European Journal of Pharmacology 765 (2015) 209–216

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Endocrine pharmacology

Treatment with curcumin alleviates sublesional bone loss following spinal cord injury in rats

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ARTICLE INFO

Article history:
Received 3 April 2015
Received in revised form
19 August 2015
Accepted 19 August 2015
Available online 20 August 2015

Keywords:
Curcumin
Spinal cord injury
Vitamin D receptor
Oxidative stress
Wnt/β-catenin pathway

ABSTRACT

This work aimed to investigate the therapeutic effect of curcumin on sublesional bone loss induced by spinal cord injury (SCI) in rats. SCI model in this work was generated in rats by surgical transaction of the cord at the T_{10-12} level. After the surgery, animals were treated with curcumin (110 mg/kg body mass/day, via oral gavages) for 2 weeks. Treatment of SCI rats with curcumin prevented the reduction of bone mass in tibiae and femurs, preserved bone microstructure including trabecular bone volume fraction, trabecular number, and trabecular thickness in proximal tibiae, and preserved mechanical properties of femoral midshaft. Treatment of SCI rats with curcumin increased osteoblast surface and reduced osteoclast surface in proximal tibiae. Treatment of SCI rats with curcumin increased osteocalcin mRNA expression and reduced mRNA levels of tartrate-resistant acid phosphatase and mRNA ratio of receptor activator of NF-κB ligand/osteoprotegerin in distal femurs. Treatment of SCI rats with curcumin reduced serum and femoral levels of thiobarbituric acid reactive substances. Treatment of SCI rats with curcumin had no significant effect on serum 25(OH)D, but enhanced mRNA and protein expression of vitamin D receptor (VDR) in distal femurs. Treatment of SCI rats with curcumin enhanced mRNA levels of Wnt3a, Lrp5, and ctnnb1 and upregulated protein expression of β-catenin in distal femurs. In conclusions, treatment with curcumin abated oxidative stress, activated VDR, and enhanced Wnt/β-catenin pathway, which might explain its beneficial effect against sublesional bone loss following SCI in rats, at least in part.

1. Introduction

Spinal cord injury (SCI) is a life long and life changing injury that results in severe immobilization, muscle atrophy, paralysis, severe sublesional bone loss and increased fracture risk (Edwards et al., 2014). Long-term follow-up data revealed that up to 50% of patients with SCI fractured at some point following their injury (Szollar et al., 1998), which complicated rehabilitation, recovery, and activities of daily living. The regions most suffered from SCI are the proximal tibia and distal femur, where reduction in bone mineral density (BMD) may exceed 50% (Gaspar et al., 2012; Eser et al., 2005). Following SCI, bone remodeling becomes uncoupled with an initial reduction in bone formation and steadily increases in bone resorption (Uebelhart et al., 1994). Various interventions (including physical activity and electrical stimulation) have been applied to patients with SCI for treatment, but less sufficient positive effects were reported (Ben et al., 2005; Giangregorio et al., 2005; Needham-Shropshire et al., 1997). Regarding pharmacologic treatments, bisphosphonates emerge as the regimen most-studied and have been tested in paraplegic patients since 1981 (Minaire et al., 1981), but their effects on bone mass of SCI patients remain controversial (Bryson and Gourlay, 2009).

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a phenolic natural product derived from the yellow-pigmented fraction of turmeric. A great deal of attention on curcumin was due to that it exhibited a wide range of biological activities, including anti-inflammation, lipid-lowering effects, and antioxidant properties (Zingg et al., 2013). Laboratory

Abbreviations: SCI, spinal cord injury; BMD, bone mineral density; Tb.N, trabecular number; BV/TV, trabecular bone volume fraction; Tb.Th, trabecular thickness; Tb.Sp, trabecular separation; MAR, mineral apposition rate; BFR/BS, bone formation rate/bone surface; ES/BS, eroded surface/bone surface; Oc.S/BS, osteoclast surface/bone surface; TBARS, thiobarbituric acid reactive substances; TRAP, tartrate-resistant acid phosphatase; RANKL, receptor activator of NF-κB ligand; OPG, osteoprotegerin; Lrp5, low-density lipoprotein-related protein5; GAPDH, glyceraldehyde phosphate dehydrogenase; VDR, vitamin D receptor; 25(OH)D, 25-hydroxyvitamin D

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http://dx.doi.org/10.1016/j.ejphar.2015.08.036
0014-2999/

investigations revealed that curcumin exhibited neuroprotective effect in SCI rat model (Jin et al., 2014; Kim et al., 2014). Curcumin has attracted considerable interest because of its beneficial effects on events associated with osteoporosis. Curcumin prevented bone loss in ovariectomized mature rodent model of postmenopausal osteoporosis (Hussan et al., 2012; Kim et al., 2011; French et al., 2008) and experimental periodontitis bone loss model (Zhou et al., 2013). Curcumin analogue UBS109 prevented bone loss in breast cancer bone metastasis mouse model by stimulating osteoblastic mineralization and suppressing osteoclastogenesis (Yamaguchi et al., 2014). Curcumin improved bone microarchitecture and enhanced BMD in APP/PS1 transgenic mice, a model of alzheimer's disease (Yang et al., 2011). Recently, it was reported that curcumin treatment attenuated hind-limb suspension-induced bone loss in rats (Xin et al., in press).

In this work, curcumin was applied for treatment of SCI-induced sublesional bone loss in rats, and underlying mechanisms were investigated.

2. Materials and methods

2.1. Animals and study design

Male Sprague-Dawley (SD, 8-week old) rats were purchased from the Vital-Aiver Animal Ltd (Beijing, China). All the rats were housed in a controlled environment at 22 ± 3 °C with a 12 h light/dark cycle, and free access to tap water and food. All animal studies were performed according to ethical guidelines for animal care. SD rats were randomly divided into four groups (n=30–32 total in each group) and treated as follows: (1) sham-operated rats (sham); (2) sham-operated rats received curcumin treatment (sham+C); (3) SCI rats (SCI); (2) SCI rats received curcumin treatment (SCI+C). At the end of the experimental period, femurs and tibiae were removed and blood was collected for measurements. All protocols were conducted in accordance with the *Guidance Suggestions for the Care and Use of Laboratory Animals*, formulated by the Ministry of Science and Technology of China.

2.2. Rat model of SCI and treatment protocol

Briefly, under deep anesthesia (ketamine, 75 mg/kg+xylazine, 10 mg/kg body weight, intraperitoneal injection), the dorsum of the animals were shaved and a longitudinal midline incision was made through the skin. After the back muscles were infiltrated, the dorsal surface of the spinal cord was exposed by laminectomy at the T_{10-12} levels, and the lower thoracic cord was subsequently completely transected with microscissors under magnifying lenses. The sponge gel was injected into the site of the transection to fill the gap. All sham-operated rats underwent a similar operation to those in the SCI group, except that the lower thoracic cord was exposed but not transected. Manual evacuation of the bladder was regularly done 3 times one day until reflex micturition was restored.

The 110 mg/kg dose of curcumin was freshly dissolved in 1.0 ml of palm oil (Hussan et al., 2012; Taty et al., 2011). Same amount of palm oil (Merck, Germany) was used as vehicle. 12 h after surgery, the treatment was given via oral gavages daily for 2 weeks.

2.3. Measurement of serum 25-hydroxyvitamin D (25(OH)D)

At the end of the experimental period, blood was collected from dorsal aorta under anesthesia. After centrifugation, serum was harvested and kept at −20 °C until analysis. Serum level of 25(OH)D was determined with a ELISA kit (IBL-America, Minneapolis, MN, USA).

2.4. Measurement of thiobarbituric acid reactive substances (TBARS) levels

At the end of the experimental period, the femurs were excised and soft tissue including cartilage, tendon and ligament was cleaned. The distal femurs were ground to a fine powder in liquid nitrogen. The frozen powder was then transferred into 1 × RIPA buffer (150 mM NaCl, 1.0% IGEPAL® CA–630, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris, pH 8.0, Sigma). The total protein content was measured using a protein assay kit (Bio-Rad Laboratories; Hercules, California, USA) utilizing bovine serum albumin (BSA) as the protein standard. TBARS levels in bone homogenates and serum were determined by using a ELISA kit (Beyotime, Jiangsu, China).

2.5. Biochemical analysis

Serum osteocalcin levels were determined with an Osteocalcin EIA kit (Xinqidi bio-Technology, Inc., China) as described in the manufacturer's directions. Serum CTX levels were quantified by an ELISA kit (Sunbio, Inc., China). DPD excretion was quantified by using an EIA kit (Xinqidi Biological Technology, Hubei, China), and the data were corrected for the urinary creatinine levels, which were determined with a Kit (West-tang Bio-Tech, Shanghai, China).

2.6. Measurement of bone mass

After the rats were killed, the tibiae and femurs were excised from each rat and cleaned soft tissue including cartilage, tendon and ligament. bone mineral density (BMD) and bone mineral content (BMC) were measured using small-animal special Dual Energy X-ray Absorptiometry (DEXA, Hologic, Inc. USA). Tibiae and femurs were placed on an acrylic platform of uniform (thickness, 38.1 mm).

2.7. Micro-CT analysis

Trabecular bone morphometry within the proximal tibial metaphysis was measured by using micro-CT (μCT). Bones were stored in parafilm to prevent dehydration during the scan. Samples were scanned in a μCT device manufactured and developed by SkyScan (SkyScan1072, Kontich, Belgium). The X-ray system is based on a micro focus tube (20–80 kV, 0–100 mA) reaching a minimum spot size of 8 mm at 8 W generating X-rays in cone-beam geometry. The metaphyseal region of proximal tibiae were scanned in 250 slices (13 μm thickness) in the dorsoventral direction. Three-dimensional reconstruction of bone was performed using the triangulation algorithm. Histomorphometric analysis was performed by pathologists (Liang Yan and Ming Yang) in a blind fashion.

2.8. Histomorphometry analysis

Eroded surface, osteoclast surface, and osteoblast surface were obtained from the histomorphometric results collected with the Bioquant Bone Morphometry System (R&M Biometrics Corp., Nashville, TN, USA). Bone formation rate (BFR) and mineral apposition rate (MAR) was determined by the calcein double-labeling method as described (Wang et al., 2013). All these histomorphometric indices in this work were reported in accordance with the guidelines on histomorphometry terminology and reporting conventions (Dempster et al., 2013).