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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).

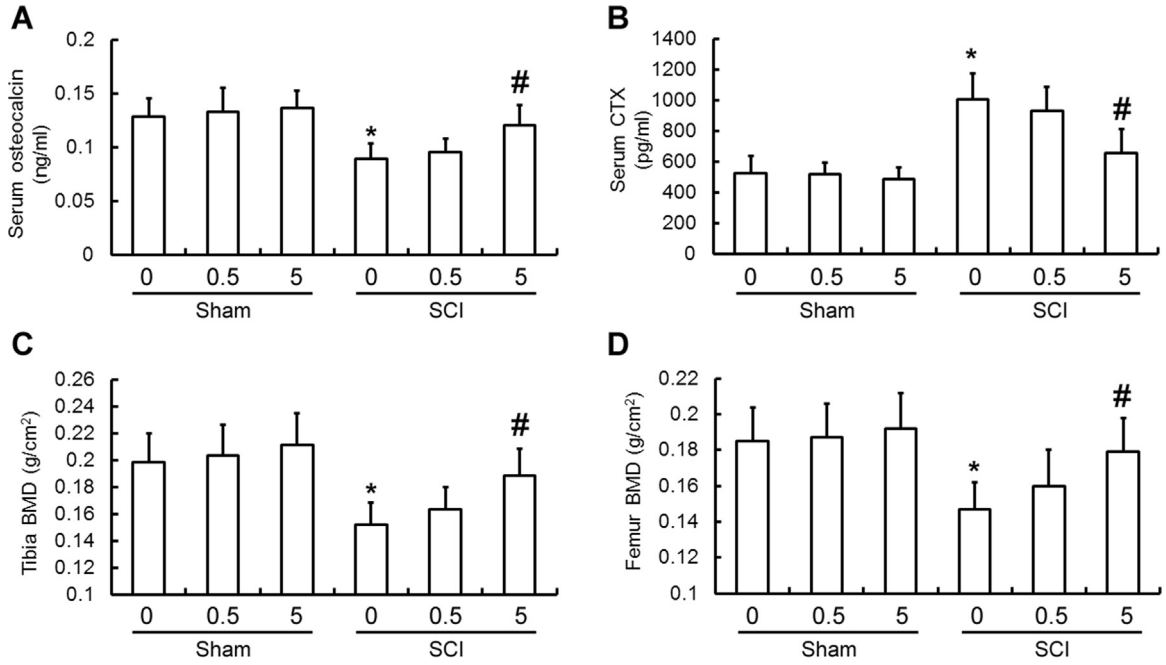

Fig. 1. Treatment with cannabidiol attenuated sublesional bone loss in rats with SCI. The adult male rats were exposed to surgical transection of the cord and treated with cannabidiol (CBD, 0.5 and 5 mg/kg/day, i.p.) for consecutive 14 days. Graphs showed the serum levels of osteocalcin (A) and CTX (B) and BMD in tibiae (C) and femurs (D) of rats. SCI, spinal cord injury; CTX, collagen type I cross-linked C-telopeptide; BMD, bone mineral density; Data are expressed as mean ± S.D.; n=9 in each group; * $P < 0.05$ compared with sham-operated group received vehicle; # $P < 0.05$ compared with SCI group received vehicle.

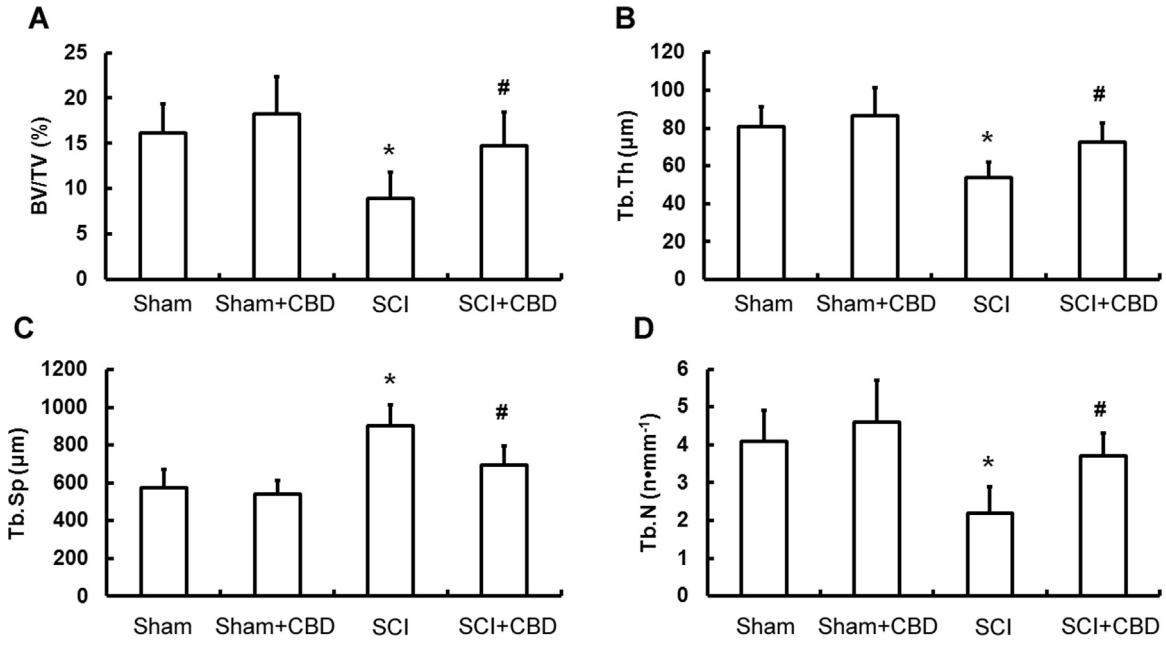

Fig. 2. Treatment with cannabidiol attenuated disruption of bone structure of proximal tibiae of rats with SCI. The adult male rats were exposed to surgical transection of the cord and treated with cannabidiol (CBD, 5 mg/kg/day, i.p.) for consecutive 14 days. Graphs showed the bone volume fraction (bone volume/total volume, BV/TV, A), trabecular thickness (Tb.Th, B), trabecular separation (Tb.Sp, C), and trabecular number (Tb.N., D). SCI, spinal cord injury; Data are expressed as mean ± S.D.; n=9 in each group; * $P < 0.05$ compared with sham-operated group received vehicle; # $P < 0.05$ compared with SCI group received vehicle.

2.6. Statistics

All values are represented as means ± standard deviation (n=9 in each group). Statistical comparisons among groups were made by one-way ANOVA followed by Newman-Keuls post-hoc analysis. Probability values of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Treatment with CBD attenuated sublesional bone loss in rats with SCI

Fourteen days following surgical transection of the cord, the serum levels of osteocalcin (Fig. 1A) were lower and levels of CTX (Fig. 1B) were higher in rats with SCI, than that in sham-operated rats. Furthermore, the BMD in tibiae (Fig. 1C) and femurs (Fig. 1D) was lower in rats with SCI, than that in sham-operated rats. Treatment of

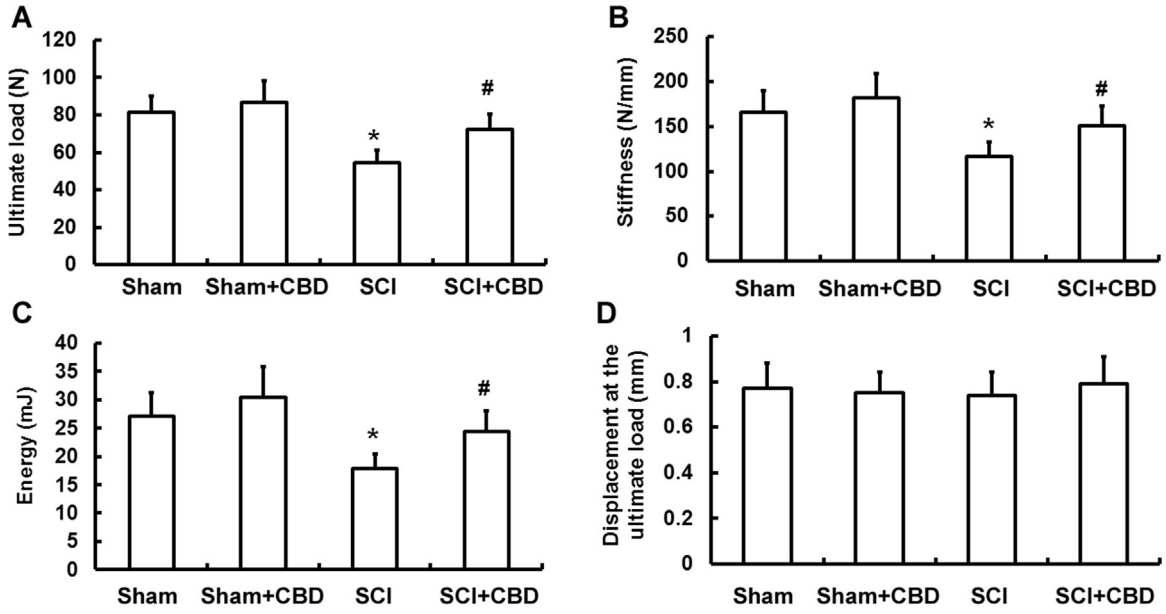

Fig. 3. Treatment with cannabidiol enhanced mechanical properties in the femoral diaphysis of rats with SCI. The adult male rats were exposed to surgical transection of the cord and treated with cannabidiol (CBD, 5 mg/kg/day, i.p.) for consecutive 14 days. Graphs showed the ultimate compressive load (A), stiffness (B), energy to max force (C), and displacement at the ultimate load (D). SCI, spinal cord injury; Data are expressed as mean ± S.D.; n=9 in each group; * $P < 0.05$ compared with sham-operated group received vehicle; # $P < 0.05$ compared with SCI group received vehicle.

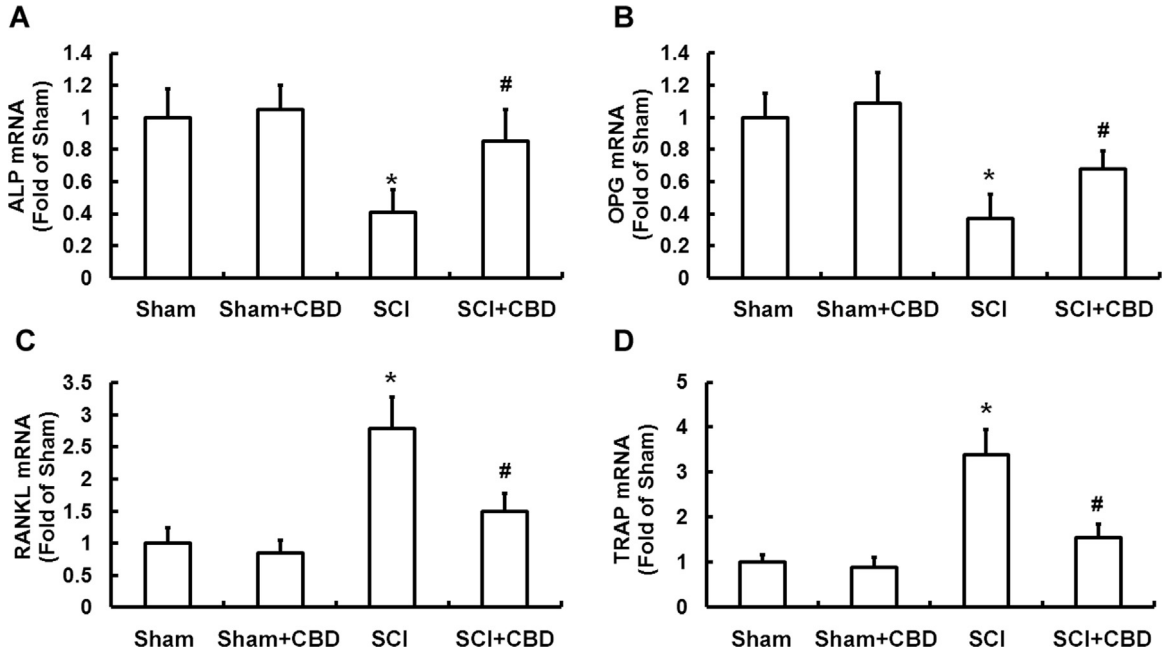

Fig. 4. Treatment with cannabidiol had beneficial effects on mRNA expression of genes involved in osteoblastogenesis and osteoclastogenesis in femurs of rats with SCI. The adult male rats were exposed to surgical transection of the cord and treated with cannabidiol (CBD, 5 mg/kg/day, i.p.) for consecutive 14 days. Graphs showed the mRNA expression of ALP (A), OPG (B), RANKL (C), and TRAP (D) in femurs of rats. ALP, alkaline phosphatase; TRAP, tartrate-resistant acid phosphatase; RANKL, receptor activator of NF-κB ligand; OPG, osteoprotegerin; SCI, spinal cord injury; Data are expressed as mean ± S.D.; n=9 in each group; * $P < 0.05$ compared with sham-operated group received vehicle; # $P < 0.05$ compared with SCI group received vehicle.

SCI rats with CBD (5 mg/kg/day, i.p.) significantly elevated the serum levels of osteocalcin, reduced the serum levels of CTX, and enhanced BMD of tibiae and femurs. In sham-operated rats, treatment with CBD had no significant effect on serum levels of osteocalcin and CTX and BMD of tibiae and femurs. 0.5 mg/kg/day of CBD had no significant effect on serum levels of osteocalcin and CTX and BMD of tibiae and femurs in sham-operated rats or SCI rats.

3.2. Treatment with CBD attenuated disruption of bone structure of proximal tibiae of rats with SCI

SCI decreased the bone volume (BV/TV, Fig. 2A), trabecular thickness (Tb. Th, Fig. 2B) and trabecular number (Tb. N, Fig. 2D), and increased the trabecular separation (Tb. Sp, Fig. 2C), suggesting that SCI caused the cancellous trabecular bone to become thin and sparse, and disrupted the bone structure. Treatment with CBD (5 mg/kg/day) for 14 days enhanced BV/TV, Tb. Th, and Tb. N, and reduced Tb. Sp in proximal tibiae of rats with SCI, indicating that treatment

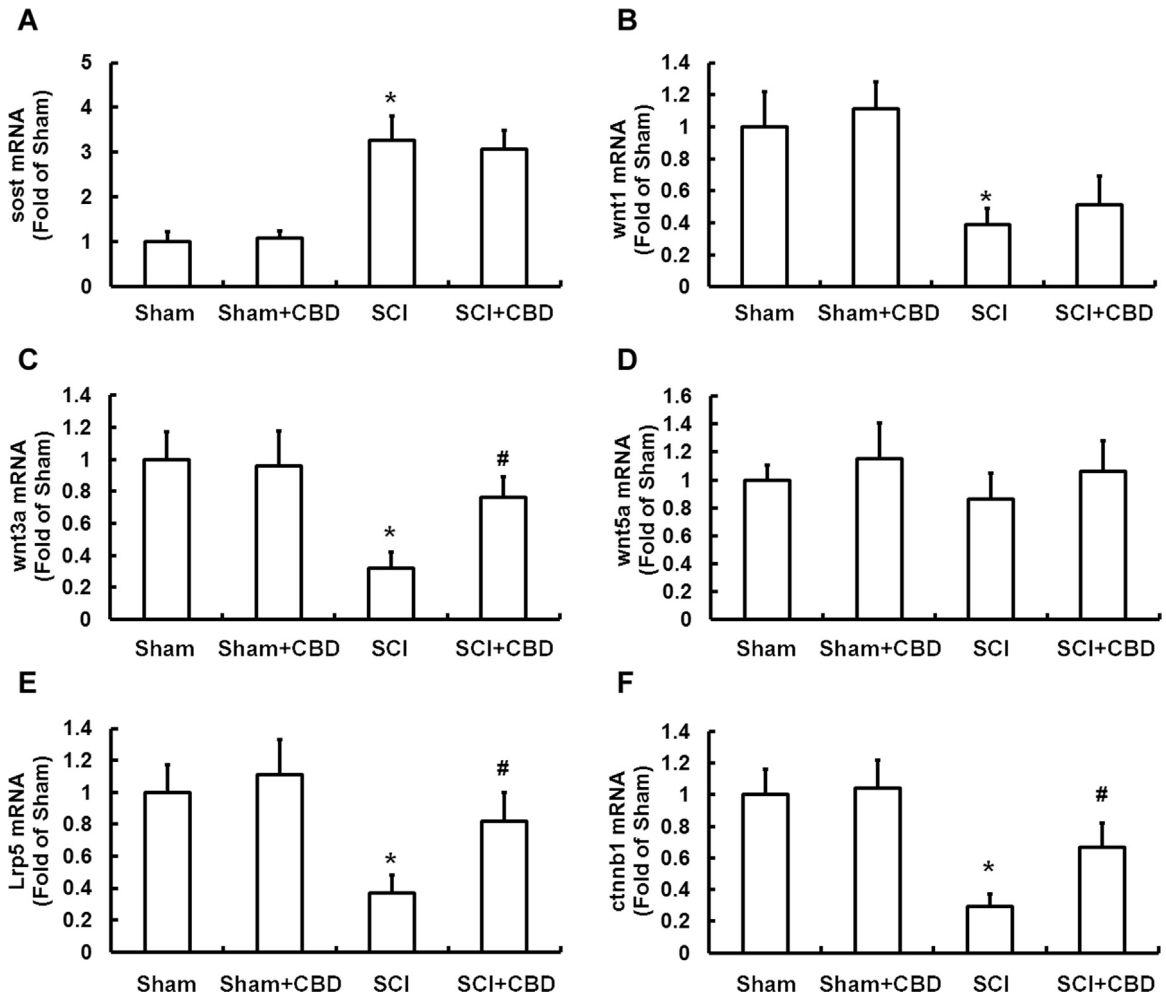

Fig. 5. Treatment with cannabidiol had beneficial effects on mRNA expression of genes involved in Wnt/beta-catenin pathway in femurs of rats with SCI. The adult male rats were exposed to surgical transection of the cord and treated with cannabidiol (CBD, 5 mg/kg/day, i.p.) for consecutive 14 days. Graphs showed the mRNA expression of sost (A), wnt1 (B), wnt3a (C), wnt5a (D), Lrp5 (E) and ctnnb1 (F) in femurs of rats. SCI, spinal cord injury; Data are expressed as mean ± S.D.; n=9 in each group; * $P < 0.05$ compared with sham-operated group received vehicle; # $P < 0.05$ compared with SCI group received vehicle.

with CBD attenuated the disruption of sublesional bone structure following SCI.

3.3. Treatment with CBD enhanced mechanical properties of the femoral diaphysis of rats with SCI

When compared with the sham-operated rats, ultimate compressive load (Fig. 3A), stiffness (Fig. 3B), and energy to max force (Fig. 3C) of femoral diaphysis were lower in rats with SCI. Treatment with CBD (5 mg/kg/day) for 14 days increased ultimate compressive load, stiffness, and energy to max force of femoral diaphysis of SCI rats, indicating that CBD enhanced mechanical properties of the femoral diaphysis of rats with SCI. Treatment of sham-operated rats with CBD had no significant effect on these mechanical parameters. The displacement at the ultimate load (Fig. 3D) was similar among four groups.

3.4. Treatment with CBD had beneficial effects on mRNA expression of genes involved in osteoblastogenesis and osteoclastogenesis in femurs of rats with SCI

At the end of the experiment, femurs were removed for RT-PCR measurement. The mRNA expression of ALP (Fig. 4A) and OPG (Fig. 4B) was lower and the mRNA expression of RANKL (Fig. 4C) and TRAP (Fig. 4D) was higher in femurs of rats with SCI than that in sham-operated rats. Treatment with CBD (5 mg/kg/day) for 14 days upregulated mRNA expression of ALP and OPG and downregulated mRNA expression of RANKL and TRAP in femurs of rats with SCI, indicating that CBD promoted genes involved in osteoblastogenesis and suppressed genes involved in osteoclastogenesis in femurs of rats with SCI. In sham-operated rats, CBD treatment had no significant effect on mRNA levels of these genes.

3.5. Treatment with CBD had beneficial effects on mRNA expression of genes involved in Wnt/beta-catenin pathway in femurs of rats with SCI

When compared to the sham-operated rats, the mRNA expression of sost (Fig. 5A) was higher and mRNA expression of wnt1 (Fig. 5B), wnt3a (Fig. 5C), Lrp5 (Fig. 5E), and ctnnb1 (Fig. 5F) was lower in femurs of rats with SCI. Treatment with CBD (5 mg/kg/day) for 14 days had no significant effect on mRNA expression of sost and wnt1, but enhanced mRNA expression of wnt3a, Lrp5 and ctnnb1 in femurs of rats with SCI. The mRNA expression of wnt5a (Fig. 5D) was similar among four groups.

4. Discussion

To our knowledge, our results provide the first direct evidence indicating that CBD treatment prevents sublesional bone loss and

deterioration of trabecular bone subsequent to SCI.

The accepted paradigm for sublesional skeletal deterioration following SCI involved two phases: acute phase (rapid, acute bone loss that plateaus approximately between 18 and 24 months post-injury) and chronic phase (ongoing bone loss that is more gradual in nature for decades after injury (Morse et al., 2009; Tan et al., 2013). Analysis of patients post-SCI revealed an initial decrease in bone formation and steadily increasing bone resorption (Uebelhart et al., 1994). This uncoupling of osteoblast/osteoclast function in response to SCI was supported by our finding in the changes of serum levels of osteocalcin and CTX 14 days post-SCI. Bone formation and resorption were coupled through the RANKL/OPG axis. RANKL could stimulate the recruitment and activity of osteoclast, and hence enhanced bone resorption. OPG could competitively bind to RANK and inhibit RANKL-stimulated osteoclast activation (Maimoun et al., 2005; Kobayashi et al., 2009). In this work, treatment with CBD suppressed RANKL expression and upregulated OPG expression in femurs of SCI rats. In experimental periodontitis, CBD inhibited RANK/RANKL expression and decreases bone resorption (Napimoga et al., 2009). In addition, it was reported that long-term stimulation with CBD induced differentiation of mesenchymal stem cells (MSCs) into the osteoblastic lineage as evidenced by increased mineralization assessed and enhanced activity of ALP (Schmuhl et al., 2014). CBD attenuated the imbalance between osteoblastogenesis and osteoclastogenesis in response to SCI, which contributed to the beneficial effect of CBD on bone loss in SCI.

In this work, the mRNA expression of sost was upregulated and mRNA expression of wnt1, wnt3a, Lrp5, and ctnnb1 was downregulated in femurs of rats with SCI, indicating that sclerostin/Wnt/β-catenin might be involved in the sublesional bone loss following SCI. Wnt/β-catenin signaling stimulated the generation of osteoblasts by promoting commitment and differentiation of MSCs towards the osteoblast lineage and decreased osteoclast differentiation by upregulating the production and secretion of OPG (Manolagas, 2014). Sclerostin inhibited the Wnt pathway by competitive binding to Lrp5 and was a key mediator of SCI-induced bone loss (Qin et al., 2015; Beggs et al., 2015). It has been demonstrated that sclerostin inhibition prevented SCI-induced cancellous bone loss (Qin et al., 2015; Beggs et al., 2015) and mice with sclerostin gene deletion were resistant to the severe sublesional bone loss induced by SCI (Qin et al., 2016). CBD was reported to activate the wnt/β-catenin in Abeta-stimulated PC12 cells and inhibit tau protein hyperphosphorylation (Esposito et al., 2006). In this work, treatment with CBD had no significant effect on sost expression, but upregulated expression of wnt3a and ctnnb1. It was reported that liposomal Wnt3a reestablished the osteogenic capacity of bone grafts from aged animals (Leucht et al., 2013). Therefore, CBD-induced activation of Wnt/β-catenin signaling following SCI might contribute to its beneficial effect against sublesional bone loss.

CBD was known to act with several receptors including CB1 receptor, CB2 receptor and GRP55. Mice with CB1 deficiency had high peak bone mass because of an osteoclast defect but develop age-related osteoporosis because of impaired bone formation. Mice with CB2 deficiency had relatively normal peak bone mass but developed age-related osteoporosis because of uncoupling of bone resorption from bone formation. Mice with GPR55 deficiency had increased bone mass because of an osteoclast defect, but bone formation was not affected (Idris et al., 2010). Whether these receptors were involving in the protection of CBD against sublesional bone loss following SCI requires further investigation.

Treatment of male mice for 8 weeks with 10 mg/kg CBD (3 times per week) significantly decreased the levels of serum CTX, a biochemical marker of bone resorption (Whyte et al., 2009). In this work, treatment of male rats for 2 weeks with CBD (5 mg/kg/day) had no significant effect on serum CTX levels and we did not observe the potential effect of CBD treatment on bone in healthy animals, which might be due to the low dose and short treatment time. Further studies are needed to clarify effect of higher-dose and longer-time exposure to CBD on bone in healthy animals.

In conclusion, CBD administration attenuated SCI-induced sublesional cancellous bone loss.

Conflicts of interest disclosure

The authors declare that we have no conflict of interest.

References

Akhigbe, T., Chin, A.S., Svircev, J.N., Hoenig, H., Burns, S.P., Weaver, F.M., Bailey, L., Carbone, L., 2015. A retrospective review of lower extremity fracture care in patients with spinal cord injury. J. Spinal Cord. Med. 38, 2–9.

Beggs, L.A., Ye, F., Ghosh, P., Beck, D.T., Conover, C.F., Balaez, A., Miller, J.R., Phillips, E.G., Zheng, N., Williams, A.A., Aguirre, J.I., Wronski, T.J., Bose, P.K., Borst, S.E., Yarrow, J.F., 2015. Sclerostin inhibition prevents spinal cord injury-induced cancellous bone loss. J. Bone Miner. Res. 30, 681–689.

Burstein, S., 2015. Cannabidiol (CBD) and its analogs: a review of their effects on inflammation. Bioorg. Med. Chem. 23, 1377–1385.

Campos, A.C., Moreira, F.A., Gomes, F.V., Del Bel, E.A., Guimaraes, F.S., 2012. Multiple mechanisms involved in the largespectrum therapeutic potential of cannabidiol in psychiatric disorders. Philos. Trans. R. Soc. Lond. B Biol. Sci. 367, 3364–3378.

Carbone, L.D., Chin, A.S., Burns, S.P., Svircev, J.N., Hoenig, H., Heggeness, M., Weaver, F., 2013. Morbidity following lower extremity fractures in men with spinal cord injury. Osteoporos. Int.: a J. Establ. Result Coop. Eur. Found. Osteoporos. Natl. Osteoporos. Found. USA 24, 2261–2267.

Cirnigliaro, C.M., Myslinski, M.J., La Fountaine, M.F., Kirshblum, S.C., Forrest, G.F., Bauman, W.A., 2017. Bone loss at the distal femur and proximal tibia in persons with spinal cord injury: imaging approaches, risk of fracture, and potential treatment options. Osteoporos. Int. 28, 747–765.

Esposito, G., De Filippis, D., Carnuccio, R., Izzo, A.A., Iuvone, T., 2006. The marijuana component cannabidiol inhibits beta-amyloid-induced tau protein hyperphosphorylation through Wnt/beta-catenin pathway rescue in PC12 cells. J. Mol. Med. (Berl.) 84, 253–258.

Idris, A.I., Ralston, S.H., 2010. Cannabinoids and bone: friend or foe? Calcif. Tissue Int. 87, 285–297.

Jiang, S.D., Jiang, L.S., Dai, L.Y., 2006. Spinal cord injury causes more damage to bone mass., bone structure., biomechanical properties and bone metabolism than sciatic neurectomy in young rats. Osteoporos. Int. 17, 1552–1561.

Jiang, S.D., Shen, C., Jiang, L.S., Dai, L.Y., 2007. Differences of bone mass and bone structure in osteopenic rat models caused by spinal cord injury and ovariectomy. Osteoporos. Int. 18, 743–750.

Kobayashi, Y., Udagawa, N., Takahashi, N., 2009. Action of RANKL and OPG for osteoclastogenesis. Crit. Rev. Eukaryot. Gene Expr. 19, 61–72.

Kogan, N.M., Melamed, E., Wasserman, E., Raphael, B., Breuer, A., Stok, K.S., Sondergaard, R., Escudero, A.V., Baraghithy, S., Attar-Namdar, M., Friedlander-Barenboim, S., Mathavan, N., Isaksson, H., Mechoulam, R., Müller, R., Bajayo, A., Gabet, Y., Bab, I., 2015. Cannabidiol, a Major Non-Psychotropic Cannabis Constituent Enhances Fracture Healing and Stimulates Lysyl Hydroxylase Activity in Osteoblasts. J. Bone Miner. Res. 30, 1905–1913.

Leucht, P., Jiang, J., Cheng, D., Liu, B., Dhamdhere, G., Fang, M.Y., Monica, S.D., Urena, J.J., Cole, W., Smith, L.R., Castillo, A.B., Longaker, M.T., Helms, J.A., 2013. Wnt3a reestablishes osteogenic capacity to bone grafts from aged animals. J. Bone Jt. Surg. Am. 95, 1278–1288.

Liu, D., Zhao, C.Q., Li, H., Jiang, S.D., Jiang, L.S., Dai, L.Y., 2008. Effects of spinal cord injury and hindlimb immobilization on sublesional and supralesional bones in young growing rats. Bone 43, 119–125.

Maimoun, L., Couret, I., Mariano-Goulart, D., Dupuy, A.M., Micallef, J.P., Peruchon, E., Ohanna, F., Cristol, J.P., Rossi, M., Leroux, J.L., 2005. Changes in osteoprotegerin/RANKL system., bone mineral density., and bone biochemicals markers in patients with recent spinal cord injury. Calcif. Tissue Int. 76, 404–411.

Manolagas, S.C., 2014. Wnt signaling and osteoporosis. Maturitas 78, 233–237.

Morse, L.R., Battaglino, R.A., Stolzmann, K.L., Hallett, L.D., Waddimba, A., Gagnon, D., Lazzari, A.A., Garshick, E., 2009. Osteoporotic fractures and hospitalization risk in chronic spinal cord injury. Osteoporos. Int. 20, 385–392.

Napimoga, M.H., Benatti, B.B., Lima, F.O., Alves, P.M., Campos, A.C., Pena-Dos-Santos, D.R., Severino, F.P., Cunha, F.Q., Guimarães, F.S., 2009. Cannabidiol decreases bone resorption by inhibiting RANK/RANKL expression and pro-inflammatory cytokines during experimental periodontitis in rats. Int Immunopharmacol. 9, 216–222.

Qin, W., Zhao, W., Li, X., Peng, Y., Harlow, L.M., Li, J., Qin, Y., Pan, J., Wu, Y., Ran, L., Ke, H.Z., Cardozo, C.P., Bauman, W.A., 2016. Mice with sclerostin gene deletion are resistant to the severe sublesional bone loss induced by spinal cord injury. Osteoporos. Int. 27, 3627–3636.

Qin, W., Li, X., Peng, Y., Harlow, L.M., Ren, Y., Wu, Y., Li, J., Qin, Y., Sun, J., Zheng, S., Brown, T., Feng, J.Q., Ke, H.Z., Bauman, W.A., Cardozo, C.C., 2015. Sclerostin antibody preserves the morphology and structure of osteocytes and blocks the severe skeletal deterioration after motor-complete spinal cord injury in rats. J. Bone Miner. Res. 30, 1994–2004.

Rajesh, M., Mukhopadhyay, P., Bátkai, S., Patel, V., Saito, K., Matsumoto, S., Kashiwaya, Y., Horváth, B., Mukhopadhyay, B., Becker, L., Haskó, G., Liaudet, L., Wink, D.A., Veves, A., Mechoulam, R., Pacher, P., 2010. Cannabidiol attenuates cardiac

dysfunction., oxidative stress., fibrosis., and inflammatory and cell death signaling pathways in diabetic cardiomyopathy. J. Am. Coll. Cardiol. 56, 2115–2125.
Rohleder, C., Müller, J.K., Lange, B., Leweke, F.M., 2016. Cannabidiol as a Potential New Type of an Antipsychotic. A Critical Review of the Evidence. Front Pharmacol. 7, 422.
Schmuhl, E., Ramer, R., Salamon, A., Peters, K., Hinz, B., 2014. Increase of mesenchymal stem cell migration by cannabidiol via activation of p42/44 MAPK. Biochem Pharmacol. 87, 489–501.
Seeman, P., 2016. Cannabidiol is a partial agonist at dopamine D2High receptors., predicting its antipsychotic clinical dose. Transl. Psychiatry 6, e920.
Silveira, J.W., Issy, A.C., Castania, V.A., Salmon, C.E., Nogueira-Barbosa, M.H., Guimarães, F.S., Defino, H.L., Del Bel, E., 2014. Protective effects of cannabidiol on lesion-induced intervertebral disc degeneration. PLoS One 9, e113161.
Sun, L., Pan, J., Peng, Y., Wu, Y., Li, J., Liu, X., Qin, Y., Bauman, W.A., Cardozo, C., Zaidi, M., Qin, W., 2013. Anabolic steroids reduce spinal cord injury-related bone loss in rats associated with increased Wnt signaling. J. Spinal Cord. Med. 36, 616–622.
Tan, C.O., Battaglino, R.A., Morse, L.R., 2013. Spinal cord injury and osteoporosis: causes., mechanisms., and rehabilitation strategies. Int. J. Phys. Med. Rehabil. 1, (pii: 127).
Troy, K.L., Morse, L.R., 2015. Measurement of Bone: diagnosis of SCI-Induced Osteoporosis and Fracture Risk Prediction. Top. Spinal Cord. Inj. Rehabil. 21, 267–274.
Uebelhart, D., Hartmann, D., Vuagnat, H., Castanier, M., Hachen, H.J., Chantraine, A., 1994. Early modifications of biochemical markers of bone metabolism in spinal cord injury patients. A preliminary study. Scand. J. Rehabil. Med 26, 197–202.
Vuolo, F., Petronilho, F., Sonai, B., Ritter, C., Hallak, J.E., Zuardi, A.W., Crippa, J.A., Dal-Pizzol, F., 2015. Evaluation of serum cytokines levels and the role of cannabidiol treatment in animal model of asthma. Mediat. Inflamm. 2015, 538670.
Whyte, L.S., Ryberg, E., Sims, N.A., Ridge, S.A., Mackie, K., Greasley, P.J., Ross, R.A., Rogers, M.J., 2009. The putative cannabinoid receptor GPR55 affects osteoclast function in vitro and bone mass in vivo. Proc. Natl. Acad. Sci. USA 106, 16511–16516.