Prolonged Heavy Metal Exposure Triggers Cellular Senescence And Mechanical Allodynia In The Lower Back

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INTRODUCTION: IVD degeneration is a leading cause of chronic low back pain. It is a multifactorial disease, characterized by the degeneration of IVD, inflammation, endplate sclerosis, cell death and hyperinnervation of nociceptive nerve fibers leading to persistent pain. This innervation consists of sensory fibers arising from neurons localized in the Dorsal Root Ganglions that express high affinity neurotrophins receptors known to transmit nociceptive information (pain) from the periphery towards the central nervous system. There is growing evidence suggesting a relationship between heavy metal exposure and intervertebral disc degeneration (IVDD). While the exact mechanisms are still under investigation, several studies have documented altered concentrations of metal elements—including heavy metals—in degenerated disc tissues. For example, research measuring element levels in intervertebral discs has found statistically significant differences in metals such as copper, iron, cadmium, mercury and lead between healthy and degenerated discs. The altered levels and relationships among these elements (for instance, correlations between zinc and lead) hint at the possibility that environmental heavy metal exposure may upset the disc's metabolic balance, ultimately contributing to degeneration. The heavy metal tungsten has been increasing in demand for use in manufacturing and recently, medical devices, as it imparts flexibility, strength, and conductance of metal alloys. Given the surge in tungsten use, our population may be subjected to elevated exposures. We previously determined that when mice are exposed to tungsten [15 ppm] in their drinking water, it bioaccumulates in the intervertebral disc (IVD) and vertebrae tissues leading to disc degeneration (ref). This study was performed to determine the mechanism(s) and physiological effects following tungsten exposure in the IVD.

METHODS: *In vitro* experiments: Bovine nucleus pulposus (hNP) (passages P0–2) were cultured as micropellets in disc cell medium supplemented with tungsten in the form of sodium tungstate [0, 1, 5 and 15 ug/mL] or H_2O_2 [100 μM]. QPCR was performed to measure changes in the expression of matrix, inflammatory and pain markers. Assessment of oxidative stress was performed using H2DCFDA to measure ROS cellular levels. DNA damage was measured using γH2AX fluorescence. Cellular senescence was determined by measuring changes in β-galactosidase activity. Co-culture experiments were performed with bNPs and RAW 264.7 cells seeded in chambers in a 6-well plates. Medium supplemented with either 0 (control) or 1,5 or 15 ppm tungsten was used and cells were incubated for 72 hrs. RAW 264.7 cell mRNA was collected and measured for markers of M1 and M2 polarization.
In vivo experiments: IHC for macrophages using MOMA staining was performed on the lower back IVDs from C57BL/6 mice on drinking water supplemented with 15 ppm tungsten for 4 weeks or tap water as control. Von Frey performed on mice following 6 months of tungsten exposure. All animal experiments were approved by the McGill University Animal Care Committee (Montreal, Quebec, Canada).

RESULTS: Tungsten and H_2O_2 significantly increased the expression of *IL1B*, *TNFA*, *NGF* and *COLII* in NP cells ($p < 0.01$, n=4). On the contrary, the matrix protein aggrecan (*ACAN*) was downregulated suggesting a degenerative phenotype ($p < 0.01$, n=4). Oxidative stress was found to be increased in a dose-dependent manner in NP cells ($p < 0.05$; n=4). Interestingly, there were no changes in DNA damage; however, we did observe an induction of cellular senescence following tungsten exposure. In our co-culture experiments, RAW macrophages demonstrated a dose-dependent increase in markers for M1 (IL1B, CD80, CD86; $p < 0.05$; n=4) versus M2 (IL10, ARG, CD206; n=4) polarization. Tungsten significantly increased the infiltration of macrophages at lumbar IVD sites ($p < 0.05$, n=4). Mice exposed to tungsten for 6 months in their drinking water showed significant changes in mechanical allodynia (Fig. 1; $p < 0.05$, n=5).

DISCUSSION: Tungsten, widely used in industry and medicine, has long been considered inert but is now emerging as a toxicant of concern. Our preliminary data show that tungsten persists in bone and IVDs after exposure, where it induces hallmark degenerative changes, disc height loss, proteoglycan depletion, fibrosis, matrix remodeling, upregulation of catabolic enzymes, inflammatory cytokines, and neurotrophic factors associated with pain. The mechanism for tungsten-induced changes in the IVD appear to be Redox driven, inducing disc cell senescence leading to the release of inflammatory factors that can drive immune and neuronal cell activation resulting in allodynia. Limitations in this study remain, for instance, it is unclear if removal from tungsten exposure allows the IVD to regenerate. It is also unknown if tungsten exposure can exacerbate the degeneration of an IVD that is already degenerate, and if other mechanisms aside from Redox potential exist that tungsten uses to induce disc degeneration and pain.

SIGNIFICANCE/CLINICAL RELEVANCE: We provide evidence that chronic tungsten exposure induces disc degeneration and pain behaviour in mice and may play a role in disc degeneration disease.

REFERENCES: European Cells and Materials Vol. 41 2021 (pages 517-530)

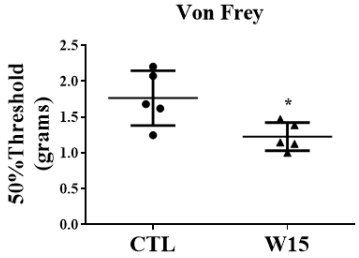

Figure 1. Effect of chronic tungsten exposure on back pain. Mice were given water with or without 15 ppm tungsten for 6 months. Von Frey was used to measure changes in mechanical allodynia in the hind paws.