A Comprehensive Study of Long-term Skeletal Changes after Spinal Cord Injury in Adult Rats

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Introduction

Osteoporosis is a well-known secondary complication of spinal cord injury (SCI).[1-3] Shortly after the injury, sublesional bone density and mass decline rapidly and linearly. This is particularly deleterious within cancellous bone located in metaphyseal-epiphyseal areas of the distal femora and proximal tibiae, which experience a decline in bone mass of 1-4% per month for the first 6-12 months. This bone loss rate is 4, 10, and 30-fold greater than that observed during microgravity, prolonged bed rest, and early menopause, respectively. Hence, severe osteoporosis, with at least a 40% reduction in bone mineral content, is common in SCI patients. In the present study, we performed a comprehensive analysis of long-term structural and mechanical changes in axial and appendicular bones in skeletally mature SCI rats to delineate the SCI damage on the entire skeleton. Olfactory ensheathing glia (OEG) transplantation recently emerges as one of the most promising approaches for nervous system repair, in particular, in the various forms of SCI. Preclinical and clinical data have demonstrated the efficacy and robustness of this treatment in promoting motor function recovery after transection of spinal cord. In this study, we also investigated whether this OEG transplantation treatment has beneficial effects on bone after SCI.

Material and Methods

SCI surgery and tissue harvest: T13 spinal cord of four-month-old male Fischer 344 rats received modest injury delivered by a 10-gram rod dropped from a 25 mm height. After injury, rats (n = 5/group) were randomly assigned to receive either vehicle (DMEM) or OEG (400,000 cells/rat) injection at 1 mm rostral and caudal to the contusion site. Sixteen weeks later, sublesional bones, including femurs and tibiae, and supralesional bones, including 4th lumbar vertebrae (L4) and humeri, were collected and fixed further in 4% PFA for subsequent measurements.

*Evaluation of bone microarchitecture by micro-computed tomography (*μCT*):* All bones were scanned by a compact fan-beam-type tomograph μCT 40 (Scanco Medical AG, Bassersdorf, Switzerland) at 15 μm nominal voxel size.

Bone histology analysis: After μCT scans, right tibiae were decalcified for 21 days followed by paraffin embedding. Five-μm longitudinal sections were stained either by hematoxylin and eosin (H&E) for counting the plump bone lining osteoblast number or the number of TRAP-positive multinucleated osteoclasts within secondary spongiosa.

Serum chemistry: Osteocalcin and TRACP 5b level were determined by Rat Osteocalcin EIA Kit and RatTRAPTM Assay.

Results

To study the SCI effects on the knee joint in details, we scanned all three regions using high-resolution μCT. In the distal femoral site, as shown in Fig. 1A, we observed that the most drastic bone loss occurred in the secondary spongiosa immediately followed by the primary spongiosa at 16 weeks post-surgery, while, surprisingly, there was only modest bone loss in the subchondral region (data not shown). 3D analysis of μCT data revealed striking 54% and 65% reductions in vBMD and BV/TV, respectively, in the secondary spongiosa of SCI-vehicle group compared to controls (Fig. 1B). In the primary spongiosa, similar but relatively reduced changes in vBMD (47%) and BV/TV (56%) were observed in SCI-vehicle rats compared to controls (Fig. 1C). Among all regions, OEG-treated rats had comparable trabecular bone phenotypes as vehicle-treated ones (Fig. 1). We next analyzed sublesional axial bones and to clarify this, L4 vertebrae were harvested for μCT analysis. Compared to controls, SCI rats apparently lost a lot of trabecular bone, specifically in the central region of the L4 (Fig. 2A). Trabecular vBMD and BV/TV in L4 from vehicle-treated SCI rats were 31% and 37%, respectively, less than those from controls (Fig. 2B). Injection of OEG marginally but significantly improved several parameters, such as vBMD and BV/TV. To understand the underlying cellular mechanisms of long term SCI damage on bone, we performed histological analysis on right tibiae of all three groups. As shown in Fig. 3A, even after 16 weeks, SCI still strongly suppressed the number of osteoblasts by 71% and enhanced the number of osteoclasts by a 3.7-fold. The effects of long term SCI on bone formation and resorption were further

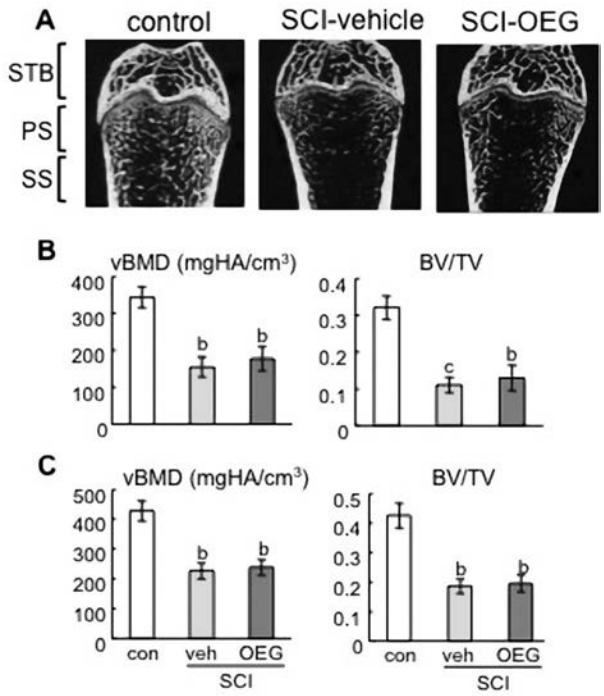

Figure 1. SCI causes drastic trabecular bone loss and structural deterioration. (A) Representative longitudinal μCT images of distal femurs in control, vehicle-treated SCI, and OEG-treated SCI rats at 16 weeks after injury. STB: Subchondral trabecular; PS: Primary spongiosa; SS: Secondary spongiosa; (B) μCT measurement of trabecular bone structural parameters in the secondary spongiosa area. a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$ vs control.

confirmed by trends of decreased amount of bone formation marker (osteocalcin) and increased amount resportion marker (TRAP) in serum (Fig. 3B). OEG treatment did not significantly affect these changes.

Discussion

Bone is a dynamic organ that undergoes constant remodeling and a balance between osteoblastic and osteoclastic activities is required for bone homeostasis. After SCI, this balance is tipped strongly toward bone resorption causing drastic bone loss particularly in the sublesional appendicular bones, which frequently leads to low impact fractures in these bones. Interestingly, we found that SCI has site-specific effects on trabecular bone even within the same appendicular bone. Based on the injury during SCI, therapeutics can include that improve the nerve function or directly improve the bone integrity. OEG treatments require further investigation and their efficacy needs to be critically evaluated.

Conclusion

In conclusion, SCI deteriorates the entire skeleton with severe bone loss and structural deterioration at the lower extremities as well as sublesional vertebrae. The upper extremities suffer bone damage to a less extent. Further, bone formation is inhibited and bone resorption is elevated in rats with chronic SCI. The drastic loss of bone mass and the continuous suppression of bone formation suggest that anabolic treatments, such as PTH1-34, sclerostin-antibody,

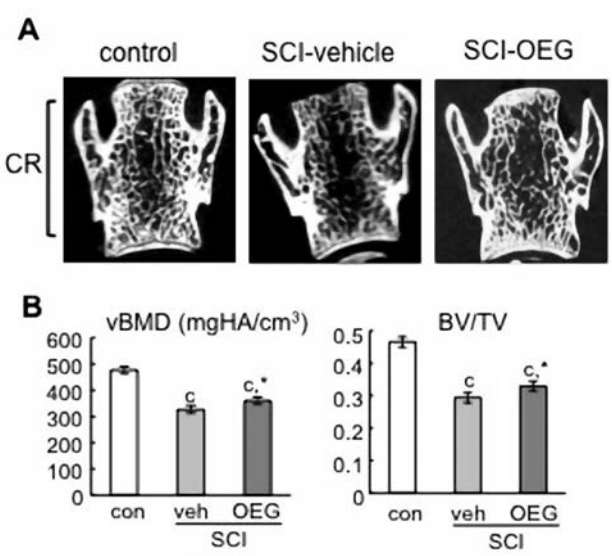

Figure 2. The trabecular bone in vertebral body is greatly damaged by SCI. (A) Representative longitudinal μCT images of L4 vertebra in control, vehicle-treated SCI, and OEG-treated SCI rats at 16 weeks after injury. CR: Central region. (B) μCT measurement of trabecular bone structural parameters inside the vertebra. a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$ vs control. *: $p < 0.05$ & : $p < 0.01$ vs veh.

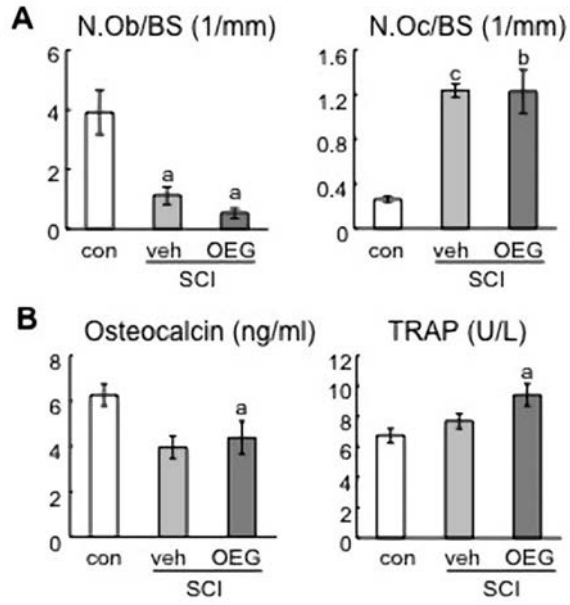

Figure 3. Chronic SCI inhibits bone formation and stimulates bone resorption. (A) Histological analysis was performed to count the numbers of osteoblasts and osteoclasts in control, vehicle-treated SCI, and OEG-treated SCI rats at 16 weeks after injury. (B) Biochemistry assays of osteocalcin and TRAP level in all three groups. a: $p < 0.05$; b: $p < 0.01$; c: $p < 0.001$ vs control.

might be the more suitable therapy to promote bone health and to prevent bone fracture in SCI patients.

References

1. Battaglino, RA, et al. Spinal cord injury-induced osteoporosis: pathogenesis and emerging therapies. *Curr Osteoporos Rep.*, 10(4): p. 278-85 (2012).

2. Jiang, SD, LY Dai, and L.S. Jiang. Osteoporosis after spinal cord injury. *Osteoporos Int.*, 17(2): p. 180-92 (2006).

3. Juknis, N., JM Cooper, and O. Volshteyn. The changing landscape of spinal cord injury. *Handb Clin Neurol.*, 109(doi): p. 149-166 (2012).