An Experimental Study on the Inhibition of Wear Particle-Induced Osteolysis by Lycium barbarum Polysaccharide In Vivo

Zige Liu,1 Lin Feng,2 Sai Kiang Yeow,3 and Desheng Chen4

1Department of Clinical Medicine, Guangxi Medical University, Nanning, China
2Department of Painology, Affiliated Hospital of North Sichuan Medical College, Nanchong, China
3Department of Orthopedic Surgery, Sengkang General Hospital, Singapore
4Department of Orthopedic Surgery, People’s Hospital of Ningxia Hui Autonomous Region, Yinchuan, China

Correspondence should be addressed to Desheng Chen; chendesheng@nxmu.edu.cn

Received 9 March 2022; Accepted 3 May 2022; Published 16 June 2022

Objective. To investigate the effect of Lycium barbarum polysaccharide (LBP) in a mouse calvarial osteolysis model and to explore the potential mechanisms involved.

Methods. Eighty C57BL/6J mice were randomly assigned to four groups: Sham control (PBS treatment), Vehicle (titanium/PBS treatment), Low-LBP (titanium/50 mg kg⁻¹ day⁻¹ LBP), and High-LBP (titanium/100 mg kg⁻¹ day⁻¹ LBP). After 2 weeks, mouse calvariae were collected for microcomputed tomography (micro-CT) and histomorphometry analysis. The levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 were measured by enzyme-linked immunosorbent assay (ELISA).

Results. LBP significantly reduced titanium-particle-induced osteolysis compared with the Vehicle group as confirmed by micro-CT and histomorphometry data. Additionally, high osteoprotegerin (OPG) and low receptor activator of nuclear factor kappa-Β ligand (RANKL), TNF-α, IL-1β and IL-6 were noted in LBP treatment groups.

Conclusion. LBP inhibited wear particle-induced osteolysis in mice and suppressed the expression of inflammation-related factors; this inhibitory effect of LBP may be achieved with the regulation of OPG/RANKL pathway and inhibition of inflammatory factor production.

1. Introduction

Peri-implant osteolysis (PIO) remains the most common complication following total joint arthroplasty (TJA), which can lead to loosening and failure of the artificial joint prosthesis [1, 2]. It is generally accepted that this pathophysiology is caused by a chronic inflammatory response to wear debris, including polyethylene, polymethylmethacrylate, cobalt, chromium, and titanium (Ti), which are produced on the surface of the prosthesis [3]. In general, wear debris induces the recruitment of macrophages, fibroblasts, osteoblasts, and osteoclasts and stimulates the secretion of high levels of cytokines and chemokines by these cells, including interleukin-6 (IL-6), IL-1β, tumor necrosis factor-α (TNF-α), and prostaglandin E2 [4]. These factors can directly or indirectly stimulate osteoclast overproliferation and bone resorption. Considering the importance of inflammation and osteoclast formation in the pathophysiology of PIO, inhibitors that inhibit inflammatory osteoclast formation or function would be potential candidates for the prevention of wear debris-induced osteolysis and subsequent aseptic loosening.

Recently, researchers have turned their attention to plant-based bioactive derivatives with immunomodulatory abilities. Lycium barbarum, also known as wolfberry, is a common traditional Chinese medicine and an edible food; it has been used throughout history to treat and prevent diseases such as insomnia, liver dysfunction, diabetes, and visual degeneration. One of its bioactive components is the Lycium barbarum polysaccharide (LBP), a polysaccharide-protein complex extracted from Lycium barbarum [5], which has been shown to have anti-inflammatory properties that inhibit chronic inflammation-related damage [6], as well as other promising therapeutic properties, including enhanced immunomodulation [7], antitumor [8], antistress [9], antiaging [10], and antioxidant [11]. As PIO is a chronic
inflammatory polyarthritis, we hypothesized that LBP could modulate the inflammatory response and provide therapeutic protection. Therefore, the aim of this study was to determine the potential of LBP as a protective agent and to investigate the potential mechanisms using a well-established mouse model of murine calvarial model of Ti-particle-induced osteolysis.

2. Materials and Methods

2.1. Ti Particles. Ti particles were obtained from Alfa Aesar Company (Ward Hill, MA, USA). A scanning electron microscope (Hitachi FESEM S-4800, Hitachi, Kyoto, Japan) and a transmission electron microscope (Hitachi H-7650; Hitachi; Kyoto, Japan) were used to measure particle size, and more than 90% of particles were <10μm, which is the most common clinical size range (Figure 1). Ti particles were soaked in 75% ethanol for 48 h and rinsed four times with sterile ultrapure water at room temperature, then soaked in 75% ethanol for 48 h and rinsed four times with sterile ultrapure water at room temperature, then fired at 180°C for 6 h. Limulus assays (Chromogenic End-point TAL with a Diazo coupling kit, Xiamen Houshiji, Fujian, China) were performed to ensure that the pellets were free of endotoxin (<0.25 EU/ml). Subsequently, the pellets were mixed with sterile phosphate-buffered saline (PBS) in the appropriate ratio, then tightly sealed and stored at 4°C until use.

2.2. Surgery Procedure. All animals were treated according to the Institutional Animal Care and Use Committee of Ningxia Medical University. The Ti particle-irritated calvarial model was established as previously described [12]. In brief, eighty C57BL/6j female mice were randomly assigned to four groups: Sham control (PBS treatment), Vehicle (Ti/PBS treatment), Low-LBP (Ti/50 mg kg-1 day-1 LBP (Sigma, St. Louis, MO, USA), and High-LBP (Ti/100 mg kg-1 day-1 LBP). Mice were anesthetized with pentobarbital (50 mg/kg). Then a 10 mm midline sagittal incision over the parietal bone was cut. Mice in the sham group were sutured without any intervention. Mice in the other groups received 30 mg of Ti particles on the calvariae of each mouse. Mice in the LBP treatment groups underwent intraperitoneal administration of LBP during 3:00 pm–5:00 pm for consecutive 14 days, and mice in untreated groups were given PBS only. During the experiment, dark/light was set as 12 h/12 h, and water and food were given ad libitum. Peripheral blood was collected prior to sacrifice on day 14 postoperatively for serological assessment, and the calvariae were harvested for radiological and histological analyses.

2.3. Radiological Analyses. Fixed specimens (n = 5 per group) were analyzed with high resolution micro CT (SkyScan1176; SkyScan, Kortich, Belgium). The calvaria were scanned with 9 μm per layer. The X-ray parameters were set at voltage of 50 kV with a current of 500 μA together with 0.7° rotational step. Then, a round region of interest (ROI), 3 mm in diameter through the midline suture, was chosen to perform related analysis including the number of pores, bone volume (BV), bone volume/tissue volume (BV/TV), and bone mineral density (BMD).

2.4. Histological and Immunohistochemical Analysis. After being fixed in 4% polyoxymethylene (pH7.4) for 2 days, calvariae (n = 5 per group) were decalcified in 10% Ethylene Diamine Tetra acetic Acid (EDTA, Sigma) for 4 weeks, and then specimens were processed for dehydrating in graded alcohols, cleared in dimethyl benzene, and embedded in paraffin. Calvariae were cut in 5μm thick to perform hematoxylin and eosin (H&E) staining and tartrate-resistant acid phosphatase (TRAP) staining. Section images (4 consecutive sections per calvaria) were captured microscopically. The ROI was set around the center of the middle suture as previously described [13]. The eroded bone surface area (mm²) and bone thickness (BT, mm) were quantified and determined with the protocols introduced by Kanis et al. [14]. Dark-purple stained granules intensely distributed at the osteolytic sites were deemed as osteoclasts. Additionally, the percentage of osteoclasts per bone surface (OcS/BS, %) was determined using the method established previously [15]. To identify the expression of RANKL, OPG (all purchased from Abcam, Cambridge, UK), immunohistochemistry staining was performed. After dewaxing and gradient hydration, sections were immersed with corresponding primary antibodies for 12 h at 4°C. After rinsing, the sections were incubated for 30 min with a biotin-conjugated secondary antibody (Sigma-Aldrich, St. Louis, MO) at 37°C. Streptavidin-horseradish peroxidase conjugate (SA-HRP) was applied to the sections for 30 min at 37°C; then 3,3′-diaminobenzidine tetrahydrochloride (DAB) was added for color development with counterstaining using hematoxylin. The digital photomicrographs were obtained and analyzed using Image-Pro image analysis software. To observe finer pathological structures, the specimens were washed 3 times in sodium arsenic buffer and then fixed in 1% osmic acid fixation solution for 2 h. Then, they were washed 2 times in dimethyl sodium arsenic buffer at 20 min intervals, with different concentrations. After alcohol dehydration and propylene oxide penetration treatment, the resin was embedded in an oven at 42°C overnight. Continuous ultrathin sectioning was conducted under an optical microscope, and the slices were placed on a copper mesh coated with formvar film. The sections were then observed under a transmission electron microscope (TEM, Hitachi H-7650, Hitachi, Kyoto, Japan).

2.5. Serum Analysis. Blood specimens (n = 10 per group) were collected just prior to euthanasia. After centrifuging, serum was collected and deposited at -80°C before further assessment. ELISA analysis was performed with kits specific for TNF-α, IL-1β, and IL-6 secretion (all purchased from eBioscience, San Diego, CA, USA) according to the manufacturer's instructions. Optical density was measured at 450 nm using a microplate reader (Thermo Fisher Scientific, Inc.)

2.6. Statistical Analysis. All data are measured by the mean ± standard deviation. Prior to further evaluation, the Kolmogorov-Smirnov test was used to verify data normality. Two-tailed Student's t-test was utilized to make comparisons between groups and one-way analysis of variance...
(ANOVA) to perform multiple comparisons. SPSS 22.0 was used to perform related calculations. The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Micro-CT Evaluation of Osteolysis. Micro-CT images revealed that clear bone resorption had occurred mainly in the midline suture of calvaria in the Ti group (Figure 2(a)). In the LBP treatment groups, particle-induced osteolysis was inhibited in a dose-dependent manner, whereby bone resorption in mice treated with the High-LBP concentration was much less than in mice treated with the Low-LBP concentration, BMD and BV/TV increased, and the number and percentage of pores within ROI decreased (Figures 2(b)–2(e)).

3.2. Bone Histomorphometry. Histological assessment and histomorphometry analysis further confirmed that LBP treatment protected against Ti particle-induced bone resorption. H&E staining revealed that a large number of inflammatory cells infiltrated into the region of Ti particle-stimulated calvarial osteolysis (Figure 3(a)). In addition, TRAP staining also suggested that multiple TRAP-positive cells were lined up along the erosion surface in vehicle group (Figure 3(b)). At the transmission electron microscope results, it was observed in this experiment that the number of osteoclasts in the PBS group was small, and their nuclei were small. There was no obvious abnormal change in the organelles in the cytoplasm, and the number of lysosomes was relatively small. In the Vehicle group, the number of osteoclasts was significantly increased, wrinkled edges were seen on the surface of the cell.
membrane, the nuclei were enlarged, the cytoplasm was abundant, and large amounts of rough endoplasmic reticulum and mitochondria were seen in the cytoplasm. Osteolytic changes can be seen on the surface of the bone tissue. In the LBP-treated group, the number of osteoclasts was small, while the rough endoplasmic reticulum, lysosomes, and mitochondria were relatively reduced, and osteoclast function decreased (Figure 3(c)). However, the area of bone erosion, the number of osteoclasts, and the OCs/BS in both the Low-LBP and High-LBP treated groups were all decreased when compared with those in the vehicle group (Figures 3(d)–3(g)). These results indicate that treatment with LBP effectively alleviated the wear debris induced inflammatory response and bone loss in the mouse calvarial model.

3.3. Immunochemical Analysis of RANKL and OPG. The analysis of immunohistochemical staining in mouse calvariae revealed a modest increase in the number of OPG-
positive cells after intracranial injection of Ti particles, while a large excess of RANKL-positive cells was observed. The LBP-treated group showed a marked decrease in the number of RANKL-positive cells compared with the control group, but a marked increase in the density of OPG-positive cells compared with Ti-stimulated mice (Figure 4).

3.4. ELISA Analysis of TNF-α, IL-1β, and IL-6. We also determined the level of serum infection marker TNF-α, IL-1β, and IL-6. The levels of TNF-α, IL-1β, and IL-6 were elevated in the Vehicle group when compared to the Sham group. Treatment with LBP reduced TNF-α, IL-1β, and IL-6 expression in a dose-dependent manner (Figure 5).

4. Discussion

Periprosthetic wear and complex biological reactions caused by wear particles remain the most common complications, limiting postarthroplasty, and are the main cause of secondary revision surgery. Many efforts have been made by scholars all over the worlds, such as improving prosthesis design and biomaterials as well as additional fixation and improved surgical techniques. However, none of these methods can completely eliminate the production of abrasive particles on the surface of the prosthesis [16]. Furthermore, new materials are ineffective in the treatment of osteolysis that has been produced. Although the exact
Figure 4: LBP modulated the expression of RANKL and OPG in the calvariae of mice. (a) Immunostaining for RANKL (scale bar = 100 μm). (b) Immunostaining for OPG (scale bar = 100 μm). (c) RANKL-positive cells (mm²). (d) OPG positive cells (mm²). (e) RANKL/OPG ratio was determined. *p < 0.05; **p < 0.01. The black arrows indicate positive expression, respectively, and the red arrows indicate Ti particles.

Figure 5: LBP reduced inflammatory cytokine expression, including TNF-α, IL-1β, and IL-6. *p < 0.05; **p < 0.01.
mechanism is unknown, wear-induced chronic inflammation, activation of osteoclast activation, and enhanced bone resorption by osteoclasts are important factors in the etiology of aseptic loosening [17]. Therefore, inhibition of osteoclast activation is considered a viable therapeutic strategy at its core. Despite extensive efforts by researchers, the current search for drugs is unsatisfactory, and many potentially serious side effects limit their further application [18]. Recent studies have identified natural compounds in therapeutic development due to their multiple functions and relatively safe properties and because of their natural origin and affordability [19].

Since the beginning of ancient China, *Lycium barbarum* has been a familiar medicinal herb to the common people, in which LBP, betaine, and Lycium pigment are the main active ingredients. The pharmacological value of LBP is more prominent, with functions such as antiaging, antihypoxia, and immunity regulation, and it is most appreciated for its strengthening effect on tendons and bones [7]. It is not difficult to understand that the pathological basis of any disease involving osteolysis and osteoporosis lies in (1) the enhancement of osteoclastic effect and (2) inhibition of osteogenesis. Although the pathogenesis of osteoporosis and aseptic loosening are different, both of them have abnormal osteoclast function and imbalance between osteoblast and osteoclast functions. Our study takes LBP as the research object, intends to further investigate its osteoprotective, antiosteolysis mechanism of action, provides the animal experimental basis for developing the medicinal value of LBP, and also looks for new therapeutic drugs for the clinical treatment of aseptic loosening.

In accordance with this study, Ti particle suspension was injected directly on the surface of calvarial bone, and excessive bone resorption was observed by using micro-CT and H&E staining in the calvariae of the Vehicle group. In contrast, histological analyses revealed that LBP decreased the eroded bone surface of the calvariae. Similarly, increased BMD and bone volume and a decreased number of pores further confirmed that the absorptive action was hindered in the LBP treatment group, which suggests that LBP exerts a protective effect on osteolytic disease. TRAP staining showed that there were some inflammatory reactions around the surface of the calvarial with the Ti particles, the number of osteoclasts increased, a few clusters of macrophages and lymphocytes and other inflammatory cells infiltrated, and there was more osteolysis. Previously, micron-sized particles were considered the main particles that induce osteolysis, but the introduction of laser capture microdissection technology and TEM has led to the detection of various nanosized particles in loosened boundary membrane tissues, including metal, ceramic granular, and polymer polyethylene nanoparticles. Because nanoparticles are more easily engulfed by phagocytes than other particles, they may play a considerably significant role in aseptic loosening [20]. In this study, we used particles including more than 90% of particles were <10 μm, which is the most common clinical size range. The results of transmission electron microscopy showed that the number of osteoclasts was significantly increased in the Vehicle group, the nucleus was enlarged, and a large number of rough endoplasmic reticulum and mitochondria were visible in the cell pulp; the lysosomes were also significantly increased. Osteoclasts were functionally active and attached to the bone tissue surface, and osteolytic changes were visible on the bone tissue surface. In contrast, the number of osteoclasts in the LBP-treated group was reduced, while the number of rough endoplasmic reticulum, lysosomes, and mitochondria was also relatively declined, indicating the function of osteoclasts was decreased.

RANKL is a ligand for RANK and is a key regulator of osteoclast differentiation and maturation. OPG is an inducible receptor for RANKL and can also bind to RANKL. Osteoclastic bone resorption is accomplished by the interaction of RANK and RANKL, and OPG can inhibit this process. The three of them regulate each other, thus maintaining the normal bone metabolic process of the body [21]. The RANKL/OPG ratio is used to determine the effect of RANKL/OPG on osteoclasts, and the change in the RANKL/OPG ratio is consistent with the level of osteoclast differentiation and proliferation and opposite to apoptosis. Periprosthetic boundary membrane cells induced by wear particles can release a variety of cytokines associated with the inflammatory response, which can ultimately contribute to aseptic loosening of osteoclast precursor cells by acting on the OPG/RANKL/RANK system and further disrupting the balance between RANKL and OPG. Activation of the OPG/RANKL/RANK system was found to exist in bone tissue in multiple osteolysis models. After gene knockdown of RANKL, osteoclast activation is reduced, bone resorption is decreased, and significant osteosclerosis occurs. Our immunohistochemical results observed a slight increase in OPG expression, a significant increase in RANKL expression, and a decrease in OPG/RANKL ratio in cranial bone tissue after stimulation with Ti particles, suggesting enhanced osteoclast activity, which is consistent with the experimentally observed destruction of cranial osteolysis induced by titanium particles and with previous findings. In contrast, the administration of LBP resulted in a significant increase in OPG expression, a decrease in RANKL expression, and an increase in the OPG/RANKL ratio, which is consistent with the improvement of cranial osteolysis after LBP treatment. Thus, the role of OPG and RANKL in Ti particle-induced osteolysis was verified. Cytokines and chemokines are important immune modulators released from immune cells in response to infection, and the proinflammatory cytokines, including TNF-α, IL-1β, and IL-6, are known to be potent immunomodulators in activated macrophages, and they are also the key cytokines involved in periprosthetic osteolysis induced by wear debris [22]. Given that LBP can modulate the OPG/RANK/RANKL pathway in bone tissue, we hypothesized that LBP inhibits Ti-induced associated inflammatory cytokines, thereby reducing the production of osteoclasts in calvaria, resulting in a significant reduction in the area of osteolysis. After we determined the level of serum infection markers TNF-α, IL-1β, and IL-6. The levels of TNF-α, IL-1β, and IL-6 were elevated in the Vehicle group when compared to the Sham group. Treatment with LBP reduced TNF-α, IL-1β, and IL-6 expression in a dose-dependent manner. Considering the role of inflammatory
factors in joint implantation-induced osteolysis, we hypothesized that the protective effect of LBP on bone and joint might be related to its inhibition of inflammatory response and reduction of inflammatory cytokine release. Therefore, reducing the inflammatory response and inhibiting the release of inflammatory factors may be one of the mechanisms by which LBP inhibits wear particle-irritated calvarial model.

5. Conclusion

Our study confirms that LBP has an inhibitory effect on wear particle-irritated in mice, and this inhibitory effect may be related to the modulation of OPG/RANKL system and inhibit the release of inflammatory factors. It can be seen that LBP may be a potential one of the future drug candidates for the treatment or prevention of wear particle-induced osteolysis.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

Authors’ Contributions

Zige Liu and Lin Feng contributed equally to this work.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (grant no. 82060408).

References


