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Berberine Inhibits Lipopolysaccharide- and Polyethylene Particle-Induced Mouse Calvarial Osteolysis *In Vivo*

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Background. **Wear particle-induced osteolysis could lead to the aseptic loosening of implants. Studies have suggested that endotoxins, such as lipopolysaccharides (LPS), may be the primary causes of wear particle-mediated osteolysis, and that osteolysis may originate from subclinical levels of bacterial infection. However, effective therapies against wear particles and gram-negative bacterial or LPS-induced bone resorption are limited.**

Materials and Methods. **In the current study, the effect of berberine on LPS- and polyethylene (PE) particle-induced osteolysis *in vivo* was investigated using a mouse calvarial model. Osteoclast number per bone perimeter and eroded surface per bone surface were measured.**

Results. **Berberine (10 mg/kg), injected either simultaneously with LPS or 3 d after LPS (25 mg/kg) treatment, blocked LPS-induced osteoclast recruitment and bone resorption in the mouse calvarial model. A daily single-dose of berberine (10 mg/kg), injected either simultaneously with PE particles or 4 d after treatment with PE particles, blocked PE particle-induced osteoclast recruitment and bone resorption. Berberine treatment markedly decreased LPS and PE particle-induced osteoclast recruitment and bone resorption in the murine calvarial model.**

Conclusion. **These results suggest that berberine may have therapeutic effect for osteolysis induced by wear particles and LPS in gram-negative bacteria.**

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INTRODUCTION

Total joint arthroplasty (TJA) is a widely successful procedure that reduces pain, restores mobility, and enables arthritis patients to engage in daily activities. Wear particle-induced osteolysis, characterized by osteoclastic osteolysis and excessive bone resorption, leads to aseptic loosening. This condition is the major cause of TJA failure, resulting in approximately 50,000 revision surgeries per year in the Unites States [1].

Studies have suggested that the biological responses induced by wear particles may be caused by adherent endotoxins because these chemicals present the same effects as wear particles [2]. Another study has suggested that endotoxins adhering to orthopedic implants are not the major causes of bone resorption considering that the endotoxins that adhere to the implant during the manufacturing process could be eliminated or deactivated after 3 wk *in vivo* [3]. However, systemic circulating endotoxins derived from intestinal flora, minor infections, or dental procedures may bind to wear particles and continuously provoke bone resorption [4]. A classic bacterial endotoxin is lipopolysaccharide (LPS), a biologically active substance found at the outer cell membrane of gram-negative bacteria. Gram-positive bacteria also produce molecules, such as lipoteichoic acid and peptidoglycans, with very similar biological effects [5] to those of LPS.

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Numerous microbiological studies have focused on aseptic loosening, which partly show subclinical levels of bacterial infection from low-virulent microorganisms. However, a number of studies have also documented the presence of bacteria on the surface of various orthopedic implants without any signs of clinical infection [6]. More sensitive methods have indicated that these microbiological studies have likely underestimated the incidence of bacteria in aseptic loosening [7]. The potential importance of subclinical levels of bacteria is emphasized by a study of over 10,000 TJAs, illustrating that the inclusion of antibiotics in the bone cement used for implant fixation reduces the frequency of aseptic loosening by approximately 50% [8]. In most cases, infectious bone resorption caused by bacteria is clinically indistinguishable from wear particle-induced bone resorption.

The mechanisms of osteolysis caused by LPS and wear particles are well documented, but effective therapies remain limited. Identifying drugs that inhibit osteolysis caused by wear particles and LPS in gram-negative bacteria remains a major goal in the prevention of bone resorption in infectious and aseptic bone diseases.

Renewed interest in natural substances has currently drawn attention toward plants rich in bioactive compounds because of their antimicrobial properties. Plants produce an enormous array of secondary metabolites, and a significant part of this chemical diversity serves to protect plants against microbial pathogens [9]. Berberine is a major isoquinoline alkaloid and an active constituent of clinically important medicinal plants, including *Coptis chinensis*, *Phellodendron japonicum*, *Berberis aquifolium*, *B. aristata*, and *Coptidis rhizoma* [10]. The extracts and decoctions of these plants have been used to treat diarrhea, gastroenteritis, and inflammation for at least 3000 years [11]. Evidence suggests that berberine can treat diabetes [12] and may reduce blood cholesterol levels of hypercholesterolemia patients [13]. This compound presents various pharmacologic advantages, including antineoplastic effects [14] from apoptosis induction in several types of cells [15], antibacterial effects [16], ability to prevent glucocorticoid-induced osteoporosis [17], and potential clinical applications in inflammatory diseases as well as other similar disorders [15, 18, 19]. Accumulated evidence also shows that berberine has potential immunomodulatory effect and anti-chronic inflammatory model of adjuvant arthritis activity [20–22]. These findings suggest that this alkaloid may be useful for the treatment of infectious or non-inflammatory bone diseases.

Little attention has been given to the effects of berberine on osteolysis caused by wear particles and LPS. Thus, the effect of berberine on LPS- and polyethylene (PE) particle-induced bone osteolysis was investigated to ascertain the importance of this alkaloid in infectious and aseptic osteolysis.

MATERIALS AND METHODS

Particles

The PE particles were provided by Dr. Paul H. Wooley (Wayne State University School of Medicine and the John D. Dingle VA Medical Center, Detroit, MI). The particles sizes were between 1.10 and 7.60 μm, 70% of which were smaller than 4.35 μm. These particles can stimulate rapid and extensive phagocytosis in macrophages *in vitro* and may potentially induce periprosthetic osteolysis *in vivo* [23]. The PE particles were processed according to a previous study [24]. To reduce endotoxin contamination, the particles were washed in 100% ethanol for 48 h. The endotoxin levels of the particles were less than 0.1 EU/mL, as determined by a commercial detection kit (Chromogenic End-Point TAL with Diazo Coupling Kit; Xiamen Houshiji, Ltd., Xiamen, China). The particles were washed thrice in phosphate-buffered saline (PBS) and centrifuged. Particles were resuspended in PBS to a final volume of 1 mL and stored at 4°C (200 mg/mL).

LPS-Induced Bone Resorption *In Vivo*

Fifty-six 12-wk-old C57BL/J6 male mice (Shanghai SLAC Laboratory Animal Co., Shanghai, China) were obtained. Prior approval was obtained from the Institutional Animal Care and Use Committee of Shanghai Jiaotong University School of Medicine.

Twenty-eight 12-wk-old C57BL/J6 male mice were equally randomized into four groups. The animal model was established as described in literature [25]. The animals received local calvarial injections of LPS (25 mg/kg, *Escherichia coli* 055:B5, Sigma-Aldrich, St. Louis, MO) with and without berberine (10 mg/kg, Sigma-Aldrich). The drug concentrations for the treatments were selected based on our preliminary findings and on reference [26]. The injection site was located over the calvarial sagittal midline suture between the two ears, and PBS was injected as control. The treated mice were divided into four groups, as follows: group 1, PBS alone; group 2, 25 mg/kg LPS; group 3, 25 mg/kg LPS and injected intraperitoneally with a single dosage of 10 mg/kg berberine immediately post surgery; and group 4, 25 mg/kg LPS followed by a single dosage injection of 10 mg/kg berberine 3 d post operation.

PE Particle-Induced Bone Resorption In Vivo and Surgical Procedure

The calvarial model of PE particle-induced osteolysis was used in 28 12-wk-old C57BL/J6 male mice. The animals were randomly and equally divided into four groups. Before surgery, the mice were anesthetized with 70 mg/kg ketamine by intraperitoneal injection. A 10 mm incision was made over the calvarial sagittal midline suture. A 1.0 cm $\times$ 1.0 cm periosteum area was exposed and left intact. In the sham control (group I), the incision was closed without any further intervention. Groups II–IV received 20 mg of PE particles. Group III received a daily single-dose injection of 10 mg/kg berberine immediately post surgery, whereas group IV received a daily single-dose injection of 10 mg/kg berberine 4 d post surgery. The incision was then sutured. The mice had free access to water and food. No complications occurred, and all wounds healed uneventfully.

Mice were sacrificed with an overdose of phenobarbital sodium 5 d after initiating treatment in the LPS group or 7 d post operation in PE particle mice. The calvaria of all animals were removed as described previously [27]. The specimens were sectioned (7 μm thick) on the sagittal plane and stained with hematoxylin-eosin (HE) and tartrate-resistant acid phosphatase (TRAP) (Shanghai Rainbow Medical Reagent Research, Shanghai, China) as described previously [24].