Biochemical and Biophysical Research Communications xxx (xxxx) xxx

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Cepharanthine ameliorates titanium particle-induced osteolysis by inhibiting osteoclastogenesis and modulating OPG/RANKL ratio in a murine model

Leming Liao*, Yongpei Lin, Qunhua Liu, Zhiwei Zhang, Yuanhong Hong, Jianguo Ni, Sai Yu, Ye Zhong

Department of Orthopedics, The First People's Hospital of Fuyang, Hangzhou, 311400, PR China

ARTICLE INFO

Article history:
Received 14 July 2019
Accepted 29 July 2019
Available online xxx

Keywords:
Cepharanthine
Osteolysis
Osteoclast
OPG
RANKL

ABSTRACT

Periprosthetic aseptic loosening, caused by wear debris, is one of the most severe complications, generally resulting in implant failure. Extensive osteoclast formation and activation are considered as the cause for periprosthetic osteolysis. However, few approaches have been approved to be used for preventing early-stage periprosthetic osteolysis. In this study, we investigated the preventive effects of CEP on titanium particles-induced osteolysis in a murine calvaria model. This inhibitory effect was confirmed to be realized by attenuating osteoclastogenesis in vivo. In addition, CEP markedly reduced wear particles-induced elevation of receptor activator of nuclear factor kappa B ligand (RANKL)/Osteoprotegerin (OPG) ratio in vivo. In conclusion, these data concluded that CEP demonstrated a preventive effect of CEP on titanium particles induced osteolysis, suggesting that CEP might be a novel therapeutic for periprosthesis loosening.

1. Introduction

Periprosthetic aseptic loosening is the major cause resulting in implant failure of arthroplasty, and eventually needs a revision surgery. It would lead to a heavy economic burden to the society [1]. Generally, implant-generated wear particles, such as cobalt chromium, titanium (Ti) and ultra-high-molecular-weight polyethylene (UHMWPE), are considered to be response for the initiation of periprosthetic osteolysis. These wear particles would induce an inflammatory response by recruiting cells to secrete chemokines and pro-inflammatory cytokines, such as TNF-α and IL-1β [2,3]. Subsequently, the inflammatory response stimulated osteoclastogenesis and elevated bone resorption in the periphery of implanted prostheses, resulting in periprosthetic osteolysis [4]. Thus, inhibition of excessive osteoclast formation is a potential way to prevent periprosthetic loosening.

Osteoprotegerin (OPG)/receptor activator of nuclear factor kappa B ligand (RANKL) system is known to play a crucial role on balancing bone formation and bone resorption during bone remodeling process [5]. RANKL is one of the only two indispensable mediators for osteoclast formation while OPG is a "decoy" receptor competitively binding with RANKL to impair its effects on osteoclastogenesis [6]. Although the particular mechanisms of wear particle-induced osteolysis remains uncertain, increasing evidence has been provided that OPG/RANKL system is included in particle-induced osteolysis [2,6]. Briefly, generated particles from implant surface disrupt the balance of RANKL and OPG, and promote osteoclastgenesis in vivo [7].

Cepharanthine (CEP) is a monomer extracted from a natural herb, Stephania cepharantha Hayata. It has been approved as an adjuvant drug for chemotherapy-induced leukopenia in the clinic and there is no serious adverse side-effect reported so far [8,9]. CEP has exhibited a variety of pharmacological activities, such as anti-inflammatory, anti-viral and anti-allergic effects [10–12]. Recently, several studies have demonstrated that CEP could suppress RANKL-induced osteoclast formation and osteoclastic bone resorption in vitro, and prevent OVX-induced bone loss in vivo [13,14]. However, there is little knowledge of CEP treatment on periprosthetic aseptic loosening. Considering that osteoclast plays an important role in periprosthetic osteolytic process, we hypothesize that CEP might have the ability to prevent periprosthetic osteolysis by inhibiting osteoclastogenesis. Here, we utilized a

* Corresponding author.
E-mail address: liaoleming@126.com (L. Liao).

https://doi.org/10.1016/j.bbrc.2019.07.115
0006-291X/

titanium particles-induced calvaria osteolysis model to explore the effects of CEP on periprosthetic osteolysis.

2. Methods and materials

2.1. Preparation of reagents and Ti particles

CEP was supplied by Selleck Chemicals (Houston, USA), and stored in −20 °C after being dissolved in DMSO. Prior to animal injection, the CEP stock solution was diluted with phosphate buffered saline (PBS). Commercial Ti particles were purchased from Johnson Matthey (Ward Hill, MA, USA), and processed to remove endotoxins as previous reported [15]. To test the residual endotoxin level, we utilized a Limulus Amoebocyte Lysate assay (Biowhittaker, Walkersville, MD, USA) and only chose the endotoxin-free particles for animal experiments.

2.2. Titanium particle-induced mouse calvaria osteolysis model establishment

The animal experiment procedures were approved by the Animal Care and Use Committee of Zhejiang Academy of Medical Sciences following the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH).Sixty 8-week-old male mice were obtained and divided into 4 groups randomly, including Sham (Sham surgery & PBS injection), vehicle (Ti particles & PBS injection), Low Dose CEP (Ti particles & 5 mg/kg CEP injection), and High Dose CEP (Ti particles & 20 mg/kg CEP injection). The animal model was established as previously described [14]. Briefly, 20 mg Ti particles or PBS were used and embedded on the murine calvarial surfaces after being anesthetized with 50 mg/kg sodium pentobarbital. CEP or PBS injection Intraperitoneally was initiated one day after surgery. Buprenorphine (0.3 mg/kg) and carprofen (4 mg/kg) were used for analgesia pre- and post-operatively. All calvaria were harvested after 2-week of CEP or PBS treatment for further analysis.

2.3. Micro-CT analysis

Calvaria (n = 5/group) were collected and fixed in 4% paraformaldehyde (PFA), followed by being analyzed using a Scanco μCT100 scanner (Scanco Medical, Bassersdorf, Switzerland). The isometric resolution was 14 μm, and the X-ray energy was 80 kV and 100 μA. Three dimensional (3D) reconstructions were achieved using Cone Beam Reconstruction software (SkyScan). The thresholds were ranging from 220 to 520 to remove Ti particles and other tissues from 3D reconstructed images [15]. A 3 mm × 3 mm × 1 mm square region around the midline suture were used for quantitative analysis, including bone volume to tissue volume ratio (BV/TV), number of porosity, and percentage of porosity, as previously reported [15].

2.4. Histomorphological analysis

The calvaria were decalcified in 10% (w/v) ethylenediaminetetraacetic acid for a period of 2 weeks after completing micro-CT scanning, and subsequently embedded in paraffin. Five μm sections were cut, followed by being stained with hematoxylin and eosin (H&E) and tartrate-resistant acid phosphatase (TRAP) staining. The images were observed using a light microscopy (Olympus BX51, Tokyo, Japan). The BV/TV and erosion area were measured with H&E staining, and the number of TRAP-positive osteoclasts, and osteoclast surface (Oc.S)/bone surface (BS) were quantified with TRAP staining.

2.5. RNA isolation and quantitative polymerase chain reaction (qPCR)

Five calvaria from each group were frozen in −80 °C immediately after dissection. Total RNA was extracted using an RNAeasy Mini Kit (Qiagen, Valencia, CA, USA) following to the manufacturer's protocol. The cDNA was synthesized using a Double-Strand cDNA Synthesis Kit (Takara, Dalian, China) and qPCR was performed as previously reported [14]. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was utilized as the housekeeping gene and all experiments were performed at least three times. The primer sequences in this study were as follows: GAPDH, forward 5′-ACCCAGAAGACTG TGGATGG-3′ and reverse 5′-CACATTGGGTAGGAACAC-3'; Cathepsin K, forward 5′-CTTCCAATACGTGCAGCAGA-3′ and reverse 5′-TCTTCAGGGCTTTCTCGTTC-3'; Calcitonin receptor (CTR), forward 5′-TGCAGACAACTCTTGGTTGG-3′ and reverse 5′-TCGGTTTCTTCTCCTCTGGA-3'; TRAP, forward 5′-CTGGAGTGCACGATGCCAGCGACA-3′ and reverse 5′-TCCGTGCTCGGCGATGGACCAGA-3'; Nuclear factor of activated T cells c1 (NFATc1): forward 5′-GGCTGCCTTCCGTCTCATAGT-3′ and reverse 5′-CAACGCCCTGACCACCGATAG-3'; OPG, forward 5′-TGAAGCACCGGAGCTGTCCCC-3′ and reverse 5′-AGGCCAAATGTGCTGCAGTTCG-3'; RANKL, forward 5′-TCCTGAGACTCCATGAAAACG-3′ and reverse 5′-CCCACAATGTGTTGCAGTTC-3'.

2.6. Enzyme-linked immunosorbent assay (ELISA) analysis

As reported previously, calvaria (n = 5/group) were collected and placed into a 6-well plate with 2 mL of Dulbecco's modified Eagle's medium (DMEM) containing 1% penicillin and streptomycin separately [16]. After 24 h culture, medium from each well was harvested and measured with RANKL and OPG ELISA kit (R&D Systems, Shanghai, China) according to the manufacturer's instructions.

2.7. Statistical analysis

All data were collected and analyzed by two investigators independently. Data were shown as mean ± standard deviation (SD). All data were analyzed using SPSS 16.0 software (SPSS, Chicago, IL, USA). One-way ANOVA with post hoc Newman−Keuls test was used for statistical analysis to carry out the differences in group comparisons. The value of $^{*}p < 0.05$ and $^{**}p < 0.01$ were considered as statistical significance.

3. Results

3.1. Treatment of CEP suppresses Ti-induced osteolysis in vivo

To explore the effects of CEP treatment on osteolysis, we set up a Ti particles-induced model in murine calvaria and treated mice with PBS, 5 mg/kg CEP or 20 mg/kg CEP. Our micro-CT results showed that extensive bone erosions were observed on the surface of calvaria in the vehicle group, as a result of the presence of Ti (Fig. 1A). However, bone erosions were significantly attenuated with CEP treatment in a dose-dependent manner (Fig. 1A). Quantification of bone parameters, such as BV/TV, number of porosity, and percentage of porosity, also confirmed that CEP could prevent Ti particles-induced bone erosions, particularly in the high dose group (20 mg/kg CEP) (Fig. 1B).

To further confirm the protective effects of CEP on osteolysis, all calvaria were decalcified and prepared for histological assessment. H&E staining exhibited that abundant infiltrated lymphocytes and macrophages were observed in calvaria in the vehicle group, as well as a large number of eroded surface (Fig. 2A). On the contrary, both

Please cite this article as: L. Liao et al., Cepharanthine ameliorates titanium particle-induced osteolysis by inhibiting osteoclastogenesis and modulating OPG/RANKL ratio in a murine model, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2019.07.115

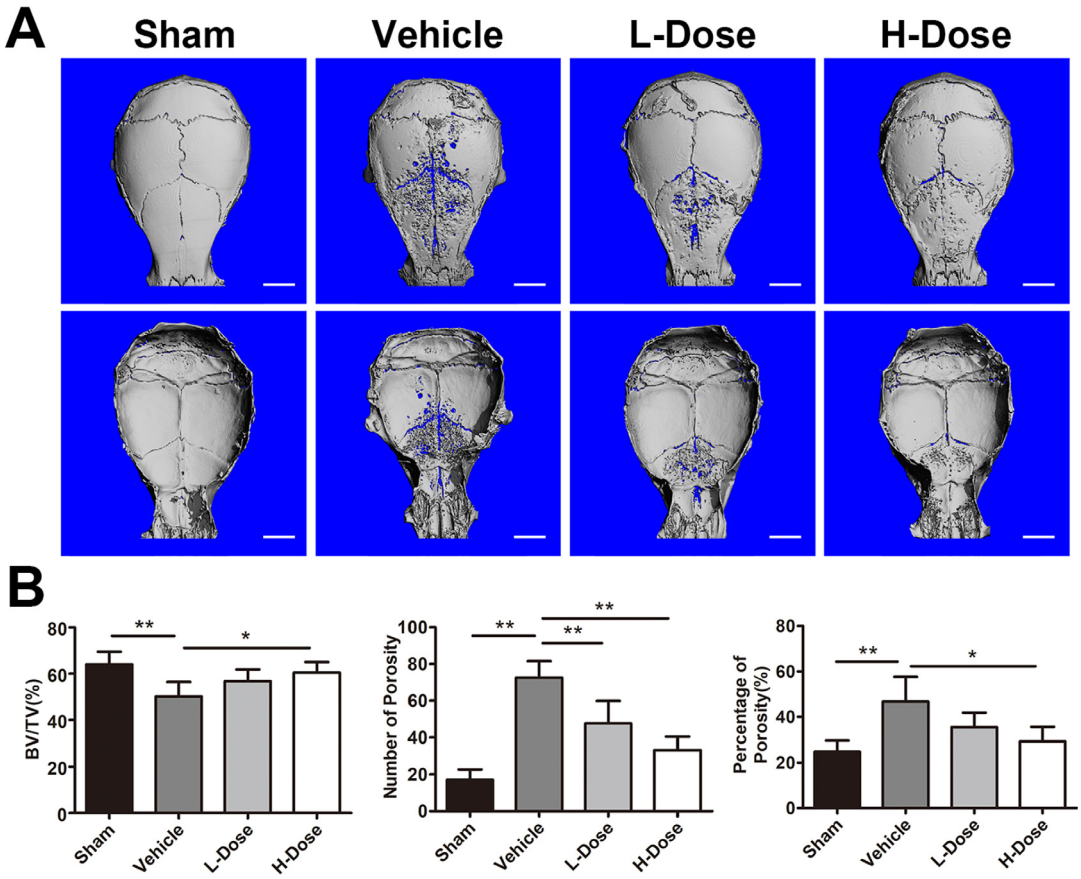

Fig. 1. CEP prevented Ti particles-induced osteolysis radiologically. (A) Representative micro-CT images of calvaria from each group were demonstrated (Scar bar = 5 mm). (B) Bone volume/Tissue volume (BV/TV), the number of porosity, and percentage of porosity were calculated. (n = 5/group). *p < 0.05 and **p < 0.01 were significantly different from the vehicle group.

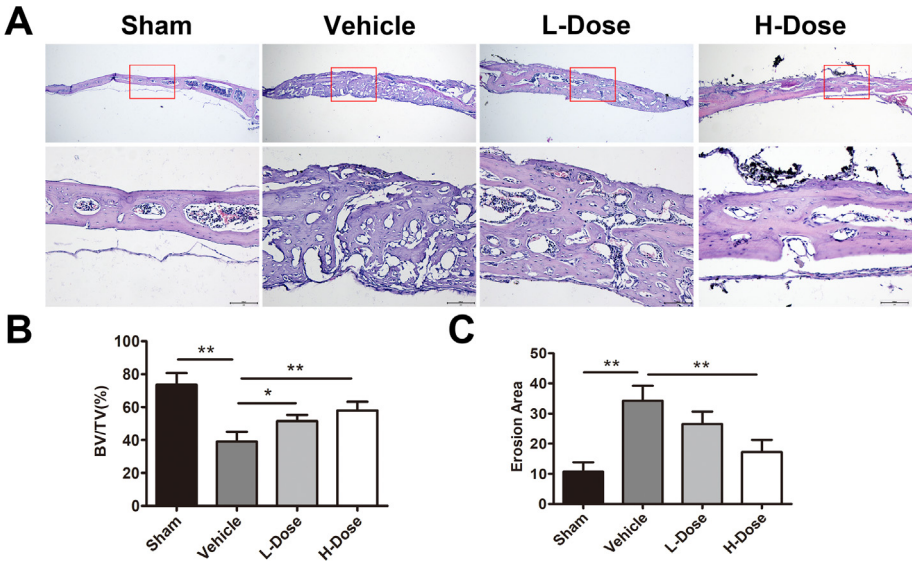

Fig. 2. CEP inhibited Ti particles-induced osteolysis histologically. (A) Representative images of H&E staining were gained from each group. (Scar bar = 100 μm) (B) BV/TV and erosion area of sections from each group were analyzed. (n = 5/group). *p < 0.05 and **p < 0.01 were significantly different from the vehicle group.

the 5 mg/kg dose and 20 mg/kg dose groups impaired lymphocytes, macrophages, and the area of eroded region (Fig. 2A). Quantification of BV/TV and erosion area revealed the preventive effect of CEP on osteolysis in a dose-dependent manner (Fig. 2B–C).

3.2. CEP inhibited osteoclastogenesis in vivo

As the important role which osteoclast plays in wear-induced osteolysis, we next evaluated the osteoclast number and activity

Please cite this article as: L. Liao et al., Cepharanthine ameliorates titanium particle-induced osteolysis by inhibiting osteoclastogenesis and modulating OPG/RANKL ratio in a murine model, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2019.07.115

using TRAP staining in the animal model. As Fig. 3A shown, increased number of osteoclasts was found in the vehicle group, compared with the sham group. Quantifications of TRAP-positive osteoclast number and OC.s/BS further confirmed the bone disruption in the vehicle group (Fig. 3B–C). On the contrary, CEP injections significantly reduced Ti particles-induced osteoclast elevation in murine calvaria dose-dependently (Fig. 3A–C).

3.3. CEP attenuates the gene expression of CTR, TRAP, cathepsin K and NFATc1

As histological data has indicated the suppressive effects of CEP on osteoclastogenesis in Ti particles-induced osteolysis, to further confirm the results, we performed qPCR to explore the mRNA expression of osteoclast marker genes, including CTR, cathepsin K, TRAP and NFATc1. As demonstrated in Fig. 3D–G, all mRNA expressions of these genes were increased in the vehicle group. By contrast, the up-regulated mRNA levels were significantly inhibited with the treatment of CEP in a dose-dependent manner (Fig. 3D–G).

3.4. CEP regulates the expression of RANKL and OPG

As the imbalance between OPG and RANKL plays a crucial in periprosthetic osteolysis [15], we subsequently explore the expression changes of OPG and RANKL with the treatment of CEP. Our data demonstrated that Ti particles markedly elevated the mRNA and protein levels of RANKL, but significantly reduced the mRNA of OPG (Fig. 4A–B). As a consequence, the ratio of RANKL to OPG increased obviously by the induction of Ti particles (Fig. 4A–B). However, the up-regulated mRNA and protein levels of RANKL, induced by Ti particles were reversed after 2-week CEP treatment, as well as the decreased mRNA and protein levels of OPG in murine calvaria (Fig. 4A–B). Thus, reduced ratio of RANKL to OPG, induced by Ti particles, was also reversed by the treatment of CEP (Fig. 4A–B).

4. Discussion

Total joint arthroplasty has been considered as the most effective treatment for end-stage joint disease currently [17]. However, aseptic implant loosening remains to be a major concern and challenge for long-time implant survival, leading to prosthetic failure only with the treatment of revision surgery. As osteoclast plays a crucial role during periprosthetic osteolysis, attention has been paid to developing new potential treatments aiming at suppressing osteoclastogenesis and subsequently inhibiting early-stage periprosthetic osteolysis in recent years. A number of agents have demonstrated their potentials on inhibiting wear particles-induced osteolysis, including sclerostin antibody, bisphosphonates and parathyroid hormone (PTH) [18–20]. Nevertheless, adverse effects of these agents limit the application in clinic

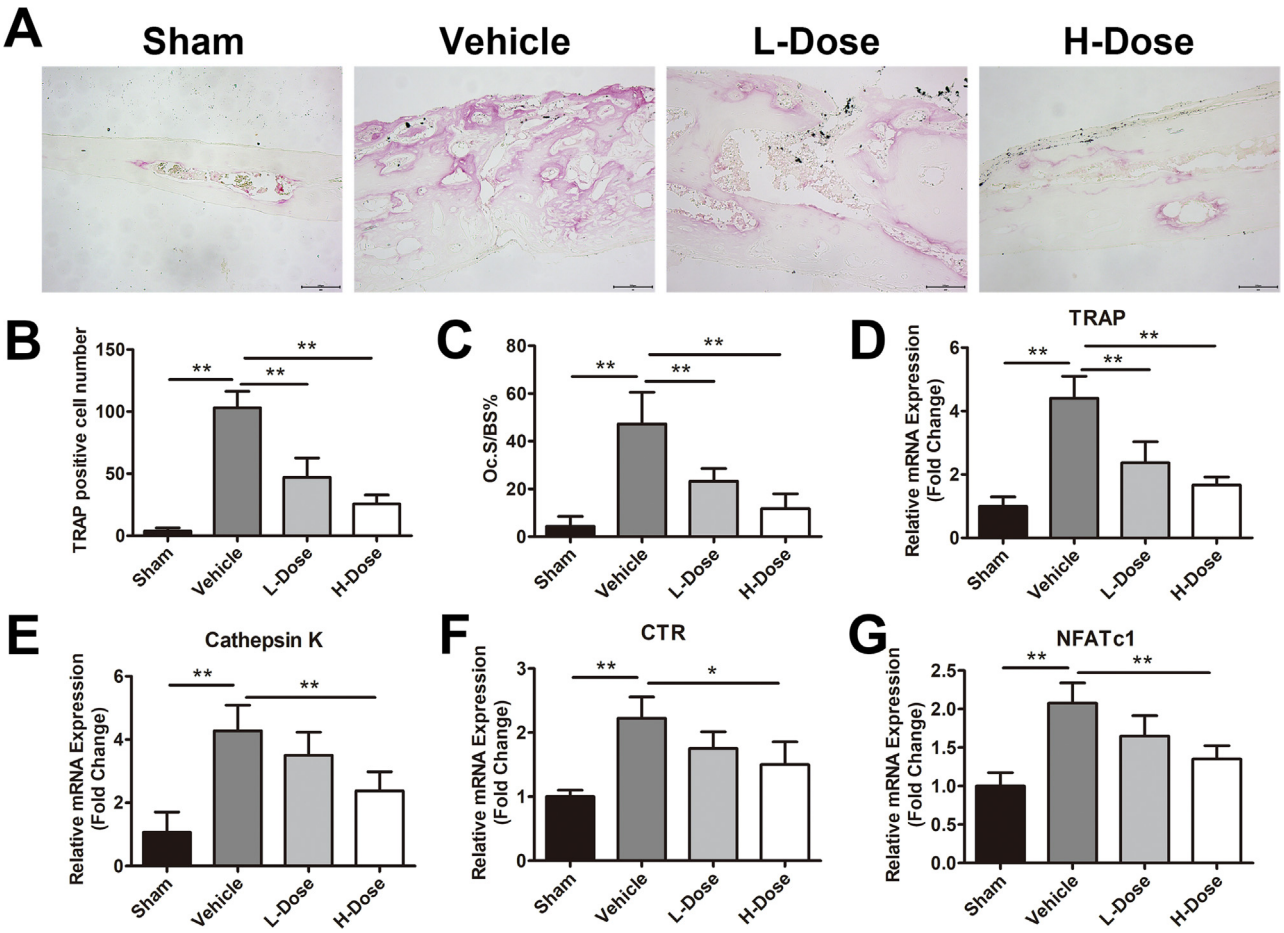

Fig. 3. CEP suppressed Ti particles-induced osteoclastogenesis in vivo. (A) Representative images of TRAP staining from each group were observed. (Scar bar = 100 μm) (B–C) The number of TRAP positive cells and osteoclast surface/bone surface (Oc.S/BS) were analyzed. (n = 5/group). (D–G) The mRNA levels of Cathepsin K, CTR, TRAP and NFATc1 were detected using qPCR. (n = 5/group). *p < 0.05 and **p < 0.01 were significantly different from the vehicle group.

Please cite this article as: L. Liao et al., Cepharanthine ameliorates titanium particle-induced osteolysis by inhibiting osteoclastogenesis and modulating OPG/RANKL ratio in a murine model, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2019.07.115

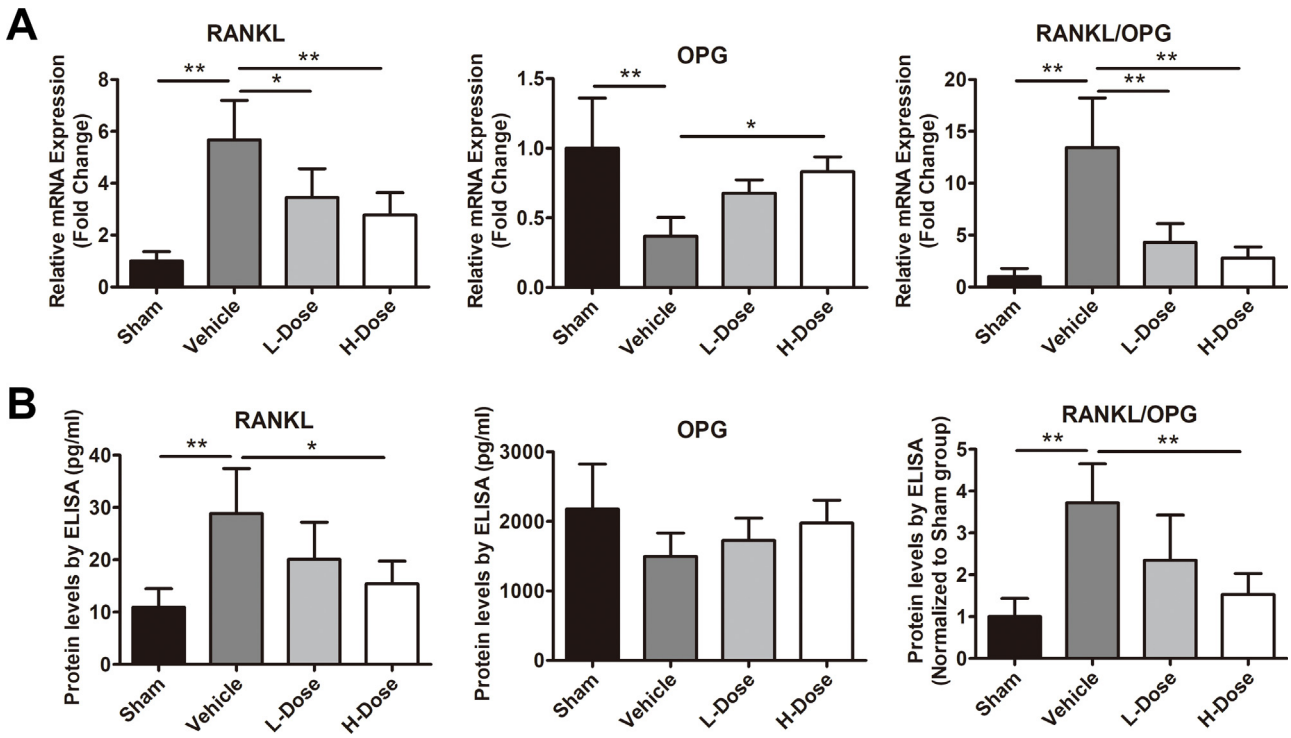

Fig. 4. CEP modulated the expression of receptor activator of nuclear factor kappa B ligand (RANKL), Osteoprotegerin (OPG) and the ratio of RANKL/OPG in vivo. (A) The mRNA levels of RANKL and OPG were detected using qPCR with calvaria from each group. (n = 5/group). The ratio of RANKL/OPG was calculated. (B) The protein levels of RANKL and OPG were detected using ELISA assay with calvaria from each group. (n = 5/group). The ratio of RANKL/OPG was calculated. $^{*}p < 0.05$ and $^{**}p < 0.01$ were significantly different from the vehicle group.

for the treatment of periprosthetic osteolysis, such as jaw necrosis, pathologic fractures and extension of fracture healing [21,22]. Thus, it is highly required to develop a therapeutic with effective action and fewer side effects. Recently, a number of natural compounds have been found to be potential candidates for periprosthetic osteolysis treatment [15,23]. In this study, we have demonstrated that CEP exhibited a suppressive effect on wear particles-induced bone loss by inhibiting osteoclastogenesis in a murine calvaria model. Further, we confirmed that CEP attenuated osteoclastogenesis through modulating OPG/RANKL ratio in the model. Therefore, our study shows that CEP might be a novel agent for preventing and treatment of wear particles-induced periprosthetic osteolysis.

CEP has been used in clinical therapy for at least 40 years in Japan and no adverse effects have been found so far, which provides the evidence that it is safe for human disease therapy [24]. CEP has been proven to exhibit a strong suppressive effect on osteoclast formation and osteoclastic bone resorption in vitroMoreover, a prophylaxis effect on ovariectomy induced bone loss has been confirmed with the treatment of CEP in mice, suggesting that CEP might have the potential to prevent and treat osteoclastic related disease. In this study, we established a Ti particles-induced murine calvarial osteolytic model, which has been widely used as a classical animal model in osteolytic research [25]. As expected, the existence of Ti particles significantly induced bone loss of calvarial bone in mice, as shown by severe bone resorption utilizing micro-CT and H&E staining, while an anti-resorptive effect was demonstrated upon CEP treatment in a dose-dependent manner in the same model. Wear particles-induced osteoclastic bone resorption has been proven to play a key role in osteolytic process [26].Therefore, the inhibition of osteoclastogenesis was considered as one of the main explanations for the effect of CEP treatment on osteolytic model. In our study, the number and the size of TRAP-positive osteoclasts on bone surface were significantly increased in Ti-induced mice model, while CEP treatment reduced Ti-induced osteoclastogenesis in vivo. In addition, CEP inhibited the expression of osteoclastic specific genes in vivo, such as TRAP, Cathepsin K, CTR and NFATc1, which in line with our histomorphological data in the study. These results indicated that CEP protected Ti particle-induced osteolysis by suppressing osteoclastogenesis directly.

In addition to the direct inhibitory effects of CEP on osteoclastogenesis, another underlying mechanism is that CEP reduced Ti-induced bone resorption by regulating the balance of OPG and RANKL in this osteolytic model. RANKL initiates osteoclast differentiation when binding to RANK on bone marrow macrophages in the presence of M-CSF, subsequently leading to bone resorption. OPG serves as a decoy receptor of RANKL to block the binding between RANKL and RANK, resulting in the inhibition of osteoclast formation and bone resorption. Thus, the modulation of OPG and RANKL affects the formation and activation of osteoclasts, and thereby bone resorption [27]. Increased level of RANKL and reduced level of OPG have been reported in the presence of wear particles in the periprosthetic tissue and osteolytic animal models, and subsequently promotes osteoclast formation and activation, leading to bone resorption [2,6].In our study, the ratios of RANKL and OPG were significantly elevated both in protein and mRNA levels upon the stimulation of Ti particles, which were consistent with studies previously [15]. However, the treatment of CEP markedly decreased the expression of RANKL and increased the expression of OPG in vivo. Additionally, the RANKL/OPG ratios were also down-regulated with CEP treatment, as well as the number and size of osteoclasts on bone surface, providing the evidence that CEP suppressed Ti-induced osteolysis partly by down-regulating the ratio of RANKL/OPG in vivo.

Please cite this article as: L. Liao et al., Cepharanthine ameliorates titanium particle-induced osteolysis by inhibiting osteoclastogenesis and modulating OPG/RANKL ratio in a murine model, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2019.07.115

While showing these interesting findings, our study still had some limitations. Clinically, UHMWPE debris is much more common than metal debris in the initiation of periprosthetic osteolysis [28]. However, Ti particles could be purchased easily while no commercial UHMWPE is available. Additionally, both metal and UHMWPE have been observed to induce osteoclastogenesis and osteolysis. Therefore, we used Ti particles to establish the animal model.

In conclusion, this study demonstrates that CEP inhibits Ti-particles induced osteolysis by suppressing osteoclast formation and activity in vivo. We also show that CEP exhibits its efficacy partly by regulation the ratio of RANKL and OPG. Therefore, our results indicate that CEP might be a novel and effective compound for periprosthesis loosening prevention.

Conflicts of interest

The authors declare that they have no conflict of interest.

References

[1] K.L. Ong, F.S. Mowat, N. Chan, et al., Economic burden of revision hip and knee arthroplasty in Medicare enrollees, Clin. Orthop. Relat. Res. 446 (2006) 22–28, https://doi.org/10.1097/01.blo.0000214439.95268.59.

[2] W.P. Ren, S.Y. Yang, H.W. Fang, et al., Distinct gene expression of receptor activator of nuclear factor-kappa B and rank ligand in the inflammatory response to variant morphologies of UHMWPE particles, Biomaterials 24 (2003) 4819–4826, https://doi.org/10.1016/S0142-9612(03)00384-3, 2003.

[3] J. Pajarinen, T.H. Lin, T. Sato, et al., Interaction of materials and biology in total joint replacement - successes, challenges and future directions, J. Mater. Chem. B Mater. Biol. Med. 2 (2014) 7094–7108, https://doi.org/10.1039/C4TB01005A.

[4] D.P. Pioletti, A. Kottelat, The influence of wear particles in the expression of osteoclastogenesis factors by osteoblasts, Biomaterials 25 (2004) 5803–5808, https://doi.org/10.1016/j.biomaterials.2004.01.053.

[5] B.F. Boyce, L. Xing, Biology of RANK, RANKL, and osteoprotegerin, Arthritis Res. Ther. 9 (Suppl 1) (2007) S1, https://doi.org/10.1186/ar2165.

[6] T. Zhang, H. Yu, W. Gong, et al., The effect of osteoprotegerin gene modification on wear debris-induced osteolysis in a murine model of knee prosthesis failure, Biomaterials 30 (2009) 6102–6108, https://doi.org/10.1016/j.biomaterials.2009.07.032.

[7] E.R. Kapasa, P.V. Giannoudis, X.D. Jia, et al., The effect of RANKL/OPG balance on reducing implant complications, J. Funct. Biomater. 8 (2017), https://doi.org/10.3390/Jfb8040042.

[8] K. Asukai, A. Kimura, I. Gorai, et al., Effects of massive administration of cepharanthin on chemotherapy-induced leukopenia, Gan to kagaku ryoho Cancer Chemother. 16 (1989) 2583–2587.

[9] R. Suzuki, M. Hara, J. Shindoh, et al., [Effects of cepharanthin on leukopenia and thrombocytopenia induced by chemotherapy in lung cancer patients], Gan to kagaku ryoho Cancer Chemother. 19 (1992) 647–652.

[10] M. Okamoto, M. Ono, M. Baba, Suppression of cytokine production and neural cell death by the anti-inflammatory alkaloid cepharanthine: a potential agent against HIV-1 encephalopathy, Biochem. Pharmacol. 62 (2001) 747–753.

[11] Y.B. Zhou, Y.F. Wang, Y. Zhang, et al., In vitro activity of cepharanthine hydrochloride against clinical wild-type and lamivudine-resistant hepatitis B virus isolates, Eur. J. Pharmacol. 683 (2012) 10–15, https://doi.org/10.1016/j.ejphar.2012.02.030.

[12] K.R. Paudel, R. Karki, D.W. Kim, Cepharanthine inhibits in vitro VSMC proliferation and migration and vascular inflammatory responses mediated by RAW264.7, Toxicol. In Vitro : An Int. J. Publ. Assoc. BIBRA 34 (2016) 16–25, https://doi.org/10.1016/j.tiv.2016.03.010.

[13] X.X. Lin, F.M. Song, L. Zhou, et al., Cepharanthine suppresses osteoclast formation by modulating the nuclear factor-B and nuclear factor of activated T-cell signaling pathways, J. Cell. Biochem. 120 (2019) 1990–1996, https://doi.org/10.1002/jcb.27495.

[14] C.H. Zhou, J.H. Meng, Y.T. Yang, et al., Cepharanthine prevents estrogen deficiency-induced bone loss by inhibiting bone resorption, Front. Pharmacol. 9 (2018) 210, https://doi.org/10.3389/fphar.2018.00210.

[15] H. Shao, J. Shen, M. Wang, et al., Icariin protects against titanium particle-induced osteolysis and inflammatory response in a mouse calvarial model, Biomaterials 60 (2015) 92–99, https://doi.org/10.1016/j.biomaterials.2015.04.048.

[16] Z. Zhai, X. Qu, H. Li, et al., The effect of metallic magnesium degradation products on osteoclast-induced osteolysis and attenuation of NF-kappaB and NFATc1 signaling, Biomaterials 35 (2014) 6299–6310, https://doi.org/10.1016/j.biomaterials.2014.04.044.

[17] R.M. Urban, D.J. Hall, C. Della Valle, et al., Successful long-term fixation and progression of osteolysis associated with first-generation cementless acetabular components retrieved post mortem, J. Bone Joint Surg. Am. Vol. 94 (2012) 1877–1885, https://doi.org/10.2106/JBJS.J.01507.

[18] F.B. Zhu, X.Z. Cai, S.G. Yan, et al., The effects of local and systemic alendronate delivery on wear debris-induced osteolysis in vivo, J. Orthop. Res. Off. Publ. Orthop. Res. Soc. 28 (2010) 893–899, https://doi.org/10.1002/jor.21062.

[19] S. Liu, A.S. Virdi, K. Sena, et al., Sclerostin antibody prevents particle-induced implant loosening by stimulating bone formation and inhibiting bone resorption in a rat model, Arthritis Rheum. 64 (2012) 4012–4020, https://doi.org/10.1002/art.37697.

[20] F. Bi, Z. Shi, C. Zhou, et al., Intermittent administration of parathyroid hormone [1-34] prevents particle-induced periprosthetic osteolysis in a rat model, PLoS One 10 (2015), e0139793, https://doi.org/10.1371/journal.pone.0139793.

[21] M. O'Halloran, N.M. Boyd, A. Smith, Denosumab and osteonecrosis of the jaws - the pharmacology, pathogenesis and a report of two cases, Aust. Dent. J. 59 (2014) 516–519, https://doi.org/10.1111/adj.12217.

[22] S.B. Goodman, E. Gibon, J. Pajarinen, et al., Novel biological strategies for treatment of wear particle-induced periprosthetic osteolysis of orthopaedic implants for joint replacement, J. R. Soc. 11 (2014) 20130962, https://doi.org/10.1098/rsif.2013.0962. Interface/the Royal Society.

[23] C.H. Zhou, Z.L. Shi, J.H. Meng, et al., Sophocarpine attenuates wear particle-induced implant loosening by inhibiting osteoclastogenesis and bone resorption via suppression of the NF-kappaB signalling pathway in a rat model, Br. J. Pharmacol. 175 (2018) 859–876, https://doi.org/10.1111/bph.14092.

[24] M. Ita, H.D. Halicka, T. Tanaka, et al., Remarkable enhancement of cytotoxicity of onconase and cepharanthine when used in combination on various tumor cell lines, Cancer Biol. Ther. 7 (2008) 1104–1108.

[25] S. Chen, G. Jin, K.M. Huang, et al., Lycorine suppresses RANKL-induced osteoclastogenesis in vitro and prevents ovariectomy-induced osteoporosis and titanium particle-induced osteolysis in vivo, Sci. Rep. 5 (2015) 12853, https://doi.org/10.1038/srep12853.

[26] W.H. Harris, Wear and periprosthetic osteolysis: the problem, Clin. Orthop. Relat. Res. (2001) 66–70.

[27] W.J. Boyle, W.S. Simonet, D.L. Lacey, Osteoclast differentiation and activation, Nature 423 (2003) 337–342, https://doi.org/10.1038/nature01658.

[28] K. Hirakawa, T.W. Bauer, B.N. Stulberg, et al., Comparison and quantitation of wear debris of failed total hip and total knee arthroplasty, J. Biomed. Mater. Res. 31 (1996), https://doi.org/10.1002/(SICI)1097-4636(199606)31:2<257::AID-JBM13>3.0.CO;2-I, 257–63.

Please cite this article as: L. Liao et al., Cepharanthine ameliorates titanium particle-induced osteolysis by inhibiting osteoclastogenesis and modulating OPG/RANKL ratio in a murine model, Biochemical and Biophysical Research Communications, https://doi.org/10.1016/j.bbrc.2019.07.115