Morindia officinalis polysaccharide attenuates osteoporosis in rats underwent bilateral ovariectomy by suppressing the PGC-1α/PPARγ pathway

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Abstract

Objective: Osteoporosis (OP) is a widespread disease that causes risks of spine and hip fractures. Morindia officinalis polysaccharide (MOP) shows therapeutic potential in OP. This article intended to understand the mechanism by which MOP impacts bone mineral density (BMD) and serum trace elements in OP rats.

Methods: OP rat models were established by bilateral ovariectomy (OVX). Rats were intragastrically administered with MOP or ZLN005 [the activator of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α)] since the first day after operation for 8 weeks. Microstructural changes in OP rats were analyzed using micro-computed tomography system. Contents of serum Zn, Cu, Fe, and Mg in rats were measured. Levels of serum superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), GSH, and malondialdehyde (MDA) in rats were determined by Enzyme-linked immunosorbent assay. Protein levels of PGC-1α and peroxisome proliferator-activated receptor γ (PPARγ) in cartilage tissues of rats were determined via Western blotting.

Results: MOP enhanced BMD, bone volume per trabecular volume (BV/TV), Tb.N, and Tb.Th and reduced Tb.Sp in the distal femur of OVX rats, elevated levels of serum Cu, Fe, and Mg and contents of SOD, GSH, and GSH-PX and decreased MDA content. Moreover, MOP suppressed the PGC-1α/PPARγ pathway. Activation of PGC-1α partially abolished the action of MOP on ameliorating OP in OVX rats and strengthening anti-oxidation ability.

Conclusion: MOP mitigated OP in OVX rats by inhibiting the PGC-1α/PPARγ pathway.

Keywords
Morindia officinalis polysaccharide, osteoporosis, PGC-1α, PPARγ, bilateral ovariectomy, bone mineral density, trace element, oxidative stress

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Introduction

Osteoporosis (OP) refers to a systemic metabolic bone disease featured by low bone mass, damaged bone microstructure, and risks of increased bone fragility and susceptibility to fractures.¹ OP is more frequently reported in postmenopausal women owing to menopause-associated
estrogen deficiency, and its ever-increasing incidence has caused public concern. Further investigation has shown a correlation between oxidative stress and postmenopausal OP. In other words, a high oxidative balance score is correlated with a low risk of lumbar spine OP. The interplay between oxidative stress and bone resorption could potentially trigger postmenopausal OP. Current therapies for OP include hormone therapy, application of bisphosphonate, and supplement of trace elements. However, limitations or side effects come along with the long-term use of medications such as gastrointestinal intolerance and increased risks for certain types of cancers. Therefore, effective drugs with fewer side effects are in urgent need. With recent advances in clinical research of OP treatment using traditional Chinese medicine, traditional Chinese medicine containing active ingredients for OP treatment has become a hot topic in clinical research, which is the starting point of this article.

Morinda officinalis How is a plant of the Rubiaceae, Morinda family abundant in tropic and sub-tropic areas such as Guangdong, Guangxi, Fujian, and Hainan provinces in China. Morinda officinalis How consists of multiple active ingredients and possesses the capabilities of strengthening muscles and bones, anti-oxidation, anti-inflammatory, and kidney protection. Morinda officinalis polysaccharide (MOP), an active ingredient extracted from Morinda officinalis How, has a promising prospect in OP prevention and management. As suggested, MOP not only yields protection against bone loss in ovariectomized rats but improves the meat quality of chickens with tibial dyschondroplasia by reducing oxidative injury. Nonetheless, whether MOP produces any effects on bone mineral density (BMD) and serum trace elements in OP rats remains a mystery. Peroxisome proliferator-activated receptor γ (PPARγ) and its coactivator-1α (PGC-1α) are implicated in OP treatment. The application of telmisartan influences bone density and microarchitecture in rats with preexisting osteoporotic bone disorders through PPARγ. Nicotinamide mononucleotide mitigates glucocorticoid-induced OP via the SIRT1/PGC-1α signaling pathway. Nevertheless, the function of MOP in BMD and serum trace elements in OP rats upon the basis of the PGC-1α/PPARγ pathway remains elusive so far. Thus, this article attempted to expound on the mechanism of MOP in affecting BMD and serum trace elements in OP rats.

Materials and methods

Ethics statement

All experimental procedures and protocols related to animals were granted by the Medical ethics committee of Xinjiang Medical University (IACUC-20211104–31) and carried out in strict accordance with the national guidelines for animal experiments. Significant endeavor was contributed to reducing the number of animals used and their suffering.

Experimental animals

Totally 40 female Sprague-Dawley rats (12 weeks, 250–280 g) procured from Shanghai Jiesijie Laboratory Animal Co., Ltd. [SCXK(Shanghai) 2018–0004, Shanghai, China] were housed at 20–25°C and 45–50% humidity with a 12/12 light/dark cycle and free access to feed and drinking water.

Establishment of OP rat models

The OP rat model was established by bilateral ovariectomy (OVX). Rats were intraperitoneally administered with 2% pentobarbital sodium (40 mg/kg) for anesthesia. Two 10-mm incisions were made on the bilateral lateral lumbar skin, and bilateral ovaries were removed after separating the exposed muscles and peritoneum using a blunt apparatus. Rats in the sham group were operated in the same way without removal of bilateral ovaries. After suture, rats were injected with 40,000 IU/mL penicillin (1 mL/kg) for three consecutive days.

One day after operation, rats were treated with MOP. Rats were randomized into five groups: (1) the sham group (rats were given an equal amount of normal saline by intragastric administration), (2) the OVX group (rats were given the equivalent amount of normal saline by intragastric administration), (3) the OVX + MOP group (rats were given 300 mg/kg MOP each day by intragastric administration for eight consecutive weeks), (4) the OVX + MOP + DMSO group [rats were intragastrically administered with an equal amount of dimethyl sulfoxide (DMSO) for eight consecutive weeks after daily intragastric administration of 300 mg/kg MOP], (5) the OVX + MOP + ZLN005 group (rats were intragastrically administered with ZLN005 (15 mg/kg body weight) for eight consecutive weeks after daily intragastric administration of 300 mg/kg MOP). MOP (98%) was supplied by Fufeng Ciyuan Biotechnology (Shaanxi, China). ZLN005, an agonist of PGC-1α, was provided by MedChemExpress (MCE, Monmouth Junction, NJ, USA). After the experiment, all rats were euthanized by an intraperitoneal injection of 180 mg/kg pentobarbital sodium, and the body weight of each rat was recorded. The uterus was isolated from each rat and immediately weighed. Meanwhile, the success of ovariectomy was confirmed by failure to detect ovarian tissues and by observation of marked atrophy of the uterine horns at necropsy.
**Micro-computed tomography**

The microstructural changes of the femur and the formation of new bones in the defect area of rats were analyzed 8 weeks later using a micro-computed tomography (micro-CT) system (mCT-80, Scanco Medical, Brüttisellen, Switzerland). Samples (0.018 mm) were scanned using 1024 reconstruction matrices and 200-ms integration time under a medium resolution. After three-dimensional reconstruction, BMD, bone volume per trabecular volume (BV/TV), number of trabeculae (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) values were automatically identified to verify the OP model. BMD and BV/TV were employed to assess new bone formation utilizing the auxiliary software (Scanco Medical, Bassersdorf, Switzerland).

**Measurement of serum Zn, Cu, Mg, and Mn**

Blood samples were collected from the abdominal aorta of rats after experimentation. The mixture of 0.1% HNO₃ (Analpure, Analytika), 0.1% Triton X-100 (for gas chromatography, Merck), and 3% 2-propanol (SupraSolv, Merck) was used for optimal dilution of the whole-blood samples. Subsequently, the samples were centrifuged and the supernatant was collected to determine minerals via inductively coupled plasma mass spectrometry (ICP-MS, NexION 350X; Perkin-Elmer, Waltham, MA, USA). The concentrations of ²⁴Mg, ⁶³Cu, and ⁶⁴Zn were quantified using the standard mode and the collision mode, respectively under the aforementioned conditions. Next, ⁴⁵Sc and yttrium (⁸⁹Y) (10 μg/L) were utilized as the internal standard elements. FAAS with a hollow cathode lamp was adopted for measurement of Fe concentration in the supernatant following serum deproteinization (1:15) with 1.1M HClO₄ (Analpure, Analytika) at 248.3 nm. The detection limits for Fe and Mg were 0.19 mg/L and 1.19 mg/mL, respectively, along with 0.40 μg/L for Cu and 3.30 μg/L for Zn.

**ELISA**

Blood samples were collected from the abdominal aorta of rats and centrifuged at 4°C and 4000 ×g for 10 min to harvest the serum. The levels of superoxide dismutase (SOD, ml059387), glutathione peroxidase (GSH-PX, ml097316), glutathione (GSH, ml531010), and malondialdehyde (MDA, ml077384) were determined using corresponding enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-linked Biotechnology, Shanghai, China) as per the manufacturer’s protocols. The concentration of serum estradiol (E₂) in rats was measured using the ELISA kit (ml002871).

**Western blotting**

Cartilage tissues were collected, lysed in lysis buffer, and centrifuged for 10 min at 10,000 ×g and 4°C. Protein concentration in the supernatant was quantified using a BCA™ protein assay kit (Beyotime Institute of Biotechnology, Hainan, China). Total protein (50 μg) was subjected to 8–10% SDS-PAGE and electrotransferred to PVDF membranes (EMD Millipore, Bedford, MA, USA). After blocking the membranes in 5% skim milk at 37°C for 1 h, primary antibodies PGC-1α (ab188102, 1/1000, Abcam, Cambridge, MA, USA), PPARγ (ab272718, 1/1000, Abcam), and β-actin (ab8227, 1/2000, Abcam) were added for overnight incubation at 4°C. Following three TBST washes, the membranes were supplemented with horseradish peroxidase-labeled secondary antibody IgG (ab6721, 1/5000, Abcam) for 1 h at room temperature. Enhanced chemiluminescence (Thermo Fisher Scientific, Rockford, IL, USA) was used for color development. Finally, immunodetection was carried out using Image Lab 3.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) with β-actin as an internal control.

**Statistical analysis**

SPSS 22.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.01 (GraphPad Software Inc., San Diego, CA, USA) were adopted for data analysis and plotting. Data were confirmed to be normally distributed by Shapiro-Wilk test. The results obtained were depicted as mean ± standard deviation. Pairwise comparison was processed utilizing the t test, while multi-group comparison was processed utilizing one-way analysis of variance (ANOVA) and Tukey’s multiple comparisons test. The value of p < .05 represented statistical significance.

**Results**

**OVX rat models are successfully established**

To understand the influence on BMD of rats produced by MOP, the OP rat model was established by OVX. The OVX rats had increased body weight and decreased uterine weight and estrogen concentration compared with sham-operated rats (all p < .01, Figure 1(a)–(c)). These results elucidated that OVX rat models were successfully established.

**MOP increases BMD in OVX rats by inhibiting the PGC-1α/PPARγ pathway**

There is evidence that MOP has anti-osteoporotic effects. There is evidence that MOP has anti-osteoporotic effects. Moreover, PGC-1α and PPARγ are active players in the development of OP. Thus, we first treated OVX rats with...
MOP. The quantitative analysis of microstructure parameters revealed lower BMD, BV/TV, Tb.N, and Tb.Th and higher Tb.Sp in the distal femur of OVX rats than those of sham-operated rats (all \( p < .01 \)). After 8 weeks of MOP treatment, OVX rats exhibited higher BMD, BV/TV, Tb.N, and Tb.Th and lower Tb.Sp in the distal femur (all \( p < .01 \), Figure 2(b)–(f)), suggesting that MOP could increase BMD in OVX rats and attenuate OP to some extent. We subsequently detected the protein levels of PGC-1\( \alpha \) and PPAR\( \gamma \) in the cartilage of OVX rats by Western blotting, which demonstrated elevated protein levels of PGC-1\( \alpha \) and PPAR\( \gamma \) (all \( p < .01 \)), and declined protein levels of PGC-1\( \alpha \) and PPAR\( \gamma \) after MOP treatment (all \( p < .01 \), Figure 2(a)), indicating that MOP could suppress the activity of the PGC-1\( \alpha \)/PPAR\( \gamma \) pathway.

To explore the functional mechanism by which MOP improves OP in OVX rats, we measured the contents of Zn, Cu, Fe, and Mg in the serum of rats. As illustrated in Figure 3(a)–(d), OVX rats exhibited decreased levels of Cu, Fe, and Mg relative to sham-operated rats (all \( p < .01 \)), and raised levels of Cu, Fe, and Mg after MOP treatment (all \( p < .01 \)). Additionally, the activation of the PGC-1\( \alpha \) pathway partially annihilated the action of MOP on elevating levels of serum Cu, Fe, and Mg in rats (all \( p < .05 \)). However, Zn level presented no prominent changes among different groups (all \( p > .05 \)). Overall, MOP could increase serum trace elements in OVX rats, whereas the activation of the PGC-1\( \alpha \) pathway could partially nullify the alleviating function of MOP on OP in OVX rats.

**MOP alleviates oxidative stress in OVX rats by suppressing the PGC-1\( \alpha \)/PPAR\( \gamma \) pathway**

Oxidative stress is proved to be connected with postmenopausal OP. To investigate whether MOP can reduce oxidative stress in OVX rats by inhibiting the PGC-1\( \alpha \)/PPAR\( \gamma \) pathway, we measured levels of serum SOD, GSH-PX, GSH, and MDA in rats through ELISA, which revealed diminished levels of serum SOD, GSH-PX, and GSH and augmented MDA level in OVX rats (all \( p < .01 \)), and the opposite trend in OVX rats after MOP treatment (all \( p < .01 \)). In addition, the activation of the PGC-1\( \alpha \) pathway partly abrogated the effects of MOP on increasing levels of serum SOD, GSH-PX, and GSH and decreasing MDA level (all \( p < .01 \), Figure 4(a)–(d)). Collectively, MOP could enhance the anti-oxidant capacity of OVX rats, while the activation of the PGC-1\( \alpha \) pathway could partially avert the promotional effects of MOP on the anti-oxidant capacity in OVX rats.

**Discussion**

OP has become a huge challenge and public health issue in need of therapeutic strategies with long-term efficacy and fewer side effects. The preservation of BMD is one of the
goals of OP treatment. The supplementations with trace elements also have a role to play in the reversal of OP progression. Intriguingly, MOP produces surprising results in OP prevention and treatment. It was unveiled in this article that MOP increased BMD and serum Cu, Fe, and Mg contents and thus palliated OP in OVX rats by suppressing the PGC-1α/PPARγ signaling pathway.

Low BMD is indicative of OP and high risks of fracture. Thus, we firstly established a rat model of OP via OVX and assessed BMD. BMD shows positive correlations with BV/TV and Tb.N and a negative correlation with Tb.Sp in patients with hip osteoarthritis. As indicated in our findings, OVX rats presented elevated BMD, BV/TV, Tb.N, and Tb.Th along with decreased Tb.Sp after MOP treatment, quite opposite to the observations prior to treatment. MOP has been shown to increase BMD of the distal femur in OVX rats. Likewise, MOP could improve BMD in OVX rats. Increased PPARγ expression is implicated in senile OP. As a PPARγ-associated transcriptional co-activator, PGC-1α is downregulated in skeletal muscles of OVX rats. We thereafter detected their protein levels in OVX rats and found enhanced protein levels before MOP treatment and decreased protein levels after MOP treatment, which was indicative of the suppression of MOP on the PGC-1α/PPARγ pathway. Next, we investigated whether the PGC-1α pathway is engaged in the regulation of MOP on OP. PGC-1α expression in OVX rats was enhanced using the PGC-1α pathway agonist ZLN005. PGC1α depletion negatively modulates bone mass in mice. Our results indicated the reversal of MOP function on increasing BMD of OVX rats mediated by activation of the PGC-1α pathway. Collectively, MOP could improve BMD in OVX rats by inhibiting the PGC-1α/PPARγ pathway.

In addition to BMD, trace elements, Zn and Cu in particular, are nonnegligible during skeleton development. At the same time, we measured the contents of serum trace elements in rats. The decreased serum Cu, Fe, and Mg contents in OVX rats were considerably increased after MOP treatment. MOP administration can remarkably enhance the concentrations of Mg, Cu, and Fe in OP rats in a dose-dependent manner. Similarly, MOP could elevate the contents of trace elements in the serum of OVX rats.

![Figure 2. MOP increases BMD in OVX rats by inhibiting the PGC-1α/PPARγ pathway.](image-url)
Upregulation of PGC-1α has been documented in copper-deficient rat hearts. Consistently, activation of the PGC-1α pathway posed a reversal effect on MOP-mediated elevation of serum Cu, Fe, and Mg in OVX rats. Thus, it could be inferred that MOP could increase serum trace elements in OVX rats by repressing the PGC-1α/PPARγ pathway.

Moreover, oxidative stress plays a potentially causal role in OP. The expressions of SOD and GSH-PX are low and MDA expression is high in femur tissues of OP rats. Our results evinced that MOP treatment raised levels of SOD, GSH, and GSH-PX and declined MDA levels in OVX rats. There is evidence that MOP reduces oxidative stress in chickens with tibial dyschondroplasia, which further confirms our finding that MOP could reduce oxidative stress in OVX rats. Furthermore, activation of PGC-1α expression is often associated with oxidative stress. In OVX rats, serum levels of SOD, GSH, and GSH-PX upregulated by MOP and MDA downregulated by MOP manifested the opposite trend after activating the PGC-1α/PPARγ pathway.

Taken together, the activated PGC-1α pathway could compromise the alleviating effect of MOP on OP in OVX rats. In other words, MOP could hinder oxidative stress in OVX rats through the inhibition of the PGC-1α/PPARγ pathway.

All in all, this article highlighted that MOP extenuated OP in OVX rats by suppressing the PGC-1α/PPARγ pathway. Nevertheless, there have been imperfections in this article. What defects this study is the lack of further animal experiments for in vivo verification and clinical data to probe into the concrete mechanism by which MOP modulates the PGC-1α/PPARγ pathway or other pathways in OP and the influential mechanism of other active
ingredients in traditional Chinese medicines on OP in OVX rats, which necessitates further studies in the future.

**Author contributions**

KR contributed to the study concepts, study design; KR and PH contributed to the literature research; KR, PH, YL and YZ contributed to the experimental studies and data acquisition; KR, LL, ZW and FW contributed to the data analysis and statistical analysis; KR contributed to the manuscript preparation and LL contributed to the manuscript editing and review; All authors read and approved the final manuscript.

**Declaration of conflicting interests**

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Ethical approval

All experimental procedures and protocols related to animals were granted by the Medical ethics committee of Xinjiang Medical University (IACUC-20211104–31) and carried out in strict accordance with the national guidelines for animal experiments. Significant endeavor was contributed to reducing the number of animals used and their suffering.

Data availability

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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