

Osteoactivin/GPNMB as a Therapeutic for Post-Traumatic Osteoarthritis

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INTRODUCTION: Osteoarthritis is a degenerative disease characterized by cartilage destruction, synovitis, and osteophyte formation and is the most common disease of the joint, affecting approximately 530 million individuals worldwide. Currently no disease modifying therapeutic options are available to treat osteoarthritis. Nonsteroidal anti-inflammatory drugs are frequently utilized for pain control and physical exercise and weight loss are recommended. Other treatments include corticosteroid, hyaluronic acid, and protein rich plasma injections, though none of the aforementioned treatment options regenerate cartilage to our knowledge. Our laboratory is interested in investigating osteoactivin, also known as glycoprotein nonmetastatic melanoma protein B (GPNMB), an anti-inflammatory glycoprotein, as a potential therapeutic. Our preliminary unpublished data suggests that GPNMB is chondroprotective, shown *in vitro* and *in vivo*. Cartilage explants treated with IL-1 β , to induce extracellular matrix (ECM) degradation, showed decreased ECM degradation with GPNMB treatment. Additionally, DBA/2J mice with an inactivating point mutation in the GPNMB gene were found to have increased cartilage degradation following the induction of post-traumatic osteoarthritis, supporting the hypothesis that GPNMB is playing a chondroprotective role *in vivo*. We seek to investigate the therapeutic potential of GPNMB in osteoarthritis utilizing intra-articular recombinant GPNMB (rGPNMB) and GPNMB related-peptide (GPNMBp) injection in a mouse model of post-traumatic osteoarthritis. This study will further investigate the potential of a future therapeutic in the use of osteoarthritis.

METHODS: All animal studies were approved by the institutional animal care and use committee (IACUC) of NEOMED. C57BL/6 male mice were purchased from Jackson Laboratories at 9 weeks of age and were given 1 week to acclimate prior to destabilization of medial meniscus (DMM) surgery of the right knee. Treatment groups included control (no DMM surgery or treatment, n=4), sham surgery (n=3), DMM surgery with PBS injection (n=3), DMM surgery with rGPNMB injection (2 μ g, Thermo Fisher Scientific, n=4) and DMM surgery with GPNMBp injection (120 μ M, Anaspec, n=5). GPNMBp is a synthetic 18-amino acid peptide comprised of a portion of the C-terminus domain of GPNMB. DMM or sham surgery occurred at 10 weeks of age, and injections were performed at 6 weeks post-DMM surgery. Animals were sacrificed at 14 weeks post-DMM surgery and knees were decalcified, fixed, and paraffin embedded. Sections (7 μ m) were either stained with Safarin-O and fast green and scored per the OARSI scoring system, or immunostained for aggrecan, matrix metalloproteinase-13 (MMP-13), or GPNMB. To assess the mechanism of GPNMB in inflammation, mouse primary chondrocytes were treated with either IL-1 β (10ng/mL) or rGPNMB (100ng/mL) and IL-1 β (10ng/mL). Catabolic and inflammatory chondrocyte markers were assessed utilizing RT-qPCR analysis.

RESULTS SECTION: Success of DMM surgery was confirmed via Safarin-O and fast green staining. Results showed that intra-articular injection led to significantly less severe osteoarthritis in the medial femoral condyle (MFC) of animals treated with rGPNMB or GPNMBp compared to those treated with PBS (Figure 1A). Immunostaining results showed that rGPNMB or GPNMBp treatment led to increased GPNMB staining in articular chondrocytes and decreased MMP-13 staining compared to PBS treated group. Additionally, immunostaining for aggrecan showed increased staining with rGPNMB or GPNMBp treatment compared to PBS treatment (Figure 1B, C). RT-qPCR analysis of primary chondrocytes treated with either IL-1 β or rGPNMB and IL-1 β showed decreased catabolic gene expression (MMP-9, MMP-13) and IL-6 expression with rGPNMB treatment compared to IL-1 β treatment alone.

DISCUSSION: Overall our preliminary results suggest that rGPNMB and GPNMBp prevent continued cartilage damage following joint injury. This shows rGPNMB and GPNMBp's potential therapeutic use in the treatment of post-traumatic osteoarthritis. To ensure continued bioavailability of rGPNMB and GPNMBp, thermoresponsive hydrogel preparation is being investigated to ensure sustained release following intra-articular injection. Future studies are underway to assess the potential therapeutic use of rGPNMB and GPNMBp at the time of injury. Further analysis will be performed to assess safety of this therapeutic. With no disease modifying treatment options for post-traumatic osteoarthritis available, this chondroprotective effect of rGPNMB and GPNMBp shows positive potential for their use as therapeutics.

SIGNIFICANCE/CLINICAL RELEVANCE: Our results indicate the potential use of GPNMB in the treatment of post-traumatic osteoarthritis and propose it as an alternative to current therapies.

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IMAGES:

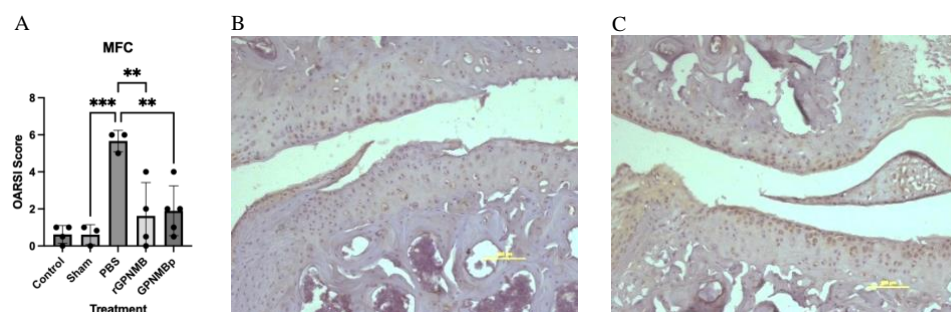


Figure 1: OARSI scoring of joints following Safarin-O and fast green staining (A). Aggrecan immunostaining of animal subjected to DMM surgery and PBS (B) or rGPNMB (C) treatment. **p<0.01, ***p<0.001.