Assessing the Effect of PROTAC 753b Senolytic on Intervertebral Disc Aging

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INTRODUCTION: Low back pain (LBP) is one of the most common musculoskeletal conditions requiring medical care and contributing to patient impairment and disability costing an estimated $100 billion annually in the US alone.[1] Age is the leading risk factor for intervertebral disc degeneration, which is characterized by progressive degradation of matrix proteoglycans (PGs) in the nucleus pulposus (NP).[2] Cellular senescence is a major driver of aging and senescent cells are known to accumulate in the nucleus pulposus and aging intervertebral disc (IVD) tissues.[3] Cellular senescence is defined by irreversible cell cycle arrest and an altered cell phenotype, the senescence-associated secretory phenotype (SASP), which is characterized by tissue specific factors that both reduce tissue function and propagates the SASP to neighboring tissues. Senescent cells are known to be resistant to apoptotic cell death, up-regulating "senescent cell anti-apoptotic pathways" (SCAPs) such as the BCL/BAX pathways.[4] PROTAC 753b is an engineered peptide senolytic designed to knock-down the expression of Bcl-x_L/Bcl2, a prominent SCAP of the NP.[5] The properties of 753B suggest that it can function at a lower concentration, with greater specificity and duration such that many of the harmful side effects of the PROTACS can be avoided. The objective of this study is to assess whether systemic 753b can reduce age-related degenerative changes of the intervertebral disc in an aging mouse model.

METHODS: 20 mice of equal mixed sexes and 16 months of age were treated with 753b was delivered intra-peritoneally 2x/week for 2 weeks per cycle with an interval of two weeks of rest between the cycles for 6 months, the duration of the experiment. Mice were divided into 1 of 4 groups: aged untreated mice, male or female and 753b-treated aged mice, male or female. A cohort of 10 untreated young mice 6 months of age (5 male and 5 female) were used as comparatives. Mice were euthanized at 22 months of age (after 6 months of treatment), the spines harvested and tissues processed for histology (paraffin embedding), protein or RNA isolation. Serial sections were stained with Safranin O/Fast Green and scored by 3 independent investigators according to Melgoza et al.[6] Western blots were carried out for AGG degradation products including neo-epitopes DIPEN (MMP) and NITEGE (ADAM-TS). Gene expression was analyzed using two-step RT-PCR for key genes: p16 and p21 (senescence regulators), Aggrecan (AGG; NP matrix), MMP13 (a SASP mediator of AGG degradation), and TIMP1&3 (regulators of MMP activity). Results are expressed as means +/- S.D, where $p < 0.05$ and Tukey post-hoc analysis.

RESULTS: In this pilot study (n=5 per group), histopathological analysis of Safranin O/Fast Green-stained sections revealed that both male and female aged mice have significantly degenerated IVDs than young mice (Fig 1A&B). Aged male mice showed 4-point decrease in histological degenerative disc score ($p < 0.05$) with 753b treatment, while female mice merely trended lower. Immunohistochemistry revealed a partial recovery of aggrecan deposition in the NP of aged male mice treated with 753b, but no significant change in the aged females.(Fig 1C&D) Similarly, western blot demonstrated a decreased of the DIPEN neo-epitope (indicative of MMP activity) in aged male mice treated with 753b, but no significant change in NITEGE neo-epitope detection (resulting from ADAMTS aggrecance activity). 753b treatment of aged females resulted in no significant changes in the presence of either aggrecan neo-epitope. RT-PCR showed that aggrecan gene expression was unchanged by 753b treatment in both male or female aged mice. Interestingly, RT-PCR revealed no change in p16 expression with 753b treatment, but a significant decrease in p21 in aged female mice only.

DISCUSSION: PROTAC 753b is designed to traffic BCLX and BCL2 to E3-ligase ubiquination and degradation.[5] This potentiates the BAX-driven release of cytochrome-c and the initiation of apoptosis by caspase-driven mechanisms in affected cells.[3] This activity has been shown to have senolytic activity in several cell types and tissues in both mouse and humans. Histolopathological sccoring and western blot analysis indicate that 753b can partially ameliorate age-related IVD degeneration in aged male mice, in part by preserving nucleus pulposus aggecan. This improvement in IVD histopathological score in male mice was not accompanied by decreases in p16 or p21 expression, key drivers of cell senescence. This indicates that 753b-mediated therapeutic effects on IVD is due to systemic effect of the drug or senescent disc cells are driven by pathways other than p16/p21. The inability of 753b to completely block IVD degeneration indicates that other SCAPs may still be active in IVD senescent cells and/or that senescence only accounts for part of the degeneration observed with aging.[5] Further work is needed to verify that 753b has reached the IVD and is causing the degradation of BCL2 before further conclusions can be drawn with respect to the ability of 753b to slow IVD age-related degeneration locally or systemically. The results of this pilot study show a sex-dependent response to 753b treatment that warrants further investigation.

SIGNIFICANCE/CLINICAL RELEVANCE: Our study provides proof of concept the therapeutic potential of using senolytics to treat IDD and LBP.

REFERENCES: [1] Manchikanti L et al. (2014) 17 Suppl 2:3-10. doi: 10.1111/ner.12018; [2] Davies C et al. (2014) Physiother Theory Pract. 30(6):399-408; [3] Silwal P (2023) Biomolecules 13(4):686; [4] Hu L et al (2022) Front Cell Dev Biol. 10:822816; [5] Negi A (2022) Chembiochem 23(12):e202100689; [6] Melgoza IP et al. (2021) JOR Spine 4(2):e1164.

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LEGENDS: Fig. 1: Effect of PROTAC 753b on intervertebral disc aging. (A&B) Histopatholigical scores in (A) male and (B) female mice; (C&D) Immunohistochemical staining for Aggrecan in (C) male and (D) female mice. There are 5 animals per group. The asterix represents a p value less than 0.05. The only significance shown is that between the vehicle and 753b-treated animals.