroke 10, 11. We hypothesized that intravenous, short-term tail vein injections of raloxifene increase the structure and strength of tail IVDs and vertebrae.

METHODS: This study was approved by IACUC. 2.5-month-old female (C57Bl/6J, n=7-9/sex/group) mice were SQ injected daily 5x/week for 6 weeks (6wks_SQ) with PBS (VEH) or 0.5 mg/kg of raloxifene hydrochloride (SIGMA, RAL). This SQ 6-week dosing regimen was aligned with established protocols in the bone research field 12. A separate cohort of mice, matched by age and sex, received intravenous tail vein (TV) injection of PBS or raloxifene (n=5/treatment) 5x/week for 4 weeks (4wks_TV) or daily for 4 days (4day_TV). Tissues were harvested at the end of the injection trial. CC9-11 was designated for Safranin-O & elastin receptor alpha (er-α) immunohistochemistry staining. CC7-CC9 IVD was designated for gene expression analysis by qPCR. CC6-CC7 IVD was designated for mechanical testing. CC6 was imaged by μCT to determine vertebral bone structure. For the trabecular analysis, the growth plate was used as a landmark and trabecular bone analysis consisted of 30 consecutive slices. For IVD mechanical testing, spine segments (bone-IVD-bone) were subjected to 20 sinusoidal loading cycles, alternately applying ± 0.8N of tension and compression at a frequency of 1Hz. IVD morphology and histological score was determined from the histological images. A Student’s-test compared the effect of treatment, with statistical significance defined at a p<0.05.

RESULTS: In our previous study, we found that in young-adult females, SQ injection of raloxifene reduces lumbar IVD degeneration scores, increases IVD compressive stiffness, and promotes vertebral bone volume fraction 7. However, daily, SQ injection of raloxifene for 6 weeks imparts no structural or mechanical benefit to tail IVDs or vertebrae (Fig. 1A-C). By contrast, TV injection of raloxifene for a shorter duration of 4 weeks increased the vertebral trabecular bone volume fraction in the tail by 27% (Fig. 1A). This shorter (6wk vs 4 wk) and more direct (SQ vs TV) approach increased IVD compressive stiffness by 48% (Fig. 1B) and reduced IVD degeneration scores by 64% (Fig. 1A, A'). In gene expression, 4 weeks of TV injection of raloxifene upregulated er-α by 9-fold, aggrecan by 4-fold and collagen 2 by 6-fold in tail IVDs (data not shown). Surprisingly, a much shorter dosage strategy of 4 days of TV similarly increased most of the same metrics tested with the 4-wks-dosing regimen (Fig. 1A-C'). Investigations are ongoing on the effect of raloxifene in males and aged mice.

DISCUSSION: Our findings suggest that a shorter duration (4wks vs 6wks) and more direct approach (TV vs SQ) of injecting raloxifene increased the tail vertebral bone volume fraction, increased IVD compressive stiffness and reduced IVD degeneration score. While 6-week SQ raloxifene injection contributes to the increase in the lumbar vertebral bone volume fraction and IVD compressive stiffness 7, it does not exert a significant effect on tail structures. This may be attributed to a lower magnitude of complex mechanical loading in tail IVDs compared to lumbar IVDs, which compounded by the natural avascularity of IVDs, may limit the transport of raloxifene when administered via subcutaneous injection. Therefore, the limited bioavailability of raloxifene of ~25% 10 encourages a more direct delivery method for rodent studies. Nevertheless, it is imperative to acknowledge the existing limitations of raloxifene, including its association with thromboembolism and stroke, which restrict its application in patients with related medical conditions. Overall, these data suggest that a short duration and more direct injections of raloxifene can improve the structure and strength of the IVD and bone.

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Figure 1. (A) Trabecular bone volume fraction (Tb. BV/TV) of tail vertebral bone and, (B) compressive stiffness of tail IVD, (C) histological images and (C') IVD degeneration scoring of young-adult female mouse injected with PBS (vehicle, VEH) or raloxifene (RAL) for 6 weeks subcutaneously (6wks_SQ), 4 weeks intravenously (4wks_TV) and 4 days (4days_TV) intravenously. *: p<0.05, Scale bar: 100 µm.