Bisphosphonates Nanoparticles Loaded Microparticles for Immunomodulatory Osteoarthritis Therapy.

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INTRODUCTION: Osteoarthritis (OA) is a painful disease of the joint with no disease modifying drug. OA affects over 600 million people globally and has an annual socioeconomic burden of around $200 billion in the United States. Traditionally, OA is considered a disease of the wear and tear of the joint, but recent evidence indicates chronic inflammation in the synovium as a key driver of disease progression and pain. This inflammation is usually marked by infiltration and activation of macrophages in the joint which secrete pro-inflammatory mediators that accelerate joint degeneration. Bisphosphonates (BPs) are a class of drugs known for their high macrophage affinity and chondroprotective benefits. However, at elevated doses, BPs prove to be cytotoxic. Moreover, being hydrophilic and anionic, BPs often suffer rapid joint clearance, limiting their clinical application. Additionally, intra-articular delivery of BPs often results in off-target delivery to bone, as BPs exhibit a strong affinity for bone. To overcome these limitations, we have developed a novel drug delivery system, bisphosphonate nanoparticles loaded microparticles (NiM) for OA therapy. This formulation can effectively target macrophages and sustainably deliver zoledronate (Zol), a potent third-generation BP having anti-inflammatory properties to modulate inflammation while minimizing cytotoxicity. The NiM system, controlled drug-release, reduced cytotoxicity, reprogrammed macrophage to reparative phenotypes, inhibited NF-κB activation and reduced reactive oxygen species. In this work we evaluated the therapeutic efficacy of a single intraarticular injection of NiM in treating OA in a clinically relevant mouse model.

METHODS: NiM formulation and characterization: Zol was complexed to calcium to form nanoparticles (CaZol-NP) via a reverse emulsification technique. CaZol-NP were loaded into polymeric microparticles (MP) of polyethylene glycol-polylactic-co-glycolic acid (PEG-PLGA; Sigma Aldrich) via coaxial flow phase separation technique, generating nanoparticle loaded microparticles (NiM). The release kinetics of Zol from NiM, at predetermined time intervals under neutral and acidic pH conditions (7.4 and 5.0, 5 mM PBS) were determined by measuring Zol concentration spectrophotometrically at 215 nm. Mouse model of OA: A non-surgical mouse model of OA was established by rupturing the anterior cruciate ligament (ACL) of the right knee by applying 60 cycles of 12 N axial compressive load at 54 N/sec with a 10 sec hold at 0.5 N between cycles (Instron 5966). This process repeatedly leads to ACL rupture (n=10) as examined by blind joint laxity test. Additionally, age matched sham animals were subjected to axial compressive load of 0.5 N. Two weeks after ACL-rupture (n=4; age 10-12 weeks; male mice were used for the this initial investigation) macrophage from the synovium and inguinal lymph node was isolated and analyzed for macrophage number and phenotype using flow cytometer. Furthermore, mice that received ACL-rupture injury were divided into three groups (n=4-6) and received a single intraarticular injection of 1) saline 2) free Zol (25 µM) 3) NiM (loaded with 25 uM Zol). Pain assessments: All groups were longitudinally analyzed for pain sensitivity for 7 weeks. A hindpaw pain assessment was conducted as per Chaplan up-down method using von-frey filaments and normalized pain withdrawal threshold was determined. Mice were assessed for changes in stride length using gait assessment. Hindpaw of the mice were stained with washable paint and footprint were recorded on a white paper runway. The distance between consecutive footprints of the same limb was then manually measured to determine the stride length. Endpoint Assessments: At terminal endpoints synovium and inguinal lymph nodes were extracted and analyzed for macrophage number and phenotypes using flow cytometer. A cohort of mice was also utilized for microCT analysis and histology. Statistical analysis was performed using Graph pad via two-way ANOVA. This study was approved by IACUC.

RESULTS: The NiM particles were sized 6.9 ± 2 µm, with encapsulation efficiency of ~56%. CaZol-NP showed pH sensitive release from NiM and exhibited minimal burst release with ~60% drug releasing over 5 days at acidic pH and ~80% release over 12 days. Analysis of the ACL-ruptured joint at two weeks showed increased macrophage infiltration with a significant increase in activated macrophages co-expressing folate receptor- β (FR-β). Detection of FR-β+ macrophages in injured knees, guided us to design a folate-conjugated targeted system (NiM-fol). Notably, a single intra-articular injection of NiM-fol sustainably delivering Zol over seven weeks produced therapeutic effects comparable to sham control, outperforming saline and free Zol treatment of equivalent dose. Particularly, NiM reduced pain sensitivity as indicated by increase in pain threshold in mouse receiving the treatment (Fig 1A). NiM treatment also showed a trend in restoring stride length in NiM treated animals equivalent to sham controls. Thus, overall showing reduced pain behaviors. Endpoint assessment indicated that NiM treatment reprogrammed activated synovial macrophages toward a reparative phenotype, as assessed by flow cytometry (Fig. 1 B). Histological analysis and microCT studies are ongoing to determine the effect of NiM treatment on cartilage and subchondral bone.

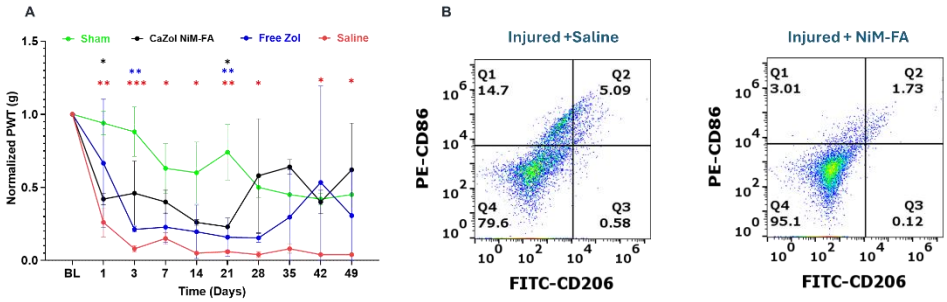

Figure1: (A) Increased paw withdrawal threshold was observed in ipsilateral loaded limbs treated with NiM. (B) Reduced M1 activated macrophages were observed in Nim treated samples as analyzed by flow cytometery. N=4-6 mouse.

DISCUSSION: We have developed a sustained release, pH-sensitive delivery system for Zol for effective targeting of activated macrophages and modulate inflammation in OA affected joint. NiM system offers several advantages such as minimal cytotoxicity, ability to suppress inflammation by targeting macrophages, suppressing NF-κB and spatially tattered delivery only under acidic conditions thus minimizing off target release. These unique characteristics allow repurposing a clinically approved drug as immunomodulatory therapy for OA. Our initial data in clinically relevant animal model of OA shows the pain reducing and immunomodulatory nature of single intraarticular injection of NiM at extended time points.

CLINICAL RELEVANCE: We developed and tested an immunomodulatory sustained-release formulation of Zol with strong potential for intra-articular delivery. Incorporation of Zol into NiM potentially improved its joint residence time and increased the therapeutic efficacy, reducing the need for repeated injections and high doses. We further evaluated the therapeutic effect in a non-surgical model of OA that more closely recapitulates human injury and disease progression. In this model, NiM treatment demonstrated potential pain-modifying effects, supporting the possibility of repurposing Zol for clinical application in OA treatment.

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