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# Cannabidiol administration reduces sublesional cancellous bone loss in rats with severe spinal cord injury

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ABSTRACT

Patients with spinal cord injury (SCI) undergo severe loss of bone mineral below the level of lesion, and data on available treatment options after SCI is scarce. The aim of this work was to investigate the therapeutic effect of cannabidiol (CBD), a non-psychoactive cannabis, on sublesional bone loss in a rat model of SCI. The adult male rats were exposed to surgical transection of the cord and treated with CBD for consecutive 14 days. It was found that CBD treatment elevated the serum levels of osteocalcin, reduced the serum levels of collagen type I cross-linked C-telopeptide, and enhanced bone mineral density of tibiae and femurs. Treatment of SCI rats with CBD enhanced bone volume, trabecular thickness, and trabecular number, and reduced trabecular separation in proximal tibiae, and increased ultimate compressive load, stiffness, and energy to max force of femoral diaphysis. Treatment of SCI rats with CBD upregulated mRNA expression of alkaline phosphatase and osteoprotegerin and downregulated mRNA expression of receptor activator of NF-κB ligand and tartrate-resistant acid phosphatase in femurs. Furthermore, treatment of SCI rats with CBD enhanced mRNA expression of wnt3a, Lrp5 and ctnnb1 in femurs. In conclusion, CBD administration attenuated SCI-induced sublesional cancellous bone loss.

## 1. Introduction

Spinal cord injury (SCI) is associated with low bone mass and deterioration of the skeletal architecture, resulting in severe osteoporosis and eventual fracture in as many as half of all affected individuals (Troy and Morse, 2015). The distal femurs and proximal tibiae appear most susceptible to bone loss after SCI, and fractures usually occur at these sites (Cirnigliaro et al., 2017). The skeletal deterioration resulting from SCI is much more severe than that resulting from early menopause (Jiang et al., 2007) or from other disuse/injured models including microgravity, prolonged bed rest, and sciatic neurectomy (Liu et al., 2008; Jiang et al., 2006). Importantly, the extensive bone loss and high fracture risk within this population lead to limiting mobility and add significant medical costs to rehabilitative care (Carbone et al., 2013; Akhigbe et al., 2015). Despite these serious health implications, there are currently relatively few treatment options available to minimize SCI-induced osteoporosis.

Cannabidiol (CBD), a major nonpsychotropic constituent of *Cannabis sativa*, presented multiple pharmacological actions, including anxiolytic, antipsychotic, sedative, antiemetic, anti-inflammatory, and neuroprotective properties (Rohleder et al., 2016; Burstein, 2015). Cannabidiol was known to act either as the cannabinoid-1 (CB1) receptor antagonist, CB2 receptor inverse agonist, transient receptor potential vanilloid-1 (TRPV1) and TRPV2 agonist, G protein-coupled receptor 55 (GPR55) antagonist, 5-hydroxytryptamine (5HT) 1A and 2A receptor agonist and 5-HT3A receptor antagonist, and partial agonist at dopamine D2High receptors (Campos et al., 2012; Seeman, 2016). In a rat model of mid-femoral fractures, CBD treatment led to improvement in fracture healing (Kogan et al., 2015). In a coccygeal intervertebral disc degeneration induced by the needle puncture model, CBD treatment by intradiscal injection mitigated lesion-induced intervertebral disc degeneration (Silveira et al., 2014). CBD treatment also decreased alveolar bone loss in a rodent experimental periodontitis induced by a ligature placed around the mandible first molars (Napimoga et al., 2009).

Therefore, we hypothesized that CBD may be beneficial to minimize SCI-induced osteoporosis and we tested its effect in a rodent model of SCI.

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## 2. Materials and methods

### 2.1. Animals

Male Wistar rats aged 3 months were obtained from the Vital-Aiver Animal Ltd (Beijing, China). All rats were housed in clean plastic cages under a 12 h light/dark cycle in a controlled environment with a temperature of 23 ± 2 °C and humidity of 50–60%. They had free access to a standard rodent chow and water ad libitum. All experiments were performed according to the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China. All efforts were made to minimize suffering.

### 2.2. Animal model of spinal cord injury (SCI) and drug treatment

All rats were anesthetized by inhalation of isoflurane and the thoracic cord was transected at the interspace between the third and fourth vertebral bodies as described (Qin et al., 2015; Sun et al., 2013). The sham-operated animals underwent an identical operation to those in the SCI group, except that the spinal cord was not transected. Urine was voided three times daily until spontaneous voiding returned, then at least once each day as need. Each rats also received prophylactic gentamicin sulfate (15 mg/kg IM) on the day of surgery and again 3 and 5 days post-injury. Body temperatures were maintained at 36 °C by using a water-filled heating pad for the duration of the surgery and until they were recovered from the anesthetic fully.

Rats were treated with cannabidiol (CBD, 0.5 and 5 mg/kg/day) (Vuolo et al., 2015; Rajesh et al., 2010) by intraperitoneal injection from 12 h following the surgery and over 14 subsequent days. CBD (Tocris Bioscience, United Kingdom) was suspended in 2% of polyoxyethylenesorbitan monooleate (Tween 80) dissolved in 0.9% saline solution. The drug was prepared immediately before use and protected from light.

At the end of the experiment, all animals were euthanized by a lethal dose of thiopental (100 mg/kg). The femurs and tibiae were removed for subsequent RT-PCR assay and morphometric and biomechanical analysis.

### 2.3. Quantitative real-time PCR (RT-PCR) analysis

The distal right femurs were placed in an RNase-free mortar and pestle which contained liquid nitrogen and ground to a fine powder immersed in liquid nitrogen. Then, the frozen powder was transferred into a tube containing Trizol (Thermo Fisher Scientific, MA, USA). Extracted RNA was quantified using spectrophotometry (NanoDrop 2000C; Thermo Scientific, Inc.). Total RNA was then reverse-transcribed to cDNA using the Super-Script II (Invitrogen, CA, USA) and the target gene was amplified using the standard RT-PCR kit (Qiagen, The Netherlands, Venlo). GAPDH was used as an internal control. Primers used for amplification of target genes were listed in Table 1.

**Table 1**
Sequences of oligonucleotides used as primers.

| Target gene | | Sequence (5′-3′) |
|---|---|---|
| TRAP | Sense | AATTGCCTACTCCAAGATCTCCAA |
| | Antisense | GCGGAACTTTGAAACGCAAA |
| RANKL | Sense | CGTACCTGCGGACTATCTTCA |
| | Antisense | GTTGGACACCTGGACGCTAA |
| OPG | Sense | CATCGAAAGCACCCTGTA |
| | Antisense | CACTCAGCCAATTCGGTAT |
| ALP | Sense | CTCTCCGAGATGGTGG |
| | Antisense | TGGAGACATTCTCTCGTT |
| Wnt1 | Sense | GGGTTTCTGCTACGTTGCTACT |
| | Antisense | GGAGGTGATTGCGAAGATAAAC |
| Wnt3a | Sense | TGAATTTGGAGGAATGGTCTCT |
| | Antisense | TGGGCACCTTGAAGTATGTGTA |
| Wnt5a | Sense | TCATGAACTTGCACAACAATGA |
| | Antisense | CCGTCTTAAACTGGTCATAGCC |
| Ctnnb1 | Sense | AACGGCTTTCGGTTGAGCTG |
| | Antisense | TGGCGATATCCAAGGGCTTC |
| Lrp5 | Sense | CTGCTGGGGGACTTCATCTACTGGAC |
| | Antisense | GGGAGGAGTGGAACACCAGGATGTC |
| sost | Sense | GGCAAGCCTTCAAGAATGATGCCA |
| | Antisense | TGTACTCGGACACGTCTTTGGTGT |
| GAPDH | Sense | TATCACTCTACCCACGGCAAG |
| | Antisense | ATACTCAGCACCAGCATCACC |

ALP, alkaline phosphatase; Lrp5, low-density lipoprotein-related protein5; TRAP, tartrate-resistant acid phosphatase; RANKL, receptor activator of NF-κB ligand; OPG, osteoprotegerin; GAPDH, glyceraldehyde phosphate dehydrogenase.

**Table 2**
Initial and final weights in each groups.

| Group | Initial weights (g) | Final weights (g) |
|---|---|---|
| Sham-operated rats received vehicle | 371 ± 25 | 385 ± 30 |
| Sham-operated rats received CBD (0.5 mg/kg/day) | 375 ± 31 | 391 ± 28 |
| Sham-operated rats received CBD (5 mg/kg/day) | 369 ± 28 | 383 ± 35 |
| SCI rats received vehicle | 373 ± 21 | 383 ± 36 |
| SCI rats received CBD (0.5 mg/kg/day) | 371 ± 29 | 384 ± 24 |
| SCI rats received CBD (5 mg/kg/day) | 372 ± 34 | 386 ± 33 |

CBD, cannabidiol; SCI, spinal cord injury; Data are expressed as mean ± S.D.

### 2.4. Measurements of bone mass, structure, and mechanical properties

Bone mineral density (BMD) of tibiae and femurs were determined by using small-animal special Dual Energy X-ray Absorptiometry (DEXA, Hologic, Inc. USA). The bone microarchitecture within the proximal tibial metaphysis was evaluated *ex vivo* using a high-resolution micro-CT (μCT40, Scanco Medical AG, Zurich, Switzerland, 10.5 μm voxel size, 55 kVp, 145 μA) with a threshold value of 240. The proximal tibial metaphysis was scanned in 250 slices (thickness, 13 μm) in the dorsoventral direction. For trabecular bone properties, a 1 mm thick volume of interest was analyzed beginning 0.5 mm distal to the proximal tibial growth plate. Mechanical properties of midshaft of left femurs were evaluated using three-point bending tests by using a BOSE ElectroForce 3520 biological material testing system (Minnesota, USA). Quasi-static loading was applied to the femoral head in a direction parallel to the femoral shaft (vertical) at a displacement rate of 2 mm/min until complete fracture. Table 2.

### 2.5. Measurements of osteocalcin, and C-terminal cross-linked telopeptides of type I collagen (CTX) in sera

After an overnight fast, blood was collected via cardiac puncture at euthanasia and left at room temperature for at least 0.5 h before centrifuging at 200×*g* for 10 min to separate serum. Levels of osteocalcin in sera were determined with a rat OC radioimmunoassay kit (Xinqidi Biological Technology). Levels of CTX, a bone resorption marker, in sera were determined by using an ELISA kit (Uscn Life Science Inc, Wuhan, china).