Systemic Monocyte Depletion Attenuates Injury-Induced Intervertebral Disc Degeneration

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INTRODUCTION: Intervertebral disc (IVD) degeneration (DD) is a major contributor to low back pain, one of the most disabling conditions worldwide [1]. Despite its high prevalence, treatments that repair or heal the IVD are limited due to inadequate understanding of disease mechanism. DD is a complex multi-factorial process, ultimately leading to structural and functional failure. This degenerative cascade initiates local tissue inflammation and immune cell recruitment [2]. Importantly, the role of inflammation in the pathophysiology of DD is poorly understood, including the limited understanding of IVD-immune cell crosstalk that contribute to tissue degeneration. Although previous studies have identified both resident and recruited macrophages / monocytes in the healthy and degenerated IVDs [3-4], the mechanism of how circulating monocytes contributes to tissue inflammation and DD remains elusive. The aim of this study was to assess the effects of systemic depletion of circulating monocytes during puncture injury-induced DD. We hypothesized that clodronate-mediated monocyte depletion will lead to decreased inflammatory and degenerative response during acute IVD puncture injury.

METHODS: C57BL/6 mice at 3 months of age received intraperitoneal injection of either clodronate liposome or saline placebo liposome (200 µL/animal, FormuMax Clophosome). At 24 hours following treatment, animals were anesthetized, and fluoroscopy-guided percutaneous caudal IVD puncture procedure was performed (total of 16 animals used; n=8/injection group; n=4/sex/group). The Co7/8 and Co8/9 IVDs were sequentially punctured with a 27-gauge needle inserted through the skin into the center of the disc using a custom sleeve to standardize the depth of puncture. The needle was held in position for 30 seconds for NP depressurization. Following the procedure, animals in clodronate and placebo groups were evenly distributed for 2 study endpoints: at 1 week or 4 weeks post-injury (n=2/sex/injection group/time point; 1wpi, 4wpi). Uninjured IVDs adjacent to the punctured IVDs were assessed for the response to aberrant loading (Co6/7, Co9/10) and uninjured Co5/6 and Co10/11 served as internal controls. In order to maintain systemic macrophage depletion, animals received additional 100 µL of clodronate or placebo injections every 3-4 days until the study endpoint for both treatment groups. Upon euthanasia, fluoroscopic images of caudal spine at Co5-Co11 levels were obtained for disc height index (DHI) assessment. IVDs from Co5-Co7 were individually dissected for RNA isolation and gene expression analysis, and caudal spine of Co7-Co11 were grossly dissected for histological analysis. All procedures involving the use of animals in this study were approved by the IACUC.

RESULTS: At 1wpi, puncture injury to placebo treated IVDs showed a loss in DHI relative to control IVDs. Clodronate treated animals at 1wpi had significantly less DHI loss in puncture (Co8/9, $p<0.05$) and adjacent (Co9/10, $p<0.01$) IVDs compared to placebo group (Figure 1A). By 4wpi, DHI loss was comparable between clodronate and placebo groups at all levels. Histological analysis of placebo treated punctured IVDs at 1wpi showed increased cellular accumulation in the peripheral AF region and increased Safranin-O staining (indicative of sulfated glycosaminoglycans) compared to the clodronate treated punctured IVDs (Figure 1B). Clodronate treatment reduced evidence of cellular accumulation in AF periphery at 1wpi. At 4wpi, there was no cellular accumulation in the AF periphery in placebo treated punctured IVDs, however there were decreased NP cells and loss of NP/AF tissue boundary compared to 1wpi. Clodronate treated IVDs showed greater number of NP cells, and a more intact NP/AF boundary compared to placebo treated injury group at 4wpi (Figure 1 C). Clodronate treatment in punctured IVDs significantly decreased gene expression of macrophage markers (*Adgre1*, *Ccr2*), pro-inflammatory cytokine (*Il1b*), and inflammatory chemokine (*Mcp1*) compared to placebo treated punctured IVDs at both 1wpi and 4wpi (Figure 1D). While clodronate treated punctured IVD showed significantly decreased *Mcp1* expression levels compared to placebo treated punctured IVD, the average fold change was significantly higher in the clodronate treated punctured IVDs compared to placebo treated uninjured internal control IVDs at both 1wpi (fold change 5.22±1.70) and 4wpi (fold change 2.54±0.30; data not shown).

DISCUSSION: Our findings demonstrate that systemic depletion of monocytes attenuates injury-induced DD. Clodronate-mediated depletion of circulating monocytes led to delayed onset of disc height loss in both puncture and adjacent IVD levels, decreased cellular accumulation at the site of injury, promoted persistence of NP cells, and decreased macrophage marker gene expression levels following injury. These findings suggest that recruited monocytes are directly involved in mediating the degenerative response of IVD to injury. Given that previous studies identified C-C chemokine receptor 2 (*CCR2*) as a crucial receptor for monocyte recruitment to the injured IVD [5-6], we assessed the changes in *Mcp1* expression levels, gene encoding for CCL2, a potent ligand for CCR2 [7]. Interestingly, while clodronate-injection led to reduction in macrophage markers and *Il1b* inflammatory cytokine to baseline levels, only partial reduction was observed in *Mcp1* levels, suggesting that macrophage-IVD crosstalk is necessary to potentiate monocyte chemotactic axis at the injury site. This study also demonstrates for the first time that prolonged monocyte depletion can be achieved using clodronate injections for the study of DD. We acknowledge that 1 week and 4 weeks timepoints capture acute injury responses, and a longer time point is needed to evaluate the role of circulating monocytes in chronic DD and injury-induced IVD tissue fibrosis.

SIGNIFICANCE/CLINICAL RELEVANCE: The current study highlights the importance of crosstalk between systemic immune cells and IVD during the progression of DD. Specifically, our findings help position circulating systemic monocyte and CCL2 as potential immune-modulatory therapeutic targets to treat or prevent DD.

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IMAGES AND TABLES:

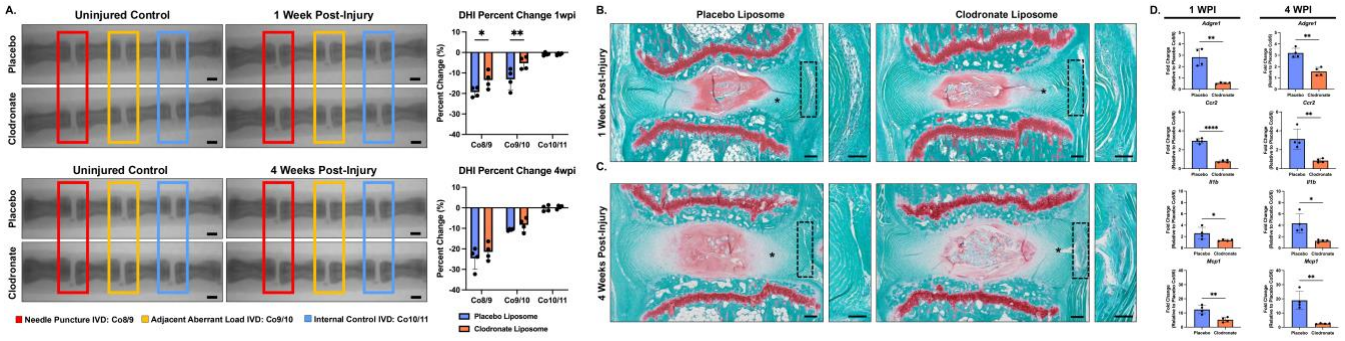

Figure 1. Assessment of intervertebral disc injury response following systemic macrophage depletion. A) Fluoroscopic images and percent changes in disc height index between clodronate- and saline placebo-injected groups at 1 week or 4 weeks post-injury. B-C) Safranin-O/Fast Green histological images of punctured Co8/9 IVDs of clodronate- and saline placebo-injected groups at 1 week or 4 weeks post-injury. D) Gene expression analysis of *Adgre1*, *Ccr2*, *Mcp1*, and *Il1b* in punctured IVDs isolated from clodronate- and saline placebo-treatment groups at 1 week or 4 weeks post-injury.