Development of a Novel Therapeutic, anti-Siglec-15 Antibody, to Reduce Bone Loss and Enhance Bone Integrity after Acute Spinal Cord Injury-induced Immobilization.

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ABSTRACT

INTRODUCTION: The rapid and extensive sublesional bone loss after spinal cord injury (SCI) is a difficult medical problem that has been refractory to available interventions [1-4] except the anti-resorptive agent, denosumab [5]. While denosumab has shown some efficacy in inhibiting bone loss, its concurrent inhibition of bone formation limits its use. Sialic acid-binding immunoglobulin-like lectin (Siglec)-15 is expressed on the cell surface of mature osteoclasts [6, 7]. Anti-Siglec-15 antibody (Ab) has been shown to inhibit osteoclast maturation and bone resorption while maintaining osteoblast activity and bone formation, which is distinct from current anti-resorptive agents that inhibit the activity of both osteoclasts and osteoblasts [8] [9]. **PURPOSE:** The goal of the present study is to test Siglec-15 Ab as a new treatment option to prevent bone loss in an acute SCI model. **RESULTS:** To this end, 12-week-old male Wistar rats underwent complete spinal cord transection. Immediately after SCI, the rats were treated with either vehicle or Siglec-15 Ab (NP159) at 20 mg/kg/biweekly for 8 weeks. Eight weeks after SCI, bone mineral density (BMD) of the distal femur and proximal tibia were diminished by -16.5% and -13.9%, respectively. Of note, NP159 treatment completely prevented the loss of BMD at these two skeletal sites after acute SCI. High-resolution microCT analysis revealed that after SCI, trabecular bone volume at the distal femur was reduced by -43% due largely to decreased trabecular number with an increase in trabecular space; trabecular connectivity was greatly reduced associated with transformation from plate-like to rod-like structures. Administration of the NP159 almost completely prevented the declines in trabecular bone volume and connectivity, primarily by increasing trabecular number and preserving trabecular space. SCI resulted in significantly decreased bone stiffness (-76.3%) by finite element analysis, whereas NP159-treated SCI rats dramatically increased bone stiffness by +207.7% compared to SCI-vehicle animals. Blood and histomorphometric analyses reveal that NP159 is able to greatly inhibit bone resorption while maintaining bone formation after acute SCI. In *ex vivo* cultures of bone marrow cells, SCI increased the number of TRAP$^+$ multinucleated cells, as well as mRNA levels of osteoclast differentiation markers (e.g., TRAP and calreticulin), and reduced the number of osteoblasts and the mRNA levels of the osteoblast differentiation markers (e.g., osteocalcin and BSP). None of these deleterious changes in the expression of skeletal markers was observed in the NP159-treated group. Notably, NP159-treated rats compared to control rats displayed a significant increase in the numbers of osteoblasts, mineralized nodules cells, as well as mRNA levels of osteocalcin and BSP. Our findings suggest that NP159 induced osteoblastogenesis while reducing osteoclastogenesis. **CONCLUSION:** In summary, treatment with NP159 fully prevented sublesional loss of BMD and metaphysis trabecular bone volume and preserved bone strength in a rat model of acute SCI. Because of its unique ability to reduce osteoclastogenesis and bone resorption while promoting osteoblastogenesis to maintain bone formation, Siglec-15 Ab may hold greater promise than prior anti-resorptive agents alone to mitigate the striking bone loss associated with SCI or other forms of severe immobilization.

EXPERIMENTAL PROCEDURES

Animals, surgery, treatment, bone mass and microarchitecture measurement and bone histomorphometry: 12-week old male male Wistar rats underwent a complete spinal cord transection (T3-T4). Sham controls underwent only a laminectomy at the same site. Immediately after SCI, the rats were treated with either vehicle or Siglec-15 Ab (NP159; 20 mg/kg) once every 2 weeks for 8 weeks. The bones were labeled with fluorochromes by the subcutaneous injection of calcein (10 mg/kg body weight) and xylenol orange (90 mg/kg body weight), respectively, on day −6 and day −2 before euthanasia. Bone mineral density was measured using a Lunar Piximus. The femur was used for micro-CT analysis and bone histomorphometry. ***Osteoclast development and osteoblast progenitor assays:*** These experiments were conducted as described previously (Qin *et al.,* 2013, Qin *et al.,* 2015, Zhao *et al.,* 2018).

RESULTS

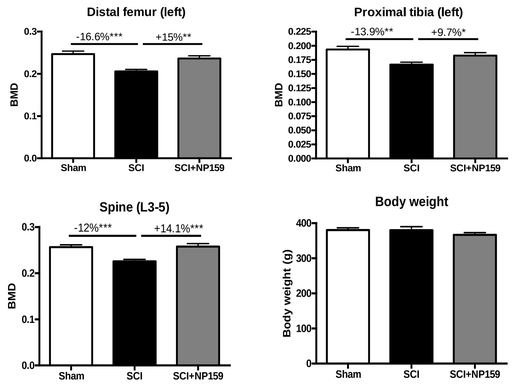

Fig 1. Areal BMD (aBMD) for the indicated sites determined by analysis of images acquired by DXA scanning. Data are expressed as mean ± SEM. N = 11–13 per group. Significance of differences was determined using one-way analysis of variance with a Newman–Keuls test post hoc. The same statistical analysis method will also be used for the rest of studies. **P < 0.01 and ***P < 0.001 versus the indicated group.

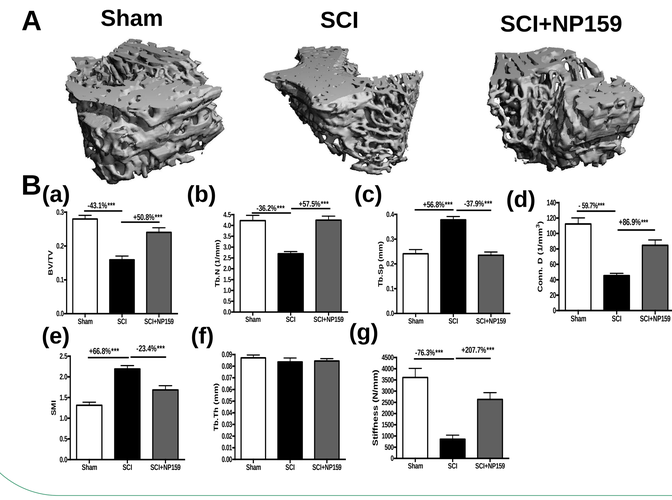

Fig 2. The effects of NP159 on trabecular architecture of the distal femur as assessed by micro-CT. A, representative 3D images of trabecular microarchitecture. Measurement are shown for: **B (a),** trabecular bone volume over total volume (BV/TV%); **(b)** trabecular number (Tb.N, mm^{-1}); **(c)** trabecular separation (Tb.Sp, μm); **(d)** connectivity density (Conn.Dn, mm^{-3}); **(e)** structure model index (SMI, ranges from 0 to 3 with 0 = platelike and 3 = rodlike); **(f)** trabecular thickness (Tb.Th, μm); and (g) bone Stiffness by finite element analysis, respectively. Data are expressed as mean ± SEM. N = 10 per group. *P < 0.05, **P < 0.01 and ***P < 0.001 versus the indicated group.

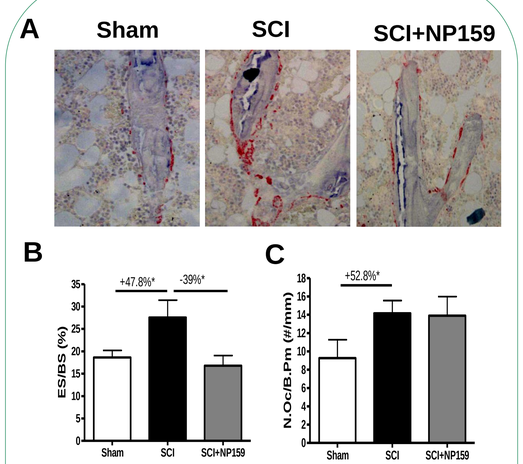

Fig 3. The effects of NP159 on bone resorption of trabecular bone at the distal femur. (A) Representative sections of trabecular bone from the femoral metaphysis immunostained for TRAP (20x). The reddish areas of TRAP staining on trabecular surfaces represent osteoclasts. (B–C) Parameters of trabecular bone resorption by histomorphometric quantification: (B) Eroded surface/bone surface (ES/BS) and osteoclast number over bone perimeter (N.Oc/B. Pm). Data are expressed as mean ± SEM. N= 6-7 per group. *p<0.05 *versus* the indicated group.

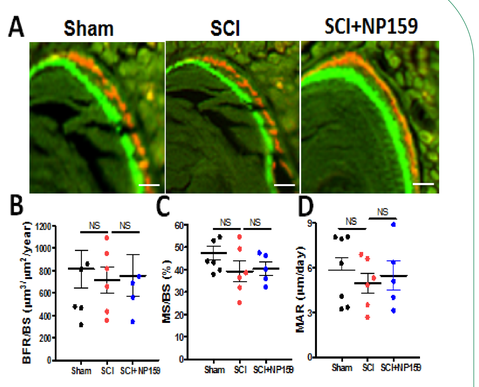

Fig 4. Effects of NP159 on bone formation of trabecular bone at the proximal tibia. (A) Representative 6-mm-thick bone specimen showed double-labeling of calcein green and xylenol orange under fluorescence microscopy (X20). Measurement is shown for **(B)** BFR/BS (mm^3/mm^2/year), **(C)** MS/BS (%), and **(D)** MAR (mm/day). These were all measured/taken in the area 1mm underneath the growth plate. Bar=50μm. Data are expressed as mean±SEM. N= 5-7/group. NS, no significance. BFR/BS, bone formation rate; MS/BS, mineralizing surface/ bone surface; MAR, mineral apposition rate.

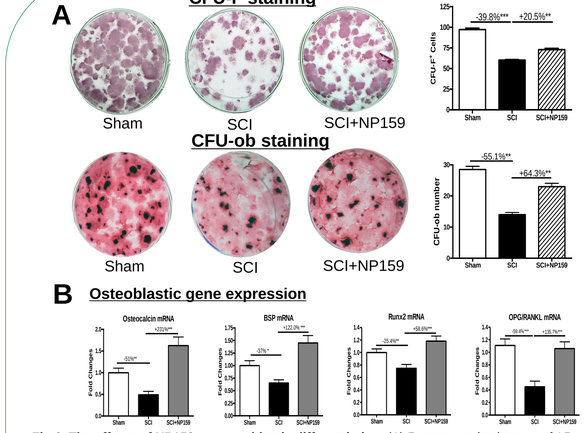

Fig 6. The effects of NP159 on osteoblastic differentiation. (A) Representative images of AP staining (CFU-F) and von Kossa staining (CFU-ob) of cultured marrow stromal cells, and the quantification of CFU-F$^+$ and CFU-ob cells. (B) The gene expression of bone formation markers by qPCR: osteocalcin and BSP. Data are expressed as mean ± SEM. N = 4/group. **p<0.01, ***p<0.001 *versus* the indicated group.

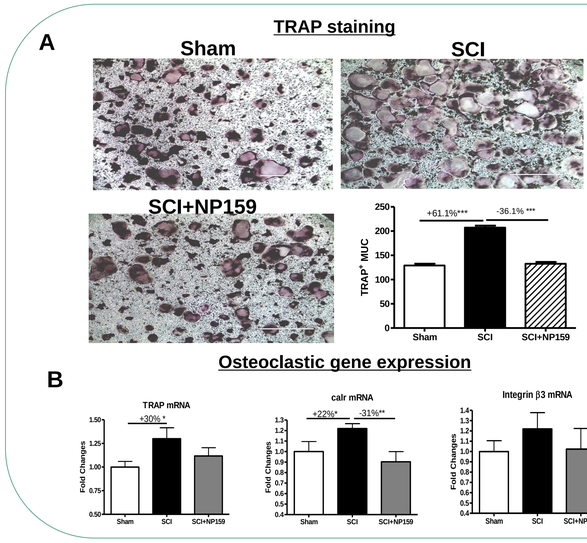

Fig 5. NP159 inhibited the osteoclastogenesis of marrow stem cells. (A) TRAP staining of cultured osteoclasts, and the quantification of TRAP$^+$ multinucleated cells. (B) The gene expression of bone resorption markers in cultured osteoclasts determined by qPCR: TRAP, calreticulin (Calr), and integrin β3. N=4-5/group *p<0.05, **p<0.01, ***p<0.001 *versus* the indicated group.

IMPACT TO THE WARFIGHTER/SIGNIFICANCE

There is currently significant unmet need for therapeutics to address the bone loss in Veterans or other military service personnel after SCI. At the present time, there is no practical intervention to safely restore a sufficient fraction of the bone loss to be of clinical relevance, highlighting the need for novel therapeutics. If skeletal integrity can be improved in persons with chronic SCI, this approach would hold the promise to increase the number of individuals who would have been denied access, but may become eligible, for rehabilitation strategies (e.g., exoskeletal-assisted walking, spinal cord stimulation) or other modalities for gait to allow greater functional independence. Implementation of such a therapeutic could significantly improve to improve healthcare, function and quality of life in Veterans or other military service personnel with SCI and other conditions of osteoporosis associated with neurologic etiologies (e.g., stroke, Parkinson's disease, ALS and multiple sclerosis), chronic immobilization and disuse (e.g., spaceflight), or activity-limiting rheumatological diseases.

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