Superoxide dismutase 2 deficiency accelerates age-related spontaneous intervertebral disc degeneration in mice

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INTRODUCTION: The etiology of intervertebral disc (IVD) degeneration is multifactorial and involves aging, mechanical stress, genetics, and other external stimuli. Emerging evidence suggests that oxidative stress contributes to IVD degeneration. Although studies suggest that oxidative stress plays a role in IVD degeneration by triggering inflammation and cellular senescence, the precise underlying mechanisms remain not fully understood. Superoxide dismutase 2 (SOD2), an antioxidant enzyme located within the mitochondria, acts to regulate the generation of reactive oxygen species (ROS), which predominantly originates from the mitochondria themselves. The degree of oxidative stress is determined by the balance between ROS production and antioxidant enzyme activity. We have shown that the expression of SOD2 and oxidative stress markers in human IVD increases with the progression of IVD degeneration and aging.1 However, it remains unclear whether an imbalance between SOD2 and mitochondrial ROS in the IVD causes disc degeneration. As such, the purpose of this study was to investigate whether SOD2 deficiency in the IVD accelerates age-related spontaneous disc degeneration in a mouse model.

METHODS: All animal studies were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Tokai University. We have previously generated chondrocyte-specific SOD2 conditional knockout (cKO) mice (Col2a1-Cre; SOD2loxPloxP) using a cre-loxP system.2 Lumbar and caudal IVD of 6-, 12-, and 18-month-old SOD2 cKO and SOD2loxPloxP (WT) mice were analyzed (n=6 each). L1/2 and L2/3 discs were prepared for fresh frozen sections in the axial plane and evaluated for intracellular and mitochondrial ROS by dihydroethidium (DHE) and MitoSOX fluorescence staining, respectively. Mean fluorescence intensity (MFI) in the nucleus pulposus (NP) and annulus fibrosus (AF) was quantified using Image J. L3/4-L6/S1 and Co2/3-Co4/5 discs were prepared for 10% neutral buffered formalin-fixed paraffin-embedded sections in the sagittal plane, and morphological changes were evaluated by hematoxylin/eosin and safranin-O/fast green staining. Histological scoring was performed using the ORS spine histopathological scoring system in a blinded manner.3 Multiple sets of data were confirmed normally distributed via Shapiro–Wilk test and analyzed with 2-way ANOVA, followed by Tukey's post hoc test via GraphPad Prism. A p value <0.05 was considered statistically significant.

RESULTS: Successful deletion of SOD2 in SOD2 cKO mice was verified by immunohistocchemistry in the entire IVD including NP, AF, and cartilaginous endplate cells in the lumbar and coccygeal spine. MFI of DHE in NP cells was 1.66-fold higher in 12-month-old (12M) WT mice (P = 0.0074) and 1.27-fold higher in 18-month-old (18M) WT mice compared to 6-month-old (6M) WT mice. MFI of DHE in AF cells was 1.55-fold higher in 12M WT mice (P = 0.0238) and 1.63-fold higher in 18M WT mice (P = 0.0090) compared to 6M WT mice. MFI of MitoSOX in NP cells was 1.31-fold higher in 12M WT mice and 1.69-fold higher in 18M WT mice (P = 0.0259) compared to 6M WT mice. MFI of MitoSOX in AF cells was 1.56-fold higher in 12M WT mice (P = 0.0058) and 2.02-fold higher in 18M WT mice (P < 0.0001) compared to 6M WT mice. Furthermore, MFI of DHE and MitoSOX in SOD2 cKO mice were significantly higher than those of WT mice at all ages. Histological analysis showed that SOD2 deficiency accelerated age-related spontaneous disc degeneration in both lumbar and coccygeal IVD (Figure 1). Specifically, the WT mice showed only mild disc degeneration at 18M (average score; lumbar: 10.2, coccygeal: 9.6), whereas the SOD2 cKO mice displayed significantly expedited disc degeneration with moderate degeneration occurring at 18M (average score; lumbar: 16.9, coccygeal: 17.2).

DISCUSSION: Previous studies have not yet elucidated whether oxidative stress accelerates disc degeneration or is just a product of aging. Our study demonstrated that ROS in the IVD increases with aging and IVD-specific SOD2 deficiency accelerates disc degeneration through dysregulated ROS turnover. The fact that elevated ROS in the IVD (observed in 12M WT mice) precedes histologically evident disc degeneration (observed in 18M WT mice) suggests that oxidative stress is a pivotal cause of disc degeneration. Further research is ongoing to assess the potential of targeting oxidative stress as a treatment for IVD degeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: Targeting mitochondrial superoxide may provide a novel treatment approach for IVD degeneration.


Figure 1. Representative histological outcomes showing hematoxylin/eosin and safranin-O/fast green staining for lumbar (A) and coccygeal (B) IVD and their respective ORS Spine histopathological score.